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Establishing homology criteria for regulatory gene networks: prospects and challenges

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Abstract. One of the most remarkable discoveries to emerge from the field of developmental genetics is the observation that many regulatory genes and segments of their interactive networks (pathways) appear to have been conserved in several metazoan phyla. Determining whether these conserved regulatory networks are homologous, i.e. derived from an equivalent network in the most recent common ancestor, is critical to understanding comparisons between model system studies, and the evolution of metazoan body plans. To this end, I outline some of the evolutionary properties of regulatory networks, and propose both similarity and phylogenetic criteria that can be used to test the hypothesis that two regulatory networks are homologous. Furthermore, I propose that genetic networks can be treated as a distinct level of biological organization, and can be analysed together with other hierarchical levels, such as genes, embryonic origins and morphological structures, in a comparative framework. Examples from the literature, particularly the genetic regulatory networks involved in patterning arthropod and vertebrate limbs, are examined using the proposed criteria and hierarchical approach.

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The last two decades of research in the field of developmental genetics has revealed that many of the genes which control embryonic development are remarkably conserved across a broad range of metazoan phyla (De Robertis 1994). Recent comparisons between arthropods, nematodes and chordates suggest that this conservation not only applies to developmental genes, but also to some of the regulatory interactions between them (Gerhart & Kirschner 1997). The discovery of evolutionarily conserved developmental genes and their interactive regulatory networks (pathways) has helped to revitalize the study of the connection between development and evolution, and has facilitated comparisons between distantly related model organisms.

Comparisons between conserved genes and networks must, however, be placed within an evolutionary framework (Abouheif 1997, Abouheif et al 1997). The concept of homology, herein defined as the derivation of an attribute from the most recent common ancestor of two organisms (Mayr 1982), provides such a framework and guides the observations, interpretations and conclusions drawn from any comparison made between two regulatory gene networks. Although there exist criteria for identifying homology among genes, as well as embryonic and morphological structures (Hall 1994), there has been little or no discussion of criteria for establishing the homology among regulatory gene networks. Genetic networks play an important role in the evolution of the genes which compose them, as well as the embryonic and morphological structures to which they give rise (Zuckerkandl 1994). Furthermore, genetic networks can be recognized as a distinct level of biological organization separate from genes, embryonic origins and morphology. Thus, the objective of this chapter is to outline some evolutionary properties of regulatory gene networks, and to establish both similarity and phylogenetic criteria for recognizing whether two genetic networks are homologous.

Searching for and discovering homologies among developmental genes and their interactive networks marks a new era of investigation. Although there currently exist few comparative data on regulatory gene networks, recent advances in molecular and developmental biology are allowing rapid progress in this area. This next century promises to be as exciting as the one in which classical morphologists, such as Etienne Geoffroy Saint-Hilaire, George Cuvier and Richard Owen, first began their search for the homology of anatomical structures across the animal kingdom (Mayr 1982).

Evolutionary properties of genetic networks

Any regulatory gene network is composed of a set of interacting genes (Fig.1; Arnone & Davidson 1997). Therefore, any consideration of whether two networks are homologous must be explicitly based on both the component genes and their regulatory interactions. In contrast to criteria for recognizing homology at other levels of biological organization, such as genes and morphology (Patterson 1988), understanding the interactions and linkages between the component genes in a network is of crucial importance. Considering the homology of both genes and their interactions is a unique property of regulatory networks.

Therefore, when evaluating comparisons between regulatory gene networks, one must distinguish between three alternative hypotheses: homology, convergence and partial homology (Fig. 2; Zuckerkandl 1994). For two regulatory networks to be homologous, all the genes and their interactions must be derived from a network in the most recent common ancestor (Fig. 2A). In

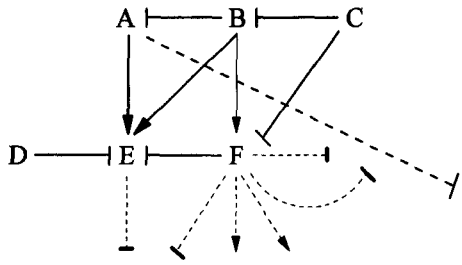


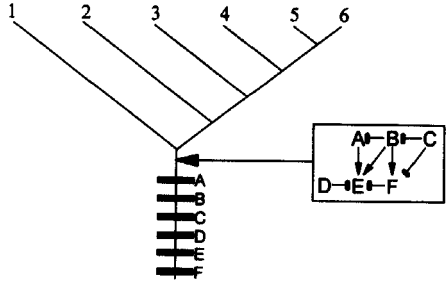
FIG. 1. A hypothetical regulatory gene network composed of six genes. The solid black lines indicate interactions between the genes A, B, C, D, E and F, whereas the dotted lines indicate interactions with genes in another region of the hypothetical regulatory network. Arrow heads indicate that the gene is activating another gene in the network, whereas the short solid lines indicate a repressive interaction.

contrast, for networks to be convergent all the genes and their interactions must have been recruited into a regulatory network after the divergence from a most recent common ancestor (Fig. 2B). Finally, for networks to be partly homologous, some genes and their interactions must be derived from the most recent common ancestor, whereas others must have been recruited into the pathway since the divergence of the species being compared (Fig. 2C).

At least three kinds of regulatory linkages can be involved in the evolution of genetic networks: obligate, facultative and novel linkages. Some members of a genetic network may be obligately linked. For example, tissues or cells expressing the signalling molecule *hedgehog* can, as far as known, only be perceived by the receptor molecule *patched* (Goodrich et al 1996). Currently, the only clear examples of obligate linkage are between genes that transmit signals within and between cells, particularly ligands and their receptors. The more obligate linkages there are in a regulatory gene network, the more likely it is to be conserved.

A particular gene in a regulatory network is facultatively linked if it can be functionally substituted by another gene through evolutionary time (Shubin et al 1997). Although a functional substitution event can occur between two unrelated genes, it may be more likely to occur between two members of a multigene family (i.e. between paralogues — gene copies produced through gene duplication; Xue & Noll 1996). For example, some members of the vertebrate Wnt gene family play important roles in limb morphogenesis. In the chick, the gene *Wnt3a* is essential for the formation of the apical ectodermal ridge (AER), a specialized ectodermal structure running along the distal margin of the developing limb bud (Kengaku et al 1998). The mouse orthologue of *Wnt3a* (i.e. a gene copy derived from the ancestral chick and mouse *Wnt3a* gene) is not expressed in the mouse AER, and the disruption of this gene does not affect AER formation (Greco et al 1996).

(A)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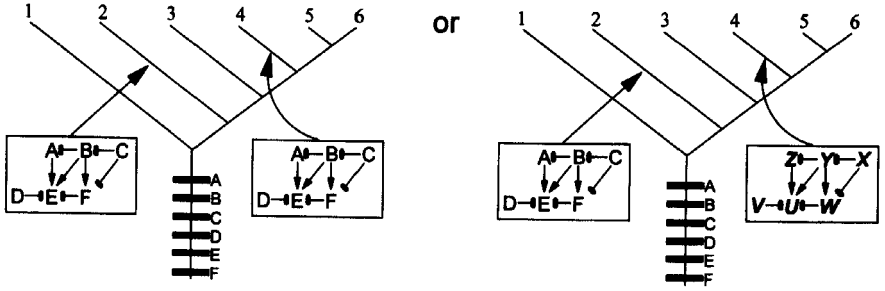
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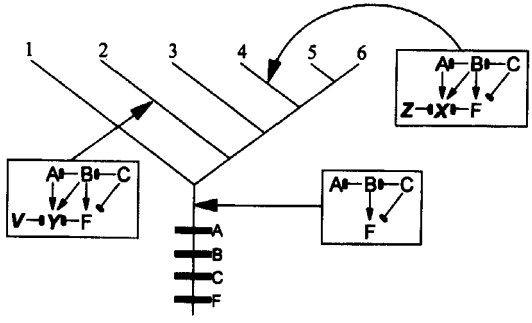
(C)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Interestingly, it is the mouse *Wnt10b* gene which has an expression domain similar to that of chick *Wnt3a* (Wang & Shackleford 1996). Depending on what the ancestral condition was, there may have been a functional substitution of *Wnt3a* by *Wnt10b* in the mouse, or a functional substitution of *Wnt10b* by *Wnt3a* in the chick. The existence of facultative linkages within regulatory gene networks, as in the above example, will tend to make networks partly homologous.

Finally, an inherent property of developmental regulatory genes is their ability to acquire novel regulatory linkages and developmental roles through evolutionary time (Gerhart & Kirschner 1997, Lowe & Wray 1997, Duboule & Wilkins 1998). This can increase the number of components of an existing regulatory network (Zuckerkandl 1997). For example, the transcription factor *distalless* has presumably acquired its multiple developmental roles—such as proximodistal axis formation of limbs in arthropods, echinoderms and chordates (Panganiban et al 1997), pattern-forming functions of the eyespots on butterfly wings (Carroll et al 1994) and branchial arch development in chordates (Akimenko et al 1994)—through the creation of novel regulatory linkages. Thus, the recruitment of novel genes into an existing regulatory gene network will also tend to make genetic networks partly homologous.

It is now well established that both morphological structures and genes can be recruited (co-opted) into new functions through evolutionary time (Gould & Vrba 1982, Lowe & Wray 1997). Thus, it is appropriate to ask whether regulatory gene networks, as a whole, can also be recruited to function in different developmental contexts and unrelated morphological structures. Current evidence suggests that this is indeed possible. For example, the Ras-RTK (receptor tyrosine kinase) pathway is a ubiquitous signal transduction network that has been highly conserved in nematodes, arthropods and chordates (Gilbert 1997). It is composed of approximately six core proteins that transmit signals between the cell surface and genes within the nucleus. This regulatory network appears to have been recruited to function in strikingly different morphological structures, such as mammalian skin, the nematode vulva and the *Drosophila* eye (Gilbert 1997). This implies that homology among networks cannot always be taken as strong evidence to support homology among

FIG. 2. Homology, convergence and partial homology among regulatory gene networks depicted on hypothetical six taxon trees, where the presence of the genes are indicated by black boxes. The interactions between these genes are shown in the little boxes. (A) A scenario of homology among the genes and their functional interactions. (B) A scenario of convergence by either recruitment of old genes into new regulatory interactions, or recruitment of new genes into an ancient set regulatory interactions. (C) Partial homology. Some elements of the regulatory network are homologous, whereas others have been recruited into the network since the divergence of the two species being compared.

embryonic origins and morphological structures. Although the Ras-RTK signal transduction network is homologous in fruit flies and nematodes, this clearly cannot be taken as evidence to support the homology between the nematode vulva and the *Drosophila* eye. Thus, recruitment is an evolutionary phenomenon that can be extended to regulatory gene networks, and further supports the notion that networks can be considered a distinct level of biological organization.

Establishing similarity and phylogenetic criteria for regulatory gene networks

The first step in testing whether two regulatory gene networks are homologous is to use a set of established similarity criteria to *propose* a hypothesis of homology, partial homology or convergence (Shubin 1994). In the following section, I suggest three similarity criteria for regulatory gene networks. Once a hypothesis has been proposed on the basis of these similarity criteria, the hypothesis must then be *tested* by mapping the individual elements of the regulatory gene network on a phylogenetic tree using the principles of parsimony (Patterson 1982, 1988). This phylogenetic criterion, discussed in more detail below, is used to test whether some or all the elements in a regulatory gene network were present in the common ancestor of the taxonomic groups being compared (homology), or whether some or all of the elements in the network have independent evolutionary origins (convergence).

Similarity criteria: proposing hypotheses of homology, partial homology and convergence

Defining regulatory gene networks. Arnone & Davidson (1997) define a genetic network as the regulatory regions of all the component genes and the interactions between them. Regulatory interactions between developmental genes are remarkably complex, and at present there exist only limited data on the operation of genetic networks (Yuh et al 1998). Therefore, it is important to be explicit about defining which subset of a genetic network one is interested in comparing when proposing a hypothesis of whether two regulatory gene networks are homologous, partly homologous or convergent.

For instance, the regulatory gene network shown in Fig. 1 is, in fact, a small subset of a much larger regulatory network (Fig. 3). This means that one is arbitrarily choosing or defining boundaries around a non-linear and continuous network. Because some regions of a regulatory network may be completely conserved, whereas others may be extensively modified over evolutionary time, it is important to attempt to define several different boundaries in order to see how sensitive the conclusions are to the boundaries chosen. Explicitly defining subsets of regulatory gene networks, although arbitrary, is currently the only

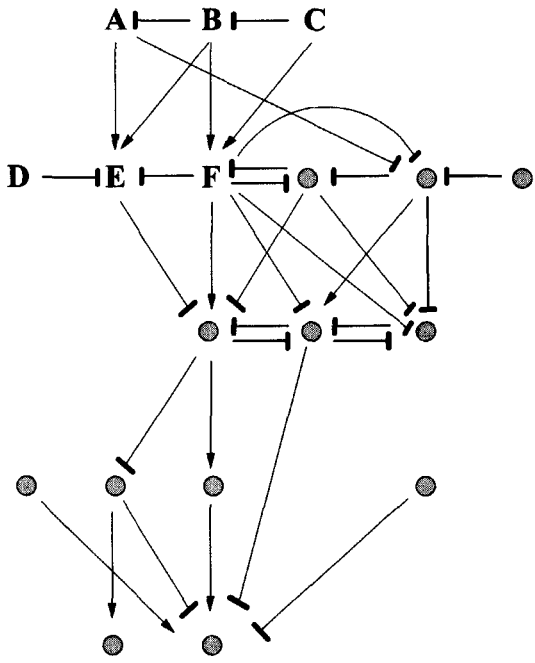


FIG. 3. The hypothetical gene network in Fig. 1 is shown with its connections to other genes to demonstrate the continuity and non-linearity among regulatory gene networks.

option available if we are to attempt to identify homologous elements and make meaningful comparisons across taxonomic groups.

Similarity of genes in a regulatory network. In order for two regulatory gene networks to be homologous, the genes within each network being compared must be orthologues of one another (gene copies produced through speciation; Abouheif et al 1997). Inferences of orthology should not be based on functional or sequence similarity, because unrelated genes can converge on similar genetic functions through evolutionary time, and measures of sequence similarity cannot distinguish between orthologues and paralogues (gene copies produced through gene duplication; Zardoya et al 1996). Determining the orthology between genes requires reconstructing the evolutionary history of the gene families being compared. Determining orthology in this way, however, becomes complicated when gene duplication events have occurred after the divergence of the species being compared (Holland 1999, this volume). For instance, there is only one copy of the *distalless* gene in *Drosophila*, but approximately seven copies of *distalless* in vertebrates. In such cases, one to one comparisons between genes in the regulatory networks of interest must be made with caution (Abouheif et al 1997).

Similarity of gene interactions. Similarity between (1) the biochemical function of the genes, (2) the developmental function of the genes and (3) the relative spatial position in which the genes are expressed, may indicate homology of the interactions among genes between two regulatory networks. For instance, the biochemical role of the *Drosophila hedgehog* gene is as a signalling molecule, and its developmental function in the developing wing imaginal disc is to specify anteroposterior fate by stimulating the secretion of Dpp protein (Brook et al 1996). This is accomplished by binding of Hedgehog protein to the patched receptor, alleviating the repression of Dpp by patched. These functional interactions are conserved in the chick (Shubin et al 1997), as the vertebrate gene *Sonic hedgehog*, is also a signalling molecule which stimulates the secretion of *BMP2*, a homologue of the *Drosophila dpp* gene. Furthermore, both *hedgehog* and *Sonic hedgehog* are expressed in the same relative spatial position, i.e. the posterior compartment in the developing imaginal disc and developing chick limb bud (Shubin et al 1997).

Phylogenetic criteria: testing hypotheses of homology, partial homology and convergence

Testing hypotheses of homology, partial homology and convergence proposed on the basis of the above similarity criteria requires use of the principles of parsimony to map the genes and their interactions onto a phylogenetic tree (for in depth discussion of how to apply this phylogenetic criterion see Kitching et al 1998; Fig. 2). For each species, all of the genes and interactions in the network should be analysed individually and defined as discrete taxonomic characters. When the characters (i.e. the genes and their interactions) are mapped onto a phylogenetic tree, they may support a hypotheses of homology—conservation dating back to their latest common ancestor (Fig. 2A). Alternatively, all or some of the characters may support a hypothesis of convergence (Fig. 2B) or partial homology (Fig. 2C).

The power of testing hypotheses of homology in this way depends upon the veracity of the phylogenetic tree used for the test, the number of taxa sampled and on the quality of the comparative data that are being mapped onto the tree. Furthermore, as knowledge on the operation of regulatory gene networks accumulates, it will be possible to incorporate this knowledge into the evolutionary assumptions made to map the characters (i.e. the genes and their interactions) onto the tree. For example, based on the limited amount of comparative data, it appears that pure convergence among regulatory networks may be improbable (Shubin et al 1997). The evolutionary gain of an entirely new regulatory gene network from its component genes appears less likely than the evolutionary loss of a network. This is due to the fact that only one upstream repressive interaction has to evolve for the regulatory network to be lost,

whereas many new interactions must evolve to assemble an entirely new regulatory network. Thus, it is important to incorporate and account for these types of biological realities when applying the phylogenetic criterion to regulatory gene networks.

Hierarchical analyses of homology and regulatory gene networks

It is now reasonably well established that homology is a phenomenon that can be expressed independently at several distinct levels of biological organization (de Beer 1971, Roth 1988, Dickinson 1995), such as genes, gene functions, gene networks, as well as embryonic origins and morphological structures. Furthermore, homology at one level of biological organization does not necessitate homology at other levels (de Beer 1971, Dickinson 1995, Abouheif 1997, Abouheif et al 1997, Wray 1999, this volume). Because regulatory gene networks can be considered as a distinct level of biological organization, they can be analysed together with the other hierarchical levels using the principles of parsimony and the phylogenetic criterion described above (Lauder 1994, Abouheif 1997). This hierarchical approach can potentially reveal several interesting evolutionary scenarios. Ten, of many possible, scenarios and their implications are outlined in Table 1, which is presented in the hope that it will serve as a method of classifying and interpreting some of the more complex examples in the literature.

Comparing the regulatory network for patterning the dorsoventral axis of the wings of *Drosophila* and chick will demonstrate the utility of Table 1, and provide an example of the hierarchical approach. *Drosophila* and chick wings are convergent as morphological structures, and are derived from different embryological origins (Shubin et al 1997). The *Drosophila* wing is derived from imaginal disc cells in the larvae, whereas the chick wing is derived from a limb bud. Furthermore, the cell interactions that give rise to the *Drosophila* wing occur within the ectoderm, whereas the interactions that give rise to the chick wing are derived from both the ectoderm and mesoderm (Brook et al 1996).

Based on the similarity criteria listed above, I propose that the genetic regulatory network that patterns the *Drosophila* and chick wings may be partly homologous. Presently, there are insufficient comparative data to test this hypothesis using the phylogenetic criterion. Thus, alternative hypotheses, such as homology and convergence, should continue to be considered until enough comparative data are accumulated to distinguish between these possibilities. The dorsoventral network in *Drosophila* wings is shown in Fig. 4 (reviewed in Brook et al 1996). The selector gene *apterous* is expressed in and specifies the dorsal compartment of the imaginal wing discs (Fig. 4a). Both *apterous*- and *fringe*-expressing cells in the dorsal compartment then activate the gene *serrate* at the boundary of cells

TABLE 1 Ten evolutionary scenarios resulting from mapping different levels of biological organization, including gene networks, on a phylogenetic tree

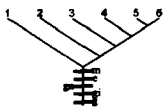
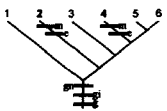
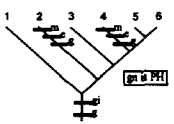
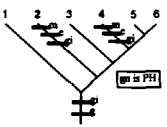
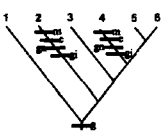
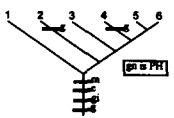
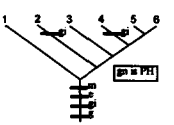
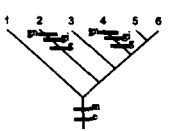
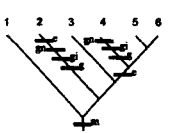
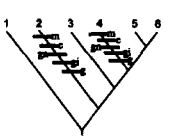
Biological level / *Scenario*	*Genes*	*Gene interactions*	*Gene network*	*Embryonic origin*	*Morphological structure*	*Phylogenetic representation*[a]	*Evolutionary implications*
1	H	H	H	H	H		Homology at all levels of biological organization.
2	H	H	H	C	C		Recruitment of homologous genes and network to function in convergent embryonic and morphological structures.
3	some H some C	H	PH	C	C		Recruitment of novel genes into an ancestral genetic network, and recruitment of this network to function in convergent embryonic and morphological structures.
4	H	some H some C	PH	C	C		Recruitment of novel regulatory interactions into an ancestral network, and recruitment of this network to function in convergent embryonic and morphological structures.
5	H	C	C	C	C		Ancient and homologous genes recruited to interact in a convergent network as well as convergent embryonic origins and morphological structures.

TABLE 1 *(continued)*

Biological level / *Scenario*	*Genes*	*Gene interactions*	*Gene network*	*Embryonic origin*	*Morphological structure*	*Phylogenetic representation*[a]	*Evolutionary implications*
6	some H some C	H	PH	H	H		Recruitment of novel genes into an ancestral network that gives rise to homologous embryonic and morphological structures.
7	H	some H some C	PH	H	H		Recruitment of novel regulatory interactions into an ancestral network that gives rise to homologous embryonic and morphological structures.
8	C	C	C	H	H		A convergent regulatory gene network giving rise to homologous embryonic and morphological structures.
9	C	C	C	C	H		Homologous morphological structure maintained, in spite of other levels being convergent.
10	C	C	C	C	C		Convergence on all levels of biological organization.

[a]Different levels of biological organization are mapped on a hypothetical six-taxon tree. Each character is represented by a black box, and is labelled according to which level of biological organization it represents. C, convergent; e, embryonic origins; g, genes; gi, gene interactions; gn, gene network; H, homologous; m, morphological structure; PH, partly homologous.

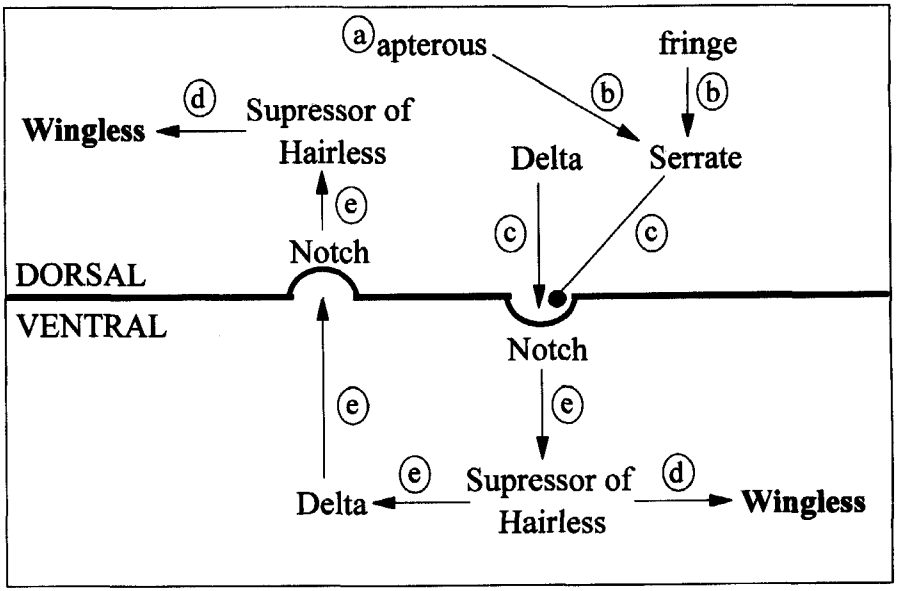


FIG. 4. The regulatory gene network involved in setting up the dorsoventral axis of the *Drosophila* wing. See text for description of this network. (Adapted from Gerhart & Kirschner 1997. Reprinted by permission of Blackwell Science, Inc.)

expressing and not expressing *apterous* (Fig. 4b). Serrate protein is then secreted into the ventral compartment and associates with Delta protein to activate Notch receptor in the ventral cells (Fig. 4c). This stimulates symmetrical *wingless* expression (Fig. 4d), via *supressor of hairless* (Fig. 4e), in both the dorsal and ventral compartments of the imaginal wing disc. The boundary between these two compartments, where wingless protein is being secreted, becomes the margin of the wing blade, and is the major source of cell proliferation and growth of wing imaginal disc cells.

In comparing this regulatory network to that of the chick (Shubin et al 1997), one finds that both the chick *Lmx-1* and *Drosophila apterous* genes are expressed in the dorsal compartment of the developing wing imaginal disc and the dorsal compartment of the mesoderm in the developing limb bud. Both genes are necessary for specifying dorsal fate. As in *Drosophila*, the *Radical fringe* gene is expressed at the boundary of the dorsal and ventral compartments and activates the chick *Serrate-2* gene (Rodriguez-Esteban et al 1997). In turn, *Serrate-2* activates the *Notch–Delta* signalling pathway which leads to the formation of the AER, a structure analogous to the *Drosophila* wing margin where cell proliferation and growth takes place (Laufer et al 1997).

Based on these remarkable similarities, it is tempting to propose that these two regulatory networks are homologous. There are, however, several differences between these networks that support the hypothesis that they are only partly homologous. For example, *apterous* and *Lmx-1* may not be true orthologues of one another, and although they both play similar functional roles in specifying the dorsal fate in the developing *Drosophila* and chick wing, *apterous* has been shown to regulate *fringe* expression, whereas the chick *Lmx-1* gene does not (Irvine & Wieschaus 1994, Shubin et al 1997, Rodriguez-Esteban et al 1997). Furthermore, there appear to be novel functional linkages in the chick regulatory network that are absent from *Drosophila*. In *Drosophila*, wingless protein mediates patterning activity at the wing margin in the developing imaginal disc. Although the chick *Wnt7a* gene is expressed and required for the development of the AER, there is currently no evidence to suggest that any member of the vertebrate *wingless* gene family, including *Wnt7a*, possesses exactly the same function as the *Drosophila wingless* gene in the development of the AER (Brook et al 1996). Instead, patterning of the AER is carried out by two members of the fibroblast growth factor family, *Fgf4* and *Fgf8* (Cohn et al 1995, Crossley et al 1996). Interestingly, no members of the fibroblast growth factor family have been identified in *Drosophila* (Brook et al 1996).

Therefore, although there are many similar, perhaps homologous, elements in the regulatory networks that pattern the dorsoventral axis in *Drosophila* and chick limbs, there have also been evolutionary modifications and additions of elements, supporting the hypothesis that these networks are partly homologous. This example can be tentatively classified under scenarios 3 and 4 in Table 1, where the embryonic origins and morphological structures are convergent, but the gene regulatory network is partly homologous.

Prospects and challenges

Homology, one of the most crucial but complex biological concepts, can provide a framework to interpret comparisons between regulatory gene networks. As comparative data on regulatory gene networks accumulate, the similarity and phylogenetic criteria outlined in this chapter can serve as a methodology to distinguish whether regulatory networks are homologous, partly homologous or convergent. Setting up alternative hypotheses when interpreting comparisons for this class of data will advance our understanding of how regulatory gene networks evolve, as well as facilitate comparisons between the genetic networks of distantly related model organisms. Furthermore, since regulatory gene networks can be treated as a distinct level of biological organization, they can be incorporated into a hierarchical concept of homology. Perhaps one of the greatest challenges

of the future will be to produce enough comparative data to analyse different levels of biological organization — such as genes, gene functions, gene networks, as well as embryonic origin and morphological structures — together in a phylogenetic framework. This will provide an integrated approach to the study of development and evolution.

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DISCUSSION

Wagner: You seemed to be pessimistic about the ability to delineate networks, but your pessimism may be unwarranted. An approach similar to the one that Georg Striedter outlined yesterday could be used to look for co-variation of gene deployment in different organs and different species, and this would generate a variational definition of what usually goes together and what doesn't. This approach would avoid the arbitrariness of individuating a subnetwork from a large network.

Striedter: I also picked up on this point. It might be difficult to delineate gene networks in some cases, but it may often be possible. You said that the problems associated with homologizing gene networks are unique, but your diagrams are similar to neural networks. Neural networks suffer from the same problems you pointed out, namely that they may be hopelessly diffuse, but within this interconnected morass there may be some modules that are relatively individualized. For other analogies, you might also look toward metabolic control theory, where investigators are similarly struggling to identify functional modules within a complexly interconnected system (e.g. Rohwer et al 1996). Along these lines, what puzzled me about your presentation was that you seemed to be saying that networks can be individualized modules but that if they're not identical across species then they can't be 'fully' homologous. I don't understand how you can make these two points in the same chain of thought.

Abouheif: You have to be able to identify the same components. If some components have been added on, then you have some components that you can identify as homologous and some components as convergent. It's the mixing of these components that makes a network partly homologous.

Striedter: It sounds to me that you are requiring 'fully' homologous networks to be identical. But how would you permit a network to change during evolution?

Abouheif: Identical in what sense? It just has to have homologous genes, and these genes have to be doing similar things.

Wray: What you're saying is that this is similar to divergence, because divergence in morphology encompasses homologues that are not identical, but we don't use the term 'partial homology'.

Striedter: Yes. An evolving neural network can contract or expand, by co-opting other neural structures into itself. If I recognize networks at the network level of analysis, then I don't have to say that they're 'partially' homologous. They may be 'fully' homologous and simply change by incorporating new elements or deleting old ones.

Abouheif: But there's a difference between things that were present in the common ancestor and then diverged, and completely new things that have been added on after divergence. I'm trying to differentiate between those two situations.

Panchen: Is your definition of partial homology the same as that for transformational homology of structures?

Abouheif: No. At the structural level, the lower jaw of lizards and the ear ossicles of mammals are the same structures that have transformed. We recognize them because they have the same connections.

Panchen: But they're not the same.

Abouheif: They perform different functions, but it's the same elements that have transformed.

Panchen: That's transformational homology. Isn't that the same for your networks?

Abouheif: No. I would call it transformational homology if the same elements had transformed in one way or another. It is not transformational homology if new elements have just been added on.

Wagner: In the case of morphological characters, there are certain character transformations that follow inherent degrees of freedom, e.g. bones can become longer, shorter or can fuse, and there are others that lead to new constraints and new variational properties. The question is, can a similar way of thinking also be applied to these networks? For instance, are there intrinsic degrees of freedom in, say, rates of transcription, that can change without changing the basic structure of the network, and are there other properties that are more likely to be conserved, for instance as direct protein–protein interactions? If so, is it possible to sort out the intrinsic degrees of freedom for change from other properties that are intrinsically stable because of their biochemistry?

Abouheif: Once we understand more about the evolutionary properties we will be able to do this.

Wagner: The partial homology problem will then disappear if you think of homologues as being different states of the same element. It will be possible to distinguish between stages where some otherwise conservative feature is changed, and occasionally this will lead to the formation of new entities. This would be a way to distinguish between simple modifications and real novelties.

Hall: We want to be able to distinguish between the basic properties at each level. For example, you can tell a cartilage in the lower jaw of a reptile is the same as the cartilage in a middle ear because they share basic properties as cartilages, but they are completely different elements in terms of what they transform into.

Maynard Smith: I worry about this. One type of change in a gene network is the recruitment of a completely new gene with a different ancestry. One could recognize such an event by homology of gene sequences. It is not clear to me that if I was looking at a set of morphological structures, I would be able to recognize an analogous process in which a morphological structure had been altered by the recruitment of a new structural component. Genes are not like bones.

Wagner: One theory of the origin of the neocortex is that it is a composite structure composed of cell populations that in non-eutherian amniotes reside in different nuclei of the telencephalon and are integrated into the modules of the neocortex. This would be an example of several independent anatomical entities that had become integrated into something completely new, namely the neocortex.

Carroll: You didn't get as far as the phylogeny. Structures of the wings of the chick and *Drosophila* are not homologous. However, if there is a control network, then this must be partially homologous and have some other function. What would that other function for *engrailed*, *wnt*, *notch*, etc. be in the putative common ancestor or even in wing-less insects and dinosaurs?

Abouheif: This *wnt* gene pathway is not only involved in the setting up of the dorsoventral axis of the wing discs, but also the dorsoventral axis of the leg discs, the brain discs and the genital discs. Therefore, the system is used repeatedly in different discs.

Akam: There probably isn't a patterning process that doesn't use at least half of the genes in this pathway. What we are looking at is the homology of metazoan cells. Signalling is probably involved in every process where two cells become different from one another. We happen to have studied two developmental events that are similar, in terms of generating wings, and we have found that many of the same genes are involved.

Tautz: It is important to find out why particular cells carry out this signalling pathway in certain locations of the body. Therefore, we should look for the network of genes that cause the cells to be placed at this location.

Akam: To talk about, say, the Ras/GTPase signalling pathway as a significant developmental homology, a developmental network, seems nonsense. It is a bit of metazoan cell biology, and probably almost all cells have that pathway functioning at some level or another. In *Drosophila*, *dpp* expression in the wing is regulated by the *hedgehog*/*engrailed* signalling pathway in the third instar disc, but 10 hours later it is regulated by a completely different regulatory network. How can a pathway be conserved between the *Drosophila* wing and the chick wing, if it is not conserved 10 hours later in the *Drosophila* wing. In many of these comparisons we are dealing with complete nonsense.

Panchen: There is a word for it, i.e. analogy, and this doesn't commit you to any hypothesis.

Wagner: The confusion with analogy stems from the fact that in biology there are two definitions of 'function': (1) the biological role that the thing is adapted to do; and (2) what it is causally effective at doing.

Wray: When we deal with gene products, there is another distinction in the way people use the term 'function' in addition to that which you just described. For example, the biochemical function of a transcription factor is simply to bind to DNA, whereas its function in a developmental sense is defined by the specific

piece of DNA to which it binds and the cell biological effects of that binding. Therefore, we need to be careful to distinguish which kinds of molecular 'function' we are talking about.

Rudolf Raff: Another complicating factor is that the networks which we find scattered throughout phylogeny have different downstream consequences, i.e. highly conserved networks can feed into different downstream functions and will produce the different consequences we observe. In this sense, 'function' has a different meaning.

Roth: Is there such a thing as homology or conservation of a role without the things playing that role actually being conserved?

Wray: We don't have enough data to make clear judgements in some of these cases. However, we have to be open to the possibility that this can occur, and we have to try to discriminate among alternative possibilities, or least keep an open mind about what the possibilities are.

Akam: The structure of promoter modules is relevant. Arnone & Davidson (1997) find that a typical promoter module involves about six transcription factors working together to give an output. No single site is critical, and the different transcription factors can be substituted for one another. Therefore, one can envisage a promoter module with a historical continuity of function, but the individual transcription factors that drive this module change rapidly in evolution, resulting in homologous modules that have less than half of their input factors in common.

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