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Cells as strain-cued automata

Brian N. Cox^{a,*} and Malcolm L. Snead^b

^aArachne Consulting, Sherman Oaks, CA 91423, USA

^bCenter for Craniofacial Molecular Biology, Ostrow School of Dentistry of USC, Los Angeles, CA 90033, USA

Abstract

We argue in favor of representing living cells as automata and review demonstrations that autonomous cells can form patterns by responding to local variations in the strain fields that arise from their individual or collective motions. An autonomous cell's response to strain stimuli is assumed to be effected by internally-generated, internally-powered forces, which generally move the cell in directions other than those implied by external energy gradients. Evidence of cells acting as strain-cued automata have been inferred from patterns observed in nature and from experiments conducted in vitro. Simulations that mimic particular cases of pattern forming share the idealization that cells are assumed to pass information among themselves solely via mechanical boundary conditions, i.e., the tractions and displacements present at their membranes. This assumption opens three mechanisms for pattern formation in large cell populations: wavelike behavior, kinematic feedback in cell motility that can lead to sliding and rotational patterns, and directed migration during invasions. Wavelike behavior among ameloblast cells during amelogenesis (the formation of dental enamel) has been inferred from enamel microstructure, while strain waves in populations of epithelial cells have been observed in vitro. One hypothesized kinematic feedback mechanism, "enhanced shear motility", accounts successfully for the spontaneous formation of layered patterns during amelogenesis in the mouse incisor. Directed migration is exemplified by a theory of invader cells that sense and respond to the strains they themselves create in the host population as they invade it: analysis shows that the strain fields contain positional information that could aid the formation of cell network structures, stabilizing the slender geometry of branches and helping govern the frequency of branch bifurcation and branch coalescence (the formation of closed networks). In simulations of pattern formation in homogeneous populations and network formation by invaders, morphological outcomes are governed by the ratio of the rates of two competing time dependent processes, one a migration velocity and the other a relaxation velocity related to the propagation of strain information. Relaxation velocities are approximately constant for different species and organs, whereas cell migration rates vary by three orders of magnitude. We conjecture that developmental processes use rapid cell migration to achieve certain outcomes, and slow migration to achieve others. We infer from analysis of host relaxation during network formation that a transition exists in the mechanical response of a host cell from animate to inanimate behavior when its strain changes at a rate that

*Corresponding author. brianlcox@outlook.com (B.N. Cox).

Appendix D. Supplementary data

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exceeds 10^{-4} – 10^{-3}s^{-1} . The transition has previously been observed in experiments conducted in vitro.

Keywords

Strain stimulus; Strain cue; Automaton; Patterning; Morphogenesis; Waves; Migration; Kinematics; Enamel; Ameloblasts; Decussation; Innervation; Angiogenesis

1. Introduction

The depiction of a cell as a “strain-cued automaton” is defined for the purposes of this article by the multi-scale paradigm of Fig. 1. All the chemical and physical actions that occur within a single cell (cell-scale phenomena) are subsumed in an elementary response function for the cell (the “single-cell response function”) (Cox, 2010). The response function prescribes the next action of an individual cell given only the mechanical boundary conditions (tractions and displacements) acting on its surface, which serve as stimuli for the cell’s next migratory action. The mechanical boundary conditions are computed as functions of time by simulating the behavior of a large population of cells (population-scale phenomena), with each cell governed by a common single-cell response function; the mechanical boundary conditions for one cell are determined from the relative motions of all its neighboring cells in the population-scale simulation.

The multi-scale paradigm has the virtue that it couples intra-cell and population-scale phenomena, both of which may be extremely complex, via a response function that can be very simple. In several of the examples reviewed in the following, the single-cell response function is controlled by just one or two scalar parameters. The plausibility of such a simple coupling between scales is rooted in the integral nature of cells.

In the strain-cue theories discussed in this work, chemical factors are absent from the variable set (boundary conditions) used to define the current state of a cell via the single-cell response function. Nevertheless, they are always present implicitly in the multi-scale paradigm. For example, at the population scale, a chemical factor might uniformly permeate a population of cells at some time, causing either the single-cell response function or the constraints on cell motions (e.g., the resistance to motion or history effects) to change for all cells. However, such a chemical effect need not in itself generate a pattern in the population; we will show in the following that cells can form patterns when guided by a single-cell response function that refers to each cell’s mechanical boundary conditions alone, is common to all cells that actively participate in forming the pattern, and is time-invariant over the duration of the pattern forming process. Further remarks on the role of chemical factors in a system of cells acting as strain-cued automata in the paradigm of Fig. 1 can be found in the document “soldiers.pdf”, a thought experiment, published online as Supplementary Material.

This article can be regarded as addressing two ideas. The first is the proposition that:

For certain time intervals and over certain spatial domains, individual cells act as strain – cued automata .

(1a)

The second is the conditional statement:

If each cell in a large population of cells acts as a strain – cued automaton, governed by a common, time – invariant response function, then the population can develop spatial patterns similar to a number of patterns observed in nature .

(1b)

The conditional statement Eq. (1b) can be proven by deductive reasoning: computer simulations of the development of patterns constitute a mathematical proof, executed by numerical methods. On the other hand, the proposition Eq. (1a), that cells do indeed act as strain-cued automata during development or in disease, is not easily proven. However, considerable credence can be found in the extent to which simulations of strain-cued automata match details of morphological development in the cases studied. Furthermore, at the intuitive level, one might foresee that a system known to be as rich in its behavioral potential as the living cell should be capable of acting as a strain-cued automaton, if that were to offer an advantage.

In the proposition Eq. (1a), the phrase “for certain time intervals and over certain spatial domains” is key. Generally, cells are known to respond to chemical, strain, and electromagnetic stimuli, not strain stimuli alone. But in this work, we hypothesize that, during the formation of patterns by certain types of migrating cell, a cell might determine its next migratory choice using only (or predominantly) the information contained in the local strain, or the time rate of change of the local strain, that it senses.

1.1. The breaking of symmetry

In the most widely cited depiction of pattern forming in biological systems, symmetry is broken in a cell population when a spatial pattern arises in some chemical factor: the concentration c_1 of chemical species 1 changes from being uniform in space to being some function $c_1(\mathbf{x})$ of the spatial variable $\mathbf{x}$. The seminal theory for this transition is the reaction–diffusion model of Turing: two or more species, 1 and 2, diffuse through space while simultaneously reacting with each other in reactions that modify their concentrations, c_1 and c_2 (Turing, 1952). Turing’s model needs consider only the concentrations of the chemical species, independently of other factors, to break symmetry and form templates for a wide range of patterns.

In recent theoretical work (Cox, 2010, 2013, 2011; Serra-Picamal et al., 2012), a fundamentally different mechanism for symmetry breaking is demonstrated: if cells in a population migrate by responding to variations in the strain fields $\epsilon(\mathbf{x})$ that arise around them, with each cell governed by the same rule for choosing the direction of its migration given its local strain environment, then the population can spontaneously develop either wavelike disturbances (Cox, 2010; Serra-Picamal et al., 2012), sliding layered patterns (Cox, 2013), or branched, network patterns (Cox, 2011). In these theories, symmetry breaking and patterning result from the development of local strain variations due to the cells' own motions; as the migration of individual cells changes in response to sensed strains, patterns emerge. Just as Turing's model can be formulated entirely in terms of the concentration fields $c_1(\mathbf{x})$, $c_2(\mathbf{x})$, etc., the origin of patterning in strain cues can be demonstrated entirely in terms of the strain fields $\epsilon(\mathbf{x})$ and their time rates of change.

1.2. Cases studied

This review comprises two parts. In Part I, we review demonstrations of wavelike phenomena and the formation of layered patterns in homogeneous populations. The single-cell response function that accounts for wavelike phenomena refers to variations in cell–cell separations, while the single-cell response function that generates layered patterns embodies a feedback effect acting on relative cell sliding or shear displacements. The feedback effect is then compared to other theories of mechanical feedback in systems of only two cells. We finally note that traveling fronts of cell maturation, including the determination fronts that govern many instances of development, can be regarded as traveling strain pulses.

In Part II, we review how strains developed in a host cell population when it is invaded by cells from a separate population might guide the invaders to form cell network patterns.

In the theories of the formation of sliding layered patterns and of the formation of network patterns, the strain fields that guide an individual cell are local to it. The strain fields that guide a cell are generated in its immediate environment largely by the motion of the cell itself. The local nature of the guiding strains makes the strain-cue mechanism robust.

While we demonstrate that strain cues acting alone can generate patterns, more generally strain-cues may act in concert with gradients in chemical factors that act as a second stimulus on an individual cell's membrane. In Part II, we exemplify the potential for generating closed-loop network patterns with diverse character by combining short-range strain cues and long-range chemical gradients in the boundary conditions to which the single cell responds.

The theory of pattern formation via kinematic feedback and the theory of network formation have simple structures, with morphological outcomes depending on only one or two scalar parameters. A central factor in both theories is the ratio of a migration velocity to a relaxation velocity, which describes the rate at which cells move to eliminate density perturbations. In the case of amelogenesis, the migration velocity refers to the rate of advance of the ameloblast population normal to the population sheet, while in the case of invasion, the migration velocity is the rate of advance of invading cells into the host cell

population. The value of the ratio of rates is critical to the desired morphological outcome in both examples.

1.3. Cells as automata vs. cells governed by thermodynamic gradients

When a cell moves, there are two possibilities: it can move as an inanimate object driven by external forces (energy gradients) or it can move as an animate entity using energy generated within itself as part of its own metabolic function. In this article, the term “automaton” applied to a cell means that its motion is enacted by energy that is internally derived and practically unlimited within the context and is not that of an inanimate object impelled by an external energy gradient. The cell may also proliferate (i.e., undergo cell division), differentiate (i.e., change its gene expression profile), or die (i.e., undergo apoptosis) as an automaton (i.e., perform these actions using internal power).

Others have addressed the possibility that gradients in strain energy (either stress in a volume or surface energy) that are generated by cells as they migrate or proliferate might be the symmetry-breaking mechanism underlying pattern formation. In the “differential adhesion hypothesis,” cells of different type are sorted spatially if their cell-adhesion properties differ (Steinberg, 1963; Townes Holtfreter, 1955; Lecuit and Lenne, 2007). Static analysis of the related phenomenon of cell surface tension can account for some patterns among cells of single type (Hilgenfeldt et al., 2008). Mechanical buckling of layers of cells on a soft substrate has been hypothesized to produce periodic patterning in organs (Chen and Yin, 2010).

In the theories of differential adhesion, cell surface tension, and buckling, cells respond inanimately to an energy gradient. The energy gradient acts to provide a pattern-forming mechanism. The theories described in this article differ from such energy-gradient patterning theories in the essential feature that cells, while sensing strain fields, move autonomously, choosing directions guided by the strain fields but often inconsistent with the direction that minimizes strain energy.

1.4. “Cellular automata” models vs. models of cells as strain-cued automata

Many prior physical models of cells behaving as automata have been based on the “cellular automata” concept, where the appellation “cellular” alludes not to the application of the theory to living cells, but rather to the fact that space variables in cellular automata models are represented by a discrete lattice of points; i.e., space is divided for computational purposes into spatial “cells” (Wolfram, 1994, 1983; Olami et al., 1992; Nagel and Schreckenberg, 1992). When interpreted as simulations of living tissues, the spatial cells in a cellular automata model often approximate the locations where individual living cells may be present (Wikipedia, 2014; Wolfram, 2002; Meinhardt, 2003; Simpson, 2007; Treweek and Landman, 2009).

While many structures occurring in nature have been mimicked by cellular automata models, the phenomenon of strain-cued motility, as well as other possible cases of kinematic instabilities, are not appropriately represented on a discrete spatial grid. In the theory of amelogenesis provided herein, the use of a continuous space–time representation permits

stability conditions to be studied in cell populations that are governed by infinitesimal changes in local strains and strain rates.

On the other hand, the theory of network formation by invading cells that act as strain-cued automata, discussed in Part II, is based on a discrete-cell representation of space and can therefore be considered a type of cellular automata model. However, the novel elements are added that strain fields are computed and continually updated for each spatial element in the model (using the mechanics theory of inclusions that contain mismatch strains), while strain-based rules guide the evolution of the system, i.e., guide the change in occupancy of spatial cells when invading cells migrate (Cox, 2011).

1.5. Other continuous-space models

A significant literature deals with the spatial development of cell populations using formulations in which spatial variables are continuous, rather than discretized. Formulations based on variants of the Smoothed Particle Hydrodynamics formulation (Gingold and Monaghan, 1977) are similar in structure to the formulation used to simulate amelogenesis (Section 3.9). Cases analyzed include systems of discrete living cells (Hester et al., 2011) and homogenized populations in which individual cells are not represented (Cristini et al., 2003; Hatzikirou, 2012; Kuhl et al., 2003). However, such studies have not considered the possibility of cells behaving as automata guided by changing local strain fields.

Others have shown that network structures similar to trabecular bone can be generated if cell secretion is correlated with stress variations that arise because of local fluctuations in the fraction of volume occupied by trabecules (Weinkamer et al., 2004). Similarly, the combination of changes in local stresses and the accretion of specialized extracellular matrix can provide a mechanism for the development of nodules and branch-like structures (Cristini et al., 2003; Weinkamer et al., 2004). The network theory discussed in Part II, in contrast, deals explicitly with motility changes at the cell scale, rather than homogenized populations; and neither surface tension nor other energy gradient guides the cells during morphological development.

2. The metabolic energy vs. mechanical strain energy

2.1. Energy estimates

A recent analysis made a direct comparison of the metabolic energy per unit volume per unit time generated in a cell (the metabolic energy density rate, or, for brevity the “metabolic energy rate” $\dot{G}_{\text{met}}$, with the dot indicating time differentiation) and the mechanical energy per unit volume per unit time associated with the strain caused by the migration of cells (the “strain energy rate” $\dot{G}_{\text{el}}$) in several cases of organogenesis (Cox and Smith, 2014). The metabolic energy rate was derived by combining the measured rate of production of ATP from glucose per cell, using data for osteoblasts, with the measured change in free energy for that reaction. Converting into a volume energy density by taking account of the cell’s volume, one finds the range $\dot{G}_{\text{met}} = 4 - 7 \times 10^{-15} \text{ J } \mu\text{m}^{-3} \text{ s}^{-1}$. An earlier estimate for cells in a growing tumor yielded the corroborating value $\dot{G}_{\text{met}} = 7 \times 10^{-15} \text{ J } \mu\text{m}^{-3} \text{ s}^{-1}$ (Narayanan et

al., 2010). The metabolic energy rate for different cells will show some variability, tending to be higher in cells such as osteoblasts or ameloblasts that are actively secreting matrix and in cells in proximity to dense vascular supply.

The strain energy rate $\dot{G}_{el}$ depends on the product of the cell stiffness, the strain in the cell, and the strain rate (time derivative of strain in the cell). Data for cell stiffness from the literature were combined with estimates of strain and strain rate that were either inferred from recent theoretical simulations of cell migration during amelogenesis (Fig. 2a and b) (Cox, 2010, 2013) or network formation (Fig. 2c) (Cox, 2011), or from new, simple appraisals of osteoblast movement in the bone multicellular unit (BMU) at the site of osteoid formation (Fig. 2d) (Cox and Smith, 2014).

The estimates of strain energy rate are likely to be reasonably accurate upper bounds to order of magnitude. Strains pertinent to organogenesis are unlikely to depart in magnitude far from 0.1; given the strain magnitude, the strain rate follows from knowledge of the cell migration velocity, which is relatively easily measured. The stiffness value used is an upper bound, being the maximum stiffness of aggregates of cells and extra-cellular matrix (ECM) that could plausibly suffer strains of magnitude 0.1 (if the ECM is too stiff, the cells cannot do work by moving it) (Cox and Smith, 2014).

The energy comparison is summarized in Table 1. The strain in each case is represented by the typical gauge-averaged value ϵ_0 , with the gauge comparable to the cell size for local strains and to the organ size for global strains. Of the cases studied, those of amelogenesis and the BMU involve cells migrating at moderate velocities ($1\text{--}10\ \mu\text{m d}^{-1}$)¹ (see Cox and Smith (2014) for details of the particular cell motions that were analyzed). In these cases, approximately uniform, slowly changing global strains develop over populations of $100\text{--}10^6$ cells and generate strain energy rates that are 5 orders of magnitude less than $\dot{G}_{met}$. Slightly more rapidly varying local strains develop over gauge lengths comparable to a single cell and generate strain energy rates that are 4 orders of magnitude less than $\dot{G}_{met}$. In contrast to amelogenesis and the BMU, innervation and angiogenesis involve cells with migration velocities among the highest of all cells ($1\text{--}3\ \text{mmd}^{-1}$). For these cells, strains vary relatively rapidly, yet the strain energy rates for local strains remain 2–3 orders of magnitude less than $\dot{G}_{met}$.

Estimates were also made of the rate of change of the cell–cell adhesion energy for a cell migrating at velocities of $100\ \mu\text{md}^{-1}$ (a high, bounding estimate for Paneth-like cells in the intestinal crypt), using recently published data on the force-separation relationship for adhering cells (Benoit and Selhuber-Unkel, 2011). The surface energy change was averaged over the interior volume of a cell that moves from contact with one cell to contact with another, yielding an average rate of change of energy per unit volume for the cell. This quantity has the same units as the strain energy rate $\dot{G}_{el}$ and we represent it by the same

¹In this paper, non-italicized “d” stands for “day”, while italicized “d” refers to cell diameter

symbol. For the gradient in adhesion energy believed to exist in the crypt, the rate of change of adhesion energy per unit cell volume lies 7 orders of magnitude below $\dot{G}_{\text{met}}$.

The maximum change in adhesion energy for the Paneth-like cell in the crypt was assumed to occur over a distance of approximately 25 μm . If instead it is assumed to occur over a distance equal to the size of a single cell (as it might during cell sorting via the differential adhesion hypothesis (Glazier and Graner, 1993)), the adhesion energy density rate rises but is still 6 orders of magnitude below $\dot{G}_{\text{met}}$.

Other entries in Table 1 are estimates for the pulsating heart and one in vitro experiment in which cells were subjected to cyclic strain of magnitude up to 10% at 1 Hz.

For further details, the reader is asked to consult Cox and Smith (2014).

2.2. Rate effects

The results in Table 1 strongly support the depiction of living cells as automata in many common situations in development. When $\dot{G}_{\text{el}}/\dot{G}_{\text{met}}$ is very small, a migrating cell need not move in the direction indicated by a spatial gradient in mechanical energy; it has abundant power to move by other rules.

The values of $\dot{G}_{\text{el}}/\dot{G}_{\text{met}}$ in Table 1 also question the validity of assuming linear elasticity (Hooke's law), or any other simple constitutive law, to relate observable strain to putative mechanical stress. In Part II, we review theoretical and experimental evidence that suggests that in fact a transition occurs in cell behavior on passing from high to low strain rates (or approximately equivalently, using the data of Table 1, from high to low values of $\dot{G}_{\text{el}}/\dot{G}_{\text{met}}$): at strain rates below a critical value $\dot{\epsilon}_{\text{cr}}$, cells maintain low levels of stress that do not change significantly when the cells are strained; whereas at strain rates above $\dot{\epsilon}_{\text{cr}}$, cell reconfiguration during strain application is not fast enough to prevent the stress in the cell rising as the applied strain increases, i.e., the cell responds as though it is an inanimate material. An estimated value of $\dot{\epsilon}_{\text{cr}}$ based on analysis of cases of network formation is in the range 10^{-4} – 10^{-3} s^{-1} (see Section 15). The existence of a transition in cell response at approximately this value of strain rate is confirmed by recent independent experiments and analysis (De et al., 2007; Webster et al., 2014). According to Table 1, this transitional range of strain rates correlates with a critical value of $\dot{G}_{\text{el}}/\dot{G}_{\text{met}}$ in the range 10^{-3} – 10^{-2} .

2.3. Evidence for cells acting as automata

That cells might move against energy gradients, i.e., autonomously, has been illustrated beyond doubt in recent in vitro tests (Weber et al., 2012). A single explanted *Xenopus* mesendoderm cell, bonded to a substrate of fibronectin, was pulled in one direction via an attached magnetic bead. The cell responded by developing a protrusion on the side opposite to the point of application of the force and then migrating in that direction, i.e., in opposition to the applied force. Such behavior of cells in a population cannot be modeled by energy-

conserving constitutive laws relating cell motion to external forces, of the type used to model inanimate engineering materials; in response to the external forces, the cell generates shear tractions between cell membrane and substrate that add energy to the mechanical system and thereby qualitatively changes the

I. PATTERNS IN HOMOGENEOUS POPULATIONS

Part I, comprising Sections 3 – 11, reviews wavelike behavior and the formation of sliding, layered patterns by cells acting as strain-cued automata within a homogeneous population. All theories reviewed follow the multi-scale paradigm of Fig. 1, in which complex infra-cell phenomena are coupled to complex population-scale phenomena (where the outcome of cell–cell interactions is expressed) via relatively simple single-cell response functions. The single-cell response functions specify the next action of a cell using only the information contained in the mechanical boundary conditions (tractions and displacements) prevailing on the cell's membrane.

An exemplary case is pattern-forming among secretory-stage ameloblasts, during the phase of their lives in which they create dental enamel. Attention to enamel is warranted partly by the fact that enamel, alone among all organs, contains a permanent, mineralized record of the trajectory of every migrating cell, which is the essential information for the inverse problem that is solved; and partly by the importance of dental enamel in the fossil record.

But ameloblasts also represent the potential for pattern-forming among any strain-cued cells that inhabit an expanding curved surface with an evolving metric. Cell populations that share these conditions and are involved in symmetry-breaking that is incompletely understood include epithelial cells in an embryo that transforms its shape during gastrulation and any mesenchymal/epithelial sheet that is on the cusp of invagination.

Furthermore, the maturation of ameloblasts into their secretory stage is timed by the arrival of a propagating “commencement front”, across which a stepwise change occurs in the state of strain of cells. The interpretation of the commencement front as a propagating strain pulse may have implications for the “determination front” observed in other cases of morphogenesis (Hester et al., 2011; Baker et al., 2006).

3. Autonomous shear enhancement

That patterns can be formed by a homogeneous population of cells in which each cell responds to local strain variations as a self-energized automaton was recently demonstrated in a theoretical study of amelogenesis (formation of dental enamel) in the mouse incisor (Cox, 2010, 2013). The pattern in the mouse incisor is formed by cells that have organized themselves spontaneously into rows that slide past each other in alternating manner, thus creating a cross-plyed laminar microstructure in the enamel, which is beneficial to tooth function (Boyde, 1989).

In the theory, the layered pattern arises as a kinematic instability, with rules of cell motion, detailed below, expressed entirely in terms of relative cell motions (the relative motions being surrogates for strain and strain rate – see Appendix A of Cox (2013)); neither stress

nor chemical gradients are computed. Predictions of patterns require a continuous rather than cellular (discretized space) representation, because the pattern-forming mechanism relies on the enhancement by feedback of initially infinitesimal variations. The governing equations are coupled nonlinear differential equations in space and time, similar to wave equations with strongly nonlinear terms. Solutions to the governing equations exhibit a transition, i.e., the solution can pass in a discontinuous manner from a trivial spatial ordering to a layered spatial ordering, as the values of two parameters are continuously varied.

In the case of the mouse incisor, a pattern of layers arises in which each layer is orientated approximately normal to the long axis of the tooth, is a single cell thick ($3.5\text{ }\mu\text{m}$), and spans the width of the tooth ($600\text{ }\mu\text{m}$). The pattern is approximately invariant along the long axis and forms in an endless recursion as the tooth erupts during the life of the animal. A gradient in strain energy (or other potential gradient, e.g., electrical) is unlikely to be the pattern forming factor: the cell motions, detailed below, are such that if the cells followed energy gradients, the energy field would have to increase along each layer but in opposite directions from one layer to the next, an improbable situation. Furthermore, the cells are of one type, negating the likelihood of differential cell–cell adhesion (Steinberg, 1963, 2007; Townes and Holtfreter, 1955) guiding the pattern.

3.1. Pattern-forming during amelogenesis in the mouse incisor

Amelogenesis is carried out by a large population of ameloblasts. Secretory-stage ameloblasts (those actively laying down the amelogenin template for enamel) are long and thin and arrayed densely as a monolayer in a curved sheet that displaces outward as enamel forms underneath the cells. In the image of ameloblasts in a rat incisor in Fig. 3a, the overall, expansive migration of the sheet, necessitated by the formation of enamel beneath it, is upwards. The speed of the cell migration normal to the population sheet will be assumed uniform in time and space in the following (which is approximately true (Lacruz and Bromage, 2006)) and denoted v_m . The lower halves of the cells, which stand on the secreted enamel ECM composed largely of amelogenin protein, are parallel to one another and aligned approximately perpendicular to the sheet. In contrast, the upper halves of the cells have already begun to deviate from their initial alignment into alternating rows: scanning across Fig. 3a, alternating cells in the foreground can be seen to tip to left or right. Thus pattern forming (the breaking of symmetry and the forming of rows) is taking place in the upper halves of the cells, away from the secreted amelogenin. As the migration proceeds, cells in different rows slide past one another within the sheet (Fig. 3b), from left to right or right to left in the orientation of Fig. 3a. The top ends of the cells, again in the orientation of Fig. 3a, must also slide or crawl across the lower surface of the stratum intermedium, which is a layer two to three cells thick that forms a carapace over the tips of the ameloblast cells (upper layer of cells in Fig. 3a).

The sliding motion of ameloblasts is presumed to continue and results in the cross-plyed microstructure seen in mouse incisor enamel (Fig. 4a). The pattern is embodied in the arrangement of so-called enamel “prisms” (sometimes called “rods”), each of which represents the mineralized trajectory of a single cell, extending continuously from the inner

to the outer surface of the enamel organ. The enamel organ constitutes an enamel bioceramic that protects the living dentine of the tooth and permits slow wear with chewing.

