Simulation of 3 Dimensional Turing Patterns Related to Early Biological Morphogenesis

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Abstract

Biological pattern formation is a process which so far has gone largely unexplained. Segment formation is a fundamental process found even in very primitive multicellular organisms. Experimentally working biologists favour mechanisms based on morphogenetic gradients thus giving rise to positional information. Specific combinations of genes (cues) are supposed to direct the activation of each particular stripe. Among theoreticians the pattern forming processes are beleived to some extent to be dependent upon truly symmetry breaking processes known to be possible in nonlinear control systems. This latter approach is explored here to discuss the early morphogenesis in *Drosophila* and early evolutionary insect morphogenesis in general. In segment formation in insects found much lower on the evolutionary ladder than *Drososphila*, stripe formation seems to have evolved based on an ancient mechanism not obviously related to the cue model proposed for *Drosophila*. It is argued here that the fundamental process may be a reaction-diffusion (Turing) prepattern, which is explored in an increasingly complex manner during evolution.

Introduction

Drosophila has emerged as one of the currently most important species for which detailed experimental data have accumulated to such an extent that a beginning is made in understanding the processes which govern early embryogenesis. A hierarchy of genes seems to control the initial transition from the egg to a segmented embryo.

In this hierarchy the primary pair-rule genes appear. These genes are each expressed in a series of 7 stripes. The mechanism for the formation of these 'zebra' stripes is unknown. Activation by a combination of maternal and gap genes above in the hierarchy seems to be involved in the expression of particular stripes. This requires a sufficient number of gradients provided by the gap level to define at least 7 distinct stripes. How a number of independent particular stripe generators (cues) could cooperate to form the observed equally spaced stripes is a much more difficult problem. Theoreticians have pointed out that the 'zebra' stripes alternatively may be generated by a truly symmetry breaking mechanism such as

Turings mechanism, that is, by an autocatalytic reaction-diffusion system which is known to be capable of producing such stripes autonomously. The particular pair-rule stripes could then be activated by a combination of maternal, gap and Turing pattern interactions.

In recent experiments on morphogenesis in insects much lower on the evolutionary ladder than *Drosophila* it appears, that the cues are absent at least in the form they have in *Drosophila* and this again opens the question as to what is the fundamental pattern (stripe) forming mechanism, see (French, 1993).

Recent reviews have appeared by Ingham (1988), Pankratz & Jäckle (1990) and Nüsslein-Volhard (1991). Recent models of the gap level appear in Struhl, Johnston & Lawrence (1992), and references to Turing type models may be found in Hunding, Kauffman & Goodwin (1990). For information on Turing structures in general see Turings original work (1952), (Nicolis & Prigogine, 1977) and (Murray, 1989).

The Hierarchy of Gene Control in *Drosophila*

Genes containing a sequence class known as the homeobox are videspread and many of these classes are strongly conserved in higher multicellular animals. The evolutionary establishment of some of these classes seem to be at least as ancient as the flatworm. It has been speculated that the homeobox class of genes somehow is connected to the origin of pattern formation in multicellular systems, like establishment of bilateral symmetry, and later segmentation.

Many of these genes are involved in segmentation processes in the evolutionary advanced insect *Drosophila*, and as this system is one of the best known systems from a genetic point of view, the control mechanism leading to segmentation in *Drosophila* has been the subject of intense research recently in the hope to bring forward insight in the fundamental pattern forming mechanisms governing early embryonic development (see Wright *et al.*, 1989; Riddihough, 1992).

Drosophila has a fast establishment of its body plan, in contrast to evolutionary lower insects. The egg contains *maternal genes* and thus an asymmetry from the very start as the anterior part of the egg contains the gene *bicoid* and consequently a gradient of *bcd* protein. Similarly the posterior part contains the gene *nanos* and presumably a similar gradient from this end in *nos* gene products. It is beleived that *nos* represses *bcd*.

Maternal *Hunchback* is present as well and the function of *nos* is to suppress expression of *hb* in the posterior part. The gene *hb* is however activated in the eggs own DNA and thus this zygotic *hb* places this gene among the first level in the hierarchy activated by the maternal genes: the gap genes. Activation of zygotic *hb* is due to *bcd*. Recently a control mechanism for the genes *hb, Kr, kni* and *gt* in the gap level has been proposed by Struhl, Johnston & Lawrence (1992) in which it is argued that *hb* has a key role in organizing gap gene expression in the posterior half. *hb* activates the gap gene *Krüppel*. The result is an expression of *Kr* in the middle of the embryo. Gene *hb* represses the next gap gene *knirps* so the result is expression of *kni* on the posterior side of the *Kr* region with some overlap. The gene *giant* has emerged as a genuine gap gene as well, and *gt* is expressed in two broad bands. The expression of *gt* and *kni* is beleived to be due to global

activation (by a so far unknown factor) followed by repression of *gt* and *kni* by *hb*. The repression of *gt* is overruled by *bcd* anteriorly. The wild type maternal *hb* is sufficient to depress *gt* and activate *Kr*. Thus this work supports the view that, say, *hb* protein may be crucial even in regions of the embryo where current techniques barely are able to detect it, a feature which further complicates the use of available expression patterns for model building.

