Published in final edited form as:

Curr Opin Genet Dev. 2017 August ; 45: 22–27. doi:10.1016/j.gde.2017.02.010.

Distributing signaling proteins in space and time: the province of cytonemes

Thomas B. Kornberg

Cardiovascular Research Institute, University of California, San Francisco, CA 94143

Abstract

During development, cells use specialized filopodia called cytonemes to deploy the signaling proteins that coordinate growth and direct morphogenesis. Cytonemes are dynamic structures that can extend long distances across tissues to either deliver or take up signaling proteins. Signaling proteins transfer between cells at the tips of cytonemes where specific contacts termed morphogenetic synapses form. This review summarizes our current understanding of the roles and functions of cytonemes, and it explores some of the conceptual issues relevant to the cytoneme mechanism of contact-dependent cellcell signaling.

Keywords

morphogen; signaling; cytoneme

Introduction

The idea that cells make substances that can induce measured, position-dependent responses in other cells dates to the early 20th century when experiments identified a particular region of an amphibian embryo that could, when transplanted to an ectopic location in another embryo, cause host cells to adopt new fates and create new structures [1]. The apparent “action at a distance” was taken to indicate that cells in this particular region impart information about relative position and direction by secreting a substance that diffuses in order to contact adjacent cells. Experimental support for “action at a distance” is now strong, and work during the past several decades has identified inducers such as the signaling proteins Wnt/Wg, Bone Morphogenic Protein (BMP), Hedgehog (Hh), Fibroblast growth factor (FGF) and Epidermal growth factor (EGF). In addition, observations in many systems indicate that these proteins do distribute across fields, forming concentration gradients, and that they elicit concentration-dependent responses. However, available evidence does not support the idea that dispersal is by secretion into extracellular space followed by some type of passive diffusion. Rather, the strongest experimental evidence shows that the signaling proteins move along cytonemes and exchange between producing and receiving cells at sites of direct contact [2]. These contacts are transient synapses that form at the tips of dynamic

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

cytonemes that can extend long distances [3,4]. This basic signaling mechanism has been documented in flies [5–9] (and reviewed in [2]), chick [10], and zebrafish [11,12], and it has many fascinating attributes.

Why not diffusion?

The complexity of biological forms is vast, and although diffusion may appear to be the simplest way to disperse signals, the informational content of diffusion-generated distributions is limited by temporal and quantitative imprecision. Lewis Wolpert, who developed the concept of positional information and was an early champion of the roles that gradients might play in development [13], has more recently argued that concentration gradients of diffusible molecules cannot adequately provide the precision and robustness that development requires of positional specification [14,15]. I will not repeat his arguments here, but point out that Wolpert now embraces the idea that the dispersion of morphogen signaling proteins is cytoneme-mediated [15]. I will also not revisit the many issues I have raised previously to argue against the idea that diffusion-generated gradients have instructive roles in either tissues or embryos [3,16–18]. Instead, the following offers alternate interpretations to several types of experiments that have heretofore been offered to support the idea of dispersion by diffusion.

Freely released signals

An experimentally placed source of signaling protein such as an FGF-soaked bead can release FGF into surrounding tissue of an injected host embryo, and assays of signal transduction targets show that the FGF that is released from a bead acts at a distance in host cells (Fig. 1). It seems likely that the released FGF reaches target cells by diffusing in the extracellular milieu, but this behavior may be irrelevant to the normal context. An electrophysiologist who measures responses to neurotransmitter added by pipet to a brain slice (Fig. 1B) does not infer that neurotransmitter is normally released to freely diffuse from its source to surrounding neurons. The electrophysiologist understands that neurotransmitter is normally released in the context of a synapse where neurotransmitter can diffuse across a synaptic gap whose dimensions and constitution circumscribe its activity both quantitatively and spatially. In experiments with the brain slice, the electrophysiologist can take advantage of the programmed responsiveness of the post-synaptic cells, irrespective of the source of neurotransmitter. Similarly, for the embryo that responds to the FGF-containing bead, the activity of the FGF is indicative of the competence of cells to respond to FGF, and no inferences should be made about the normal route or mode of FGF dissemination.

