

Homologies of Process and Modular Elements of Embryonic Construction

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ABSTRACT There are several signal transduction pathways that integrate embryonic development. We find that both within species and between species, these pathways constitute homologous modules. The processes, themselves, can be considered homologous, just as structures can be considered homologous. Just like vertebrate limbs, these pathways are composed of homologous parts (in this case, the proteins of the pathway) that are organized in homologous ways. These pathways are conserved through evolutionary time, and they undergo descent with modification. Such homologies of processes become critical to the discussion of evolution and development when we consider (1) that evolution depends on heritable changes in development, (2) that development is modular such that different modules can change without affecting other modules, (3) that modules can be co-opted into new functions, and (4) that modules depend on intercellular communication. *J. Exp. Zool. (Mol. Dev. Evol.)* 291:1–12, 2001. © 2001 Wiley-Liss, Inc.

THE RETURN OF EMBRYONIC HOMOLOGIES

From pattern to process

Biologists have been arguing about homology since the 1830s, and despite enormous advances in molecular, evolutionary, and developmental biology, the debate continues. In his aptly titled pamphlet *Homology: An Unsolved Problem*, Gavin de Beer ('71) drew a clear line between structure, which he viewed as the only appropriate thing to be homologized, and function: "An organ is homologous with another because of what it is, not because of what it does." Distinguishing structure from function was straightforward when we knew little about the developmental mechanisms responsible for the emergence of anatomical structures. Recent progress in developmental genetics, however, has given us remarkable insights into the molecular mechanisms of morphogenesis but has at the same time blurred the clear divide between structure and function. At the genetic or molecular level, it is difficult to tell where one ends and the other begins. One scientist's "cause" is another scientist's "phenotype."

De Beer's analyses of structural homologies excluded not only function, but also generative processes. In justifying this approach, he cited several classic cases of homologous structures that develop in different ways. In salamanders, lenses derived

from the dorsal iris and those derived from the surface ectoderm were homologous, although they arose from different tissues. Similarly, vertebrate neural tubes produced by primary neurulation were homologous to those produced by secondary neurulation. Both examples demonstrate the independence of structural homology from developmental processes.

The entry of developmental genetics into the realm of morphogenesis has changed this situation. The original aim of developmental genetics had been to explain the problem of cell differentiation (see Gilbert, '97): How does one cell become different from other cell types and from its progenitors; how does an epidermal skin cell become different from a nerve cell and from the ectodermal cell that had the potency to become either? However, by the early 1990s, genes were found whose expression determined not merely cells but entire body units: the homeotic gene complex in *Drosophila* was shown to specify the identity of body segments, and mutations of similar genes in mice caused region-specific (rather than

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cell-type specific) defects (Nüsslein-Volhard et al., '87; Chisaka and Capecchi, '91). At the same time, the intercellular and intracellular pathways active in forming organs were being elucidated. This program began with studies of genes whose misregulation produced cancers, but these oncogenic pathways were soon found to be the same ones embryonic cells use to regulate their growth and coordinated differentiation (see Gilbert, '96). The similarities of these developmental genes and pathways between evolutionarily separated organisms and between different organs within the same organism have reopened the debate about the relevance of development to homology and to evolution. One issue concerns the role of "process" characters in assessing the homology of the structures they help build. We are not going to deal with that in this chapter (see Bolker and Raff ['96] for discussion). Instead, we will focus on a second, more fundamental question: whether and how we can homologize the processes themselves.

This is an important exercise. Homologies of processes become critical to the discussion of evolution and development when we consider (1) that evolution depends on heritable changes in development, (2) that development is modular such that different modules can change without affecting other modules, (3) that modules can be co-opted into new functions, and (4) that modules depend on intercellular communication transduced by families of paracrine factors, signaling cascades, and transcription factors. Thus, the signal transduction pathways themselves can constitute modules that can be used for several different developmental and physiological functions. Like intracellular biochemical pathways, they can be conserved through evolution and modified both within and between species. However, unlike the metabolic biochemical modules, those pathways of intercellular communication can act to create new morphological entities during development.

The embryo and homology

The notion that homologies were best studied in embryos can be traced back to Martin Barry (1837) who claimed that structural homologies were best recognized without the "embarrassment of function," and, moreover, that the "manner of development" was similar between different animal groups. Later, structural embryonic homologies were considered some of the most important evidence for evolutionary theory: the larval tunicate showed the ascidians to be chordates, the larval barnacle demonstrated that the cirripedes

were modified crustaceans (Kowalewski, 1871; Müller, 1864), and the embryonic mammalian jaw, not its adult structure, showed the affinity of mammals to the reptiles (Reichert, 1837). Wilson (1898) used homologous cleavage and cell-partitioning patterns to show the affinity of mollusc, annelid, and flatworm phyla. Such homologies indicated common descent. They were *evidence* of evolution but shed no light on evolutionary *mechanisms*—natural and sexual selection. In the early twentieth century, developmental mechanics separated embryology from evolutionary biology, genetics replaced embryology as the perceived motor of evolution, and arguments over homology and homoplasy frustrated attempts to create phylogenetic trees (Bowler, '96; Gilbert et al., '96). From this point, embryonic homologies were little studied.

