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Review

How the diversity of the faces arises

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ABSTRACT

Background: The evolution of the face is crucial for each species to adapt to different diets, environments, and in some species, to promote social interaction. The diversity in the shapes of the face results from divergence in the process of facial development that begins during early embryonic development.
Highlights: Here we review the recent advancements in the understanding of the genetic, epigenetic, molecular, and cellular basis of facial diversity. We also review the robustness of facial development and how it relates to the evolution of the face. Finally, we discuss the current challenges in achieving a deeper understanding of facial diversity.
Conclusion: We have gained much knowledge with respect to *cis*-regulatory elements, gene expression, cellular behavior, and the physical forces in facial development in the past two decades. Significant interdisciplinary work is needed to integrate these varied pieces of information into a complete picture of how the diversity of faces arises.

Contents

1. Introduction

The morphology of the face is critical for each species to adapt to different diets and environments; a classic example is Darwin's finches, in which different morphologies of the beak allow the birds to feed on different types of food. In humans, the importance of facial morphology goes beyond adaption to the environment and diet; it also plays a critical role in establishing individual identity. Despite great advances in molecular biology and genetics, we are

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just starting to understand the genetic basis of facial morphology. In this review, we aim to summarize the recent understanding of variation in faces and the molecular and cellular mechanisms associated with facial morphology.

Although the face is made of multiple tissue types including the skin, muscle, skin appendages, cartilage, and so on, the most fundamental determinant of facial shape is the skeleton; therefore, in this review, we focus on the variation in skeletal components and their evolution.

The skeletal components of the face include the maxillae, the premaxillae, the mandibles, the nasal bones and cartilage, and the cranial bones, all of which build the framework of the face; the shape and relative size and position of these bony components largely defines the facial morphology. For example, in avian species, the maxillae are reduced and the premaxillae are highly developed to form a prominent beak, whereas in rodents, the premaxillae are relatively small compared to the maxillae, resulting in the appearance of a snout.

2. Embryogenesis of the face

The skeletal components of the face originate from cranial neural crest cells. These cells arise from the neural folds during early embryogenesis and subsequently undergo epithelial–mesenchymal transition (EMT) and delamination, and migrate to each facial primordium where they proliferate, differentiate, and interact with the facial epithelium. The major primordia of an embryonic mouse face include a pair of median processes (MNPs), a pair of lateral nasal processes (LNPs), a pair of maxillary processes, and a pair of mandible processes. The facial primordia interact with each other and eventually fuse.

At the onset of facial primordium fusion, the faces of different amniotes reach maximum similarity. This stage is termed as the phylotypic stage [1]. The presence of the phylotypic stage may imply some general constraints such as requirement of the fusion of facial prominences despite great differences in the final structure of the face [1]. The morphological trajectory begins to diverge after the phylotypic stage [1].

During facial embryogenesis, the epithelium and underlying mesenchyme of the facial primordia interact to control the morphogenesis process. To investigate which tissue plays a major role in determining the facial morphology, Schneider and Helms transplanted the neural crest cells from a duck to one side of a quail and obtained a quail that had a beak similar to a duck (which they called a “quck”) [2]. This result suggests that the neural crest cells carry most of the information about the morphology of the beak in birds, whereas the epithelium plays mainly a permissive role [3]. Although no comparable experiment has been performed in mammals, we may expect that this may also be the case for mammals.

Forming the face involves orchestration of multiple signaling pathways including Hh signaling [4–6], Wnt signaling [7–9], Bmp signaling [10–12], retinoid signaling [13,14], and Fgf signaling [15–18]. Despite the great diversity of the craniofacial complex across different animals, the molecular building blocks of the face are strikingly conserved, as most of the genes expressed in the developing chicken face are also expressed in the developing mouse face. This implies that the diversity in facial morphology results primarily from the quantitative differences in gene expression levels rather than the identities of genes that are expressed during facial morphogenesis. This also suggests that we can gain insights into craniofacial defects by studying animal models.