Role of the ECM

In part because tissues are not flat and some (such as the *Drosophila* leg imaginal disc) are compressed by tight folds, restricting morphogen protein movement to epithelial surfaces has been proposed as a means of constraining and molding their distributions [19–23]. Experimental evidence cited in support of restricted diffusion includes the failure of signaling proteins to traverse over cells that are unable to make cell surface heparan sulfate proteoglycans (HSPGs) [24–32]. Models suggesting how HSPGs might sculpt morphogen

distributions include direct binding of morphogens or morphogen-containing lipoprotein particles to heparan sulfate chains [27,29,33,34], with repeated cycles of binding and release leading to surface-restricted diffusion (Fig. 2). An alternative (and more likely) explanation is provided by experiments showing that cytonemes do not extend over cells defective in HSPG production [5,35]. This finding shows that the movement of signaling proteins and the responses they induce in their targets are dependent on HSPGs because of the essential roles that cytonemes have in trafficking the signaling proteins. Although the interactions between cytonemes and HSPGs have not been characterized and are not understood, they appear to be specific and to contribute to the contours of signaling protein distributions: cytoneme sub-types that are dedicated to different signaling systems have specific requirements for different HSPGs [35].

Particles moving in space

Signaling proteins have been reported to traffic in various types of extracellular particles, such as argosomes [36], lipoprotein particles [34], exosomes [37,38] and exosome-like particles [39]. Images of fixed and live tissue preparations have been interpreted to indicate that protein-containing particles move in extracellular space between producing and target cells. However, particles that appear to move freely between producing and target cells may not actually be untethered. Signaling proteins move along cytonemes in vesicles [5,6,8,9,38], and if cytonemes are not marked, the vesicles they traffic would appear to be untethered when in fact they have not actually been released and are not extracellular.

Studies of signaling protein produced by cultured cells have characterized both particulate and soluble forms that are present in culture medium, but there is no evidence that cells in culture release proteins by the same processes that constrain them in vivo. Therefore the presence of protein in cell culture medium may not be relevant to the systems that normally control secretion and release, and therefore should not be interpreted as evidence for in vivo behavior.

Turing models

The mathematics developed by Turing describes how reactive and diffusible molecules might produce the kinds of patterns that biological systems create [40], and studies of pigment stripe formation in the adult zebrafish have revealed precisely the type of short and long range interactions that are consistent with a Turing reaction-diffusion process [41]. However, such correlations are not evidence of a diffusion-based mechanism. Stripe generation in the zebrafish is in fact dependent on short range signals transmitted by cells that extend short processes that appear to directly contact their targets, and long range signaling is mediated by long projections that also appear to make direct contacts [42]. To quote from Hamada et al, 2014, “Gierer and Meinhardt noted that essential conditions for autonomous pattern formation are the combination of short-range activation and long-range inhibition [43]. “Diffusion” itself is dispensable and can be replaced *by any other means that can transmit signals over the appropriate spatial distance* [italics added].” The studies of Hamada et al, 2014 suggest that the signals that pattern zebrafish stripes are transmitted by means of cell extensions that make direct contacts, not by diffusion. These studies also implicated the Notch/Delta signaling system in stripe pattern formation, implying that Notch

signaling may be dependent on juxtacrine cell-cell contacts made by cell extensions that enable binding of the Notch membrane receptor to its Delta membrane ligand. Studies of Notch/Delta-dependent patterning in *Drosophila* have also identified an essential role for direct contacts made by cell extensions (cytonemes) [35,44].

Properties of cytonemes

Although studies of filopodia have a long history, we still have little understanding of how their growth initiates and what guides their orientation, or for the most part, even what they do. Their purported roles include force generation, migration and environment sensing, but because they share structural elements with the cytoskeleton, functional characterizations have mostly been limited to descriptions of their distribution and behavior. Relevant examples include neuronal growth cone filopodia, which extend and retract at the forefront of a growing neurite, appearing to search for attractive “guidance cues”, and sea urchin embryo “thin filopodia” that appear to be used as sensors for patterning [45]. Although there is no reason to suggest that these filopodia do not carry out these functions, we still lack genetic or molecular evidence that directly establishes the role of growth cone filopodia in guidance cue signaling or identifies the signaling systems “thin filopodia” might mediate. Studies of cytonemes provide the first definitive evidence for a specific role: these specialized filopodia mediate paracrine signaling by directly linking signaling cells and by moving signaling proteins from cells that produce signaling proteins to cells that receive them. The experiments that establish their roles are of several kinds.