3.2. Multi-scale approach to simulating migration during amelogenesis

The theory of pattern formation among ameloblasts describes the cells as automata responding to changing mechanical boundary conditions alone (Fig. 1). In the particular case of ameloblasts, where the population-scale behavior results from kinematic feedback, it proves sufficient to include only displacement boundary conditions; tractions must exist, acting between cells that slide relative to one another and also between each cell and the stratum intermedium, but are assumed to be maintained by the cell to be just those required to achieve the displacements specified by the response function and therefore need not be considered as independent variables. That cells can adjust tractions in this manner is supported by energy arguments (Cox and Smith, 2014) and is consistent with their depiction as automata (Section 2).

In the particular multi-scale formulation used to study amelogenesis (Cox, 2010), each cell is represented by a single point x_i identified with its leading tip (Fig. 3b).² Since the rest of the cell, including the amelogenin-secreting Tomes' process at its base, must follow the leading tip, the pathway traced by the leading tip of any cell parallels the motion of its Tomes' process. The trajectory of the tip $x_i(t)$, where t is time, should therefore correspond to the locus of the mineralized prism that is observed in the microstructure of the mature enamel organ; the set of all such prism trajectories defines the pattern that the theory seeks to replicate (Fig. 4a).

The theory solves an "inverse problem" (Tarantola, 2005), namely: find a single-cell response function governing the response of an individual cell to changes in its local strain environment that will lead, when attributed to each cell in the population, to predictions of the observed microstructure of Fig. 4a. A successful rule is not necessarily a unique solution of the inverse problem: other rules might also be successful. However, the existence of a successful rule does prove that the strain-cue mechanism is indeed capable of predicting the pattern observed in tooth enamel.

3.3. Pattern formation by ameloblasts exhibiting strain-cued shear enhancement

The rule (response function) that successfully generates layered patterns (Fig. 4b) describes the autonomous enhancement of shear motion: when a cell detects a shearing or sliding motion relative to any cell it currently contacts within the population, the rule states that it will move to enhance that relative sliding motion (Cox, 2013). The rule operates as a positive feedback mechanism, amplifying initially infinitesimal variations in strain and strain rate (relative cell displacement and relative cell motion) into macroscopic cell velocities.

The question of how such a feedback mechanism might originate within the single cell is discussed in Section 4. Mechanical feedback mechanisms are central in the field of soft

²In (Cox, 2013), the point x_i representing cell i was associated with its center of mass. We are indebted to Dr. A. Boyde for pointing out his image reproduced in Fig. 3a, which implies that a more rational association for x_i is the tip of the cell. The required change to the theory is entirely semantic; all computations and predictions in Cox (2013) remain as they stand.

matter physics (e.g., Marchetti et al., 2013). We also note that the concept that feedback in chemical signaling might play a role in patterning in biology has been recognized (Brandman and Meyer, 2008; Kholodenko, 2006).

The shear enhancement rule is expressed in terms of the positions $\mathbf{x}_i$, $i=1, \dots, N$, of the tips of individual ameloblast cells (Fig. 3b) and the corresponding velocities $\mathbf{v}_i = d\mathbf{x}/dt$, $i = 1, \dots, N$, where N is the size of the population. The relative shear velocity vector between a cell i and any of its contacting neighbors j is defined by (Fig. 5a)

$$\mathbf{v}_{ij}^{(\text{shear})} = \mathbf{v}_j - \mathbf{v}_i - \left\{ (\mathbf{v}_j - \mathbf{v}_i) \cdot \hat{\mathbf{r}}_{ij} \right\} \hat{\mathbf{r}}_{ij}, \quad \hat{\mathbf{r}}_{ij} = (\mathbf{x}_j - \mathbf{x}_i) / |\mathbf{x}_j - \mathbf{x}_i|. \quad (2a)$$

This is the relative velocity of the tip points of the two cells in the plane of the population sheet, projected onto the direction that lies perpendicular to the line joining the cells' tips. Since the cells are constrained to have equal speeds, v_f , in the direction normal to the plane of the population sheet, the relative velocity is affected only by the velocity components within the plane of the sheet (lateral velocities).

The shear stimulus acting on cell i is defined to be the vector sum of the stimuli due to its interaction with each contacting neighbor, weighted by a factor w_j that is proportional to the contact area with neighbor j . The set of cells that contact any particular cell, and the contact areas between cells, change continually during a simulation; they are determined from the current positions of the cell tips $\mathbf{x}_i$, using the Voronoi construction (Fig. 5b) (Voronoi, 1907). The changing neighbor set is a source of strong nonlinearity that is peculiar to a discrete-cell representation.

The stimulus is expressed (in a form simplified slightly from that in Cox, 2013) as

$$\mathbf{S}_i^{(\text{shear})} = \sum_j c_1 w_j \frac{\mathbf{v}_{ij}^{(\text{shear})}}{v_f} \quad (2b)$$

with c_1 an enhancement strength, or gain factor. The presence of the normalizing speed v_f in Eq. (2b), which equals the known rate of advance of the growth front, i.e., the rate of thickening of the enamel layer, minimizes the number of independent parameters in the theory - only the ratio of rates matters.

Given the vector-summed shear stimulus, the shear enhancement rule specifies the acceleration of cell i , which is the rate of change of its lateral velocity:

$$\frac{d\mathbf{v}_i}{dt} = \beta \mathbf{S}_i^{(\text{shear})} \quad (2c)$$

The factor β is discussed in Sections 5 and 6. The lateral motions control pattern formation.

In the early stages of enamel formation, illustrated for the human molar in Fig. 6a, enamel accumulates first at the location where the DEJ has its highest curvature, or smallest radius of curvature ρ . The cell population, which sits first on the DEJ and then rides on the surface of the enamel formed to the current time, experiences initially tensile strain in all directions in the plane of the population sheet as the enamel domain expands. The DEJ is doubly-curved, and the enamel domain has non-uniform thickness, and therefore the tensile strain is non-uniform. Relative cell motions must arise in the plane of the population, including relative shearing displacements between pairs of contacting cells. These relative displacements, initially infinitesimal, are the input that cells sense. The shear enhancement response of Eq. (2) augments the displacements, which increases the strength of the stimulus $S_i^{(\text{shear})}$, which increases the lateral acceleration of the cells, and the feedback mechanism is established.

The shear enhancement rule is applied isotropically, i.e., it enhances relative sliding between cells in any direction. Patterning therefore nucleates in simulations at different locations in an irregular manner (ordering is short-range only, with varying local sliding directions). However, after a relatively brief transient regime, ordering spreads and the population enters a steady-state configuration, arranging itself into a single system of layers spanning the whole tooth, with a universal sliding direction or global ordering (see the movie “decussating cells” published online) (Cox, 2013).

To generate the layered pattern, the shear enhancement rule must express active augmentation of sliding motion. A rule that merely weakens the resistance to mutual slip of successive rows of ameloblasts, which would be analogous to lowering the Peierls’ stress to create planes of easy slip in crystals (Hirth and Lothe, 1982), is unsuccessful (Cox, 2013). The shear enhancement rule represents the action of a living entity, using its internal energy to generate an amplified response to a small stimulus.

3.4. The balance of the driving force of shear enhancement and constraining factors

The shear enhancement effect destabilizes the kinematics of the population, which opens the possibility of transitions in the spatial arrangement of the population but, unchecked, leads quickly to meaningless noise in cell trajectories. Useful patterns are obtained by balancing the driving force of shear enhancement against a number of constraining factors.

One constraint is cell–cell adhesion: the lateral velocities of cells that have been in contact with one another for a long time will tend to be the same. A second constraint is a history effect, representing a lag time in a cell’s ability to change direction (to reconfigure its cytoskeleton). The history effect has a natural explanation in the fact that mRNA levels, once elevated, tend to stay elevated for some time (Oyler et al., 2014). Neither the presence of cell–cell adhesion or history effects, nor the choice of parameters used to quantify them, has any effect on the condition for the onset of patterning, since neither rule causes symmetry breaking, but they are necessary to the stability of patterns (Cox, 2013). Constraints allow attainment of the steady-state configuration and maintain the integrity of the layers: even when large-scale perturbations in the population geometry cause the layers to develop

substantial curvature, cells moving laterally within a layer follow the curved layer shape while maintaining their sliding arrangement.

3.5. The “commencement front”

Ameloblasts at different locations on the tooth mature into the secretory stage in a sequential manner, rather than simultaneously: a “commencement front” separates those that are still stationary on the DEJ from those that have begun to migrate away from it (e.g., Warshawsky and Smith (1976)). Since cells that have begun to migrate also begin to develop strains, the commencement front is associated with a pulse of strain that travels across the cell population, as discussed further in Section 6.

In the mouse incisor, the commencement front propagates along the long axis of the tooth at an approximately constant velocity. In simulations, the front is assumed to be a straight line traversing the width of the tooth (but see Section 6). The fact that the beginning of migration is timed by the passage of the commencement front has some important effects on the outcome of the simulations.

3.6. Details of initiation and the transient regime

Three movies “transient c055”, “transient c060”, and “transient c065” (published online; see also Appendix C) illustrate the emergence of a sustained decussation pattern with global ordering from a transient regime in which layered patterns nucleate at multiple sites with generally different orientations. The simulations are of amelogenesis in the mouse incisor, with cells exhibiting enhanced shear motility (the rule of Eq. (2)), as well as being governed by a tendency to maintain uniform area density (detailed in Section 5). The movies follow a window that is fixed to the propagating commencement front, which appears as a white line near the top of the field of view.

The following should be read in conjunction with the details of the movies that are provided in Appendices C.1 and C.3.

3.6.1. Characteristics of pattern forming—The feedback mechanism of enhanced shear motility creates variations in lateral velocity that are at first small in magnitude. Therefore, ordering appears first in the azimuthal angle β formed by a cell’s trajectory (which measures the direction of the lateral velocity), while the polar angle γ (which measures the magnitude of the lateral velocity) departs visibly from zero a short time later. This sequence is observed in the transient regime at random sites where local ordering first initiates; and, in the steady-state regime, at the onset of decussation near the commencement front.

Simulations are shown for three different values of the shear enhancement factor, $c_1 = 0.055$, 0.06, and 0.065 in the movies “transient c055”, “transient c060”, and “transient c065”, respectively. The value $c_1 = 0.055$ is not far above a critical minimum value at which a transition occurs from invariant ordering to decussation (Section 5). In this case, even in the steady-state regime, initiation of decussation involves large fluctuations, with initiation events occurring in bursts between the commencement front and the front of the established pattern, which are relatively well separated.

As c_1 increases, the decussation pattern in the steady-state regime initiates closer to the commencement front. In fact, for $c_1 = 0.065$, the pattern initiates almost at the commencement front; and the extension of the existing pattern is no longer in bursts, but occurs as a uniform advance.

The large fluctuations in ordering as the transition is approached ($c_1 = 0.055$) are reminiscent of the large fluctuations in thermodynamic systems upon approaching a phase transition (Stanley, 1987), although in the present simulations, in which cells are represented as lumped entities that can be energy sources, there is no meaningfully defined temperature.

The sequential commencement of the ameloblasts' secretory-stage migration contributes to the emergence of global ordering from the transient regime: see a further example of the relation between the orientation of the commencement front and the orientation of sliding layers for the mouse incisor in the steady-state regime in the movie "incipient decussation", published online.

3.6.2. Defects—Dislocations appear in all these simulations: the rows of layered ordering in the steady-state regime do not always line up all the way across the population. With the present rules for cell behavior, such dislocations lead to regions of disorder that grow from top to bottom in the field of view (i.e., with time for a fixed group of cells). In another simulation (see the movie "dislocation pair (vor.06 48 292 annih disl 5.28.12).wmv" published online with Cox, 2013), a pair of dislocations annihilates. Once a dislocation-free pattern arises, further dislocations tend not to arise. Therefore, if simulations were continued to the age of a mature mouse, say 10^2 – 10^3 d, rather than the 2–4 days represented by the present simulations, a defect-free decussation structure may well set in and remain stable.

Whether dislocations exist in the decussation pattern in the mouse incisor is not known. However, mouse incisor enamel does exhibit a relatively complex structure at the location of highest curvature across the tooth, departing from the simple planar pattern of Fig. 4a (S. White, private communication).

3.7. The absence of stress and energy gradients in the theory

The strain rates experienced by cells migrating within the population sheet during amelogenesis are easily estimated from histological records and are very small (10^{-7} – 10^{-6} s $^{-1}$) (Cox, 2010, 2013). Thus one may assume that a cell can generate whatever tractions are required to execute the motions dictated by its response to strain stimulus (Section 2), and represent the results of such cell action as kinematic activity, while leaving the associated stresses in the cell indeterminate.

The strain in an individual cell varies in a cyclic manner as it passes cells in adjacent layers of the layered decussation pattern (observe the shapes of cell cross-sections depicted in Fig. 2b or in the movie "decussating cells" published online). Therefore the strain energy density in the cell will also vary in a cyclic manner and, during at least part of the cell's motion, the cell must move in an energetically unfavorable manner, i.e., the cell must move as an automaton.

3.8. Molecular signaling

While the information a strain-cued autonomous cell needs to decide how to move correctly in a pattern-forming population is confined to its mechanical boundary conditions, it may propitiate the execution of its specified motion by the exchange of signaling molecules, e.g., between the ameloblasts and the cells of the stratum intermedium, as well as fluxes of molecules within individual cells (e.g., rho proteins). One key participant is PERP, which is correlated with the strength of attachment of the cells and successful amelogenesis (Jheon et al., 2011), and Notch, which regulates PERP (Espinoza et al., 2012). Sliding movement between pairs of ameloblasts is mediated partly by metalloproteinases (Bartlett and Smith, 2013). This short list of chemical factors undoubtedly understates the complexity of the molecular signaling occurring during the sliding migratory motions of ameloblasts.

3.9. Similarity to other formulations

3.9.1. Smoothed Particle Hydrodynamics (SPH)—In its computational structure, but not in the physics it represents, the discrete-cell formulation used to deal with large populations of ameloblasts resembles the Smoothed Particle Hydrodynamics (SPH) method that was first proposed as a computationally efficient way of analyzing Newtonian fluid flow in stars (Gingold and Monaghan, 1977). In the theory of amelogenesis, all field quantities and the equations of motion refer to a system of moving points (cell tips) that move in continuous space and time, analogously to SPH. The neighbors of any one cell are deduced from all cells' positions using the Voronoi construction (Voronoi, 1907), whereas in SPH neighbors are usually determined by a simple distance criterion. Given the neighbor sets, local strain fields and strain rates (or their surrogates, neighbor distances and relative neighbor velocities, which are better variables to use in computations) can be constructed from the point data of neighbor sets using interpolative formulae that are analogous to those used in SPH to compute density (Appendix A of Cox, 2013). Cells can move long distances relative to one another, as in SPH, and therefore the set of neighbors of any cell is continually updated, using a sector search to enhance computational speed (Appendix IV of Cox, 2010).

In the theory of Cox (2010, 2013), the ameloblast population moves according to equations of motion that incorporate autonomous shear enhancement as a rule for cells, which appears to have no precedent in applications of SPH. This rule would be effected in an SPH formulation by pairs of body forces (force couples), acting in opposite directions on a pair of neighboring points in the system. The shear-enhancing body force pairs should have magnitudes that depend on the existing relative sliding velocity of the pair of points, but, unlike dissipative frictional forces in a viscous fluid, the force couple would add energy into the system, powered by cell metabolism acting as an energy source. In a continuum model of the flow of a cell population, enhanced shear behavior would be represented by a negative viscosity (a thermodynamic impossibility for an inanimate fluid).

Finally, mass density plays a central role in SPH applied to fluid dynamics, being the source of inertia, whereas mass inertia is not present in the theory of amelogenesis, where inertial effects arise from the competition between rate processes.

3.9.2. Control systems with jump disturbances—The governing equations for ameloblasts expressing shear enhancement while subject to constraints and experiencing discontinuities associated with changes of contacting neighbors are isomorphic to those that arise in certain problems in control theory, in which a system responds to continuous feedback mechanisms combined with discontinuous jumps. The stability domains of such hybrid control systems are a topic of current research (Cox et al., 2014), which may bear on the stability of patterns due to strain cues in cell populations.

4. Motility feedback at the cell scale

How a cell might generate enhanced shear motility as its response to relative motion, i.e., generate the single-cell response function of Eqs. (2) and (3), is resolved within the single cell.

An early paper by Bois et al. presented a mechanism related to the dynamics of the actomyosin cell cortex for forming patterns within a single cell. Symmetry can be broken within a cell in which molecules are transported in a nonlinear manner, within a so-called “active fluid” (Bois et al., 2011). The stress that drives fluid flow includes a term that is proportional to the spatial gradient of the chemical potential of a regulator molecule, while the regulator is continuously redistributed in space by a combination of diffusion and advection (i.e., the transport of the regulator within the fluid, when the fluid flows). Steady-state solutions exist in which balanced gradients in the concentration of the regulator and the velocity of the fluid maintain a periodic spatial pattern within the cell.³

Two later theories, extending the spirit of the work of Bois et al., it have also analyzed nonlinear transport mechanisms within the cell, but with the transport mechanisms explicitly coupled to changes in the mechanical boundary conditions imposed on the cell (Leong, 2013; Camley et al., 2014). The theories demonstrate feedback mechanisms that initiate and sustain rotating sliding motion of the cells across a substrate. The theories address experimental observations of a two-cell problem, in which a pair of cells form a circular shape in vitro on a flat substrate, with the adhered cell–cell interface assuming an “S” shape, so that the pair resembles the Yin–Yang symbol (Fig. 7a). The cells rotate on the substrate in “persistent rotational motion (PRM)”, with the direction of rotation always such that the pointed extremity of either cell advances around the perimeter of the pair (Brangwynne et al., 2000). Rotating multi-cellular groups have also been observed in three dimensions and in vivo (Tanner et al., 2012), where they might be regarded as a form of collective migration (Rørth, 2012).

In their theory of the two-cell phenomenon, Camley et al. examined how factors that influence cell polarity might control whether the two cells can attain a stable state of PRM (Camley et al., 2014). The factors considered were neighbor alignment (NA), i.e., the tendency of neighboring cells to align their polarity; velocity alignment (VA), i.e., the

³One might conjecture that a similar mechanism operating at the population scale could account for the appearance of layers in the decussation patterns of ameloblast populations. However, the feedback mechanism of the active fluid requires advective flow (mass transport) in the direction perpendicular to the layers. Since the cells themselves do not move perpendicular to the layers they have formed, the required advective flow perpendicular to the layers must occur through the cell membranes, which seems improbable.

tendency of a cell to align its polarity with its own velocity; and contact inhibition of locomotion (CIL), i.e., the tendency of contact pressure to reorient a cell's polarity. Cell polarity is defined in terms of a spatial gradient in Rho GTPase, whose distribution was found by solving a reaction-diffusion problem. The persistence of motion of a cell (which depends on the concentration of Rho GTPase) was varied through an inhibitory agent, whose effect could be manipulated. Terms in the equations describing the time variations of both the concentration of Rho GTPase and the inhibitory factor were assumed coupled to the mechanical boundary conditions acting on the cell membrane. Thus cell shape and global cell motion were associated with mechanisms internal to the cell. The main outcome of the study was that PRM is most easily achieved when the tendency for alignment of a cell's polarity with its own velocity is strong. Both the tendency for neighbor alignment (NA) and CIL frustrate PRM, with the exception that, if CIL operates only when the fronts of the two cells (defined as the locations of highest concentration of Rho GTPase) collide, PRM can still be achieved. As intuition suggests, relatively strong persistence of cell motion is also required for PRM.

While the theory of Camley et al. addresses a problem with different geometry from the case of ameloblasts sliding past one another in rows, and is unlikely to replicate the details of the intra-cell mechanisms that will ultimately account for ameloblast behavior, it is nevertheless suggestive of a form of enhanced shear motility analogous to Eq. (2): a feedback mechanism converts a small initial disturbance into persistent sliding motion of the cells, which the cells accomplish using their internal power (i.e., act as automata). In PRM, the sliding motion builds up relative to a substrate; in decussation during amelogenesis, cells slide relative to the stratum intermedium and also slide past each other.

A minimal formulation of the cell-scale problem in the case of amelogenesis would consider the activity within individual cells on a cross-sectional plane parallel to the population sheet (Fig. 5b). This is the plane containing the lateral cell velocities that give rise to the decussation pattern. A three dimensional model would include the sliding interaction of the tips of the ameloblasts with the stratum intermedium (Fig. 3a).

In planes parallel to the population sheet, spatial variations of the cell's internal structure, whether scalar fields (concentrations) or vector fields (concentration gradients or particle velocities), are expected to have the symmetry elements indicated in Fig. 7b, when fluctuations are removed by time averaging. The symmetry elements specify characteristics of intra-cell structure. The symmetry elements are dictated by kinematics, i.e., the fact that the layered pattern exists in cell motions, rather than comprising variations in cell phenotype.

5. Wave motion: the response of autonomous cells to density variations

Cells acting as strain-cued automata can create wavelike density disturbances that propagate across the population. In this section and Section 6, we review wavelike phenomena that have been inferred from the study of dental enamel; in Section 8 we review the direct observation of wavelike disturbances in a population of epithelial cells in vitro.

Besides decussation, another universal feature of the microstructure of mammalian dental enamel is waviness in the prism loci (Fig. 8) (Macho et al., 2003), which suggests that waves of strain disturbance have propagated across the ameloblast population during the outward migration of the population sheet. Waviness can be predicted by a single cell response function that embodies a tendency to restore a preferred cell density. A stimulus function is defined by

$$S_i^{(\text{lin})} = \sum_j \frac{|x_j - x_i| - d}{d} \hat{r}_{ij} \quad (3)$$

where d is the center-to-center distance between cells that corresponds to the preferred density. This rule, in conjunction with Eq. (2c), creates a tendency for a cell to restore a preferred center-to-center distance relative to a contacting neighbor, i.e., to exert a countering response to strain along the line that joins the centers of the pair.

This rule was first used in a study of prism waviness and the onset of instabilities during ameloblast migration (Cox, 2010), where it was termed the “linear-stimulus rule”. When used alone, $S_i^{(\text{lin})}$ is substituted for $S_i^{(\text{shear})}$ on the right hand side of Eq. (2c). In the simulations of Cox (2013), $S_i^{(\text{lin})}$ acts simultaneously with shear enhancement, in which case it is added to $S_i^{(\text{shear})}$.⁴

The rule of Eq. (3) can generate waves that traverse the cell population at a velocity v_p that depends on the choice of the parameter β in Eq. (2c). Long-range oscillations in cell velocities can occur when waves reflect off the population boundaries (see the experimental data of Section 8 and the movie “pulse” published online).

In the case of ameloblast migration, the convex curvature of the population front leads initially to tensile strains in the plane of the population sheet as the population expands outwards, at speed v_m . The rule of Eq. (3) causes a tendency back towards compression after sufficient time and the predicted trajectories of cell migration are therefore wavy (Fig. 8c). The strain variations occur approximately uniformly over the population, rather than manifesting as boundary reflections. Nevertheless, the predicted wavelength λ in simulations is found numerically to scale with the width of the population, as well as the factor β of Eq. (2c). With an independent calibration of β , described in Section 6, predictions of wavelength match measured wavelengths in the human molar and the mouse incisor (Cox, 2010); and smooth, wavy variations of nearly parallel trajectories that are qualitatively similar to those in Fig. 8c are observed in the dental enamel of many species (Macho et al., 2003).

⁴When enhanced shear is added as a second rule, decussation patterns emerge, as demonstrated in Cox(2013). However, the decussation creates noise at the boundaries of the population, due to numerical issues associated with the simplicity of the cell representation. The boundary noise interferes with global strains and waviness is therefore not clearly present in Fig. 4b, for example. A refined theory, with better treatment of boundary effects, is expected to yield waviness and decussation simultaneously. The preferred-density rule still has an important role in decussation, partly determining patterning transitions (Section 7).

The wavelength, λ , and the rate of propagation of a pulse, v_p , are not determined by mass inertia, which is exceedingly small in amelogenesis (Cox, 2010), but by rate inertia: cells that are already moving apart from each other (or towards each other) will continue to move apart (or towards each other) for some time after their equilibrium separation has been reached.

6. Moving fronts of cell maturation (determination fronts)

One type of wave disturbance that can be sustained by strain-cued automata consists of a traveling pulse of high strain rate, which can arise when the cells' response to density variations or shear strains acts in conjunction with a transition, induced by strain, to a different state. In this section, we review the inference of such a traveling pulse from the histology of amelogenesis, the possible relation of the strain pulse to "determination fronts", and a recent theory that describes wavefronts propagating in a mechanically excitable medium. In Section 8, we review the direct observation of strain-activated traveling fronts in epithelial populations in vitro.

From histological images of the first formed enamel, we can deduce that a strain front propagates across all ameloblast populations. The propagating strain front is a result of the fact that, as remarked in Section 3.5, ameloblasts assembled as a sheet on the dentin–enamel junction (DEJ) prior to their secretory-stage migration do not all mature into their secretory stage simultaneously. Instead, cells at one location, typically the location of highest curvature on the DEJ, mature first, followed in succession by those at increasing distances from this location. Thus cell maturation spreads as a "commencement front", in the terminology of Cox (2010): ahead of the front, cells are still stationary while, behind the front, they have begun to move away from the DEJ as enamel forms beneath them.

Because of this timing sequence, the newly formed enamel is wedge-shaped, as illustrated for the human molar in Fig. 6 (Nanci, 2003). The wedge is thickest where cells have secreted the longest (the location of highest curvature of the DEJ in Fig. 6a) and vanishes at the current location of the commencement front. The wedge geometry implies a surge of shear strain in cells as the commencement front passes them (Fig. 6b). The magnitude of the engineering shear strain suffered by each cell as the front passes equals the wedge angle, which is roughly 0.05 rad in the human molar. The front can also be regarded as a step change in the state of strain of ameloblasts that have just been passed by the front relative to those that have not yet been reached by it, reminiscent of a soliton (Bullough, 1978).

