ly occurs. Depletion of two factors requisite for NMD, Upf1 and Upf2, abrogated NMD of mutant T cell receptor mRNAs. In contrast, reading frame–dependent NAS proved resistant to Upf2 depletion (*1, 2*). Yet, because both processes do require Upf1, there is clearly some mechanistic overlap between them.

In their study, Mendell *et al.* used RNA interference (RNAi) to eliminate Upf1 and Upf2 from their test cells. RNAi can be initiated in mammalian cells by incubating them with small pieces of double-stranded RNA, one strand of which is complementary to a target mRNA. These small double-stranded RNAs cause the targeted mRNA to be degraded. RNAi allows the researcher to "knock down" individual mRNAs, and thereby remove the proteins they encode, without having to introduce mutations at the DNA level. After determining a knockdown phenotype, one generally wants to know what specific part of the target protein is responsible for the eliminated activity. Thus, having shown that Upf1 is necessary for both NMD and reading frame–dependent NAS, Mendell *et al.* (*1*) wondered whether Upf1 plays the same role in both processes. To address this question, they used a strategy known as "allele-specific RNAi." By changing the sequence of the Upf1 gene encoded on a plasmid, they created a version of Upf1 that was not subject to RNAi by the double-stranded RNAs used to knock down the endogenous protein. They could then rescue both NMD and NAS in the RNAi-induced cells by introducing this altered plasmid. This strategy allowed them to determine the effects of two different substitution mutations in conserved regions of Upf1. One of these mutations supported NAS, but failed to rescue NMD. Thus, the functions of Upf1 in NAS and NMD are genetically separable, again demonstrating that these two processes are related yet mechanistically different.

Although the two new studies rule out one possible mechanism for reading frame–dependent NAS, we are left with many more unanswered questions. Of utmost interest is the unknown mechanism by which recognition of the mRNA reading frame can feed back to alter pre-mRNA splicing. One way to address this problem is to identify the proteins required. Identification of Upf1 as the first such factor thus represents a crucial step toward this goal.

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PERSPECTIVES: EVOLUTION

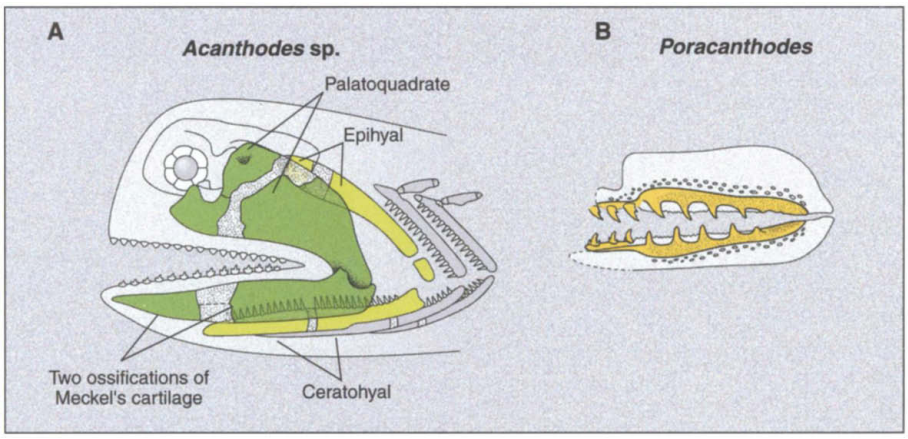
Jaws of the Fates

Georgy Koentges and Toshiyuki Matsuoka

In his poem about the lonely "Maldive Shark," Herman Melville describes the daunting jaws of a serious meat-eater, which serve as an asylum for the sleek little pilot fish, azure and slim, hiding in his "jaws of the Fates." The "jaws of the Fates" may act rather unpredictably in the uncharted oblivion of the Indian Ocean, but Depew *et al.* (*1*) report a remarkable catch—the genes that dictate the fates of jaws—on page 381 of this issue. These authors turn lower jaws into upper jaws by simultaneously inactivating the homeobox genes *Dlx5* and *Dlx6* of mice. Such a spectacular transformation of "jaw identity" unveils a family of genes that are crucial for directing formation of the vertebrate face. This gene family may have been subject to profound modifications during vertebrate evolution and in certain human congenital diseases. The *Dlx5* and *Dlx6* genes are now implicated in the elaboration of vertebrate lower jaws, from the ferocious feeding machinery of the great white shark to the sophisticated hearing system of mammals.