3. Genetics of craniofacial diversity

Considering that craniofacial diversity arises primarily from quantitative expression levels, efforts were made to identify the molecules that control the shape of the face in a dose-dependent manner. Bmp4 was the first molecule found to be related to the size of the beak in Aves. Wu et al. found that chick embryos expressed significantly less *Bmp4* in the frontonasal process (FNP) compared to duck embryos and that *Bmp4* overexpression dramatically increased the size of the beak in chicken [19]. By studying the different sizes of beaks in Darwin's finches, Abzhanov et al. also found that the *Bmp4* expression level was positively correlated with the size of the beaks in different groups of Darwin's finches [20]. These studies support the idea that different traits could be obtained from the differential expression of certain genes. The list of genes that could modulate the shape and size of the beak has been increasing. Dkk3, TGFβIIr, β-catenin [21], Calmodulin [22], and Runx2 [23] are also involved in controlling different aspects of beak morphology. The amount of SHH from the brain has also been shown to regulate the mid-facial morphology in a continuous manner by regulating cell proliferation and the *Shh* expression pattern in the frontonasal ectoderm zone (FEZ) [24]. Shh is believed to play an important role in the covariance of the brain and face [25]. It may also play a role in controlling the species-specific size of the lower jaw [26]. A summary of the current understanding of the genetic basis of facial morphology is shown in Fig. 1. A high-throughput comparative study on the transcriptome of the facial mesenchyme during embryonic face development in chicks, ducks, and quail revealed multiple differentially expressed genes [27]. More detailed studies are needed to gain a mechanistic understanding of the roles of these genes in facial diversity.

Most of our knowledge about facial diversity comes from avian species, perhaps because of the difficulty in manipulating gene expression in mammals compared to birds. However, the diverse facial phenotypes in different breeds of domestic animals offers a great opportunity to investigate the mechanisms of facial diversity in mammals. The length ratio of repetitive glutamine to repetitive alanine in RUNX2 protein is associated with the length of midface and the degree of dorsoventral nose bend among different breeds of dogs [28], presumably because of the opposing effect of glutamine repeats and alanine repeats on transcriptional activity [29]. Tandem repeats of glutamine and alanine can also explain the midfacial length of carnivores [30]. The brachycephalic phenotype (short snout, flat face and rounded head) in dog breeds such as the Pug and Bulldog was found to be associated with a mutation in Bmp3 [31]. The brachycephalic phenotype in the Burmese cat is associated with a 12 bp frame deletion in one copy of the Alx1 gene [32].

4. Cis-regulatory elements and evolution

Many of the genes that control facial morphology such as Bmp4 are involved in functions other than facial development; Bmp4 is not only involved in the regulation of beak size, but also plays critical roles in the development of many other organs, such as the heart [33,34]. The rapid evolution of beak size in species such as Darwin's finches requires a dramatic change in Bmp4 expression levels during beak development, whereas the expression and function of Bmp4 in the development of other organs should not be disturbed. Thus, the Bmp4-associated evolution of the beak is unlikely to be driven by the evolution of Bmp4 protein itself but would rather be driven by the evolution of tissue-specific regulatory sequences.

Cis-regulatory elements are thought to be favored by morphological evolution [35], as evolution through *Cis*-regulatory elements such as enhancers allows each tissue to evolve independently despite the shared molecular pathways and genes during development. This evolutionary decoupling of different tissues would allow rapid evolution of certain tissues while avoiding undesirable changes in other tissues.

Dramatic examples of enhancer-driven evolution have been identified, such as the loss of limbs in snake, which was found to be associated with an Shh enhancer called ZRS [36]. Substitution of the mouse ZRS into a snake resulted in truncated limbs, whereas the other Shh-regulated organs remained unaffected. Other examples include the pelvis of stickleback fish [37], the armor plates of stickleback fish [38,39], and so on.

One of the challenges of studying enhancers is identifying them. Several studies have been carried out to screen for enhancers that are active during embryogenesis of the face. Attanasio et al. screened for distant-acting enhancers using the transcriptional coactivator, P300 by performing ChIP-seq [40] during craniofacial development. Thousands of putative enhancers related to craniofacial development were identified and many of them were flanked by known craniofacial development-associated genes; knocking out some of these enhancers caused subtle but measurable changes in the craniofacial morphology of the mouse.

