copy servicing the plastid and the other taking over the role of the pre-existing (noncyanobacterial) cytosolic protein (Brinkmann and Martin, 1996). This phenomenon is known as *endosymbiotic gene replacement*, and while a few cases have been well documented, its overall contribution to the nuclear genome of plants has not been clear. Armed with the complete set of proteins encoded in the nuclear genome of *Arabidopsis* (The *Arabidopsis* Genome Initiative, 2000), Martin and co-workers were able to tackle this question on a large scale.

The researchers compared 24 990 *Arabidopsis* proteins to those encoded in a set of completely sequenced archaeal, bacterial, and cyanobacterial genomes, as well as those of yeast. From a set of 9368 proteins that produced a significant match in at least one reference genome, about 1700, or 18%, of the genes were most similar to a cyanobacterial homologue. Extrapolating to the genome as a whole, they estimated that about 4500 *Arabidopsis* nuclear genes are of cyanobacterial origin. Regardless of whether this is an overestimate or underestimate (there are arguments for both), this is an unexpectedly large number. Indeed, the estimated 4500 cyanobacterial genes in the *Arabidopsis* nucleus is over 1000 more genes than the total gene complement of the cyanobacterium *Synechocystis* (Kaneko *et al*, 1996) and over 60% of the number of genes encoded in the largest sequenced cyanobacterial genome, that of *Nostoc* (Meeks *et al*, 2001). While subsequent analysis and new data are certain to revise this estimate somewhat (eg, see Rujan and Martin, 2001), it is clear that the cyanobacterial endosymbiont gave vastly more of its genome to the host than previously appreciated.

However, the significance of this observation lies not so much in the sheer number of genes involved, but rather in the diversity of cellular functions predicted for the proteins they encode. Metabolism, cell growth and division, intracellular transport, cell organization, and transcription are all implicated. Even more remarkable, fewer than half of the cyanobacterial-like proteins in *Arabidopsis* are predicted to be targeted to the plastid, leading the authors to conclude that the impact of plastid endosymbiosis on the host was far greater than just acquiring an organelle (Martin *et al*, 2002). One of the steps in the textbook explanation of endosymbiotic organelle origins is the severe reduction of the endosymbiont and its genome. This may still be true in a fashion, but at least in plastids it appears that much of the endosymbiont genome has survived this reduction by relocating and finding a new role in the cell. Apparently endosymbiosis creates an influx of raw genetic material, and the mixing and matching of this material with existing host genes fosters a period of invention for the host.

Decades after the general acceptance of an endosymbiotic origin for plastids, various aspects of the process and its implications remain to be fully understood. One aspect of plastid evolution that may be interesting to consider in the light of these new findings is secondary endosymbiosis. While all plastids are ultimately derived from the original endosymbiosis between a eukaryote and a cyanobacterium, plastids have also spread laterally among eukaryotes. Secondary endosymbiosis occurs when a eukaryotic alga is swallowed by a second, heterotrophic, eukaryote and the two integrate to form a new algal lineage (Archibald and Keeling, 2002). This phenomenon accounts for much of algal diversity, and the genetic contribution of these endosymbionts to their hosts is particularly interesting since the endosymbiont brings with it a large, eukaryotic genome. The integration of endosymbiont nuclear genes into the secondary host nucleus should be easier, because the eukaryotic genes and the proteins they encode may be more easily incorporated into their new eukaryotic background than prokaryotic genes. However, such replacements will be far more difficult to detect since the host and the endosymbiotic alga are both eukaryotes, and therefore much more closely related to each other than to cyanobacteria. To date, little sequence information exists from the nuclear genomes of most of these organisms, but in time these genomes should provide another new glimpse into the effects of endosymbiotic mergers at the molecular level. ■

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Evolution and development

Making jaws

T Schilling

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Until now, it was unclear as to which genes control differences between the upper and lower jaw of the vertebrate head. Depew and his colleagues take a bite out of this problem by creating mutant mice in which the lower jaw is transformed into a copy of the upper jaw (right down to the whiskers).

Jawed vertebrates evolved from jawless ancestors over 400 million years ago, and the evolution of a biting lower jaw was a critical step in vertebrate evolution. Comparative studies of vertebrate embryos suggest that lower jaws arose during evolution through changes in patterning along the proximodistal (PD) axis of the jaw as it forms.

The jaw is one of a series of segmentally reiterated structures called pharyngeal arches, and its PD axis, like appendages such as limbs, extends from the base of the arch to its tip (Figure 1). How this axis is established is still debated, but like limbs it clearly involves *Dlx* homeobox transcription factors related to *distalless*, a key regulator of appendage development in Drosophila.