One reason for the recent revival of interest in developmental homology is its central importance to the contemporary (re)synthesis of developmental and evolutionary biology. Three research programs have converged to make developmental homology a critical issue. First, paleontology's punctuated equilibrium model of evolution requires rapid morphological change, and its supporters have proposed developmental phenomena such as allometry, heterochrony, and homeosis as candidate mechanisms (Gould, '77). Homologous structures in different lineages became the evidence for the historical occurrence of these processes, so criteria for recognizing homologies are central to this program.

In molecular biology, nucleic acid and protein sequencing have shown that gene structures could be homologous (Britten, '67; Fitch, '70). While this program originally centered on proteins such as globins, whose structures were homologous both within the organism and between species, the most interesting homologies came from a third program, the analysis of developmental regulatory genes. E.B. Lewis and colleagues pioneered the genetic analysis of a region on *Drosophila* chromosome 3 that contained several homeotic genes, and in the 1980s the 180-base-pair homeobox was found in these genes. These fly "Hom-C" genes were thus identified as serially homologous. Shortly thereafter, molecular hybridization studies showed that these genes were also specially homologous: the same genes were found in vertebrates (Carrasco et al., '84; for review, see Gilbert et al. '96). Not only did the genes appear to be homologous within a species, but the same genes also appeared to reside on the vertebrate chromosomes in the same order as

they did on the fly chromosomes. The complexes were, themselves, homologous.

Other highly conserved developmental regulatory genes have since been identified, and it is clear that analogous structures are often made from homologous genes. (We do not subscribe to the view that the expression of homologous genes renders the structures themselves homologous; Dickinson, '95; Bolker and Raff, '96.) The *PAX6* (*eyeless*) gene is expressed during the development of photoreceptors throughout the animal kingdom, the *Tinman* (*Csh*) gene appears to be the basis for both the *Drosophila* and the vertebrate hearts, and the expression of the *fringe* gene apparently defines the region of limb outgrowth in both flies and birds, even though the mechanisms of limb formation in arthropods and vertebrates are completely different. In none of these cases does the expression of a highly conserved gene confer homology on the resulting structures.

A final reason to reconsider homologies of process is the wealth of new data concerning the importance of modularity in both evolution and development. The notion of modularity is attracting increasing interest from developmental and evolutionary biologists as well as from philosophers (Raff, '96; Wagner, '96; Van Dassow and Munro, '99). Biologists have long recognized that structures (or parts of structures) can act as modules, distinct but potentially interacting developmental or evolutionary units. We are now realizing that dynamic entities such as morphogenetic fields (Gilbert et al., '96; Raff, '96), developmental equivalence groups, or signal transduction pathways may also act as modules.

WHAT ARE HOMOLOGIES OF PROCESS?

Extending the concept of homology

Extending the concept of homology to cover genetic characters as well as aspects of morphology has been straightforward. Genes or DNA sequences are still physical entities, with a defined structure, and the original homology criteria derived from comparative anatomy (relative position, transformation, special quality) readily apply. Recognizing "homologies of process," however, represents a qualitative change. If we are to homologize processes—as indeed is already common practice—we must consider whether we can apply the original criteria of homology, and what complications arise from applying them to a new sort of character.

Process homology confronts head-on two central issues of homology debates. The first is the prob-

lem of function, and the importance of excluding functional criteria from homology definitions or assessments. It is not at all easy to define a process independent of its function. The second is that although hypotheses of homology must specify a particular level of biological organization (Striedter and Northcutt, '91; Bolker and Raff, '96; Abouheif, '97; Dickinson, '95), gene expression bridges two such levels: the genetic and the phenotypic. These are nontrivial problems. Before we go on to address the difficulties with homologies of process, however, we need to explain what we mean by the term.

The concept of homologies of process has its origin in Howard Schneiderman's observations of "homologous specification" in *Drosophila*. Here, transdifferentiating cells or the tissue resulting from homeotic gene mutations retain positional information that reflects their position in the imaginal disc (Postlethwait and Schneiderman, '71). Antennal imaginal disc tissue misdirected to differentiate as leg tissue will differentiate according to its location in the antennal disc. Thus, if the disc region destined to become the most distal portion of the antenna forms leg structures instead, it becomes a claw (not a coxa or trochanter).

Schneiderman's observations had three central implications for the homology problem. First, they reinforced the idea that homology had to be defined at a particular level. Second, they showed that homology at the structural level was separable from that of process. Third, and most importantly, they demonstrated that structures with no *anatomical* homology—the eye and the leg—could nevertheless have an underlying homology of *process* in their construction.