6.1. Human molar

The arrival of the shear strain pulse was conjectured in Cox (2010) to be the triggering signal for cell maturation: a still-stationary cell is instructed to secrete and migrate when the motion of the cell next to it induces shear in the still-stationary cell, with a delay in the commencement of migration from cell to cell that is controlled by the rate of propagation of strain information, v_p . The wedge angle must equal v_m/v_p and, since v_m is known ($2.5\text{--}4\ \mu\text{m d}^{-1}$ for ameloblasts in the human molar (Lacruz and Bromage, 2006)), v_p can be calibrated to the value $v_p \approx 70\text{--}100\ \mu\text{m d}^{-1}$ (Cox, 2013).

The shear strain rises from 0 to 0.05 when the commencement front passes one cell, which takes time $d/v_p \approx 0.1 d$, given that the cell width $d=6 \mu\text{m}$. The average strain rate is therefore $\sim 10^{-5} \text{s}^{-1}$, similar to the estimates for local strains in decussating cells in Table 1.

Predictions using the preferred-density rule of Eq. (3) depend on a single adjustable parameter, which is the ratio v_p/v_m . With v_p/v_m calibrated by the wedge angle, the theory has no adjustable parameters and can be used to predict the wavelength of the wavy trajectories. The predicted wavelengths are correct for the human molar (Cox, 2010).

6.2. Mouse incisor

No image comparable to Fig. 6 is available for the mouse incisor. However, calibration of v_p can be achieved by a different argument. The mouse being a rodent, its incisor erupts continuously during the life of the animal. In the mature animal, the entire spatial pattern formed by the different stages of the odontogenic process achieves an approximately steady state configuration, translating in a self-similar manner back along the erupting tooth towards the tooth organ. If the zone of odontogenesis remains stationary relative to the tooth organ, the whole zone, including the commencement front for secretory-stage maturation, must have a velocity equal in magnitude to the velocity of eruption, but opposite in sign. If the commencement front is a propagating strain pulse, then $v_p=250 \pm 40 \mu\text{m d}^{-1}$, the measured rate of eruption in a mature wild-type mouse (Hwang and Tonna, 1965). With this calibration, the strain-cue theory predicts the measured wavelength of waviness in mouse incisor enamel within 50% (Cox, 2013). (Some uncertainty in correlating waviness and the speed of the commencement front is inevitable due to the difficulty of measuring wavelengths precisely, the variability in the speed of migration of cells during amelogenesis, and idealizations in the theory.)

The interpretation of the commencement front as a propagating strain pulse gains further credence from the shape of the front in the mouse incisor. In simulations in which the commencement front is assumed to be straight, the local strain state in the population is predicted to exhibit “C” shaped isostrain contours; the strain builds up more slowly near the edges of the population after the commencement front passes a fixed location and the cells begin to migrate, because the presence of the free edge allows the cells to relax into unstrained configurations (Fig. 9a). If a critical magnitude of the local strain is required to trigger cell migration, then the commencement front should not be straight but “C” shaped. This is indeed the case in the mouse incisor (Fig. 9b).

6.3. Traveling strain pulses as timing signals

If a propagating strain pulse governs cell maturation in the erupting mouse incisor, then it may also control the rate of eruption of the tooth. Traveling wavefronts have long been considered pivotal in development: the clock and wavefront theory of Cooke and Zeeman generates regular morphological sections and has been used to explain the formation of somites (precursors of vertebral segments) (Hester et al., 2011; Baker et al., 2006; Cooke and Zeeman, 1976). In such work, the wavefront is known as the “determination front”. The physics of the determination front and the factors governing its velocity, which is crucial, e.g., in determining somite size, remain poorly understood. Yet cells lying anterior and

posterior to the determination front in somitogenesis are known to differ in morphology, implying that the front is indeed associated with a step-function change in strain. Thus we conjecture that the velocity of the determination front in somitogenesis, like that of the commencement front in amelogenesis, is that of a propagating strain pulse.

Theories of mechanical wavefronts in biology have begun to appear. Idema and Liu show that a traveling front can be maintained in a relatively robust manner within a “mechanically-excitable medium”: discrete nodes generate additional stresses within the medium when the stress they experience exceeds some threshold (Idema and Liu, 2014). Among other results, their analysis proves that a wavefront can be maintained by entities that respond in a spatially and temporally discrete manner. Others have addressed traveling waves in media whose activity is continuous (Section 8). In the theory of Idema and Liu, the additional stress or motion that triggers the wavelike response requires energy from the cells, i.e., the cells must act as automata; and the response is cued by stress or, equivalently, since the material is assumed linear elastic, by strain. The resemblance of the theory of Idema and Liu to a theory of invasion fronts in cell invasion processes appears in Part II, Section 14.

The commencement front resembles a soliton wave, which describes the stable propagation of a configuration in which a variable sustains a step-like change in value within a small interval (Bullough, 1978; Ablowitz et al., 1973; Newell, 1985). Soliton wave motion has been proposed to describe other phenomena in biology (Davydov, 1979; Yakushevich, 1989). In a simplifying limit, the governing equations for the simulations of amelogenesis of Section 3 are similar to the Sine–Gordon equation (Appendix SE of Cox, 2013), because of the presence of a linear term (preferred density restoration) and a nonlinear driver (shear enhancement) (Buttiker and Landauer, 1981).

6.4. Robustness of a wavefront

As Cooke and Zeeman pointed out (Cooke and Zeeman, 1976), a requirement of the mechanism responsible for the traveling wavefront in the clock and wavefront mechanism is that it should generate a front of stable shape. A front driven by the response of cells to strains that are also constrained by cell–cell adhesion would indeed tend to be geometrically stable.

In the case of amelogenesis, a perturbation in the shape of the front would lead to a corresponding perturbation in the thickness of the enamel layer. Consider the arrangement of ameloblasts illustrated in Fig. 6. Irregularity in the thickness of the enamel would lead to fluctuations in the shear strain among the cells (a component of shear strain in a plane that includes the long axis of the cells). Cell–cell adhesion will tend to limit such shear fluctuations and therefore limit the perturbation in the triggering signal felt by cells ahead of the commencement front. Thus the shape perturbation will tend to be damped out. (An analogous shape stabilizing effect due to correlation of local strains with local shape perturbations is seen in the growth of cracks in structural materials (Cox and Morris, 1988). If the crack front is retarded locally, e.g., due to a variation in the local alloy grain structure, the crack driving force, i.e., the local strain field, becomes elevated at that point; and conversely, if the crack front spurts ahead locally, the crack driving force diminishes at that point. The net effect is that the front remains relatively uniform in shape even though the

driving mechanism might have quite strong spatial fluctuations, e.g., Fig. 11 of Cox and Morris, 1988).

7. Curvature

Cells acting as strain-cued automata will be strongly affected by curvature, which is ubiquitous in development, e.g., from the beginning of invagination of an epithelial-mesenchymal bilayer, or in the developing embryo before the onset of gastrulation. In the numerical simulations of Cox (2010, 2013), strain in the population, and thus the first infinitesimal relative cell motions, arise because the cells are migrating away from a doubly-curved surface (the DEJ). In all mammalian teeth, the migration away from the DEJ begins where the DEJ has the lowest value of its radius of curvature, ρ (the “dentin horn”) (Fig. 6a) (Nanci, 2003). The rate at which strain develops for a given increment of enamel thickness increases as ρ decreases.

7.1. Simulations

In simulations in which cells obey the preferred density rule of Eq. (3) alone, a critical value ρ_c exists for ρ (Cox, 2010). If $\rho > \rho_c$, cells adjusting their locations according to Eq. (3) can maintain invariant ordering; the changes in strain imposed by curvature are sufficiently slow that the cells can move to cancel them while remaining ordered. If $\rho < \rho_c$, cell motions cannot cancel the strains and the population breaks symmetry, transitioning into a pattern of patches of cells within any one of which the lateral velocity has a nearly uniform value, but between any pair of which the lateral velocity varies discontinuously (Figs. 8 and 9 of Cox, 2010). The value of ρ_c determined by simulations is numerically close to the measured minimum radius of curvature of the human molar, approximately 100–300 μm (Cox, 2010). The transition at critical ρ does not occur in small populations, due to the rapid relaxation of strains that occurs at free edges; nor in the presence of strong degrees of constraints such as cell–cell adhesion or history effects.

In simulations in which cells obey both the rules of Eqs. (2) and (3), the population transitions from invariant ordering into a pattern of layers of cells with common lateral velocity, rather than patches. The transition is governed by only two parameters (like many automata models, the theory is remarkably simple): one is the gain factor c_1 for the shear enhancement effect (Eq. (2b)); the other is the rate ratio, v_p/v_m (Fig. 10). The transition to decussation is favored by high c_1 and high v_p . The transition condition is approximately independent of the radius of curvature, ρ , but curvature still plays a role: the first cells to show the beginnings of decussation after the commencement front has passed are those at the location of minimum ρ , as can be seen for steady-state conditions in the movie incipient decussation (published online).

7.2. Histological data

High curvature of the population sheet is correlated with at least two observations in the histology of enamel. First, anomalies occur in the prism structure at the onset of the secretory-stage migration of ameloblasts, which is at the dentin horn. In the human molar, a region of relatively short-wavelength oscillations occurs in cell trajectories above the dentin

horn, creating “gnarled enamel” (Fig. 6 of Cox, 2010) (Nanci, 2003). In this same region, a relatively disordered pattern arises in so-called enamel tufts (Fig. 11) (Amizuka et al., 1992), which are protein-rich ribbon-like structures that rise up from the DEJ. Since the tufts lie between prisms, they indicate disorder in the patterning of ameloblast trajectories.

Second, transitions in cell behavior, including the transition to the secretory stage, and from the decussation pattern to invariant ordering (Fig. 4), occur first at the location of highest curvature of the population sheet, e.g., Fig. 9b and other maps of cell stages made by Warshawsky and Smith (1976).

8. Waves in vitro

8.1. Experiments

Serra-Picamal et al. have recently made direct measurements of propagating strain pulses in vitro, during research into wound healing (Serra-Picamal et al., 2012). In their experiments, epithelial cells were seeded on a flat gel substrate that was coated with collagen I. The cells were initially confined to a rectangular domain, which was formed as a rectangular aperture cut into a membrane that masked the gel substrate. When the constraining membrane was removed, the cells began to spread into the newly accessible space. At any subsequent time, cells in an outer domain had acquired outward migratory velocities, while cells in an inner domain were still essentially stationary. The boundary between the inner and outer domains propagated from the edge of the population towards its center.

Such behavior is analogous to the commencement front of amelogenesis: a moving boundary separates cells that have begun to migrate from those that are still stationary; and the boundary is associated with a propagating, relatively sudden change in the state of strain of the cell, and appears to be strain activated. The directions of motion are different in amelogenesis and in the experiments of Serra-Picamal et al. (2012), the former involving cell migration that is mainly normal to the population sheet and therefore creating a front of predominantly shear strain (Fig. 6b), the latter involving cell migration that is in the plane of the sheet and therefore creating a front of predominantly tensional strain. However, the phenomena are otherwise similar and we will use the term commencement front for both.

The velocity of the commencement front observed in Serra-Picamal et al. (2012) was approximately $600 \mu\text{m d}^{-1}$ (inferred from Fig. 3 of Serra-Picamal et al., 2012), which is approximately 2.5 times the velocity of the commencement front in the mouse incisor. The higher velocity may be associated with the fact that the cells in the experiments of Serra-Picamal et al. (2012), which migrated into open space, were less constrained than cells confined within the context of a living organ.

Another observation in the experiments of Serra-Picamal et al. (2012) was that of a propagating strain pulse (“crest of strain” or “X wave” in the terminology of Serra-Picamal et al., 2012), moving at approximately twice the speed of the commencement front. The strain pulse propagated back and forth across the population (Fig. 12a). Such a propagating pulse is consistent with the concept of propagating waves that account for the observed

waviness in the microstructure of mammalian enamel (e.g., the movie “pulse” published online and Fig. 3 of Cox, 2010).

In the experiments of Serra-Picamal et al. (2012), the strain rate (first time derivative of strain), which was reported for the middle of the population, oscillated in sign, consistent with the reflection of waves from the population boundary (Fig. 12b). The strain rate had magnitude mostly less than $4 \times 10^{-5} \text{ s}^{-1}$, with a brief peak of $8 \times 10^{-5} \text{ s}^{-1}$. These strain rates are at least an order of magnitude greater than those deduced for ameloblasts; the peak value in fact approaches the strain rate in the host cells during innervation (Table 1). The higher strain rates are consistent with the fact that the commencement front and the X waves in the experiments of Serra-Picamal et al. (2012) move faster than their counterparts in amelogenesis.

Serra-Picamal et al. also report the measurement of the tractions T exerted by cells on the substrate across which they migrate. In a one-dimensional system (plane conditions), the traction at any point equals the spatial derivative $d\sigma/dx$, where σ is the stress component in the direction in which the tractions act, averaged over the thickness of the cells, which is assumed uniform. Thus the stress in the population can be deduced in principle by integrating the tractions from the edge of the population, where $\sigma=0$ by assumption. The stress rises monotonically from the edge of the population to values of 200 Pa or more. The deduced values at the center of the population oscillate in time consistently with the oscillations observed in strains, although, unsurprisingly, the latter offer the less noisy data, being direct observations of cell displacements.

8.2. Wave representations based on strain or stress

Serra-Picamal et al. present a theoretical account of their observations that depicts cells as autonomous entities responding to changing mechanical boundary conditions in an idealized one-dimensional array (Serra-Picamal et al., 2012). Indeed, the experiment begins when the mechanical boundary condition imposed on the peripheral cells of the population is suddenly changed by the removal of the constraining template. Like the theory of wave motion among ameloblasts (Section 5), their theory represents individual cells by single points, among which excitations arise on the population scale (large number of cells). Thus the theory may be considered to parallel the multi-scale paradigm of Fig. 1. Contacting pairs of cells tend to move along the axis between their centers to maintain a preferred center-to-center separation. This action appears in (Serra-Picamal et al., 2012) as a force-reaction coupling between the two cells via their membranes, where the force magnitude is related to the strain between the pair by a stiffness parameter, k . The force-pair terms in Serra-Picamal et al. (2012) share the form of Eq. (3) here, but k is made history-dependent to replicate the observed shapes of strain and stress waves that move across the population (Supplement 3 of Serra-Picamal et al., 2012).

In the theory of motility in amelogenesis and the theory of Serra-Picamal et al., cell motion is influenced by additional motility terms. In the latter theory, the cell applies an additional force F to the substrate (Serra-Picamal et al., 2012). This second force (or traction) expresses a form of enhanced motility. The stress waves result from the combined effect of the driving force F , which pushes the cells out of displacement balance, and the restoring

effect of the force–reaction coupling between cell pairs, which tends to return them to their preferred separation. In the theory of motility in amelogenesis (Cox, 2010, 2013), two terms drive the cells out of displacement equilibrium: their migration out of the plane of the doubly-curved population sheet, and the enhanced shear motility response of Eq. (2). Thus, in both theories, symmetry is broken by terms that express the cell's autonomous nature.

9. Correlations of strain-cued motility to global strain and cell activity

9.1. Switching in and out of the shear-enhancing state

In the theory of amelogenesis, decussating patterns arise when the population is either expanding early in amelogenesis, or contracting but still in global tensile strain in the mid-stages of amelogenesis. Decussation then ceases when the population goes into global compression (compare Fig. 2a or 8c with Fig. 4a and b). Consistently, in vitro laboratory tests on sheets of a variety of cell types reveal relatively high relaxation velocities when the sheet is held in tension and relatively low relaxation velocities in compression (Angelini et al., 2011; Zhou et al., 2009). (Fig. 3a of (Angelini et al., 2011) shows inverse relaxation time falling with average cell density; the inverse relaxation time is proportional to the relaxation velocity of this article.)

We also note that odontoblasts, the mesenchymal cells that are arrayed opposite ameloblasts (in a bi-layer) in the odontogenic environment, move into compression during their formation of dentin, because they move away from the concave side of the DEJ (Fig. 6a); and these cells exhibit invariant ordering, indicating the absence of lateral migration within the population sheet. The resulting dentin microstructure consists of parallel (non-decussating) tubules (Marshall, 1993).

9.2. Global compression correlates with size determination

The fact noted above that the onset of global compression correlates with the transition from decussating enamel to invariant ordering (“outer enamel” in the mouse incisor in Fig. 4a and in the simulation of Fig. 4b), together with the fact that enamel formation ceases shortly after that transition, imply that compressive global strains are available as a signal for limiting organ size (Cox, 2010). A similar observation has been made in the case of the fly wing, where compressive strain develops due to cell proliferation in a confined area (Hufnagel et al., 2007).

9.3. Correlation of shear enhancement and amelogenin secretion

A correlation exists between an ameloblast executing shear enhancement to achieve the decussation pattern and its ability to form dense enamel with the correct microstructure by secreting the amelogenin protein scaffold. While shear enhancement implied by a decussating pattern and normal enamel formation are concurrent through most of the formation of decussating enamel in healthy wild-type mice, at two times the absence of orderly decussation and the formation of no or poor enamel occur simultaneously.

First, in healthy wild-type mice, the first-formed enamel, which occupies a small domain on the extremity of the tooth, lacks the decussating microstructural pattern and is characterized

by enamel mineralization of inferior density (Sehic et al., 2009; White et al., 2001). The presence of this imperfect, first-formed enamel morphology correlates with the transient regime in simulations, where long-range order is absent (Section 3).

Second, in nature, when decussating cells reach the boundaries of the ameloblast population, they cease to generate the enamel matrix that is converted into biomineralized enamel. In simulations, when cells reach a boundary, they can no longer maintain ordered decussating rows; the absence of the correct set of contacting neighbors creates disorder in trajectories.

10. Patterns of cell shape among non-motile, strain-cued cells

In a recent theoretical study, Ben-Yaakov et al. demonstrate the existence of attractions or repulsions between cells, caused by their mutual mechanical interactions and their interactions with an elastic substrate to which they adhere (Ben-Yaakov et al., 2015). While the cellular assemblies studied were very symmetric, the results suggest that in less symmetric arrangements, the mechanical interactions could lead to changes in cell shapes. In contrast to the models of patterning during amelogenesis and wavelike motion described in the foregoing sections, the cells remain non-motile.

The theory of Ben-Yaakov et al. echoes concepts from the physics of atoms or molecules adhering to the surface of a crystalline substrate. The deformation fields induced in the substrate by two atoms that are sufficiently close to one another will interact in a generally nonlinear manner, leading to an effective force of repulsion or attraction between them (Stanley, 1987; Bruch, 1983). The essential novelty of the theory of Ben-Yaakov et al. is that any two adhering cells are also alive; in particular, they maintain homeostasis of either tractions or strain. The capacity for doing the required work to maintain homeostasis changes the forces between cells, thus changing the available solutions for mutual attraction and repulsion, and for cell shape change, in interesting ways; the population will cooperate in forming certain repeated shape patterns. A possibly related experimental observation has been reported of populations of smooth muscle cells arranging themselves into rows when the substrate upon which they are proliferating is subjected to cyclic strain (Mack et al., 2013).

In accord with the multi-scale paradigm of Fig. 1, each cell in the theory of Ben-Yaakov et al. responds to changes in the mechanical boundary conditions that prevail at its membrane, where the membrane contacts either another cell or the substrate. No chemical gradient is required external to an individual cell. In the language of the present article, the propensity for homeostasis could be considered a particular single cell response function, which Ben-Yaakov et al. derive from simple models of the mechanical equilibrium of the forces cells experience and generate. To deal with cell shape, the cell response function acts on a set of independent variables that includes more degrees of freedom to represent the single cell than were necessary to study the decussation patterns or wavy patterns reviewed above.

11. Summary remarks on homogeneous populations

That strain waves exist among cell populations is clear: wavy prism loci, which are the records of cell trajectories, are ubiquitous in mammalian dental enamel, the commencement

front in amelogenesis is a traveling strain pulse, and strain waves have been observed in vitro. To infer that strain waves exist from these cases is merely a characterization of directly observable geometry: the strain waves consist of observed systematic spatial variations in cell positions.

In contrast, the question of the origin of the strain waves is challenging. The theories reviewed here attribute the formation of strain waves to cells' responding to changes in the mechanical boundary conditions that are present at the cell membrane. At least some aspects of the patterns formed by the strain waves might alternatively be predicted using a reaction–diffusion mechanism that generates propagating and oscillating variations in the concentration of one or more chemical factors: cells that can serve as sources or sinks of chemical factors, and maintain a clock measuring time elapsed from an event, can create a wide range of spatial distributions. However, a number of characteristics of the wavelike patterns are appealingly interpreted as caused by cells acting as strain-cued automata, rather than as diffusion phenomena, including:

1. global waviness in cell trajectories, including the transition from global tension to global compression (Fig. 2a);
2. the relaxation of strain fields near free edges (Fig. 9a);
3. propagating pulses, including the reflection of a tensional pulse as a compressive pulse (Fig. 12 and the movie “pulse” published online);
4. the existence of soliton-like propagating strain fronts, which can serve as triggers for cell maturation (Appendix SE of Cox, 2013 and Section 14 below); and
5. the coupling of transitions to curvature (Fig. 11 and Fig. 8 of Cox, 2010).

A set of reaction-diffusion events that would replicate the observation of strain pulses that switch from tension to compression when they reflect from a boundary requires a fairly complex set of timed releases of agents. Similarly, the replication via a reaction-diffusion mechanism of the symmetry elements present within cells during decussation (Fig. 7b) remains to be demonstrated, whereas they could develop very simply via kinematic feedback, as demonstrated by the analyses of PRM (Section 4).

The commencement front of amelogenesis, while unquestionably associated with a strain rate pulse (Fig. 6b), could in principle have a speed governed not by that of the strain pulse but by that of a propagating front in the concentration of a chemical factor. The sharpness of such a front in concentration can be maintained by “self-reinforcement”, i.e., a cell reached by the front emits the same signaling molecule itself (Zaikin and Zhabotinsky, 1970). But a more elaborate argument is required to account for the curved shape of the commencement front (Fig. 9b) using a reaction–diffusion mechanism.

The induction of a transition in cell ordering at the location of maximum curvature of the population sheet (Section 7) might be interpreted as a focusing phenomenon if modeled by reaction–diffusion mechanisms; if cells on a curved surface emit uniformly, the concentration of the emitted molecule will be largest at the focal point of the surface (see

related concepts in Abbas et al., 2009). However, in the case of ameloblasts, the focal point lies within the body of enamel formed so far, which is not accessible to diffusing molecules.

The nature of the strain waves predicted by the theories reviewed here is that of strain disturbances that are propagated by changes in cell motility, i.e., with energy supplied by cells acting as automata. Cell motility is governed by response functions that could in general express many possible relationships between strain stimuli and changes in motility. Much richer solutions are possible than for common acoustic strain waves, in which a pulse of energy supplied at a source propagates through a lifeless medium, governed by its elasticity.

The potential richness of solutions is demonstrated by pattern forming during amelogenesis, where strain-cued automata induce a transition from spatial uniformity to layered patterns via a kinematic feedback phenomenon. The successful rule for the response of an individual cell to strain cues is the rule of shear enhancement (loosely analogous to a negative viscosity), which attributes to a cell the tendency to augment relative sliding motions. The theory presented by Serra-Picamal et al. (2012) hypothesizes a similar enhancement of motion, but along the line joining cells.

The theory of amelogenesis contains only two parameters, one of which is independently calibrated, yet it succeeds in:

1. predicting the wavelength of the waviness in enamel microstructure
2. predicting the shape of the commencement front in the mouse incisor
3. offering an account of the transition from the decussating microstructure of inner enamel to the invariant ordering of outer enamel that is observed in all mammalian teeth
4. associating potential transitions in cell ordering with the evolving curvature of the population sheet, and
5. offering a possible cue to the cessation of amelogenesis, which determines the thickness of the enamel organ.

The representation of cells as discrete entities in a population that communicate with each other via mechanical stimuli passing between them has an interesting parallel at the scale of the organism: populations of ants, gathered into packed bodies, exhibit population-scale response to global displacement or force boundary conditions that may be viewed as those of “an active granular system, in which particles can sense their surroundings and adjust their bodies accordingly” (Foster et al., 2014).

Examination of common factors in pattern forming during amelogenesis and network formation, and possibly other instances of organogenesis, appear in Part II.

II. INVASIONS

Part II, comprising Sections 12 – 21, reviews strain-cue concepts for a heterogeneous case, namely the formation of network patterns when cells of one type, acting as strain-cued

automata, invade a distinct host population. Using their internally powered motility, the invaders migrate relatively rapidly through the host population, generating relatively high strain rates in the host cells and thus conditions in which mechanical stress in the host may be an important factor in shaping developmental organization. Symmetry is broken by the spatial distribution of stress (or elastic strain) fields: the stress induced in the host cells by the motion of the invading cells is always largest in magnitude immediately ahead of the tip of an invading branch of cells. This fact offers a mechanism for stabilizing the slender geometry of the branch.

Strain cues can act as an adjunct to chemical signaling factors in controlling network morphology. As in the kinematic feedback mechanism reviewed in Part I, strain cues in network formation act in a local manner: the key strains are generated in the host population in the immediate environment of the invading cells. We examine the hypothesis that desirable network morphology is achieved by a combination of external or long-range gradients, which arise in chemical concentrations, and local or short-range gradients, arising in strain fields. Consideration of the details of how stress fields are distributed spatially around an advancing branch of a network, and of the rate at which mass transport in the displaced host medium relaxes the stresses, reveals pathways by which strain information could be exchanged between the invader cells and the host population. Thus a model emerges of invader cells forming network features by migrating as self-powered automata guided by variations they sense in the mechanical boundary conditions that act on their membranes.

The theoretical model we use to investigate the possible role of strains in guiding invader cells assumes that the invaders, when forming a network, maintain a continuously connected domain. More generally, while cells exhibiting collective migration are sometimes observed to remain contiguous, in other cases they constitute a more loosely connected group: individual cells interact to maintain the group's integrity but repeatedly and dynamically lose contact and then re-attach with one another and swap their positions in the invading body (Rørth, 2012). Further remarks on how the theory developed for a connected group of invaders might offer insight into the behavior of a dynamic group of invaders appear in Section 18.