Depew *et al* (2002) and his colleagues in a new article published in *Science* take our understanding a step further. They demonstrate that two closely related *Dlx*

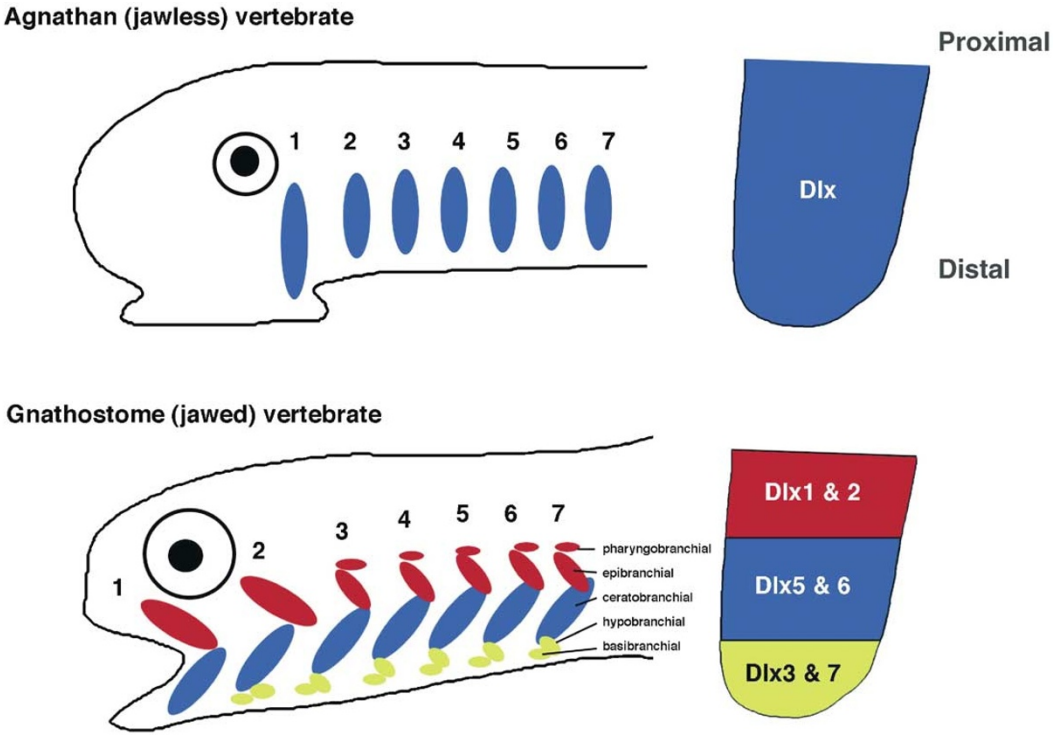

Figure 1 Roles for *Dlx* transcription factors in patterning along the proximal–distal axis of the pharyngeal arches in agnathan and gnathostome vertebrates. Homologous pharyngeal arch segments are numbered 1–7; skeletal elements and the *Dlx* genes they express are color-coded (red = dorsal; blue = intermediate; yellow = ventral).

genes play synergistic roles in specifying the lower jaw.

At first glance, jaw formation seems extremely complex. There are cellular contributions from all three embryonic germ layers: pharyngeal mesoderm, endoderm and neural crest that migrates out of the ectoderm (Noden, 1983). Extensive migration of the neural crest must occur and crest cells then form over 20 different bones of the jaw skeleton in a mouse, each of which has a distinct size and shape. Little is known about how individual bones are specified during these cellular rearrangements or how their formation is coordinated with surrounding tissues.

What we do know is that homeotic (*Hox*) transcription factors are early developmental switches that specify the segmental identities of these neural crest cells (Trainor and Krumlauf, 2001). Closely related *Hox* genes are expressed in overlapping or 'nested' sets of segments along the anterior-posterior (AP) axis, forming a combinatorial code that is unique for every arch (intersegmental patterning). This relatively simple code of positional information determines the emergence of the segmental complexity of the head skeleton.

The formation of jaws, like limbs, is mediated by a second phase of patterning. After the neural crest migrates into the pharyngeal region, it subdivides into distinct arch fields that have their own axes (intrasegmental patterning). Again, at this stage there are nested patterns of transcription factors expressed within an arch field, notably genes of the *Dlx* family.