Compared to protein coding sequences, enhancers are generally less conserved and many enhancers that have a role in craniofacial development may not be conserved between rodents and primates. As a result, using mice as a model, we are likely to miss out many enhancers that are involved in determining the craniofacial morphology in primates. However, experiments using embryos of humans and other primates cannot be carried out because of ethical issues. To overcome this difficulty, Prescott et al. utilized iPSCs and differentiated them *in vitro* into cranial neural crest cells [41]. As neural crest cells are the major determinates of craniofacial structures [2], the species divergent *cis*-regulatory elements (CRE) detected in the *in vitro* induced neural crest cells may play roles in defining the differences in the craniofacial features between human and chimpanzee. Among the many CREs identified *in vitro*, most showed enhancer activity *in vivo* in mice, as shown by transgenic enhancer reporters, and many of them showed biased enhancer activities between the chimpanzee version and human version. These studies provided a pool of candidate CREs involved in the regulation of facial diversity. Further detailed studies of these CREs will provide great insights into the diversity of the face.

For humans, facial characters are very important in their social identity. However, compared to facial variations among different species, variations between individuals are much more subtle and hard to model. Many loci have been identified via genome-wide association studies (GWASs) [42]. Although these studies established the association between genotype and phenotype using statistical methods, they did not prove causality or reveal the underlying biological mechanisms [43]. Nevertheless, many of the genes flanking these loci—that were revealed by GWASs—such as *GLI3*, *RUNX2* [44], *PAX9* [45,46], and *PAX3* [46], are known to play a role in the craniofacial development of mice and chicken, suggesting that despite the great difference in facial appearance, the basic mechanisms of facial development are similar between birds and humans, and most of the diversity of the face may arise from the modulation of these genes.

5. Facial diversity and developmental robustness

Facial forms are highly evolvable to adapt to different diets or to different skull morphologies [47,48]. However, facial development needs to be sufficiently robust to ensure the generation of consistent facial morphology despite noise in gene expression [49] and small perturbations from the environment. Although the abundance of the gene products of many genes is known to regulate facial appearance, knocking out a single copy of many craniofacial development-related genes such as Fgf8 [16] and Bmp4 [50], does not significantly change the facial morphology, suggesting a remarkable robustness. Although seems counterintuitive, people have argued that robustness can actually enhance evolvability [51–54], as structures with high developmental robustness are less sensitive to mutations and thus allow accumulation of genetic variations at the population level.

Several mechanisms have been proposed to explain the robustness of the developmental process. Functional redundancy is one of these mechanisms. For example, knocking out the transcription factor Alx3 has no obvious effect on the mouse face [55], and mice lacking Alx4 only show minor craniofacial defects [56]. However, mice with Alx3 and Alx4 compound mutations exhibit severe frontonasal dysplasia [55]. A similar phenomenon is also found with Alx1/Alx4 [57], Six1/Six2 [58], and others. Another mechanism is the topology of the gene regulation network, including feedback loops [59–61]. This mechanism plays an important role in buffering the noise in gene expression. One example in facial development is the Bmp-regulated negative feedback loop [62].

Robustness can also arise from the nonlinear relationship between genotype and phenotype [63]. By using mice in which the expression level of Fgf8 in the developing face was genetically modified, Green et al. concluded that variation in Fgf8 has a nonlinear relationship with the morphometrics of the developing face, thus contributing to the robustness of facial morphogenesis [63]. This observation demonstrates that in addition to the buffering systems that resist perturbations in gene expression, the system of the developing face can also tolerate a wide range of gene expression variance with minimum variation in the phenotype, even for genes that play a critical role in facial morphogenesis. This also implies that the association between gene expression and facial phenotype is complex, and that the relatively linear genotype–phenotype relationships observed in Darwin's finches [20,22] may be special cases instead of representing a general principle of facial diversity.