12. Symmetry breaking in network formation

The problem of identifying mechanisms by which invading cells can sustain stable branch structures, or stable “chains”, has received much attention, especially in relation to vasculature, or angiogenesis, and innervation of the gut. In these developmental processes, cells invade an existing tissue to form closed loop networks of relatively fine, slender branches (Figs. 13 and 14). Summaries of the biology involved, which are particularly welcoming for a physicist or mathematician because they segue into the inference of the spatial and temporal principles by which the cells might organize themselves into such structures, can be found in the papers by Newgreen et al. (2013), McLennan et al. (2012) and Wynn et al. (2012) (re innervation of the gut), Plank and Sleeman (2004) (re angiogenesis induced by a tumor), and Ji et al. (2007) and Liu et al. (2011) (re neo-vascularization induced by hypoxia). Here we review the concepts that have been proposed

in these and other theoretical works to explain network formation, emphasizing aspects in which strain cues, examined in detail in subsequent sections, may possibly make an important contribution.

For a group of motile invading cells to form a desirable network structure, they must accomplish a number of actions whose correct execution demands that individual cells, both within the invading group and within the host population, can sense their position at any time relative to features of the advancing network. Given positional information, the cells must maintain the slender geometry of advancing branches, create bifurcations of branches at a desirable rate, and execute coalescence of branches. In this section, we will focus on the question of how cells might maintain slender branch geometry, because this single question invokes many of the competing concepts in the literature. The other actions required of cells forming networks will be discussed only briefly here, but more fully in the remainder of the article.

Consider the generic problem sketched in Fig. 15, in which a line of cells invades a host cell population. If the cells depicted are to form a stable branch geometry as part of a network, they must advance at least approximately in the direction of the line they have already established (the x-direction in Fig. 15); the tip cell, labeled T, must advance to the right. The advance can be achieved by the tip cell migrating, or the tip cell splitting in two along the axis of the branch, or by a cell to the left of the tip cell migrating to overtake the tip cell. However, regardless of which of these events occurs, the key question is how the cells involved in the branch extension, including the invading cells and the host cells that surround them, can sense the preferred direction of branch extension.

Suppose the next increment of branch extension is achieved by the tip cell T migrating. Cell T can migrate at any angle θ to the x-direction, with $\theta=0$ desired. The possible means by which cell T can identify the direction $\theta=0$ include:

1. following a pre-existing template;
2. reference to the direction vector defined by its own internal polarity;
3. following a gradient dC/dx in the concentration C of a chemical factor that is established by far-field conditions, e.g., a chemical factor diffusing away from hypoxic tissue or a tumor that is located at a large distance along the x-axis;
4. establishing a local, oriented gradient in a chemical factor by exchanging chemical signals with host cells or other invading cells;
5. sensing strain fields.

12.1. Pre-existing templates

Endothelial cells forming retinal vasculature in the mouse exemplify guidance by a pre-existing template (Dorrell et al., 2002; Gerhardt, 2008). The template is constructed by astrocytes, which precede the endothelial cells in emerging from the optic nerve; the endothelial cells then migrate along the branches of the astrocyte network (Fig. 13a). However, in this example, the mechanism of branch stabilization remains imperfectly resolved in two regards. First, the question remains of how the astrocytes themselves form

geometrically stable branches during their own migration. Second, the endothelial cells form a network that is sparser than the astrocyte network, following only a fraction of the available branches in the astrocyte network (Fig. 13a); how does an endothelial cell, encountering a bifurcation in the denser branch structure of the astrocyte network, make a choice about which branch to follow? In particular, why do the endothelial cells prefer to follow astrocyte branches that tend to align with the line they have formed so far, rather than following branches in the astrocyte network that point to the sides or backwards?

A second example in which a template may stabilize slender branch structures is provided by the case of innervation of the gut by neural crest cells, which invade the pre-existing gut, migrating along its length while confined to thin layers within the gut wall (Fig. 13b–d) (Young et al., 2004; Druckenbrod and Epstein, 2005; Epstein et al., 1991). The outcome of innervation in the mouse gut is exemplified in Fig. 13b: a closed network of cells forms behind a propagating front, which is defined by the furthest migration of neural crest cells to that time. During this process, neural cells may extend long, thin axons in the general direction of the migration. The neural crest cells form “chains” (Newgreen et al., 2013; Landman et al., 2011), which exhibit strong spatial correlation with the axons, raising the possibility that the axons act as templates to assure the stability of slender branches (Fig. 13d). However, while the axons are present in the mouse, they are absent in the chick, and therefore cannot be the universal mechanism for stabilizing slender branch geometry. Further, the question of whether the neural cells follow the axons, or vice versa, is not entirely resolved (Landman et al., 2011).

12.2. Polarity of the tip cell

The polarity of the tip cell is tantamount to a directional vector pointing in the desired direction of further migration, $\theta=0$ (Fig. 15). If the tip cell migrates in the direction of its existing polarity vector and the polarity vector remains stable, then the branch geometry will be stable.

In a mechano-biological model of capillary network formation, Checa and Prendergast included a “persistence ratio”, an ad hoc rule that explicitly biased capillary extension in the direction already established (Checa and Prendergast, 2008); and, in simulations of network formation by enteric neural crest cells, Landman et al. included a similar “persistence” weight in determining the next movement of a cell (Landman et al., 2011). (The term “persistence” regarding the direction of cell motion has been used in the literature for some time (Davis and Trinkaus, 1981).) Such rules could be interpreted as the assumption that the tip cell tends to follow its own stable polarity vector.

The common observation of extending branch tips in, e.g., vascularization, is that the tip cells do indeed maintain a stable direction in their polarity vector; the vector points forward. However, the observation leaves unanswered the question of how a tip cell achieves that condition. Pairs of cells experiencing contact inhibition of locomotion (CIL) (Abercrombie and Heaysman, 1953) undergo re-orientation of their polarity vectors as the formation of protrusions is inhibited in the contact zone (Woods et al., 2014). In fact, contact inhibition of locomotion has been described as “contact promotion of locomotion in a different direction” ((Newgreen, 1990) and D. F. Newgreen, private correspondence). Thus it may be surprising

that the polarity vector of the tip cell in a propagating branch, which propels itself into contact with host cells that already occupy the space it desires for itself, remains constant. This is particularly so in light of the fact that, as we show in Section 17.2, the contact pressure between a tip cell and the host population has its maximum value (i.e., maximum compressive traction) for the direction $\theta=0$. In the theory of persistent rotational motion (PRM) of a two-cell system due to Camley et al. (2014) (reviewed in Section 4), which successfully replicates the observed cell geometry in PRM, the tendency for the polarity vector to rotate away from a contact area increases with the contact pressure.

Indeed, during collective migration, the polarity of cells can become randomized in direction to a degree that is sensitive to a number of conditions, including cooperative mechanical cell–cell interactions and whether cells remain spatially confined during their migration (Weber et al., 2012; Woods et al., 2014; Carmona-Fontaine et al., 2011). We note especially recent experiments demonstrating correlation between the degree of ordering of cell polarity vectors in a population of tens of cells both in vitro and in vivo and confinement of the cells to “permissive” or “restricted” regions, defined either by natural conditions in vivo or by fibronectin templates in vitro (Woods et al., 2014). The question of how the shapes of the restricted or permissive regions are established in vivo remains open.

The stress fields predicted in the following (Section 13) present a type of spatial confinement around a branch of invading cells: the host population envelopes the invader domain in a sleeve of compressive stress, which moves along with the branch as it propagates. The compressive sleeve correlates spatially with the desired restricted region and might therefore contribute to maintaining invader cell polarity.

12.3. Chemical gradient due to far-field conditions

Spatial gradients in chemical factors are present in angiogenesis. In tumor growth, factors diffuse radially away from the tumor (e.g., Owen et al., 2009 and references therein). In normal developing or re-modeling tissue, gradients in oxygen arise when tissue is insufficiently vascularized (e.g., Mac Gabhann et al., 2007 and references therein).

A number of theories of angiogenesis in the vicinity of a tumor have explored the possibility that a spatial gradient in a tumor angiogenesis factor (TAF) breaks symmetry and stabilizes branches (Anderson and Chaplain, 1998; Plank and Sleeman, 2003). The source region of the TAF is the environs of the tumor, which is a relatively large object. The gradient in the TAF is therefore characterized by length scales much larger than the dimensions of a single cell. Slender branch geometry is maintained if tip cells follow the gradient; the maintenance of branch geometry relies on the tip cell sensing the difference between the concentration of the chemical factor ahead of it, C_1 , and the concentration behind it, C_2 . Since the gradient in TAF exists in one direction (towards the tumor), the observed propagation of branches in multiple directions remains unexplained; theories tend to generate branches that propagate directly towards the tumor. An elaboration of the theory in Anderson and Chaplain (1998) resolves this by introducing a weighting factor for advance of a tip in different directions (Plank and Sleeman, 2004), but this appears to be an ad hoc assumption, not related to any predicted variation in the local concentration of a chemical factor.

Another line of theoretical research has predicted the spatial distributions of oxygen and VEGF during the phenomenon of neovascularization, which occurs in normal mature tissue, e.g., after an uptick in exercise level (Ji et al., 2007; Liu et al., 2011; Mac Gabhann et al., 2007). The theory considers realistic, complex configurations of blood vessels: a model is built in which existing blood vessels, down to the capillary scale, are positioned in correspondence with observations of normal tissue. Local VEGF production is assumed to be correlated with the level of hypoxia. Reaction–diffusion calculations, which may be regarded as derivatives of Turing’s hypothesis (Turing, 1952), predict the degree of hypoxia for all positions in space and thence the spatial distribution of VEGF (Mac Gabhann et al., 2007).

While the longer-range gradients in predicted VEGF levels correlate with the expected general directions of neovascularization, the calculations of Mac Gabhann et al. (2007) raise issues that militate against the hypothesis that VEGF gradients govern growth directions at the scale of a single cell, e.g., the tip cell of a branch. First, while sprouting into hypoxic domains can be explained by an assumed response of invaders to gradients in VEGF, growth towards blood vessels that already exist, and the formation of connections with such vessels, requires growth in directions along which the VEGF concentration is predicted to decrease, not increase (Mac Gabhann et al., 2007). This difficulty is overcome in simulations by adding ad hoc the condition that a connection (anastomose) is formed when a tip cell comes within 5 μm of an existing vessel (Liu et al., 2011). Second, because the oxygen concentration and therefore the predicted VEGF concentration tend to be axially symmetric around the existing vessel, sprouts guided by the VEGF gradient should always tend to grow perpendicularly away from the vessel (Mac Gabhann et al., 2007). In reality, the angles of new vessels from the parent vessel are distributed over some range.

These results suggest that the information content in chemical gradients emanating from far-field conditions, i.e., generated by entities removed from the invading cells, is not the complete set of information required to guide the invading cells in the formation of desired network morphology. It would appear that the required gradients need to be established at least in part by the invading cells themselves and the host cells that are in immediate contact with the invading cells. Only at this scale, the scale of the single tip cell, can the required spatial variations in guiding factors be completed.

The stress and strain fields formed by cells invading an occupied spatial domain exhibit spatial variations over exactly this scale. The fields translate with the motion of the tip of the branch and are always present, no matter what the current direction of advance is relative to far-field gradients.

12.4. Chemical gradient originated by invading cells

At the scale of the tip cell, the spatial distribution of a chemical factor that will maintain the slender geometry of the branch should be such that an extremum exists ahead of the tip cell at orientation $\beta=0$ in the schematic of Fig. 15, i.e., the required gradient in concentration C is C/β with a maximum or minimum at $\beta=0$. To establish such a gradient, the cells in the branch must communicate with the host cells that are currently positioned around them. If instead the invading cells near the branch tip simply emit a chemical factor that diffuses into

the host medium from the surface of the branch, the spatial distribution of that factor in the host medium remains essentially uniform around the periphery of the branch.

Invading cells can also establish a chemical gradient by absorbing (or internalizing) some factor that is already present in the host. If the factor is initially uniformly distributed throughout the host, a diffusion gradient will arise directed towards the absorbing invaders. This mechanism has been examined in simulations of directed migration of neural crest cells emerging from the dorsal neural tube (McLennan et al., 2012), and of innervation of the gut (Newgreen et al., 2013). In both studies, the chemical gradient gave a mechanism for sustaining directed migration over long times and distances, but whether the gradient established local conditions that would specifically aid in maintaining stable tip geometry remained unclear.

More generally, one might seek the symmetry-breaking required for branch stabilization in a sequence of communications between invader and host cells, building on the fact that one available factor for identifying host cell position relative to a branch tip is the time at which the host cell is encountered by an invading cell for the first time. If it has only just been contacted, or closely approached, then it is at or near the branch tip. However, the details of such signaling that establish the required spatial pattern around a branch tip to favor extension in the direction $\theta=0$ are apparently still lacking.

Furthermore, identifying host cell position by the time since being approached by an invading cell may be vulnerable to noise: cells involved in collective migration or growth tend to exhibit substantial positional fluctuations, reminiscent of Brownian motion. Using again the example of neural cells innervating the gut, “the cells forming [branches] are in a constant flux with rapid bursts of movement, slower gradual movements, pauses and constant exchange of neighbors, which are unpredictable at the level of each individual cell” (from Newgreen et al., 2013; see also Young et al., 2004; Druckenbrod and Epstein, 2005¹, 2007).

In contrast, the strain fields predicted below need not be strongly influenced by the history of such random cell motions; for the conditions of the invasions studied, the strain fields change essentially immediately upon the movement of an invading cell to a new position. They can therefore provide cues that may be less vulnerable to the noise of recent history.

12.5. Strain fields

In summary, cell guidance during collective migration, including network-forming invasions, remains a complex and imperfectly resolved mystery.

In the following, we will demonstrate that many of the ambiguities of host cell position can be removed if the cells respond to the state of stress (or elastic strain) that they currently experience. Strain is necessarily present in any invasion, by the very fact that the invaders displace host cells as they invade. We will show that strain cues are well suited to aiding cells in forming network structures by their spatial and temporal variations.

13. Cells guided by strain cues can form networks

In this section, we review computer simulations of cells that form closed loop networks when their migratory choices are guided solely by the strain fields they themselves induce in their surroundings (Cox, 2011). The cells move as automata, forcing their way into energetically unfavorable positions. In Section 17, we illustrate the expanded variety of network morphology that can be achieved if invading cells are assumed to be guided by a combination of local strain fields and a long-range gradient in a chemical factor arising from a far-field source.

13.1. Symmetry breaking

When an existing host cell population is invaded by a second type of cell, stresses are induced in the host cells as they move to accommodate the volume of the invading cells (Fig. 2c). The stresses, or, equivalently, the elastic or reversible part of the induced strains (e.g., wikipedia, 2014), can be analyzed using the mechanics theory of “inclusions”, which are misfitting bodies contained within another body (Eshelby, 1957; Mura, 1982). The arrival of an invader in a computational spatial element causes an increase in the average density $\bar{\rho}$ of cells in that element (Fig. 16a), which is represented as an “eigenstrain” (Mura, 1982) or misfit strain $\bar{\epsilon}$ (a change in the stress-free volume of the material in the element, which constitutes a misfit). Strain and stress fields that arise at any position as a result of the eigenstrain can be computed using Green’s functions (Eshelby, 1957; Mura, 1982; Li and Anderson, 2001).

As time passes, any density perturbation is reduced by mass transport: the spike in cell density that is created in a spatial element when it is first invaded slowly dissipates, and therefore the magnitude of the eigenstrain attributed to the element diminishes (Fig. 16b). Thus, while the inclusion theory is derived within the framework of linear elasticity (stress and strain related by Hooke’s law) (Eshelby, 1957; Li and Anderson, 2001), linear behavior is present only for times approaching zero. Relaxation via mass transport implies that the stresses generated by the invasion decay in time; the host medium retains large irreversible strains, because it is still stretched around the invaders, but the stress (reversible strain) in it relaxes over time. Plausible mass transport mechanisms are cell migration, fluid flow, molecular fluxes, and cytoskeletal depolymerization within host cells.

Spatial elements that have not been invaded but are adjacent to invaded elements may also have elevated density (and therefore an eigenstrain), because the mass transport mechanism that relieves the elevated density in an invaded element will do so by transferring mass to adjacent elements (mass must be conserved). This is an important phenomenon in the theory.

Given the set of eigenstrains in all elements at any time, the strains induced in the host can be easily computed (Li and Anderson, 2001). The strain induced in the host due to the eigenstrains in all elements is computed at points in the host just outside each boundary of each invaded cell. These are the strains in the host that might cue an invading cell and guide its next migratory choice. The question of how an invader might sense a strain cue that is present in the host is discussed below.

The strength of a strain cue is taken to be proportional to the sum of two strain components, $\epsilon_1 + \epsilon_2$, which are the components of elastic strain evaluated in the host medium along local axes 1 and 2 that are parallel to the surface of an invaded spatial element. At point P in Fig. 16c, for example, axes 1 and 2 identify with the y and z axes, while at point Q₁ they identify with the x and z axes.

The sum $\epsilon_1 + \epsilon_2$ measures the stretch of the host cells, tangential to the surface of an invading cell, as they dilate around the invader. The sum $\epsilon_1 + \epsilon_2$ is checked at every location in the host that is adjacent to the mid-point of a side of an invaded element and is just outside the invaded element. Several such locations, P, Q₁, Q₂, ... are marked in Fig. 16c for the case in which a line of invaded elements has already formed.

The strain fields in the host break symmetry (Fig. 16d): the maximum value of the elastic strain metric $\epsilon_1 + \epsilon_2$ always occurs in the host immediately ahead of the tip of the invasion branch (point P in Fig. 16c, which lies at $\theta=0$ in the notation of Fig. 15). The strain concentration is analogous to that found at the tip of a crack in fracture mechanics (Neuber, 1946).

An important consequence of the mass-transport relaxation mechanism is that the sum $\epsilon_1 + \epsilon_2$ develops a spatial gradient along the flanks of the branch, decreasing with distance from the tip, for reasons that can be understood as follows. First, the strain in the host at a point along the flank is dominated by the magnitude of the eigenstrain in the nearest invaded elements in the branch; although the eigenstrains in other invaded cells contribute, their contribution falls as the cube of distance (Eshelby, 1957). Second, the time over which relaxation has taken place, and therefore the degree of diminution of the eigenstrain in an invaded element, increase with distance from the tip.

The steepness of the spatial gradient along the flank depends on the ratio of the invasion velocity v_m to the rate at which mass transport relaxes density spikes in the host, which can also be quantified by a relaxation velocity, v_r (Fig. 16b). As the ratio v_r/v_m increases, relaxation due to mass transport becomes more complete for invaded elements at a given distance from the branch tip.

Thus the strain field has exactly the spatial variation necessary to stabilize the branch morphology: if the host strain acts as a cue for subsequent invasion, progression of the migration will be most probable from the tip of the branch, in the direction already established by the axis of the branch ($\theta=0$).

Even further behind the branch tip, the sum $\epsilon_1 + \epsilon_2$ becomes negative (compressive strains), for reasons discussed below. Fig. 16d shows values of $\epsilon_1 + \epsilon_2$ relatively near the branch tip, where they remain positive, or only slightly negative, for the cases drawn; the complete spatial distribution of larger negative values will be illustrated below (Section 13.6).

13.2. Stochastic rules yield rich solutions

In the strain-cue theory, the event of further invasion depends only on the local value of the strain metric $\epsilon_1 + \epsilon_2$, but in a probabilistic manner, with high probability of invasion across

an element boundary for a high value of $\epsilon_1 + \epsilon_2$, and low probability for a low value of $\epsilon_1 + \epsilon_2$. Invasion occurs if the condition

$$pS - X \geq 0 \quad (4a)$$

is satisfied, where X is a random number uniformly distributed over $[0,1]$, p is a velocity parameter, and

$$S = \frac{1}{2} \{1 + \text{erf}[g(\epsilon_1 + \epsilon_2)]\}, \quad g(\epsilon) = \frac{1}{\chi} \left(\frac{\epsilon}{\epsilon_0} - 1 \right) \quad (4b)$$

with $\text{erf}[\dots]$ the error function, ϵ_0 a reference strain, and χ a strain sensitivity parameter.⁵ Values of the parameters p , ϵ_0 , and χ are discussed below. The strain cue S is bounded by zero and unity, with larger values occurring when $\epsilon_1 + \epsilon_2 > \epsilon_0$.

The fact that the process is stochastic has important consequences. While further migration of invaders that have formed a branch is most probable through the tip, it can also occur from points along the flank of the branch, but with diminished probability. At any time step, the question of where invasion will occur next is tested for every surface of every element that has been invaded to date. The test for new invasion out through any invaded element surface is exactly the same, with no bias introduced other than the different value of the strain cue $\epsilon_1 + \epsilon_2$ that has been computed at a point in the host just outside that surface; migration is favored from the tip of the branch, but can have a non-negligible probability elsewhere. Migration out through a surface of the invader domain along the flank of a branch constitutes a bifurcation event.

The frequency of bifurcation cannot be specified in the model of Eq. (4); it is a predicted outcome determined by probabilistic dependence on the spatial variations in strain. More realistically, bifurcation rates are governed by a combination of strain and chemical cues.

Branch coalescence can also occur: in fact, when two branches approach one another, the value of $\epsilon_1 + \epsilon_2$ at points between them tends to be high and therefore their coalescence is promoted.

13.3. Simplicity of the strain-cue mechanism

In Eq. (4), ϵ_0 is a reference strain, set equal to the value of $\epsilon_1 + \epsilon_2$ outside a single, isolated, newly invaded element (this is a hypothetical configuration, requiring the spontaneous appearance of an invader at some point within the host medium, and is used only to establish a reference for normalization). With ϵ_0 fixed, the remaining parameters of the model are p and χ of Eq. (4), together with a parameter β , which appears in the model for mass transport and is proportional to the relaxation velocity, v_r , illustrated in Fig. 16b. Statistical analysis of many simulations reveals that the mean predicted rate of advance of the front of the forming

⁵In Cox(2011), the strain sensitivity parameter was denoted c_1 , but that symbol is reserved here for the strength of the shear enhancement effect in the theory of amelogensis.

network, which approximates the migration velocity of the invaders, v_m , is a numerically determined function of p and β only. Thus the model parameters p , β , and χ can be mapped onto v_r , v_m , and χ , with v_r controlled via β and v_m then controlled via p .

Furthermore, due to scaling relations in the formulation, the morphology of predicted networks depends only on the ratio v_r/v_m and not on the separate values of v_r and v_m (the absolute values of v_r and v_m determine only how much time it takes to form the network). Thus morphology depends only on v_r/v_m and χ .

The fact that the governing equations are invariant under certain re-scalings also proves that the predicted morphology is independent of the size assumed for the spatial elements and the size of the time step in the computations. In other words, it is the spatial distribution of computed strains alone that determines morphology in this model.

The dependence of morphological outcome on χ is relatively weak. The value of χ is confined to the range 0.2–0.3 by a form of anthropic principle: if χ were much smaller than 0.2–0.3, the system would be so responsive to strain deviations that noise would overwhelm pattern forming, while if χ were much larger than 0.2–0.3, the strains required for stimulus would be above those that can be generated in the host by invaders. For values of χ in or near the range 0.2–0.3, substantial changes occur in the value of the cue S of Eq. (4b) when the individual strain components ϵ_1 and ϵ_2 vary over plausible ranges, e.g., from zero to approximately 0.1. Furthermore, calculations show that the outcome of the theory is insensitive to the choice of χ provided χ takes a value in or near the range 0.2–0.3. With χ set, the rate ratio v_r/v_m remains as the single scalar parameter available for choice.

13.4. Mechanical role of ECM in the host tissue

In the theory of Cox (2011), the elasticity of the invading cells was assumed to be the same as that of the host medium, which is likely to be a valid assumption when the host medium has a low content of ECM and the ECM has not formed a connected structure. Such conditions are most likely to exist during development, when an organ is immature. Given equality of the stiffness of the invading cells and the host medium, the particular value of the stiffness does not influence the morphological outcome, because the strain cues $\epsilon_1 + \epsilon_2$ at all points are related to the current set of eigenstrains by factors that depend on geometry alone (Eshelby, 1957).

In the case of invasion of a mature tissue, such as neovascularization, the ECM in the host medium will be substantial and may be connected into an organized structure. The pertinent stiffness of the host medium is then that of a composite of cells and ECM, which will be considerably stiffer than the invading cells. In fact, such mature tissue may be stiff enough that the question arises of whether the invading cells are capable of displacing it; weakening of the ECM in the host tissue, e.g., by enzymes or Ca ions emitted by an invader, may then propitiate the invasion.

13.5. Quantitative predictions of network morphology

The dependence on v_r/v_m can be summarized as follows. For a narrow range of values, $0.03 < v_r/v_m < 0.06$, networks are formed that resemble some that occur in nature, e.g., those

formed in innervation of the mouse gut (Fig. 17). If $v_r/v_m > 0.06$, branches form but tend to terminate; the host relaxes rapidly around a formed branch, reducing the cue for further invasion to low values after a brief time, and, if by chance no invasion occurs in that time, the strains become even smaller and the branch tends to be permanently arrested. At the other extreme, if $v_r/v_m < 0.03$, strains relax very slowly, further invasion remains possible for long times not only from branch tips but also from the flanks of branches, to the extent that massive bifurcation events lead to the filling of nearly all space by invaders. The network morphology is lost.

With branch extension, spontaneous bifurcation, and coalescence all possible, simulations with $0.03 \leq v_r/v_m \leq 0.06$ typically generate patterns of random closed networks. One instantiation can be seen in the movie “network d05 gch0” published online. In this simulation, invaders are confined to a two dimensional (2D) domain, which is representative of networks such as those formed in innervation of the gut, where the 2D domain is a thin cylindrical layer within the gut wall (Fig. 13c) (Druckenbrod and Epstein, 2005). Nevertheless, the computation of strain cues must be, and is, solved as a three-dimensional (3D) problem, to assure the correct prediction of the variation of the induced strain with distance from an invaded spatial element (i.e., the simulation finds solutions in a 3D space, but with invaders obliged to choose a direction of migration that keeps them within a planar sheet of computational elements).

