Developmental Regulatory Genes and Echinoderm Evolution

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Abstract.—Modified interactions among developmental regulatory genes and changes in their expression domains are likely to be an important part of the developmental basis for evolutionary changes in morphology. Although developmental regulatory genes are now being studied in an increasing number of taxa, there has been little attempt to analyze the resulting data within an explicit phylogenetic context. Here we present comparative analyses of expression data from regulatory genes in the phylum Echinodermata, considering the implications for understanding both echinoderm evolution as well as the evolution of regulatory genes in general. Reconstructing the independent evolutionary histories of regulatory genes, their expression domains, their developmental roles, and the structures in which they are expressed reveals a number of distinct evolutionary patterns. A few of these patterns correspond to interpretations common in the literature, whereas others have received little prior mention. Together, the analyses indicate that the evolution of echinoderms involved: (1) the appearance of many apomorphic developmental roles and expression domains, some of which have plesiomorphic bilateral symmetry and others of which have apomorphic radial symmetry or left–right asymmetry; (2) the loss of some developmental roles and expression domains thought to be plesiomorphic for Bilateria; and (3) the retention of some developmental roles thought to be plesiomorphic for Bilateria, although with modification in expression domains. Some of the modifications within the Echinodermata concern adult structures; others, transient larval structures. Some changes apparently appeared early in echinoderm evolution (>450 Ma), whereas others probably happened more recently (<50 Ma). Cases of likely convergence in expression domains suggest caution when using developmental regulatory genes to make inferences about homology among morphological structures of distantly related taxa. [Echinodermata; evolution of development; homeobox gene; homology; Metazoa; regulatory gene.]

The genetic and developmental bases for morphological change in evolution are poorly understood (Raff, 1996; Arthur, 1997; Hall, 1992). The importance of understanding this relationship is clear, but the means to study it has long remained elusive. Until recently, it was difficult to imagine a way of identifying specific changes in particular genes responsible for a given morphological transformation or novelty. Metazoan genomes contain on the order of $10^4$ genes scattered through ~0.1 to 3 gigabases of DNA (Collins, 1995), making such a search seemingly impossible. This situation is changing rapidly, however. Progress in understanding the developmental genetics of “model” organisms during the past two decades is generating information and technical tools that provide increasingly direct access to the underlying bases of morphological evolution. It will probably never be possible to compile a complete list of the genetic changes responsible for any but the simplest evolutionary transformations in morphology. However, we are now able to identify some of the genes involved in even very complex transformations (e.g., Averof and Cohen, 1997; Lowe and Wray, 1997; Shubin et al., 1997).

Most regulatory proteins that function in animal embryos derive from a small number of gene families that predate the origin of the Bilateria (Schubert et al., 1993; Duboule, 1994; Atchley and Fitch, 1997; Gerhart and Kirschner, 1997) or even the Metazoa (Bharathan et al., 1997). These proteins have become famous for regulating similar developmental processes in dissimilar and distantly related animals (DeRobertis and Sasai, 1996; Gerhart and Kirschner, 1997). However, it is also evident that these proteins regulate a wide range of developmental processes that must have evolved...

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eral regulatory genes during echinoderm evolution. These changes underscore how evolutionarily malleable developmental regulatory genes can be, a contrast to their famously conservative roles.

REGULATORY GENES AND THEIR EVOLUTION

Most studies of molecular evolution have examined changes in the sequences of metabolic enzymes, structural proteins, and ribosomal RNAs (Nei, 1987; Gillespie, 1991; Li, 1997). Relatively little attention has been paid to regulatory genes, or to changes in the noncoding cis-regulatory regions surrounding genes. Yet there are compelling reasons for expecting changes in the expression of regulatory genes, and changes in interactions among them, to be the loci of many mutations that produce phenotypic change (Britten and Davidson, 1969; Wilson, 1975; Raff and Kaufman, 1983; Arthur, 1997; Gerhart and Kirschner, 1997). It follows that comparative analyses of regulatory genes, their interactions, and mechanisms of transcriptional regulation in general will form an important component of our understanding of the genetic basis for morphological evolution.

To understand how regulatory genes might contribute to modifications in morphology, it is important to realize that changes in the coding sequence of a gene are unlikely to be the only factor involved, and perhaps not even a major factor. This prediction is supported on both theoretical and empirical grounds. First, a theoretical consideration: It is possible for any gene to come under the control of a new regulatory circuit without a change in the coding sequence of any gene. All that this requires is the appearance of a cis-regulatory element that serves as a binding site for a transcription factor. Many binding sites of this nature are only 6 to 10 nucleotides long, tolerate some sequence variation, can lie in either orientation, and may reside anywhere within thousands of bases of the transcriptional start site (Arnone and Davidson, 1997; Gerhart and Kirschner, 1997). The appearance of new cis-regulatory elements by random mutation is therefore inevitable. In some cases, the result is that an existing
gene becomes entrained into a new regulatory circuit. Second, an empirical observation: The coding region of a regulatory gene from one species can often functionally substitute for its ortholog in another species. This is true of many cases, even when such “gene swaps” have been made between phyla (e.g., Vaux et al., 1992; Tomarev et al., 1997). In each case, the transplanted gene product activates or regulates developmental processes in the host species that they do not normally regulate in the donor species. The fact that they can do so demonstrates the relatively minor role that changes in coding sequences of regulatory genes need play in mediating morphological change.

Thus, a focus of attention in evolutionary comparisons of regulatory genes must lie in the interactions among genes and gene products. Unfortunately, these interactions are exceedingly complex (Arnone and Davidson, 1997). Individual regulatory genes are components in extensive networks of interacting genes (Fig. 1). The products of several other genes regulate when, where, and at what level a particular regulatory gene is expressed. The protein produced from that regulatory gene in turn modulates either the expression of other “target” genes or the function of their products. These regulatory circuits remain poorly understood, even in “model” organisms, but involve feedback, autoregulation, and integration of multiple signals (Kirchhamer and Davidson, 1996; Arnone and Davidson, 1997). Regulatory genes interact with each other indirectly, through cis-regulatory regions, protein–protein binding, phosphorylation, proteolytic cleavage, hormonal intermediaries, second-messenger systems, and a variety of other mechanisms that have rarely been considered in an evolutionary context (but see Gerhart and Kirschner, 1997).

The simplest and most direct interactions between regulatory genes occur when one gene encodes a transcription factor that modulates the expression of another. The evolution of these interactions can readily be studied indirectly, using in situ hybridization and immunolocalization, which are techniques that reveal where and when a particular gene or gene product is expressed during development. Data of this kind for multiple species can reveal evolutionary changes in gene interactions. An apomorphic time, location, or level of expression implies changes in linkages that lie genetically upstream of the gene (Fig. 1b). An apomorphic expression domain in a tissue or cell type in which expression in other species is lacking implies recruitment to a derived developmental role, which in turn implies interactions with different downstream targets (Fig. 1c). Many studies have used this approach to document evolutionary changes in the expression domains and roles of regulatory genes in a variety of metazoan phyla (e.g., Garcia-Fernández et al., 1991; Salser and Kenyon, 1992; Shenk et al., 1993; Wedeen and Weisblat, 1991; Degnan and Morse, 1993; Master et al., 1996; Savage and Shankland, 1996; Corbo et al., 1997; Grenier et al., 1997; Lowe and Wray, 1997; Panganiban et al., 1997; Pilon and Weisblat, 1997; Wada et al., 1997; Telford and Thomas, 1998; Abzhanov and Kaufman, 1999; Ogasawara et al., 1999).

**REGULATORY GENE EXPRESSION IN ECHINODERMS**

Echinoderms are among the more bizarre products of the metazoan radiation. Although they are radially symmetrical as adults, they begin development as bilaterally symmetrical embryos and larvae. They are generally considered to lack a head and brain (but see Jefferies et al., 1996), yet they apparently evolved from deuterostome ancestors that probably had both (Brusca and Brusca, 1990; Nielsen, 1995). They also possess a suite of distinctive synapomorphies: the water vascular system, an organ system involved in locomotion, respiration, sensation, and feeding; a mineralized endoskeleton with a characteristic porous microstructure; and a central nervous system that lies perpendicular to the gut (Brusca and Brusca, 1990; Nielsen, 1995). Besides being highly derived, the phylum Echinodermata encompasses an unusually broad range of morphological disparity, particularly in terms of body organization. This disparity encompasses some transformations that are exceedingly unusual elsewhere within the Metazoa: changes in symmetry number, the origin of bilateral symmetry superimposed
Figure 1. Evolution of interactions among regulatory genes. The most direct interactions among regulatory genes occur when one gene encodes a transcription factor that influences the expression of another gene within the same cell (Arnone and Davidson, 1997). Interactions are often less indirect, involving numerous intermediate processes such as cell–cell signaling and signal transduction (Gerhart and Kirschner, 1997). For the sake of simplicity, this diagram illustrates interactions among loci encoding transcription factors. (a) Various transcription factors bind to specific cis-regulatory sites within the promoter of the gene of interest, thereby modulating its expression. These genes are said to lie genetically upstream of the gene. The product of the gene is itself a transcription factor, which binds to sites within the promoters of genetically downstream "target" genes, in turn modifying their expression. Several kinds of evolutionary changes can evolve in such systems. (b) The expression of the gene of interest can change in various ways, including the timing, location, and level of expression. These changes can arise from mutations in cis-regulatory regions of the gene itself, or from changes in the presence or relative abundance of transcription factors in the nuclei of particular cells. In either case, an altered array of transcription factors now interacts with its regulatory sequences. (c) Changes can also evolve in the target genes with which the protein product of the gene interacts, including new interactions and losses of existing interactions. These changes can arise from changes in the coding region of the gene of interest, altering the specificity of its protein product, or from changes in the target genes or their products. Thus, functionally equivalent changes could have very distinct genetic bases.
## Table 1. Expression domains of some developmental regulatory genes in echinoderms.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phasea</th>
<th>Locationb</th>
<th>Taxac</th>
<th>Referenced</th>
<th>Evolutionary inferencee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachyury</td>
<td>E, L</td>
<td>secondary mesenchyme</td>
<td>E</td>
<td>A, B</td>
<td>recruited role</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>oral ectoderm</td>
<td>A</td>
<td>C</td>
<td>recruited role</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>coeloms</td>
<td>B</td>
<td></td>
<td>recruited role</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>prospective adult ectoderm</td>
<td>E</td>
<td>B</td>
<td>recruited role</td>
</tr>
<tr>
<td>β-catenin</td>
<td>E</td>
<td>vegetal plate of embryo</td>
<td>E</td>
<td>D</td>
<td>recruited role</td>
</tr>
<tr>
<td>distal-less</td>
<td>L</td>
<td>ciliated band (neurons?)</td>
<td>E</td>
<td>E</td>
<td>recruited role</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>prospective adult ectoderm</td>
<td>E</td>
<td>E</td>
<td>recruited role, ~250 Ma</td>
</tr>
<tr>
<td></td>
<td>R-P</td>
<td>podia, during outgrowth</td>
<td>A, E, H</td>
<td>E, F, G</td>
<td>recruited role, &gt;450 Ma</td>
</tr>
<tr>
<td>engrailed</td>
<td>P</td>
<td>ectoderm in radial nerves</td>
<td>O</td>
<td>F</td>
<td>plesiomorphic/recruited role</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>ectoderm, near skeletogenesis</td>
<td>O</td>
<td>F</td>
<td>recruited role</td>
</tr>
<tr>
<td>ets-4</td>
<td>E</td>
<td>nonvegetal cells of embryo</td>
<td>E</td>
<td>H</td>
<td>recruited role</td>
</tr>
<tr>
<td>FGF receptor</td>
<td>L, P</td>
<td>muscle cells</td>
<td>E</td>
<td>I</td>
<td>recruited role</td>
</tr>
<tr>
<td>forkhead</td>
<td>L</td>
<td>posterior gut</td>
<td>E</td>
<td>J</td>
<td>recruited role</td>
</tr>
<tr>
<td>hedgehog</td>
<td>L</td>
<td>coelom</td>
<td>E</td>
<td>K</td>
<td>recruited role</td>
</tr>
<tr>
<td>Hox-3</td>
<td>R</td>
<td>tooth sacs</td>
<td>E</td>
<td>L</td>
<td>recruited role</td>
</tr>
<tr>
<td>Hox-7</td>
<td>L</td>
<td>posterior ectoderm</td>
<td>E</td>
<td>M</td>
<td>recruited role</td>
</tr>
<tr>
<td>MEF-2</td>
<td>L</td>
<td>larval muscle cells</td>
<td>E</td>
<td>N</td>
<td>plesiomorphic role</td>
</tr>
<tr>
<td>not</td>
<td>E</td>
<td>secondary mesenchyme</td>
<td>E</td>
<td>B</td>
<td>recruited role</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>prospective adult ectoderm</td>
<td>E</td>
<td>B</td>
<td>recruited role</td>
</tr>
<tr>
<td>Notch</td>
<td>E</td>
<td>secondary mesenchyme</td>
<td>E</td>
<td>O</td>
<td>recruited role</td>
</tr>
<tr>
<td>orthodenticle</td>
<td>E-L</td>
<td>ectoderm, oral ectoderm</td>
<td>E</td>
<td>P</td>
<td>recruited role</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>ectoderm, ciliated band</td>
<td>H</td>
<td>F, Q</td>
<td>recruited role, &lt;100 Ma</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>podia</td>
<td>O, E, A, H</td>
<td>F, G, R</td>
<td>recruited role, &gt;450 Ma</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>Aristotle’s lantern</td>
<td>E</td>
<td>R</td>
<td>recruited role</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>circum oral ectoderm</td>
<td>E</td>
<td>R</td>
<td>recruited role</td>
</tr>
<tr>
<td>runt</td>
<td>E</td>
<td>blastula</td>
<td>E</td>
<td>S</td>
<td>recruited role</td>
</tr>
<tr>
<td>snail</td>
<td>E</td>
<td>all mesenchyme</td>
<td>E</td>
<td>T</td>
<td>plesiomorphic/recruited role</td>
</tr>
<tr>
<td>twist</td>
<td>E</td>
<td>mesoderm</td>
<td>E</td>
<td>U</td>
<td>plesiomorphic/recruited role</td>
</tr>
<tr>
<td>Wnt-7</td>
<td>P</td>
<td>neurons, radial nerves</td>
<td>E</td>
<td>V</td>
<td>recruited role</td>
</tr>
</tbody>
</table>

*aE = embryo, L = larva, R = imaginal rudiment during preparation for metamorphosis, P = postmetamorphic juvenile or adult (or both). Note that expression may occur at other times that have not been examined (in particular, during and after metamorphosis for most genes).

*bCell type, tissue, or region where expression has been documented. Note that expression may occur in other regions that have not been examined.

*cA = Asteroidea (seastars), O = Ophiuroidea (brittle stars and basket stars), E = Echinoidea (sea urchins and sand dollars), H = Holothuroidea (sea cucumbers). Note that the expression domain in question may exist in other taxa besides those listed, because in most cases only one echinoderm species has been examined (usually an echinoid).

*dA = Harada et al. (1995); B = Peterson et al. (1998); C = Shoguchi et al. (1999); D = Logan et al. (1999); E = L. Issel-Tarver and G. Wray, unpubl.; F = Lowe and Wray (1997); G = Paganian et al. (1997); H = Wei et al. (1999); I = McCoon et al. (1998); J = Harada et al. (1996); K = Hertzler et al. (1996); L = Arenas-Mena et al. (1999); M = Angerer et al. (1989); N = Venuti et al. (1993). J. Venuti, pers. comm.; O = Sherwood and McClay (1997, 1999); P = Gan et al. (1995); Q = Lowe and Wray, unpubl.; R = Wray, Lowe, Wikramanyake, and Klein, unpubl.; S = Coffman et al. (1996); T = J. Hardin, pers. comm.; U = Weng et al. (1998); V = Ferkowicz and Raff, pers. comm.

*eSimplest evolutionary interpretation, given the limited taxonomic sampling available at the time of writing. Expression in a structure apomorphic for or within the phylum is interpreted as recruitment. Expression in a plesiomorphic structure is provisionally interpreted as recruitment if its expression has not been described in that structure in other phyla. Expression that bears vague similarity to that in another phylum is interpreted equivocally, as possibly plesiomorphic and possibly apomorphic. Expression that bears a specific resemblance to that in another phylum is interpreted as plesiomorphic. In the few cases in which expression data from multiple echinoderm species are available and are similar, the expression domain is regarded as homologous, and minimum estimates of when the role evolved are listed, based on phylogenetic distribution of character states and stratigraphic ranges of taxa (Smith, 1984).
Nearly all of the similarities in regulatory gene expression that do exist between echinoderms and other phyla are rather general (e.g., *engrailed* in neurons, *snail* and *twist* in mesodermal cells) instead of specific (as in the nested domains of Hox expression shared by arthropods and chordates). Because these similarities involve only a general localization to a germ layer or organ system rather than to a specific structure, it is not clear that the expression domains in echinoderms that are similar to those in chordates and arthropods share a common evolutionary origin.

During the past few years, many regulatory genes originally identified from genetic screens in “model” organisms have been isolated from and characterized in echinoderms. Table 1 summarizes the expression domains of some of these genes in echinoderms. Before examining these data in detail, note some of the biases and limitations in this data set. From the perspective of evolutionary studies, the most severe limitation is that the expression of most genes has been examined in only a single echinoderm species, usually an echinoid. This restricts most comparisons to the interphylum level and reveals nothing about variation within the Echinodermata. A second important bias is that most studies have examined only embryonic and early larval stages of development. This limits the inferences that can be made about evolutionary changes in expression domains and developmental roles associated with the most unique part of the life cycle in echinoderms, the radially symmetrical adult. Together, these limitations mean that the number of expression domains listed in Table 1 is almost certainly an underestimate, in that additional domains of expression for each of these genes may well exist during and after metamorphosis and in other echinoderm species.

Despite these limitations, the existing expression data from echinoderms provide a wealth of important information about the evolution of developmental regulatory genes in general, and, more specifically, about the evolution of the phylum. First, and most apparent to anyone familiar with the roles of these genes in arthropods and chordates, the majority of expression domains listed in Table 1 will seem unfamiliar. Indeed, there are few obvious similarities to known developmental roles of these genes in flies and mice (reviewed in Duboule [1994] and Gerhart and Kirschner [1997]).
plesiomorphically absent in echinoderms; and (3) roles and expression domains are homologous in arthropods and chordates but have been secondarily lost in echinoderms. In the last two cases, expression domains in echinoderms would represent apomorphic developmental roles. Although we cannot yet distinguish with confidence among these possibilities, it seems likely that a combination of all three patterns of change are represented by the genes listed in Table 1. Sampling additional taxa, characterizing expression in more detail, and gathering functional data on developmental roles in echinoderms should help distinguish among these possibilities. Also important will be integrating information about the evolution of morphology within the phylum, including data from the fossil record.

A second generalization that emerges from Table 1 is that many of these regulatory genes have multiple, distinct expression domains in echinoderms. For example, Brachyury and orthodenticle both have spatially and temporally distinct phases of expression during embryogenesis and metamorphosis (Gan et al., 1995; Harada et al., 1995; Lowe and Wray, 1997; Peterson et al., 1999). As mentioned earlier, multiple phases of expression are characteristic of all metazoan regulatory genes that have been examined in detail. In general, distinct expression domains within a species are expected to reflect distinct developmental roles, based on detailed functional studies in "model" taxa (e.g., for engrailed, see Kornberg et al., 1985; Doe et al., 1988; Duboule, 1994; Rogers and Kaufman, 1996). Distinct developmental roles imply that the regulatory gene in question is interacting directly or indirectly with different sets of effector genes or gene products to mediate its different roles. Exceptions include reiterated expression domains corresponding to serially homologous structures (e.g., reiterated stripes of engrailed and wingless expression associated with forming segment boundaries in insect embryos) and expression corresponding to a specific cell type that is distributed in different organ systems (e.g., myo-D and SUM-1 expression in muscle cells).

A third generalization is that many of the expression domains listed in Table 1 occur
in structures that are apomorphic for the Echinodermata as a whole or for a clade within the phylum. This implies that new developmental roles have been acquired by these genes during the origin and diversification of the phylum (Lowe and Wray, 1997). For the more extensively studied genes (e.g., Brachyury and orthodenticle), more than one apomorphic expression domain is known. As mentioned previously, the genetic basis for role recruitment is not known, but it probably includes interactions with the promoters of additional target genes (Fig. 1c). The implications of recruitment for understanding the origin of evolutionary novelties will be discussed later.

A fourth generalization is that the genes listed in Table 1 have at least one radially symmetrical domain of expression (with the possible exception of those genes that have been examined only at stages prior to radial body patterning). Of the genes examined so far, the only one that might actually be involved in establishing radial symmetry is distal-less. All of the others are expressed too late to have a radial patterning role, since the onset of their expression follows overt morphological manifestations of radial symmetry. Regardless of what processes these genes regulate during echinoderm development, the existence of radially symmetrical expression domains can only have evolved through changes in the regulatory inputs governing their expression. Virtually nothing is known about how regulatory inputs evolve in any group of organisms. In principle, however, these changes must reflect altered interactions between transcription factors and the regulatory regions of the gene in question. Thus, the genetic basis for altered gene expression may lie adjacent (cis), in the sequences of promoter elements (Fig. 1b), or may be genetically distant (trans), in the spatial distribution of transcription factors that either activate or inhibit their expression.

A fifth, and puzzling, feature of the data summarized in Table 1 concerns larval expression domains. Plesiomorphically, echinoderm larvae are bilaterally symmetrical, have a well-defined anteroposterior axis, and contain structures homologous to those in tornaria larvae of enteropneust hemichordates, such as a circumoral ciliated band and trimerous coeloms (Nielsen, 1987; Strathmann, 1988; Wray, 1992a). Echinoderm larvae are thus the most conserved phase of the life cycle, and one might have expected regulatory gene expression domains in echinoderm larvae to most closely resemble those in other, bilaterally symmetrical, animals. The available data, however, reveal few similarities, even in this relatively conserved phase of development (for the one known example, see Peterson et al., 1999). For example, the Hox genes are not responsible for patterning the early embryo (Arenas-Mena et al., 1998), and orthodenticle is not responsible for patterning the anterior region of the embryo (Gan et al., 1995; Lowe and Wray, 1996). These observations are consistent with the hypothesis that large adult bodies in metazoans evolved independently on several separate occasions from ancestors with much smaller, larva-like adults (Davidson et al., 1995; Nielsen, 1995; La calli, 1997).

**Evolution of Regulatory Genes in Echinoderms**

In studying the evolution of regulatory genes, it is important to distinguish between changes in the structure of genes, their developmental roles, their expression domains, and the structures to which they give rise (Abouheif, 1997; Abouheif et al., 1998; Wray, 1999). Placing the data summarized in Table 1 into a phylogenetic context reveals several distinct evolutionary patterns. Below we discuss six such patterns (Fig. 3), focusing on the implications of the expression data from echinoderms but also incorporating available data from other phyla. We do not expect these six patterns to provide an exhaustive list of the ways in which developmental regulatory genes evolve, although they do expand considerably on the range of possibilities generally discussed in the literature. Furthermore, we expect these evolutionary patterns to be general to the Metazoa, and we discuss likely cases from other phyla.

**Plesiomorphic Role and Expression Domain Retained**

Most comparisons of regulatory gene expression among metazoan phyla have
Figure 3. Distinct patterns of regulatory gene evolution. Four features directly relevant to understanding the evolution of a regulatory gene are the gene itself (G), its developmental roles (R), its expression domains (E), and the structures or aspects of phenotype to which it contributes (S). Because these are evolutionarily dissociable features, it is important to reconstruct their histories separately (Abouheif, 1997; Abouheif et al., 1997; Wray and Abouheif, 1998). In this paper, we discuss six distinct patterns of evolutionary change (a–f); presenting probable examples of each in the text. It seems unlikely that these six patterns exhaust the possible range of changes. This diagram is intended to be as general as possible, but for a concrete image, one could imagine the terminal taxa to be metazoan phyla, from left to right being Cnidaria, Annelida, Arthropoda, Chordata, and Echinodermata. In each case, the gene itself is ancient, predating the origin of bilaterian animals (Gerhart and Kirschner, 1997). (a) Plesiomorphic role and expression domain conserved: The expression domain, developmental role, and structure are homologous among all taxa where they have been observed. This is the most common evolutionary interpretation in the literature. (b) Plesiomorphic role retained, expression domain modified: An ancient and conserved developmental role (R) is associated with a conserved expression domain (E). Within one clade, this expression domain has been modified (E'), in association with a change in morphology (S'). (c) Plesiomorphic role lost: An ancient and widely conserved role (R) has been lost in one clade (~R). This is associated with the loss of the associated expression domain (~E) and structure (~S). (d) Apomorphic role gained: Recruitment (also known as co-option) has resulted in a new expression domain (E) and developmental role (R) that are associated with a new structure (S). (e) Apomorphic role gained for plesiomorphic structure: Recruitment (E, R) occurred after the structure first evolved; the gene of interest originally had no part in the development of the structure but subsequently acquired such a role. (f) Plesiomorphic role, independently deployed: The gene had an ancient developmental role (R) that was associated with a particular expression domain and structure, which may or may not have been subsequently lost. On independent occasions, this ancient role has been deployed in novel expression domains (E2 and E3) in association with independent morphological transformations (S2 and S3).
A conserved developmental role need not be associated with a conserved expression domain (Fig. 3b). Examples are known from insects, in which a gene is expressed in a strikingly different temporal pattern in long- and short-germ embryos (Patel, 1994). A possible instance of this in echinoderms involves the expression of engrailed in the central nervous system. In arthropods, chordates, and annelids, one of the expression domains of engrailed is a reiterated pattern within paired ganglia along the axis of the central nervous system (Patel et al., 1989; Davis et al., 1991; Wedeen and Weisblat, 1991). This has been hypothesized to represent the ancestral role of engrailed (Patel et al., 1989; Davis et al., 1991). In an ophiuroid echinoderm, engrailed is also expressed in paired, repeated ganglia along the five radial nerves that lie within the arms (Lowe and Wray, 1997). In all metazoans that have been examined so far, engrailed expression begins too late to have a role in patterning the central nervous system, and a role in neuronal differentiation seems more plausible (Patel, 1994).

It is not at all certain that these similarities in engrailed expression domains among phyla reflect a common evolutionary origin. The absence of data on engrailed function during neurogenesis in any metazoan limits the evidence for homology to similarities in expression. In addition, the possible homology of engrailed-expressing neurons between protostomes and deuterostomes has not been investigated. Three plausible interpretations of engrailed expression within the central nervous system of echinoderms are as follows: Both the developmental role of engrailed and the cells in which it is expressed are homologous to those of chordates (and perhaps arthropods); the role is homologous, but the cells expressing engrailed are not; or neither is homologous. If either of the first two possibilities turns out to be true, it would imply some interesting spatial shifts in gene expression following the divergence of echinoderms and chordates (Fig. 4a). If each radial nerve in an echinoderm is homologous to the single
dorsal nerve chord of chordates, this would imply a pentuplication (fivefold multiplication) of *en¬grailed* expression domains. However, whether any portion of the echioderm central nervous system is homologous to the dorsal nerve cord of chordates is not at all clear (Bullock and Horridge, 1969; Brusca and Brusca, 1990). Thus, it is also possible that *en¬grailed* is playing a conserved role in nonhomologous cells.

A different evolutionary change in expression domains may have occurred during the diversification of the extant classes of echinoderms. The presence of well-defined ganglia and the organization of ganglia within radial nerves vary among classes (Bullock and Horridge, 1969). Only in ophiuroids and asteroids are cell bodies in the radial nerves organized into well-defined ganglia; in most other living echinoderms, ganglia are either absent or not as prominent (Bullock and Horridge, 1969; Byrne, 1994). In addition, the bilateral organization of cell bodies and fiber tracks within each radial nerve is unique to ophiuroids and asteroids; in other echinoderms, the organization of repeated structures within the radial nerves is staggered, reflecting a staggered organization of the water vascular system and other sites of innervation (Fig. 4b). Based on their limited phylogenetic distribution within echinoderms, both the presence of well-defined ganglia and their bilateral organization are very likely to be derived conditions within the phylum, unique to ophiuroids and asteroids (Fig. 4c). Thus, the paired organization of *en¬grailed*-expressing ganglia within the radial nerves of ophiuroids almost certainly reflects differences from the plesiomorphic condition in echinoderms. This suggests that the similarity in expression domains between ophiuroids and other phyla is due to convergence rather than conservation (Fig. 4c).

This example provides a lesson that may be broadly relevant: Superficial similarity in gene expression domains does not always reflect evolutionary conservation. Comparative anatomists have long been attuned to the possibility of convergence in morphology, but the same caution is not al-

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**Figure 4.** Evolution of central nervous system *en¬grailed* expression in echinoderms. (a) One possible interpretation of the superficially similar expression of *en¬grailed* within the central nervous systems of three phyla. If the developmental roles are homologous in all three phyla, and if the cells in which expression occurs are also homologous, these data would imply a “pentuplication” of the plesiomorphic expression domain in the lineage leading to echinoderms. Other possible interpretations of the data differ in details but still require that *en¬grailed* comes under the direct or (more likely) indirect control of the developmental processes that establish fivefold radial symmetry. (b) The anatomy of the central nervous system differs among echinoderms in ways that are relevant to understanding the evolution of *en¬grailed* expression domains. In most classes, the radial nerves have a staggered organization and distinct ganglia are absent; in ophiuroids and asteroids, ganglia are present and radial nerve organization is bilaterally symmetrical. (c) The phylogenetic distribution of these traits implies that the similarity in expression domains between ophiuroids and chordates is at least in part convergent. If the role of *en¬grailed* within the central nervous systems of echinoderms and chordates is homologous, the existing expression data imply three evolutionary changes in spatial expression domains during echinoderm evolution: entrainment into fivefold symmetry, change to staggered organization, and change to bilateral (paired) organization. Phylogenetic framework from Sumrall and Sprinkle (1998).
Plesiomorphic Role Lost

There are several good examples of a regulatory gene losing a particular developmental role (Fig. 3c). For instance, the Hox-3 ortholog in *Drosophila* (known as *zen*) has lost a homeotic patterning role during insect evolution (Falciani et al., 1996) and *even-skipped* has lost a pair-rule patterning function in the polyembryonic wasp *Copi-dosoma floridanum* (Grbic and Strand, 1998). Part of the reason that the expression domains of regulatory genes look so different in echinoderms may be the loss of plesiomorphic roles. Recognizing such cases requires first gathering evidence that similar expression domains in arthropods and chordates are homologous rather than convergent, and then demonstrating their absence in echinoderms. Both points can be difficult to document convincingly. Although similar expression domains in arthropods and chordates are usually considered homologous (e.g., Duboule, 1994; Finkelstein and Boncinelli, 1994; DeRobertis and Sasai, 1996; Kimmell, 1997), this interpretation is not always based on a careful consideration of gene orthology and morphological homology (Dickinson, 1995; Abouheif et al., 1997), and alternative hypotheses such as convergence are rarely explored. In addition, the apparent absence of a developmental role in echinoderms as inferred from expression patterns may not be real, but instead may be due to technical problems, such as biologically relevant but undetectable levels of expression, sampling the wrong developmental stages, or poor binding between the probe used and the molecule of interest.

Nonetheless, loss of a developmental role seems the most reasonable interpretation for the apparent absence of some gene expression domains in echinoderms. For instance, *orthodenticle* is involved in anterior specification and is expressed in broad regions of anterior ectoderm in the embryos of both arthropods and chordates (Finkelstein et al., 1990; Simeone et al., 1992). This has been interpreted as a conserved developmental role (Finkelstein and Boncinelli, 1994). No obviously comparable anterior expression domain has been detected dur-
The only vague similarity is expression in the ectoderm surrounding the adult mouth of *Strongylocentrotus droebachiensis* (Echinoidea), which could perhaps be interpreted as expression at the “anterior” end of the adult animal. However, this phase of expression begins after the adult oral/aboral axis has already formed, which suggests that *orthodenticle* plays no role in patterning the adult “head” in urchins. Nor is there evidence of a role for *orthodenticle* in patterning the anterior end of the embryo or larva in echinoderms. In *Strongylocentrotus purpuratus* (Echinoidea), expression initially occurs throughout the ectoderm, and is later restricted to the oral (anterior) ectoderm (Gan et al., 1985). Again, expression commences too late to play a role in patterning the anterior end of the larva. In any case, this phase of expression is apparently not present in larvae from other echinoderm classes. In *Dermasterias imbricata* (Asteroidea) there is no detectable ectodermal expression (Lowe and Wray, 1997), whereas in *Psolus chitonoides* (Holothuroidea) expression within the larval ectoderm is restricted to ciliated cells, which occur in circumferential bands (Lowe and Wray, 1997, and unpubl.). If the anterior specification role of *orthodenticle* is homologous in arthropods and chordates, then it has probably been lost, at least in some echinoderms (Fig. 5a).

A second possible case of role loss concerns the expression of *distal-less* in the central nervous system. This gene appears to play a similar role in the development of the anterior central nervous system in arthropods and chordates (Price et al., 1991; Kaphingst and Kunes, 1994). Expression data suggest a much wider phylogenetic distribution for this role—in annelids, onychophorans, nematodes, and cephalochordates (Holland et al., 1996; Panganiban et al., 1997). This broad phylogenetic distribu-

**Figure 5.** Possible evolutionary losses of developmental roles. (a) In arthropods and chordates, ectodermal expression of *orthodenticle* is required for anterior patterning (Finklestein et al., 1990; Finklestein and Boncinelli, 1994). In at least one echinoderm (*Strongylocentrotus purpuratus*; Echinoidea), *orthodenticle* is expressed in the anterior ectoderm of larvae, but this expression begins long after embryonic patterning is complete (Gan et al., 1995). In the larvae of some other echinoderms, *orthodenticle* is not expressed in anterior ectoderm at all (e.g., *Dermasterias imbricata*, Asteroidea; *Psolus chitonoides*, Holothuroidea). Even if the anterior expression in *S. purpuratus* reflects a developmental role homologous to that in arthropods and chordates (for which there is no evidence), this role has probably been lost more than once elsewhere during the echinoderm radiation. Alternatively, the anterior patterning role of *orthodenticle* could have been lost much earlier in echinoderm evolution, and the anterior expression domain in *S. purpuratus* could represent a recruited role with a convergently similar expression domain to that in arthropods and chordates. Phylogenetic framework from Sumrall and Sprinkle (1998). (b) In several phyla, *distal-less* is expressed within the anterior central nervous system. There is no evidence of expression within the central nervous system of any echinoderm examined so far. The most likely explanations are loss of a plesiomorphic role in echinoderms or convergence in deuterostomes and protostomes; the former is the scenario diagrammed here. Phylogenetic framework from Aguinaldo et al. (1997) and Sumrall and Sprinkle (1998).
tion has been interpreted as evidence of conservation dating back at least to the protostome–deuterostome ancestor (Panganiban et al., 1997). No \textit{distal-less} expression has been detected anywhere in the central nervous system of echinoderms, although several species in three different classes have been examined and expression has been found in other structures (Lowe and Wray, 1997, and unpublished data) (Fig. 5b). Again, the most reasonable interpretation is that this expression domain has been lost during echinoderm evolution. For both \textit{orthodenticle} and \textit{distal-less}, the apparent absence of a putatively conserved role is probably not due to lack of probe binding, because other expression domains are easily detected, nor to sampling the wrong stages, because a wide range of developmental stages have been examined in several different species. It thus seems likely that the anterior developmental roles of \textit{orthodenticle} and \textit{distal-less} are homologous in arthropods and chordates and that these roles have been lost in echinoderms.

Why might this have happened? It is perhaps not a coincidence that the two most plausible cases of role loss concern “anterior” developmental roles. All living echinoderms lack a head at all phases of the life cycle. Even the larvae, which have an anteroposterior axis, lack an anterior brain and are not cephalized. The fossil record suggests that echinoderms lost cephalization very early in their evolutionary history (Paul and Smith, 1984), and possibly the developmental roles associated with patterning the head were lost at about the same time. This hypothesis requires that the common ancestor of chordates and echinoderms was cephalized, a point that remains controversial (see Gee, 1997). If evidence emerges that this ancestor was not cephalized, then the scenarios become more complex. In the case of \textit{orthodenticle}, a reasonable interpretation would be that the developmental roles in arthropods and chordates are convergently similar, given the unlikelihood that a role in head development could be conserved if an intermediate ancestor lacked a head. In the case of \textit{distal-less}, interpretations would be less clear. Species from several phyla, including one without extensive cephalization (Neomatoda), have a superficially similar expression domain (and, by inference, developmental role) in anterior central nervous system development (Panganiban et al., 1997).

\textbf{Apomorphic Role Gained}

Nearly all the developmental regulatory genes listed in Table 1 have at least one expression domain that differs from any known domain in arthropods or chordates. It is possible that these are phenotypically neutral and are expression domains with no associated developmental roles. This is unlikely, however, to be the case for all, or even a majority, of the expression domains listed in Table 1. Phenotypically neutral expression is implausible for genes that have similar expression domains in more than one species, particularly if they belong to different classes. For instance, \textit{orthodenticle} is expressed in similar patterns in the podia of asteroids, ophiuroids, and echinoids (Lowe and Wray, 1997), which last shared a common ancestor \~5 billion years ago (Smith, 1988). If these are homologous expression domains, which seems plausible, they have been conserved far too long to be phenotypically neutral.

The alternative explanation is role recruitment: The gene has acquired an apomorphic developmental role in echinoderms, distinct from any previously described role in other phyla (Fig. 3d). The process of role recruitment is a well-documented phenomenon (Holland, 1990; Patel et al., 1992; Averof et al., 1996; Gerhart and Kirschner, 1997), but it is not generally considered a common process—at least, not as common as conservation of roles. Most of the expression domains listed in Table 1, however, are likely to represent recruited roles. The rationale for this interpretation is based on the fact that they occur in structures that are apomorphic for the Echinodermata or some clade within it. In principle, it seems unlikely that an expression domain could evolutionarily predate the structure in which it is expressed. Thus, if the structure in which expression occurs is apomorphic for echinoderms (based on fossil evidence or comparisons among extant taxa), then the expression domain is likely
to be apomorphic as well. For example, homeobox genes are clearly older than the Metazoa (Bharathan et al., 1997), so the role of Hox genes in patterning the central nervous system of chordates must represent a recruited role.

We interpret most of the known expression domains of regulatory genes in echinoderms as recruited roles, apomorphic for either the Echinodermata as a whole or some clade within it. Any hypothesis of role recruitment can be tested in a variety of ways. The simplest test is to gather additional comparative expression data. For example, comparisons of Brachyury expression among an asteroid, an echinoid, and a hemichordate have shown that one expression domain (oral ectoderm) probably predates the divergence of the two phyla, whereas two others (secondary mesenchyme and coelom) seem to be unique to echinoderms (Harada et al., 1995; Peterson et al., 1999; Shoguchi et al., 1999). None of these expression domains corresponds to any that have been described in chordates (Smith, 1999). Because hemichordates and echinoderms are clearly closely related phyla (Brusca and Brusca, 1990; Nielsen, 1995), the one expression domain they do share may be a synapomorphy of the two phyla rather than a feature conserved from the ancestor of the Bilateria. The second means of testing a hypothesis of recruitment is to examine the function of the gene in the particular expression domain. The prediction is that this function in a recruited gene should differ from its function in other organisms. This is technically difficult to test but has been done for some of the genes listed in Table 1. For example, dominant negative and overexpression experiments have demonstrated that Notch is directly involved in specifying the fate of secondary mesenchyme in the early embryo of a sea urchin (Sherwood and McClay, 1999). This is a developmental role that the gene does not carry out in other phyla, according to available evidence.

There are at least two reasons, not mutually exclusive, why so many developmental roles might be apomorphic in echinoderms (Lowe and Wray, 1997). The first possibility is that role recruitment has been an important mechanism in the origin of the extensive morphological novelties that characterize echinoderms. Interestingly, many of the putative derived developmental roles are associated with structures that are themselves apomorphic in echinoderms, such as the water vascular system and endoskeleton. For instance, several genes are ex-

![Figure 6. Probable apomorphic developmental roles in echinoderms. Several expression domains in echinoderms occur in structures that are unique to the phylum or to particular clades within it. These expression domains are likely to represent role recruitments that evolved after echinoderms diverged from chordates, because the structures in which they occur apparently did not exist before this time. Two examples are orthodenticle (Otx) expression in podia and distalless (Dlx) expression within the hydrocoel. Both structures are components of an organ system, the water vascular system, that is an echinoderm synapomorphy. In both cases, expression occurs in species belonging to different echinoderm classes, which suggests a common evolutionary origin. Given the divergence times between the classes (Smith, 1988), a single origin would have occurred at least 490 million years ago. Other expression domains probably evolved more recently, given their more restricted phylogenetic distribution. For example, orthodenticle is also expressed within ectodermal cells that make up the ciliated bands of the larva of the holothurian Psolus chitonoides but not in the larvae of other species from the order Dendrochirotida (see text and references cited in Table 1 for details). Phylogenetic framework from Sumrall and Sprinkle (1998), divergence times from Smith (1988). The divergence time between the two holothuroid species is very poorly resolved.](image-url)
pressed in the imaginal adult rudiment during metamorphosis, when many of these structures are constructed. Examples include *distal-less*, *not*, and *Brachyury* (Lowe and Wray, 1997; Peterson et al., 1999). That several regulatory genes seem to have been recruited into the development of echinoderm-specific structures suggests that the evolutionary origin of novel structures can involve extensive recruitment of preexisting genes.

A second possibility is that some derived roles were associated with the diversification of larval morphology and life history. As with most metazoan phyla, extant echinoderms exhibit a wide range of larval morphology (Mortensen, 1921; Wray, 1992b) and encompass diverse reproductive modes (McEdward and Janies, 1997). The larvae of many asteroids, for example, uniquely contain structures used for settlement called brachiolar arms. During late larval development, *distal-less* is expressed within these structures (Lowe and Wray, 1997). Asteroid larvae also contain a distinct population of mesenchyme cells, called subtrochal cells, that serve to anchor muscles to the body wall (Lacalli, 1996). Uniquely within the mesoderm, these cells also express *distal-less* (Lowe and Wray, 1997). Both of these expression domains may be linked to the origin of novelties in larval morphology.

Comparisons of gene expression among several echinoderm species suggest that role recruitments have occurred throughout the history of the phylum. As noted above, some similar expression domains are shared by more than one echinoderm class, implying an origin >450 million years ago (Ma) (Smith, 1988). Podial expression of *orthodenticle* is a likely case (Fig. 6). Other expression domains appear to have evolved much more recently and may have been associated with the diversification of echinoderm morphology or life history. A likely example is the expression of *distal-less* within the invaginating vestibule of echinoids (Lowe and Wray, 1997). Throughout the Acroechinoidea (the clade that contains the majority of extant echinoids), one of the first morphogenetic events leading to the radial adult body is an invagination of the ectoderm overlying the left hydrocoel (Mortensen, 1921; Hyman, 1955). This invagination forms a pocket of ectoderm called the vestibule, within which the imaginal adult rudiment develops. Before and during invagination, *distal-less* is expressed throughout the vestibule (Lowe and Wray, 1997). Because the vestibule does not occur in the two closest outgroups, the Echinothuroidea and Cidaroida (Emlet, 1988; Parks et al., 1989; Amemiya and Emlet, 1992), it is probably an apomorphic structure. Given the stratigraphic ranges of these groups (Smith, 1984), the vestibule probably originated around the end of the Paleozoic, ~250 Ma. In echinoderms from other classes, no comparable *distal-less* expression has been detected. Together, these observations suggest that the role of *distal-less* in vestibule formation evolved during the history of the class Echinoidea. A more precise estimate of when this occurred will require examining additional taxa within the class Echinoidea.

**Apomorphic Role Gained for Plesiomorphic Structure**

In some cases, a developmental role appears to have evolved long after the structure or cell type in which it is expressed (Fig. 3e). An example is *fushi-tarazu*, a pair-rule gene required for segmentation in *Drosophila*, which is expressed in a “pair rule” pattern in alternate segments. In *Tribolium* (Coleoptera), *fushi-tarazu* also has a pair-rule expression pattern, but out of register with that in *Drosophila* (Brown et al., 1994). Remarkably, however, in an out-group taxon, *Schistocerca* (Orthoptera), the *fushi-tarazu* ortholog is not expressed in a pair-rule pattern at all and apparently does not play a role in segmentation (Dawes et al., 1994). Thus, the involvement of *fushi-tarazu* in segment patterning likely evolved after segments themselves. Another pair-rule gene, *even-skipped*, provides a second example. Pair-rule expression is present in *Drosophila* (Diptera) and in *Tribolium*, *Dermestes*, and *Callosobruchus* (Coleoptera) but absent in *Schistocerca* (Orthoptera) (Patel et al., 1992, 1994). So far, there are no data from an outgroup to polarize this difference: Either a pair-rule role in segmentation was lost without losing segments them-
selves, or a pair-rule role in segmentation was gained long after segments evolved. In either case, there is a clear decoupling between the evolution of a structure and the involvement of some genes in the development of that structure.

A similar case from echinoderms concerns the expression of orthodenticle in the ciliated bands of some holothurid larvae. Within the Echinodermata, larvae pleiomorphically have a single, convoluted ciliated band that is used for both feeding and locomotion (Nielsen, 1987; Strathmann, 1988). In independently derived nonfeeding larvae, cilia are used exclusively for swimming and are often arranged into circumferential bands (Emlet, 1991). In Psoluschitonoides, a holothurid with nonfeeding larvae, orthodenticle is expressed in ectodermal cells that bear large cilia (Lowe and Wray, 1997, and unpubl.) (Fig. 7). This suggests a role in either patterning ciliation or in the differentiation of ciliated cells.

Interestingly, orthodenticle expression is apparently not present within the ectoderm of other holothurid larvae during rearrangements of ciliation patterns (Lowe and Wray, unpubl.) (Fig. 7). The phylogenetic sampling includes three other species, two of which have nonfeeding larvae and one of which has circumferential ciliated bands. Given a lack of phylogenetic resolution, it is not clear whether these holothurids with nonfeeding larvae represent independent life history shifts. If they do, the lack of orthodenticle expression in their ciliated bands would suggest that parallel morphological transformations can occur with at least partially different underlying genetic bases. Like the fushi-tarazu example, orthodenticle has apparently become involved in the development of a structure long after that structure first evolved.

Although such cases may initially seem surprising, they make sense in retrospect. Consider how the genetic basis for a particular aspect of phenotype changes through time. One extreme possibility is that all evolutionary changes in that aspect of phenotype result from mutations in a fixed, unchanging set of genes. In other words, once a structure evolves, the genes that build that structure are forever the only ones that can do so. It seems unlikely that a structure could be genetically “insulated” from the rest of the genome in this manner. An alternative possibility, supported by the cases just described, is that other genes can become involved in the development of an existing structure after that structure first evolves. The history of animals abounds with examples of structures that have changed in size, shape, number, position, orientation, and complexity (to name a few of the possibilities) during the course of evolution. Some of these phenotypic transformations may be the result of a change in regulatory linkages among genes, such that there is a change in the complement of genes that directly influence the structure in question.

We hypothesize that the evolutionary history of the genetic basis for many struc-
tures has involved both the recruitment of regulatory genes that were previously not part of the development of that structure and the loss of regulatory genes that were at one time part of its development. In such cases, most of the genetic basis for the structure is probably unchanged, and the gain or loss of a particular regulatory gene linkage may be the genetic basis for some change in phenotype. The frequency with which these kinds of regulatory gains and losses happen is not clear. However, other likely cases of postorigin recruitment are known. An example is the role of Sex-lethal in sex determination within the Diptera. This gene, the “master regulatory gene” of sex determination in several species of Drosophila, does not have a role in sex determination in several other Dipteran genera (Meise et al., 1998; Caccone et al., 1998). Sex determination is clearly much older that the involvement of Sex-lethal in this process.

Plesiomorphic Role, Independently Deployed

One of the more surprising evolutionary patterns that has emerged recently concerns the expression of a homologous gene, carrying out an apparently similar developmental role, but in structures that are clearly not homologous (Fig. 3f). The first clues to this phenomenon emerged from similarities in gene expression during limb development in Drosophila and chordates. Several genes are now known to be involved in limb patterning in both phyla, including distal-less, hedgehog, serrate, and fringe (Panganiban et al., 1997; Shubin et al., 1997). Yet it seems extremely unlikely that the limbs of insects and tetrapods share a common evolutionary origin (e.g., Brusca and Brusca, 1990; Nielsen, 1995; Panganiban et al., 1997; Shubin et al., 1997).

Two scenarios can explain these comparative expression data. The first posits coincidence: Orthologous genes were independently recruited into functionally similar, perhaps identical, developmental roles in two different phyla entirely by chance. The second hypothesis proposes that these genes are part of a developmental “module” that carries out a homologous, generic task (in this case establishing the axes of an appendage) and have been deployed in a new (i.e., nonhomologous) expression domain. The first possibility might be reasonable if only one such case were known, but it seems less attractive when the expression domains (and perhaps developmental roles) of several different genes show this phylogenetic distribution (Shubin et al., 1997). Examining additional taxa and genes are obvious ways to distinguish between these possibilities.

The first kind of analysis has now been done for distal-less, by examining expression in representatives of five additional phyla: Onychophora, Annelida, Nematoda, Urochordata, and Echinodermata (Panganiban et al., 1997; Lowe and Wray, 1997). The results were surprising. Several additional appendages that are almost certainly not homologous to the limbs of either arthropods or chordates also express distal-less distally during their outgrowth, including the podia of echinoderms, the spines of adult ascidians, and the ampullae of ascidian larvae. Even the vestibule of echinoids, which is an ingrowth rather than an outgrowth, also expresses distal-less at its distal end during elongation. Expression is also present in the distal portion of onychophoran lobopods and in the possibly homologous parapodia of annelids. There are no experimental data on distal-less function in any of these “non-model” taxa, but the spatial and temporal pattern in each case is strikingly similar to that during limb development in arthropods and chordates, in that expression commences before outgrowth and persists at the distal end of the appendage throughout its outgrowth.

A plausible interpretation of these data follows (see also Fig. 8). The common ancestor of protostomes and deuterostomes had a structure (or structures) that lay orthogonal to the anteroposterior axis. This structure may have been a limb, but that is not required by the hypothesis; nor is it necessary that homologs of this structure exist in any living animal. The essential point is that distal-less was involved in patterning the proxiomodistal axis of this structure. During the subsequent radiation of metazoan phyla, various other protruding structures evolved, including the nonhomologous appendages of echinoderms,
arthropods, and chordates. Some, but not necessarily all, of these morphological innovations involved taking advantage of the proximodistal patterning machinery that already existed in the genome. From the existing comparative data, we posit a minimum of seven such independent reapplications (Fig. 8).

This scenario makes several testable predictions. First, at least some of the genes that interact with *distal-less* in patterning the limbs of arthropods and chordates should also be expressed in nonhomologous appendages during their development. This test would involve cloning the orthologs of genes such as *fringe* and *hedgehog* from representatives of additional phyla and then characterizing their expression. Second, experimentally disrupting *distal-less* expression during the outgrowth of these nonhomologous limbs should produce a phenotype consistent with a role in proximodistal patterning. Various methods for disrupting gene expression or function in nonmodel organisms are becoming available, and some pioneering studies demonstrate how fruitful this approach can be for understanding the evolution of regulatory genes and their developmental roles (e.g., Swalla and Jeffery, 1996; Wada et al., 1997; Sherwood et al., 1999). Third, analyses of additional phyla should reveal more cases of *distal-less* expression in nonhomologous appendages. The number of independent reapplications of *distal-less* to proximodistal patterning implied by the few taxa that have been examined to date suggests that others await discovery.

The notion of a developmental “module” that has been redeployed as part of the development of nonhomologous structures grows out of a considerable body of empirical data. Examples include the genes encoding proteins that precisely regulate the timing of cell division (Murray and Hunt, 1993), carry out programmed cell death (Vaux et al., 1992), initiate the differentiation of myocytes (Olson, 1992), and are involved in cell–cell signalling (Greenwald and Rubin, 1992). In each case, systems of interacting proteins encoded by homologous genes regulate functionally conserved processes in organs that are clearly not homologous. Many other examples could be cited, and new cases are being discovered at a rapid pace. The existence of “portable” developmental modules that carry out generic developmental functions has important implications for understanding the evolution of morphological novelties, a topic of much speculation (Müller and Wagner, 1991). The evolutionary origin of a novel structure, such as an appendage, requires a large number of developmental processes, including axial patterning, cell-
cell communication, precisely regulated cell proliferation, and so forth. These are all generic processes, in the sense that they are used at many other times and places during development of all triploblastic metazoans. It seems much more likely that the origin of a novel structure will be based on this existing machinery, rather than reinventing all of these complex developmental processes de novo.

CONCLUSIONS

Until recently, most comparative analyses of the expression domains of metazoan developmental regulatory genes sampled two distantly related species, usually *Drosophila melanogaster* and *Mus musculus*. This situation is changing rapidly, as expression data for many more taxa are being published. This expanded taxonomic sampling allows for more rigorous comparative analyses and is providing a wealth of information about the polarity and timing of evolutionary changes in genes, their expression domains, and their developmental roles. Echinoderms are one of the most extensively studied metazoan phyla outside the Arthropoda and Chordata in terms of developmental regulatory gene expression. By placing these data into a phylogenetic framework, it has been possible to identify several interesting evolutionary processes that have not been discussed in the literature. Although the emphasis in this paper has been on echinoderms, an informal consideration of the existing data from other phyla suggests that these evolutionary processes are widespread in metazoans. The available data suggest the following tentative conclusions.

1. These genes are extraordinarily flexible. Before much information was available, it was easy to imagine that important developmental regulatory genes would be among the most highly constrained of all genes (e.g., Arthur, 1988). Empirical studies have clearly demonstrated that, far from being highly constrained, developmental regulatory genes are evolutionarily quite flexible. Even from the rather limited phylogenetic sampling to date, evolutionary changes in roles and expression domains appear rather common: many new roles have been acquired, others have been lost, and expression domains have changed in size, position, timing, and even symmetry. Because many regulatory genes are older than the Metazoa (Atchley and Fitch, 1997; Bharathan et al., 1997; Gerhart and Kirschner, 1997), few of their current roles in extant animals are likely to be the original ones. Indeed, reconstructing the nature of those original roles may prove quite difficult.

2. These genes provide insights into the origin of novelty. Little is known about the genetic and developmental bases for the origin of novel phenotypes, despite the obvious importance of this issue (Müller and Wagner, 1991). It is now possible to identify genes that may have been involved in mediating phenotypic transformations by analyzing the expression of several regulatory genes in a variety of organisms (e.g., Averof and Cohen, 1997; Lowe and Wray, 1997; Shubin et al., 1997). At least in some cases, the origin of a morphological novelty has been accompanied by novel expression domains and (very likely) recruited developmental roles for preexisting genes. These results favor a “new roles for old genes” explanation for the origin of novelties, although they do not rule out the participation of novel genes (e.g., through gene duplication or assembly from existing exons). The parallel recruitment of *distal-less* into appendage development on several separate occasions suggests that whole developmental modules may on some occasions be recruited to build a novel structure. This seems more feasible than evolving a new developmental pathway from scratch.

3. These genes are fickle indices of morphological homology. Similar expression domains of regulatory genes are usually interpreted as resulting from common ancestry (Slack et al., 1993; Duboule, 1994; DeRobertis and Sasai, 1996; Kimmel, 1996; Holland et al., 1997). Although this may often be the case, in at least some cases similarity may be the result of coincidence or convergence rather than conservation (Wray and Abouheif, 1998). Similarities in gene expression domains can be the result of several distinct, and distinguishable, evolutionary histories, likely examples of which were discussed above. The very real
possibility of convergent similarity in expression domains means that caution should be exercised when using developmental regulatory genes as indices of homology among morphological structures (Dickinson, 1995; Abouheif et al., 1997; Wray and Abouheif, 1998). In particular, the existence of developmental modules that are reapplied in functionally similar contexts in nonhomologous structures poses a very real problem for testing hypotheses of homology among morphological structures.

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