First, signaling proteins have been observed exchanging between signaling cells that are linked by cytonemes. Studies in *Drosophila* have provided two examples. In the columnar epithelial layer of the wing disc, Hh produced by cells in the posterior compartment is transported along cytonemes that extend from posterior compartment cells across the compartment border to anterior compartment cells that take it up [6]. In the air sac primordium (ASP), a tracheal tube that associates with the wing disc, cytonemes extending from the ASP contact wing disc cells that produce Decapentaplegic (Dpp; a BMP ortholog), take up Dpp and transport Dpp to the ASP [8,9]. These experiments establish that cytonemes are conduits that transport signaling proteins between signaling cells. In other contexts, the presence of cytonemes has been correlated with paracrine Wnt [12,35], FGF [8,46], EGF [7] and Notch signaling [11,35,42,44].

Second, paracrine signaling by Hh, Wnt/Wg, FGF and Notch/Delta is reduced by genetic conditions that impair cytonemes. Use of morpholino oligomers to target expression in zebrafish embryos has shown that cytonemes that regulate gene expression in the neural plate are Cdc42-dependent [12]. In *Drosophila*, analysis of RNAi-induced downregulation, expression of dominant negative constructs and genetic mutants have revealed essential roles for genes that encode several actin binding proteins (i.e. Capping protein a, SCAR, Pico, Diaphanous), membrane proteins (i.e., Neuroglian, Capricious), a GTPase (i.e., Shibire), two HSPGs (Dally and Dally-like), four planar cell polarity components (Prickle, Van Gogh and Grunge), three integrin subunits (Myospheroid, Multiple edematous wings and Inflated), Integrin-linked kinase, and the laminin Wing blister [5,9,35,47]. Signaling is affected by genetic conditions that reduce the function of these proteins, and although most of these

conditions also reduce the number of cytonemes, the effect on cytoneme number is not universal - the number of cytonemes is not reduced in cells that lose Capricious function. However, the cytonemes these cells make do not synapse with target cells, revealing that the direct contacts - morphogenetic synapses [4] - are essential for paracrine signaling [9].

Many of the proteins that are essential for cytoneme-mediated paracrine signaling are also necessary for cell viability, but the attributes and flexibility of the *Drosophila* system make it possible to tailor the knock-down conditions in order to reduce function in only selected groups of cells and to levels that do not compromise cyto-architecture or viability. For example, the cytonemes that ASP cells extend cytonemes to pick up Dpp or FGF from the wing disc are sensitive to reduced levels of Diaphanous [9]. Diaphanous is a formin that is essential for viability, but the knock-down conditions that essentially eliminate cytonemes and paracrine signaling do not measurably change cell shape, cell division or cell viability. This key virtue of the *Drosophila* system has been exploited to establish that cytonemes function as conduits for signaling proteins that are essential to paracrine signaling, providing the first direct experimental evidence for a function of filopodia in any context.

Reconsidering the paracrine mechanism of cell-cell signaling

Paracrine signaling is considered to be a form of signaling that is effected by secreted molecules that diffuse in extracellular space and whose range of action is limited by uptake into neighboring cells, degradation by extracellular enzymes, and adsorption to components of the extracellular matrix. The presence of cytonemes that move signaling molecules in the space between cells through which paracrine signals are said to diffuse requires re-evaluation of the paracrine model. Extant histological evidence does not resolve whether in-transit signaling molecules are extracellular, or are external and at or near the plasma membrane, and therefore we lack evidence that might distinguish between secreted molecules that diffuse in extracellular space and molecules that are at the cell surface or are associated with a cytoneme. Moreover, cytonemes can be sensitive to chemical fixatives and are highly sensitive to shear, and they are not visible unless marked in a way that differentiates them from the cells that provide a substrate for their extension. Given these uncertainties, it is not clear whether current experimental evidence establishes a context for the classic paracrine mechanism. For proteins such as Wnt/Wg, BMP/Dpp, Hh, FGF, EGF, and Notch/Delta whose dispersion is cytoneme-mediated, the classic paracrine mechanism appears to be irrelevant.