The next level in the gene hierarchy is the primary pair-rule genes *hairy, runt* and *even-skipped*. After the gap genes start to be expressed but before they reach quasistationary spatial positions the primary pair-rule genes generate 7 zebra stripes in the middle of the embryo. The zebra stripes were first seen in the gene *fushi tarazu* (or *ftz* for short) (Hafen, Kuroiwa and Gehring, 1984). Subsequently it was inferred that the seven stripes were controlled as a single unit (Hiromi, Kuroiwa and Gehring, 1985). This prompted speculations that the underlying mechanism was connected to Turing structures. However the *ftz* gene activation is now beleived to be preceded by genes *hairy, runt* and *eve* for which reason these genes are known as the primary pair-rule genes. Turings mechanism for these genes is at present not the prevailing model after the discovery that partial deletions in the *hairy* DNA caused particular *hairy* stripes to vanish. The discovery of these region specific *hairy* alleles (Howard, Ingham and Rushlow, 1988) have given rise to the model most videly used at present. It is beleived that gradients formed above in the gene hierarchy activate particular stripes. This idea has recently been exploited to give a detailed model for control of *eve* stripe 2. (Small, Blair & Levine, 1992). However if this kind of interactions were the only ones necessary for generating specific stripes, this interpretation raises the very serious question how 7 individual stripes could be generated with *equal spacing*. It is inferred that *hairy* and *runt* depress each other and *eve* depress *ftz*. Also *hairy* depress *ftz* and *runt* depress *eve*. Such interactions and evolutionary selection are held responsible for the eventual appearance of the final regular pattern.

This picture of specific cues for each stripe is not without its problems though. The formation of stripes begin before the gap genes reach their final positions. One would expect to see the initial stripes move around until they found their final position but this is not observed. Models of the refinement required to yield an equally spaced pattern by mutual interactions between primary pair-rule genes must meet the experimental observation that *hairy* yields some stripes in the absence of all the other primary pair-rule genes. The same holds for *eve*.

It has thus been argued (by experimentalists) that the pair-rule genes may not just respond to maternal and gap genes and other pair-rule genes but to some general, redundant striping mechanism. Carroll and Vavra (1989) point out however that the very different effects of *hb, kni* double mutants on *eve* and *hairy* indicate that the mechanisms for *eve* and *hairy* control from the gap level are widely different. This indicates that the two genes dont read the same cues from the gap level, but then it becomes a sinister question how they manage to resolve into 7 stripes which very nearly *overlap* in the wild type.

It is difficult however to see what common striping mechanism could be present if it does not have its origin in cues set up by the gap (and maternal) genes. A common striping mechanism for the pair-rule genes could be of Turing type, but then it should be sought in low molecular weight components which could diffuse rapidly enough. It has been pointed out by Lacalli and Harrison

(1991) that a feasible mechanism could be the interaction of such factors with the class of promiscuous proteins, which interact with many gene promoter regions. Thus defiency in this control system could have lethal effects much before the pattern forming processes start, and such factors would thus not have emerged in the screening of genes responsible for generation of pattern.

Numerical Treatment of Pattern Formation

Our efforts to develop efficient software for the calculation of spontaneous pattern formation in biological systems have led to fast codes for vector supercomputers. Recently a truly parallel computer architecture has been investigated. Much of the problems arising on such machines are connected to the need to transmit results calculated on one processor to some of the other processors before the next sweep of iterations occur. Thus many parallel codes become communications bound and interconnect technology in such machines is the bottleneck. Data allocation to each processor must be so that communication is minimized.

Most parallel architectures has a rather small fast local memory, with an additional substantially larger, but much slower, secondary memory. If substantial data traffic is needed from the local secondary memory to the CPU during a specific computational loop, the performance may be reduced. To achieve high local computational speed on each processor, this suggests allocating a relatively small amount of data to each processor, to fit into the fast memory, and thus to spread out the data on a large number of processors. This however conflicts with the above requirement to minimize communication among processors after a typical fast computational sweep through the data.