In mice with facial development genes knocked out, in addition to changes in morphology, a dramatic increase in developmental variation was also observed frequently [16,64], suggesting that these genes stabilized the developmental process. However, the mechanisms of how these genes contribute to the robustness of facial development remain poorly understood and should be addressed in future studies.

6. Current challenges

To understand how we obtain our unique facial appearance, we need to understand facial development at different levels including the genome, regulatory sequences, gene expression, signaling pathways, cell behaviors, and physical forces that shape the facial morphology. Although we have obtained the information at different levels (Fig. 2), integrating this information remains a great challenge. For example, we have discovered many associations between the genomic variations in facial phenotypes [65–67]. It has also been found that the expression levels of many genes could determine certain facial features [19,22,68]. The cellular mechanisms of facial diversity have also been reported [3,26]; however, we know very little about how the genomic variations are “translated” into differential gene expression, and how the differential gene expression leads to differential cellular behavior. Although sequencing techniques and bioinformatics tools have rapidly

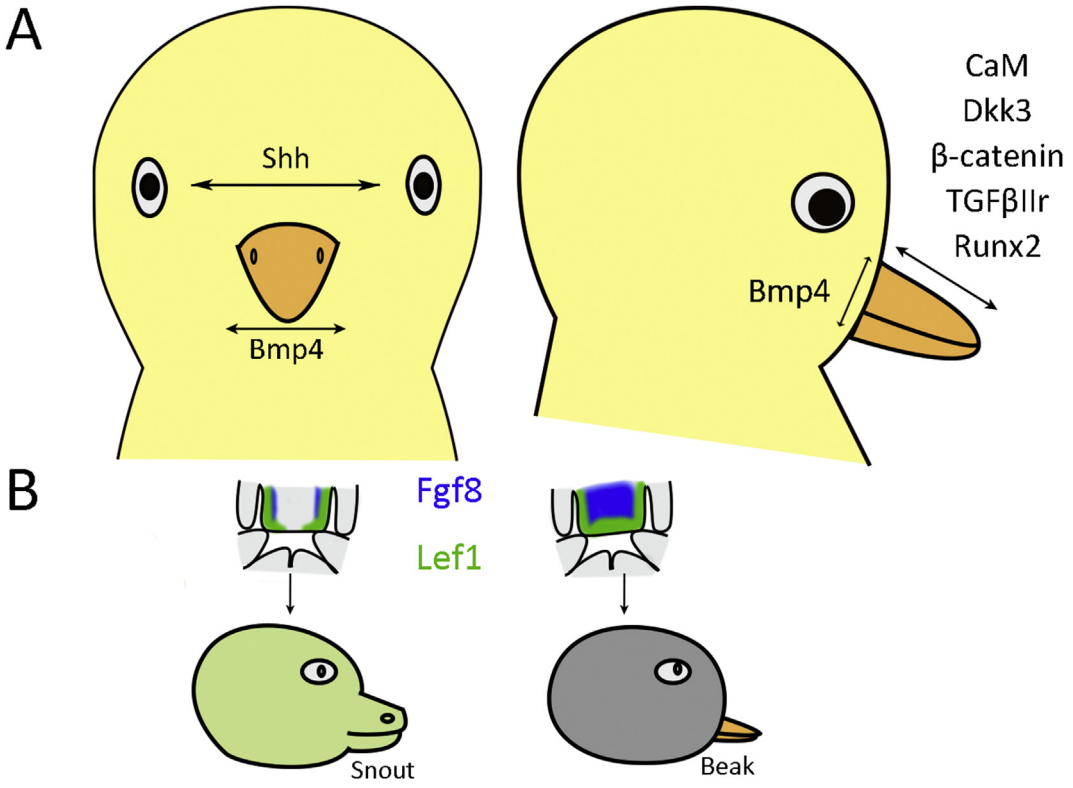

Fig. 1. The molecular regulators of facial morphology. **A** Genes that can regulate facial morphology quantitatively in a dose-dependent manner. **B** A case in which the expression pattern of certain genes during development determines the phenotype. The expression patterns of Fgf8 and Lef1 in the frontonasal process during embryogenesis are associated with the evolutionary formation of a snout or a beak [74].

