New insights into craniofacial morphogenesis

Jill A. Helms[1,*], Dwight Cordero[2] and Minal D. Tapadia[1]

[1]Department of Plastic and Reconstructive Surgery, Stanford University, Stanford, CA 94305, USA
[2]Department of Obstetrics and Gynecology, Montefiore Medical Center/Albert Einstein, College of Medicine, Bronx, NY, USA
*Author for correspondence (e-mail: jhelms@stanford.edu)

Development 132, 851-861
Published by The Company of Biologists 2005
doi:10.1242/dev.01705

Summary

No region of our anatomy more powerfully conveys our emotions nor elicits more profound reactions when disease or genetic disorders disfigure it than the face. Recent progress has been made towards defining the tissue interactions and molecular mechanisms that control craniofacial morphogenesis. Some insights have come from genetic manipulations and others from tissue recombinations and biochemical approaches, which have revealed the molecular underpinnings of facial morphogenesis. Changes in craniofacial architecture also lie at the heart of evolutionary adaptation, as new studies in fish and fowl attest. Together, these findings reveal much about molecular and tissue interactions behind craniofacial development.

Introduction

For all intents and purposes, craniofacial development is initiated as soon as the anteroposterior axis of an embryo is established. The ability to specify a head structure, rather than reiterate another body segment, was a crucial step in vertebrate evolution that corresponded to the acquisition of two cell populations: the neural crest and the ectodermal placodes (reviewed by Basch et al., 2004; McCabe et al., 2004). In recent years, new data have begun to reveal how the neural crest cell population is actually generated, what types of controls are in place to modify neural crest cell migration and, ultimately, the role that this cell population plays in establishing the pattern of the craniofacial skeleton.

Although the neural crest receives a significant amount of attention, it is not the only craniofacial tissue with patterning information. New studies have further clarified the contribution of epithelia as a source of patterning information for the face. Regardless of whether epithelia are ectodermal in origin [covering the facial prominences (Hu et al., 2003)], or are neural ectoderm (Cordero et al., 2004; Creuzet et al., 2004; Walshe and Mason, 2003), or are of endodermal origin and line the pharynx (Ruhin et al., 2003), these tissues can no longer be viewed as being bystanders in the process of craniofacial morphogenesis. In a growing number of cases, epithelial tissues are actually the instigator of morphological change. Our review focuses on innovative studies that have addressed these issues, sometimes with new and unexpected results. Several other reviews are also available that provide excellent summaries of related work (Francis-West et al., 2003; Le Douarin et al., 2004; Manzanares and Nieto, 2003).

In the beginning

Although the postnatal vertebrate head exhibits an exceedingly intricate and varied morphology, the craniofacial complex initially has a much more simple geometry, consisting of a series of swellings or prominences that undergo growth, fusion and expansion (Fig. 1). There are seven prominences that comprise the vertebrate face: the midline frontonasal prominence and three paired structures derived from the first pharyngeal (branchial) arch (Fig. 1). The frontonasal prominence contributes to the forehead, the middle of the nose, the philtrum of the upper lip and the primary palate, while the lateral nasal prominence forms the sides (ala) of the nose (Larson, 2001) (Fig. 1). Until recently, it was thought that the ventral region of the first pharyngeal (branchial) arch gave rise to the mandibular prominence and therefore the lower jaw, and that the dorsal region of the first arch gave rise to the maxillary prominences, which form the sides of the middle and lower face, the lateral borders of the lips, and the secondary palate (Fig. 1). Two new studies, carried out in avians and axolotls, contest this view and demonstrate that at least part of this fate-map is incorrect. Using DiI labeling to track the fates of cells, both groups show that the ventral region of the first arch actually gives rise to both maxillary and mandibular skeletal elements, rather than to only the mandibular elements, as previously thought (Cerny et al., 2004; Lee et al., 2004) (Fig. 1). Which cell populations in the first arch actually contribute to a particular skeletal element, however, is still not known. These new studies also indicate the need for much more detailed fate maps of these latter stages of craniofacial development; remarkably, this information is only now coming to light after decades of study.

When considering the origami-like process of tissue folding, flexure and growth that ultimately results in a face, one must also bear in mind that the cells comprising the face have undergone a massive relocation, owing to both active neural crest cell migration and the passive displacement of tissue that is associated with neurulation and head flexure (Figs 2, 3). Consequently, cells from different lineages end up forming composite tissues and, conversely, cells that were initially from a single developmental field can be found in very distant locations as a result of these migratory events (Fig. 1). As one might imagine, both types of cellular displacement profoundly impact facial morphogenesis, and therein lies at least one reason for the generation of meticulous fate maps of the craniofacial region (Couly and Le Douarin, 1988; Couly et al., 1996; Hall, 1980; Imai et al., 1996; Köntges and Lumsden, 1996; Rubenstein et al., 1998).

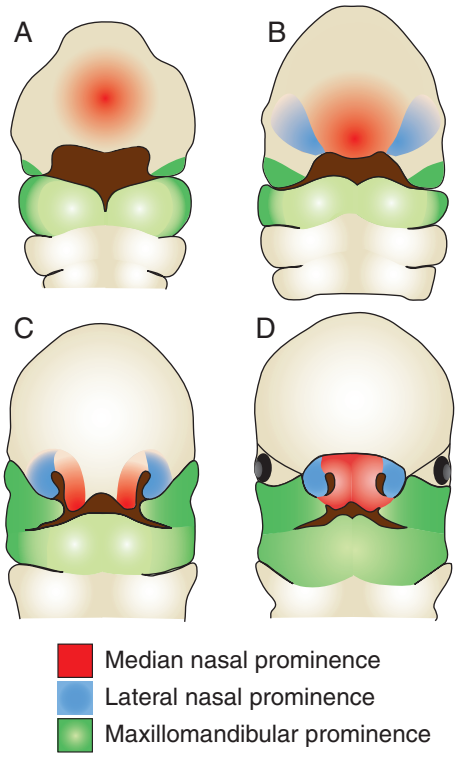

Fig. 1. Development of the craniofacial primordia. (A-D) A frontal view of the prominences that give rise to the main structures of the face. The frontonasal (or median nasal) prominence (red) contributes to the forehead (A), the middle of the nose (B), the philtrum of the upper lip (C) and the primary palate (D), while the lateral nasal prominence (blue) forms the sides of the nose (B,D). The maxillomandibular prominences (green) give rise to the lower jaw (specifically from the mandibular prominences), to the sides of the middle and lower face, to the lateral borders of the lips, and to the secondary palate (from the maxillary prominences).

Creating the neuroectoderm-surface ectoderm boundary

One of the first crucial steps in craniofacial development occurs when head ectoderm is subdivided into non-neural and neural regions, because this effectively establishes which head epithelium will lie outside of the cranial neural crest and which will lie inside it (Fig. 2B-D). A subset of epithelial cells located at this neural/non-neural boundary separate from the epithelium, adopt a mesenchymal character and come between these two epithelia as they start their migration (Fig. 2B-D). The epithelial-mesenchymal transition that marks the birth date of the neural crest has been shown to depend upon cells shifting from G1 to S phase and, at least for trunk neural crest cells, this shift is dependent upon bone morphogenetic protein (Bmp) signaling (Burstyn-Cohen et al., 2004). When Bmp signaling is inhibited by the overexpression of noggin, a Bmp antagonist, the G1/S transition is blocked and neural crest cells are no longer generated from the margins of the neural folds (Burstyn-Cohen et al., 2004). Bmp signaling achieves this effect in part by regulating *Wnt1* transcription (Burstyn-Cohen et al., 2004). In turn, Wnt signaling appears to be essential for the generation of neural crest cells as inhibition of its activity can block the production of neural crest cells (Garcia-Castro et al., 2002).

In addition to Bmp and Wnt proteins, several new molecules have also been implicated in the generation or early migration of neural crest cells. Sox transcription factors, which are well known for their roles in skeletogenic cell fate and sex determination, are also involved in generating neural crest cells (Cheung and Briscoe, 2003; Honore et al., 2003; Perez-Alcala et al., 2004). These studies indicate that the overexpression of Sox genes lengthens the developmental window during which cranial and trunk neural crest cells can be induced, and then promotes neural crest-like characteristics in those cells. Are these same molecular programs operating during the generation of cranial neural crest cells? And is this molecular model of neural crest induction species specific or can it be generalized to all vertebrates (Streit and Stern, 1999)? Addressing the latter question is particularly relevant to craniofacial biology because several craniofacial malformations, collectively referred to as neurocristopathies, can be attributed to defects in the generation, migration or survival of neural crest cells (reviewed by Bolande, 1997). If there is species-specific variability in the model of neural crest generation, this will have a profound impact on the interpretation of these neurocristopathic anomalies.