On the Kendall Square Res. machine a typical configuration is 32 processors, each running at a nominal peak speed of 40 MFLOPS. This requires local data to be in the subcache which holds at most 0.25 MB of data. Our results so far indicate the possibility of achieving speeds of the order 4-11 MFLOPS per processor (communication included) and thus up to 3 GFLOPS on a large machine. If the same job runs on a 32 processor machine, the data cannot be stored in the fast subcache, and the local slower 32 MB cache of each processor must be invoked. The bottleneck of this medium size parallel configuration is thus the data transfer speed from the 32 MB local cache to the CPU. This bottleneck may be eased in near future updates of the machine though.

The numerical methods we have used is largely the same as earlier described (Hunding et al., 1990). The method of lines was used and thus the system of nonlinear partial differential equations was converted to a large system of ordinary differential equations by discretization of the Laplacian in three curvilinear coordinates. The resulting system is stiff and solved accordingly (modified Gear code).

The Jacobian used in the corrector step is a sparse banded matrix which may be rearranged (chessboard numbering of meshpoints) to yield large blocks within which the solution vector elements may be iterated in parallel (RBSOR method). Implementation on vector computers results in a huge speed up: The RBSOR code runs efficiently and close to the top speed of vector machines like

the Fujitsu/Amdahl VP1200 (410 MFLOPS sustained speed) and 690 MFLOPS have been recorded on a single processor CRAY C90.

The actual rates of the chemical interactions involved in *Drosophila* are unknown. From the proposed interactions for a particular model it is possible however to write up matching rates. This may be illustrated by the rate for the pair-rule gene *eve*:

$$\frac{d(eve)}{dt} = \frac{(A)^n}{1 + K_2(A)^n} \times \frac{1}{1 + K_3(runt)^m} \times f(gap, mat) \qquad (1)$$

The substance A is one of the components of a stripe defining Turing system, which is activating *eve* expression. Both A and *runt* repression are here taken to be cooperative with Hill constants n and m greater than two. The last term $f(gap, mat)$ may be included to define activation or repression by cues defined by combinations of gap (or maternal) genes.

The rate for *ftz* is analogous to that of *eve*, with *hairy* replacing *runt*, and an additional term for *eve* repression. The interaction with the Turing system is now through the second component B of this Turing system. As B is high where A is low and *vice versa* this yields activation of *ftz* in stripes positioned between those of *eve*. Thus the influence on the stripe stabilizing Turing system from the maternal and gap levels may be taken through position dependent rate constants. Essentially the present model is then a model of spatial coupling in a selforganizing system of the global field type.

Discussion

The study of feasible cues is only in a preliminary state, both experimentally and theoretically. The idea that gap genes may influence rate constants in a Turing system which generates stripes is common for recent models (Lacalli 1990; Hunding *et al.*, 1990). The studies so far indicate that a possible Turing mechanism should explain experimentally recorded distorted zebra stripes in gap mutants not as altered Turing patterns, but rather with an almost intact Turing zebra stripe pattern which is then read out to the pair-rule level in a distorted manner due to the missing gap gene.

Thus the model proposed on the basis of the present numerical study may be said to combine the two current rivaling models, as it indicates that cues are necessary to explain the experimentally observed results on zebra stripes caused by gap mutants, but Turing stripes are necessary to provide a stable underlying stripe generator.

The stabilization of the Turing zebra stripes in the present study is mainly due to the gradients from the *bcd, nos* system. This model is robust towards gap gene mutants as the zebra stripe pattern is now basically undistorted.

This may actually be taken to mean that the cues in *Drosophila* are not the actual fundamental system for segment formation. One may speculate that the cues are evolutionary late additions to a fundamental segmentation mechanism, which has been obscured by the addition of gap and pair-rule genes. Neither the gap nor the pair-rule genes seem to take part in generating one segment after another in less evolutionary advanced insects like the grasshopper (Patel, Ball &

Goodman, 1992). An intermediate control system with some gap and pair-rule genes seems to be present in beetles (Sommer and Tautz, 1993), (French, 1993). Here a pair of *hairy* stripes form and vanish, but subsequent pairs appear posteriorly, which also fade away as if a progress zone was active over a robust short wave length stripe generator througout the posterior part of the elongating embryo. This process does not take place in a syncytium though, and thus stripe control by combinations of diffusing large molecules as proteins (cues) seems unlikely. This recent discovery has then reopened the debate as to the evolutionary significance of the cue control system found in *Drosophila.*

The much faster overall development rate required in the life cycle of insects like *Drosophila* may have favoured the observed simultaneous triggering of all 7 zebra stripes. The addition of *bicoid* and the gap genes may then be seen as stabilizers of this process, and the subsequent cues as an intricate system to make sure that each generated stripe lines up properly with the local gap genes. Another role for the gap genes during evolution may have been to map out some coarse regions within which simultaneous triggering of stripes could occur, such as a *hb* region first, then perhaps addition of a *Kr* region etc. something which may actually play a role in the observed beetle morphogenesis.