Recent examples

Since Schneiderman's initial description of "homologous specification," molecular biologists and developmental geneticists have discovered many additional examples that illustrate these principles. We now use the term "process homology" (or, more formally, "homology of process") to describe the relationship between pathways that are composed of homologous proteins and that are related by common ancestry. These homologous pathways need not form anatomical homologs.

Along with applying a new label—"process homology"—to the relationship uniting the eye, wing, and leg discs, we can now identify its molecular basis: the interaction of the hedgehog and Wnt pathways. These two paracrine factors interact within the disc to specify the proximal/distal axes of the respective organ (see Ingham, '94). The

same molecules that specify these axes in the eye also specify them in the leg and wing discs, constituting a serial process homology.

Moreover, the same pathways exist in vertebrates. For instance, in the Wnt signaling cascade, every element in the insect pathway has a homologue in the vertebrate embryo, and the same interactions that transmit the *Drosophila* Wingless signal to the nucleus occur in vertebrates. The genes and the protein interactions are the same: only the “readout” (the target genes) is changed between tissues and species (Cadigan and Nusse, '97; Fig. 1). The Wnt pathways in vertebrates, nematodes, and arthropods comprise homologous proteins arranged in similar fashions: we therefore consider them homologous pathways. The Wnt pathway (or the set of homologous Wnt pathways), then, is a homologous cassette of information, a homologous module of gene–protein interactions—hence, a homology of process.

A second example of pathway and process homology is the Rel protein pathway. In the *Drosophila* blastoderm, the binding of ligand (Spätzle protein) from the ventral follicle cells to its receptor (Toll protein) transduces a phosphorylation signal that separates a potential transcription factor (Dorsal protein) from its cytoplasmic inhibitor (the cactus protein). Once released, the factor enters the nucleus and regulates the transcription of particular genes, a critical step in forming the ventral portion of the fly embryo. This *Drosophila*

pathway corresponds protein for protein with the pathway that liberates NF- κ B from its inhibitor, I κ B, when interleukin-1 coactivates mammalian B lymphocytes through its cell surface receptor (Shelton and Wasserman, '93). This pathway is also used in the formation of the fly's immune system and for the vertebrate limb (Lemaitre et al., '96; Bushdid et al., '98; Kanegae et al., '98; Wu and Anderson, '98). While there is no homology being postulated among the mammalian B lymphocyte, the fly fat body, the insect blastoderm, and the avian limb, there is a remarkable homology in the pathways that help construct these three structures.

GENERAL PRINCIPLES

Processes and the nature of homology

We are used to thinking of pathways or signal transduction systems as temporal series; indeed, we usually write them with arrows showing the sequential activation of each part. The molecules of a pathway, however, are also physically linked: the surface of one protein fits into the surface of the next. In either the anatomical or the process case, homologous structures (the proteins in a pathway, or the bones in a skeleton) are linked together in the same physical organization. Thus, the standard criteria for anatomical homology remain relevant to homologies of process. Just as “the forelimb” is a homologous entity (between species) because it is composed of parts that are

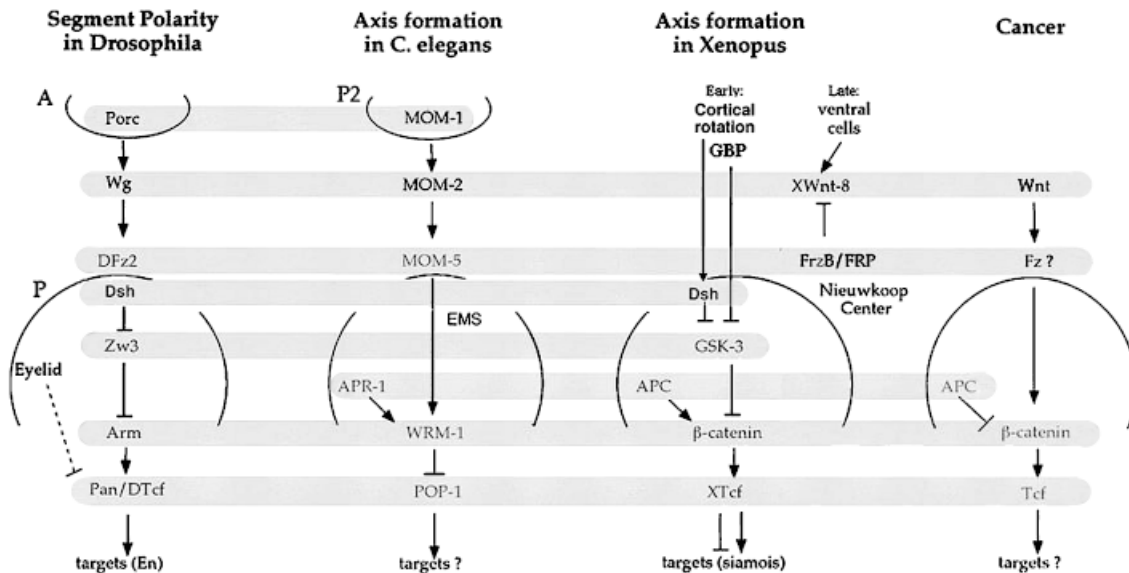


Fig. 1. Comparison of Wnt pathways in embryogenesis and mammalian carcinogenesis. Related genes are highlighted across the different systems. Potential differences in the path-

ways are shown shaded. Broken lines indicate alternative pathways (after Cadigan and Nusse, '97).