Enhanced online at www.sciencemag.org/cgi/content/full/298/5592/371

A complex series of cellular and molecular interactions underlies the assembly of the vertebrate face. Most structures are formed by the neural crest, a tissue that emanates from the early embryonic brain and populates the so-called branchial arches (*2*). Branchial arches are a segmental series of bulges in the embryonic head and are predecessors of all facial elements. Within the first (mandibular) branchial arch, jaw elements develop from three bulges: the mandibular, maxillary, and frontonasal processes that are filled by neural crest cells from different origins (midbrain and hindbrain) (*3*). Widespread mixing between them supports the notion that jaw neural crest is exposed to instructive signals from its environment that establish a proximodistal axis to the jaw-forming branchial arch (*3*). Such external cues are translated into a code of neural crest proximodistal "identity," leading to the precisely orchestrated formation of skeletal and muscular elements. Much evidence implicates the *Hox* homeobox genes as the encoders of "rostrocaudal identity" in all branchial arches posterior to the jaw-forming arch. However, until now no genes have been proven to act as true selector genes for proximodistal identity of neural crest cells in branchial arches.



A more symmetrical smile. (**A**) Skeleton of the head of a jawed vertebrate, *Acanthodes*, showing symmetry between upper and lower jaw elements (green) and hyoid elements (yellow). The upper jaw (palatoquadrate) and lower jaw are both subdivided in two by a cartilaginous bridge. (**B**) Jaws of the acanthodian *Poracanthodes* with symmetrical jaws and dentition (yellow). The success of jawed vertebrates is partly attributable to the morphological independence (that is, asymmetry) of upper and lower jaws encoded by differentially expressed *Dlx* genes. Elaboration of this developmental code imbued vertebrates with hearing machinery and a wide variety of feeding capabilities. [Adapted from (*8*)]

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Jawed vertebrates have three pairs of *Dlx* homeobox genes—*Dlx1/2*, *Dlx5/6*, and *Dlx3/7*—that are expressed in restricted domains across the proximodistal axis of the branchial arches (*4*). Their nested expression within the branchial arches and the fact that their *Drosophila* homolog *distalless* is a master regulator of distal leg identity make the *Dlx* genes excellent candidates for encoding distal identity in vertebrates. In all bilateral organisms, *distalless* genes appear to be involved in controlling the outgrowth of body appendages (*5*). Thus, the idea that the vertebrate *Dlx* homologs serve a similar function is attractive. Rather disappointingly, mice missing a single *Dlx* gene exhibit only piecemeal changes in the identities of isolated skeletal elements and teeth. This finding suggested that *Dlx* genes act as "micromanagers" rather than as "master regulators." Now, Depew and colleagues report the striking phenotype of the *Dlx5/6* double mutant mouse (*1*). They provide evidence that *Dlx5* and *Dlx6* are indeed the selectors of distal branchial arch identity. Their work suggests that the absence of a clear phenotype in mice lacking one *Dlx* gene is due to compensation by other coexpressed *Dlx* genes. Thanks to their discovery, the concept of a proximodistal molecular identity code is alive and well.

The phenotype of the *Dlx5/6* double mutant mouse is complex but remarkably clearcut. All of the skeletal elements below the primary jaw joint (the joint between the malleus and incus of the mammalian middle ear) are missing. Even more intriguing, in the region of the lower jaw, the mutant mice possessed a second complete set of bona fide upper jaw elements. During the early evolution of mammals, the major upper jaw element (the so-called palatoquadrate) became fragmented. Parts of the palatoquadrate became fused to the braincase—the "tennis racket"-shaped alisphenoid bone in figure 3E and supplementary figure 3D of the Depew *et al.* paper—or gave rise to elements of the mammalian pharynx (pterygoid). Other parts such as the quadrate turned into the mammalian incus, and the hyomandibula became the third middle ear bone (the stapes)—all elements of a new hearing apparatus (*6*). In the *Dlx5/6* double mutant mice all of these elements are duplicated, resulting in a symmetrical instead of an asymmetrical mouth.