The results found in the morphogenesis of the beetle may be tentatively taken in support of this view. One may envisage a comprehensive model for early, medium and late evolutionary segment formation based upon a robust global Turing stripe generator in which one stripe after another is activated as an inhibitor gradually vanish posteriorly (grasshopper), then an intermediate form in which the global Turing pattern is activated in a zone comprising a few stripes and this progress zone moves posteriorly as an inhibitor gradually vanish (beetle), and finally the rapid all at once stripe activation in *Drosophila* in which the global Turing stripe generator is activated over a large region in several somewhat overlapping subregions provided by the gap genes (Fig. 1). In this model the evolutionary conserved essential stripe generator is the global Turing pattern which ensures a prepattern of equally spaced stripes which is then exploited in a progressively more and more complex manner in different species as these move up the evolutionary ladder. The cues found in *Drosophila* are thus not the fundamental stripe generating elements but evolutionary late additions to a more ancient mechanism.

The Turing based control system just described is then a beautiful example of a selforganizing mechanism, which has developed from a simple rule to global complexity.

Such speculations however must await further experimental results about the role of gap genes during the evolution of insect morphogenesis. Whatever new experimental findings about the pattern forming processes this reveals may now be tested numerically with methods as they are described above for *Drosophila.*

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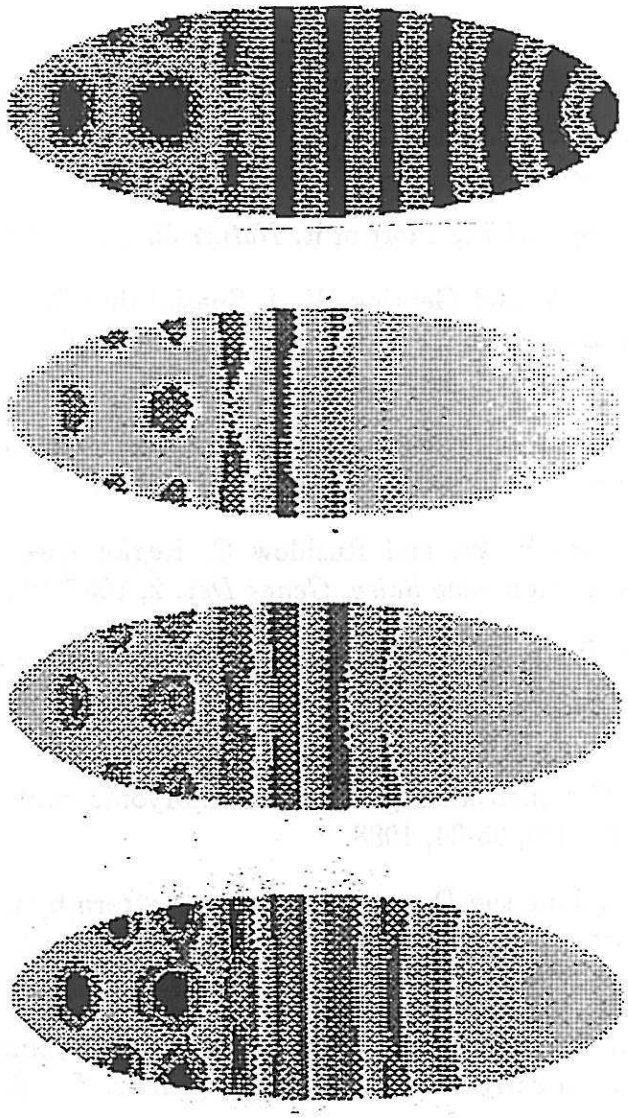

Figure 1: Computer simulation of stripe prepattern in a three dimensional region resembling an early insect embryo. Spontaneous pattern formation occurs in reaction-diffusion systems. In the model shown here a gradient is imposed posteriorly (right) and the resulting space dependent rate constant in the RD system creates a Turing pattern (a) which yields stripes posteriorly, but anteriorly the stripes break up to yield a highly symmetrical pattern which may play a role in head formation. Once established, this RD prepattern governs the read out of genes related to segment formation in the embryo. If an inhibitor is present with high concentration posteriorly, such genes are prohibited from being activated posteriorly and no segments form. When the inhibitor gradually vanish, stripes are activated one after another (b-d). If the inhibitor gradient triggers another activator gene in a zone along the inhibitor gradient, this progress zone would activate a few stripes simultaneously along the Turing pattern, and eventually all stripes become activated from left to right. Finally several such distinct activator regions may form, as realised with the gap genes in *Drosophila*, to yield activation of all the stripes along the Turing pattern simultaneously.

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