themselves homologous, “the Wnt pathway” is a unit of anatomical homology. However, because pathways have temporal as well as physical organization, “the Wnt pathway” is more than a unit of structure: it is also a modular unit of process.

We recognize homologies of process in the common cassettes or modules that occur in different lineages, and these can be used in different ways. The structure and expression patterns of one set of genes may not indicate homology. The coordinated assembly of several genes and gene products into a functional module, and the sharing of such modules between species or between tissues of an organism, is more significant. Such shared modules embody homologies of process.

They need not, however, imply homologies of resulting structures. For example, the presence of the Rel pathway in both the *Drosophila* and the mammalian immune systems does not mean that the immune systems are homologous (i.e., derived from a common ancestral immune system). Rather, it means that both immune systems use a common modular subunit in their respective constructions. The Rel pathway is also used in *Drosophila* segmentation and in vertebrate limb development, and these developmental events are certainly not homologous.

Processes as characters

Processes, or assemblies of processes into pathways or cassettes, are themselves characters. This idea has not penetrated discussions of homology in the evolutionary literature, perhaps because evolutionary biology and systematics have traditionally focused on adult structures. It is, however, essential for evolutionary analyses of development: many of the key characters of embryos are processes. Moreover, many embryonic structures are themselves transient (i.e., time dependent). Thus if we seek homologies in embryos, they should be homologies of processes rather than of structures. This is not a new conclusion, at least among those who have sought to integrate developmental and evolutionary biology.

Waddington ('75) emphasized the importance of processes as characters, citing Whitehead's ('33) “...replacement of ‘things’ by processes which have an individual character which depends upon the ‘conrescence’ into a unity of very many relations with other processes.” Waddington envisioned these “conrescences” in embryos as assemblies of many genes and their products into stabilized pathways or modules. Bonner ('88) has associated modularity with “gene nets” that can participate

in many different aspects of development, and Wagner ('96) identifies homologs as units of evolutionary transformation.

Embryonic induction, a central mechanism of metazoan development, neatly illustrates the usefulness of considering pathways as characters. As Waddington pointed out, a cell's competence to respond to a particular inducer depends on the prior existence of a response pathway in that cell. Altering the receptor allows the cell to respond to a new inducer, using the same internal pathway. Similarly, altering the transcription factors activated by the pathway could cause a cell to express a new set of genes.

This seems to have occurred in the classic locus of induction, the amphibian organizer. In *Xenopus*, the organizer-inducing activity of the Nieuwkoop Center appears to result from the activation of β -catenin proteins (Brannon et al., '97; Larabell et al., '97). The normal activator of β -catenin is a Wnt protein, but some other protein apparently activates the distal portion of the Wnt pathway in the dorsal vegetal cells of the *Xenopus* blastula (Yost et al., '98; Miller et al., '99; Fig. 1). Similarly, the Rel cascade initiated by the binding of Toll and Spätzle during *Drosophila* development is also used by the fly's immune cells to activate genes necessary for fighting fungal infections (Lemaitre et al., '96). In both these cases, a conserved pathway serves different functions as it is initiated by different activators in distinct contexts.

These two examples also illustrate other properties of homologies of process. As mentioned earlier, homologous pathways are composed of the products of homologous genes. We expect such homologous pathways to represent variations on a common theme. Figure 1 shows some of the variations played upon the Wnt pathway. Homology is not identity, and the existence of pathways allows for evolutionary modulation at each step.

What process homology is not: function as criterion

Two of the major, and most common, misuses of developmental processes in homology assessments are (1) the use of processes as criteria for homology, rather than as characters, and (2) the conflation of processes with function. First, in contrast to the common practice of using processes to help assess the homologies of genes or structures, we ask whether we can establish homologies between the processes themselves, and what such homologous relationships might reveal about the evolution of developmental pathways. In our

view, developmental processes are *characters in and of themselves*, not merely criteria for assessing structural homology.

Second, process homology has nothing to do with so-called “functional homology.” Process homology treats processes as characters, that is, as parts of organisms that can be compared and homologized, just as the classical homology concept treats morphological characters. The term “functional homology,” on the other hand, represents a fundamental misunderstanding of the central concept of homology. Homology is a relationship of “sameness” due to descent from a common ancestral precursor. Similarity of function carries no implication of evolutionary relationship (indeed it can arise by numerous means other than phylogenetic continuity), and relationships based on functional resemblance are analogies, not homologies. It is therefore critical in discussing process homology to distinguish process from function as clearly as possible.