In the simulations shown in the movie “network d05 gch0” and in Fig. 17, source invaders are placed randomly at time zero in a statistically homogeneous manner in a narrow band at one boundary of the host medium. The stochastic rule for new invasion triggers migratory advance of a few invaders at random locations, resulting in fresh local density variations. Continued application of the stochastic rule for migration, alternated with calculations of the relaxation of density fluctuations, creates a diffuse front of network formation, which propagates away from the band of initial invader sites. In the wake of the front, a stable, steady state random network pattern forms, replete with branching and coalescence. In the movie and in a still shot (Fig. 17a), branch points are marked as black elements.

The branching frequency and therefore the density of the network structure roughly match those observed in innervation. Perhaps this is fortuitous: no distinct mechanism for branching has been introduced and the branching frequency cannot be independently controlled in the model. The bifurcation occurs spontaneously, without external prompt, by application of the same probabilistic, strain-cue rule for further invasion from an occupied element that accounts for tip extension. If a different functional form were assumed for the probabilistic dependence on strain embodied by Eq. (4), a different bifurcation frequency would be expected. Furthermore, the occurrence of bifurcation events (or sprouts) is certainly influenced by other factors, such as VEGF gradients in vascularization.

Other outcomes of simulations that are consistent with observations for innervation of the mouse gut are: the diffuse nature and width of the invasion front; the prevalence of non-right-angled junctions due to the tendency of bifurcations to occur at elbows; the termination of some branches, even in well-formed networks; occasional clusters that form among invader cells; and back-filling of void areas in the wake of the advancing invasion front.

13.6. Strain patterns during network formation

Fig. 18 exemplifies the evolution of strain distributions around key features of a randomly propagating network, using color coding to exhibit the value of the elastic strain metric $\epsilon_1 + \epsilon_2$ in each spatial element that is currently occupied only by host cells and is adjacent to an invaded element.⁶ The image is built from the simulation that appears in the movie “network d05 gch0”. The evolution in time of $\epsilon_1 + \epsilon_2$ over the entire domain of the simulation can be seen in a second movie, “strains d05 gch0”.

In Fig. 18, the blue spectrum indicates positive or tensile tangential strains $\epsilon_1 + \epsilon_2$ in a host element. The most intense blue (highest tension) occurs in host elements immediately ahead of each propagating branch tip, encouraging further branch extension into those elements. The tangential elastic strains diminish in tensile magnitude (paler blue) for host elements that are to the side of or immediately behind a branch tip, as mass transport causes stress relaxation.

Further back along the flanks of a branch, the tangential elastic strains switch into compression (negative values), indicated by the yellow-red part of the spectrum. This phenomenon indicates further progression of the mass transport mechanism: when excess cell density diffuses out of an invaded spatial element into the adjacent host elements, those host elements swell and cause significant stretching of the next shell of host elements, i.e., host elements that are further removed from the invaded elements. The tensile stretching of the further-removed host elements drives the host elements adjacent to the invaded elements into compression. In terms of the mechanics of inclusions, material inside an inclusion that bears a positive eigenstrain ϵ_{ii} is always in compression, while the material outside the inclusion is always in tension in the tangential directions. Mass transport has led to the inclusion, defined as the spatial domain in which positive eigenstrain exists, to effectively expand to incorporate the host elements around the branch. Because those host elements now also bear positive eigenstrain, they switch from tensile to compressive values of $\epsilon_1 + \epsilon_2$.

Where a bifurcation has recently occurred, the magnitude of the compressive values of $\epsilon_1 + \epsilon_2$ tends to be greatest: the presence of two branches implies a greater average eigenstrain in that locality and therefore higher stress magnitudes.

In the far wake of branch tips, compressive values of $\epsilon_1 + \epsilon_2$ tend to fade as continuing mass transport removes the remaining non-uniformity in cell density.

13.7. Prior work

The ability of strain variations in developing organs to generate dendritic fractal structures has been previously suggested, but without demonstration of branch stabilization by the strain field variations specific to different cell configurations, or consideration of the role of time-dependence in the fields (Ingber, 2006).

⁶Fig. 18 can be constructed from Fig. 4, partscandd, of Cox(2011), which show spatial distributions of the strain measures $(\epsilon_x + \epsilon_z)/\epsilon_0$ and $(\epsilon_y + \epsilon_z)/\epsilon_0$. Depending on the orientation of the interface between a host element and the invaded element it abuts, one of $(\epsilon_x + \epsilon_z)/\epsilon_0$ or $(\epsilon_y + \epsilon_z)/\epsilon_0$ will become $\epsilon_1 + \epsilon_2$ in Fig. 18.

14. The invasion front as a self-reinforcing mechanical stress wave

In the simulations of Fig. 17a and the movie “network d05 gch0”, the network formation advances across the host domain as a front in a diffusive process, driven by a gradient in the density of invaders. There is no external gradient present, such as in a chemical factor. Under these conditions, branches can propagate just as readily in the reverse direction as in the forward direction; but those propagating in the reverse direction soon encounter an existing branch and are thereby arrested, while those advancing in the forward direction can advance indefinitely. The probability of a reverse-propagating branch encountering an existing branch is high when invader cells are confined to a single layer of computational elements, as in the simulation shown in Fig. 17a; but even if the invader cells were permitted to migrate in all directions, the distance a reverse-propagating branch will travel before an encounter is limited.

The propagating network front can be viewed as an example of wavefront propagation in a mechanically-excitabile medium, a concept recently developed by Idema and Liu (2014). In the theory of Idema and Liu, a stress pulse propagates through a population of cells, creating wavelike cell motions. Cell motions are damped by viscosity, but the front is sustained by work that is added into the system when the cells that are newly reached by the front are activated; activated cells generate new sources of localized forces that act on the cells’ environment. The theory of Idema and Liu is an example of the paradigm of Fig. 1: cells act as self-powered automata, responding to changing mechanical boundary conditions. Idema and Liu demonstrate the ability of such a system to maintain a well-defined stress pulse, propagating at a unique velocity, which are conditions necessary for its possible function as a timing signal in development.

The theory of Idema and Liu and the theory of network formation share similarities. In the theory of Idema and Liu, a discrete element is activated when the stress in it exceeds a critical level; in the network theory, an analogous critical level is represented by the reference strain ϵ_0 . In both theories, a cell when activated perturbs its environment by imposing an additional force: in the theory of Idema and Liu, the force may be either a dipole or a hydrostatic stress; in the network theory, it is generated by the change in the eigenstrain of an activated (i.e., invaded) element. In both theories, local stresses decay after they have been generated: in the theory of Idema and Liu, the decay is associated with damped motion of material elements; in the network theory, the decay is effected by mass transport (Fig. 16b). The primary distinction of the two theories is that, in the network theory, the activation of elements is statistically anisotropic, favoring activation of elements immediately ahead of lines of elements that have already been activated (Fig. 16c), whereas in the theory of Idema and Liu, activation does not include such symmetry-breaking and thus wavefronts are approximately uniformly dense rather than consisting of branches.

The viewpoint that the front in a forming network is one case of a self-reinforcing wavefront in a population of cells activated by strains (or stresses) forms an important conceptual link to cases of wavefronts propagating through homogeneous populations, such as the commencement front in amelogenesis or the waves observed propagating through epithelial cells in vitro, which were reviewed in Section 8.

15. Crucial role of relative rates

While the morphologies they predict and the mechanisms by which they act are very different, the theories of pattern formation during amelogenesis and the theory of network formation reviewed in Section 13 have common elements. The outcome in each depends on the ratio of two rates, which must take a value confined to a fairly narrow range. In the amelogenesis theory, the two rates are the velocity v_m of the advance of the population sheet (i.e., of the advance of the growth front) and the rate of propagation of strain information across the population, v_p . The strain propagation velocity v_p can be shown by analysis to be equivalent to the relaxation velocity v_r for the cells in an invaded host (see Cox (2011, especially Section 5.1 and Appendix F). Thus, like the theory of network formation, the theory of amelogenesis can be considered to depend on the ratio of a relaxation velocity and a migration velocity, v_r/v_m .

In the network theory, the value of v_r/v_m controls the question of whether a good network can be formed. In the theory of pattern forming in amelogenesis, the values of v_r/v_m (or v_p/v_f in the notation of Cox, 2013) and c_1 , i.e., the strength of the shear enhancement phenomenon (Eq. (2b)), control the correct formation of the decussation pattern: values of v_r/v_m and c_1 must lie above a transition boundary, but not too far above it (Fig. 10). A particular value of v_r/v_m is also required to predict the waviness of dental enamel correctly (Section 6).

The ratios of velocities that we discuss here could alternatively be expressed as the ratios of two time scales, one characterizing the time over which a driving force changes and the other the time over which the material responds. Such a ratio is known in rheology as the Deborah Number (Reiner, 1964); see also Angelini et al. (2011). Referring to migration velocities and to the propagation velocity of a determination front or a cell density wave, has the advantage that these quantities are measured directly.

15.1. Migration and relaxation

Table 2 summarizes the relaxation and migration velocities for a number of cases. The migration velocities are all published, measured quantities. That for the case “epithelial cells in vitro” refers to the velocity of the leading edge in the experiments on expanding populations of cells reported by Serra-Picamal et al. (2012). The number quoted for osteoblasts in the BMU reflects the rate at which the radius of the interior surface of the osteoid formed within a BMU decreases (Fig. 2d and Cox and Smith, 2014; Buenzli et al., 2010); the cells reside on this inner surface. The relaxation velocities for cases of amelogenesis were determined as described in Section 7. The relaxation velocities for cells in the host medium involved in innervation and angiogenesis are estimated by combining the typical migration rates of the invading cells (neural cells in innervation and endothelial cells in angiogenesis) with the condition $0.03 < v_r/v_m < 0.06$. The relaxation velocity for the case “epithelial cells in vitro” refers to the velocity of propagation of so-called X-waves in Serra-Picamal et al. (2012).

Even though the cases listed span different organs and species, the relaxation velocities are quite similar; most lie within a factor of two of their mean, and the extreme values are within one order of magnitude of each other. The high extreme is for the epithelial cells moving in

vitro, which may be associated with the fact that the degree of spatial constraint is much less in this experiment than in vivo. In contrast, the migration velocities vary over three orders of magnitude, the values for either case of network formation being 100–1000 times greater than the values for other processes of organogenesis. Indeed, invading cells that form networks move faster than all cells except cancer cells, which have similar velocities (Maiuri et al., 2012).

15.2. Critical strain rate conjecture

Migration velocities straddle the approximately common relaxation velocity in Table 2. Migration velocities for amelogenesis and in the BMU, and most other values reported in the data of Maiuri et al. (2012), are less than the relaxation velocities; whereas the cells that form networks, which must satisfy the condition $0.03 < v_m/v_r < 0.06$ according to the theory reviewed in Section 13, and cancer cells (Maiuri et al., 2012) have migration velocities that are much greater than the relaxation velocity. Thus network-forming invaders and cancer cells are exceptional.

We hypothesize that cells forming networks, e.g., in innervation of the gut or angiogenesis, migrate unusually fast precisely so that the cells in the host domain that they invade will not have time to reconfigure and, by reconfiguring, avoid the creation of significant stresses within the host cells. We also infer that the invading cell must migrate just fast enough for this purpose, and not much faster: for the elastic strains induced in the host to vary spatially as required for network formation, i.e., as depicted for the condition $0.03 < v_m/v_r < 0.06$ in Fig. 16d, the velocity v_m of the invading cells must be high enough, relative to v_r , to generate significant stresses in the host ahead of the branch tip, but not too high, because the stress must diminish in relatively short times along the flanks of the branch.

Since the velocity of the invaders dictates the strain rate (time rate of change of strain) generated in the host, the fact that invaders move just fast enough to generate significant host stresses before relaxation can occur implies that a critical strain rate $\dot{\epsilon}_{cr}$ exists for the host cells: significant stresses arise in a host cell if it is subjected to a strain rate $\dot{\epsilon} > \dot{\epsilon}_{cr}$, whereas the host cell reconfigures and maintains low stress if it is subjected to a strain rate $\dot{\epsilon} < \dot{\epsilon}_{cr}$.

We can estimate $\dot{\epsilon}_{cr}$ from the strain rates predicted in the host by the theory of network formation. If the invading cells move just fast enough to generate stresses in the host ahead of the branch tip, then the strain rates computed in the host ahead of the branch tip will equal $\dot{\epsilon}_{cr}$. The computed average of one component of the elastic strain over a gauge length extending the width of a host cell, $d \approx 10 \mu\text{m}$, ahead of the branch tip is $\epsilon_{av} \approx 0.07$ (Cox, 2011). Since the elastic strains further ahead of the branch tip are close to zero, the strain rises from zero to its maximum value in the time d/v_m it takes the branch tip to advance a distance d . Given $v_m \approx 1 \text{ mmd}^{-1}$ for innervation, the strain rate in the host as it is approached by the branch tip is $\dot{\epsilon} \approx 10^{-4} \text{ s}^{-1}$. For angiogenesis, v_m is somewhat higher and therefore $\dot{\epsilon} = \epsilon_{av} v_m / d \approx 3 \times 10^{-4} \text{ s}^{-1}$ (Table 2). Thus we infer that the value of the critical strain rate lies, to order of magnitude, in the range $10^{-4} < \dot{\epsilon}_{cr} < 3 \times 10^{-4} \text{ s}^{-1}$.

The existence of a critical strain rate and the estimate of its value presented above are supported by two sources of data for individual cells. First, a transition is observed in a single cell subjected to cyclic straining, from alignment with the direction of applied strain at low frequencies to alignment orthogonal to that direction at high frequencies (De et al., 2007). Theoretical analysis explains the transition as a manifestation of the fact that the cell's "reorganization can only occur if the temporal variation of the force is slower than the time required for the focal adhesions and stress fibers to reform" (De et al., 2007). Re-examination of the analysis in (De et al., 2007) shows that the transition in cell response occurs for an applied strain rate in the range $3 \times 10^{-5} \leq \dot{\epsilon}_{cr} \leq 2 \times 10^{-4} \text{ s}^{-1}$ (Appendix A).

The second source of data is recent measurements on single cells under monotonic (non-cyclic) loading (Webster et al., 2014). A single fibroblast was allowed to attach itself to the arm of a cantilever beam and simultaneously to a fixed plate. The cantilever was then displaced at a constant displacement rate, imposing a constant strain rate on the cell. Strain rate values can be obtained by simple manipulations of the pertinent data from Webster et al. (2014) (Appendix A). At a strain rate of 10^{-4} s^{-1} , the total force in the cell did not change substantially, even as the cell was stretched through a strain of 0.1. In contrast, at a strain rate of 10^{-3} s^{-1} , the total force increased substantially. The measured transition implies a value of $\dot{\epsilon}_{cr}$ in the range $10^{-4} \leq \dot{\epsilon}_{cr} \leq 10^{-3} \text{ s}^{-1}$. This range overlaps that estimated from the cell re-orientation analysis and approximately coincides with the range deduced from the theory of strain-cued network formation.

Consistently, the relatively high strain rates predicted in the host cells (i.e., $\dot{\epsilon} > \dot{\epsilon}_{cr}$) imply relatively high strain energy rates, which are not far below the hosts' metabolic energy rate: $\dot{G}_{el}/\dot{G}_{met}$ is in the range 10^{-3} – 10^{-2} , which is substantially higher than for other cases of organogenesis (Table 1). We infer a possible critical value of $(\dot{G}_{el}/\dot{G}_{met})_{cr}$ in the range $10^{-3} \leq (\dot{G}_{el}/\dot{G}_{met})_{cr} \leq 10^{-2}$, such that significant stresses can develop within cells that are subjected to strain due to cell migration during organogenesis if $\dot{G}_{el}/\dot{G}_{met} \geq (\dot{G}_{el}/\dot{G}_{met})_{cr}$.

However, while this may be a sufficient condition for significant stresses arising, it is not necessary: non-migrating cells (cells with zero strain rate) can exert large forces over long times when attached to stiff substrates (Discher et al., 2005; Harris et al., 1980).

15.3. Strain rates and mechanical stress

Last, we note the difficulty of predicting the magnitude of mechanical stress in cases of organogenesis other than network formation. For the case of amelogenesis, one has the condition $v_r/v_m \approx 20$. Although the geometry of cell deformation during amelogenesis is different to that of host cells that are being invaded during network formation, nevertheless the curves of Fig. 16d will be qualitatively indicative of how fast elastic strains might form and relax during amelogenesis. For $v_r/v_m \approx 20$, the elastic strains can dissipate essentially as soon as they form (indicated by the dashed red curve in Fig. 16d⁷). No doubt, stresses must

⁷The numerical methods used to generate Fig. 16d are not accurate for density fluctuations that decay in less than one time increment, but the implications for large v_r/v_m are as indicated.

be present within ameloblasts as they slide past one another and across the stratum intermedium (Fig. 3); tractions must be exerted by the ameloblasts on one another and on the stratum intermedium to achieve relative motion and the tractions must be reacted through the cell volume. Consistently, the significant presence of desmosomes in the stratum intermedium implies that substantial stresses are present in that layer (Bartlett and Smith, 2013). However, the high values of v_r/v_m characteristic of amelogenesis, or equivalently, the low values of $\dot{G}_{el}/\dot{G}_{met}$, in the range 10^{-4} – 10^{-5} (Table 1 and Cox and Smith, 2014), which is well below the conjectured critical value $(\dot{G}_{el}/\dot{G}_{met})_{cr}$, warn that the inference of the state of stress from the observed state of strain in the system is very difficult. For a given strain and typical strain rate, the cells have abundant metabolic energy and time to assume any state of stress they wish; we note especially that the stress state in the cell interior inferred from boundary tractions, even where the tractions are fully known, is non-unique. Accordingly, the theory of amelogenesis makes no reference to stress; the entire pattern-forming mechanism is defined in terms of strains alone.

The exceptional situation with host cells during angiogenesis or innervation is that they are strained at such a rate that the assumption of linear elasticity, i.e., the mechanical response of inanimate material, is probably approximately valid for times that are short, yet long enough to be relevant in branch stabilization.

16. Possible roles of strains in establishing cues for cell behavior

Cell behavior during an invasion is governed by a complex set of chemical factors, operating within individual cells and exchanged between cells. Prior theories of the achievement of network morphology, e.g., in angiogenesis, neovascularization, and innervation, have been based exclusively on analysis of chemical factors and their spatial distribution. Yet the local strain fields generated during an invasion in both the host and the invading cells have symmetry-breaking characteristics that are ideally suited to guiding the invasion. Here we consider mechanisms by which the strains might contribute to cell behavior, as well as some apparent gaps in models that refer only to soluble factors or chemical adhesion.

16.1. Force required for host cell separation

When averaged over gauge lengths equal to the typical width of a single cell, e.g., 10 μm , the two components of tensile elastic strain induced in the host immediately ahead of a branch tip and tangential to the surface of the invaded domain have magnitudes of approximately $\epsilon_1 = \epsilon_2 = 0.07$ (Cox, 2011; Cox and Smith, 2014). If the host cells are assumed to be governed by Hooke's law (for short times and high strain rates), then the stress in the host cells in the direction that is tangential to the surface of the invaded domain is

$$\sigma_t = C_{11}\epsilon_1 \quad (5)$$

where $C_{11} = E(1-\nu)/((1+\nu)(1-2\nu))$ and E and ν are Young's modulus and Poisson's ratio, respectively, for the host cells. With Young's modulus given a typical value $E \approx 100$ Pa for cells without ECM and $\nu=0.3$, Eq. (5) yields $\sigma_t \approx 10$ Pa. Using Young's modulus for cells

without ECM is appropriate for estimating the stress in the cell, even if ECM is present, provided the cells and the ECM share the same average strain, which is a reasonable assumption for typical spatial distributions of ECM.

From data obtained in experiments on epithelial cells conducted in vitro using a type of force microscope (Benoit and Selhuber-Unkel, 2011), the maximum traction (force per unit area), σ_{ad} , required to overcome cell–cell adhesion is estimated to be in the range 5–30 Pa (Appendix B). From data from the same reference, the traction required to overcome cell–matrix adhesion is estimated to be approximately 10 Pa (Appendix B). Thus the adhesion strengths are similar to the estimate for σ_t in the host ahead of a branch tip, i.e. $\sigma_{ad} \approx \sigma_t$.

In the experiments on spreading epithelial cells reported by Serra-Picamal et al. (Section 8), adhesive tractions of 200 Pa or more were inferred by the analysis of measured tractions in the substrate over which the cells were migrating, which are much higher than the estimates of Appendix B. However, the spreading epithelial cells were participating in collective migration via plithotaxis (Serra-Picamal et al., 2012), during which cells entrain and pull one another along by force. Such cells are likely to have assembled dense, aligned stress fibers and dense focal adhesions for this purpose, and may have significantly higher adhesive strengths than host cells that have been relatively inert mechanically prior to being encountered during an invasion.

Thus we conclude that any invader that migrates fast enough to induce Hookean response in host cells, and that generates elastic strains of approximately 0.07, will thereby induce stresses σ_t in the host cells that can be comparable to the adhesive strength σ_{ad} of either pairs of host cells, or the host cells and the ECM. The interfaces that are most affected are those that stand normal to the surface of the branch of invaders, because they therefore lie normal to the direction of tensile strain. The distance from the branch tip over which interfaces may be affected, i.e., over which the tangential stresses have not yet significantly decayed, is comparable to the width of the branch tip, typically one cell diameter.

16.2. Surface traction acting on invaders: contact inhibition of locomotion?

The invaders themselves are always in a state of compression, because the host cell medium reacts back on the invasion domain when it is displaced by the invaders. This reaction can be quantified by the computed stress component σ_n in the direction normal to the host/invader interface, evaluated at the interface. The normal stress σ_n is also the contact pressure experienced by the invader cells.

We consider the corresponding component e_n of elastic strain, which is proportional to σ_n , for a single propagating branch of invaders (Fig. 19). At all locations, e_n is negative (compressive). The maximum magnitude of e_n is always found immediately ahead of the branch tip (point P of Fig. 16c). The magnitude of e_n declines along the flank of the branch at a rate that increases when the ratio v_f/v_m increases.

The fact that the contact pressure has maximum magnitude immediately ahead of the branch tip has interesting implications if the tip cell of the branch is susceptible to contact inhibition of locomotion (CIL). One might expect that the polarity of the tip cell would rotate away

from the location of greatest contact pressure (Newgreen, 1990), i.e., away from the orientation $\beta=0$ of Fig. 15. This would disrupt the stability of the branch tip geometry.

16.3. Host medium stress and invaders' migration

However, a different possibility regarding the directional stability of an invasion branch, and the stability of the polarity vector of the tip cell, emerges from considering how the energetics of candidate invader cell actions would be influenced by the tangential stresses that are present in the host cells.

Consider an increment of tip cell migration to be executed in two distinct steps: the extension of a filipodium between two host cells, during which process the host cells need not deform extensively because the filipodium is thin; followed by the movement of the entire tip cell between the two host cells, during which the pair of host cells in question must displace significantly and the host cells that surround that pair will also be significantly strained by the incursion of the tip cell.

16.3.1. Filipodium insertion—Immediately ahead of a newly arrived branch tip (Fig. 20a), $\delta_t \approx \delta_{ad}$ (Section 16.1). Therefore the additional work the invader must do to de-adhere the interface, a requirement for inserting a filipodium between the host cells, is effectively zero in such locations. The adhesion energy can be characterized by the product $\delta_{ad}\delta_c$, where δ_c is proportional to the separation of the two host cell membranes at which the adhesive force between them vanishes. The tangential stress contributes work $\delta_t\delta_c$ during the same separation and therefore the effective adhesive energy, i.e., the work the filipodium must do to separate the host cells, is $W_{\text{interface}} = \delta_{ad}\delta_c - \delta_t\delta_c \approx 0$. Energy is conserved: the tip cell already did the work required to create the stress δ_t during the migratory step that brought it to the position shown in Fig. 20a, before it extended a filipodium into the stressed host medium. A few cell–cell adhesions that are candidates for weakening by stress in the host medium are labeled “F1” etc. in Fig. 20b.

Thus the usual expectation of the suppression of filipodia during CIL at continuous contacting surfaces might not occur: the tip cell encounters a contacting surface that is discontinuous, with lines of weakened adhesion between the host cells.

In contrast, along the flanks of the branch, where the tangential stress in the host is compressive ($\delta_t < 0$), $W_{\text{interface}} > \delta_{ad}\delta_c$; filipodia must do increased work to penetrate the host medium. The inferred spatial variation in filipodium number density (Fig. 20b) is consistent with the fact that, during the migration of neural crest cells in chain assemblies, the tip or leading cells are observed to be densely covered in filipodia, whereas trailing cells extend relatively few filipodia (Wynn et al., 2012; Teddy and Kulesa, 2004).

16.3.2. Migratory step—The displacement of the host cells remains small during the insertion of a filipodium, which is very thin. Therefore, the stress in the host medium, δ_c , will remain approximately unchanged during this event. Since the filipodium is now intervening between two host cells, the host cells are imposing an outward pointing traction of magnitude δ_t on the invader's membrane (Fig. 20c). This traction has its maximum value for filipodia immediately ahead of the invader branch tip, i.e., at $\theta=0$ (Figs. 16d and 20a).

For directions of tip cell migration that are increasingly removed from the direction $\theta=0$, the traction at first diminishes and then changes sign when δ_l become compressive.

The tractions acting on the filipodium must change sign as the invader undertakes its migratory step, becoming compressive (inward pointing) with the spatial distribution of Fig. 19 when the migratory step is completed. Nevertheless, the fact that the tractions are initially tensile (outward pointing) offers the cell a mechanical boundary condition that discriminates directions; invader migration along the direction that maintains stable branch tip geometry, $\theta=0$, is favored energetically during the first swelling of the filipodium.