Acknowledgments

Funding from National Institutes of Health: GM030637 and GM105987

References

1. Spemann H, Mangold H. Induction of embryonic primordia by implantation of organizers from a different species. *Roux Arch Entw Mech*. 1924; 100:599–638.
- 2**. Kornberg TB. Cytonemes and the dispersion of morphogens. *Wiley Interdiscip Rev Dev Biol*. 2014; 3:445–463. This offers a perspective and review contact-based cell-cell signaling. [PubMed: 25186102]

3. Kornberg TB, Roy S. Cytonemes as specialized signaling filopodia. *Development*. 2014; 141:729–736. [PubMed: 24496611]
4. Kornberg TB, Roy S. Communicating by touch—neurons are not alone. *Trends Cell Biol*. 2014; 24:370–376. [PubMed: 24560610]
- 5**. Bischoff M, Gradilla AC, Seijo I, Andres G, Rodriguez-Navas C, Gonzalez-Mendez L, Guerrero I. Cytonemes are required for the establishment of a normal Hedgehog morphogen gradient in *Drosophila* epithelia. *Nat Cell Biol*. 2013; 15:1269–1281. This article is the first to describe cytoneme dynamics in vivo and to show how a Hh gradient forms in relation to cytoneme-mediated Hh transport. [PubMed: 24121526]
6. Callejo A, Biloni A, Mollica E, Gorfinkel N, Andres G, Ibanez C, Torroja C, Doglio L, Sierra J, Guerrero I. Dispatched mediates Hedgehog basolateral release to form the long-range morphogenetic gradient in the *Drosophila* wing disk epithelium. *Proc Natl Acad Sci U S A*. 2011
7. Hsiung F, Ramirez-Weber FA, Iwaki DD, Kornberg TB. Dependence of *Drosophila* wing imaginal disc cytonemes on Decapentaplegic. *Nature*. 2005; 437:560–563. [PubMed: 16177792]
8. Roy S, Hsiung F, Kornberg TB. Specificity of *Drosophila* cytonemes for distinct signaling pathways. *Science*. 2011; 332:354–358. [PubMed: 21493861]
- 9**. Roy S, Huang H, Liu S, Kornberg TB. Cytoneme-mediated contact-dependent transport of the *Drosophila* decapentaplegic signaling protein. *Science*. 2014; 343:1244624. This paper presents evidence that cytonemes traffic signaling proteins and transfer them at synapses. [PubMed: 24385607]
10. Sanders TA, Llagostera E, Barna M. Specialized filopodia direct long-range transport of SHH during vertebrate tissue patterning. *Nature*. 2013; 497:628–632. [PubMed: 23624372]
11. Eom DS, Bain EJ, Patterson LB, Grout ME, Parichy DM. Long-distance communication by specialized cellular projections during pigment pattern development and evolution. *Elife*. 2015; 4
- 12*. Stanganello E, Hagemann AI, Mattes B, Sinner C, Meyen D, Weber S, Schug A, Raz E, Scholpp S. Filopodia-based Wnt transport during vertebrate tissue patterning. *Nat Commun*. 2015; 6:5846. This paper reports the role and importance of cytoneme-mediated Wnt signaling in zebrafish. [PubMed: 25556612]
13. Wolpert L. Positional information and the spatial pattern of cellular differentiation. *J Theor Biol*. 1969; 25:1–47. [PubMed: 4390734]
14. Kerszberg M, Wolpert L. Specifying positional information in the embryo: looking beyond morphogens. *Cell*. 2007; 130:205–209. [PubMed: 17662932]
- 15**. Wolpert L. Positional Information and Pattern Formation. *Curr Top Dev Biol*. 2016; 117:597–608. The communication explores the issues relevant to dissemination of signaling proteins. [PubMed: 26970003]
16. Kornberg TB. The imperatives of context and contour for morphogen dispersion. *Biophys J*. 2012; 103:2252–2256. [PubMed: 23283223]
17. Kornberg TB, Guha A. Understanding morphogen gradients: a problem of dispersion and containment. *Curr Opin Genet Dev*. 2007; 17:264–271. [PubMed: 17643982]
18. Ali-Murthy Z, Kornberg TB. Bicoid gradient formation and function in the *Drosophila* pre-syncytial blastoderm. *Elife*. 2016; 5
19. Lander AD, Nie Q, Wan FY. Do morphogen gradients arise by diffusion? *Dev Cell*. 2002; 2:785–796. [PubMed: 12062090]
20. Schwank G, Dalessi S, Yang SF, Yagi R, de Lachapelle AM, Affolter M, Bergmann S, Basler K. Formation of the long range Dpp morphogen gradient. *PLoS Biol*. 2011; 9:e1001111. [PubMed: 21814489]
21. Tabata T, Takei Y. Morphogens, their identification and regulation. *Development*. 2004; 131:703–712. [PubMed: 14757636]
22. Yan D, Lin X. Shaping morphogen gradients by proteoglycans. *Cold Spring Harb Perspect Biol*. 2009; 1:a002493. [PubMed: 20066107]
23. Zhu AJ, Scott MP. Incredible journey: how do developmental signals travel through tissue? *Genes Dev*. 2004; 18:2985–2997. [PubMed: 15601817]