Dlx genes are physically linked to the *Hox* clusters, and like them have undergone several duplication events in the vertebrate lineage, resulting in multiple genes with similar patterns of expression in the arches (Simeone *et al*, 1994; Stock *et al*, 1996). However, different pairs of *Dlx* genes differ in where they are expressed, that is, their expression domains. It is these expression domains that divide up the arch field into zones, which appear to correspond to individual bones (Figure 1). Thus, it seems that pairs of closely related *Dlx* genes, acting in combination, enable programmed differentiation of the bones of the jaw.

One means of investigating the precise roles these genes play is, like Depew and his colleagues, to see what happens when they do not work. Previous studies like this in mice showed that *Dlx1* and *Dlx2* were both required for the development of a normal proximal arch skeleton, including the upper jaw (Qiu *et al*, 1995, 1997). However, despite these genes also being distally expressed, mice without *Dlx1* and *Dlx2* function still had normal distal structures.

One explanation for this, which Depew and his co-authors investigated, was that two other distally expressed *Dlx* genes, *Dlx5* and *Dlx6*, are the key to differentiation of the distal structures, including the lower jaw. They confirmed this hypothesis, showing for the first time that in mice without *Dlx5* and *Dlx6* function (*Dlx5/6*$^{-/-}$ double mutants), lower jaw bones are replaced by small distal bones that resemble the upper jaw (Acampora *et al*, 1999; Depew *et al*, 1999, 2002; Robledo *et al*, 2002).

The mechanism underlying this amazing transformation is still unclear. Most evidence suggests that distal arch cells are transformed into proximal cells in these mice, but this is hard to determine from the skeletal information alone. Strong evidence in favor of this explanation comes from the correlated changes in the soft tissues of the lower jaw, even down to the duplication of whisker barrels. Formally proving, however, that a transformation is taking place will require following the fates of distal arch cells directly in mutants. Furthermore, interpretations in this new study are limited by a lack of very good molecular markers for proximal cells.

The new study also revealed exciting new evidence for at least two important signaling centers in an arch field. One lies in mesenchyme that underlies

growth zones in the arch; *Bmp7* and *Wnt5A* expression in the mesenchyme both require *Dlx5* and *Dlx6*. Signals from this mesenchyme are required to maintain signals in the surrounding epithelia (Ferguson *et al*, 2000). In many respects, these interactions resemble those that occur between the apical ectodermal ridge and mesenchyme of the limb bud, where *Dlx* genes are also required.

A second, unexpected signaling center must lie near the center of the arch field, since the transformations produced by loss of *Dlx5* and *Dlx6* functions are mirror-image duplications along the PD axis (intrasegmental duplicates of the upper jaw). Significantly, mice that lose the function of some *Hox* genes undergo intersegmental mirror-image duplications along the AP axis, suggesting that this may be a common aspect of homeotic duplications of adjacent fields of cells in vertebrates.

Perhaps there is an organizing center at the junction between proximal and distal cells that polarizes the arch field. *Fgf8*, which is maintained in the *Dlx5/6*$^{-/-}$ mutants, is one prime candidate for the signal (Trumpp *et al*, 1999). Thus, a combination of cell intrinsic (*Dlx*) and extrinsic (*Fgf8*) factors is probably required to establish the skeletal pattern. This is the first genetic demonstration of the existence of such an organizing center in the jaw.

Biological processes are seldom simple, but this work shows that even something as complex as the development of a jaw can be understood as a series of much more simple developmental steps. Pharyngeal arches are evolutionarily ancient, predating vertebrates, and our jawless, agnathan ancestors clearly had homologous head segments patterned by *Hox* genes. Studies in living agnathans, such as lampreys, however, suggest that primitively *Dlx* genes were expressed more uniformly throughout the arches and this correlates with a lack of any obvious PD subdivisions (Kimmel *et al*, 2001; Neidert *et al*, 2001) (Figure 1).

One tempting hypothesis is that the duplication and divergence into nested expression of the *Dlx* gene family allowed differentiation to occur along the proximal–distal axis within arches and thereby drove jaw evolution. Alternatively, agnathans may have secondarily lost what was primitively a more regionalized pattern in the arches. This can be addressed by examining outgroups to the vertebrates, such as amphioxus, for these aspects of arch patterning including putative signaling centers that may have played an important role in arch evolution.

The work by Depew and his collaborators may also have much more practical implications than these. One question the new study suggests is: What are the molecular pathways through which *Dlx* genes confer positional identities to cells in the jaw? Defects in any component of these pathways may cause congenital defects in humans such as micrognathia and deafness. Thus, future research that untangles this mechanism could be vital if we are to understand and effectively treat such defects. ■

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