By a “process” we refer to an action (*what* happens), not to its functional outcome (*why* it happens). For instance, making a phone call is a process that can be described entirely without reference to why one might make a phone call, or the effect the call might produce. The process of making the call is the same whether its function is to remind one’s spouse to buy milk or to accept a job offer. The result of the call may be to ensure that there is milk for breakfast, awaken someone from a nap, or set paperwork in motion, but it is in any case entirely distinct from the process of telephoning (or the equipment used to transmit one’s voice).

Function can be an organismal trait (forming a leg, inducing a neural tube, etc.) or it can be a molecular trait (such as phosphorylating a particular protein or transcribing a particular gene). Both are causal but at different levels. Homologies of process are seen at the molecular level. This distinction between levels is essential when analyzing homologous pathways with respect to function.

In this regard, we should distinguish homologies of process from the concept of homodynamy. Homodynamy (Baltzer, ’50, ’52) concerns developmental inductions that are followed in the same manner between two organisms. For instance, homodynamy is invoked when embryonic frog epidermis, transplanted into that region of the newt embryo destined to become jaw, produces a frog jaw in the newt head (see Riedl, ’78). There is a similarity in the ability to respond to the same inducer to “form a jaw.” It indicates that the same “readout system” can respond to the same inducer

even in diverse species. Thus, homodynamy is a phenomenon at the organismal level, not at the molecular level. Moreover, it can easily be counterfeited by other organs producing the same signal. The newt otic vesicle or nose rudiment will cause the formation of ectopic limbs when transplanted beneath the flank ectoderm of newt embryos (Balinsky, ’33). This is probably because they secrete FGF8, an excellent inducer of limb formation, although probably not the actual inducer used during embryogenesis to form limbs (Crossley and Martin, ’95; Ohuchi et al., ’97).

The function problem

Objections, explanations, and pleas notwithstanding (e.g., de Beer, ’71; Dickinson, ’95; Bolker and Raff, ’96; Abouheif et al., ’97), the notion that function is linked to homology refuses to die. One reason for its persistence is the common use of “homology” as an impressive-sounding synonym for “similarity” in the molecular literature (Hillis, ’94); a second is that many developmental geneticists are unaware of the history and established meanings of the term. A third reason, however, may be that evolutionists have failed to explain clearly why the notion of “functional homology” is problematic. This flat refusal to consider function in any way when assessing homologies has been particularly unconvincing to biologists working at the molecular level, where structure and function are so intimately intertwined.

The ultimate problem with including function as a criterion (or definition) of homology is that function is not evolutionarily transmitted in the way that structure—or process—is. There is no genetic basis for the function of a structure, or a gene, or a signal cascade, only for the phenotypic character itself. The ends for which the character is used, its functions, are not heritable, and evolutionary homology is a relationship based on inheritance of a character (whether morphological or process) from a common ancestor.

We believe that there are important insights to be gained into development, evolution, and their relationship from examining homologous processes. At the same time, we contend that it is essential to exclude function from assessments or assignments of homology. So how can we include processes among the developmental “characters” we compare without conflating process and function, or homology and analogy?

One answer is simply to be careful. The current trend in developmental and molecular genetic studies is to focus on apparently universal and

deeply conserved features of organisms. It is essential that we remember that even seemingly universal similarities can arise in more than one way. Our first, and often correct, assumption is that such similarities represent synapomorphies. However, extraordinarily similar structures and processes, even with identical functions, can also arise through convergence. (This has happened, for example, in lysozyme sequences, see Stewart and Wilson, '87; Swanson et al., '91.)

A second approach is to exploit the concept of modules to help distinguish processes as such from their results or functions. Homologous pathways, which act as modules, are composed of homologous proteins arranged in a homologous manner. These modules can function in several capacities during development. For instance, the RTK-RAS pathway can be used as a module for the morphogenesis of a *Caenorhabditis elegans* vulva or for the determination of a *Drosophila* photoreceptor cell. The Wnt pathway can be used for the development of fly wings or mouse kidneys. Gene level homologies cannot be determined solely by patterns of gene expression. The case for homology at the gene level can, however, be bolstered by the gene product's similarity to the product of another (putatively homologous) gene that belongs to a homologous pathway. As with organismal-level homologies, evidence for the homology of the whole strengthens the evidence for the homologies of the corresponding parts.

Tricky as it may be, careful discrimination between processes and their results can yield crucial evolutionary (and even therapeutic) insights. In their analysis of recent biomineralization research involving combinations of bone and nacre (Atlan et al. '97; Westbroek and Marin, '98) conclude that

although nacre and bone are not homologous as such, parts of the complex machinery that directs their formation may be. Logically, this would imply that, before the Cambrian diversification, these homologous parts served some function other than biomineralization and that they were co-opted for the latter purpose when the different metazoan stocks began producing skeletons (p. 862).