If *Dlx5* and *Dlx6* are distal selector genes, then where does the initial patterning information for the branchial arch proximodistal axis come from? A recent elegant paper by Couly *et al.* sheds light on this difficult question (*7*). By transplanting pharyngeal endoderm into different locations in the developing chick head, Couly *et al.* generated jaw duplications that are remarkably similar to those obtained by Depew *et al.* (*1*). A growing body of evidence suggests that initial cues from the pharyngeal endoderm impose a first proximodistal patterning axis onto the adjacent branchial arch neural crest cells. This prepattern is then "interpreted" by neural crest cells, resulting in the nested expression of *Dlx* gene pairs, *Dlx5/6* and *Dlx3/7*, by these cells. If this is the case, what does the *Dlx5/6* mutant phenotype tell us about the evolution of jaws?

Although upper and lower jaw elements have never been completely symmetrical during vertebrate history, early jawed vertebrates related to the ancestors of bony fish (such as acanthodians) experimented with the shape and symmetry of their jaws. Acanthodians, for example, sometimes displayed remarkable symmetry between their upper and lower jaw elements (see the figure, part A, green) as well as in their dentition (see the figure, part B, yellow) (*8*). Such symmetrical features, which disappeared later in evolutionary history, now reappear in the *Dlx5/Dlx6* double mutant mice.

The Depew *et al.* work suggests that lower jaw patterning that is dependent on *Dlx5/6* expression may have been elaborated and embellished between the phylogenetic nodes of jawed vertebrate and bony fish ancestors. Going back one step further in evolutionary history, jawless vertebrates such as lampreys only have four *Dlx* genes with unclear homologies to their jawed vertebrate counterparts (*9*). All lamprey *Dlx* genes are expressed in branchial arches, but a nested expression pattern appears to be the invention of the jawed vertebrates (*10*). Our knowledge of the enhancer organization that controls this nested *Dlx* gene expression in jawed vertebrates is still rudimentary. Comparative genomic and functional studies of the regulatory elements controlling *Dlx* gene expression in lampreys, sharks, bony fish, coelacanths, and tetrapods will reveal the molecular evolution of the proximodistal code that underlies the shapes and fates of jaws.

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PERSPECTIVES: OPTICS

A New Low for Nonlinear Optics

M. C. Downer

In the first nonlinear optics experiment, Franken *et al.* (*1*) focused a 3-joule red ruby laser pulse into a quartz crystal to generate a few nanojoules of ultraviolet light at exactly twice the incident frequency. The photographic recording of the signal was so weak that the editor mistook it for a blemish and erased it before publication. The memory of nonlinear optics might have been erased, too, except that since then, a series of breakthroughs has increased the efficiency of many nonlinear optical frequency conversion processes by orders of magnitude, transforming them into useful tools for science and technology.

In the 1960s, researchers discovered a class of crystals, now standard in laser laboratories, that could convert pulses one-thousandth as strong as Franken's ruby pulse to another color with as much as 50% efficiency, simply by matching the index of refraction of the input and output frequencies (*2*).

In the 1990s, a new class of synthetic nonlinear optical multilayer structures was developed and commercialized that efficiently converted still weaker, fixed-frequency beams from small solid-state lasers to tunable visible and infrared radiation for applications in materials processing, remote sensing of environmentally sensitive gases, and interferometry (*3*).

Along an independent line, chemists discovered in the 1970s that another notoriously weak nonlinear optical process, spontaneous Raman scattering, used to fingerprint molecular vibrations (*4*), could be enhanced dramatically by attaching molecules to rough metal surfaces or metal nanoparticles (*5*). With this approach, Raman spectra of single molecules can now be measured (*6*).

On page 399 of this issue, Benabid *et al.* (*7*) report a breakthrough in the nonlinear optics of molecular gases that adds a new milestone to these historical examples. After pressurizing the hollow core (HC) of a meter-long glass photonic-crystal fiber (PCF) with hydrogen gas, the authors

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