24. Belenkaya TY, Han C, Yan D, Opoka RJ, Khodoun M, Liu H, Lin X. Drosophila Dpp morphogen movement is independent of dynamin-mediated endocytosis but regulated by the glypican members of heparan sulfate proteoglycans. *Cell*. 2004; 119:231–244. [PubMed: 15479640]
25. Desbordes SC, Sanson B. The glypican Dally-like is required for Hedgehog signalling in the embryonic epidermis of Drosophila. *Development*. 2003; 130:6245–6255. [PubMed: 14602684]
26. Fujise M, Takeo S, Kamimura K, Matsuo T, Aigaki T, Izumi S, Nakato H. Dally regulates Dpp morphogen gradient formation in the Drosophila wing. *Development*. 2003; 130:1515–1522. [PubMed: 12620978]
27. Han C, Belenkaya TY, Wang B, Lin X. Drosophila glypicans control the cell-to-cell movement of Hedgehog by a dynamin-independent process. *Development*. 2004; 131:601–611. [PubMed: 14729575]
28. Lin X, Buff EM, Perrimon N, Michelson AM. Heparan sulfate proteoglycans are essential for FGF receptor signaling during Drosophila embryonic development. *Development*. 1999; 126:3715–3723. [PubMed: 10433902]
29. Lin X, Perrimon N. Dally cooperates with Drosophila Frizzled 2 to transduce Wingless signalling. *Nature*. 1999; 400:281–284. [PubMed: 10421372]
30. Bellaiche Y, The I, Perrimon N. Tout-velu is a Drosophila homologue of the putative tumour suppressor EXT-1 and is needed for Hh diffusion. *Nature*. 1998; 394:85–88. [PubMed: 9665133]
31. The I, Bellaiche Y, Perrimon N. Hedgehog movement is regulated through tout velu-dependent synthesis of a heparan sulfate proteoglycan. *Mol Cell*. 1999; 4:633–639. [PubMed: 10549295]
32. Tsuda M, Kamimura K, Nakato H, Archer M, Staatz W, Fox B, Humphrey M, Olson S, Futch T, Kaluza V, et al. The cell-surface proteoglycan Dally regulates Wingless signalling in Drosophila. *Nature*. 1999; 400:276–280. [PubMed: 10421371]
33. Callejo A, Culi J, Guerrero I. Patched, the receptor of Hedgehog, is a lipoprotein receptor. *Proc Natl Acad Sci U S A*. 2008; 105:912–917. [PubMed: 18198278]
34. Panakova D, Sprong H, Marois E, Thiele C, Eaton S. Lipoprotein particles are required for Hedgehog and Wingless signalling. *Nature*. 2005; 435:58–65. [PubMed: 15875013]
35. Huang H, Kornberg TB. Myoblast cytonemes mediate Wg signaling from the wing imaginal disc and Delta-Notch signaling to the air sac primordium. *Elife*. 2015; 4:e06114. [PubMed: 25951303]
36. Greco V, Hannus M, Eaton S. Argosomes: a potential vehicle for the spread of morphogens through epithelia. *Cell*. 2001; 106:633–645. [PubMed: 11551510]
37. Beckett K, Monier S, Palmer L, Alexandre C, Green H, Bonneil E, Raposo G, Thibault P, Le Borgne R, Vincent JP. Drosophila S2 cells secrete wingless on exosome-like vesicles but the wingless gradient forms independently of exosomes. *Traffic*. 2013; 14:82–96. [PubMed: 23035643]
38. Gradilla AC, Gonzalez E, Seijo I, Andres G, Bischoff M, Gonzalez-Mendez L, Sanchez V, Callejo A, Ibanez C, Guerra M, et al. Exosomes as Hedgehog carriers in cytoneme-mediated transport and secretion. *Nat Commun*. 2014; 5:5649. [PubMed: 25472772]
39. Matusek T, Wendler F, Poles S, Pizette S, D'Angelo G, Furthauer M, Therond PP. The ESCRT machinery regulates the secretion and long-range activity of Hedgehog. *Nature*. 2014; 516:99–103. [PubMed: 25471885]
40. Turing AM. The chemical basis of morphogenesis. *Phil Tran R Soc Lond*. 1952; 237:37–72.
41. Yamaguchi M, Yoshimoto E, Kondo S. Pattern regulation in the stripe of zebrafish suggests an underlying dynamic and autonomous mechanism. *Proc Natl Acad Sci U S A*. 2007; 104:4790–4793. [PubMed: 17360399]
- 42**. Hamada H, Watanabe M, Lau HE, Nishida T, Hasegawa T, Parichy DM, Kondo S. Involvement of Delta/Notch signaling in zebrafish adult pigment stripe patterning. *Development*. 2014; 141:318–324. This paper reports data that supports the contact-based signaling model and explores the role of the reaction-diffusion Turing mechanism. [PubMed: 24306107]
43. Gierer A, Meinhardt H. A theory of biological pattern formation. *Kybernetik*. 1972; 12:30–39. [PubMed: 4663624]
44. Cohen M, Georgiou M, Stevenson NL, Miodownik M, Baum B. Dynamic filopodia transmit intermittent Delta-Notch signaling to drive pattern refinement during lateral inhibition. *Dev Cell*. 2010; 19:78–89. [PubMed: 20643352]

