

Syntagms in development and evolution

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ABSTRACT The genetic analysis of segmentation, neurogenesis, appendage formation and other developmental processes has revealed that the development of *Drosophila* can be broken down into discrete elementary operations. Thus development can be viewed as a stepwise process where each step is driven by a small group of genes working interactively. García-Bellido proposed that each of these groups be called a "syntagm". In this review, we will describe a series of developmental syntagms, and explore the consequences of this discontinuous organization of the developmental program on evolution.

KEY WORDS: *evolution, syntagma, segmentation, dorso-ventral axis, limb formation, axonal guidance, neurogenesis*

"Nous ne connaissons de l'univers que des éléments discontinus"
(Henri Laborit)

Introduction

The basis of species diversity

With the advent of molecular biology we are gaining more and more insight into the mechanisms that underlie major developmental processes such as axis formation, segmentation, neurogenesis and limb formation. Interestingly, this analysis has uncovered impressive similarities between flies, vertebrates and other species in terms of both the genes involved and their interactions. Starting from a point where any functional conservation between arthropods and vertebrates seemed ludicrous, we are now coming to an almost opposite situation: the amount of genetic conservation is so large that one wonders where exactly is the difference between a fly and a mouse, and what is the basis for evolution?

In this paper we propose to explore the different aspects of the concept of syntagm, a word originally coined by A. García-Bellido to describe any group of genes that interact to perform a discrete developmental operation. We propose that differences in the connectivity between syntagms may be an important source of species diversity.

The notion of syntagm

The word "syntagm" is derived from the French word "*syntagme*" which itself is based on the Greek "*syntagma*" (organized set, e.g., the Constitution as an organized set of laws). The Robert dictionary defines the word as: "*groupe de morphèmes ou de mots qui se suivent avec un sens - ce groupe formant une unité dans une organisation hiérarchisée de la phrase*" (a succession of words

making a meaningful group - this group as a unit within the hierarchical organization of a sentence).

This definition could be applied to any group of two or more interacting elements working together in a given process, e.g., amino acids in a protein, or neurons in a brain. As used by García-Bellido, however, the word applies specifically to genes, or gene products. The notion of syntagm applies to any number of genes or gene products that are involved in a given developmental operation, and are linked together by direct interactions. Whether two elements belong to a syntagm can be operationally defined: modifying their interaction (e.g., by severing the interaction, or more subtly by altering the relative dosage of the two genes) must lead to an alteration (a mutant phenotype).

The structure of the developmental program

Jacob and Monod, in their study of the bacterial adaptation to lactose, proposed the operon model. In this model, some genes have the capability to regulate the activity of other genes by acting on regulatory sequences (operators) that are adjacent to the target gene: the first example of a regulatory syntagm (Jacob and Monod, 1961). This discovery led to the idea that the control of development may rely heavily on the function of regulatory genes. A major challenge during the following decades has been to understand how genes regulate development, what is the nature of the operations that they control, and whether they act independently of each other, in hierarchy or in combination.

One possibility would be that developmental programs show no recognizable regularity. In this view, the differentiation of each cell represents the outcome of a large number of regulatory interac-

Abbreviations used in this paper: BX-C, bithorax complex; A-P, antero-posterior; D-V, dorso ventral; AER, apical ectodermal ridge.

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tions that finally adjust all metabolic, cytoskeletal, etc., functions to the levels that correspond to that particular histotype. In this generalized version of the prototypic Jacob-Monod system, the developmental program has no identifiable structure - *stricto sensu*, there is no program at all (Stent, 1981, 1985). Development is seen as the outcome of an intricate set of metabolic regulations, each of which involves a low-complexity syntagm where each interaction can be individually tuned to satisfy the demands of selective pressure. Multiple interactions between individual regulatory loops form a diffuse and very plastic network. Disentangling this network may be possible in bacteria, but attempts at doing so in more complex organisms are hopeless and indeed pointless, for each particular set of gene activities in a particular cell of a particular animal would be contingent.

Alternatively, one might imagine that there is an intrinsic hierarchy in the regulatory network that underlies biological development. Thus the connectivity within the regulatory system would somehow parallel the obvious impression of orderly process that one gains from looking at the development of any organism. This is indeed the essence of the Britten and Davidson (1969, 1971) models: each regulator is connected to a battery of target genes, and each target gene is itself controlled by a combination of regulators, those whose products can bind to its various enhancer elements (updated and reviewed in Arnone and Davidson, 1997). With the Britten and Davidson model, we are moving from a loose, highly plastic association of low complexity syntagms to a hierarchical organization of individual gene interactions where no simple subset can be identified: the program of development becomes a single, very large syntagm.

The picture that is now emerging in the case of *Drosophila* suggests a third picture, where development is genetically subdivided into discrete steps. This picture is rooted in the pioneering work of Ed Lewis, Antonio García-Bellido, Eric Wieschaus and Christiane Nüsslein-Volhard. Lewis showed that the bithorax complex (BX-C), a set of genes linked not only in position but also by common regulatory rules (colinearity and cis-overexpression), is required for the acquisition of segment identities (Lewis, 1978). These genes were later demonstrated to act upon each other at the transcriptional level. García-Bellido analyzed the specificity of the BX-C mutant phenotype and proposed that this specificity could be due to differential activation of the BX-C genes along the antero-posterior axis of the fly embryo. He implicated the regulators of the BX-C, not the BX-C itself, as responsible for the differences along the antero-posterior axis by defining which genes of the BX-C will be activated where (García-Bellido, 1981). Nüsslein-Volhard and Wieschaus in their mutational analysis of segmentation discovered that the mutations affecting the segmentation uncover genes that belong to either of three clear-cut classes: the gap genes, the pair-rule genes and the segment polarity genes (Nüsslein-Volhard and Wieschaus, 1980). As it turned out, the gap and pair-rule genes are the regulators of the BX-C anticipated by García-Bellido.

In the next section, we will describe a series of developmental syntagms to illustrate the variety of their composition.

Developmental syntagms

Segmentation

The segmental patterning along the A-P axis is best understood in *Drosophila*. Three groups of genes (gap genes, pair-rule genes

and segment polarity genes) are involved in a temporal cascade to progressively subdivide the embryo into discrete segments. A fourth group, the homeotic genes, makes the segments different from each other.

The gap genes activate the pair-rule genes, which themselves control the expression of the segment-polarity genes. The final segmentation results from the establishment of compartment boundaries marked by the limits of expression of the segment-polarity gene *engrailed* (*en*). The gap and pair-rule genes also control the expression of the homeotic genes which make the segments different from each other. The control cascades leading to the appropriate expression of *en* and of each homeotic gene correspond to the original definition of the bithorax syntagm (García-Bellido, 1981). Each cascade comprises (1) a particular selector gene responsible for selecting which part of the genome will be active in the cells where it is expressed, e.g., one of the BX-C genes, (2) its activators (gap and pair-rule genes), responsible for the activation of the selector in the appropriate region of the embryo, and (3) its realizers (targets of BX-C genes). This type of syntagm can be called "temporal" since its constituents are activated in a temporal sequence: first activators, then selector, then realizers.

In addition, however, each of the four groups of genes mentioned above also forms a syntagm: gene interactions within each group are instrumental in translating quantitative information (e.g., gradients) provided by the genes of the previous group into qualitative information (boundaries, discrete population of cells). For example, the interactions between the products of the gap genes help transform overlapping bell-shaped distributions of gene products into sharply defined abutting domains of gene expression (French, 1988). Likewise, the interactions between the pair-rule genes contribute to the transformation of the patchwork of gap gene domains into a spatial organization based on metameric reiteration (Ingham and Gergen, 1988). The segment polarity genes also turn out to be a set of interacting genes that translate the alternating expression of the pair-rule genes into a segmentally repeated set of boundaries (Howard, 1988 and references therein). Finally, the proper expression of each homeotic gene depends not only on the gap and pair-rule genes but also on its interactions with other homeotic genes. The four genetic teams can therefore be defined as syntagms. We will call such syntagms "spatial" since the interactions define spatial, rather than temporal, relationships. The interactions between these four teams create yet another syntagm of higher complexity.

At this level it is worth recalling the classical definition of the syntagm as any group of interacting elements that forms a meaningful unit, a definition that allows syntagms to be part of higher level syntagms. This seemingly confusing aspect of the notion of syntagm will be discussed further at the end of this review.

While individual genes or sets of genes belonging to the three segmentation syntagms are conserved among various arthropod groups (Orthoptera, Coleoptera, Lepidoptera, Hymenoptera), there are significant differences which could account for large differences between the modes of development among these groups (e.g., long germ band vs short germ band, review Patel, 1994). In other phyla, the search for members of segmentation syntagms as well as the analysis of their function or connectivity is only beginning; yet the first results suggest that there may be at least partial conservation between arthropods and other phyla (Love

and Tuan, 1993; Akasaka *et al.*, 1996; Hobert *et al.*, 1996; Holland *et al.*, 1997).

Dorso-ventral axis

In *Drosophila*, *short-of-gastrulation (sog)* defines the ventral pole of the embryo and counteracts the product of the *decapentaplegic (dpp)* gene, which is necessary for dorsal identity (Biehs *et al.*, 1996). In *Xenopus*, *chordin* and *BMP4* are counterparts and functional homologs of *sog* and *dpp* respectively, and interact much in the same way as *sog* and *dpp* do to establish the dorso-ventral axis of the embryo (Sasai *et al.*, 1994, 1995; Holley *et al.*, 1995; Schmidt *et al.*, 1995). Interestingly, however, the D/V axis as defined by the domains of activity of *chordin* and *BMP4* in vertebrate is inverted relative to the V/D axis defined by *sog* and *dpp* in the fly. Since the ordering of successive germ layers following the onset of *chordin/sog* and *BMP4/dpp* activity is also conserved, it follows that the axis has been conserved but its direction has been inverted in vertebrates relative to flies, e.g., the nerve cord is dorsal in vertebrates, but ventral in arthropods.

Formation of sense organs

In *Drosophila*, the formation of sense organs depends on a cascade of discrete operations (for review, Ghysen and Dambly-Chaudière, 1989; Vervoort *et al.*, 1997). In a first step, a set of prepattern genes (e.g., some pair-rule genes in the embryo, *iroquois* and *pannier* in the adult) defines heterogeneities in the undifferentiated ectoderm. A second set of genes, the proneural genes (*achaete-scute* complex, *atonal*, *daughterless*), are locally activated in response to particular combinations of prepattern gene products, and thereby define groups of cells competent to form a sense organ. The proneural genes activate *Delta*, a member of the neurogenic syntagm (including *Notch*, *Delta*, *Suppressor of Hairless*, *Enhancer of split*) which sets up a system of lateral inhibition. This system presumably involves a negative feedback of *Notch* on the proneural genes. This system allows only one of the competent cells to become a precursor. The precursor then divides according to a fixed lineage to generate the four cell types that will form the bristle. Cell fate allocation in the lineage depends on a fourth set of interacting genes. This set of genes includes again *Notch* and possibly *Delta*, but it differs from the neurogenic group in that it includes other genes, forming a new syntagm in which the gene *numb* is involved (Frise *et al.*, 1996; Spana and Doe, 1996).

Many of these genes have been widely conserved during evolution. Homologs of the *achaete-scute* genes have been found in hydra (Grens *et al.*, 1995), nematodes (Zhao and Emmons, 1995) and various vertebrates (Lo *et al.*, 1991; Guillemot and Joyner, 1993; Zimmerman *et al.*, 1993; Hatten *et al.*, 1997) and are in general involved in neural determination. Homologs of *Notch* and *Delta* have been found in vertebrates (Chitnis *et al.*, 1995) and in nematodes, where they appear to regulate neural differentiation much in the same way as in *Drosophila* (for review, Kopan and Turner, 1996; Lewis, 1996). A *numb* homolog has been identified in murine species (Verdi *et al.*, 1996; Zhong *et al.*, 1996) where it acts in concert with the *Notch/Delta* syntagm to control the acquisition of neural fate (for review, Huttner and Brand, 1997). Finally, homologs of the prepattern gene *iroquois* have recently been found in the mouse (see review by Modolell and Campuzano, this issue). Although more work needs to be done to define the connectivity between *iroquois*, *achaete-scute*, *Notch/Delta* and *numb* homologs

in vertebrates and worms, the fact that all or most of the genes are involved in the acquisition of neural fate suggests that at least parts of the syntagms that operate in flies have been conserved in other phyla.

Appendage formation

Limb formation is another example where syntagms are conserved between fly and vertebrate. In *Drosophila* wings and vertebrate limbs, outgrowth in the distal direction occurs from a margin at the border between dorsal and ventral portions of the appendage. In the fly, this margin occurs at the border between *fringe*-expressing cells in the dorsal compartment and non-*fringe*-expressing cells in the ventral compartment (Irvine and Wieschaus, 1994). At this border, *Serrate (Ser)* is upregulated (Kim *et al.*, 1995) and activates syntagms in which *N/Dl*, *vestigial (vg)* and *wingless (wg)* promote further patterning and distal growth (Couso *et al.*, 1995; Neumann and Cohen, 1996). In vertebrates, the border between dorsal cells expressing a *fringe (fg)* homolog, *Radical fringe (R-fg)*, and the ventral cells not expressing it, signals the formation of the apical ectodermal ridge (AER) (Laufer *et al.*, 1997; Rodriguez-Esteban *et al.*, 1997). This ridge is similar to the *Drosophila* wing margin in that it expresses the homologs of fly *Ser*, *N*, *Dl*, and *wg* and is required for limb outgrowth. Thus the syntagms defining limb margins and wing outgrowth appear conserved between flies and vertebrates. In addition, both in flies and vertebrates, limb compartmentalization involves the genes *engrailed/engrailed-1*, *wingless/Wnt-7a* (Dealy *et al.*, 1993) and *apterous/lmx* (Vogel *et al.*, 1995). However, the compartments in which these genes are expressed in relation to the A-P and D-V axes show some differences, which will be discussed below.

Axonal guidance

The cues for the dorsalward and ventralward guidance of axons seem to have been conserved between nematodes, vertebrates and insects. In the nematode, the current model is that the *unc6* gene product is concentrated ventrally in the animal. This product appears to be both attractive for neurons with dorsally located cell bodies which project their axons ventrally, and repulsive for neurons with ventral cell bodies that project dorsally. The attraction is mediated by binding of UNC6 to a receptor encoded by *unc40*, which is found on ventrally projecting axons. Similarly, the repulsion is mediated by binding of UNC6 to a receptor complex encoded by products of the *unc40* and *unc5* genes which is found on the dorsally projecting axons (Culotti, 1994; Chan *et al.*, 1996).

The vertebrate homologs of *unc6*, *unc5* and *unc40* have been isolated and called *netrin*, *unc5H1-4* and *dcc* respectively. Netrins are chemoattractants for commissural axons that extend ventrally in vertebrate spinal cord. The *unc5H* has been shown to encode a netrin-binding protein (Leonardo *et al.*, 1997), while *dcc* is expressed on the axons and anti-DCC antibody treatment blocks netrin dependent outgrowth (Keino-Masu *et al.*, 1996). Although further examination of the interactions between these products is needed, the present data, strongly suggest that the guidance mechanisms are at least partly conserved between nematodes and vertebrates.

In the fly, two of the three components have been isolated so far, netrin and the *unc40* homolog, *frazzled*. Both are involved in motoneuron guidance. *frazzled* is expressed on motoneurons that use a netrin source for proper orientation (Kolodziej *et al.*, 1996).

Thus, at least one syntagm for axon guidance seems to have been conserved between worms, flies and vertebrates.

The properties of syntagms

The examples given above illustrate how the program of development is assembled from discrete genetic subunits, the syntagms. A syntagm may comprise as few as two members, but typically includes more genes. By definition, each gene of the group (or its product) must interact directly with at least one other member. It usually happens, however, that there are multiple direct interactions, such that every member of the group can interact with several partners. Thus the connectivity within one syntagm is often very high, making it extremely resistant to change (see below).

In its original definition the syntagm referred to a sequential structure including a selector gene, its upstream activators and its downstream realizators. In addition, however, many developmental operations depend on "spatial" syntagms, groups of interacting genes acting in parallel. Thus, the same gene can be considered in the context of different syntagms. This is conveniently illustrated by the case of *Ultrabithorax (Ubx)*, the very gene that motivated the adoption of the word by García-Bellido. *Ubx* can be studied from the point of view of the "bithorax-syntagm", stressing control and target genes, and also from the point of view of the "homeotic-syntagm", stressing its interactions with the other homeotic genes.

Besides the distinction between temporal and spatial, syntagms can also be classified as "elementary" or "complex" according to whether they can be broken down into smaller units or not. Elementary syntagms can be part of different complex syntagms: for example, the *N/D1* elementary syntagm is found in various complex syntagms such as those underlying lateral inhibition, cell lineage, wing margin development and oocyte determination.

The notion of syntagm provides a new and manageable way to analyze development and evolution. The modular nature of the developmental program provides a potential explanation for diversity, as illustrated by a comparison of fly and vertebrate limb development. Both involve conserved syntagms as illustrated above; however, limb formation also involves the Hox syntagm in vertebrates, but not in flies. Thus, while the syntagms themselves have been conserved, their connectivity has not.

Syntagms and the individual gene

Code genes

We have considered so far that most of the interactions within a syntagm control the level of expression or activity of individual genes, each of which acts either as a regulator modifying other genes' activities, or as an effector modifying cell shape, function or behavior. In this view, each gene has its own effect and if several genes are active in the same cell, their effects are additive. A somewhat different view, the "code" hypothesis, has been proposed in the case of the homeotic genes in flies and in vertebrates (McGinnis and Krumlauf, 1992, Lawrence and Morata, 1994, see also Castelli-Gair, this issue). In the code view, the effect of combining different gene activities is not additive but combinatorial: the effect of genes a and b acting together would code for, or determine, a special property or fate different from those coded for by genes a and b independently.

The difference between the additive and the combinatorial views can be illustrated by the difference between a mosaic of green and red tiles, which is green and red (additive), and a mixture of green and red light, which is yellow (combinatorial). In the few cases where it has been rigorously tested, the "code" hypothesis does not seem to hold and Lewis' view (1978, 1982) that each segment is a mosaic of specific structures and functions, each of which is under a specific homeotic genes' control, seems much closer to the truth (Castelli-Gair and Akam, 1995). It certainly remains possible that in some cases homeotic genes do act in concert but combinatorial coding of metamere identity is probably not the rule - at least not in *Drosophila*.

Key genes

In all the examples illustrated above, each developmental step or operation depends on a "spatial" syntagm. There is, however, experimental evidence pointing to the existence of genes that would by themselves trigger the development of a specific type of structure. Such genes have been defined as "key" or "master" genes. One of the most prominent examples of a master gene is *MyoD1* which, when transfected into fibroblasts, induces them to undergo myogenesis (Goswami *et al.*, 1993). A similar case is the *eyeless* gene of *Drosophila*, which promotes eye development in tissues where it is ectopically expressed in flies (Halder *et al.*, 1995). Thus it would seem that some developmental operations are controlled by a single gene, rather than by a syntagm. The case of *MyoD1* suggests that this conclusion may be deceptive, however.

Extensive analyses of the determinants of myogenesis in vertebrates have amply demonstrated that *MyoD1* is embedded in a set of interacting genes, and that its "master" function derives from the experimental set-up that has been used (Thayer MJ and Weintraub, 1990). The fact that *eyeless* (or its mouse homolog *pax-6*) can exert its eye-determining effect only in some tissues of the fly suggests that here again the master gene can operate only in a given genetic background, and therefore requires other elements to fulfill its function. In this view, gene A in one species would substitute for gene A' in another species whenever it is capable of interacting with the other elements of the syntagm. We need to know more about the elements with which *eyeless/pax6* interacts before we can decide whether or not these "master" genes are embedded in "eye-determining" syntagms.

Syntagms and development

One way to assign a function to a gene is to consider the terminal phenotype that results for its loss of function. For example, the loss of *Ubx* function leads to a fly with two nota and two pairs of wings. One might therefore conclude that *Ubx* has a wing-suppressing and notum-suppressing function. This, however, would be a deceptive statement because in butterflies, where hind wings are prominently present, *Ubx* is expressed much like it is in flies (Warren *et al.*, 1994), reminding us that fly and butterfly T3 wings are just modified version of their T2 counterpart. Thus the function of *Ubx* is not to repress wing formation, but to allow the modulation of mesothoracic elements (wing, notum) in more posterior segments. This modulation may result in minor differences in wing pattern, in some insects, or in near disappearance, in flies. Homeotic genes are used to modulate a set of properties that are repeated

along the antero-posterior axis -their expression in defined domains simply creates regulatory possibilities that may or may not be exploited, and will be so in different ways by different cells and in different species.

In the wing imaginal disc of *Drosophila*, the gene *engrailed* is expressed in the posterior compartment. We could conclude that *en* is responsible to give an A-P polarity and more specifically to define the posterior compartments. Again, this could be a deceptive statement because in vertebrates, *en-1* is expressed in the ventral compartment of the ectodermal layer and the underlying mesoderm of the budding limb. In addition, mutational analysis of *engrailed* during limb development in fly shows that *en* function is not solely restricted to compartmental identity but to growth and patterning as well (Hidalgo, 1994). The case of *engrailed* illustrates the difficulty of assigning a function to a gene, reminds us that final structures in development do not depend on single genes but on gene assemblies, and illustrates how trying to associate a specific function to an isolated gene may often be pointless.

The nature and shape of a given structure is the outcome of the progressive increase in organizational complexity and in positional resolution created by the connected activities of many syntagms. A corollary to this conclusion, and possibly the major contribution of developmental genetics, is therefore that instead of equating one gene to one end result, one has to analyze the program itself, identify its functional subunits (syntagms), and work out how these subunits are interconnected.

Syntagms and evolution

Inertia

If syntagms are the building blocks of development, it seems plausible that they are also the basic material of evolution. The complex set of interactions within a syntagm will endow it with a very large inertia, however, since any change will likely disrupt the function of the entire set. Except in their most primitive forms, therefore, when the connectivity is low, syntagms are expected to be as resistant to change as complex multimolecular assemblies such as ribosomes and transcription complexes.

Furthermore, since we argue that the functional unit of the developmental program is the elementary-syntagm rather than the individual gene, we would expect that once a syntagm has been exploited to perform a given developmental operation it will remain associated with that operation. Its elements will therefore appear to have conserved the same developmental function in widely different species, as illustrated above. The fact that closely conserved, and even interchangeable, syntagms are found in morphologically very different species argues against variations in the syntagms being responsible for these differences.

More than the elementary syntagms themselves, which are largely invariant, it must therefore be the connectivity between syntagms that defines the developmental program, and the origin of developmental differences is probably to be found in changes in this connectivity. In the following sections we will examine how the constraints linked to syntagm rigidity may facilitate rather than hinder evolution.

Recycling syntagms: cassettes

Even if entire syntagms appear to have been conserved in association with a given developmental function, in the examples

given above, there is no reason why they could not be exploited for other purposes if the possibility arises. The products of some of the proneural genes in *Drosophila*, which by virtue of forming active or inactive heterodimers can efficiently measure relative concentrations, are involved in the measuring of the ratio of X to autosomal chromosomes in flies (Younger-Shepherd *et al.*, 1992). Signaling pathways such as the *Notch/Delta* or the *wingless/hedgehog/patched* system are used in oogenesis, neuroectoderm determination, sensory organ emergence, segmentation and appendage formation (Doherty *et al.*, 1996). Such "recycled" syntagms that can be used again and again to fill a number of diverse functions can be considered as developmental cassettes (Younger-Shepherd *et al.*, 1992; Jan and Jan, 1993).

Another spectacular example of recycled syntagm is the homeotic genes in vertebrates, which are involved in the specification of regional differences in several tissues (including the antero-posterior axis of the CNS, the proximo-distal and dorso-ventral axes of limbs. The case of the homeotic complexes also illustrates a variation on the theme of recycling cassettes: syntagm duplication, which has the advantage that it allows modification of the syntagm without affecting its original function.

Combining syntagms into higher-order syntagms

As syntagms can serve as developmental cassettes, their effect on development varies according to the other components with which they are combined, or according to their position in the hierarchy of developmental instructions. An illustration of the first situation, differences in the combination of syntagms, is provided by a comparison of limb development in flies and vertebrates. Both involve the N/DI syntagm and at least part of the segment-polarity syntagm, but limb formation in vertebrates also includes the homeotic complex which is not involved in the growth and patterning of the fly appendages (Tabin, 1991).

An illustration of the second situation, changes in the hierarchical position of a syntagm, is the use of the segment-polarity syntagm in both segmentation and limb formation in *Drosophila*. In segmentation, however, the activity of the segment-polarity genes depends on the previous deployment of the gap and pair-rule syntagms. On the contrary, in limb formation, the segment-polarity genes seem to be at the origin of the process.

Playing with syntagms: neoteny and other abrupt changes

Comparing limb formation between fly and vertebrate, it seems that development is essentially discontinuous: a syntagm can be integrated in a complex-syntagm, it can be disconnected from the complex-syntagm where it belongs or it can even change hierarchical position within the complex-syntagm to create a new complex-syntagm and innovating new developmental processes. Since blocks can be added, deleted or rearranged from an extant complex-syntagm, the resulting evolutionary changes in development will be dramatic and sudden rather than progressive. This is illustrated by the following three examples.

Neoteny results in reaching reproductive maturity during the larval stage, prior to adulthood. This presumably occurs by alteration of the time where sexual maturation complex-syntagm is active. In neotenic species, the adult development program may be preserved if it presents some advantage, but it may also be discarded. More generally, changing the time or space where a syntagm is active may drastically effect morphogenesis, as is now

emerging in the case of segmentation and the cascade of segmentation syntagms (Patel, 1994).

Molgula oculata and *Molgula oculata* are closely related ascidian species. As their confusing names do not indicate, *M. oculata* has a tail while *M. Oculata* does not. The basis of this difference is the level of expression of one gene, *manx*, which is down regulated in the tailless species such that tail formation is prevented. When *manx* is experimentally upregulated in the tailless species, a complete tail is formed (Swalla and Jeffery, 1996) suggesting that the entire, higher-order tail syntagm is still present in the genome of the tailless species. It is simply not used anymore, with dramatic morphological consequences, but without much effect on the other parts of the developmental program (due to the modularity of this program), and therefore without impairing species survival.

Another case of change in connectivity between syntagms is what we might call a short cut. An example is that of the two closely related sea urchins *Heliocidaris erythrogramma* and *Heliocidaris tuberculata*. The two adult forms are morphologically similar but the embryonic development is very different. In *H. tuberculata* the embryonic development is of the indirect type, with a pluteus stage, whereas *H. erythrogramma* develop directly from egg to adult. One difference lies in the heterochrony of expression of the *msp130* gene between the two species due to changes in the regulatory region of this gene (Klueg *et al.*, 1997). Since the two urchin species diverged 10 million years ago (Smith *et al.*, 1990; McMillan *et al.*, 1992), chances are that the genetic program allowing for the pluteus stage has been lost in the directly developing species. This would make it impossible to revive a pluteus stage in *H. erythrogramma* as could be done with *Molgula's* tail. Nevertheless, it will be of interest to attempt such an experiment to define what portion of the unused pluteus syntagm have been conserved.

These examples illustrate the difficulties that arise when one tries to understand the genetic program in terms of its end result. Indeed it would seem that the opposite attitude, trying to understand the end result in terms of the mechanisms that generate it, makes more sense. This attitude, however, is difficult to reconcile with the idea that the driving force of evolution is natural selection, for this bears only on the end result of the developmental program. Is it possible, then, that natural selection plays a more limited role in shaping evolution than is usually assumed? Could internal constraints play a major role in the evolutionary process by defining the realm of possible changes?

Darwin and the syntagm

Darwin's view of evolution is founded on the observation of morphological diversity among species and their adaptation to the outside world. According to him, the driving force for species divergence and evolution is "natural selection" or "survival of the fittest," and the consequence is that "as natural selection acts solely by accumulating successive, favourable variation, it can produce no great or sudden modification..." (Darwin, 1859). He was aware that morphological intermediates between two different species are never or seldom found, contrary to his own rule that modifications must be small and accumulate gradually. He thought that this was due to the imperfection of geological record. However, in light of the data reviewed in this paper, it seems likely that in most cases Darwin would never have found the intermediate forms, for they did not exist. This is because changes in syntagm connectivity result in discontinuous changes. If development is modular evolu-

tion must also proceed by discrete steps. This new insight does not detract from Darwin's theory; it simply changes the type of variation expected from a given mutation. Changes need not be gradual and subtle but may proceed by leaps and can result dramatic changes.

Thus, the syntagmatic nature of the genetic program of development is a source of major constraints on evolution, as the very structure of syntagms makes them resistant to change. Yet at the same time this modular structure provides an unprecedented capability for discrete, "saltatory" changes due to rearrangements among the existing syntagms, and becomes an essential feature of any evolutionary process.

We have considered so far only the inertia associated with the multiple interactions within one syntagm. The connectivity between syntagms is more flexible, because it involves simpler interactions -maybe as simple as a one gene, one target interaction. In spite of a larger flexibility, however, there is also undoubtedly some inertia in the connectivity, and syntagms within a given developmental program are probably so adjusted to one another that the room for changes and evolution is reduced. Indeed, the average survival time of a species suggests that once a new, developmentally coherent rearrangement of syntagms has been identified by chance, this new combination remains stable for millions of years. Presumably this length of time is what is required to develop in a population a sufficient array of polymorphism and variations in connectivity. Random reassortment of these variations will constantly provide possible new solutions to the connectivity problem, and viable variants can then be put to the test.

Conclusion

We presently view development as a concatenation of discrete operations, each of which results from the action of a small set of interacting genes called a syntagm. The operations themselves are largely invariant, due to the high inertia of the underlying syntagms. The connections between operations, however, are more flexible since changes at this level do not impair the workings of each operation but rather create more possibilities and serve as a source of species diversity. Thus we expect evolution to be essentially as discontinuous as the developmental program itself. Major aspects of the evolutionary process, such as its saltatory nature and the highly discontinuous morphologies of the major phyla, are consistent with this view.

The idea that evolution and the creation of new forms stem from within and not from without, and that the changes reflect a continuous reassortment of existing, highly invariant operations, makes it necessary to reconsider the belief that natural selection and adaptation is the driving force for species diversity. Darwin himself was well aware that the driving force of evolution was variation: "...[Natural selection] implies only the preservation of such variations as arise and are beneficial to the being under its conditions of life..." (Darwin, 1859). What we have now discovered is that the syntagmatic structure of the developmental program imposes strong constraints on the type of variation that can arise and be viable.

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