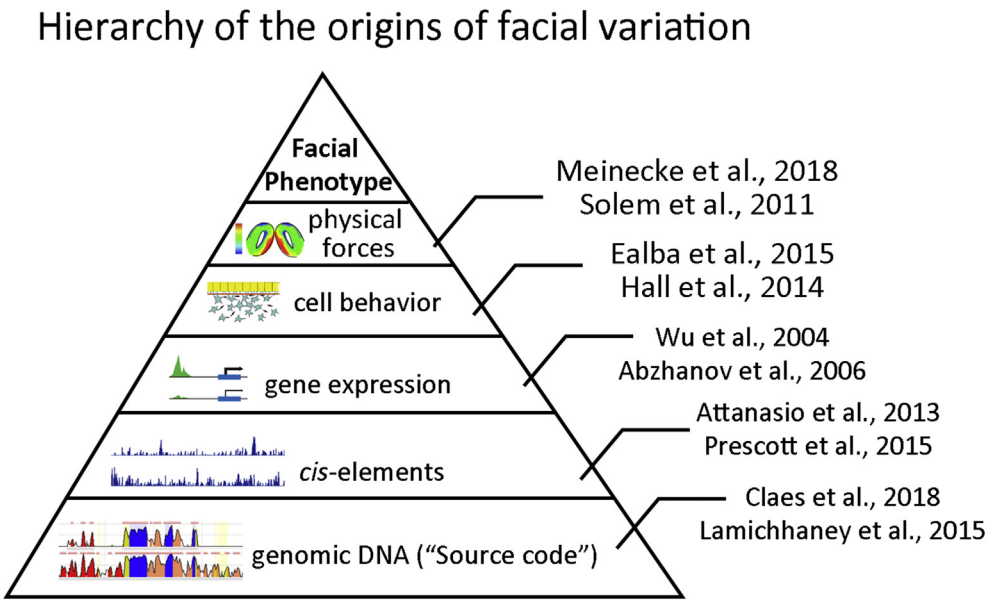

Fig. 2. Schematic of different levels of factors that cause diversity in facial morphology. The factors at higher levels could be derived from the factors at lower levels. The publications cited in this figure are included in the references [19,22,40,41,46,65,65,71–73].

improved in recent years and genotype–phonotype associations are much easier to identify, identifying the underlying biological mechanisms remains challenging. These challenges arise from different aspects. First, many phenotypes of the face result from the combinatorial effect of many loci, whereas each locus may have only a very subtle effect, and the combined effect of multiple loci could be non-additive. Moreover, facial development involves complex physical and genetic interactions between multiple facial prominences, which renders some of the gene expression-facial morphology relationships nonlinear (e.g., Young et al., 2010 [24]). Another challenge is that if we were to look at the regulation of facial variation in humans, most of our knowledge of facial

morphogenesis is derived from animal models; however, the facial appearance and embryogenesis processes in humans are different from those in mice, chicks or zebrafish, indicating that many of the human facial variations could not be easily modeled using animal models. Furthermore, many *cis*-regulatory sequences involved in human facial morphogenesis are also not conserved and cannot be directly investigated in animal models.

Without a detailed understanding of facial development, from the DNA sequence to facial morphology, can we predict the facial appearance of an individual based solely on the genetic information of an individual? Recent advances in machine learning techniques have provided hope for this possibility [69]; if this is achieved, it could have a revolutionary impact in fields such as forensic science. However, it is also argued that the great complexity of the genomic sequence-facial appearance association renders such prediction impossible [70].

7. Conclusion

Facial development is extremely complex involving interactions between different primordia and the involvement of many molecular pathways. This complexity has made great diversity possible, while it also prevented us from gaining a comprehensive understanding of facial diversity using traditional approaches. Interdisciplinary research is needed to understand how *cis*-regulatory elements, gene expression, cellular behaviors, and physical forces are integrated to shape the face. The robustness of the facial developmental process also needs to be maintained during evolution, Significant work is needed to understand how this evolvability and robustness is balanced.

Ethical statement

Ethical approval is not required for this review.

Conflicts of interest

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.job.2019.08.001.

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