Neural crest contributions to craniofacial patterning

Which tissue controls facial patterning? The answer to this question continues to be debated, with strong data to support both sides of the controversy. In two recent studies, the contribution of the neural crest to facial patterning was assessed by swapping neural crest cells between ducks and quails. It was found that switching frontonasal neural crest cells between ducks and quails altered the countenances of the chimeras to such an extent that ducks with quail frontonasal neural crest cells had a quail-like beak, and quails carrying duck neural crest cells had a duck-like beak (Schneider and Helms, 2003). The molecular mechanisms underlying these facial transformations were hinted at when transplanted neural crest cells were found to maintain their temporal program of gene expression and to alter gene expression in the host epithelia (Schneider and Helms, 2003). Tucker and Lumsden reached a near-identical conclusion when they independently performed the same types of inter-species transplants (Tucker and Lumsden, 2004). They, too, found that the capacity to form species-specific skeletal elements in the head is an inherent property of the neural crest, and concluded that this characteristic is produced in response to signals from epithelia (Tucker and Lumsden, 2004). In fact, it appears as if the anteriormost neural crest cells acquire at least some patterning information from epithelia, as discussed in the next section. It should be emphasized that in both these studies, the extent to which facial features were transformed was directly proportional to the number of transplanted neural crest cells that made their way into the chimeric tissue. In other words, the transformation was a ‘population-dependent’ effect, as was reported in much earlier transplantation studies (Andres, 1949). So it seems that only when the contingency is large enough do neural crest cells follow molecular cues that are generated and maintained by the assemblage itself, disregarding signals emanating from the local environment. When the numbers of transplanted cells are below some crucial threshold, then they appear to respond to local cues from the surrounding epithelia. Just what these population-dependent

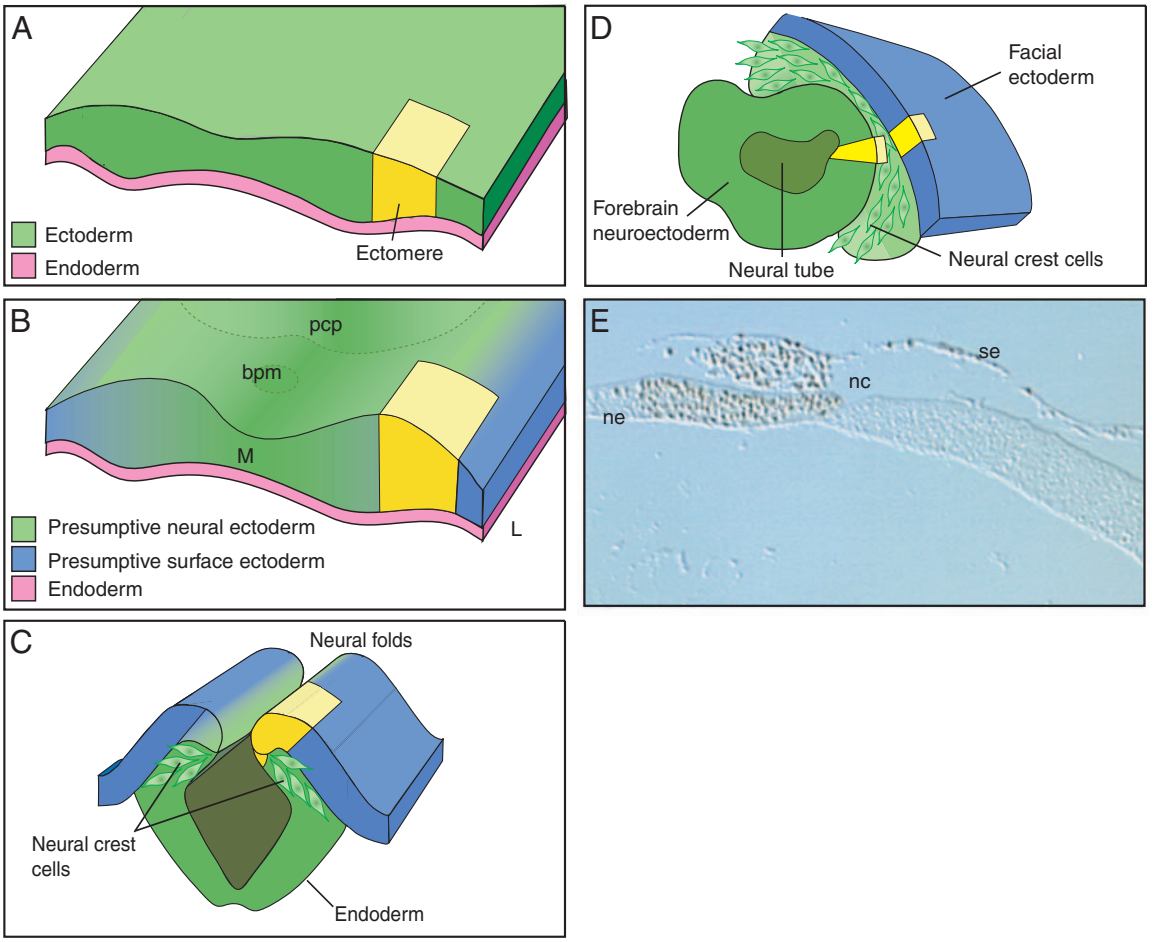

Fig. 2. Neurulation in the developing vertebrate embryo. (A) Neurulation begins with a unified layer of ectoderm, underneath which lies the endoderm. A single ectomere is shown in yellow. Ectomeres are discrete regions of superficial ectoderm that exhibit a segmented pattern of gene expression. Fate-mapping experiments suggest that, together with neural crest and neuroectoderm, they define a larger developmental unit (Couly and Le Douarin, 1999). Later, these tissues act on signaling centers in the facial prominences (Hu et al., 2003). (B) The ectoderm begins to fold upwards, giving rise to the neural folds. During this process, interactions between signaling molecules begin to delineate the medial ectoderm as being neural (green) and the lateral regions of ectoderm as being non-neural (blue). The prechordal plate mesendoderm (pcp) and the buccopharyngeal membrane (bpm) become evident at this stage. (C) The neural tube forms upon fusion of the neural folds, giving rise to discrete neuroectoderm (green) and surface ectoderm (blue). Around the same time, the border region between the neuroectoderm and surface ectoderm gives rise to neural crest cells. The surface ectoderm and neuroectoderm of single ectomeres remain aligned during this process. (D) Neurulation completes upon formation of the neural tube, and neural crest cells (nc) lie sandwiched between the facial (surface) ectoderm and the neuroectoderm. Again, the individual neuroectoderm and surface ectoderm components of the ectomere remain in register. (E) Sagittal section through neural tube of a stage 15 chick embryo, showing neural crest (nc) located between surface ectoderm (se) and neuroectoderm (ne). L, lateral; M, medial. (E) Unpublished data from J.A.H.'s laboratory.

cues are, and how many cells are required to maintain them, is unknown. What we do know, however, is that facial morphogenesis is the cumulative result of reciprocal signaling between and among all of these tissues, and that the issue of which tissue contains patterning information becomes a question of timing. We discuss these ideas in subsequent sections.

Epithelial contribution to craniofacial patterning

Oral ectoderm and tooth patterning

Perhaps no system better exemplifies the importance of reciprocal signaling between epithelia and neural crest mesenchyme in the control of craniofacial patterning than that of tooth development. The conflict over whether mesenchyme or ectoderm was responsible for tooth morphology arose because of two experimental results that, at first, appeared to be mutually exclusive (Cobourne and Sharpe, 2003). Recombinations of dental mesenchyme with non-dental ectoderm produce teeth, implicating the mesenchyme as the source of dental patterning information (Kollar and Baird, 1969). But recombinations of presumptive dental epithelium and naïve mesenchyme also result in teeth, indicating that the epithelium controls dental patterning. Which tissue contains the initial information for patterning (Lumsden, 1988; Mina and Kollar, 1991; Miller, 1969) teeth? As it turns out, it depends on when you look. If early (embryonic day, E10.5) chick oral ectoderm is used in the recombination experiments, then this tissue directs patterning. However, if the experiment is conducted with E11.0 mesenchyme (after patterning information has been transferred to the mesenchyme), then this

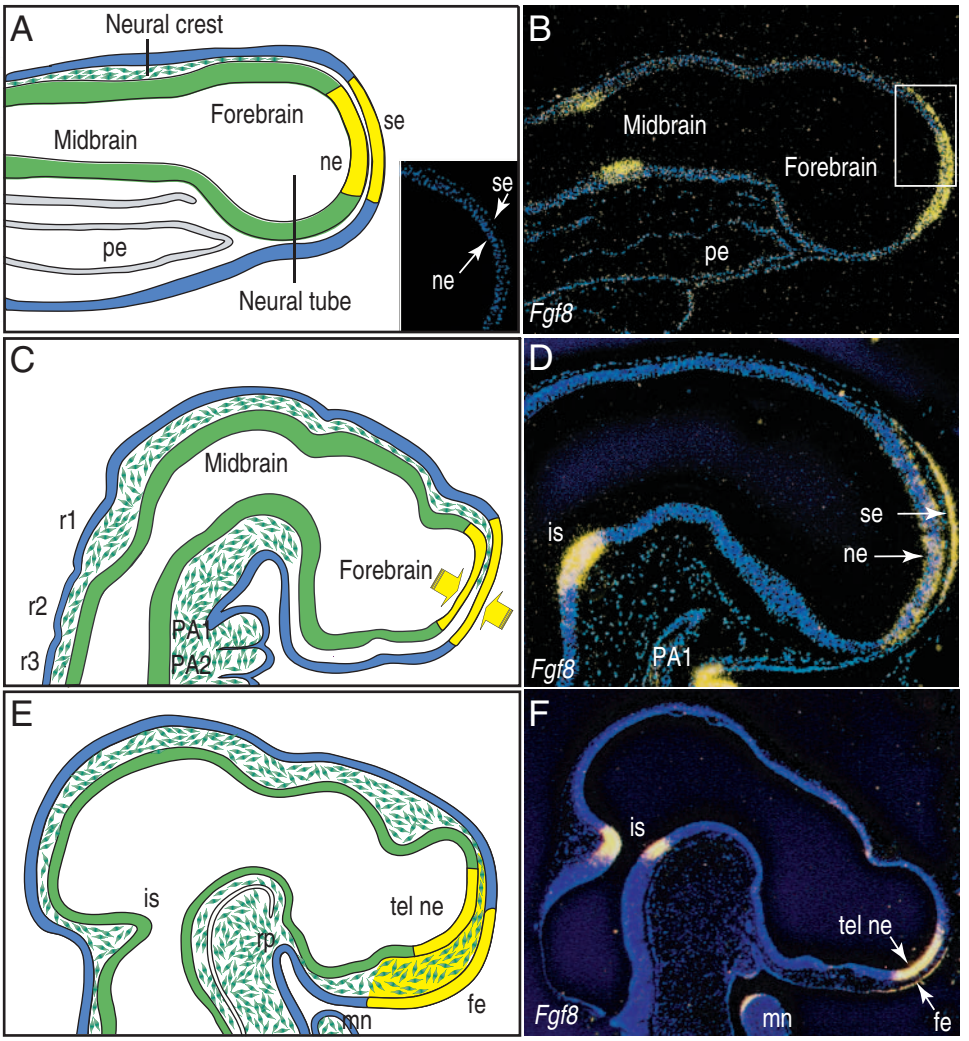

Fig. 3. Neural crest migration and ectomere alignment. (A,C,E) Schematics of a developing chick embryo illustrating neural crest migration during craniofacial development. (B,D,F) In situ hybridization showing *Fgf8* expression (yellow) during chick craniofacial development. (A-D) As the closed neural tube begins to differentiate into the central nervous system, the neural crest begins to migrate anteriorly from specific rhombomeres (r1-r3) into discrete regions of the face. During this process, the neuroectoderm (ne) and surface ectoderm (se) components of the ectomeres continue to remain aligned (yellow arrows in C). Inset in A shows higher magnification of the boxed area in B (the direct contact between the anterior neuroectoderm and presumptive facial ectoderm, prior to neural crest cell migration between those two epithelial layers). (E,F) As neural crest migration nears completion, the neuroectoderm and facial ectoderm (fe; late-stage term for surface ectoderm) components of the ectomere are no longer aligned. is, isthmus; mn, mandible; PA, pharyngeal arch; pe, pharyngeal endoderm; rp, Rathke's pouch; tel ne, telencephalic neuroectoderm. (B,D,F) Unpublished data from J.A.H.'s laboratory.

tissue controls patterning (reviewed by Miletich and Sharpe, 2003). This type of reciprocal signaling was demonstrated by transplanting cranial neural crest cells from a mouse (which develops teeth) into a bird (which does not), and resulted in the formation of tooth-like rudiments. Although the experiment was first performed over 30 years ago (Kollar and Fisher, 1980), investigators can now demonstrate that these teeth are composed of avian epithelium and murine mesenchyme (Mitsiadis et al., 2003). Therefore, despite the fact that birds have been edentulous (i.e. toothless) for nearly 100 million years, avian oral ectoderm has apparently retained its ability to induce tooth formation, provided the neural crest mesenchyme has retained its capacity to respond. The molecular mediators of this patterning information have also been identified. The general consensus in this field is that future oral ectoderm is somehow imbued with a basic 'pre-pattern' through the nested expression of fibroblast growth factors (Fgfs), sonic hedgehog (Shh) and Bmp4. These signals are then interpreted and refined by the underlying mesenchyme into spatially restricted domains of homeobox gene expression. In turn, these transcription factors regulate other signaling molecules (Bmp, Wnt and Fgf proteins) that induce the epithelial folding and invagination that signal the initiation of tooth development. Just how does the future oral ectoderm acquire this basic pre-pattern? By extending previous fate maps (Couly and Le Douarin, 1990), Sharpe and colleagues show that the regionalization of the oral ectoderm into *Fgf8*-positive (molar) and *Fgf8*-negative (incisor) domains occurs long before the pharyngeal arches have formed; the regionalization is evident as early as neurulation (Haworth et al., 2004). And what regionalizes the ectoderm? The instigator of this patterning appears to be pharyngeal endoderm (Haworth et al., 2004). This tissue does far more than set up a framework for tooth development, however, as will become evident in the next section.

Pharyngeal endoderm and arch patterning

Experiments from Le Douarin and colleagues, and Graham and co-workers demonstrate that the pharyngeal endoderm has a profound influence on the morphogenesis of the middle and lower face (Crump et al., 2004a; Crump et al., 2004b; Trokovic et al., 2003; Veitch et al., 1999). Recent work shows that Fgf signaling is an essential component of this tissue patterning. Kimmel and his colleagues used time-lapse microscopy in zebrafish to demonstrate that pharyngeal pouches form when clusters of endoderm cells migrate laterally, and that if *Fgf8* is inactivated and Fgf3 is knocked down with morpholinos, the migration of these endodermal cells is disrupted (Crump et al.,

2004a). Consequently, the pharyngeal pouches fail to form and the pharyngeal arch cartilages are disorganized (Crump et al., 2004a). Not all cartilages are equally affected, however; mandibular cartilages derived from Hox-negative neural crest cells are less affected than are Hox-positive second arch cells, a finding that has also been shown in avian embryos. In these avian studies, pharyngeal endodermal grafts were positioned adjacent to the neural tube (Ruhin et al., 2003). The response was a remarkable duplication in pharyngeal arch skeletal structures, the general morphology of which correlated with the level from which the endodermal graft was derived. Le Douarin and co-workers also showed that removing the endoderm completely blocked the formation of the pharyngeal arch skeleton. As in the zebrafish studies, they suggest that Fgf8 is a key mediator of this activity (Ruhin et al., 2003). Does the pharyngeal endoderm influence the morphogenesis of the entire facial skeleton (Ruhin et al., 2003)? Analyses of the zebrafish mutant, *casanova*, indicate not, because in this animal the pharyngeal endoderm is not required for normal development of the middle and upper part of the face (Aoki, 2002). Instead, two other epithelia, the anterior (or forebrain) neuroectoderm and the facial/stomodeal ectoderm, appear to have taken over this crucial role.

Neural and surface ectoderm: patterning the middle and upper face

When regions of facial ectoderm are transplanted to ectopic sites in the avian face, the developmental fate of underlying frontonasal neural crest cells is altered and the result is a duplication of upper beak structures (Hu et al., 2003). This same bit of facial ectoderm can elicit similar duplications when transplanted into the first, Hox-negative, arch, but has no effect when transplanted into the second, Hox-positive, arch (Hu et al., 2003). This result indirectly illustrates how neural crest plasticity is balanced against a 'pre-pattern', owing in no small part to the expression of Hox genes in the facial tissues (Creuzet et al., 2002). What types of signals imbue this facial ectoderm with the ability to re-specify the fates of neural crest cells? Both *Shh* and *Fgf8* are expressed in juxtaposed non-overlapping domains in this region of tissue, but whether they are the molecules responsible for achieving this effect, or simply molecular markers of an important boundary domain in the face, remains to be determined.

Neural ectoderm is also a source of patterning information for the middle and upper face, as has recently been shown in a series of experiments conducted in zebrafish. In these experiments, Eberhart and Kimmel found that Shh emanating from anterior ventral neuroectoderm directly patterned the ventral surface ectoderm, without requiring an intermediate signal generated by neural crest sandwiched between these two epithelia (J. Eberhart and C. Kimmel, unpublished). The loss of neuroectodermal Shh prevented neural crest cells from aggregating into condensations and eventually from forming skeletal elements. This result supports previous findings in mice (Jeong et al., 2004) and birds (Cordero et al., 2004).

Molecular mediators of craniofacial morphogenesis

Sometimes, the mechanisms that regulate normal development are best appreciated by studying cases of abnormal development. Human craniofacial malformations have been avidly catalogued since the Aristotelian era but only lately have researchers pinpointed some of the genes responsible. The next hurdle is to understand the function of the encoded proteins in craniofacial morphogenesis. This aim is complicated by the fact that these genes are invariably expressed in multiple tissues and at multiple times during facial development, and so separating their numerous functions becomes a difficult task. The case of holoprosencephaly illustrates this point perfectly.

A sonic boom

One of the best studied craniofacial abnormalities is holoprosencephaly (HPE), a syndrome that is associated with perturbations in a handful of *Shh*-related genes (Belloni et al., 1996; Brown et al., 1998; Cole and Krauss, 2003; Cordero et al., 2004a; Gripp et al., 2000; Marini et al., 2003; Ming et al., 2002; Roessler et al., 1996; Roessler et al., 2003). At one end of the HPE spectrum, fetuses exhibit cyclopia, a condition characterized by a single, central eye and no discernable nose, but a relatively normal-looking middle and lower face (Chiang et al., 2001). At the other extreme, obligate HPE carriers can have a normal facial appearance (McKusick, 2000). In an effort to explain this remarkable phenotypic variation, Traiffort and colleagues recently examined how specific human HPE mutations affected the structure and function of the SHH protein. By coupling three-dimensional modeling of fragments of the SHH protein with a series of functional assays, the researchers found that most HPE mutations fall into one of three classes: mutations that influenced zinc binding of the protein; those that affect the auto-processing of SHH; and those that adversely alter SHH stability (Traiffort et al., 2004). However, none of these mutation types could be linked to a specific phenotype (Traiffort et al., 2004), confirming previous speculations along the same lines (Dipple and McCabe, 2000).

If there is no clear genotype-phenotype correlation, then what explains the variable expressivity of this craniofacial malformation? One appealing hypothesis is that environmental agents act in conjunction with an autosomal dominant mutation to compromise Shh signaling (Cordero et al., 2004; Edison and Muenke, 2003). If this scenario were true, then varying the time in which an embryo was exposed to an environmental teratogen could elicit different disease phenotypes. We tested this hypothesis by exposing avian embryos to cyclopamine, a potent inhibitor of the Hedgehog signaling pathway (Chen et al., 2002a; Chen et al., 2002b), and found that by varying the delivery time so that it coincided with *Shh* induction in the forebrain and later in the face, we could reproduce the spectrum of HPE phenotypes (Cordero et al., 2004). Although this is unlikely to be the sole, or even the predominant, explanation for variations in HPE phenotype, experiments such as these indicate that Shh has a variety of functions in facial development. This point is well illustrated by studies showing that *Shh* initially plays a role in patterning the neural plate midline (Chiang et al., 1996), and later is critically required for the proper development of a subset of neural crest-derived facial bones (Hu and Helms, 1999; Jeong et al., 2004). These data also imply that some, but not all, cranial neural crest cells need a Hedgehog signal both to survive and, ultimately, to differentiate appropriately (Ahlgren and Bronner-Fraser, 1999; Ahlgren et al., 2002).

Fgfs and craniofacial patterning: a question of timing

Even when the source of a signal important for craniofacial

development has been identified, there are often multiple sources of a particular morphogen, and each source may control a different aspect of patterned outgrowth and cell differentiation. This has been well illustrated in recent studies evaluating the consequences of Fgf perturbation at four separate points in craniofacial development. Early in craniofacial development, Fgf signaling is involved in the production of dopaminergic neurons (Ye et al., 1998); the same signal is crucial in establishing the midbrain-hindbrain boundary (Scholpp et al., 2003). Later in development, Fgf signaling from ventral forebrain and pharyngeal endoderm is required for pharyngeal skeletogenesis, as inhibiting this pathway prevents the formation of the second arch skeleton (Creuzet et al., 2004; Walshe and Mason, 2003). Later still, blocking Fgf signaling from the surface ectoderm disrupts outgrowth of the frontonasal skeleton (A. Abzhanov, D. Hu, J. Sen, C. J. Tabin and J.A.H., unpublished). Finally, just before birth, disruptions in Fgf signaling cause premature osteogenesis in the sutures (Moore et al., 2002; Sarkar et al., 2001). Clearly then, Fgfs play multiple, fundamental roles in craniofacial morphogenesis, but unraveling this complicated molecular machinery will have to await better genetic and molecular tools that permit a more precise regulation of gene activity.

Bmp proteins and craniofacial patterning in birds

Vertebrates exhibit a marvelous range of craniofacial features that are designed to fit specialized niches and behaviors. These postnatal facial features are immediately obvious, but during the embryonic period, vertebrate faces look remarkably similar (Haeckel, 1897). The proteins that establish this basic blueprint of the craniofacial region are still unidentified but likely candidates are those same molecules that establish other developmental axes in vertebrates and invertebrates: Hedgehog and Wnt proteins, and members of the Bmp and Fgf families. Some new studies have begun to explore how different species use these pathways to create distinctive facial features.

In the Galapagos finches, Darwin had noted that ‘a nearly perfect gradation may be traced from a beak extraordinarily thick to one so fine that it may be compared with that of a warbler.’ (Darwin, 1859). We now know that these species-specific morphological variations are evident during embryogenesis, and are first evident around Hamburger and Hamilton (Hamburger and Hamilton, 1951) stage 22 (S. Brugmann and J.A.H., unpublished). Prior to that time, the faces of different avian species are indistinguishable from one another (Schneider and Helms, 2003). Tabin and co-workers set out to understand how such morphological variations might arise. They evaluated two finch species – the ground and cactus finches – that represent the extremes in Galapagos finch beak morphology (Grant, 1986) (Fig. 4A,F). At the time when ground and cactus finch embryos appear similar, in situ hybridization analyses by these investigators revealed a difference in the patterns of *Bmp4* expression (Abzhanov et al., 2004) (see Fig. 4). To test experimentally whether spatial and temporal changes in *Bmp4* expression could account for the relative size and shape differences in these finches’ beaks, the investigators mis-expressed *Bmp4* throughout the mesenchyme of a chick frontonasal prominence (Fig. 4D). This misexpression converted the narrow short chick beak into a much broader bigger beak that resembled that of the large ground finch (Abzhanov et al., 2004) (Fig. 4D).

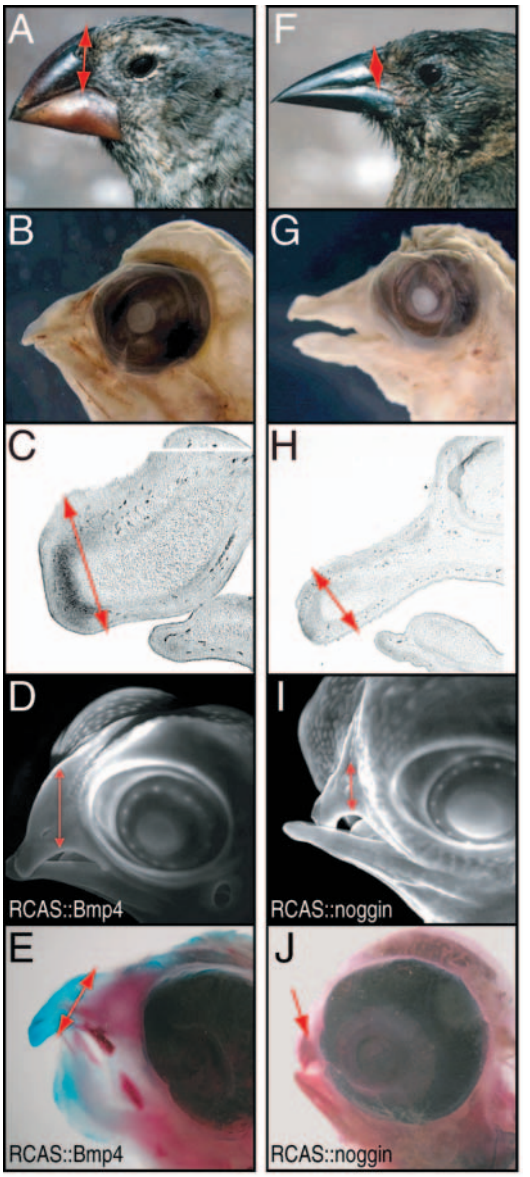

Fig. 4. Bmp4 expression levels control beak depth and height. (A,B) Large ground finches have thick, broad and long beaks. (C) The embryonic beak of a ground finch exhibits high *Bmp4* expression levels, which promote chondrogenesis and therefore increased beak height, length and depth (red arrow). (D) Misexpression of *Bmp4* in the frontonasal process mesenchyme of chick embryos produces a noticeably broader and thicker upper beak, paralleling the beak morphology of the ground finch. (E) Alcian staining of chick embryos injected with RCAS-Bmp4 reveals enlarged skeletal elements in the upper beak. (F,G) Cactus finches have thinner, shorter and narrower beaks. (H) The embryonic beak of a cactus finch exhibits very little *Bmp4* expression, and chondrogenesis of the beak is not as pronounced, which leads to an overall smaller beak. (I) Misexpression of noggin, a Bmp4 antagonist, in frontonasal process mesenchyme of chick embryos produces a noticeably thinner and narrower upper beak, paralleling the beak morphology of the cactus finch. (J) Alcian staining reveals stunted upper beak skeletal elements in chicken embryos injected with RCAS-noggin. (B-D,G-I) Reproduced, with permission, from Abzhanov et al. (Abzhanov et al. 2004). (E,J) Reproduced, with permission, from Wu et al. (Wu et al. 2004). (A,F) Courtesy of P. Grant, A. Abzhanov and C. Tabin.

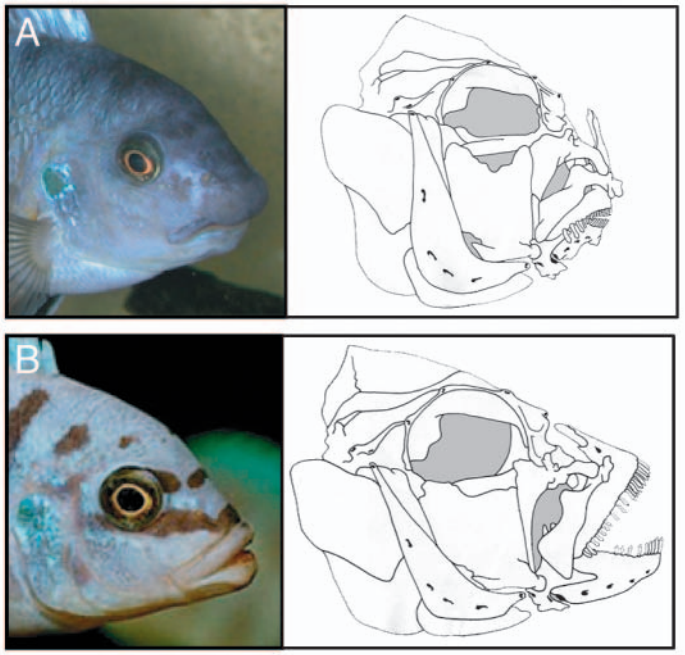

Fig. 5. Morphological differences between jaws of cichlids. (A) The river-dwelling cichlid *Metriaclima zebra* (left) has a jaw structure (right) that is well-suited for sucking. (B) The Great Lakes cichlid *Labeotropheus fuelleborni* (left) has a jaw structure (right) that is well-suited for biting. Photographs of *Labeotropheus fuelleborni* and *Metriaclima zebra* courtesy of J. Dion and F. Hagblom, respectively. Drawings reproduced, with permission, from Albertson et al. (Albertson et al., 2003a).

Chuong and colleagues also used the inherent differences in avian beak morphology to address whether spatial variations in *Bmp4* expression coincided with spatial differences in growth between two avian beaks (Wu et al., 2004). These investigators used chicks and ducks for their study, because of the obvious differences in the beaks of seed-eating avians, such as chickens, and the broad flat bills of waterfowl. The researchers focused on a region of the frontonasal prominence, the frontonasal ectodermal zone (FEZ), which functions as an organizing center for the middle and upper face (Hu et al., 2003). They found that although chicks had a single population of proliferating cells near the FEZ, ducks appeared to have two such sites situated on the lateral borders of the FEZ (Wu et al., 2004). These areas of cell proliferation coincided with sites of *Bmp4* expression in the frontonasal prominence, indicating that the localized growth zones might be responsible for producing beaks versus bills. When Bmp signaling was overexpressed, or repressed by Noggin, the size of the beaks increased or decreased, respectively (Wu et al., 2004) (Fig. 4I).

These two studies indicate that modulations in Bmp4 activity can alter beak morphology, but they do not clarify whether Bmp4 is instigating these morphological changes or whether Bmp4 is being altered in response to another molecule. Nonetheless, as studies in fish show (as discussed below), Bmp4 has also been implicated in patterning the craniofacial complex in other organisms.

Bmp4 and craniofacial patterning in fish

Cichlids are small fish found in the rivers and lakes of the East African rift valley that exhibit a dramatic variation in facial form, not unlike that of finches (Fig. 5). An astonishing 200 species are estimated to have evolved within the past 10 million years (Kocher, 2004), which certainly places cichlids on the fast track in terms of evolutionary diversity. This rapid diversification offers another advantage: as speciation occurred relatively recently, interbreeding is possible. This means that two species with dramatically different facial skeletons can be mated to generate progeny with intermediate phenotypes, and in a recent series of experiments, investigators did just that. Albertson and colleagues used the detailed description of cichlid skeletons as a starting point (Barel, 1983) for a series of morphometric analyses on two species of cichlids and their progeny (see Fig. 5). The authors then mapped genomic regions (so called quantitative trait loci – QTL) that co-segregated with specific morphological alterations to the jaw skeleton. Their findings showed that only a handful of QTL need to be modified to provide a cichlid with a unique set of jaws (Albertson et al., 2003a; Albertson et al., 2003b) (see also Box 1).

Okada and co-workers focused on *Bmp4* as a candidate gene that might underlie one of these QTL (Terai et al., 2002). They took advantage of the fact that the cichlids that occupy the East African Great Lake exhibit a higher degree of speciation relative to cichlids occupying the nearby rivers. Okada

Box 1. How malleable is the mandible?

The advantages of a hinged jaw are considerable, especially if an animal can adapt the morphology of the jaw to suit its diet. From a mechanical perspective, even small changes in morphology can affect whether jaws are suitable for chomping versus slurping. Two new studies have explored this link between form and function using the fish jaw skeleton as a model system.

Labridae are extraordinarily adaptable fish that occupy every niche available to the inhabitants of a coral reef ecosystem. Some labridae, such as the parrotfish, have jaw skeletons that allow them to eat coral; some have jaws designed for capturing elusive prey; others have jaw skeletons suited for sucking mucus from injured coral (Wainwright, 1988). These jaws differ considerably in length, width, breadth and overall form, and, theoretically, there are an infinite number of points in this 'morphospace' that could be responsible for altering the mechanical properties of the jaw (Hulsey and Wainwright, 2002). The problem lies in identifying which of these points are actually relevant. Peter Wainwright and co-workers undertook a 'needle in a haystack' search and found that muscle-attachment sites were key determinants of jaw function (Wainwright et al., 2004). Muscle-attachment sites control how rapidly the jaw can be opened and closed, which is an essential characteristic of whether an animal can have a diet of elusive prey, as well as affecting how much bite force an animal can generate (important for a diet that consists of durable and motionless quarry). Wainwright's study also raises a note of caution when considering landmarks on any facial skeleton: not all points in a given morphospace are equivalent discriminators of jaw function. In other words, although the length, depth and breadth of a lower jaw can change in accordance with the jaw's function, some of these anatomical landmarks are closely related to morphological variations (e.g. sites of muscle attachment), whereas other landmarks change as a consequence of an overall variation in shape. Fortunately for biologists, biomechanical modeling provides such insights into how morphological variations affect mechanical function.

postulated that the more highly speciated of the lake cichlids would exhibit an elevated frequency of amino acid substitutions in those genes that were involved with generating morphological variations. No significant differences in amino acid substitution rates were observed for Otx1, Otx2 and Pax9. The pro-domain of Bmp4, however, showed significant modifications (Terai et al., 2002). These findings imply that post-translational modifications of Bmp4 could account for at least some of the variations in the facial features of this fish, just as it can in birds. However, it is once again not clear whether Bmp4 is responding to, or is actually instigating, morphological change.

Evolution and patterning of the jaw

Faces have changed drastically throughout evolution and although differences in the length, width and breadth of facial features are certainly of great consequence, the most notable alteration has been the evolution of a hinged jaw. This advancement endowed its new owner with the ability to diversify its eating habits, thereby proffering a hefty leg up on the competition. Current studies in lampreys and fish are shedding new light on the molecular changes required for leaping this evolutionary hurdle.

In the portrait gallery of comparative anatomy, the larval form of jawless lampreys bear a remarkable resemblance to jawed animals, in that both possess a braincase and pharyngeal arches, which are the building blocks for much of the craniofacial skeleton. The question is, if jaw-lacking (agnathan) larvae and jaw-possessing (gnathostome) larvae have comparative facial features, then how did one species develop a hinged jaw while the other did not? Recent studies of Hox gene expression patterns may reveal the molecular mechanisms behind this transformation.

Hox genes are expressed in a nested pattern along the body axis, which has led to the speculation that they provide cells with a regional identity. A variety of functional tests, most recently by Wellik and Capecchi, have provided convincing evidence to that effect in mice (Wellik and Capecchi, 2003). Gain- and loss-of-function studies in chicks have also demonstrated that *Hoxa2* gene expression constrains the range of decisions that cranial neural crest cells can make as they differentiate into the facial skeleton (Couly et al., 2002; Hu et al., 2003; Ruhin et al., 2003). In light of these data, Cohn asked whether the loss of anterior Hox expression correlated with the acquisition of a hinged jaw apparatus, because if first-arch neural crest cells are Hox positive in a more primitive condition but become Hox negative through evolution then, theoretically, these cells would be at liberty to respond to new signals in their changing environment. Such a newly acquired flexibility might then allow for adaptive variations in the jaw structures formed by these neural crest cells.

Cohn examined jawless lamprey larvae and found that *HoxL6* was expressed in the first pharyngeal arch, which is a Hox-negative region in jawed embryos (Cohn, 2002) (see Fig. 6). Was this simply an odd twist of fate for lampreys, as opposed to being a molecular feature of a more primitive evolutionary condition? Lampreys are currently the only agnathan available for study, so Cohn turned to a more primitive animal – the cephalochordate Amphioxus, which also possesses a Hox cluster (Ferrier et al., 2000) – to support his argument. As he had found with lampreys, the Hox homolog *AmphiHox6* was also expressed in the anterior head (Cohn, 2002) (see Fig. 6), lending further support to his hypothesis that loss of Hox gene expression correlates with the gain of a hinged jaw joint.

There is, however, a caveat to this story: when examining a different species of lamprey captured in Japan, Kuratani and colleagues saw Hox expression in more posterior regions of the neural tube but did not detect Hox expression in the first arch (Takio et al., 2004). At this point in time, there is no good explanation for these different findings. The same region of the lamprey gene was used in both in situ hybridization analyses (S. Kuratani, personal communication), and although it is theoretically possible that different lamprey species show variations in Hox gene expression, this is not a likely explanation. However, comparative anatomists frequently

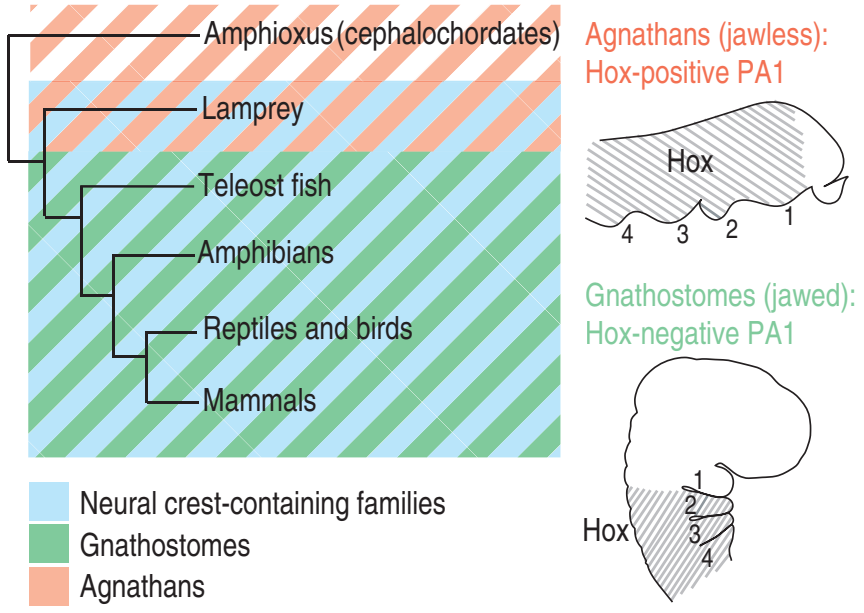

Fig. 6. Hox expression in agnathans and gnathostomes. (A) Correlations between Hox expression and jaw development in chordates. The phylum chordata can be subdivided into two groups: jawed gnathostomes (green) and jawless agnathans (red). Some organisms in both groups, including the jawless lamprey and the jawed teleost fish, possess neural crest (blue) that can be acted on by Hox genes. Recent experiments (Ferrier et al., 2000; Cohn, 2002) have demonstrated that Hox expression exists as anterior as the first pharyngeal arch (PA1) in agnathan lampreys and amphioxus. Conversely, in most gnathostome vertebrates, Hox expression is evident only up to the second pharyngeal arch (PA2), and no Hox expression is seen in PA1. As such, loss of Hox expression in PA1 can be correlated with the development of jaws in vertebrates.

point out that the highly derived morphology of the lamprey feeding apparatus makes it a less than ideal agnathan archetype to study. Therefore, comparisons between structures in lampreys and jawed vertebrates should be treated with caution. Perhaps we will soon understand how modifying Hox, or any other gene, expression patterns turned out to be one small step for agnathans but one giant leap for gnathostomes.

Conclusions

A recent meeting organized by the Anatomical Society of Great Britain and Ireland demonstrated that the field of craniofacial biology attracts scientists from a wide range of disciplines. Developmental and evolutionary biologists, reproductive toxicologists, bioengineers and genome biologists have recently contributed to our understanding of the mechanisms by which the craniofacial tissues are patterned and their outgrowth regulated. We are tackling issues first posed by Darwin and reiterated by Spemann, Wolpert and other notable scientists, as they relate to the patterning of the craniofacial complex. There remain a number of pressing issues. For example, studies conducted in a single species are oftentimes presumed to represent conserved mechanisms of patterning across all species; although this approach has some utility, there are bound to be errors made as a result of over simplification. Generalizations from one species to another may cloud subtle variations that could be responsible for certain aspects of species-specific facial morphology. And although there is much to be learned about studying conserved molecular pathways and their various functions in craniofacial development, there are no studies to date that have addressed how these same morphogens create a face and not a limb bud or other structure. Finally, although the issue of whether the neural crest or epithelium contains patterning information might be settled (they both do), how the patterning process itself is instigated remains unknown. The next few years will undoubtedly yield resolution of these issues and invariably give rise to many more.

Photographs and illustrations were kindly provided by the following individuals: J. E. Randall (labridae); P. Wainwright and the Linnean Society (labrid jaw diagrams); J. Dion and F. Hagblom (cichlids); C. Albertson and the National Academy of Sciences (cichlid jaw diagrams); and P. Grant (finches). We also thank C. Tabin, C.-M. Chuong, and *Science* for allowing us to reproduce figure panels. The authors also thank T. Schilling, P. Sharpe, S. Brugmann, S. Kuratani, J. Hanken and P. Herandez for helpful discussions regarding the manuscript, J. Eberhart and C. Kimmel for sharing data prior to publication, and C. Moreau for assistance with manuscript preparation. J.A.H. is supported, in part, by The Oak Foundation.

References

Abzhanov, A., Protas, M., Grant, B. R., Grant, P. R. and Tabin, C. J. (2004) Bmp4 and morphological variation of beaks in Darwin's finches. *Science*, **305**, 1462.

Ahlgren, S. C. and Bronner-Fraser, M. (1999). Inhibition of sonic hedgehog signaling in vivo results in craniofacial neural crest cell death. *Curr. Biol.* **9**, 1304-1314.

Ahlgren, S. C., Thakur, V. and Bronner-Fraser, M. (2002). Sonic hedgehog rescues cranial neural crest from cell death induced by ethanol exposure. *Proc. Natl. Acad. Sci. USA* **99**, 10476-10481.

Albertson, R. C., Streelman, J. T. and Kocher, T. D. (2003a). Directional selection has shaped the oral jaws of Lake Malawi cichlid fishes. *Proc. Natl. Acad. Sci. USA* **100**, 5252-5257.

Albertson, R. C., Streelman, J. T. and Kocher, T. D. (2003b). Genetic basis of adaptive shape differences in the cichlid head. *J. Hered.* **94**, 291-301.

Andres, G. (1949). Untersuchungen an chimären von Triton und Bombinator. *Genetics* **24**, 387-534.

Barel, C. D. N. (1983). Toward A Constructional Morphology Of Cichlid Fishes (Teleostei, Perciformes). *Neth. J. Zool.* **33**, 357-424.

Basch, M. L., Garcia-Castro, M. I. and Bronner-Fraser, M. (2004). Molecular mechanisms of neural crest induction. *Birth Defects Res. Part C Embryo Today* **72**, 109-123.

Belloni, E., Muenke, M., Roessler, E., Traverso, G., Siegel-Bartelt, J., Frumkin, A., Mitchell, H. F., Donis-Keller, H., Helms, C., Hing, A. V. et al. (1996). Identification of Sonic hedgehog as a candidate gene responsible for holoprosencephaly. *Nat. Genet.* **14**, 353-356.

Bolande, R. P. (1997). Neurocristopathy: its growth and development in 20 years. *Pediatr. Pathol. Lab. Med.* **17**, 1-25.

Brown, S. A., Warburton, D., Brown, L. Y., Yu, C. Y., Roeder, E. R., Stengel-Rutkowski, S., Hennekam, R. C. and Muenke, M. (1998). Holoprosencephaly due to mutations in ZIC2, a homologue of Drosophila odd-paired. *Nat. Genetics* **20**, 180-183.

Burstyn-Cohen, T., Stanleigh, J., Sela-Donenfeld, D. and Kalcheim, C. (2004). Canonical Wnt activity regulates trunk neural crest delamination linking BMP/noggin signaling with G1/S transition. *Development* **131**, 5327-5329.

Cerny, R., Lwigale, P., Ericsson, R., Meulemans, D., Epperlein, H. H. and Bronner-Fraser, M. (2004). Developmental origins and evolution of jaws: new interpretation of 'maxillary' and 'mandibular'. *Dev. Biol.* **276**, 225-236.

Chen, J. K., Taipale, J., Cooper, M. K. and Beachy, P. A. (2002a). Inhibition of Hedgehog signaling by direct binding of cyclopamine to Smoothened. *Genes Dev.* **16**, 2743-2748.

Chen, J. K., Taipale, J., Young, K. E., Maiti, T. and Beachy, P. A. (2002b). Small molecule modulation of Smoothened activity. *Proc. Natl. Acad. Sci. USA* **21**, 21.

Cheung, M. and Briscoe, J. (2003). Neural crest development is regulated by the transcription factor Sox9. *Development* **130**, 5681-5693.

Chiang, C., Litingtung, Y., Lee, E., Young, K. E., Corden, J. L., Westphal, H. and Beachy, P. A. (1996). Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. *Nature* **383**, 407-413.

Chiang, C., Litingtung, Y., Harris, M. P., Simandl, B. K., Li, Y., Beachy, P. A. and Fallon, J. F. (2001). Manifestation of the limb prepattern: limb development in the absence of sonic hedgehog function. *Dev. Biol.* **236**, 421-435.

Cobourne, M. T. and Sharpe, P. T. (2003). Tooth and jaw: molecular mechanisms of patterning in the first branchial arch. *Arch. Oral Biol.* **48**, 1-14.

Cohn, M. J. (2002). Evolutionary biology: lamprey Hox genes and the origin of jaws. *Nature* **416**, 386-387.

Cole, F. and Krauss, R. S. (2003). Microform holoprosencephaly in mice that lack the Ig superfamily member Cdon. *Curr. Biol.* **13**, 411-415.

Cordero, D., Marcucio, R., Hu, D., Gaffield, W., Tapadia, M. and Helms, J. A. (2004). Temporal perturbations in sonic hedgehog signaling elicit the spectrum of holoprosencephaly phenotypes. *J. Clin. Invest.* **114**, 485-494.

Couly, G. and Douarin, N. M. L. (1988). The fate map of the cephalic neural primordium at the presomitic to the 3-somite stage in the avian embryo. *Development* **103**, 101-113.

Couly, G. and Le Douarin, N. M. (1990). Head morphogenesis in embryonic avian chimeras: evidence for a segmental pattern in the ectoderm corresponding to the neuromeres. *Development* **108**, 543-558.

Couly, G., Grapin-Botton, A., Coltey, P. and Le Douarin, N. M. (1996). The regeneration of the cephalic neural crest, a problem revisited: the regenerating cells originate from the contralateral or from the anterior and posterior neural fold. *Development* **122**, 3393-3407.

Couly, G., Creuzet, S., Bennaceur, S., Vincent, C. and Le Douarin, N. M. (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.

Creuzet, S., Couly, G., Vincent, C. and Le Douarin, N. M. (2002). Negative effect of Hox gene expression on the development of the neural crest-derived facial skeleton. *Development* **129**, 4301-4313.

Creuzet, S., Schuler, B., Couly, G. and Le Douarin, N. M. (2004). Reciprocal relationships between Fgf8 and neural crest cells in facial and forebrain development. *Proc. Natl. Acad. Sci. USA* **101**, 4843-4847.

Crump, J. G., Maves, L., Lawson, N. D., Weinstein, B. M. and Kimmel, C. B. (2004a). An essential role for Fgfs in endodermal pouch formation

influences later craniofacial skeletal patterning. *Development* **131**, 5703-5716.
Crump, J. G., Swartz, M. E. and Kimmel, C. B. (2004b). An integrin-dependent role of pouch endoderm in hyoid cartilage development. *PLoS Biol.* **2**, E244.
Darwin, C. (1859). *The Origin of Species*. New York: The Crowell-Collier Publishing.
Dipple, K. M. and McCabe, E. R. (2000). Phenotypes of patients with 'simple' Mendelian disorders are complex traits: thresholds, modifiers, and systems dynamics. *Am. J. Hum. Genet.* **66**, 1729-1735.
Edison, R. and Muenke, M. (2003). The interplay of genetic and environmental factors in craniofacial morphogenesis: holoprosencephaly and the role of cholesterol. *Congenit. Anom. Kyoto* **43**, 1-21.
Ferrier, D. E., Minguillon, C., Holland, P. W. and Garcia-Fernandez, J. (2000). The amphioxus Hox cluster: deuterostome posterior flexibility and Hox14. *Evol. Dev.* **2**, 284-293.
Francis-West, P. H., Robson, L. and Evans, D. J. (2003). Craniofacial development: the tissue and molecular interactions that control development of the head. *Adv. Anat. Embryol. Cell Biol.* **169**, 1-138.
Garcia-Castro, M. I., Marcelle, C. and Bronner-Fraser, M. (2002). Ectodermal Wnt function as a neural crest inducer. *Science* **13**, 13.
Grant, P. R. (1986). *Ecology and Evolution of Darwin's Finches.* Princeton, NJ: Princeton University Press.
Gripp, K. W., Wotton, D., Edwards, M. C., Roessler, E., Ades, L., Meinecke, P., Richieri-Costa, A., Zackai, E. H., Massague, J., Muenke, M. et al. (2000). Mutations in TGIF cause holoprosencephaly and link NODAL signalling to human neural axis determination. *Nat. Genet.* **25**, 205-208.
Haeckel, E. H. P. A. (1897). *The Evolution of Man*. New York: D. Appleton.
Hall, B. K. (1980). Tissue interactions and the initiation of osteogenesis and chondrogenesis in the neural crest-derived mandibular skeleton of the embryonic mouse as seen in isolated murine tissues and in recombinations of murine and avian tissues. *J. Embryol. Exp. Morphol.* **58**, 251-264.
Hamburger, V. and Hamilton, H. L. (1951). A series of normal stages in the developing chick embryo. *J. Morphol*. **88**, 49-92.
Haworth, K. E., Healy, C., Morgan, P. and Sharpe, P. T. (2004). Regionalisation of early head ectoderm is regulated by endoderm and prepatterns the orofacial epithelium. *Development* **131**, 4797-4806.
Honore, S. M., Aybar, M. J. and Mayor, R. (2003). Sox10 is required for the early development of the prospective neural crest in Xenopus embryos. *Dev. Biol.* **260**, 79-96.
Hu, D. and Helms, J. A. (1999). The role of sonic hedgehog in normal and abnormal craniofacial morphogenesis. *Development* **126**, 4873-4884.
Hu, D., Marcucio, R. S. and Helms, J. A. (2003). A zone of frontonasal ectoderm regulates patterning and growth in the face. *Development* **130**, 1749-1758.
Hulsey, C. D. and Wainwright, P. C. (2002). Projecting mechanics into morphospace: disparity in the feeding system of labrid fishes. *Proc. R. Soc. Lond. B Biol. Sci.* **269**, 317-326.
Imai, H., Osumi-Yamashita, N., Ninomiya, Y. and Eto, K. (1996). Contribution of early-emigrating midbrain crest cells to the dental mesenchyme of mandibular molar teeth in rat embryos. *Dev. Biol.* **176**, 151-165.
Jeong, J., Mao, J., Tenzen, T., Kottmann, A. H. and McMahon, A. P. (2004). Hedgehog signaling in the neural crest cells regulates the patterning and growth of facial primordia. *Genes Dev.* **18**, 937-951.
Kocher, T. D. (2004). Adaptive evolution and explosive speciation: the cichlid fish model. *Nat. Rev. Genet.* **5**, 288-298.
Kollar, E. J. and Baird, G. R. (1969). The influence of the dental papilla on the development of tooth shape in embryonic mouse tooth germs. *J. Embryol. Exp. Morphol.* **21**, 131-148.
Kollar, E. J. and Fisher, C. (1980). Tooth induction in chick epithelium: expression of quiescent genes for enamel synthesis. *Science* **207**, 993-995.
Kollar, E. J. and Mina, M. (1991). Role of the early epithelium in the patterning of the teeth and Meckel's cartilage. *J. Craniofac. Genet. Dev. Biol.* **11**, 223-228.
Köntges, G. and Lumsden, A. (1996). Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. *Development* **122**, 3229-3242.
Larson, W. J. (2001). *Human Embryology*. London, UK: Churchill Livingston.
Le Douarin, N. M., Creuzet, S., Couly, G. and Dupin, E. (2004). Neural crest cell plasticity and its limits. *Development* **131**, 4637-4650.
Lee, S. H., Bedard, O., Buchtova, M., Fu, K. and Richman, J. M. (2004). A new origin for the maxillary jaw. *Dev. Biol.* **276**, 207-224.
Lumsden, A. G. (1988). Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development* **103**, 155-169.
Manzanares, M. and Nieto, M. A. (2003). A celebration of the new head and an evaluation of the new mouth. *Neuron* **37**, 895-898.
Marini, M., Cusano, R., De Biasio, P., Caroli, F., Lerone, M., Silengo, M., Ravazzolo, R., Seri, M. and Camera, G. (2003). Previously undescribed nonsense mutation in SHH caused autosomal dominant holoprosencephaly with wide intrafamilial variability. *Am. J. Med. Genet.* **117A**, 112-115.
McCabe, K. L., Manzo, A., Gammill, L. S. and Bronner-Fraser, M. (2004). Discovery of genes implicated in placode formation. *Dev. Biol.* **274**, 462-477.
McKusick, V. A. (2000). *Online Mendelian Inheritance in Man, OMIM (TM).* Vol. 2002. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD).
Miletich, I. and Sharpe, P. T. (2003). Normal and abnormal dental development. *Hum. Mol. Genet.* **12**, R69-R73.
Miller, W. A. (1969). Inductive changes in early tooth development. I. A study of mouse tooth development on the chick chorioallantosis. *J. Dent. Res.* **48**, 719-725.
Ming, J. E., Kaupas, M. E., Roessler, E., Brunner, H. G., Golabi, M., Tekin, M., Stratton, R. F., Sujansky, E., Bale, S. J. and Muenke, M. (2002). Mutations in PATCHED-1, the receptor for SONIC HEDGEHOG, are associated with holoprosencephaly. *Hum. Genet.* **110**, 297-301.
Mitsiadis, T. A., Cheraud, Y., Sharpe, P. and Fontaine-Perus, J. (2003). Development of teeth in chick embryos after mouse neural crest transplantations. *Proc. Natl. Acad. Sci. USA* **100**, 6541-6545.
Moore, R., Ferretti, P., Copp, A. and Thorogood, P. (2002). Blocking endogenous FGF-2 activity prevents cranial osteogenesis. *Dev. Biol.* **243**, 99-114.
Perez-Alcala, S., Nieto, M. A. and Barbas, J. A. (2004). LSox5 regulates RhoB expression in the neural tube and promotes generation of the neural crest. *Development* **131**, 4455-4465.
Roessler, E., Belloni, E., Gaudenz, K., Jay, P., Berta, P., Scherer, S. W., Tsui, L. C. and Muenke, M. (1996). Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. *Nat. Genet.* **14**, 357-360.
Roessler, E., Du, Y. Z., Mullor, J. L., Casas, E., Allen, W. P., Gillessen-Kaesbach, G., Roeder, E. R., Ming, J. E., Ruiz i Altaba, A. and Muenke, M. (2003). Loss-of-function mutations in the human GLI2 gene are associated with pituitary anomalies and holoprosencephaly-like features. *Proc. Natl. Acad. Sci. USA* **100**, 13424-13429.
Rubenstein, J. L., Shimamura, K., Martinez, S. and Puelles, L. (1998). Regionalization of the prosencephalic neural plate. *Annu. Rev. Neurosci.* **21**, 445-477.
Ruhin, B., Creuzet, S., Vincent, C., Benouaiche, L., Le Douarin, N. M. and Couly, G. (2003). Patterning of the hyoid cartilage depends upon signals arising from the ventral foregut endoderm. *Dev. Dyn.* **228**, 239-246.
Sarkar, S., Petiot, A., Copp, A., Ferretti, P. and Thorogood, P. (2001). FGF2 promotes skeletogenic differentiation of cranial neural crest cells. *Development* **128**, 2143-2152.
Schneider, R. A. and Helms, J. A. (2003). The cellular and molecular origins of beak morphology. *Science* **299**, 565-568.
Scholpp, S., Lohs, C. and Brand, M. (2003). Engrailed and Fgf8 act synergistically to maintain the boundary between diencephalon and mesencephalon. *Development* **130**, 4881-4893.
Streit, A. and Stern, C. D. (1999). Neural induction. A bird's eye view. *Trends Genet.* **15**, 20-24.
Takio, Y., Pasqualetti, M., Kuraku, S., Hirano, S., Rijli, F. M. and Kuratani, S. (2004). Evolutionary biology: lamprey Hox genes and the evolution of jaws. *Nature* **429**, 262.
Terai, Y., Morikawa, N. and Okada, N. (2002). The evolution of the pro-domain of bone morphogenetic protein 4 (Bmp4) in an explosively speciated lineage of East African cichlid fishes. *Mol. Biol. Evol.* **19**, 1628-1632.
Traiffort, E., Dubourg, C., Faure, H., Rognan, D., Odent, S., Durou, M. R., David, V. and Ruat, M. (2004). Functional characterization of SHH mutations associated with holoprosencephaly. *J. Biol. Chem.*
Trokovic, N., Trokovic, R., Mai, P. and Partanen, J. (2003). Fgfr1 regulates patterning of the pharyngeal region. *Genes Dev.* **17**, 141-153.
Tucker, A. S. and Lumsden, A. (2004). Neural crest cells provide species-specific patterning information in the developing branchial skeleton. *Evol. Dev.* **6**, 32-40.
Veitch, E., Begbie, J., Schilling, T. F., Smith, M. M. and Graham, A. (1999).

Pharyngeal arch patterning in the absence of neural crest. *Curr. Biol.* **9**, 1481-1484.
Wainwright, P. C. (1988). Morphology and ecology: functional basis of feeding constraints in Caribbean Labrid fishes. *Ecology* **69**, 635-645.
Wainwright, P. C., Bellwood, D. R., Westneat, M. W., Grubich, J. R. and Hoey, A. S. (2004). A functional morphospace for the skull of labrid fishes: patterns of diversity in a complex biomechanical system. *Biol. J. Linnean Soc.* **82**, 1-25.
Walshe, J. and Mason, I. (2003). Fgf signalling is required for formation of cartilage in the head. *Dev. Biol.* **264**, 522-536.
Wellik, D. M. and Capecchi, M. R. (2003). Hox10 and Hox11 genes are required to globally pattern the mammalian skeleton. *Science* **301**, 363-367.
Wu, P., Jiang, T. X., Suksaweang, S., Widelitz, R. B. and Chuong, C. M. (2004). Molecular shaping of the beak. *Science* **305**, 1465-1466.
Ye, W., Shimamura, K., Rubenstein, J. L., Hynes, M. A. and Rosenthal, A. (1998). FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. *Cell* **93**, 755-766.