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J Dent Res 88(1):12-21, January, 2009

ABSTRACT

Avian embryos, which have been studied scientifically since Aristotle, continue to persevere as invaluable research tools, especially for our understanding of the development and evolution of the craniofacial skeleton. Whether the topic is beak shape in Darwin's finches or signaling interactions that underlie bone and tooth formation, birds offer advantages for craniofacial biology that uniquely complement the strengths of other vertebrate model systems, such as fish, frogs, and mice. Several papers published during the past few years have helped pinpoint molecular and cellular mechanisms that pattern the face and jaws through experiments that could only have been done together with our feathered friends. Ultimately, such knowledge will be essential for devising novel clinical approaches to treat and/or prevent diseases, injuries, and birth defects that affect the human craniofacial skeleton. Here we review recent insights plucked from avians on key developmental processes that generate craniofacial diversity.

KEY WORDS: neural crest cells, craniofacial, development, experimental embryology, chimeras, evolutionary biology.

The Cells that Fill the Bill: Neural Crest and the Evolution of Craniofacial Development

INTRODUCTION

The development of the skeleton requires the precise orchestration of complex spatio-temporal signaling interactions among molecules, cells, and tissues. Nowhere is this complexity better demonstrated than in the head, where a great number of disparate parts come together to form a structurally intricate and functionally integrated skull. Thus, not surprisingly, congenital defects in craniofacial bones are relatively common, with individuals presenting overt phenotypes leading to distinct physical and social challenges. In fact, craniofacial mineralized tissue anomalies are involved in one-third of all congenital birth defects, which is approximately one in 300-600 live births (Gorlin *et al.*, 1990). While existing treatments can be considered adequate, the potential exists to prevent or mitigate craniofacial skeletal anomalies entirely by means of novel molecular and stem-cell-based therapies. But achieving such clinical advancements will require substantial resources and more basic science data.

Much of what is understood about the development of the craniofacial skeleton comes from more than a century of dedicated investigation of the cranial neural crest (Noden and Schneider, 2006). Neural crest cells arise from the margins of the neuro-epithelium, and they migrate laterally and ventrally to fill the facial prominences with mesenchyme. Alterations in neural crest cell migration and proliferation often lead to profound pathologies (Garg *et al.*, 2001; Vitelli *et al.*, 2002; Dixon *et al.*, 2006; Jones *et al.*, 2008). The neural-crest-derived mesenchyme eventually forms the facial and jaw skeletons (Noden and Trainor, 2005). Additionally, cranial neural crest cells contribute to the cranial ganglia, sympathetic nervous system, adrenal medulla (chromaffin cells), and melanocytes (Hall, 2005). In mammals, they also generate the dentin, dental pulp, alveolar bone, and periodontal ligament that are associated with teeth (Chai *et al.*, 2000). Trunk neural crest cells do not differentiate into skeletal cells *in vivo*, although this capacity has been realized *in vitro* (McGonnell and Graham, 2002; Abzhanov *et al.*, 2003).

Neural crest cells have been considered to be stem cells, due to their ability to self-renew and differentiate into multiple fates (Crane and Trainor, 2006). Yet the extent to which they meet these criteria *in vivo* remains unresolved. In culture, neural-crest-like cells have been isolated from human embryonic stem cells and differentiated into Schwann cells, glial cells, peripheral neurons, melanocytes, pericytes, adipocytes, osteoblasts, and chondroblasts (Lee *et al.*, 2007; Motohashi *et al.*, 2007). These studies, along with the systematic mapping of human neural crest cell streams (O'Rahilly and Muller, 2007), provide valuable information on the biology of human neural crest cells, which will surely be a part of new clinical strategies for treating individuals with patterning defects in the craniofacial skeleton. However, for obvious reasons, there are severe limitations to what can be obtained about mechanisms that pattern human neural-crest-derived skeletal

DOI: 10.1177/0022034508327757

Received April 18, 2008; Last revision July 10, 2008; Accepted July 16, 2008

A supplemental appendix to this article is published electronically only at <http://jdr.sagepub.com/supplemental>.

tissues *in vivo*. Therefore, a menagerie of animal models—including chick, quail, duck, quail-duck chimeras, Darwin's finches, and mouse-chick chimeras—has become especially useful.

CRANIOFACIAL OSTEOGENESIS

For several reasons, craniofacial osteogenesis can be considered dissimilar from the bone formation that occurs in the remainder of the skeleton (Helms and Schneider, 2003; Richman and Lee, 2003; Chung *et al.*, 2004; Abzhanov *et al.*, 2007; Wang *et al.*, 2007). First, a majority of bones in the skull develops through intramembranous ossification, which involves the direct deposition of an osteoid matrix by mesenchyme. Other bones in the skull, as well as the post-cranial skeleton, form primarily *via* endochondral ossification, which is where bone replaces a cartilaginous template. Second, most skull bones are derived from neural crest cells, whereas the rest of the skeleton is of mesodermal origin. Third, craniofacial osteogenesis seems to involve molecular osteogenic pathways that are different from those required post-cranially. For example, mis-expression of hypoxia-inducible factor- α (HIF- α) in transgenic mice has a profound effect on the axial and appendicular skeletons, but little or no effect on craniofacial bones (Wang *et al.*, 2007). From a clinical perspective, these distinct mechanisms of bone formation may explain surgical observations of why calvarial or mandibular bone grafts (*i.e.*, partially or fully derived from neural crest cells, respectively) incorporate better than iliac bone grafts (*i.e.*, mesoderm-derived) in maxillary or alveolar bone augmentations (Hall, 2005; Crespi *et al.*, 2007).

AVIAN SYSTEMS AND CRANIOFACIAL RESEARCH

While many model organisms have been used to examine the role of neural crest cells during craniofacial development and evolution, much current knowledge has been collected through the use of several bird species. Chicken development has been studied for thousands of years, with the first documented observations attributed to Aristotle, who compared embryos that were incubated for various amounts of time (Kember, 1971). These initial endeavors not only demonstrate Aristotle's prescience for the field of developmental biology, but also highlight embryo accessibility as a major strength of avian systems. Over two millennia later, Hamburger and Hamilton (1951) systematically categorized chick development into 46 stages based on morphological features. Remarkably, all bird embryos, whether precocial or altricial, can be roughly sorted into the same staging table created by Hamburger and Hamilton (HH), which allows for the direct comparison of all avian species regardless of their evolutionary divergence (Starck and Ricklefs, 1998).

The use of non-chicken avians for the study of craniofacial development and specifically for the characterization of neural crest cells was pioneered by Le Douarin and her colleagues in the early 1970s (reviewed in Le Douarin, 2004). Such an approach has been readily adopted (Le Douarin, 1973; Le Lievre and Le Douarin, 1975; Noden, 1978, 1983, 1986; Couly and Le Douarin, 1990; Couly *et al.*, 1992, 1993; Selleck and Bronner-Fraser, 1995; Kontges and Lumsden, 1996; Baker

et al., 1997, 1999; Schneider *et al.*, 1999, 2001; Olivera-Martinez *et al.*, 2000; Cobos *et al.*, 2001; Lwigale, 2001; Borue and Noden, 2004; Marcucio *et al.*, 2005; Noden and Schneider, 2006; Schienda *et al.*, 2006). The transplantation of quail neural crest cells into chicks with the critical ability to distinguish quail-donor cells from chick-host cells was a major achievement in the characterization of neural crest cells, particularly in studies of cell fate mapping (reviewed in Clarke and Tickle, 1999). From these tissue transplantation experiments, avian systems have smoothly transitioned into the genomics era. The first avian genome sequenced and assembled by the International Chicken Genome Sequencing Consortium (2004) was that of the Red Jungle Fowl (*Gallus gallus*), the ancestor of domestic chickens. Comparative mapping, which evaluates the location of homologous genes between species, suggests that organization of the human genome is more like that of the chicken than that of the mouse (Burt *et al.*, 1999). This surprising result reminds us that the study of one animal model system may be insufficient for the application of results to humans, and that an inter-specific approach can be most informative.

Currently, many molecular manipulation techniques are available for the mis-expression of genes in avian systems. These include the use of replication-competent avian sarcoma (RCAS) retroviruses (Hughes, 2004), electroporation (Itasaki *et al.*, 1999; Krull, 2004; Nakamura *et al.*, 2004), and siRNA and antisense oligonucleotides for gene repression (Kos *et al.*, 2003; Rao *et al.*, 2004). A fortuitous characteristic of RCAS vectors is their apparent inability to spread across basement membranes, thereby confining mis-expression of genes specifically to mesenchyme or epithelium (Abzhanov *et al.*, 2004). Advances in laser-capture microdissection techniques also allow specific tissues to be isolated for genomic and proteomic analyses (Espina *et al.*, 2007).

One of the conspicuous limitations of avian systems has been the inability to generate transgenic animals. Several factors have hampered stable modification of the chicken genome (Sang, 2006). Not only are the eggs difficult to manipulate, due to the abundant yolk and size, but also, by the time a fertilized egg is laid, it has already advanced to a stage of ~60,000 cells. Lentiviral vector transduction of newly laid eggs and the isolation of chick embryonic stem cells for *in vitro* modification to generate chimeric chickens have met with limited or no success (McGrew *et al.*, 2004; van de Lavoie *et al.*, 2006b). Recently, van de Lavoie *et al.* (2006a) isolated and cultured primordial germ cells, the precursors of germ cells. The cultured cells were genetically modified by the incorporation of β -actin-eGFP and injected into the bloodstream of developing embryos, which were then incubated and hatched. Hatched males were crossed with wild-type hens. The genetically modified primordial germ cells maintained their commitment to the germ line, resulting in offspring derived from GFP-expressing transgenic primordial germ cells (van de Lavoie *et al.*, 2006a). The next challenge is to demonstrate effective gene targeting in cultured primordial germ cells. However, the work by van de Lavoie *et al.* (2006a) is an exciting step toward the realization of transgenic chickens. Moreover, this method may be applied to yield high levels of therapeutic proteins that are glycosylated similarly to human proteins (Sang, 2006). Such advances in molecular and transgenic techniques will clearly enhance the experimental potential of avian systems.

MESENCHYMAL CONTROL OF BEAK MORPHOGENESIS

Birds display magnificent variation in their beak morphology. Despite this diversity, all beaks originate from equivalent embryonic primordia, tissues, and cells. The upper beak forms from the frontonasal and paired maxillary primordia, whereas the lower beak arises from the mandibular primordia (Fig. 1). Each primordium is comprised primarily of two tissues, mesenchyme and epithelium. Cranial mesenchyme consists of loosely associated spindle-shaped cells derived from either the neural crest or the mesoderm. Epithelium functions as a sheet of connected cells that surrounds the mesenchyme and forms a barrier to the environment. Epithelia around the facial primordia arise from oral ectoderm and pharyngeal endoderm. Sandwiched between the epithelium and mesenchyme is a thin, acellular basement membrane derived from mesenchyme. This basement membrane plays a regulatory role in epithelial-mesenchymal signaling, and is characterized by the presence of collagen types III, IV and VII (Paulsson, 1992).

Several studies have demonstrated the distinct roles of epithelium and mesenchyme during beak patterning (Le Douarin *et al.*, 2004; Helms *et al.*, 2005; Schneider, 2005). Epithelia, through a mixture of secreted factors such as Shh and Fgf8, establish positional cues to align the upper beak (Hu and Helms, 1999; Schneider *et al.*, 2001; Hu *et al.*, 2003; Foppiano *et al.*, 2007) and direct the outgrowth and orientation of skeletal elements (Shigetani *et al.*, 2000; Abzhanov and Tabin, 2004). Similarly, epithelium derived from pharyngeal endoderm is necessary for the formation and arrangement of facial skeletal elements (Couly *et al.*, 2002). Thus, epithelia seem to supply the positional cues and local maintenance factors required for pattern outgrowth by eliciting programmatic responses from underlying mesenchyme (Richman and Tickle, 1989; Schneider, 2005). Complementing these epithelial studies is work designed to understand the role of mesenchyme in patterning the beak. In particular, experiments have been performed in a variety of avians to identify and characterize the molecular and cellular mechanisms that generate species-specific differences. Such studies demonstrate the dominant role of mesenchyme and have identified molecular factors involved in the evolution of species-specific skeletal patterns.

Quail-Duck Chimeras

One of the newly emerging avian models for analyzing the distinct functions of neural-crest-derived mesenchyme during craniofacial development is the quail-duck chimeric transplantation system (Schneider and Helms, 2003; Tucker and Lumsden, 2004; Eames and Schneider, 2005; Schneider, 2005). The domestic duck has a long history of being included in avian chimeras as a way to study those patterning mechanisms that make embryos morphologically distinct (Waddington, 1930, 1932; Hampe, 1957; Zwilling, 1959; Dhouailly, 1967, 1970; Pautou, 1968; Sohal, 1976; Sohal *et al.*, 1985, 1990; Yamashita and Sohal, 1986, 1987). But the combination of Japanese quail (*Coturnix coturnix japonica*) and White Pekin duck (*Anas platyrhynchos*) as donors and/or hosts adds value for at least four reasons (Lwigale and Schneider, 2008). First, both species are commercially available. This makes possible a ready supply of

relatively inexpensive fertilized eggs. Second, quail and duck are morphologically distinct; among other features, quail possess short, blunt beaks, whereas ducks have long, broad bills. This provides a straightforward way for assaying the ability of donor neural-crest-derived mesenchyme to pattern the facial skeleton of the host. Third, quail and duck embryos have different rates of maturation (17 vs. 28 days to hatching, respectively), which allows for the clear identification of developmental events that are temporally interdependent between donor and host. Last, quail cells can be detected by means of a ubiquitous nuclear marker (QcPN) not present in the duck. This facilitates the unequivocal identification of donor- vs. host-derived structures.

Transplanting from quail to duck those cranial neural crest cells destined to form the beak produced chimeric "quck" embryos (Schneider and Helms, 2003). Chimeric "duail" embryos were also made by transplanting duck neural crest cells into quail (Fig. 1). Analysis of quail-duck chimeras revealed that quck embryos are essentially duck embryos with quail-like beaks, whereas duail embryos are quail embryos with duck-like beaks. Further inquiry revealed that neural crest cells convey species-specific patterning information by retaining and expressing intrinsic cues and by regulating gene expression in host epithelium. In a parallel study, neural crest cells fated to form the skeletal elements around the jaw joint were transplanted between quail and duck (Tucker and Lumsden, 2004). Similar to the results involving beak morphology, the patterning of cartilages such as the retro-articular process and entoglossum, which differ markedly between quail and duck in conjunction with their unique feeding behaviors, formed according to the donor species. Overall, these studies point to the dominant regulatory role of neural-crest-derived mesenchyme in establishing species-specific beak morphology. Such findings build upon classic transplant experiments with salamanders and frogs that were among the earliest to suggest a role for neural crest cells in patterning the jaw skeleton (Andres, 1949; Wagner, 1959).

Subsequent work has expanded these conclusions to include cranial feather development, which is considered a classic system for understanding the functioning of epithelial-mesenchymal signaling interactions like those that underlie mammalian tooth formation (Eames and Schneider, 2005; Schneider, 2005). In the same way, tissue recombination and extirpation experiments with chicken and mouse embryos have long shown that interactions between mesenchyme and adjacent epithelium underlie the intramembranous ossification of the mandible. What has remained unclear are the precise roles for epithelium and mesenchyme, as well as the specific signaling pathways through which these tissues regulate bone formation. To identify signaling events that determine when bone forms, and to elucidate the function of mesenchyme and epithelium, investigators have again used quail-duck chimeras (Merrill *et al.*, 2008). Pre-migratory cranial neural crest cells destined to form bone in the lower jaw were transplanted unilaterally from quail to duck. In resulting chimeras, quail donor mesenchyme established a significantly faster program for osteogenesis on both the molecular and histological levels, within the relatively slower duck host environment (Fig. 2). A series of subsequent experiments combining organ culture with gain- and loss-of-function approaches was used to explain the chimeric phenotype. Results revealed that neural crest mesenchyme uses BMP signaling as a molecular

mechanism for establishing the timing of intramembranous ossification in the mandibular primordia. Specifically, the osteo-inductive factor was shown to be *Bmp4*, which is expressed first in the epithelium and then in mesenchyme shortly thereafter. The chimeras revealed that neural-crest-derived mesenchyme dictates the timing of bone formation by temporally regulating its interactions with epithelium and its own expression of *Bmp4*. This role for the neural crest, and especially the involvement of BMP signaling, provides a likely link between developmental mechanisms and craniofacial evolution.

BMP/Calmodulin Regulation and Darwin's Finches

To understand the basis for species-specific differences in beak morphology between chick and duck, investigators used short pulses of BrdU (5-bromo-2'-deoxyuridine) to analyze proliferative or growth zones in the frontonasal process (Wu *et al.*, 2004, 2006). In both species, distinct, bilateral proliferative zones are present at stage HH26. Chick embryos at HH28 showed convergence of bilateral proliferative zones into one centrally localized zone, whereas in the duck, the bilaterally positioned proliferative zones persisted, widening the developing frontonasal process. Since BMPs and specifically BMP4 play a role in craniofacial osteogenesis (Francis-West *et al.*, 1994; Ashique *et al.*, 2002a,b; Merrill *et al.*, 2008), the authors examined the expression level and profile of *Bmp4* in the frontonasal process of both chick and duck. *Bmp4* was found to be expressed at much higher levels in duck compared with chick throughout the neural-crest-derived mesenchyme. Moreover, the expression profile of *Bmp4* was closely associated with the shifting proliferative zones. These observations suggest that BMP4 enhances proliferation in the growth zones, resulting in species-specific beak patterning. To test whether BMP4 drives beak growth, *Bmp4* was over-expressed with RCAS retrovirus in the mesenchyme of the chick frontonasal process. This led to significant increases in size (*i.e.*, width and depth) of the beaks compared with controls. Similar phenotypes were obtained with placement of BMP4-coated beads. Moreover, when *Noggin*, a BMP-antagonist, was expressed with RCAS retroviruses in the same region, beaks were miniaturized.

The extent to which such molecular mechanisms operate in natural systems was addressed in a well-known group of birds from the Galápagos Islands. Upon his visit to the Galápagos Islands in 1835, Charles Darwin observed the adaptive radiation of 14 closely related species of birds from a single ancestral

Figure 1. The avian head skeleton and the quail-duck chimeric system. **(A)** Neural-crest-derived mesenchyme generates much of the craniofacial skeleton (dark grey) as shown in schematic lateral view of a stage (HH) 40 chick embryo. Illustration modified from Noden (1983). Mesodermally derived mesenchyme produces parts of the calvarium and cranial base (light grey). **(B)** Neural crest mesenchyme fills the frontonasal (fnp), maxillary (mx), and mandibular (ma) primordia around the oral cavity (oc) of quail, as shown in frontal view at HH25. Modified from Schneider (2005). **(C)** Adult quail and duck skulls in lateral view. Note the species-specific differences in size and shape. The teeth-like structures observed in the duck are keratinized epidermal appendages. **(D)** Unilateral grafts of neural crest from the hindbrain (hb) and mid-brain (mb) were transplanted from quail to duck embryos, which were stage-matched at HH9.5, to generate chimeric "quack" as shown in dorsal view. Modified from Merrill *et al.* (2008). **(E)** Divergent maturation rates of quail and duck embryos. Modified from Eames and Schneider (2005). **(F)** Quail donor mesenchyme can be identified unequivocally by staining chimeras with the QcPN antibody (black dots). QcPN-positive cells can be observed throughout the frontonasal (fnp), maxillary (mx), and mandibular (ma) primordia. Note the unlabeled duck host epithelium (*i.e.*, QcPN-negative) surrounding the facial primordia and also around the forebrain (fb) as shown in the sagittal section. A color version of this Fig. is available online.

stock (Bowman, 1961). These birds, famously known as Darwin's finches, are considered by many to be the ultimate demonstration of rapid morphological evolution in response to natural selection. Some state that, "Darwin's finches are to evolutionary biology what Newton's apple is to physics" (Pennisi, 2004). The evolution of the various species of finches has historically been attributed to natural selective pressures, such as environmental niches, competition for resources, or mating signals (Schneider, 2007). In particular, the beaks of the finches are the primary distinguishing features, which are selected for in

Figure 2. Mesenchyme-dependent BMP signaling directs the timing of mandibular osteogenesis. **(A)** Whole-mount *in situ* hybridization reveals that chimeric quack mandibles containing quail donor mesenchyme (right side of dotted line) express molecular markers for osteogenesis on their own timetable. For example, mRNA transcripts for *Bmp4* are detected at high levels (arrow) in comparison with that observed on the duck host side. **(B)** *BmpR1a*, which is a receptor for *Bmp4*, is also up-regulated on the donor side (arrow), which is indicative of a signal transduction event. **(C)** A model for the role of neural-crest-derived mesenchyme in controlling mandibular osteogenesis through BMP signaling. Neural crest cells (grey) migrate from the rostral neural tube around HH9.5 and fill in the mandibular primordia by HH15. At this early stage, these cells establish the timing of interactions necessary for bone formation by signaling to overlying epithelium (black arrows). At the same time, epithelium is expressing growth factors such as *Bmp4* (dark grey arrows) and other genes (light grey arrows) that promote the autonomous progression of mesenchymal regulatory programs. **(D)** At HH23, epithelium continues to express *Bmp4* (dark grey domain) and other genes (light grey arrows) that allow mesenchyme to maintain outgrowth. **(E)** By HH25, *Bmp4* expression transitions from epithelium to mesenchyme. Mesenchymal expression of *Bmp4* is auto-regulatory (black arrows), but requires permissive signals from epithelium. **(F)** By HH27, epithelium is no longer needed to sustain *Bmp4* expression and mesenchyme autonomously differentiates into bone. Modified from Merrill *et al.* (2008). A color version of this Fig. is available online.

accordance with the differences in their respective diets (Bowman, 1961). Thus, ground finches have deep and wide beaks for crushing seeds, whereas cactus finches have long and pointed beaks for reaching into cactus flowers and fruits.

Abzhanov *et al.* (2004) compared and analyzed six species of Darwin's finches in the genus *Geospiza*: *G. difficilis* (considered to be the most basal species), with a small symmetrical beak; 3 ground finches with broad, deep beaks (*G. fuliginosa*, *G. fortis*, and *G. magnirostris*); and 2 cactus finches with long, pointed beaks (*G. scandens* and *G. conirostris*). The expression patterns of a variety of growth factors were examined (*i.e.*, *Shh*, *Fgf8*, *Bmp2*, *Bmp4*, *Bmp7*). The expression of *Bmp4* showed a striking correlation with beak morphology. Thus, all 3 ground finches with deep, wide beaks (*G. fuliginosa*, *G. fortis*, and *G. magnirostris*) showed elevated levels of *Bmp4* expression compared with the basal *G. difficilis*. As a functional test, *Bmp4* was over-expressed in the mesenchyme of chick frontonasal process by means of an RCAS retrovirus, resulting in deep, broad beaks similar to those of ground finches. By utilizing a candidate gene approach, Abzhanov *et al.* (2004) and Wu *et al.* (2004) independently arrived at the same conclusion: that over-expression of *Bmp4* in neural-crest-derived mesenchyme is sufficient to generate species-specific beak morphology.

Despite generating important information, the candidate approach failed to yield any novel factors that could be involved in the evolution of a longer beak morphology characteristic of cactus finches. Therefore, to identify molecular factors and pathways regulating beak length, Abzhanov *et al.* (2006) merged microarray technology with their previous work on Darwin's finches. A custom DNA microarray was printed with RNA isolated from multiple frontonasal processes of *G. fortis*. Four species of *Geospiza* (2 ground finches - *G. magnirostris*, *G. fortis*; and 2 cactus finches - *G. fuliginosa*, *G. scandens*) were then directly compared against a common reference sample from *G. difficilis* (Fig. 3). Microarray analysis revealed *calmodulin* (*CaM*) to be one of the most highly expressed genes in the cactus finches (compared with *G. difficilis* and the ground finches). Subsequently, a constitutively active form of a downstream effector of CaM, CaM kinase kinase (*CaMKII*), was over-expressed in the frontonasal mesenchyme of chicken embryos with an RCAS retrovirus. Infected embryos showed about a 10% increase in beak length relative to controls. Of particular interest is the finding that two different factors, *Bmp4* and *CaMKII*, over-expressed in similar domains (frontonasal process mesenchyme) during a similar period of time (HH21-24), caused independent changes in growth along different axes of the developing beak (width/depth and length, respectively).

Mouse-Chick Chimeras

The combination of mouse and chick models in neural crest-transplantation experiments has generated interesting information regarding the role of neural-crest-derived mesenchyme in tooth development. The mouse-chick chimeric system was originally designed for the study of neurogenesis (Fontaine-Perus *et al.*, 1997), but many soon realized that this system would serve as a useful model to study the development of teeth (Lwigale and Schneider, 2008). Birds are believed to have lost their teeth about 80 million years ago; however, several genes involved in tooth development continue to be expressed in the

mandibular and maxillary primordia (Francis-West *et al.*, 1998; Schneider *et al.*, 1999). For example, the homeobox gene *Pitx2*, which is the earliest known marker of presumptive dental epithelium (Mucchielli *et al.*, 1997), and the secreted factor *Fgf8* are expressed in equivalent regions of chick epithelium at HH21 (approximately mouse E10.5) (Mitsiadis *et al.*, 2003, 2006), whereas *Bmp4* and *Shh* are not expressed in these domains.

Tooth development is comprised of a complex cascade of reciprocal inductive and permissive interactions between epithelium and neural-crest-derived mesenchyme. Many of the signaling pathways regulating inductive tissue interactions have been elucidated by a range of approaches, including transgenic mice (Thesleff and Sharpe, 1997; Peters and Balling, 1999). From *in vitro* tissue recombination experiments, oral epithelium has been shown to provide the instructive signal that initiates tooth development (Mina and Kollar, 1987; Lumsden, 1988). Presumptive dental epithelium from embryonic days 9 to 11 (E9-E11) mice could initiate tooth formation in non-dental neural-crest-derived mesenchyme. Subsequently, reciprocal interaction of E12 neural crest mesenchyme instructs epithelium to form tooth-like structures. Thus, dental epithelium is generally considered to be the initiator of tooth formation, even though heterospecific *in vitro* tissue recombination experiments suggest mesenchyme to be the initiating tissue (Lemus, 1995).

By grafting E8 mouse neural crest cells into stage-equivalent chick, Mitsiadis *et al.* (2003, 2006) demonstrated the successful incorporation of mouse neural crest cells into beak mesenchyme. More importantly, mouse-chick chimeras showed ingrowths of oral epithelium with tooth bud and cap configurations. There was no evidence of epithelium-derived enamel. Furthermore, mouse neural-crest-derived mesenchyme induced and/or maintained oral epithelial expression of *Pitx2*, *Fgf8*, *Bmp4*, and *Shh* (Mitsiadis *et al.*, 2003, 2006). Grafted neural crest cells from the E8 mouse populate the upper beak (maxillary) and lower beak (mandibular) primordia, and interact with oral epithelium one day after neural crest transplantation. E9-E11 presumptive dental epithelium, which, with instructing mesenchyme, has been attributed to initiate tooth development in tissue recombination experiments (Mina and Kollar, 1987; Lumsden, 1988), appears to be pre-patterned by neural-crest-derived mesenchyme. Thus, mouse-chick chimeras indicate that neural-crest-derived mesenchyme possesses odontogenic potential to initiate tooth formation.

EVOLUTION OF CRANIOFACIAL PATTERNING

Craniofacial bones have been highly modified during evolution and, as a result, represent some of the most diversified and highly adapted anatomical structures in our bodies. Historically, much attention has been given to the role of external factors such as the environmental niche, competition for resources, or mating behavior in directing the course of craniofacial evolution, but recent studies are beginning to provide a deeper understanding of developmental mechanisms that may have facilitated the rapid diversification of the craniofacial complex (Schneider, 2005). Such developmental mechanisms are rooted deeply in the origins, abilities, and roles of the cranial neural crest cells that ultimately produce the facial and jaw skeletons. The unique properties of neural crest cells spawned the “New Head” hypothesis, to account for the evolution of vertebrates from

Figure 3. Microarray analysis of Darwin's finches. **(A)** Five species of Galápagos finches display variation in beak size and shape. **(B)** Tissues destined to form the upper beak were harvested from finch embryos to produce a microarray chip and fluorescently labeled targets. Each species was compared with the reference species. Medium grey spots signify higher levels of expression in the reference sample, dark grey spots denote higher levels in the other species, and light grey spots indicate comparable levels in both species. One of the highest signals was for the gene *calmodulin* (CaM). **(C)** To reproduce the higher levels of CaM signaling observed in the cactus finch, the frontonasal primordia of chicken embryos were infected with a retrovirus (RCAS). The retrovirus mis-expressed a downstream effector of CaM, termed CaM kinase kinase (CaMKII), which caused chicken embryos to develop longer upper beaks. Modified from Schneider (2007) and based on Abzhanov *et al.* (2006). A color version of this Fig. is available online.

passive filter feeders to active predators (Gans and Northcutt, 1983; Graham *et al.*, 2004; Northcutt, 2005).

At least three features in the developmental programs of craniofacial mineralized tissues potentially predispose them to rapid evolutionary transformations (Schneider, 2005). First, to reiterate, neural-crest-derived mesenchyme acts as the dominant source of species-specific patterning information. Thus, mesenchyme serves as a conductive target of natural selection and a driving force for generating phenotypic variation. Observations from quail-duck chimeras, the expression patterns in Darwin's finches, over-expression of *Bmp4* and *CaMKII* in chick facial mesenchyme, and mouse-chick chimeras suggest that genetic changes to mesenchyme alone during development may be sufficient to provide the material basis for evolution. Second, the program for osteogenesis operates as a module of tightly integrated molecular and histogenic events that are choreographed by the mesenchyme. This includes the series of epithelial-mesenchymal interactions underlying intramembranous ossification and the regulation of molecular pathways known to be essential to skeletal patterning and growth. By enabling developmental programs to be enacted in a variety of new contexts, modularity may be an essential element for enhancing the "evolvability" of anatomical structures. Third, developmental programs possess plasticity, a measure of the extent to which ontogenetic systems can respond to internal and external perturbations and produce an integrated and sustainable phenotype. As highlighted in quail-duck and mouse-chick chimeras, host tissues readily respond to donor neural crest mesenchyme and incorporate morphogenetic modifications (*i.e.*, molecular and histogenic events) introduced by internal stimuli. Concomitantly, neural crest mesenchyme enacts its spatiotemporal programs, which results in the creation of a donor phenotype in the host. While natural selection can enable a particular phenotype to become seamlessly fitted to its environment, the means to attain this condition is adaptability, and adaptability occurs as a by-product of the amount of spatiotemporal plasticity built into a given developmental system.

Changes in coding and non-coding DNA sequences, especially mutations in *cis*-regulatory elements, are the principal enabling events for natural selection and consequent morphological evolution (Stern, 2000; Wray, 2003; Carroll, 2005). *Cis*-regulatory mutations predominantly modify the timing, levels, and/or spatial domains of gene expression, which, in turn, affects morphology. For example, replacement of a mouse limb-specific enhancer of *Ptx1* with the orthologous bat sequence results in elevated levels of *Ptx1* expression in the developing mouse forelimb, and leads to longer forelimbs (Cretekos *et al.*, 2008). In addition, quail-duck and mouse-chick chimera experiments—which are set apart from classic frog and salamander transplant studies because they incorporate molecular biology and analyses of underlying genetic cascades—demonstrate that much of the driving force for evolutionary change is coming from the mesenchyme *via* the regulation of its own gene expression as well as that in surrounding tissues (Mitsiadis *et al.*, 2003, 2006; Schneider and Helms, 2003; Eames and Schneider, 2005, 2008; Merrill *et al.*, 2008). In contrast, mutations within coding sequences of genes can also contribute to morphological modifications. For example, there is a strong correlation among the length of Runx2 glutamine-alanine (QA) tandem-repeats, *Runx2*

transcriptional activity, and facial length in domestic dogs (Sears *et al.*, 2007). Mutations in coding sequence often lead to changes in DNA or protein interactions that affect the subsequent transcriptional or signaling properties of the gene product. However, if the gene product is expressed in multiple tissues, the coding sequence mutation is pleiotropic, and the gene locus contains multiple *cis*-regulatory elements (3 characteristics shared by most genes), then evolution is more likely to occur through changes in *cis*-regulatory sequences (Stern, 2000; Wray, 2003; Carroll, 2005; Cretekos *et al.*, 2008). A challenge in future studies is to elucidate the precise molecular mechanisms that drive genetic changes governing facial morphology and, ultimately, the evolution of species.

CONCLUSION

Avian systems have been instrumental in deciphering the roles of epithelium and mesenchyme in the development and evolution of the craniofacial skeleton. Although epithelium and mesenchyme can each take on the function of inducer with respect to craniofacial patterning (Richman and Lee, 2003), numerous experiments point to neural-crest-derived mesenchyme as the dominant instructive tissue in the initiation of craniofacial bone and tooth formation. Epithelium is intimately involved and necessary for craniofacial patterning, but tends to play a more permissive role, heeding instructions from mesenchyme.

Clearly, much work is still needed to achieve a comprehensive understanding of how craniofacial bones and teeth develop and evolve. Many questions remain. For example, what factors regulate BMP4 and CaM/CaMKII signaling during beak morphogenesis? What are the neural-crest-derived molecular and cellular mechanisms involved in pre-patterning oral epithelium before mouse E9? Considering that CaM is a calcium-interacting/sensing protein, does the amount of dietary calcium intake play a role in its regulation and subsequent effect on beak length? Last, if beak morphology can be altered and tooth formation can be initiated in birds, could this information be applied in humans to reconstruct and regenerate alveolar bones and ridges, or "awaken" tooth formation in edentulous individuals? What remains clear is that an intimate and equal partnership of basic research in avian and other model systems, along with applicable translational and clinical approaches, is currently the best method to improve therapies for the treatment of conditions that afflict the face and oral cavity.

ACKNOWLEDGMENTS

A.H.J. would like to dedicate this publication to Dr. Jaroslav Sodek, who died suddenly in August, 2007. Many in the science community, particularly the young investigators who greatly benefited and those who would have benefited from his mentorship, will miss Jaro. We lost not only a great scientist but also a great person and friend.

This work was supported by a fellowship from the Canadian Institutes of Health Research (CIHR) to A.H.J.; and by R01 DE016402-01 from the NIDCR, R21 AR052513-01 from the NIAMS, and Research Grant 5-FY04-26 from the March of Dimes Birth Defects Foundation to R.A.S.

REFERENCES

- Abzhanov A, Tabin CJ (2004). Shh and Fgf8 act synergistically to drive cartilage outgrowth during cranial development. *Dev Biol* 273:134-148.
- Abzhanov A, Tzahor E, Lassar AB, Tabin CJ (2003). Dissimilar regulation of cell differentiation in mesencephalic (cranial) and sacral (trunk) neural crest cells in vitro. *Development* 130:4567-4579.
- Abzhanov A, Protas M, Grant BR, Grant PR, Tabin CJ (2004). Bmp4 and morphological variation of beaks in Darwin's finches. *Science* 305:1462-1465.
- Abzhanov A, Kuo WP, Hartmann C, Grant BR, Grant PR, Tabin CJ (2006). The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. *Nature* 442:563-567.
- Abzhanov A, Rodda SJ, McMahon AP, Tabin CJ (2007). Regulation of skeletogenic differentiation in cranial dermal bone. *Development* 134:3133-3144.
- Andres G (1949). Untersuchungen an Chimären von Triton und Bombinator. *Genetica* 24:387-534.
- Ashique AM, Fu K, Richman JM (2002a). Signalling via type IA and type IB bone morphogenetic protein receptors (BMPR) regulates intramembranous bone formation, chondrogenesis and feather formation in the chicken embryo. *Int J Dev Biol* 46:243-253.
- Ashique AM, Fu K, Richman JM (2002b). Endogenous bone morphogenetic proteins regulate outgrowth and epithelial survival during avian lip fusion. *Development* 129:4647-4660.
- Baker CV, Bronner-Fraser M, Le Douarin NM, Teillet MA (1997). Early- and late-migrating cranial neural crest cell populations have equivalent developmental potential in vivo. *Development* 124:3077-3087.
- Baker CV, Stark MR, Marcelle C, Bronner-Fraser M (1999). Competence, specification and induction of Pax-3 in the trigeminal placode. *Development* 126:147-156.
- Borue X, Noden DM (2004). Normal and aberrant craniofacial myogenesis by grafted trunk somitic and segmental plate mesoderm. *Development* 131:3967-3980.
- Bowman RI (1961). Morphological differentiation and adaptation in the Galapagos finches. *Univ Calif Publ Zool* 58:1-302.
- Burt DW, Bruley C, Dunn IC, Jones CT, Ramage A, Law AS, et al. (1999). The dynamics of chromosome evolution in birds and mammals. *Nature* 402:411-413.
- Carroll SB (2005). Evolution at two levels: on genes and form. *PLoS Biol* 3(7):e245.
- Chai Y, Jiang X, Ito Y, Bringas P Jr, Han J, Rowitch DH, et al. (2000). Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. *Development* 127:1671-1679.
- Chung UI, Kawaguchi H, Takato T, Nakamura K (2004). Distinct osteogenic mechanisms of bones of distinct origins. *J Orthop Sci* 9:410-414.
- Clarke JD, Tickle C (1999). Fate maps old and new. *Nat Cell Biol* 1:E103-E109.
- Cobos I, Shimamura K, Rubenstein JL, Martinez S, Puelles L (2001). Fate map of the avian anterior forebrain at the four-somite stage, based on the analysis of quail-chick chimeras. *Dev Biol* 239:46-67.
- Couly G, Le Douarin NM (1990). Head morphogenesis in embryonic avian chimeras: evidence for a segmental pattern in the ectoderm corresponding to the neuromeres. *Development* 108:543-558.
- Couly GF, Coltey PM, Le Douarin NM (1992). The developmental fate of the cephalic mesoderm in quail-chick chimeras. *Development* 114:1-15.
- Couly GF, Coltey PM, Le Douarin NM (1993). The triple origin of skull in higher vertebrates: a study in quail-chick chimeras. *Development* 117:409-429.
- Couly G, Creuzet S, Bennaceur S, Vincent C, Le Douarin NM (2002). Interactions between Hox-negative cephalic neural crest cells and the foregut endoderm in patterning the facial skeleton in the vertebrate head. *Development* 129:1061-1073.
- Crane JF, Trainor PA (2006). Neural crest stem and progenitor cells. *Annu Rev Cell Dev Biol* 22:267-286.
- Crespi R, Vinci R, Cappare P, Gherlone E, Romanos GE (2007). Calvarial versus iliac crest for autologous bone graft material for a sinus lift procedure: a histomorphometric study. *Int J Oral Maxillofac Implants* 22:527-532.
- Crettekos CJ, Wang Y, Green ED, Martin JF, Rasweiler JJ 4th, Behringer RR (2008). Regulatory divergence modifies limb length between mammals. *Genes Dev* 22:141-151.
- Dhouailly D (1967). [Analysis of the factors in the specific differentiation of the neoptile feathers in the duck and chicken]. *J Embryol Exp Morphol* 18:389-400.
- Dhouailly D (1970). [Specific feather potentialities of the embryonic tegument of the chicken and duck]. *Annee Biol* 9:587.
- Dixon J, Jones NC, Sandell LL, Jayasinghe SM, Crane J, Rey JP, et al. (2006). Tcof1/Treacle is required for neural crest cell formation and proliferation deficiencies that cause craniofacial abnormalities. *Proc Natl Acad Sci USA* 103:13403-13408.
- Eames BF, Schneider RA (2005). Quail-duck chimeras reveal spatiotemporal plasticity in molecular and histogenic programs of cranial feather development. *Development* 132:1499-1509.
- Eames BF, Schneider RA (2008). The genesis of cartilage size and shape during development and evolution. *Development* 135, 3947-3958.
- Espina V, Heiby M, Pierobon M, Liotta LA (2007). Laser capture microdissection technology. *Expert Rev Mol Diagn* 7:647-657.
- Fontaine-Perus J, Halgand P, Cheraud Y, Rouaud T, Velasco ME, Cifuentes Diaz C, et al. (1997). Mouse-chick chimera: a developmental model of murine neurogenic cells. *Development* 124:3025-3036.
- Foppiano S, Hu D, Marcucio RS (2007). Signaling by bone morphogenetic proteins directs formation of an ectodermal signaling center that regulates craniofacial development. *Dev Biol* 312:103-114.
- Francis-West PH, Tatla T, Brickell PM (1994). Expression patterns of the bone morphogenetic protein genes Bmp-4 and Bmp-2 in the developing chick face suggest a role in outgrowth of the primordia. *Dev Dyn* 201:168-178.
- Francis-West P, Ladher R, Barlow A, Graveson A (1998). Signalling interactions during facial development. *Mech Dev* 75:3-28.
- Gans C, Northcutt RG (1983). Neural crest and the origin of vertebrates: a new head. *Science* 220:268-274.
- Garg V, Yamagishi C, Hu T, Kathiriyi IS, Yamagishi H, Srivastava D (2001). Tbx1, a DiGeorge syndrome candidate gene, is regulated by sonic hedgehog during pharyngeal arch development. *Dev Biol* 235:62-73.
- Gorlin RJ, Cohen MMJ, Levin LS (1990). Syndromes of the head and neck. Oxford: Oxford University Press.
- Graham A, Begbie J, McGonnell I (2004). Significance of the cranial neural crest. *Dev Dyn* 229:5-13.
- Hall BK (2005). Bones and cartilage: developmental and evolutionary skeletal biology. London: Elsevier Academic Press.
- Hamburger V, Hamilton HL (1951). A series of normal stages in the development of the chick embryo. *J. Morphol.* 88:49-92.
- Hampe A (1957). [Role of mesoderm and ectoderm of leg bud in exchanges between duck and chicken.]. *C R Hebd Seances Acad Sci* 244:3179-3181.
- Helms JA, Schneider RA (2003). Cranial skeletal biology. *Nature* 423:326-331.
- Helms JA, Cordero D, Tapadia MD (2005). New insights into craniofacial morphogenesis. *Development* 132:851-861.
- Hu D, Helms JA (1999). The role of sonic hedgehog in normal and abnormal craniofacial morphogenesis. *Development* 126:4873-4884.
- Hu D, Marcucio RS, Helms JA (2003). A zone of frontonasal ectoderm regulates patterning and growth in the face. *Development* 130:1749-1758.
- Hughes SH (2004). The RCAS vector system. *Folia Biol (Praha)* 50:107-119.
- International Chicken Genome Sequencing Consortium (2004). Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* 432:695-716; erratum in *Nature* 433:777, 2005.
- Itasaki N, Bel-Vialar S, Krumlauf R (1999). 'Shocking' developments in chick embryology: electroporation and in ovo gene expression. *Nat Cell Biol* 1:E203-E207.
- Jones NC, Lynn ML, Gaudenz K, Sakai D, Aoto K, Rey JP, et al. (2008). Prevention of the neurocristopathy Treacher Collins syndrome through inhibition of p53 function. *Nat Med* 14:125-133.
- Kember OD (1971). Aristotle and the chick embryo. *The Classical Quarterly* 21:393-396.
- Kontges G, Lumsden A (1996). Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. *Development* 122:3229-3242.
- Kos R, Tucker RP, Hall R, Duong TD, Erickson CA (2003). Methods for introducing morpholinos into the chicken embryo. *Dev Dyn* 226:470-477.

- Krull CE (2004). A primer on using in ovo electroporation to analyze gene function. *Dev Dyn* 229:433-439.
- Le Douarin N (1973). A biological cell labeling technique and its use in experimental embryology. *Dev Biol* 30:217-222.
- Le Douarin NM (2004). The avian embryo as a model to study the development of the neural crest: a long and still ongoing story. *Mech Dev* 121:1089-1102.
- Le Douarin NM, Creuzet S, Couly GF, Dupin E (2004). Neural crest cell plasticity and its limits. *Development* 131:4637-4650.
- Le Lievre CS, Le Douarin NM (1975). Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. *J Embryol Exp Morphol* 34:125-154.
- Lee H, Al Shamy G, Elkabetz Y, Schofield CM, Harrision NL, Panagiotakos G, et al. (2007). Directed differentiation and transplantation of human embryonic stem cell-derived motoneurons. *Stem Cells* 25:1931-1939.
- Lemus D (1995). Contributions of heterospecific tissue recombinations to odontogenesis. *Int J Dev Biol* 39:291-297.
- Lumsden AG (1988). Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development* 103(Suppl):155-169.
- Lwigale PY (2001). Embryonic origin of avian corneal sensory nerves. *Dev Biol* 239:323-337.
- Lwigale PY, Schneider RA (2008). Other chimeras: quail-duck and mouse-chick. In: *Methods in avian embryology*. Vol. 87. 2nd ed. Bronner-Fraser M, editor. San Diego: Academic Press, pp. 59-74.
- Marcucio RS, Cordero DR, Hu D, Helms JA (2005). Molecular interactions coordinating the development of the forebrain and face. *Dev Biol* 284:48-61.
- McGonnell IM, Graham A (2002). Trunk neural crest has skeletogenic potential. *Curr Biol* 12:767-771.
- McGrew MJ, Sherman A, Ellard FM, Lillico SG, Gilhooley HJ, Kingsman AJ, et al. (2004). Efficient production of germline transgenic chickens using lentiviral vectors. *EMBO Rep* 5:728-733.
- Merrill AE, Eames BF, Weston SJ, Heath T, Schneider RA (2008). Mesenchyme-dependent BMP signaling directs the timing of mandibular osteogenesis. *Development* 135:1223-1234.
- Mina M, Kollar EJ (1987). The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. *Arch Oral Biol* 32:123-127.
- Mitsiadis TA, Cheraud Y, Sharpe P, Fontaine-Perus J (2003). Development of teeth in chick embryos after mouse neural crest transplantations. *Proc Natl Acad Sci USA* 100:6541-6545.
- Mitsiadis TA, Caton J, Cobourne M (2006). Waking-up the sleeping beauty: recovery of the ancestral bird odontogenic program. *J Exp Zool B Mol Dev Evol* 306:227-233.
- Motohashi T, Aoki H, Chiba K, Yoshimura N, Kunisada T (2007). Multipotent cell fate of neural crest-like cells derived from embryonic stem cells. *Stem Cells* 25:402-410.
- Mucchielli ML, Mitsiadis TA, Raffo S, Brunet JF, Proust JP, Goridis C (1997). Mouse Otx2/RIEG expression in the odontogenic epithelium precedes tooth initiation and requires mesenchyme-derived signals for its maintenance. *Dev Biol* 189:275-284.
- Nakamura H, Katahira T, Sato T, Watanabe Y, Funahashi J (2004). Gain- and loss-of-function in chick embryos by electroporation. *Mech Dev* 121:1137-1143.
- Noden DM (1978). The control of avian cephalic neural crest cytodifferentiation. I. Skeletal and connective tissues. *Dev Biol* 67:296-312.
- Noden DM (1983). The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. *Dev Biol* 96:144-165.
- Noden DM (1986). Patterning of avian craniofacial muscles. *Dev Biol* 116:347-356.
- Noden DM, Schneider RA (2006). Neural crest cells and the community of plan for craniofacial development: historical debates and current perspectives. In: *Neural crest induction and differentiation*. Saint-Jeannet J, editor. Georgetown, TX: Landes Bioscience, pp. 1-23.
- Noden DM, Trainor PA (2005). Relations and interactions between cranial mesoderm and neural crest populations. *J Anat* 207:575-601.
- Northcutt RG (2005). The new head hypothesis revisited. *J Exp Zool B: Mol Dev Evol* 304:274-297.
- O'Rahilly R, Muller F (2007). The development of the neural crest in the human. *J Anat* 211:335-351.
- Olivera-Martinez I, Coltey M, Dhoubailly D, Pourquie O (2000). Mediolateral somitic origin of ribs and dermis determined by quail-chick chimeras. *Development* 127:4611-4617.
- Paulsson M (1992). Basement membrane proteins: structure, assembly, and cellular interactions. *Crit Rev Biochem Mol Biol* 27:93-127.
- Pautou MP (1968). [Determining role of the mesoderm in the specific differentiation of the leg in birds]. *Arch Anat Microsc Morphol Exp* 57:311-328.
- Pennisi E (2004). Bonemaking protein shapes beaks of Darwin's finches. *Science* 305:1383.
- Peters H, Balling R (1999). Teeth. Where and how to make them. *Trends Genet* 15:59-65.
- Rao M, Baraban JH, Rajaii F, Sockanathan S (2004). In vivo comparative study of RNAi methodologies by in ovo electroporation in the chick embryo. *Dev Dyn* 231:592-600.
- Richman JM, Lee SH (2003). About face: signals and genes controlling jaw patterning and identity in vertebrates. *Bioessays* 25:554-568.
- Richman JM, Tickle C (1989). Epithelia are interchangeable between facial primordia of chick embryos and morphogenesis is controlled by the mesenchyme. *Dev Biol* 136:201-210.
- Sang H (2006). Transgenesis sunny-side up. *Nat Biotechnol* 24:955-956.
- Schienda J, Engleka KA, Jun S, Hansen MS, Epstein JA, Tabin CJ, et al. (2006). Somitic origin of limb muscle satellite and side population cells. *Proc Natl Acad Sci USA* 103:945-950.
- Schneider RA (2005). Developmental mechanisms facilitating the evolution of bills and quills. *J Anat* 207:563-573.
- Schneider RA (2007). How to tweak a beak: molecular techniques for studying the evolution of size and shape in Darwin's finches and other birds. *Bioessays* 29:1-6.
- Schneider RA, Helms JA (2003). The cellular and molecular origins of beak morphology. *Science* 299:565-568.
- Schneider RA, Hu D, Helms JA (1999). From head to toe: conservation of molecular signals regulating limb and craniofacial morphogenesis. *Cell Tissue Res* 296:103-109.
- Schneider RA, Hu D, Rubenstein JL, Maden M, Helms JA (2001). Local retinoid signaling coordinates forebrain and facial morphogenesis by maintaining FGF8 and SHH. *Development* 128:2755-2767.
- Sears KE, Goswami A, Flynn JJ, Niswander LA (2007). The correlated evolution of Runx2 tandem repeats, transcriptional activity, and facial length in carnivorans. *Evol Dev* 9:555-565.
- Selleck MA, Bronner-Fraser M (1995). Origins of the avian neural crest: the role of neural plate-epidermal interactions. *Development* 121:525-538.
- Shigetani Y, Nobusada Y, Kuratani S (2000). Ectodermally derived FGF8 defines the maxillomandibular region in the early chick embryo: epithelial-mesenchymal interactions in the specification of the craniofacial ectomesenchyme. *Dev Biol* 228:73-85.
- Sohal GS (1976). Effects of reciprocal forebrain transplantation on motility and hatching in chick and duck embryos. *Brain Res* 113:35-43.
- Sohal GS, Knox TS, Allen JC Jr, Arumugam T, Campbell LR, Yamashita T (1985). Development of the trochlear nucleus in quail and comparative study of the trochlear nucleus, nerve, and innervation of the superior oblique muscle in quail, chick, and duck. *J Comp Neurol* 239:227-236.
- Sohal GS, Bal HS, Campbell LR, Husain I, Arumugam T, Kumaresan K (1990). Synapse formation on quail trochlear neurons transplanted in duck embryos before naturally occurring motor neuron death. *Int J Dev Neurosci* 8:9-16.
- Starck JM, Ricklefs RE, editors (1998). *Avian growth and development: evolution within the altricial-precocial spectrum*. New York, NY: Oxford University Press.
- Stern DL (2000). Evolutionary developmental biology and the problem of variation. *Evolution* 54:1079-1091.
- Thesleff I, Sharpe P (1997). Signalling networks regulating dental development. *Mech Dev* 67:111-123.
- Tucker AS, Lumsden A (2004). Neural crest cells provide species-specific patterning information in the developing branchial skeleton. *Evol Dev* 6:32-40.
- van de Lavoie MC, Diamond JH, Leighton PA, Mather-Love C, Heyer BS, Bradshaw R, et al. (2006a). Germline transmission of genetically modified primordial germ cells. *Nature* 441:766-769.

- van de Lavoie MC, Mather-Love C, Leighton P, Diamond JH, Heyer BS, Roberts R, *et al.* (2006b). High-grade transgenic somatic chimeras from chicken embryonic stem cells. *Mech Dev* 123:31-41.
- Vitelli F, Morishima M, Taddei I, Lindsay EA, Baldini A (2002). Tbx1 mutation causes multiple cardiovascular defects and disrupts neural crest and cranial nerve migratory pathways. *Hum Mol Genet* 11:915-922.
- Waddington CH (1930). Developmental mechanics of chick and duck embryos. *Nature* 125:924-925.
- Waddington CH (1932). Experiments on the development of chick and duck embryos cultivated in vitro. *Philos Trans R Soc Lond B Biol Sci* 221: 179-230.
- Wagner G (1959). Untersuchungen an Bombinator-Triton-Chimaeren. *Roux' Archiv für Entwicklungsmechanik der Organismen* 151:136-158 [article in German].
- Wang Y, Wan C, Deng L, Liu X, Cao X, Gilbert SR, *et al.* (2007). The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. *J Clin Invest* 117: 1616-1626.
- Wray GA (2003). Transcriptional regulation and the evolution of development. *Int J Dev Biol* 47:675-684.
- Wu P, Jiang TX, Suksaweang S, Widelitz RB, Chuong CM (2004). Molecular shaping of the beak. *Science* 305:1465-1466.
- Wu P, Jiang TX, Shen JY, Widelitz RB, Chuong CM (2006). Morphoregulation of avian beaks: comparative mapping of growth zone activities and morphological evolution. *Dev Dyn* 235:1400-1412.
- Yamashita T, Sohal GS (1986). Development of smooth and skeletal muscle cells in the iris of the domestic duck, chick and quail. *Cell Tissue Res* 244:121-131.
- Yamashita T, Sohal GS (1987). Embryonic origin of skeletal muscle cells in the iris of the duck and quail. *Cell Tissue Res* 249:31-37.
- Zwilling E (1959). Interaction between ectoderm and mesoderm in duck-chicken limb bud chimaeras. *J Exp Zool* 142:521-532.