45. Miller J, Fraser SE, McClay D. Dynamics of thin filopodia during sea urchin gastrulation. *Development*. 1995; 121:2501–2511. [PubMed: 7671814]
46. Snyder JC, Rochelle LK, Marion S, Lyerly HK, Barak LS, Caron MG. Lgr4 and Lgr5 drive the formation of long actin-rich cytoneme-like membrane protrusions. *J Cell Sci*. 2015; 128:1230–1240. [PubMed: 25653388]
- 47**. Huang H, Kornberg TB. Cells must express components of the planar cell polarity system and extracellular matrix to support cytonemes. *Elife*. 2016; 5 This paper describes the role of the extracellular matrix for cytonemes and contact-based signaling.
48. Neubuser A, Peters H, Balling R, Martin GR. Antagonistic interactions between FGF and BMP signaling pathways: a mechanism for positioning the sites of tooth formation. *Cell*. 1997; 90:247–255. [PubMed: 9244299]

Highlights

Cytoneme-mediated, contact-based exchange of signaling proteins is a new paradigm for cell-cell signaling that exposes many unfounded assumptions that supported diffusion-based models of morphogen dissemination.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1. Experimental addition of unbound signal

(A) Placement of a bead soaked with PBS+FGF induces *pax9* expression in surrounding cells (right), in contrast to a bead soaked with only PBS (left). (from [48]) (B) Cells in a brain slice respond to neurotransmitter added by pipet.

Figure 2. Surface diffusion model of signal protein dispersion

Movement (left to right) of signaling protein (red) across a sheet of cells with HSPGs (green column with blue strands) and receptor (black) on the cell surface is hypothesized to repeat cycles of binding to an HSPG, release and random walk, with eventual binding to receptor and uptake.

Figure 3. Cytonemes of normal and mutant cells

(A, B) Cytonemes extend across “intermediate cells” (gray), linking cells (green) that produce signal (red) with cells (beige) that receive signal. (C) Receiving cells that lack *Neuroglial (Nrg)*, *diaphanous (dia)*, *shibire (shi)*, *myospheroid (mys)*, *multiple edematous wings (mew)* or *inflated (if)* do not have cytonemes. (D) Receiving cells that lack *Capricious (Caps)* extend cytonemes that do not contact producing cells. (E) Cytonemes do not extend across intermediate cells that lack *dally*, *dally-like*, *prickled (pk)*, *Van Gogh (Vang)*, *Grunge*

(*Gug*) or wing blister (*wb*). (F) Producing cells that lack *SCAR*, *Capping protein a* (*cpa*) or *pico* do not extend cytonemes.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript