Phenotypic and dynamical transitions in model genetic networks
II. Application to the evolution of segmentation mechanisms

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ABSTRACT

Knowledge of the genetic control of segmentation in *Drosophila* has made insect segmentation a paradigmatic case in the study of the evolution of developmental mechanisms. In *Drosophila* the patterns of expression of segmentation genes are established simultaneously in all segments by a complex set of interactions between transcriptional factors that diffuse in a syncytium occupying the whole embryo. Such mechanisms cannot act in short germ-band insects where segments appear sequentially from a cellularized posterior proliferative zone. Here we compare mechanisms of segmentation in different organisms and discuss how the transition between the different types of segmentation can be explained by small and progressive changes in the underlying gene networks. The recent discovery of a temporal oscillation in expression of vertebrate homologs of the pair rule gene *hairy* during somitogenesis enhances the plausibility of an earlier proposal that the evolutionary origin of both the short and long germ band modes of segmentation was an oscillatory genetic network (Newman, 1993). An implication of this scenario is that the self-organizing pattern forming system embodied in an oscillatory network operating in the context of a syncytium (i.e., a reaction-diffusion system), which is hypothesized to have originated the simultaneous mode of segmentation, must have been replaced by the genetic hierarchy seen in modern-day *Drosophila* over the course of evolution. The strong tendency, demonstrated by the simulations in the accompanying paper, for "emergent" genetic networks, associated with self-organizing processes, to be replaced by natural selection with hierarchical networks, is discussed in relation to the evolution of segmentation.

1. Introduction

The special suitability of *Drosophila melanogaster* for genetic analysis has led to it being the best understood developmental system at the genetic level. In *Drosophila* segmentation, for example, a precise mechanistic understanding of how networks of gene products produce morphological patterns has emerged. Specifically, the formation of segments has been shown to depend on the prior establishment of spatial patterns of gene expression, including (depending on the gene) gradients or 1-7 stripes arranged perpendicular to the anteroposterior axis of the embryo (Gilbert, 2000). As discussed below, such patterns are conserved in many insects, and although they are relatively simple, the networks of gene product interactions that give rise to them are not.

In order to obtain a better understanding of the dynamics of pattern specification produced by such networks, mathematical models have been developed (Hunding et al., 1990; Reinitz and Sharp, 1995). Although all models inevitably ignore some aspects of reality, such approaches are especially useful for integrating and predicting global effects of the manipulation or mutation of single genes (Reinitz and Sharp, 1995; von Dassow et al., 2000).

During evolution, changes in gene expression patterns are produced by mutations affecting the genetic networks that generate such patterns. In the accompanying paper we have presented a strategy for simulating pathways of evolution of pattern-forming networks, as well as some results that suggest that powerful evolutionary inferences can be drawn from studying such model systems. The advantage of this approach is that it can correlate possible morphological transitions with changes at the molecular level. In addition, as we have shown, the variational properties exhibited by different types of networks are so different that strong inferences can be made about the underlying molecular bases of the origin and stabilization of patterns and forms

despite the inherent historical nature of evolution. The aim of this paper is to show how certain general results obtained by studying the evolution of model genetic networks can be applied to some paradigmatic evolutionary problems of insect segmentation.

2. Modes and mechanisms of segmentation

Long, short, and intermediate germ band insects

In *D.melanogaster* and other long germ-band insects segments are generated synchronously from a blastoderm occupying the whole surface of the embryo. The blastoderm is produced through the cellularization of a syncytium comprising the entire embryo (Anderson, 1973; Patel, 1994b). In contrast, in short germ-band insects such as *Schistocerca*, the blastoderm initially occupies only one segment, and the remaining segments are sequentially produced from a posterior proliferative zone (Fig. 1).. Short germ-band segmentation has been found only in some species within some groups (Anderson, 1973). The most widespread mode of segmentation among insects is found in the intermediate germ-band organisms, where a species-specific number of segments forms synchronously from an anteriorly restricted blastoderm, while the remainder form sequentially from a posterior proliferative zone. Although many of the genes involved in *D.melanogaster* segmentation are also involved in the segmentation of short and intermediate germ-band insects, the actual molecular mechanisms controlling the gene expression patterns in these latter two modes are less well understood.

At first sight, the mechanisms producing the presegmentation gene stripes would be expected to differ among the various modes of segmentation because the cellular contexts in which the stripes are formed are so different. In long germ-band insects, pattern formation results from the interaction between transcriptional factors diffusing in the syncytial cytoplasm. This mechanism would not seem feasible in a cellularized context. This, in turn, suggests that

two different mechanisms may be functioning in intermediate germ-band insects, one for the syncytium and one for the proliferative zone. On the other hand, the transition between these mechanisms needs to be relatively easy, since in many orders different species use different modes of segmentation. This shows, at the very least, that selection has acted not only on small details of pattern forming mechanisms, but on their global dynamic properties.

The mechanism by which presegmentation molecular stripes in the fruit fly are formed is complicated and requires many diffusing proteins interacting in rather subtle ways (Frasch and Levine, 1987a; Ish-Horowicz et al., 1989; Small et al., 1992). It seems unlikely that when the transition between intermediate and long germ-band segmentation took place in dipteran ancestors a mechanism with as complicated a molecular hierarchy as that operating in modern-day *Drosophila* was present. Indeed, this would have required that ancestral intermediate or short germ-band insects had paracrine factors and receptors regulating each of the transcriptional factors that later became diffusible morphogens once the proto-*Drosophila* syncytium was established. It would also have entailed many molecular changes for which there was no plausible selective advantage. Moreover, such transitions have appeared many times in different lineages, making scenarios requiring large numbers of mutational events even more unlikely.

One solution to this apparent paradox is that the genetic networks responsible for generating sequential stripe patterns in intermediate germ-band insects can also generate stripes in a syncytium, or at least can do it by small mutation-based changes in their structures. While genetic networks that can form stripes in both a cellular and syncytial context exist, they are members of a different dynamical category ("emergent" networks; see accompanying paper) than that found in *D.melanogaster.* If such networks were indeed present at the origin of segmentation in insects the original genetic network must have been replaced by a different

dynamical category ("hierarchic" networks) during the evolution of *D. melanogaster*.

Molecular mechanisms of segmentation in Drosophila

The formation of overt segments in *Drosophila* requires the prior expression of a stripe of *engrailed* (*en*) expression in the posterior border of each presumptive segment (Karr et al., 1989). The positions of these stripes are largely determined by the activity of the pair-rule genes *even-skipped* (*eve*) and *fuhsi-tarazu* (*ftz*) which exhibit complementary seven stripe patterns prior to the formation of the blastoderm (Frasch and Levine, 1987b; Howard and Ingham, 1986). The processes leading to the stripe patterns of the pair rule genes involve a complex set of interactions among transcriptional factors in a syncytium that encompasses the entire embryo. It has been shown, for example, that the formation of particular eve stripes requires the existence of stripe-specific enhancers in the *eve* promotor (Small et al., 1992; Small et al., 1996; Small et al., 1991). These respond to specific combinations of gap gene products expressed at the location where the eve stripe will appear. This suggests that each stripe may be produced by the presence of a stripe-specific combination of upstream transcriptional factors.

It is possible to model the diffusion of transcription factors in a syncytium using much simpler molecular mechanisms and arrive at a pattern like that seen in *Drosophila* (Meinhardt, 1982; Lacalli *et al*., 1988; Nagorcka, 1988; Hunding *et al*., 1990; Goodwin and Kauffman, 1990). Such reaction-diffusion mechanisms (Turing, 1952; Meinhardt, 1982), which are essentially the same as the emergent networks we have discussed (Salazar et al., 2000; accompanying paper), produce patterns by the reciprocal asymmetric interactions of diffusible gene products. In these mechanisms, each stripe is regulated by the same genes and in the same way. Although very different from the complicated genetic circuitry by which *Drosophila* forms pair rule stripes, reaction-diffusion mechanisms appear to be involved in the formation of pigment stripes in fish

(Kondo and Asai, 1995), eye spots on butterfly wings (Nijhout, 1991), feather germs on bird skin (Jiang et al., 1999), and precartilage mesenchymal condensations *in vitro* (Miura and Shiota, 2000a; Miura and Shiota, 2000b).

Molecular mechanisms of segmentation in species other than Drosophila

The expression of pair-rule genes and *engrailed* in many insects and arthropods other than *Drosophila* have also been explored. In *Schistocerca* a short germ-band insect, no pair-rule genes have been found to be expressed in stripes although the *en* homolog has been found in stripes marking the borders of segments (Patel *et al.*, 1989; Patel *et al.*, 1992). In *Tribolium*, an intermediate germ-band coleopteran, the homologs of *eve*, *hairy*, *ftz* and *en* are expressed in a pattern similar to that found in the fruit fly (Brown *et al*., 1994a; Brown *et al*., 1994b; Patel *et al*., 1994, Sommer and Tautz, 1993). In particular, there are stripes that appear in the syncytium, marking the presumptive segments that will arise within it. Posterior stripes appear in rows of cells arising from the posterior proliferative zone prior to the formation of corresponding segments (Fig.1).

There is considerable variability in the modes of segmentation found in different insects. Even within a single order, different species can exhibit different segmentation types. In coleoptera, three different species exhibit different types of segmentation (Patel *et al.*, 1994a), but in all cases the number of eve stripes appearing in the syncytium prefigures the number of segments. In fact, it has been suggested that long germ-band segmentation has arisen independently several times (Anderson 1973). But while the modes of segmentation may have changed, the patterns of pair-rule genes, and especially that of the segment polarity gene *engrailed* seem to be highly conserved. Parasitoid wasps (hymenoptera), represent an extreme example in which en and en-like eve (Grbic *et al*., 1996) stripes appear, although the rest of the

early development is highly derived (Development of these organisms is polyembryonic and never produces a syncytium, and stripes are produced in a rapid anterio-posterior progression).

Other arthropods also exhibit significantly conserved patterns of pair-rule and *en* genes. In crustaceans, most segments are also produced by posterior growth. In *Artemia* (Anacarida) the zone of growth consist in a disorganized blastema in the posterior extreme of the nauplius larva (Manzanares *et al*. 1993). In *Mysidium* (Malacostracea), in contrast, the nauplius exhibits two posterior teloblasts that asymmetrically divide to generate highly ordered antero-posterior lines of cells. In each case, stripes of the *en* homolog appear progressively as new cells are produced (Patel, 1994b). In chelicerates, where there is also a posterior zone of progressive addition of segments, it has been shown that the homolog of *eve* and two other pair-rule genes, *runt* and *hairy*, are expressed in a striped pattern that appears progressively as segment primordia (Damen et al., 2000).

In some annelids, such as the leech *Hirudinea* en has also been found to exhibit a pattern marking segment borders (Weisblat *et al.*, 1994). A similar pattern of *en* expression has even been found during the simultaneous formation of the eight first somites in cephalocordates (Holland *et al*., 1997). Engrailed homologs are expressed in a segmentation-like pattern of iterated stripes in chiton (polyplacophora mollusks) (Jacobs et al., 1994) and in the arms of starfish (asteroidea echinodermata) (Lowe and Wray, 1997).

Based on the conservation of these gene expression patterns throughout the insects, and their similarities with these found in other groups, it seems reasonable to assume that the last common ancestor that *Drosophila* shares with the closest intermediate germ-band insect was itself intermediate germ-band, and had a pattern of pair-rule and *en* expression similar to that found in *Drosophila*.

3. A hypothesis on modes and mechanisms of segmentation

Some tentative hypotheses have been proposed to explain how intermediate germ-band segmentation may function and how its transition to the long germ-band mode may have been be attained. Some researchers (Tautz and Sommer, 1995) suggest that segmentation gene products could be secreted and diffuse between cells, which would have specific receptors for them. However, if the interactions between such gene products are similar to those found in *Drosophila*, the number of changes required for switching from these indirect transduction routes to a diffusion-mediated mechanism seems formidable. Gap junction coupling of cells is another possibility, but although the structure of arthropod gap juctions is not well understood, it seems unlikely that whole proteins would be able to pass through them. Other investigators have instead suggested that segmentation gene products may be located in the cytoplasm of the teloblast and progressively become diluted as cells bud off (Tautz and Sommer, 1995, Patel, 1994b). None of these hypotheses has any experimental foundation, and all present difficuties of the sort discussed above in accounting for evolutionary transitions in segmentation mode.

An earlier hypothesis by one of us suggested a scenario for this transition that was not subject to the same problems (Newman, 1993). The sequential appearance of gene expression stripes from the posterior proliferative zone can be explained if it is assumed that there is an internal clock by which the level of expression of various genes oscillates periodically. It was proposed that this clock regulated, directly or indirectly, downstream genes such as *engrailed* in the proliferative zone, which became fixed when they left the proliferative zone. If this clock, moreover, had a period different from that of the cell cycle, alternating populations of cells would leave the zone with different levels of *en* expression, which would recur at intervals represented by the lowest multiple of the regulatory clock and cell cycle times (Fig.2). The

sequential appearance of stripes (e.g., eve, ftz or en) would thus arise by the extension of a temporal pattern, via growth, into a spatial pattern (Newman, 1993).

The existence of biochemical clocks based on gene regulatory networks has been well-documented, and even constructed by genetic engineering techniques (Elowitz and Leibler, 2000; Judd et al., 2000). Even more interesting for our purposes is the finding that vertebrate somitogenesis requires the expression of homologs of the pair-rule gene *hairy* in a temporally oscillatory pattern (Palmeirim et al., 1997; Dale and Pourquié, 2000; Holley et al., 2000). Significantly, the somites appear by the progressive anterior conversion of this temporally periodic pattern into a spatially periodic pattern in both chickens (Palmeirim et al., 1997) and zebrafish (Holley et al., 2000). The existence of this mechanism in vertebrates makes the clock model for short and intermediate germ band insects plausible. A similar clock model has also been proposed for the leech (Weisblat et al., 1994).

The kinetic properties that give rise to a chemical oscillation (what mathematicians refer to as a "limit cycle"), can also, when one or more of the components is diffusible, give rise to standing or travelling spatial periodicities of chemical concentration (Epstein, 1991; Boissonade et al., 1994; Muratov, 1997). This transition occurs under particular ratios of reaction and diffusion coefficients. An important requirement of both these kinetic schemes is the presence of a direct or indirect positive autoregulatory circuit, a condition satisfied in *Drosophila* by both *eve* (Harding et al., 1989) and *ftz* (Schier and Gehring, 1993). This was the basis of our proposal that the short/intermediate germ-band-long germ-band transition can be explained by the consequences of allowing a molecular clock operating in a cellular system to come to operate in a syncytium (Newman, 1993). And indeed, the genetic network model described in the accompanying paper and earlier (Salazar-Ciudad et al., 2000) has been used to show that many

networks exhibiting temporally oscillatory patterns when confined to a single cellular cytoplasm can produce stripe patterns when they are allowed to function in a synctium (e.g., Fig. 3).

This hypothesis is especially useful in resolving the apparent paradoxes in insect segmentation outlined above. Thus, in this model the transition from short/intermediate germ-band to long germ-band insects does not require many intermediate steps of implausible adaptability. Instead the transition between one mode and another requires few mutational steps (or none, depending on the network considered). Also readily explained by this hypothesis is the presence of different modes of segmentation in species of the same order. The recurrent appearance of long germ-band segmentation in many independent lineages is a consequence, under this hypothesis, of this kind of transition being a generic variational property of the networks involved in short/intermediate germ-band segmentation. Because the emergent genetic networks hypothesized to underlie segmentation can readily generate different numbers of segments with small changes in dynamical parameters, the presence of different numbers of segments in related lineages is also readily accounted for. Finally, the presence of both mechanisms in a single embryo is also easily explained from this perspective.

The evolutionary transition between modes of segmentation, in this view, moreover, does not require the recruitment of a panoply of intercellular receptors or other unusual mechanisms of cell communication. However, despite its explanatory power, this hypothesis introduces a new puzzle of its own: Why does modern-day *Drosophila* not use a reaction-diffusion mechanism to produce its segments?

4. Hierarchic networks versus reaction-diffusion mechanisms

In what follows we will use the results of the simulations in the accompanying paper to show why a periodicity-generating genetic network would tend to be replaced by an elaborate

hierarchic network like that actually found to underlie segmentation in *Drosophila*. Specifically, the tendency for one type of network to be selectively replaced by another relate to the molecular structure of the networks (on which mutations act), the phenotypes they produce (on which selection acts), and to the relationship between the genotypic and phenotypic levels. These characteristics are mainly related to the internal logic of such developmental mechanisms, and are extensively explored in the accompanying paper. Here we will apply such results to the transition between modes of segmentation.

In the accompanying paper we show that the category of gene networks encompassing reaction-diffusion ("emergent") mechanisms can produce patterns with any number of stripes. In addition, these networks require few genes. As already noted, a genetic network producing a clock (and stripes over a spatial domain when coupled with cell division) can produce simultaneously-appearing stripes when acting in a syncytium. In these networks the number of stripes can be regulated by making small changes in interaction strengths between transcription factors. We suggest in the accompanying paper that their simplicity at the molecular level and the spectrum of forms that they can generate make emergent networks good candidates for involvement in the generation of novelty in developmental systems.

This is exemplified in our simulations of an evolutionary process in which genetic networks capable of reproducing and mutating were selected according to the degree of similarity between the patterns they produce and an arbitrary pattern, defined as optimal, consisting of a variable number of equally spaced stripes. When the optimal pattern consisted of more than three stripes, the optimal was attained, most often, by an emergent network. Moreover, this model shows that networks forming stripes by hierarchic mechanisms (in which each stripe is regulated by a specific combination of upstream genes) always require a larger number of genes for forming the

same number of stripes, which is the main reason such networks tend to appear later during evolution.

Several aspects of the molecular organization of hierarchic networks, however, favor their substitution for emergent networks by selection once a particular pattern has become established. This substitution cannot occur suddenly, because a hierarchic network capable of producing the same pattern as an emergent network is likely to require many genes and connections between its gene products. However, any intermediate step in such a transition would be adaptive in its own right. The reasons for this are multiple:

Patterns produced by hierarchic networks are more stable against mutational change than patterns produced by emergent networks. In particular, our simulations show that hierarchic networks have a higher chance than emergent networks of producing the same patterns if only minor mutations occur (accompanying paper). This is evolutionarily relevant since once an optimal pattern is attained, any variation changing it may be highly maladaptive and will be eliminated by conservative selection. This kind of selection is likely to have acted on pair-rule and *en* stripe patterns, since they appear to be highly conserved. Thus, once an optimal pattern is found, the advent of a simple hierarchic network producing part of the pattern (reinforcing one stripe against developmental or environmental noise, for example) will be immediately adaptive and will increase its frequency in the population.

Another consideration in the potential for replacement of emergent networks by hierarchic networks is the question of refinement of the patterns produced. Does either or both classes of mechanism allow the generation, under mutational change, of similar patterns with only subtle differences? Or, rather, does either class of mechanism fail to allow the production of small variations on similar patterns. We note that both possibilities have been observed in the

morphological variation within populations (Alberch, 1980; Chevereud *et al.* , 1991;Nijhout, 1990). The importance of such differences has been discussed (Alberch, 1980), and they would clearly affect the maximum degree of adaptation achievable using a given mechanism. For example, in the cases of the hierarchic segmentation network employed by *Drosophila* the levels of gene expression in each stripe can be independently regulated. In contrast, in emergent networks there is a reciprocal relationship among genes that results in each change affecting the whole pattern. From the existing comparative data concerning the patterns of gene expression it seems reasonable to expect that few and small variations in the patterns of expression of segmentation genes are allowed by selection. The replacement of emergent networks is thus favored since hierarchic networks produce a type of variation more suitable for the selective requirements of segmentation patterns.

In addition we have found that such adaptations are more rapidly achieved in hierarchic than emergent networks (accompanying paper). This is because the relationship between genotype and phenotype is closer in hierarchic networks. That is, similar hierarchic networks more often produce similar patterns. This implies that patterns of a similar adaptive value are genetically close to one another, and thus that the adaptive landscapes over which optima are attained are not very rugged (Kauffman and Levin, 1987; Kauffman, 1993). In contrast, genotype-phenotype relationships are less consistent in emergent networks. But phenotypically gradual changes are more easily produced when there is a close correspondence between genotype and phenotype. Thus, hierarchic networks can adapt more rapidly to small changes in the optimal pattern since they exhibit a closer relationship between genotype and phenotype, and would therefore tend to prevail over a potentially emergent competitor. The evolutionary relevance of the relationship between genotype and phenotype has been discussed (Kauffman, et al., 1993), although the role

of different types of developmental mechanism was not explicitly considered.

5. Conclusions

From the perspective outlined here and in the accompanying paper we suggest that insect segmentation was orginally of the sequential mode seen in other arthropods. Subsequently, in many independent lineages, more posterior segments progressively appeared in the anterior syncytium. Initially the segmentation gene stripes were generated by the cellular clock mechanism coupled to growth; when syncytia emerged this clock mechanism became a standing wave-generating reaction-diffusion mechanism. Later, the mechanism forming each syncytial stripe was replaced by a hierarchic network.

While it may appear from our model that the transition from short/intermediate germ-band to long germ-band modes of segmentation could have proceeded directly, with no intermediate stages, we believe this to be unlikely. Because the change in the network generating the stripe pattern may have been one of several alterations required for the transition in segmentation mode it is reasonable to expect it to have been somewhat gradual.

On mechanistic grounds it is plausible that each time a new segment was formed from the anterior syncytial blastoderm the network forming the corresponding stripes would progressively be replaced by a hierarhic one. Whereas the hierarchic networks for generating many stripes are complicated and therefore would not be expected to arise *de novo* (see accompanying paper), the hierarchic networks implicated in generating only one stripe are simpler. On the other hand, the formation of segments from the proliferative zone may continue to use a clock mechanism similar to that seen in vertebrate somitogenesis because of the unavailability of readily achieved alternative mechanisms for generating sequential patterns.

As we suggest in the accompanying paper and in previous analyses (Newman and Comper,

1990; Newman 1993; 1994; Newman and Müller, 2000), this dynamic of substitution between types of networks may be widespread in the evolution of development and form, providing insight into the origins of developmental canalization (Waddington, 1957). Because the properties exhibited by different types of networks suggest that they will appear at different times and contexts in evolution and development, the analysis of variational properties of model genetic networks can provide an important means for interpreting and designing empirical studies on the ontogenetic and phylogenetic aspects of pattern and form.

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Figure captions

Fig. 1. Schematic summary of segmentation modes in short germ-band and long germ-band insects. *Left, A*, In short germ-band insects one or groups of a few segments appear in succession. Brown patches indicate expression of a segment polarity gene such as *engrailed*. Some patches of expression may appear later in development in head segments. *B*, More segments appear posteriorly from a zone of proliferation. *C*, The remainder of the segments form sequentially, as in *B*. *D*, Idealized insect larva showing full array of segments. *Right, A'*, Long germ-band embryo with gradients of expression of maternal genes (e.g., *bicoid* and *nanos*) (e.g., engrailed). For simplicity, the patterns of gap gene expression (e.g., hunchback, kruppel), intervening between steps A' and B' in Drosophila, are not shown. *B'*, Expression of pair-rule genes (e.g., *eve*, *ftz*, *hairy*). *C'*, Expression of segment polarity genes (e.g., *engrailed*).

Fig. 2. Model for the generation of segments in a zone of synchronized cell multiplication, by the temporal oscillation of the concentration of a molecule (e.g., en, ftz, hairy) that regulates expression of a segment polarity gene such as engrailed. The clock faces represent the phase of the cell cycle (C) and that of the periodically varying regulatory molecule (R). It is assumed in this example that the duration of the cell cycle is three hours, the period of the chemical oscillation is two hours, and that both cycles start together. During the first cell cycle, newly formed cells have a level of engrailed specified by the initial value of R (*green*). During the second cell cycle, R is in mid-cycle, and the newly formed cells have a different level of engrailed (*orange*). During the third cell cycle R is again at its initial concentration, and the new cells have the first level of engrailed. The assumption of cell synchrony is for simplification of the model; the mechanism would also give rise to segments in a zone of asynchronous cell multiplication with local cell sorting-out. (Based on Newman, 1993).

Fig. 3. An example of a network that can produce (for the same parameter values) sequential stripes when acting as an intracellular biochemical clock in a cellularized blastoderm with a potserior proliferative zone, and simulataneously-forming stripes when acting in a diffusion-permissive syncytium. The network is shown in the central box. Black arrows indicate postive regulation and white arrows negative regulation. In the upper boxes the equations governing each of the two behaviors are shown. The four genes involved in the central network diagram, as well as their levels of expression, are denoted by g1, g2, g3 and g4. In the reaction-diffusion case g1 and g 2 can diffuse between nuclei (note that the two set of equations differ only in the presence of a diffusion term in genes 1 and 2). The lower boxes indicate the levels of expression of gene 2 for the two systems. For the intracellular clock the *x*-axis represents time, while in the reaction-diffusion system this axis represents space. The patterns produced by the two different behaviors are not exactly equivalent because the patterns produced by the reaction-diffusion system has a small dependency on initial conditions. In the pattern shown the intial condition consisted of all gene values set to zero except gene 1 in the central cell which was assigned a small value (the exact value did not affect the pattern). The patterns shown were found when the following parameter values were set: k_M=0.01 ; w_{13}=0.179; w_{23}=0.716; w_{24}=- 0.704; w_{31}= 0.551; w_{34} =-0.466; w_{42}=0.831; w_{43} =-0.281; μ_1=1.339; μ_2 =2.258; μ_3=2.941; μ_4 = 2.248. For the reaction-diffusion case the same parameter values are set but in addition: D_1=0.656 and D_2=0.718.