16.4. Fracture-like process induced by invaders acting as strain-cued automata

The above description is a qualitative depiction of a fracture process: advance of a crack in the direction already established is favored because it maximizes the release of strain energy in the medium being fractured. The host cells have no active role in this process; the invader cell is merely recouping the elastic energy it generated in the host cells when it advanced into their vicinity, i.e., to the configuration shown in Fig. 20a, prior to the migratory step depicted in Fig. 20c. All the energy needed for the migration is likely to be supplied by the invader cells, acting as automata. The host cells play the role of temporarily storing some of this energy in a spatially distributed manner that favors maintaining the direction of an invasion branch.

One key to this process is the step-wise character of invader cell advance as described above: rather than the entire cell translating through the host with approximately invariant shape, the cell first extends a thin filipodium or protrusion into a region of the host that it has already placed into tensile strain, then surges forward by expanding the protrusion, initially aided by the tractions applied to it by the host.

16.5. Chemical factors as the mechanism for maintaining collective streaming during migration and the control of sprouting frequency

A very large literature describes the search for chemical factors that act as inhibitors during collective migration in such a way that desirable morphogenesis is achieved. In a small sampling: the ability of cells to maintain a relatively tight formation during collective migration over long distances, such as the cells forming a single branch within a network, rather than diffusing amorously through the host medium, has been attributed to inhibitory chemical factors ("negative taxis") that create spatial restrictions on cell movement (e.g., Theveneau and Mayor, 2011 and references therein); the suppression of sprouting from locations near existing bifurcations is attributed to the Notch signaling pathway (Siekman and Lawson, 2007; Phng and Gerhardt, 2009; Eilken and Adams, 2010); and Slit-Robo signaling has been correlated with limiting excessive migration of endothelial stalk cells during vasculature (Jones et al., 2008).

Critical to the operation of inhibitory factors is spatiotemporal sensing: cells must release such agents at the correct places and times. The means for such sensing do not yet appear to be fully clear. In a number of theoretical simulations of network formation, ad hoc rules are invoked to govern sprouting frequency, etc., in place of physical models of the timed release of factors. For example, in an attractive study of the role of gradients of hypoxia-induced

gradients in VEGF in angiogenesis, Liu et al. impose the arbitrary constraints that “the number of activated cells is constrained by a specified maximum number per unit capillary length” (Liu et al., 2011). Network density is determined by assuming that “branching occurs with a specified probability” (Liu et al., 2011). Elsewhere, in a theory of angiogenesis and vascular remodeling, a rule was arbitrarily imposed that defined an exclusion radius around the base of each sprout or incipient branch, inhibiting further spreading of the cells into that space (Owen et al., 2009). This rule ensures that branches and sprouts extend without thickening, an essential characteristic for achieving a network. The physical origin of such a rule is suggested to be in the actions of Delta-Notch signaling, but the source of the spatiotemporal information that leads to such signaling operating at the base of a sprout rather than elsewhere around a branch is undetermined.

16.6. Cell–cell contact rules – plithotaxis vs. automatus cells

Alternative mechanisms for maintaining a chain structure among cells migrating in a branch have considered the role of cell–cell contact. In one theory (a hypothetical instance of “plithotaxis”), an automatus tip cell pulls passive stalk cells along in its wake; the tractions that pass from one cell to the next serve to keep them entrained (Friedl, 2004; Szabó and Czirók, 2010). However, as pointed out by Szabó and Czirók, such a system would be vulnerable to the Plateau–Rayleigh instability when its length far exceeds its diameter: analogously to a stream of water breaking into droplets when surface tension effects amplify small variations in the stream’s cross-section (Strutt and Rayleigh, 1878), it should break up into subgroups of cells (Szabó and Czirók, 2010).

Such a physical instability can be countered if the cells act autonomously. Szabó and Czirók show that a continuous chain structure can remain as a stable possibility if the stalk cells are also assumed to actively migrate to maintain contact with other cells: the cells can remain in a narrow branch structure (sprout) if their attraction is biased towards cells that have elongated shape. In the theory of Szabó and Czirók, elongated shape is also identified with a cell polarity vector. The simulations yield encouragingly attractive outcomes in terms of shape and stability, and realistic predictions of the ability of an individual cell within the migrating group to remain attached to the group rather than migrating errantly into the host medium (Szabó and Czirók, 2010). That is, the system becomes anisotropic, spontaneously identifying the direction of the axis of the branch. However, the mechanism by which one cell might be preferentially attracted to elongated cells, which is the essential assumption of the theory, remains obscure.

Strain cues offer an alternative mechanism for maintaining the integrity of a chain-like structure, even if the chain is repeatedly broken by cells that temporarily lose contact with one another. We discuss this further in Section 18.2.

16.7. Positional and orientational information in strain fields

The strain distributions that arise in the host cells immediately adjacent to an advancing invasion branch (Section 13.6), which are illustrated in Fig. 18 and the movie “strains d05 gch0”, provide information about both the position of individual cells and the orientation of the branch. First, the components of stress (or elastic strain) in the branch-adjacent host cells

that are tangential to the surface of the invader domain switch from tension to compression, as described above. (More fully, the state of stress in the branch-adjacent cells is triaxial: the component normal to the surface of the invader domain is always compressive, while the two tangential components switch from tension to compression.) As well as providing a mechanism for suppressing filipodial extension from the invader cells along the flanks of the branch, as noted above, the compressive stress in the branch-adjacent host cells presents a constricting sleeve to the invaders within the branch. This compressive sleeve raises the energy required for an invader cell to migrate into the adjacent host and thus identifies the orientation of the branch axis.

Second, an uptick occurs in the compressive stress in the branch-adjacent cells at a bifurcation (e.g., red spatial elements in Fig. 18): bifurcation leads to locally high density fluctuations and high eigenstrains. The augmentation of compression can serve to notify a host cell that it is not merely branch-adjacent, but bifurcation-adjacent.

16.8. The effects of using a cubic mesh in the computation of strains

The strain estimates of Fig. 16d or Fig. 18 were obtained using a mesh of cubic computational elements. This choice allowed the application of a very useful formula for computing the stresses induced by density variations (Li and Anderson, 2001).

The strains computed in this way are similar to those that would be predicted if the spatial domain of a branch of invaders were assumed to elliptical (needle shaped) rather than cuboidal (blunt): the maximum strain would still lie ahead of the branch tip (Eshelby, 1957; Cox, 1989). However, cell shape will have an influence. In particular, the strain concentration factor would be greater ahead of an advancing cell that has a pointed tip than in the results reviewed here. Therefore strain cues acting on such a pointed invader cell would more strongly favor continued migration in the direction $\theta=0$.

17. Mechanical and chemical factors acting in tandem in network formation

17.1. Far-field gradient in a chemical factor

In the simulations of network formation via strain cues shown in the movies “network d05 gch0” (published online), a front of network formation advances in one dominant direction, from left to right without the aid of a global gradient in any factor. Front propagation in the absence of a far-field gradient in a chemical factor might be representative of the case of innervation of the gut (Trewenack and Landman, 2009; Newgreen et al., 2013; Simpson et al., 2006).

More generally, if a chemical factor, e.g., VEGF or TAF, is required to stimulate the invading cells to invade, then the network will tend to grow where such a factor is concentrated (e.g., Fig. 20 of Gerhardt et al., 2003). Fig. 21 and the movie “strains d085 gch-0.5” (published online) illustrate one example of morphological control by a long-range chemical gradient and short-range strain gradients acting in tandem. The effect of the chemical gradient was simulated by changing the function $g(e)$ of Eq. (4b) to

$$g(\varepsilon) = \frac{1}{\chi} \left(\frac{\varepsilon}{\varepsilon_0} - 1 \right) + g_{\text{ch}} J \quad (6a)$$

$$J = \begin{cases} 1 \\ 1/2 \\ 0 \\ -1/2 \\ -1 \end{cases} \quad \text{if } (\delta_x, \delta_y) = \begin{cases} (1, 0) \\ (1, \pm 1) \\ (0, \pm 1) \\ (0, \pm 1) \\ (-1, 0) \end{cases} \quad (6b)$$

with g_{ch} a constant and (δ_x, δ_y) the candidate invasion step normalized by the computational element size. The term J introduces directional bias in the generated branches, while bifurcation events, the random lateral fluctuations of branch directions, and branch coalescence events due to strain cues are all preserved.

17.2. Possible role of chemical factors in a strain-cue signaling pathway

Part of the motivation of the predictions of the spatial distribution of VEGF in Mac Gabhann et al. (2007) is the absence of experiments that can resolve this distribution over gauge lengths comparable to the dimensions of a branch tip; while longer-range variations in VEGF are known, the possibility that VEGF, or any other chemical factor, varies systematically around a branch tip remains untested. However, the angiogenesis signaling molecule VEGF-A tends to occur as an intercellular secretion (e.g., Ruhrberg et al., 2002, especially Fig. 17 therein) and therefore one can conjecture that its production could be augmented where pairs of host cells, or host cells and the ECM, are de-adhered by the stress induced by rapidly migrating invaders, thus echoing the pattern of the strain field.

In the case of the neural growth cone, which involves the migration of a single cell through a host, spatial variations of chemical factors acting as guidance cues are seen that are similar to those around the tip cell of a branch (e.g., wikipedia, 2014). Molecules that have been identified as factors include Netrin, Slit, Ephrins, and Semaphorins (Gordon-Weeks, 2005).

18. Migration of non-contiguous cells

18.1. Isolated cell

In the theory of cell network formation, symmetry is broken partly because an existing network branch is long and slender and strains in the host are therefore concentrated at its tip (Eshelby, 1957; Neuber, 1946). However, a second symmetry-breaking factor is also present: the relaxation of strains and stresses in the host material is more complete in the wake of a moving invader than ahead of the invader. This phenomenon would be present even for an isolated migrating cell: the strain fields will be polarized by the cell's motion and could therefore in principle stabilize the polarity of the cell and guide its motion through a host along relatively straight lines over biologically significant distances, as illustrated in the following.

Consider a single cell migrating through an initially homogeneous host cell population and guided by strains. Assume that the invading cell is somewhat longer than wide and that it moves initially to the right at a fixed velocity (Fig. 22a). Let the motion of the invader be governed by Eq. (4), modified to preserve the integrity of the cell: migration is only permitted from one of the spatial elements at the ends of the threesome, not from the central element; and, if migration occurs from one of the end elements, the other two elements occupied by the migrating cell move to follow, so that the cell always consists of three contiguous elements. The migration can occur from either the right or left end at any time increment, biased according to the relaxed elastic strains in the adjacent host (e.g., Fig. 22b⁸).

Given the condition $0.03 < v_p/v_m < 0.06$, the spatial distribution of the strain cue $\epsilon_1 + \epsilon_2$ will depend on the cell's recent motion, being larger at point P_1 than at point P_2 . The host cells are newly strained at P_1 , the migrating cell having just increased cell density in that vicinity, whereas the host cells along the flanks of the migrating cell and in its wake at P_2 were strained some time ago and density fluctuations have therefore had more time to relax. Thus the cell moves in a random walk biased toward migration in the same direction as the current motion. While direction reversals can still occur occasionally, the migrating cell increases its distance from its initial location much more efficiently (wolfram_research, 2013). The movie "loner trio" illustrates one instantiation of stochastic migration biased by host relaxation.

A single cell migrating through a host population will on average proceed significantly further if it maintains a migration velocity that is relatively high: assuming universality of relaxation velocities (Table 2), the ideal migration velocity will be comparable to that of invader cells during innervation or angiogenesis. Migrating cancerous cells also move very fast (Maiuri et al., 2012), a property that, if they exploit strain cues, would help them maintain their direction while traversing host populations, as in metastasis.

Biased random walks have previously been developed to study collective cell migration, with the bias generally attributed to a global chemical gradient (a gradient that is gradual relative to the cell size) (Huang et al., 2005; Szabó et al., 2010; Berg, 1993).

18.2. Migration of a group of cells that are not in constant contact

An interesting conjecture follows from the above regarding cells that migrate collectively but are not always in contact with one another, a commonly occurring case in collective migration (Rorth, 2012). To begin, we note that an elongated, peloton-like body of migrating cells that are all in contact with one another will develop stresses in the surrounding host whose spatial variations will have similarities to Fig. 16d: the tangential stress will be tensile immediately ahead of the peloton and decay to compressive states along its flanks (Fig. 23a).

If a cell on the periphery of the peloton should separate from the peloton, two strain-cue effects will enhance the probability that at some later time it will run into the peloton again

⁸The model of Fig. 22 is very simplistic in assuming bluff shapes for both the tip and tail of a cell. A more realistic model would include shape anisotropy as a further biasing factor, e.g., a tapered tail.

and re-unite with the peloton. First, the stress in the host that intervenes between the separated cell and the peloton will tend to be strongly tensile, at least initially, in the directions tangential to the surfaces of the cell and the peloton at their nearest approach (Fig. 23b). If the motion of the separated cell is directed towards tensile strain cues, it will tend to move back towards the peloton. This phenomenon is related to the tendency of two branch tips in network formation to jump together if they get close (Section 13.2). Second, if the separated cell becomes separated without changing the direction of its velocity vector significantly, i.e., separates from but moves approximately parallel to the peloton, it will tend to maintain a velocity that remains close in direction to that of the peloton because of the effect described above for a single migrating cell. Thus it may remain relatively close to the peloton and therefore be more likely to come back into contact with it by random motion.

Thus strain cues may provide a mechanism for maintaining communication among cells that exhibit collective migration without always being in contact with one another. The interplay of such strain-cue effects with the Plateau–Rayleigh instability, which tends to break assemblies of migrating cells into multiple smaller groups (and therefore more lethal groups in the case of glioblastomas), and with adhesion effects, esp. those of the anisotropic type studied by Szabó and Czirók (2010), is a potentially interesting research topic.

19. Characteristics of successful strain cues

1. In both the theories of amelogenesis and network formation, the magnitudes of components of local strains take values typically in the range 0.05–0.1. This range coincides with the strain amplitudes in experiments in which fibroblasts exhibited a transition in force response as the strain rate increased (Webster et al., 2014) and with the strain amplitudes at which substantial changes have been measured in the rates of proliferation and differentiation of fibroblasts (Li et al., 2009).
2. The predicted near equality of stresses induced in the host during an invasion and cell–cell and cell–matrix adhesion strengths requires satisfaction of an equality involving three physical parameters:

$$\sigma_{\text{ad}} \approx E\epsilon_0 \quad (7)$$

The gauge-averaged strain ϵ_0 is more or less fixed at $\epsilon_0 \approx 0.07$ by the geometry of the invasion. Thus Eq. (7) relates the adhesion strength, σ_{ad} , which depends on the density and strength of molecular attachments between cells, and the short-time modulus of the interior of the cell, E , which depends on the cytoskeletal density and configuration. Given the wide range of plausible orders of magnitude of adhesion strengths and moduli, the fact that Eq. (7) holds approximately provokes the conjecture that the cytoskeleton and membrane chemistry have co-evolved to achieve matched values and an advantage in natural selection. Being able to separate host cells during invasion, i.e., having $\sigma_{\text{ad}} \approx E\epsilon_0$, is an advantage to the organism, allowing the invaders to be guided. Maximizing σ_{ad} subject to

this inequality, i.e., achieving the equality, avoids compromising tissue integrity under other circumstances. Similarly, requiring rapid invasion to generate significant stresses (strain rates in excess of $\dot{\epsilon}_{cr}$ protects the developing organ from chaotic outcomes when cells move about incidentally, but at lower rates.

3. Even for invasion into tissue in a non-structural organ (e. g., kidney, liver, brain, and heart) with a matured content of connected ECM, the rate at which an invading cell must do mechanical work to strain the host medium sufficiently strongly and rapidly to trigger de-adhesion events (i.e., to approximately 7% strain at strain rates above 10^{-4}s^{-1}) remains 2–3 orders of magnitude lower than the rate at which it can produce energy via its metabolism (using data from Table 1). This suggests that invading cells could do the work necessary to move forward through mature tissue and generate strain cues. However, they may also facilitate their invasion by secreting enzymes (proteases and aggrecanases) to degrade the host ECM (Vempati et al., 2007). The spatial distribution of such weakening need not itself break symmetry; provided the host medium is weakened, allowing the invading cell to migrate rapidly, the cell's motion will generate the strain pattern that is sufficient to guide network formation.

20. Robustness of strain-cue mechanisms

A successful mechanism for pattern forming must be robust against perturbations of the system, e.g., variance in the initial conditions or a changed external boundary condition. Robustness of the Turing mechanism has been a major topic of research (e.g., Maini et al., 2012 and references therein).

The strain-cue mechanism for achieving network morphology has some attractively stable characteristics. The guiding strain field does not rely on the accurate maintenance of any long-range gradient, but is generated locally, by the invaders' own motions; in fact, significant stress perturbations in the host medium extend only a few cells' diameter, at most, from the invader domain (Fig. 18). As long as the invader has made a recent advance, the strain cues will be present in the host to guide its next migratory step. Furthermore, the local strains are much larger in magnitude than those that might reasonably be expected to arise as a far-field perturbation, e.g., due to a random external load.

Similarly, the strain-cue mechanism for forming the sliding layered pattern of decussation in dental enamel (Section 3) relies only on the interactions of each cell and its current set of contacting neighbors.

The local nature of the strain cues also implies that migration in organs with differing states of mechanical constraint might be similar. Because the local strains are larger than long-range fields that are likely to arise due to organ constraint, the strain cue mechanism will function in essentially the same way whether the organ is in a confined volume, such as the brain, or whether it is not, such as the breast. This observation might be relevant to cell migration in normal development or in metastasis.

Insensitivity to initial conditions is illustrated by the case of network generation in which the initial configuration consisted of a single spatial element occupied by an invader. This different starting condition leads to networks that are statistically identical, in terms of branch frequency, loop size distribution, etc., to those initiated from bands of invaders (compare the movie “point source d055.wmv” with the movie “network d05 gch0”, published online). Network formation from single cells acting as nucleating sites appears to play a part in innervation of the gut: enteric neural crest cells are seen to migrate ahead of the front of the forming network and generate new network patches, which later unite with the main network (Young et al., 2014).

21. Electric fields – an isomorphism

The potential field from which elastic strain energy is derived has the same form as the electric potential. Therefore, the concentration that arises in the strains at the tip of a branch of invaders (Fig. 16d) will also arise in the electric field that would be predicted if the invaders were electrically charged. The idea of electric fields as a branch-stabilizing mechanism has been noted (Levin, 2012). The concept of strain-cued automata is distinguished by the role played by the relaxation of stresses in the host during network formation, which generates the spatial distributions that are so well adapted to branch morphogenesis, and the appealing universality of the relaxation velocities inferred by analysis of different patterns across organs and species (Table 2).

22. Concluding remarks

That strain fields can act as a mechanism for the formation of patterns in living cell populations has been established partly by direct observations of cells in vitro and partly by computer simulations. The strain fields break symmetry and thus can be the trigger for a cascade of events leading to individual cells migrating in such a way that the population develops biologically relevant patterns.

The patterns are realized by cells acting as automata, i.e., migrating in directions that do not necessarily coincide with those established by energy gradients (stress, pressure gradients, cell–cell adhesion gradients). Cells have abundant energy available in their metabolism to move where they wish.

Thus the conditional statement of Eq. (1b), that cells acting as strain-cued automata can form patterns similar to those observed in nature, is proven. The proposition of Eq. (1a), that cells do indeed act as strain-cued automata, over certain time intervals and certain spatial domains, remains imperfectly tested.

22.1. Waves and wavefronts

Wavelike motion has been observed directly in vitro among epithelial cells (Serra-Picamal et al., 2012) and inferred by analysis from the wavy nature of the microstructure of dental enamel (Cox, 2010). In both the epithelial cell population observed in vitro and an ameloblast population responsible for amelogenesis, a soliton-like traveling wavefront is observed, characterized by step changes in the cell velocity and the strain state. The

traveling wavefront is proposed to act as a trigger in amelogenesis for the maturation of cells into secretory-stage migration, i.e., into the action of laying down enamel (Cox, 2010). More generally, such a wavefront is conjectured to be a determination front, e.g., in the clock and wavefront model of Cooke and Zeeman (1976). That wavefronts can remain compact and propagate as stress disturbances at an approximately constant velocity, sustained by an active medium of cells responding to changes in their mechanical boundary conditions, has been demonstrated theoretically for sparse systems in the strain-cue theory of network formation (Cox, 2011) and for continuous systems in a theory due to Idema and Liu (2014).

22.2. Patterns of sliding layers

In the theory of amelogenesis, symmetry is broken via “enhanced shear motility”, whereby cells enhance initially infinitesimal relative sliding motions (or local shear strain rates) via a feedback mechanism that operates between contacting neighbors and thus spontaneously form sliding layered patterns. At an intuitive level, one might regard enhanced shear motility as equivalent to a negative viscosity in a continuum fluid model of the population (but beware that few results reported here for the case of amelogenesis could be replicated without representing cells as discrete entities). The possibility that cells might use feedback acting via their mechanical boundary conditions to establish sliding motion has also been explored in theories of activity in the interior of cells, which account for the experimentally observed phenomenon of persistent rotational motion (Leong, 2013; Camley et al., 2014).

22.3. Network formation

That strains arise around an invader that are similar to those computed in the particular formulation of the model of Section 13 cannot be disputed: the strains describe the necessary displacement of the host medium to make room for the invaders and are bound, by the nature of mechanical inclusions, to be maximum ahead of a slender branch. That the host medium should then relax the stresses that are associated with the strains it suffers also seems very likely. The diminution of the tangential stress behind a branch tip and its possible transition from tensile ahead of a branch tip to compressive along its flanks immediately follow. That is to say, the predicted strain and stress fields are not likely to be model dependent; they are likely always to have the same qualitative form.

The most uncertain feature of the simulations of Section 13 is the value assigned to the ratio of the relaxation velocity and the migration velocity. However, the relaxation velocity required to produce lifelike networks coincides approximately with that deduced for ameloblasts by analysis of waviness in enamel microstructure, and it also leads to a rate of switching to a pattern of tensile and compressive stress around an invader branch that correlates approximately with the observed presence or absence of filipodia emerging from tip or stalk cells in angiogenesis.

Estimates of the energies involved show that an invader cell can be guided in the forward direction by de-adhesion or weakening of the bond between pairs of host cells, or between a host cell and the ECM, and the subsequent effect of tractions imposed by the host cells upon protrusions that emerge from the invader. In contrast, compressive tangential stresses along the flanks of a branch can act as an inhibitory or confining sleeve that inhibits the intrusion

of filipodia and maintains the entrainment of follower or stalk cells in the branch. Thus, in the network-forming process, the filipodia of invaders are proposed to serve partly as strain sensors.

The migration of an invader cell in response to sensed strains must be executed using internally derived energy (even if host cell relaxation aids one step of the migratory sequence). Thus the invader cell acts as a strain-cued automaton in the paradigm of Fig. 1.

Compressive tangential stresses of unusually high magnitude are predicted at bifurcation events, providing positional information for cells in that vicinity, which could be used, e.g., to trigger the emission of notch factors to inhibit further sprouting.

Thus the local elastic strain fields that are generated by the intrusion of invaders into a host medium, and the relaxation of the host medium, form spatial patterns that offer much of the positional information individual cells require to create networks correctly.

22.4. Competing rates

Pivotal to the analyses of amelogenesis and network formation is the ratio of two competing rate processes, one a migration velocity, v_m , and the other a relaxation velocity, v_r . The ratio of these two rates must have a value within a correct range for biologically relevant patterns to emerge from simulations.

The values of the relaxation velocity are relatively uniform over the organs and species in the few cases in which data and analysis are available. In contrast, migration velocities vary by three orders of magnitude, with network-forming invaders moving relatively very fast.

By analysis of rate effects in network forming, we infer a transition in cell behavior as the time rate of change of an applied strain increases: when the strain rate in host cells exceeds a critical value in the range 10^{-4} – 10^{-3} s⁻¹ the host cell response switches from “animate” behavior (able to maintain low stress when strained) to “inanimate” behavior (unable to avoid the generation of stress when strained). A transition in cell response at strain rates that are close to this range has been inferred by analysis of cyclic loading experiments (De et al., 2007) and by recent measurements on single fibroblasts in vitro (Webster et al., 2014). We propose that invaders migrate at unusually high speeds exactly for the purpose of driving host cells into an inanimate response, at least over short times.

22.5. Chemical factors acting in concert with strain cues

The cell-scale theories (Section 4) and population scale theories reviewed in this article share the feature that cells achieve the desired outcomes by interacting with each other solely via the mechanical boundary conditions present at the cell membrane. This proves the truth of the conditional statement of Eq. (1b). Yet chemical factors are ubiquitous: the ability of a cell to respond to a strain cue is actuated by chemical signals within the cell and the nature of the cell’s response to strain cues can be altered by the arrival of a chemical factor at its membrane.

Furthermore, in general cases, the motility of cells will respond to a combination of mechanical and chemical stimuli acting simultaneously on their membranes. In the multi-scale formulation, chemical stimuli would enter as additional variables in the cell's boundary conditions, which would be mapped into cell actions by an elaborated single cell response function. An example was presented of network formation governed by a combination of strain cues and a chemical gradient.

22.6. Possible relevance to cancer

The variation of tangential strains that would arise in a host population around a single invader or a cluster of invaders that is migrating through the host medium is similar to that around a branch in a forming network, being tensile ahead of the cell or cluster and compressive to the sides of the cell or cluster, or behind it. We infer that, if the single cell or detached cluster moves sufficiently quickly, it may cause de-adhesion of host cells immediately ahead of it, aiding its ability to migrate more effectively in a single direction. Individual cancer cells and clusters of cancer cells do indeed migrate unusually fast, at speeds similar to neural cells forming networks or endothelial cells forming blood vessels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Transitions in cell response vs. strain rate

A.1. Cell rotations under cyclic loading

De et al. developed a model of the dynamics of a cell's reconfiguration of its cytoskeleton and focal adhesions that accounts for an experimentally observed transition in the ability of a cell to re-orientate under the influence of an externally imposed cyclic load (De et al., 2007). In their theory, if the cell is loaded at high frequency, it aligns orthogonal to the load axis to minimize the stress that the applied load can develop across the cell; whereas, if it is loaded at low frequency, the cell aligns parallel to the load and re-configures its cytoskeleton to match the load axis. The cell response passes through this transition when the dimensionless quantity $\omega_0\tau_r$ takes a value in the range 0.1–0.4, where ω is the angular frequency of the applied stress variation and τ_r is a relaxation time that is a characteristic of the cell (a representation of the rate at which its internal dynamics operate) (see Fig. 3b of De et al., 2007). Typically, τ_r takes a value of tens of minutes; or 500 τ_r 1000 s.

Let $\epsilon_{\max}$ denote the applied strain amplitude in an experiment addressed by the theory. The average strain rate $\dot{\epsilon}$ between the points of minimum and maximum applied strain (i.e.,

between $-\epsilon_{\max}$ and $\epsilon_{\max}$ is $2\epsilon_{\max}/(T/2)$, where T is the period of the applied strain variation; or $\dot{\epsilon} = 2\pi\epsilon_{\max}\omega$. Hence

$$\omega = \frac{\dot{\epsilon}}{2\pi\epsilon_{\max}} \quad (\text{A.1})$$

and the condition for the transition in the theory of De et al. becomes

$$0.1 \leq \frac{\dot{\epsilon}_{\text{cr}}\tau_r}{2\pi\epsilon_{\max}} \leq 0.4 \quad (\text{A.2})$$

where $\dot{\epsilon}_{\text{cr}}$ denotes the value of a critical strain rate associated with the transition. The critical strain rate has the significance that the cell will have time to reconfigure its cytoskeleton in response to an applied strain that increases with rate $\dot{\epsilon}_{\text{cr}}$ if and only if $\dot{\epsilon} \leq \dot{\epsilon}_{\text{cr}}$.

Assuming $\epsilon_{\max}=0.05$ (i.e. a 5% peak strain in the experiments modeled by De et al.), one finds

$$3 \times 10^{-5} \leq \epsilon_{\text{cr}} \leq 2 \times 10^{-4} \text{ s}^{-1}. \quad (\text{A.3})$$

The range of this estimate of a critical strain rate overlaps the range estimated from consideration of the dynamics of network formation, $10^{-4} < \epsilon_{\text{cr}} < 10^{-3} \text{ s}^{-1}$.

A.2. Cell stretching

Data in Webster et al., 2014) were re-interpreted as follows, to quantify the strain rate at which a transition in cell response was observed.

In the experiment in Webster et al. (2014), a cell was stretched or compressed between a platen and a moving cantilever arm. The cell had an initial height $h = 12 \mu\text{m}$. The change in the total height of the cell was $1 \mu\text{m}$, creating a total (final) strain of 8%. This is comparable to the strains predicted in the cases of amelogenesis and invasion listed in Table 1.

The transition in response occurred between two loading cases, viz., fast loading at arm velocity $v = 1.0 \mu\text{m}/\text{min}$ and slow loading at $v = 0.1 \mu\text{m}/\text{min}$ (see Webster et al., 2014, especially Fig. 2 therein and related text). At $v = 1.0 \mu\text{m}/\text{min}$, the total force exerted by the cell rose by 19%, whereas at $v = 0.1 \mu\text{m}/\text{min}$, the total force rose by only 3%.

The average strain rate in these two cases was $\dot{\epsilon} = v/h$, or $\dot{\epsilon} = 1.4 \times 10^{-3} \text{ s}^{-1}$ for the higher velocity and $\dot{\epsilon} = 1.4 \times 10^{-4} \text{ s}^{-1}$ for the lower velocity. Thus the critical strain rate for a change in cell behavior is deduced to be in the range

$$10^{-4} \leq \dot{\epsilon}_{\text{cr}} \leq 10^{-3} \text{ s}^{-1} \quad (\text{A.4})$$

This range is higher than that estimated from the analysis of De et al. (above), but overlaps it, and also coincides with the interval for $\dot{\epsilon}_{\text{cr}}$ that was deduced from analysis of network formation.

Appendix B. Traction estimates for cells using literature data

Fig. 2 of Benoit and Selhuber-Unkel (2011) shows a maximum force of 0.8 nN between a single fibroblast and a fibronectin surface. While the contact area was not reported, a reasonable estimate is that it is approximately $100 \mu\text{m}^2$. The traction acting at the cell/fibronectin interface is therefore approximately 10 Pa, which we take as representative of cell-ECM adhesion.

Fig. 8 of Benoit and Selhuber-Unkel (2011) shows a maximum force of 10–15 nN between a layer of uterine epithelial cells on a sphere and a layer of similar cells on a flat substrate. The sphere has a radius of $30 \mu\text{m}$. While the contact area again is not reported, a reasonable estimate is $1000 \mu\text{m}^2$. The peak traction acting at the interface between the two layers of cells is therefore again approximately 10–15 Pa. With allowance for uncertainties in the assumed contact areas, we infer peak cell–cell adhesive tractions in the range 5–30 Pa.

Appendix C. Description of movies published online

Part I: Homogeneous populations

C.1. General description of the movies

The movies show the cells projected down onto the x–y plane in the coordinate system of Fig. 3b. Each cell therefore appears as its cross-section normal to its long axis (Fig. 3b). The cross-sectional shape is depicted as a Voronoi polygon (Appendix IV of Supplementary Information of Cox, 2010). Because the images are projections, only the lateral motion of cells within the plane of the population sheet are noticeable; the migration of cells away from the DEJ is mainly out of the plane of the image (curvature of the DEJ is a minor effect) and not discernible.

The left and right images present in all but one movie use color coding to exhibit the orientation of each cell's trajectory. The image at the left depicts the polar angle, γ , and the image at the right depicts the azimuthal angle, θ (Fig. C1).

Fig. C.1.

The polar and azimuthal angles defined for a cell trajectory, which has velocity $\mathbf{v}_i$. The axis $\mathbf{n}_i$ is normal to the population sheet while $\hat{\mathbf{X}}_1$ is tangential to the sheet and chosen to lie in the global x - z plane.

C.2. Typical motions of cells during decussation

“decussating cells” highlights the lateral motions of a few cells in single-row decussation. The cells are initially stationary, then move after the commencement front has passed. Cells in this particular movie are not colored according to the angles θ and γ , but instead are mainly monochrome, with just a few cells colored differently to make it easier to follow their motion. The cell cross-sections are represented by approximately hexagonal polygons (Voronoi constructs), which distort in an approximately periodic manner as the cells slide past one another.

C.3. Transition from local to global ordering in pattern development in the mouse incisor

In the three movies, “transient c055”, “transient c060”, and “transient c065”, the field of view is locked onto the commencement front, which is marked by the white line near the top of the image. Since the commencement front advances along the incisor at uniform speed, cells enter the field of view at the top, progress downwards at approximately uniform velocity, and leave the field of view at the bottom. Once passed by the commencement front, the cells begin to migrate away from the DEJ (a motion that is mainly out of the plane of the image). Since the DEJ is doubly curved, the motion away from the DEJ necessarily causes strains among the cells in the plane of the population sheet, which trigger changes in the cells’ lateral motility.

During the span of each movie, the commencement front advances approximately 0.5–1 mm. The movie therefore simulates approximately 2–4 days in the life of the mouse. The

field of view shows approximately 6000 cells at any time, extracted from a simulation of approximately 10^5 cells.

Because of the limitations of the field of view, only the first approximately 25–30 μm of growth away from the DEJ are achieved by any cell in the movie, which is approximately 3% of the total distance the cell will move from the DEJ in forming the enamel layer. Nevertheless, the patterning is already established in this initial region. Completion of the entire enamel layer can be seen in other images from simulations (Fig. 4b and Cox, 2013).

C.4. Behavior in the steady-state regime

“incipient decussation”, created following analyses reported in Cox (2013), exemplifies the development of a pattern of decussation among ameloblasts following the passage of the commencement front, which moves (unmarked in the image) from bottom to top. Thus cells at the top of the image are stationary at first, and then begin to move, spontaneously forming rows moving to the left or right. The rows of alternating motion characteristic of decussation appear first in the azimuthal angle, and first at the location of highest curvature on the DEJ (the starting surface for secretory-stage migration). The location of highest curvature is marked in the movies by a change in color of the azimuthal angle plot. (The color change arises from numerical treatment of the fact that the azimuthal angle is indeterminate initially, and has no physical meaning; but it serves to mark the location of minimum curvature.) The in-plane motions form patterns while the polar angle remains very small; the patterns grow by augmentation of infinitesimal motions. Other details of this movie may be found in Cox (2013).

C.5. Wavelike behavior

“pulse” illustrates wave-like behavior created by linear stimulus, i.e., a rule restoring a preferred cell density, and was created following analyses reported in Cox (2010), especially Fig. 3 therein. The simulated population of cells behaves somewhat like ameloblasts, with cells propagating out of the page as the population sheet advances while simultaneously migrating laterally within the sheet. A strain pulse, triggered by assigning initially perturbed velocities to a row of cells, propagates across the population with velocity v_p and reflects from a boundary, whereupon the average strain in the pulse changes from tensile (blue) to compressive (orange). In this simulation, cells do not decussate (slide past one another in rows); they only restore the preferred density after the density is perturbed.

Part II: Invasions

C.6. Network formation

“network d05 gch0” shows an instantiation of network formation, in which cells initially in a band-like source at the left of the image (e.g., cells in an idealized neural crest) migrate across the simulation domain. The movie typifies transition to a steady-state closed network, with approximately constant density, via branching, occasional branch arrest, and branch coalescence. Invaded spatial elements are gray, branch points are in black.

“strains d05 gch0” shows the evolution in time of the strain metric $\epsilon_1 + \epsilon_2$ within host spatial elements that are adjacent to elements that have already been invaded. The case shown is the same as that in the movie “network d05 gch0”. All invaded elements are shown in black. The color of a host element follows the legend of Fig. 18.

“strains d085 gch-0.5” shows a network formed with the same type of initial conditions as in the example above, but with a far-field chemical gradient contributing to the migration decision of individual invaders. All invaded elements are shown in black. The color of a host element follows the legend of Fig. 18.

“point source d055.wmv” shows an instantiation of network formation that starts with a single source element in the center of the simulation domain instead of a band-like source, but uses identical rules to those used in the movie for a band-like source. Invaded spatial elements are gray, branch points are black.

C.7. Isolated invaders

“loner trio” displays a biased random walk executed by a three-element cell migrating through a relaxing host population, guided by strain cues. The initial condition is that the cell is already moving to the right in the image.

“loner.wmv” displays a biased random walk executed by a one-element cell migrating through a relaxing host population, guided by strain cues. The initial condition is that the cell is already moving to the right in the image.

References

- Abbas L, Demongeot J, Glade N, 2009 Synchrony in reaction–diffusion models of morphogenesis: applications to curvature-dependent proliferation and zero-diffusion front waves. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences* 367 (1908), 4829–4862.
- Abercrombie M, Heaysman JE, 1953 Observations on the social behaviour of cells in tissue culture: I. Speed of movement of chick heart fibroblasts in relation to their mutual contacts. *Experimental cell research* 5 (1), 111–131. [PubMed: 13083622]
- Ablowitz MJ, Kaup D, Newell A, Segur H, 1973 Method for solving the sine-Gordon equation. *Physical Review Letters* 30 (25), 1262.
- Amizuka N, Uchida T, Fukae M, Yamada M, Ozawa H, 1992 Ultrastructural and immunocytochemical studies of enamel tufts in human permanent teeth. *Archives of Histology and Cytology* 55 (2), 179–190. [PubMed: 1497948]
- Anderson AR, Chaplain M, 1998 Continuous and discrete mathematical models of tumor-induced angiogenesis. *Bulletin of Mathematical Biology* 60 (5), 857–899.
- Angelini TE, Hannezo E, Treppe X, Marquez M, Fredberg JJ and Weitz DA, 2011 Glass-like dynamics of collective cell migration. *Proceedings of the National Academy of Sciences* 108(12), 4714–4719.
- Baker RE, Schnell S, Maini P, 2006 A clock and wavefront mechanism for somite formation. *Developmental Biology* 293 (1), 116–126. [PubMed: 16546158]
- Bartlett JD, Smith CE, 2013 Modulation of cell–cell junctional complexes by matrix metalloproteinases. *Journal of Dental Research* 92 (1), 10–17. [PubMed: 23053846]
- Ben-Yaakov D, Golkov R, Shokef Y and Safran SA, 2015 Response of adherent cells to mechanical perturbations of the surrounding matrix. *Soft Matter*.

- Benoit M, Selhuber-Unkel C, 2011 Measuring cell adhesion forces: Theory and principles Atomic Force Microscopy in Biomedical Research: Methods and Protocols, C. Braga and D. Ricci, Springer Science+Business Media.
- Berg HC, 1993 In: Random walks in biology Princeton University Press.
- Bois JS, Julicher F, Grill SW, 2011 Pattern formation in active fluids. *Physical Review Letters* 106 (2), 028103. [PubMed: 21405254]
- Boyde A, 1989 Enamel Handbook of Microscopic Anatomy. A. Oksche and L. Vollrath, 6 Springer-Verlag, Berlin 309–473.
- Brandman O, Meyer T, 2008 Feedback loops shape cellular signals in space and time. *Science* 322 (5900), 390–395. [PubMed: 18927383]
- Brangwynne C, Huang S, Parker KK, Ingber DE, 2000 Symmetry breaking in cultured mammalian cells. *In Vitro Cellular & Developmental Biology-Animal* 36 (9), 563–565. [PubMed: 11212140]
- Bruch L, 1983 Theory of physisorption interactions. *Surface Science* 125 (1), 194–217.
- Buenzli PR, Pivonka P, Smith DW, 2010 Spatio-temporal structure of cell distribution in cortical Bone Multicellular Units: a mathematical model. *bone* 48 (4), 918–926. [PubMed: 21172465]
- Bullough RK, 1978 Solitons. *Physics Bulletin*, 78–82.
- Buttiker M, Landauer R, 1981 Nucleation theory of overdamped soliton motion. *Physical Review A* 23, 1397–1410.
- Camley BA, Zhang Y, Zhao Y, Li B, Ben-Jacob E, Levine H and Rappel W-J., 2014 Polarity mechanisms such as contact inhibition of locomotion regulate persistent rotational motion of mammalian cells on micropatterns. *Proceedings of the National Academy of Sciences* 111(41), 14770–14775.
- Carmona-Fontaine C, Theveneau E, Tzekou A, Tada M, Woods M, Page KM, Parsons M, Lambris JD, Mayor R, 2011 Complement fragment C3a controls mutual cell attraction during collective cell migration. *Developmental cell* 21 6, 1026–1037. [PubMed: 22118769]
- Checa S and Prendergast PJ, 2008 Capillary network formation during tissue differentiation. A mechano-biological model ECIFMBE 2008, Springer-Verlag.
- Chen X, Yin J, 2010 Buckling patterns of thin films on curved compliant substrates with applications to morphogenesis and three-dimensional micro-fabrication. *Soft Matter* 6, 5667–5680.
- Cooke J, Zeeman E, 1976 A clock and wavefront model for control of the number of repeated structures during animal morphogenesis. *Journal of Theoretical Biology* 58 (2), 455–476. [PubMed: 940335]
- Cox BN, 1989 Surface Displacements and Stress Field Generated by a Semi-Ellipsoidal Surface Inclusion. *Journal of Applied Mechanics* 56, 564–570.
- Cox BN, 2010 A multi-scale, discrete-cell simulation of organogenesis: Application to the effects of strain stimulus on collective cell behavior during ameloblast migration. *Journal of Theoretical Biology* 262, 58–72. [PubMed: 19765593]
- Cox BN, 2011 A strain-cue hypothesis for biological network formation. *Journal of the Royal Society, Interface* 2011 (8), 377–394.
- Cox BN, 2013 How the tooth got its stripes: patterning by strain-cued motility. *Journal of the Royal Society, Interface* 10, 20130266.
- Cox BN, Morris WL, 1988 Monte Carlo simulations of the growth of small fatigue cracks. *Engineering Fracture Mechanics* 31, 591–610.
- Cox BN, Smith DW, 2014 On strain and stress in living cells. *Journal of the Mechanics and Physics of Solids* 71, 239–252.
- Cox N, Marconi L, Teel A, 2014 High-gain observers and linear output regulation for hybrid exosystems. *International Journal of Robust and Nonlinear Control* 24 (6), 1043–1063.
- Cristini V, Lowengrub J, Nie Q, 2003 Nonlinear simulation of tumor growth. *Journal of Mathematical Biology* 46, 191–224. [PubMed: 12728333]
- Davis EM, Trinkaus J, 1981 Significance of cell-to-cell contacts for the directional movement of neural crest cells within a hydrated collagen lattice. *Journal of embryology and experimental morphology* 63 1, 29–51. [PubMed: 7310293]
- Davydov AS, 1979 Solitons in molecular systems. *Physica Scripta* 20 (3–4), 387.

- De R, Zemel A, Safran SA, 2007 Dynamics of cell orientation. *Nature Physics* 3 (9), 655–659.
- Discher DE, Janmey P, Wang Y, 2005 Tissue cells feel and respond to the stiffness of their substrate. *Science* 310, 1139–1143. [PubMed: 16293750]
- Dorrell MI, Aguilar E, Friedlander M, 2002 Retinal vascular development is mediated by endothelial filopodia, a preexisting astrocytic template and specific R-cadherin adhesion. *Investigative ophthalmology & visual science* 43 (11), 3500–3510. [PubMed: 12407162]
- Druckebrod NR, Epstein ML, 2005 The pattern of neural crest advance in the cecum and colon. *Developmental Biology* 287, 125–133. [PubMed: 16197939]
- Druckebrod NR, Epstein ML, 2007 Behavior of enteric neural crest-derived cells varies with respect to the migratory wavefront. *Developmental Dynamics* 236 (1), 84–92. [PubMed: 17039523]
- Eilken HM, Adams RH, 2010 Dynamics of endothelial cell behavior in sprouting angiogenesis. *Current opinion in cell biology* 22 5, 617–625. [PubMed: 20817428]
- Epstein ML, Poulsen KT, Thiboldeaux R, 1991 Formation of ganglia in the gut of the chick embryo. *Journal of comparative neurology* 307 (2), 189–199. [PubMed: 1856323]
- Eshelby JD, 1957 The determination of the elastic field of an ellipsoidal inclusion, and related problems. *Proceedings of the Royal Society A* 241, 376–396.
- Espinoza R, Jheon AH, Wen T, Biehs B and Klein OD, 2012 Notch signalling is required for the attachment of the ameloblast-SI interface. *AADR/CADR Annual Meeting & Exhibition*, Tampa, Florida.
- Foster PC, Mlot NJ, Lin A, Hu DL, 2014 Fire ants actively control spacing and orientation within self-assemblages. *The Journal of experimental biology* 217 12, 2089–2100. [PubMed: 24920836]
- Friedl P, 2004 Dynamic imaging of cellular interactions with extracellular matrix. *Histochemistry and cell biology* 122 3, 183–190. [PubMed: 15258769]
- Gerhardt H, 2008 VEGF and endothelial guidance in angiogenic sprouting. *VEGF in Development* Springer 68–78.
- Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, 2003 VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *The Journal of cell biology* 161 6, 1163–1177. [PubMed: 12810700]
- Gingold RA, Monaghan JJ, 1977 Smoothed particle hydrodynamics-theory and application to non-spherical stars. *Monthly notices of the royal astronomical society* 181, 375–389.
- Glazier JA, Graner F, 1993 Simulations of the differential adhesion driven rearrangement of biological cells. *Physical Review E* 47, 2128–2154.
- Gordon-Weeks PR, 2005 In: *Neuronal growth cones* Cambridge University Press.
- Harris AK, Wild P, Stopak D, 1980 Silicone rubber substrata: a new wrinkle in the study of cell locomotion. *Science* 208 (4440), 177–179. [PubMed: 6987736]
- Hatzikirou H, Chauviere A, Bauer AL, Leier A, Lewis MT, Macklin P, Marquez-Lago TT, Bearer EL, Cristini V, 2012 Integrative physical oncology. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine* 4 (1), 1–14. [PubMed: 21853537]
- Hester SD, Belmonte JM, Gens JS, Clendenon SG, Glazier JA, 2011 A multi-cell, multi-scale model of vertebrate segmentation and somite formation. *PLOS Computational Biology* 7 (10), e1002155. [PubMed: 21998560]
- Hilgenfeldt S, Eriskens S and Carthew RW, 2008 Physical modeling of cell geometric order in an epithelial tissue. *Proceedings of the National Academy of Sciences of the United States of America* 105, 907–911. [PubMed: 18192402]
- Hirth JP, Lothe J, 1982 In: *Theory of Dislocations* John Wiley & Sons, New York.
- Huang S, Brangwynne C, Parker K, Ingber D, 2005 Symmetry-breaking in mammalian cell cohort migration during tissue pattern formation: Role of random-walk persistence. *Cell Motility and the Cytoskeleton* 61 (4), 201–213. [PubMed: 15986404]
- Hufnagel L, Teleman AA, Rouault H, Cohen SM and Shraiman BI, 2007 On the mechanism of wing size determination in fly development. *Proceedings of the National Academy of Science of the USA* 104(10), 3835–3840.

- Hwang WSS, Tonna EA, 1965 Autoradiographic analysis of labeling indices and migration rates of cellular component of mouse incisors using tritiated thymidine (H3TDR). *Journal of Dental Research* 44 (1), 42–53. [PubMed: 14245495]
- Idema T, Liu AJ, 2014 Mechanical signaling via nonlinear wavefront propagation in a mechanically excitable medium. *Physical Review E* 89 (6), 062709.
- Ingber DE, 2006 Mechanical control of tissue morphogenesis during embryological development. *International Journal of Developmental Biology* 50, 255–266. [PubMed: 16479493]
- Jheon AH, Mostowfi P, Snead ML, Ihrle RA, Sone E, Pramparo T, Attardi LD, Klein OD, 2011 PERP regulates enamel formation via effects on cell–cell adhesion and gene expression. *Journal of Cell Science* 124 (5), 745–754. [PubMed: 21285247]
- Ji JW, Mac Gabhann F, Popel AS, 2007 Skeletal muscle VEGF gradients in peripheral arterial disease: simulations of rest and exercise. *American Journal of Physiology-Heart and Circulatory Physiology* 293 (6), H3740–H3749. [PubMed: 17890434]
- Jones CA, London NR, Chen H, Park KW, Sauvaget D, Stockton RA, Wythe JD, Suh W, Larrieu-Lahargue F, Mukoyama Y, 2008 -s Robo4 stabilizes the vascular network by inhibiting pathologic angiogenesis and endothelial hyperpermeability. *Nature medicine* 14 (4), 448–453.
- Kholodenko BN, 2006 Cell-signalling dynamics in time and space. *Nature reviews Molecular cell biology* 7 3, 165–176. [PubMed: 16482094]
- Kuhl E, Menzel A, Steinmann P, 2003 Computational modeling of growth: A critical review, a classification of concepts and two new consistent approaches. *Computational Mechanics* 32, 71–88.
- Lacruz RS, Bromage TG, 2006 Appositional enamel growth in molars of South African fossil hominids. *Journal of Anatomy* 209, 13–20. [PubMed: 16822265]
- Landman KA, Fernando AE, Zhang D, Newgreen DF, 2011 Building stable chains with motile agents: Insights into the morphology of enteric neural crest cell migration. *Journal of Theoretical Biology* 276 (1), 250–268. [PubMed: 21296089]
- Lecuit T, Lenne P-F, 2007 Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. *Nature Reviews, Molecular Cell Biology* 8 (August), 633–644. [PubMed: 17643125]
- Leong FY, 2013 Physical explanation of coupled cell–cell rotational behavior and interfacial morphology: A particle dynamics model. *Biophysical Journal* 105 (10), 2301–2311. [PubMed: 24268142]
- Levin M, 2012 Morphogenetic fields in embryogenesis, regeneration, and cancer: non-local control of complex patterning. *Biosystems* 109 (3), 243–261. [PubMed: 22542702]
- Li B, Li F, Puskas KM, Wang JH-C, 2009 Spatial patterning of cell proliferation and differentiation depends on mechanical stress magnitude. *Journal of Biomechanics* 42, 1622–1627. [PubMed: 19482284]
- Li Q, Anderson PM, 2001 A compact solution for the stress field from a cuboidal region with a uniform transformation strain. *Journal of Elasticity* 64, 237–245.
- Liu G, Qutub AA, Vempati P, Mac Gabhann F, Popel AS, 2011 Module-based multiscale simulation of angiogenesis in skeletal muscle. *Theor Biol Med Model* 8, 6. [PubMed: 21463529]
- Mac Gabhann F, Ji JW, Popel AS, 2007 VEGF gradients, receptor activation, and sprout guidance in resting and exercising skeletal muscle. *Journal of Applied Physiology* 102 (2), 722–734. [PubMed: 17038488]
- Macho GA, Jiang Y, Spears IR, 2003 Enamel microstructure - a truly three-dimensional structure. *Journal of Human Evolution* 45, 81–90. [PubMed: 12890446]
- Mack JJ, Corrin AA, dos Santos e Lucato SL, Dunn JC, Wu BW, Cox BN, 2013 Enhanced cell viability via strain stimulus and fluid flow in magnetically actuated scaffolds. *Biotechnology and Bioengineering* 110 (3), 936–946. [PubMed: 23042257]
- Maini PK, Woolley TE, Baker RE, Gaffney EA, Lee SS, 2012 Turing's model for biological pattern formation and the robustness problem. *Interface focus* 2 4, 487–496. [PubMed: 23919129]
- Maiuri P, Terriac E, Paul-Gilloteaux P, Vignaud T, McNally K, Onuffer J, Thorn K, Nguyen PA, Goergoulia N, Soong D, Jayo A, Beil N, Beneke J, Lim JCH, Sim CP-Y, Chu Y-S, Jimenez-Dalmaroni A, Joanny J-F, Thiery J-P, Erfie H, Parsons M, Mitchison TJ, Lim WA, Lennon-

- Dumenil A-M, Piel M, Thery M, 2012 The first world cell race. *Current Biology* 22 (17), R673–R675. [PubMed: 22974990]
- Marchetti M, Joanny J, Ramaswamy S, Liverpool T, Prost J, Rao M, Simha RA, 2013 Hydrodynamics of soft active matter. *Reviews of modern physics* 85 (3), 1143.
- Marshall GW Jr, 1993 Dentin: Microstructure and characterization. *Quintessence International* 24, 9.
- McLennan R, Dyson L, Prather KW, Morrison JA, Baker RE, Maini PK, Kulesa PM, 2012 Multiscale mechanisms of cell migration during development: theory and experiment. *Development* 139 (16), 2935–2944. [PubMed: 22764050]
- Meinhardt H, 2003 In: *The Algorithmic Beauty of Sea Shells* Springer-Verlag, Berlin.
- Mura T, 1982 *Micromechanics of defects in solids*, The Hague Martinus Nijhoff.
- Nagel K, Schreckenberg M, 1992 A cellular automaton model for freeway traffic. *Journal de physique I* 2 (12), 2221–2229.
- Nanci A (Ed.), 2003 *Ten Cate's Oral Histology: Development, Structure, and Function*. Mosby, Inc, St. Louis, Missouri.
- Narayanan H, Verner S, Mills K, Kemkemer R, Garikipati K, 2010 In silico estimates of the free energy rates in growing tumor spheroids. *Journal of Physics: Condensed Matter* 22 (19), 194122. [PubMed: 21386444]
- Neuber H, 1946 *Theory of notch stresses, Principle for exact stress calculations* Edwards, Ann Arbor, Michigan.
- Newell AC, 1985 *Solitons in mathematics and physics*. SIAM.
- Newgreen DF, 1990 Control of the directional migration of mesenchyme cells and neurites. *Seminars in Developmental Biology* 1, 301–311.
- Newgreen DF, Dufour S, Howard MJ, Landman KA, 2013 Simple rules for a “simple” nervous system? Molecular and biomathematical approaches to enteric nervous system formation and malformation. *Developmental Biology* 382 (1), 305–319. [PubMed: 23838398]
- Olami Z, Feder HJS, Christensen K, 1992 Self-organized criticality in a continuous, nonconservative cellular automaton modeling earthquakes. *Physical Review Letters* 68 (8), 1244. [PubMed: 10046116]
- Owen MR, Alarcon T, Maini PK, Byrne HM, 2009 Angiogenesis and vascular remodelling in normal and cancerous tissue. *Journal of Mathematical Biology* 58, 689–721. [PubMed: 18941752]
- Oyler JE, Yaniv A, Krichevsky O and Altan-Bonnet G, 2014 mRNA as a molecular clock: how mRNA longevity controls the persistence of cellular information.
- Phng L-K, Gerhardt H, 2009 Angiogenesis: a team effort coordinated by notch. *Developmental cell* 16 2, 196–208. [PubMed: 19217422]
- Plank M, Sleeman B, 2003 A reinforced randomwalk model of tumour angiogenesis and anti-angiogenic strategies. *Mathematical Medicine and Biology* 20 (2), 135–181. [PubMed: 14636027]
- Plank M, Sleeman B, 2004 Lattice and non-lattice models of tumour angiogenesis. *Bulletin of Mathematical Biology* 66 (6), 1785–1819. [PubMed: 15522355]
- Reiner M, 1964 The Deborah number. *Physics Today* 17 (1), 62.
- Rorth P, 2012 Fellow travellers: emergent properties of collective cell migration. *EMBO reports* 13 11, 984–991. [PubMed: 23059978]
- Ruhrberg C, Gerhardt H, Golding M, Watson R, Ioannidou S, Fujisawa H, Betsholtz C, Shima DT, 2002 Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. *Genes & development* 16 (20), 2684–2698. [PubMed: 12381667]
- Sehic A, Peterkova R, Lesot H, Risnes S, 2009 Distribution and structure of the initial dental enamel formed in incisors of young wild-type and Tabby mice. *European journal of oral sciences* 117 (6), 644–654. [PubMed: 20121926]
- Serra-Picamal X, Conte V, Vincent R, Anon E, Tambe DT, Bazellieres E, Butler JP, Fredberg JJ, Trepap X, 2012 Mechanical waves during tissue expansion. *Nature Physics* 8 (8), 628–634.
- Siekmann AF, Lawson ND, 2007 Notch signalling limits angiogenic cell behaviour in developing zebrafish arteries. *Nature* 445 (7129), 781–784. [PubMed: 17259972]

- Simpson MJ, Landman KA, Hughes BD, Newgreen DF, 2006 Looking inside an invasion wave of cells using continuum models: Proliferation is the key. *Journal of Theoretical Biology* 243, 343–360. [PubMed: 16904698]
- Simpson MJ, Merrifield A, Landman KA, Hughes BD, 2007 Simulating invasion with cellular automata: connecting individual and macroscopic properties. *Physical Review E* 76, 021918–021929.
- Stanley HE, 1987 In: *Introduction to phase transitions and critical phenomena* Oxford University Press.
- Steinberg MS, 1963 Reconstruction of tissues by dissociated cells. Some morphogenetic tissue movements and the sorting out of embryonic cells may have a common explanation. *Science* 141, 401–408. [PubMed: 13983728]
- Steinberg MS, 2007 Differential adhesion in morphogenesis: a modern view. *Current opinion in genetics & development* 17 (4), 281–286. [PubMed: 17624758]
- Strutt JW, Rayleigh L, 1878 On the instability of jets. *Proc. London Math. Soc* 10, 4–13.
- Szabó A, Czirók A, 2010 The role of cell–cell adhesion in the formation of multicellular sprouts. *Mathematical modelling of natural phenomena* 5 (01), 106–122. [PubMed: 20165554]
- Szabó A, Ünnep R, Méhes E, Twal W, Argraves W, Cao Y, Czirók A, 2010 Collective cell motion in endothelial monolayers. *Physical biology* 7 4, 046007. [PubMed: 21076204]
- Tanner K, Mori H, Mroue R, Bruni-Cardoso A and Bissell MJ, 2012 Coherent angular motion in the establishment of multicellular architecture of glandular tissues. *Proceedings of the National Academy of Sciences* 109(6), 1973–1978.
- Tarantola A, 2005 Inverse problem theory and methods for model parameter estimation, siam.
- Teddy JM, Kulesa PM, 2004 In vivo evidence for short- and long-range cell communication in cranial neural crest cells. *Developmental Biology* 131 (24), 6141–6151.
- Theveneau E, Mayor R, 2011 Collective cell migration of the cephalic neural crest: the art of integrating information. *Genesis* 49 (4), 164–176. [PubMed: 21157935]
- Townes PL, Holtfreter J, 1955 Directed movements and selective adhesion of embryonic amphibian cells. *Journal of Experimental Zoology* 128, 53–120.
- Trewenack AJ, Landman KA, 2009 A travelling wave model for invasion by precursor and differentiated cells. *Bulletin of Mathematical Biology* 71, 291–317. [PubMed: 19130145]
- Turing AM, 1952 The Chemical Basis of Morphogenesis. *Philosophical Transactions of the Royal Society of London* 237, 37–72.
- Vempati P, Karagiannis ED, Popel AS, 2007 A biochemical model of matrix metalloproteinase 9 activation and inhibition. *Journal of Biological Chemistry* 282 (52), 37585–37596. [PubMed: 17848556]
- Voronoi G, 1907 Nouvelles applications des paramètres continus à la théorie des formes quadratiques. *Journal für die Reine und Angewandte Mathematik* 133, 97–178.
- Warshawsky H, Smith CE, 1976 Movement of entire cell populations during renewal of the rat incisor as shown by radioautography after labeling with 3H-thymidine. *American Journal of Anatomy* 145, 225–260. [PubMed: 1258806]
- Weber GF, Bjerke MA, DeSimone DW, 2012 A mechanoresponsive cadherin-keratin complex directs polarized protrusive behavior and collective cell migration. *Developmental cell* 22 1, 104–115. [PubMed: 22169071]
- Webster KD, Ng WP, Fletcher DA, 2014 Tensional homeostasis in single fibroblasts. *Biophysical Journal* 107 (1), 146–155. [PubMed: 24988349]
- Weinkamer R, Hartmann MA, Brechet Y, Fratzl P, 2004 Stochastic lattice model for bone remodeling and aging. *Physical Review Letters* 93 (22), 228102. [PubMed: 15601120]
- White SN, Luo W, Paine ML, Sarikaya M, Fong H, Snead ML, 2001 Biological organization of hydroxyapatite crystallites into a fibrous continuum toughens and controls anisotropy in human enamel. *Journal of Dental Research* 80, 321–326. [PubMed: 11269723]
- Wikipedia. (2014). “Growth cone.” from (http://en.wikipedia.org/wiki/Growth_cone).
- Wikipedia. (2014). “([http://en.wikipedia.org/wiki/Deformation_\(mechanics\)](http://en.wikipedia.org/wiki/Deformation_(mechanics))).”.

- wikipedia. (2014). "Life-like cellular automaton." from http://en.wikipedia.org/wiki/Life-like_cellular_automaton.
- Wolfram S, 1983 Statistical mechanics of cellular automata. Reviews of modern physics 55 3, 601.
- Wolfram S, 1994 In: Cellular automata and complexity: collected papers Addison-Wesley Reading.
- Wolfram S, 2002 A new kind of science, Wolfram Media, Inc.
- wolfram_research. (2013). "<http://mathworld.wolfram.com/RandomWalk1-Dimensional.html>".
- Woods ML, Carmona-Fontaine C, Barnes CP, Couzin ID, Mayor R, Page KM, 2014 Directional Collective Cell Migration Emerges as a Property of Cell Interactions. PLoS ONE 9 (9), e104969. [PubMed: 25181349]
- Wynn ML, Kulesa PM, Schnell S, 2012 Computational modelling of cell chain migration reveals mechanisms that sustain follow-the-leader behaviour. Journal of The Royal Society Interface 9 (72), 1576–1588.
- Yakushevich L, 1989 Nonlinear DNA dynamics: a new model. Physics Letters A 136 (7), 413–417.
- Young HM, Bergner AJ, Anderson RB, Enomoto H, Milbrandt J, Newgreen DF, Whittington PM, 2004 Dynamics of neural crest-derived cell migration in the embryonic mouse gut. Developmental Biology 270, 455–473. [PubMed: 15183726]
- Young HM, Bergner AJ, Simpson MJ, McKeown SJ, Hao MM, Anderson CR, Enomoto H, 2014 Colonizing while migrating: How do individual enteric neural crest cells behave? BMC biology 12 1, 23. [PubMed: 24670214]
- Zaikin A, Zhabotinsky A, 1970 Concentration wave propagation in two-dimensional liquid-phase self-oscillating system. Nature 225 (5232), 535–537.
- Zhou E, Treppe X, Park C, Lenormand G, Oliver M, Mijailovich S, Hardin C, Weitz D, Butler J and Fredberg J, 2009 Universal behavior of the osmotically compressed cell and its analogy to the colloidal glass transition. Proceedings of the National Academy of Sciences 106(26), 10632–10637.

Fig. 1.

Multi-scale simulation strategy (after Cox, 2010). Given a single-cell response function, which specifies the next increment of migratory motion of the cell given the displacement and traction boundary conditions currently existing around its membrane, the behavior of a large population can be computed by the iterative loop between the two modules at left. The single-cell response functions of Eqs. (2) and (3) were determined as a solution to the inverse problem of matching the results of population-scale calculations to observed enamel microstructure. The single-cell response function could also in principle be computed using a cell-scale computation (at right), where a survey of predicted cell responses to numerous potential sets of boundary conditions is summarized as the response function. The nature of possible cell-scale calculations is discussed in Section 5.

Fig. 2.

Strains in some cell populations: (a) predicted global strain ϵ_0 generated in a curved sheet of ameloblasts as the sheet displaces outward and the cells migrate within the sheet, governed by a preferred density rule, during the formation of dental enamel (details in Section 6 and Cox, 2010). The outward sheet displacement equals the accumulated enamel thickness, Z . (b) Snapshot from a simulation of ameloblasts forming the cross-plyed microstructure of mouse incisor enamel, showing cell cross-sections in the plane of the population sheet. Distortions of the initially hexagonal cross-sectional shapes of the cells (local strains) occur as the cells slide past one another within the sheet, governed by a shear enhancement rule, red cells to the right, blue to the left (details in Section 3 and Cox, 2013); (c) When a cell of one type invades an existing population of a different type, the host population cells distort as they vacate the invaded volume, generating local strains in the host (details in Section 13 and Cox, 2011). (d) Global compression develops in osteoblasts lining the inner surface of osteoid formed by a BMU. Cells at a fixed global position x_1 reside on a circle of decreasing radius as the entire BMU translates to the left (details in Cox and Smith, 2014). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3.

(a) “Secretory ameloblasts of rat incisor during development of inner enamel showing alternate inclinations of proximal ends of cells in alternating transverse rows, which are sliding past one another at this stage of development. Papillary cells are seen at the top of field of view, enamel at the bottom” (quoted caption and image from (Boyde, 1989)). (b) Schematic of cells in (a), with the cell tips marked by tip points, e.g., x_4 . The trajectory of Tomes’ process, on average, shadows the trajectory of the cell tip, as illustrated for cell 4.

Fig. 4.

(a) Enamel morphology in the mouse incisor (wild type), viewed on a section transverse to axis of tooth and inclined to coincide with prism layers. The population of ameloblasts has migrated as a curved sheet starting at the curve “DEJ” and proceeding in the direction of the block arrows to the outer enamel surface (top of image). “Prisms”, i.e., mineralized trajectories of individual ameloblasts, appear as whitish lines, arrayed in layers with alternating orientation. Part of one prism layer can be seen behind part of another (most clearly visible in area marked “decussation”). Beyond the curve marked “transition,” prisms switch to a pattern of invariant ordering. (Image courtesy Dr. Shane White, UCLA.) (b) Predictions of trajectories of cells governed by rules of shear enhancement and preferred density (simulation with sub-scale enamel thickness).

Fig. 5.

(a) Illustration of the relative shear velocity of two cells, represented by points i and j , which correspond to the tip points marked in Fig. 3b. (b) Schematic of four rows of ameloblasts sliding past one another in the alternating layered pattern of decussation in the mouse incisor. (The sliding pattern depicted is representative of the outcome of simulations, not an assumption.) Cell boundaries have been determined by the Voronoi construction. The length of one contact between two cells, which determines the weight factor w_j in Eq. (2), is marked “ l ”. Small horizontal arrows indicate the alternating average directions of sliding. Bold arrows indicate implied sliding between cell membranes (which need not be explicitly included in the population-scale model to prove the capacity for pattern forming).

Fig. 6.

(a) The migration of an ameloblast population as a continuous curved sheet of cells that expands away from the DEJ as enamel forms underneath it is typified by this image of the early bell stage of odontogenesis in the human molar (adapted by Cox (2010) from Nanci (2003)). Odontoblasts migrate down to form dentin and ameloblasts migrate up, with a thin boomerang-shaped wedge of enamel (black) already formed in their wake. Migration starts at the “dentin horn”, indicated by the label “maximum curvature”. (b) Enlargement shows how cells are sheared when the wedge of enamel forms beneath them. The “commencement front” lies at the tip of the enamel wedge (marked in (a)) and indicates the transition from stationary to migrating secretory-stage in the ameloblast’s life.

Fig. 7.

(a) In the phenomenon of PRM, a cell pair spontaneously establishes a rotating sliding motion across a substrate. The pair exhibit two-fold rotational symmetry (Huang et al., 2005). (b) Symmetry elements expected to be present in the phenomenon of decussation are depicted for two cells sliding past one another, extracted from a pattern of repeated sliding rows. The symmetry elements include reflection symmetry for variables within a single cell about the cell's mid-plane (marked O_1), inversion symmetry about the point O_2 in the plane of contact between two cells that are sliding past one another, and translational invariance between pairs of points separated by the lattice vector $\mathbf{a}$ in the x -direction.

Fig. 8.

(a) A single prism (mineralized matrix secreted by an ameloblast that records its trajectory from the dentine to the enamel surface), highlighted by a black curve, in the human molar (adapted from Macho et al., 2003). (b) Waves in the enamel of the mouse incisor, traced by black curves (image courtesy Dr. Shane White, UCLA). (c) Trajectories of individual cells predicted when a sheet of cells migrates away from a curved surface (the DEJ), which necessarily introduces strains in the population, which in turn leads to waviness in the trajectories when pairs of contacting cells tend to restore a preferred center-to-center separation (from Cox, 2010).

Fig. 9.

(a) The time rate of change of Von Mises' equivalent strain immediately behind the commencement front in a slightly sub-scale simulation of amelogenesis in the mouse incisor (arbitrary units of strain) (Cox, 2013). (b) C-shaped commencement front observed in a rat incisor (Warshawsky and Smith, 1976). The line marked "peak" corresponds to the location of maximum curvature on a section of the DEJ made in the x - z plane. (Ignore other boundaries marked in (b).).

Fig. 10.

Domains of decussation and invariant ordering in the parameter space (c_1 , v_p/v_m), where c_1 is the strength of shear enhancement, v_p is the velocity of propagation of strain information, and v_m is the rate of outward expansion of the population sheet. Points A and B mark independent estimates of v_p/v_m for the beginning and end of amelogenesis in the mouse incisor, along with a calibrated value of c_t . Point C marks a value of c_1 for which transition to invariant ordering will occur when a decussating pattern has already been established (details in Cox, 2013).

Fig. 11.

Tufts (white) in the human molar, exposed by etching away the mineralized enamel, exhibit a complex pattern in the “cuspal region” (which lies immediately above the region of highest curvature on the DEJ), whereas they exhibit a relatively simple ordering in rows away from the cusp (lower right part of image) (from Amizuka et al., 1992).

Fig. 12.

Data for a population of epithelial cells expanding on a substrate *in vitro*. The substrate lies in the x - y plane and expands, for the cells shown predominantly in the x -direction: (a) a “kymograph” of the time rate of change of the strain in the x direction, ϵ_x , in which any horizontal scan line in the image shows the variation of ϵ_x along a scan line at fixed position y in the cell population at the time t given by the ordinate. (b) The average strain rate in small region at the mid-line of the population, $x=0$ in (a), vs. time, t .

Fig. 13.

Networks growing in two dimensions: (a) endothelial cells (red/orange) migrate across a network template formed by astrocyte cells (green) during retinal angiogenesis in the mouse (from Dorrell et al., 2002). The longer branches of endothelial cells in this image have followed approximately straight paths, ignoring branches in the astrocyte network that would have led them to the side or backwards. (b) Nerve network (white) forming in the mouse ileum and cecum by invading neural crest cells (from Druckenbrod and Epstein, 2005). (c) In innervation of the gut, neural crest cells (marked “ENS”) are confined to thin layers within the cylindrical walls of the pre-existing gut tissue. (d) schematic of neural crest cells associated with an axon (from Newgreen et al., 2013). In (a) and (d), the invasion is progressing from left to right; in (b), from right to left. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 14.

Networks growing in three dimensions: (a) and (b) a simulation of neo-vascularization induced by hypoxia. The network structure at one instant will grow under the stimulus of VEGF, which is generated by tissue that is not currently well supplied with oxygen (blue or darker areas in (b)) and then diffuses to the existing blood vessels (from Liu et al., 2011). (c) and (d) Angiogenesis induced, e.g., by VEGF emitted by a tumor, showing initial sprouting from an existing vessel (vertical), assumption of slender branch geometry, branching or bifurcation of branches, and anastomosis or coalescence of branches (from Plank and Sleeman, 2004). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 15.
Branch geometry at one time instant (only near-tip invaders are shown).

Fig. 16.

Simulation of the mechanics of network formation on a cubic grid: (a) when an element is first invaded, it acquires an enhanced average density $\bar{\rho} > \rho_h$, where ρ_h is the relaxed host density. (b) As time passes, the density spike in an invaded cell dissipates as matter diffuses into neighboring spatial elements via some mass transport process. The result shown is computed for a line (branch) of invaded spatial elements of size a , with cells moving radially away in all directions at every point along the line (Cox, 2011). The change per unit time of the reciprocal of the slope of the curve marked “approximately exponential variation,” evaluated near the branch, defines the relaxation velocity v_r . (c) Locations $P, Q_1, \dots$ at which strains in non-invaded elements adjacent to invaded elements are sampled, illustrated for a

line of invaded elements. (d) Variation of strain measure $\varepsilon_1 + \varepsilon_2$ around line of invaded elements (path s in (c)). (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

Fig. 17.

- (a) A network predicted for stochastic strain-cued automata, from a simulation that was stopped while branches were still propagating (propagation from left to right) (Cox, 2011).
 (b) Network morphology of the nerves (white lines) in the mouse gut (propagation from right to left) (image from Druckenbrod and Epstein (2005), taken at a later time than that reproduced in Fig. 13b).

Fig. 18.

The predicted distribution of strain cues around network branches at one time instant. Spatial elements that have already been invaded are black. Host spatial elements are colored according to the value of the strain metric $\varepsilon_s = \varepsilon_1 + \varepsilon_2$ that is predicted in the host element at a point in the host element just outside the interface with an adjacent invaded element. This definition of strain in an element is inapplicable to host elements that are not adjacent to invaded elements and such host elements are therefore plotted with the value zero. Such elements generally have nonzero strains, but of small magnitude that falls rapidly with distance from the nearest invaded element. Typical fields around an extending branch tip are labeled T. Typical fields around a recent bifurcation are labeled B. Typical fields along the flanks of an established branch are labeled F. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

Fig. 19.

The variation of the elastic strain in the direction normal to the interface between the invader domain and the host, ε_n , for the case of a straight propagating branch. The compressive contact pressure at the interface is proportional to ε_n . The points P, Q₁, etc., refer to the locations marked in Fig. 16c.

Fig. 20.

(a) Qualitative distribution of tensile and compressive tangential stresses in the host adjacent to an invasion branch. (b) Energetically favorable locations F1, F2, F3, and F4 of filipodial protrusions from the tip cell. (c) Direction of tip cell migration that initially releases most strain energy in the host medium.

Fig. 21.

The morphology of a network formed by an algorithm that combines cues that depend on the tangential strain in the host around an invader domain and cues that depend on the gradient dC/dx in a chemical factor.

Fig. 22.

(a) A migrating cell within a medium of host cells, in a cubic spatial discretization. The cell occupies three spatial elements and moves initially to the right as indicated by the large arrow. At this instant, the points P₁ and P₂ lie just outside the occupied elements, in elements that are currently host cells. (b) One possible subsequent configuration of the migrating cell, after one computational time step.

Fig. 23.

(a) A contiguous, elongated body of cells (a "peloton") migrating to the right induces stresses in the host that are similar to those of Fig. 17d. (b) If one cell separates from the peloton, the stress in the host will initially jump to a tensile state at a point between them, because the separated cell constitutes a new mechanical "inclusion" with an eigenstrain (i.e., a mis-fitting body) surrounded by host that is newly stretched around it and has not yet had time to relax to accommodate it.

Table 1

Estimates of mechanical energy density rates (from Cox and Smith, 2014).

	Strain rate $\dot{\epsilon}_0$ (s^{-1})	Strain energy rate $\dot{G}_{el}$ (J $\mu m^{-3} s^{-1}$)	$\frac{\dot{G}_{el}}{\dot{G}_{met}}$
Ameloblasts (i) global strains (areal tension or compression) (human molar)	2.5×10^{-7}	3×10^{-20}	10^{-5}
Ameloblasts (ii) local strains (tension or compression + shear) (mouse incisor)	10^{-5}	10^{-18}	10^{-4}
BMU (global circumferential compression)	10^{-8} – 10^{-7}	10^{-19}	10^{-5}
Innervation of avian gut (biaxial tension+compression) ^a	10^{-4}	10^{-17}	10^{-3}
Angiogenesis (biaxial tension+compression) ^a	3×10^{-4}	3×10^{-17}	10^{-2}
Tumor growth ^b	–	10^{-19}	10^{-5}
Cells migrating along gradient in cell–cell adhesion (crypt)	N/A	6×10^{-21} ^c	10^{-6}
Cells being sorted by differential adhesion	N/A	10^{-20} ^c	10^{-6}
Pulsating heart muscle (primarily uniaxial strain)	2.3	10^{-14}	2^d
Cells in a typical <i>in vitro</i> experiment (tension / compression)	4π	4×10^{-16}	10^{-1}

^aStrains and strain energies in the host cells displaced by invading cells.^bQuoted from Ref. Narayanan et al. (2010).^cTime rate of change of total adhesion energy for cell, divided by the cell's volume.^dPeak ratio during pulse.

Table 2

Relaxation and migration velocities.

Case	Relaxation velocity, v_r ($\mu\text{m d}^{-1}$)	Migration velocity, v_m ($\mu\text{m d}^{-1}$)	Strain rate, $\dot{\epsilon}$ (s^{-1})
Amelogenesis – human molar	70–100	2.4–4	10^{-7} – $10^{-5}{}^a$
Amelogenesis – rat incisor	250 ± 40	13	10^{-7} – $10^{-5}{}^a$
Osteoblasts during osteoid formation	Unknown	10	$10^{-7}{}^b$
Innervation of mouse gut	30–60	1000	$10^{-4}{}^c$
Angiogenesis	90–180	3000	$3 \times 10^{-4}{}^c$
Epithelial cells <i>in vitro</i>	$1200{}^d$	$600{}^d$	$4\text{--}8 \times 10^{-5}{}^e$

^aLow end of range represents global strains and high end represents local strains.

^bGlobal strains.

^cStrains in host cells

^dEstimated from Fig. 3b of Serra-Picamal et al. (2012), together with report in Serra-Picamal et al. (2012) that wave speeds were “approximately twice the speed of the advancing front edge”

^eStrain rate crests in Fig. 3f of Serra-Picamal et al. (2012).