PATTERN FORMATION IN EARLY INSECT EMBRYOGENESIS – DATA CALLING FOR MODIFICATION OF A RECENT MODEL

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SUMMARY

A mathematical model of biological pattern formation based upon lateral inhibition has recently been applied by Meinhardt to insect embryogenesis. This model has stimulated a re-evaluation of previous results, and new experiments designed to test the validity of the model. Split u.v. dose experiments with eggs of the chironomid midge Smittia show that the effective targets for the production of the aberrant pattern 'double abdomen' are not subject to the rapid turnover which is required by the model in its currently published version. Certain types of segment pattern, and differences in the length of segments as predicted by the model could not be observed. Other data conflict with the rather unusual type of photoreversal and the particular view of determination associated with the model. The model can be reconciled with part of the conflicting data if the effective targets for double abdomen induction are regarded as morphogen-producing structures, rather than the morphogen itself which specifies the segment pattern (Meinhardt, personal communication). This version of the model, however, is still at variance with some of the data discussed here.

A complementary explanation is proposed taking into account relevant aspects of homoeotic transformations.

INTRODUCTION TO THE MODEL

A mathematical model of biological pattern formation has been devised (Gierer & Meinhardt, 1972) and applied to various problems in developmental biology (Meinhardt & Gierer, 1974). The model is based upon lateral inhibition, and involves the interactions of a substance with a short diffusion range ('activator') and its more rapidly diffusible antagonist ('inhibitor'). The activator stimulates both its own production and the production of the inhibitor, while the inhibitor inhibits the production of the activator. In an extended area, any small local peak in activator concentration causes a further autocatalytic increase of local activator concentration which proceeds to a level where further increase is balanced by losses via diffusion and/or degradation. The rapidly diffusing inhibitor which is also produced in response to the increasing activator concentration, suppresses activator production outside the activated area. Under appropriate conditions, these interactions result in a steady state with a relatively sharp activator peak and a broader inhibitor peak in the same place. If the size of the system is of the order of the activator range, only one peak of activator and inhibitor concentration will develop.

The lateral inhibition model has recently been applied to account quantitatively
for the specification of the longitudinal body segment pattern in insect embryogenesis (Meinhardt, 1977). A peak of activator concentration and thus also a maximum of inhibitor concentration are thought to develop at the posterior egg pole. It is assumed that the differentiation pathways of the embryonic cells are controlled by the local levels of inhibitor concentration. The inhibitor gradient would thus provide 'positional information', i.e. a specification of the cells' position with respect to one or more reference points in the embryo (Wolpert, 1969). The positional information is thought to be 'interpreted' by the genome of the blastoderm cells so that the orderly pattern of body segments is formed.

Abnormal patterns found by various authors after experimental interference with the embryogenesis of several insect species are interpreted by Meinhardt in terms of the lateral inhibition model. Experiments of Sander, Kalthoff, and coworkers with eggs of the leafhopper Euscelis, and a chironomid midge, Smittia, have been treated in detail. In the simulation of these experiments, Meinhardt explicitly states the numerical values of the constants involved in the mathematical model, and shows the resulting concentrations of both activator and inhibitor along the longitudinal axis of the embryo and their change within time.

These computer simulations adequately cover many experimental results. They have led us to perform new experiments designed to test the validity of the model as applied to the specification of the antero-posterior pattern in Smittia. Most of these experiments have been proposed by Meinhardt (1977) in his recent article and in the course of our ensuing personal communication. The results of these and other experiments and observations reported here do not support Meinhardt's model in its currently published version.

RESULTS

Ultraviolet-induction of the aberrant pattern 'double abdomen'

Eggs of chironomid midges have an apparent predisposition to produce, upon various types of experimental interference, embryos with longitudinal pattern duplications, which were first described by Yajima (1960, 1964). In the 'double abdomen', head, thorax, and anterior abdominal segments are replaced by an additional set of posterior abdominal segments joined in mirror image symmetry to the original abdomen (Fig. 1). In Smittia eggs, the double abdomens are always symmetrical in their external and internal morphology except that germ cells are found only in the original abdomen (Gollub & Sander, personal communication). This abnormal segment pattern has been produced in Smittia eggs by u.v. irradiation of the anterior pole, or application of RNase at the anterior pole, or other manipulations (see Kalthoff, 1976).

According to Meinhardt's (1977) model, double abdomen formation is triggered by a dramatic decrease in the inhibitor concentration (e.g. down to 5%, see his fig. 10) at the anterior pole. This can be achieved, according to the model, by u.v. damage to, or leakage of the inhibitor. The reduced inhibitor concentration allows an autocatalytic increase of activator there, provided the activator concentration exceeds a critical level. The new activator peak at the anterior pole in turn causes an increase
of the inhibitor concentration so that a symmetrical inhibitor profile with 2 maxima, one at each egg pole, may result. With appropriate parameters, the model can account for the segment patterns found after u.v. irradiation of different egg areas (see Meinhardt, 1977). However, the results of other experiments on double abdomen formation in *Smittia* eggs call for a modification of the model.

![Fig. 1. Normal larva and double abdomen in eggs of *Smittia*. Top, larva with normal body segment pattern; below, aberrant segment pattern 'double abdomen'. Length of the eggs: 250 µm. Photograph: K. Sander. From Kalthoff (1970).](image)

**Split u.v. doses on identical target area.** It is a basic requirement of the model that both production and decay of the inhibitor are fast processes. It is only under this condition that new peaks of activator and inhibitor concentration are established rapidly, whereas concentration disturbances which are not sufficient to trigger new peak formations soon disappear. In the case of *Smittia* eggs, the inhibitor life time was assumed to be 0.92 h (Meinhardt, 1977, fig. 10). If the turnover of the inhibitor were slower, the lack of asymmetrical intermediates between the normal segment pattern and the symmetrical double abdomens would be very difficult to explain. The assumed rapid turnover of the inhibitor, however, predicts that the effect of repeated subcritical reductions of the inhibitor concentration are not additive provided the time elapsing between these events permits the system to approach its normal steady state again.

This prediction was tested by double irradiation of *Smittia* eggs with u.v. doses under conditions where a single dose did not produce abnormal segment patterns. The eggs were obtained from our laboratory culture and prepared and staged as
Table 1. Effects of variously applied u.v. irradiation on Smittia eggs

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>No. of expt. series</th>
<th>No. of eggs</th>
<th>Total no.</th>
<th>No. of normal larvae</th>
<th>No. of double abdomens</th>
<th>Undifferentiated results</th>
<th>% double abdomens of total</th>
<th>% double abdomens of survivors</th>
<th>χ²-test, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>E 450 + 450 J m⁻¹</td>
<td>4</td>
<td>158</td>
<td>32</td>
<td>122</td>
<td>4</td>
<td>77.2</td>
<td>79.2</td>
<td>&gt; 20 %</td>
</tr>
<tr>
<td></td>
<td>C 900 J m⁻¹</td>
<td>4</td>
<td>162</td>
<td>26</td>
<td>130</td>
<td>6</td>
<td>80.2</td>
<td>83.3</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>E 360 + 360 J m⁻¹</td>
<td>4</td>
<td>156</td>
<td>99</td>
<td>56</td>
<td>1</td>
<td>35.9</td>
<td>36.1</td>
<td>&gt; 10 %</td>
</tr>
<tr>
<td></td>
<td>C 720 J m⁻¹</td>
<td>4</td>
<td>153</td>
<td>86</td>
<td>64</td>
<td>3</td>
<td>41.8</td>
<td>42.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>E 1-h interval</td>
<td>4</td>
<td>148</td>
<td>73</td>
<td>66</td>
<td>9</td>
<td>44.6</td>
<td>47.5</td>
<td>&gt; 20 %</td>
</tr>
<tr>
<td></td>
<td>C &lt; 10-s interval</td>
<td>4</td>
<td>146</td>
<td>85</td>
<td>60</td>
<td>1</td>
<td>41.1</td>
<td>41.4</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>E RNase + light</td>
<td>4</td>
<td>79</td>
<td>21</td>
<td>38</td>
<td>20</td>
<td>48.1</td>
<td>64.4</td>
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<tr>
<td></td>
<td>C RNase</td>
<td>4</td>
<td>86</td>
<td>21</td>
<td>35</td>
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<td>40.7</td>
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<tr>
<td>3b</td>
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<td>4</td>
<td>85</td>
<td>44</td>
<td>18</td>
<td>23</td>
<td>21.2</td>
<td>29.0</td>
<td>&gt; 20 %</td>
</tr>
<tr>
<td></td>
<td>C RNase</td>
<td>4</td>
<td>98</td>
<td>43</td>
<td>32</td>
<td>32</td>
<td>23.5</td>
<td>34.8</td>
<td></td>
</tr>
</tbody>
</table>

Expt. 1 (M. Bathe). The anterior quarter of eggs was irradiated with a split u.v. dose, the interval between the 2 doses was 1 h in the experimental series; the controls received the full (double) u.v. dose at one time.

Expt. 2 (K. Kalthoff). A split u.v. dose was applied to adjacent targets areas (second and first = anterior quarter). The interval between the 2 doses was 1 h in the experimental series and less than 10 s in the controls.

Expt. 3 (I. Kandler-Singer). After double-abdomen induction by application of RNase to the anterior pole region, experimental eggs were exposed to light while the controls were incubated in the dark. The exposed eggs faced the incident light with the dorsal side (a) or with the anterior pole (b).

The χ²-tests indicate the probability with which the different numbers of normal larvae and double abdomens in experimental and control series are expected by chance. For a different evaluation of experiment 3 see text.
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described earlier (Kalthoff & Sander, 1968; Kalthoff, Kandler-Singer, Schmidt, Zissler & Versen, 1975). In the first set of experiments, the anterior quarter of the eggs was irradiated at a wavelength of 285 nm, using the monochromator apparatus described previously (Kalthoff, 1973). The u.v. dose applied was 360 or 450 J m\(^{-2}\). As a single dose, this did not cause the formation of any double abdomens. However, a considerable yield of double abdomens resulted after twofold applications of such doses; one dose was applied at stage P\(_2\) (2 pole cells), and the same dose again 1 h later. A control group of eggs from the same batch received the 2-fold dose (720 or 900 J m\(^{-2}\)) either at stage P\(_2\) or 1 h later. In contrast to the prediction of the model, the split u.v. doses produced almost as many double abdomens as the 2-fold doses (Table 1). The small difference found could either be chance or represent the well-known phenomenon that cellular repair mechanisms for u.v. damage operate more effectively under split dose conditions (Kiefer, 1967; Harm, 1968; Maroudas & Wilkie, 1968).

Split u.v. dose on adjacent target areas. To avoid the ambiguity introduced by possibly enhanced repair under split dose conditions, another experiment was carried out in which the 2 u.v. doses were applied to adjacent target areas using the u.v. microbeam apparatus described recently (Kalthoff, Hanel & Zissler, 1977). The second quarter and the first (most anterior) quarter of the eggs were irradiated consecutively. The u.v. dose was adjusted so that neither of these irradiations alone could produce double abdomens, whereas the 2 irradiations in combination resulted in a considerable yield of double abdomens. The dynamic characteristics of the model predict that the combined irradiations should be effective if carried out in immediate succession, whereas with an interval of 1 h between the 2 irradiations the double abdomen yield should be much lower. To test this prediction, the second quarter was irradiated at stage \(M_1\), and the first quarter 1 h later. During the break, a control group from the same batch was irradiated the same way except that the interval between the irradiation of the second and the first quarter was less than 10 s. The duration of the u.v. irradiation was 5 s for each dose. With the 1-h interval between the 2 u.v. irradiations, a few more double abdomens were produced (Table 1). This result is in contrast to the prediction of the model in its recently published form (Meinhardt, 1977).

Biochemical nature of the effective targets for u.v. induction of double abdomens. The effective targets for u.v. induction of double abdomens, i.e. those egg components which must be ultraviolet irradiated in order to produce double abdomens, apparently consist of ribonucleoprotein (RNP) particles. This contention is mainly based on the action spectrum for u.v. induction of double abdomens (Kalthoff, 1973), on the cellular localization of the effective targets (Kalthoff et al. 1977), on the production of double abdomens by application of RNase (Kandler-Singer & Kalthoff, 1976), and on the correlation between photoreversal after u.v. induction of double abdomens and the disappearance of pyrimidine dimers from the RNA of u.v.-irradiated eggs (Jäckle & Kalthoff, 1977). Since u.v. induction of double abdomens is possible from egg deposition until blastoderm formation (Kalthoff, 1971a), RNP particles would have to be produced throughout this period, with a rapid turnover, in order to meet the requirements of Meinhardt's proposal. In contrast to this idea, it is generally believed that
very little if any RNA is synthesized in insect eggs during the series of rapid mitoses prior to blastoderm formation (Pietruschka & Bier, 1972; Zalokar, 1976).

Photoreversal. The u.v. induction of double abdomens in *Smittia* eggs is photo-
reversible, i.e. eggs destined to become double abdomens can be renormalized by exposure, after u.v. irradiation, to light of wavelengths between 320 and 480 nm (Kalthoff, 1973). This phenomenon which is usually termed 'photoreactivation' (i.e. mitigation of u.v. inactivation), is commonly ascribed to light-dependent repair of u.v.-induced pyrimidine dimers in nucleic acids (Cook, 1970; Gordon, Huang & Hurter, 1976). Our biological data indicate that photoreversal after u.v. induction of double abdomens is of the so-called 'direct' type, i.e. based upon repair of the u.v.-damaged site (Kalthoff, 1973). Recent biochemical experiments have also shown that exposure to visible light causes the disappearance of pyrimidine dimers from the RNA of u.v.-irradiated *Smittia* eggs (Jäckle & Kalthoff, 1977).