In this case, widely shared—and homologous—elements of developmental “machinery” or processes have a longer evolutionary history than the tissues they now help to build.

Implications of process homology for the levels issue

The importance of specifying the level at which homologies are assigned has been discussed at

length elsewhere (e.g., Dickinson, '95; Bolker and Raff, '96). In short, hypotheses of homology relate characters at the same level of the biological hierarchy, and homologies at one level—for example, that of gene sequences—do not automatically imply homologies at another level—for example, the structures in which the genes are expressed. (This is especially true when the expression pattern has already been used to classify the two similar genes as homologous.)

Homologies of process form an intermediate level between gene homologies and morphological homologies. Identifying process homologies can therefore help us assess (a) whether genetic pathways themselves are homologous, (b) whether their component parts are homologous, and (c) whether the structures in which they are expressed are homologous. Homologies of process are thus critical connections between the genetic and the morphological levels of organization.

In theory, one should keep different levels entirely distinct. In fact, life is more complicated: gene expression patterns represent an “intrusion” of the genetic level onto the morphological (or, more generally, the phenotypic) level. This complexity is reflected in the different ways evolutionary and developmental biologists describe genes. Evolutionary biologists and population geneticists treat genes as markers or patterns analogous to morphological characters—effectively, as modules of structure. In contrast, developmental geneticists and molecular biologists see genes as causal agents, the basis of specific functions, or elements in networks of functionally interconnected units: modules of process or of function.

This distinction is more than semantic: it represents a fundamental difference in the type of character one considers a gene to be. One can classify the spatial and temporal pattern of gene expression as a quasi-morphological character. Traditional homology concepts, and classical criteria, work fairly well for this. Alternatively, one can consider the gene's action as an element of a process. In this latter case, the concepts of modularity and process homology offer a way to homologize dynamic aspects of gene expression and interactions, while avoiding the morass of “functional” homology.

Expression data and gene homologies

Molecular data—sequences and/or gene expression patterns—have been used in two ways to indicate homology, and the two uses are often confused. The traditional approach is to cite ex-

pression patterns to support hypotheses of *homology between gene sequences*. The second approach is to treat similar expression patterns of homologous genes as evidence for *morphological homologies*. While we believe the former strategy is appropriate (if used carefully), the second is more problematic because homologous genes can be used in many different structures and contexts.

The designation of two genes as homologous has often been based on combinations of structural and functional properties, a procedure that risks conflating homology and analogy. Still, from a less purist and more practical perspective, similar expression patterns and/or functions usually tip the probabilistic argument in favor of a pair of genes' having a common ancestor rather than a fortuitous homoplasy. For example, the *chordin* and *short-gastrulation* genes, whose products have only 29% of their amino acid sequences in common, are considered homologs. These two genes are expressed in the same (i.e., presumably homologous) places in vertebrate and fly embryos. Moreover, their products have similar functions: binding BMP4/DPP proteins, thereby allowing neuralization of the ectoderm (Hawley et al., '95; Sasai et al., '95).

Ultimately, neither structure nor expression pattern alone is a good criterion for gene-level homology. Sequence similarity can be caused not only by divergence from a common sequence but also by (1) convergence to a shared sequence due to selection for a common function such as DNA binding (molecular homoplasy) or (2) the accumulation of exons from unrelated genes. Just because ankyrin has EGF-like domains does not mean that it evolved from a member of the EGF family.

When homologous developmental genes show similar expression patterns, it is often proposed that the structures in which they are expressed are homologous. Thus, the observation of *Distal-less* expression in *Drosophila* leg primordia and in the maxilla was widely seen as proof that the maxillary appendages were homologous to the legs (Panganiban et al., '94). This particular scenario is credible on paleontological grounds as well, but the expression data alone are not sufficient evidence for homology. This was underscored by later research (Panganiban et al., '97) demonstrating *Distal-less* expression not only in the feet of insects, but also in the tube feet of sea urchins and in just about any other structure that everts from the main body axis. A proposed synapomorphy of insect legs became a symplesiomorphy characteristic of any "stickem-outy" from the developing

animal body. The conserved genes, per se, tell us nothing about the evolutionary relationships between particular appendages.

Deep homology and its limitations

In some cases, gene-level homologies, such as the presence of the Hox cluster, can represent fundamental synapomorphies that link widely divergent groups. Such "deep homology" may exist between processes as well. Two processes have been cited as examples of deep homologies. De Robertis and Sasai ('96) suggested that the character "formation of neural ectoderm" is one such deep homology, and that every neural cord uses the same pathway. Similarly, Shubin and colleagues ('97) proposed that limb formation evolved only once, and that despite the enormous divergence between insect and vertebrate limbs, they are constructed according to the same rules and use the same homologous processes.

The chordin/BMP4 pathway that triggers neural ectoderm formation in both the vertebrate and fly ectoderms is an excellent example of a homology of process. In *Xenopus*, the paracrine factor BMP4 is secreted by ventral mesodermal cells and is activated by the combined action of BMP1 and BMP7. Subsequent binding of BMP4 to ectodermal cells causes these cells to activate transcription factors that in turn cause the ectoderm to become epidermal (i.e., skin). However, the organizer region (underlying dorsal mesoderm) secretes chordin, which binds to and blocks the action of BMP-4. This prevents the ectoderm from expressing genes specifying an epidermal fate, so that it instead becomes neural. Activation of this pathway on the dorsal side of *Xenopus* and zebrafish embryos results in the formation of a dorsal neural tube. In the fly *Drosophila*, a BMP4 homolog (the decapentaplegic protein) is activated by BMP1 and BMP7 homologs (the tolloid and screw proteins, respectively) and instructs the ectoderm to produce skin. As in vertebrates, an arthropod homologue of chordin (the short-gastrulation protein, *sog*) blocks the effects of decapentaplegic, causing the ectoderm to become neural where *sog* is secreted. In contrast to the vertebrate pattern, this takes place on the ventral surface of the fly, producing a ventral neural cord.

Despite major differences between the neural cords of flies and vertebrates, the instructions telling them where to form are remarkably similar. This similarity has been confirmed by reciprocal substitution experiments (Holley et al., '95; Schmidt et al., '95; Marqués et al., '97). Chordin

mRNA will cause neural formation in flies; injection of *sog* causes ectopic neural tube formation in the frog. BMP4 will prevent fly neural cord formation, and decapentaplegic protein will block the formation of the frog neural tube. There seems to be only one set of instructions for forming neural ectoderm, though they can be followed in different regions of the embryo. The BMP/chordin interaction specifies neural ectoderm, whether dorsally in the frog or ventrally in the fly (for review and references, see DeRobertis and Sasai, '96; Gilbert, '97).

Although the insect leg and the vertebrate leg are obviously analogous structures, they appear to share a deep homology in the way they develop and thus serve as a second example of deep homology (Shubin et al., '97). Development of vertebrate and invertebrate limbs uses homologous sets of genetic instructions, in largely—though not entirely—homologous ways. Both express the fringe protein at the distalmost end of the proximal–distal axis and use boundary conditions to activate their respective *fringe* homologs. Both use hedgehog homologues to establish the anterior–posterior axis, and ectopic expression of *hedgehog* in the posterior section of the limb disc or field causes the formation of mirror-image ectopic limbs. In both vertebrate and arthropod limbs, the dorsal–ventral axis is marked by the activation of *apterous* homologs by Wingless homologs.

However, there are significant differences as well. Insects have no equivalent of the vertebrate apical ectodermal ridge (AER), nor do they use fibroblast growth factors to mediate cell proliferation or to maintain hedgehog expression. Moreover, even though insect and vertebrate limb primordia express many similar genes, the functions of those genes can vary. While *apterous* is critical in activating *fringe* expression in the fly limb disc, its homolog, *lmx*, is not important for *fringe* homolog expression in the vertebrate limb field. The Engrailed protein confers posterior identity in the fly limb but ventral identity in the vertebrate limb. Such complications undermine the hypothesis of deep homology between limbs. Much of the “toolbox” for making limbs is also shared with nonhomologous structures such as vertebrate teeth (Vaahtokaari et al., '96), which strengthens the argument for convergence rather than true homology between developmental processes in insect and vertebrate limbs. The deep homology of limbs therefore remains controversial.

The argument from parsimony (i.e., the “small toolbox argument”) must be kept in mind when-

ever processes are claimed as homologous, since the smallness of the toolbox implies the distinct possibility of convergence. For instance, if certain fibroblast growth factors generally maintain cell division, certain bone morphogenetic proteins generally initiate apoptosis, and certain transforming growth factors generally stimulate extracellular matrix production, one would expect these proteins to be utilized in different embryos whenever processes of cell division, apoptosis, and matrix formation are required. These commonalities of process, however, do not render the various resulting structures homologous, any more than their common expression of Pax6 makes a mouse's nose homologous to its eyes.

Rewards of thinking about processes

Despite its potential for misuse, we believe the concept of process homology is a powerful tool. Extending the concept of homology from morphological to molecular characters has already shed new light on the relationships and evolution of organisms and the genes they carry. Further extension of the concept of homology to include developmental processes will illuminate relationships between different ontogenies. Ultimately it is ontogenies, not individual organisms or their genes, that evolve. Homology was initially about phenotypes, and the concept was successfully expanded to include genotypes as well; now it is time to apply it to the ontogenetic processes that link these two levels at which evolution is studied.

A carefully defined and applied concept of process homology will refine comparisons of developmental processes between divergent model species and provide a framework for integrating data from nonmodel organisms as well. For example, recent work by Minsuk ('96, '97) has shed new light on the long-studied problem of amphibian mesoderm morphogenesis by going beyond classical anatomical comparisons in traditional “model” species. Minsuk's comparative morphogenetic and experimental approach has broadened our understanding of morphogenetic mechanisms and their evolution within amphibians, and also deepened our understanding of the gastrulation processes of *Xenopus*, the best-known species.

Another merit of process homology for developmental biologists is its inclusion of a time axis, which traditional homology concepts lack. Development is ultimately a process, and its central “characters” are as much temporal as spatial. One difficulty of applying a traditional (structural) homology concept to embryos is that many of their

structures (e.g., somites) are transient or are made up of continually changing populations of cells (e.g., the dorsal blastopore lip, or Hensen's node). The most significant features of embryos are not structures. Rather, they are the processes and changes embryos undergo, and the mechanisms by which those changes occur—whether changes in gene regulation or the mechanical interactions among different regions of the embryo. Moreover, evolutionary-scale comparisons between ontogenies often describe differences in terms of heterochronies, or shifts in developmental timing. Neither sort of character—transient structures or timing differences—can be addressed satisfactorily by either a structural or a genetic homology concept. Carefully defining and identifying process homologies will give us a conceptual tool for comparing dynamic developmental characters and looking at how they have evolved in different lineages.

The concept of process homology is useful for examining not only the evolution of development but also evolution more generally, including the role of genes. Classical evolutionary theory considered evolution a subset of population genetics and explained natural selection in terms of changes in gene frequencies. Each gene was seen as an independent, autonomous unit within the genome and could be transmitted independently of any other. Natural selection occurred via the differential transmission of independent alleles through adult organisms that competed for reproductive advantage.

The present synthesis of evolutionary biology and developmental genetics provides a different, and complementary, view of the roles of genes in evolution. First, the new view focuses on the construction of embryos rather than on competition between adults, with an eye toward the kinds of developmental differences that can generate new structures. Second, the developmental synthesis highlights genetic regulatory regions rather than exonic differences (such as changes that make one enzyme variant more efficient than another). Third, this developmental approach focuses on the similarities between genes rather than on their differences.

Most significantly, we now look at genes as parts of a pathway or field, rather than as autonomous agents. By describing homologies of process between whole pathways, we begin to see genes integrated into new, larger evolutionary units. Recognizing developmental genes, processes, and pathways as modules, and as evolutionary characters in their own right, is a critical step toward

a new synthesis of evolution and development. Evolution ultimately depends on heritable changes in development, and the modular organization of development facilitates evolutionary change in several ways.

First, this organization allows different modules to change without affecting others. Second, modules can be co-opted into new functions. Third, since modules depend on the intercellular communication network of paracrine factors, signaling cascades, and transcription factors, these signal transduction pathways, themselves, can constitute modules that can be used for several different developmental and physiological functions. They can be conserved through evolution and modified both within and between species.

We repeatedly find homologies of process among the signal transduction pathways that operate between embryonic cells. What we now call paracrine factors and ligands turn out to be the long-sought inducers of classical experimental embryology. Embryologists now recognize receptors and signal transducing molecules as components of the competence apparatus that enables certain cells to respond to specific inducers. These signaling pathways are the bases of embryonic induction, which is in turn the core of organogenesis. If macroevolution involves changing morphological features, then the alteration of signal transduction pathways becomes critical for any discussion of large-scale evolution.

CONCLUSIONS: PROCESS HOMLOGY, DEVELOPMENT, AND VIEWS OF EVOLUTION

Identifying the ways in which homologous processes are regulated, replicated, and changed over time will enable us to better understand how changes in development generate changes in morphology and, ultimately, the evolution of new groups of animals.

Waddington ('53) noted that natural selection worked in two distinct modes. The traditional one concerned the elimination of adult phenotypes ("normative selection"), while the less studied mode ("stabilizing selection") eliminated individuals with unstable systems of epigenetic interactions. Concepts of modularity and process homology give us a way to begin exploring the latter mode, which focuses on developmental processes rather than adult phenotypes. Such exploration opens up valuable ways of looking not only at the history of phylogenesis but also at its mechanisms.

The developmental approach to homology and evolution complements the classical genetic ap-

proach. Both are needed. Before this decade, we did not know enough about the mechanisms of development to understand how changes in developmental processes could explain evolution. We are now beginning to acquire that knowledge through our understanding of homologous genes and processes. Looking at the Modern Synthesis in 1953, J.B.S. Haldane used a developmental analogy to express a hopeful sense of things to come: "The current instar of the evolutionary theory may be defined by such books as those of Huxley, Simpson, Dobzhansky, Mayr, and Stebbins. We are certainly not ready for a new moult, but signs of new organs are perhaps visible." The structure of a more inclusive, developmentally oriented evolutionary synthesis is beginning to emerge, and we echo Haldane's conviction that the current diversity of views on the form it should take "...is not a bad sign. It points forward to a broader synthesis in the future."

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