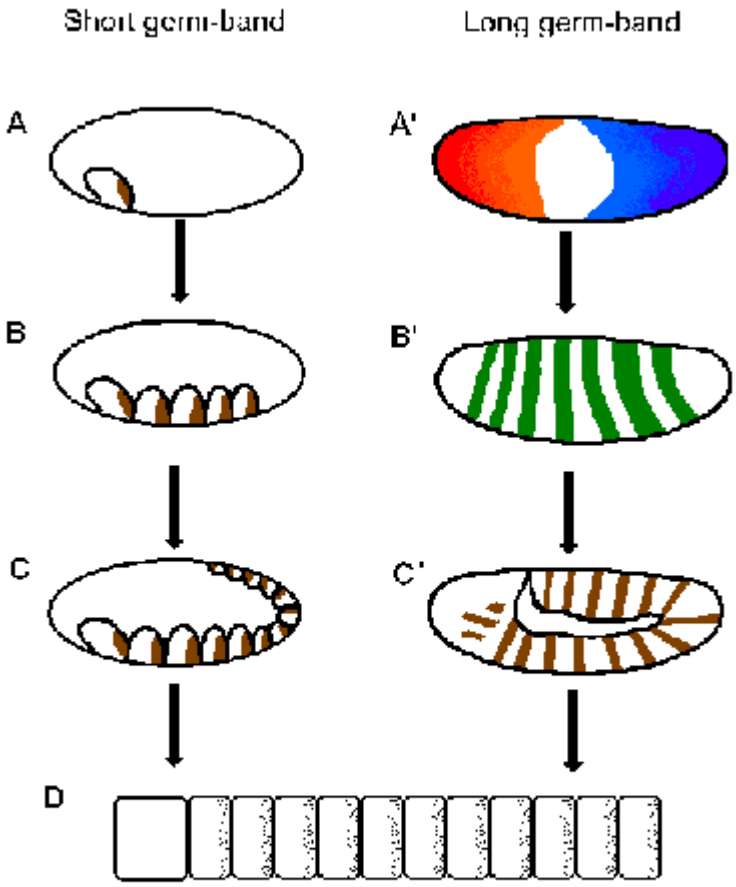

Fig. 1

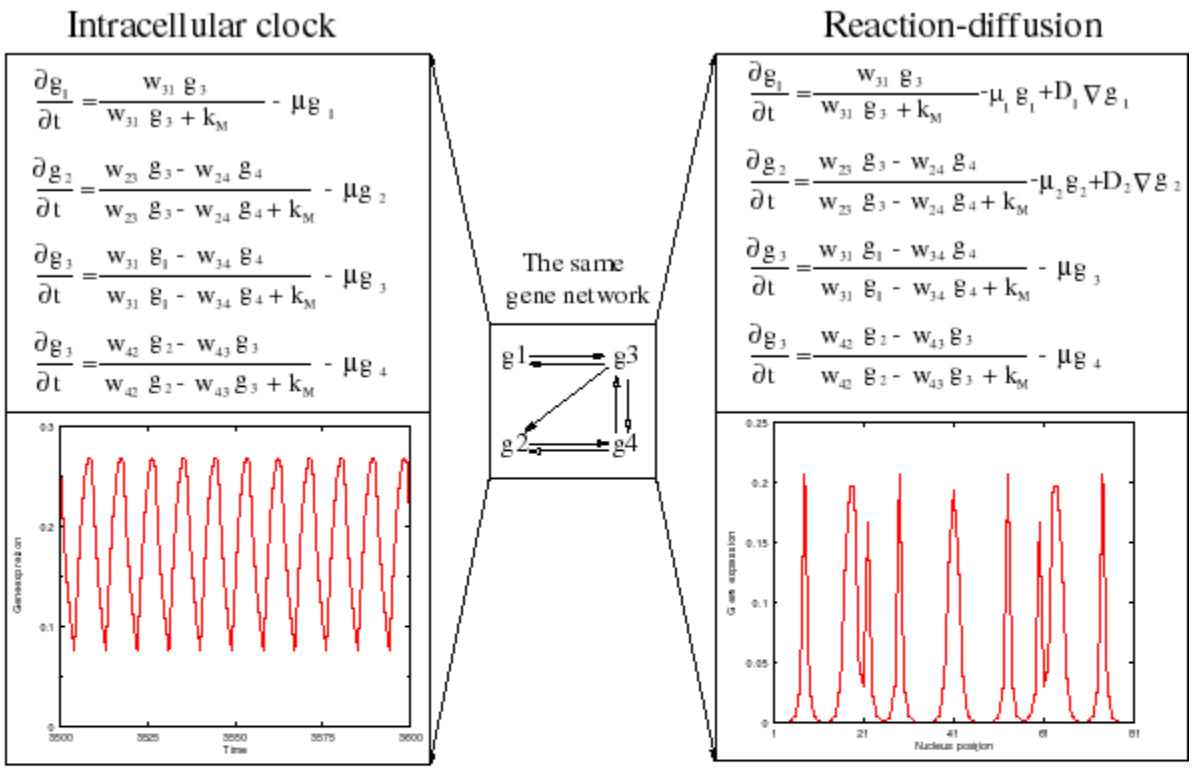
Intracellular clock
Reaction-diffusion
The same gene network
g1
g2
g3
g4
Time
Nucleus position

Fig. 2

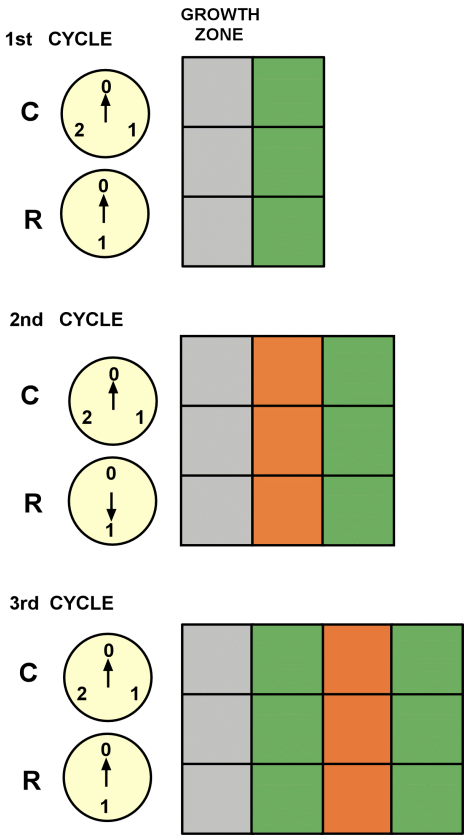
GROWTH ZONE
1st CYCLE
C
0
2
1
R
0
1
2nd CYCLE
C
0
2
1
R
0
1
3rd CYCLE
C
0
2
1
R
0
1

Fig. 3