This interpretation of our data on photoreversal, which is in agreement with common understanding among photobiologists (Cook, 1970; Harm, 1976), is at variance with Meinhardt's (1977, p. 125) interpretation. He proposes that 'the light irradiation does not reverse the u.v. damage to the inhibitor but blocks the activator production which normally follows, and that the activator concentration will then decrease to a sub-threshold value'. This unorthodox view of a particular instance of photoreversal appears to be an inevitable consequence of Meinhardt's current proposition. On one hand, the life time of the inducer, i.e. the presumed target of u.v. is less than 1 h; on the other, photoreversal after induction of double abdomens by u.v. irradiation of the anterior egg quarter can be delayed for 2–3 h without much detriment to its efficiency (Kalthoff et al. 1975). Meinhardt's explanation is that the 'autocatalytic activator increase (peak formation) is time-consuming, especially if the amount of activator produced after u.v. irradiation is just above the threshold for the activation of a new source' (Meinhardt, 1977, p. 125 and fig. 4A). The assumption that photoreversal is achieved *indirectly* by interference with the activator production thus helps to explain the delayed photoreversal in a way which conforms with the model. On the other hand, if this assumption is correct indirect photoreversal should also be possible in eggs where the inhibitor concentration has been reduced by other means than ultraviolet light.

Application of RNase to the anterior pole of *Smittia* eggs causes double abdomen production with considerable yields (Kandler-Singer & Kalthoff, 1976). To test whether this effect was photoreversible, eggs were exposed to light after application of RNase. Monochromatic light from our monochromator (440 nm wavelength, 8 W m⁻² dose rate) was applied for 1 h at 20 °C, since these conditions lead to maximum photoreversal after u.v. induction of double abdomens (Kalthoff, 1973). The eggs were oriented so that they faced the incident light with either the anterior pole or the whole dorsal side. Regardless of orientation, photoreverting treatment was ineffective (Table 1), which is again in marked contrast to the prediction of the model in its currently published form (Meinhardt, 1977). It may be objected that the numbers of surviving eggs in these experiments were rather small. Yet our conclusion seems reasonably safe. If double abdomen yields as found in these experiments had
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been produced by u.v. irradiation, photoreverting treatment under the conditions described above would have reduced the double abdomen yields to about 10% in experiment 3a, and 0% in experiment 3b (Kalthoff, 1971, fig. 2). With this expectation as an a priori hypothesis, a χ²-test indicates that the deviations of the results from the hypothesis are highly significant (P < 0.001).

**Combined fragmentation and u.v. irradiation.** Meinhardt (1977, p. 124) has predicted that after a ligation of *Smittia* eggs at 40% EL (% EL = per cent egg length, 0% = posterior pole) and u.v. irradiation of the 40–60% EL area, a set of posterior abdominal segments should be formed in both the posterior and the anterior egg fragment, with both abdomens in normal antero-posterior orientation. To test this prediction, eggs were fragmented by pinching them between the edges of 2 razor blades at levels ranging from 45 to 55% EL. The anterior quarter of the eggs (about 75–100% EL) was shielded while the remainder was u.v. irradiated with a dose sufficient to kill the posterior fragment. Of 61 treated eggs, 25 anterior fragments developed beyond blastoderm formation. All of these showed clearly head but no abdominal structures (Ritter & Sander, personal communication).

**Symmetry and invariance of the segment pattern in double abdomens.** Double abdomens produced in *Smittia* eggs display a remarkable symmetry and invariance in the numbers of segments. The normal abdomen has 10 segments, two of which fuse during embryogenesis (Kalthoff & Sander, 1968). Counting these separately, and regarding the large element usually found in the middle of the double abdomens (Fig. 1) as equivalent to 2 segments, the most frequent double abdomen pattern comprises 14 or 16 segments (7 or 8 per abdomen) while extreme cases with 13 or 19 segments are very rare (< 5%). However, the frequency distribution of the number of segments, and the symmetry of the double abdomens, are almost unaffected by parameters which strongly affect the yield of double abdomens, such as u.v. dose and wavelength, size and position of the target area, the age of the egg during irradiation (Bathe, Kalthoff & Sander, unpublished). These observations can be explained in terms of Meinhardt’s (1977) model provided there is sufficient time to build up first a new peak of activator and then the inhibitor maximum required for the specification of the terminal abdominal segments. Taking into account that the autocatalytic activator increase alone may take hours (see above), asymmetrical double abdomens with the terminal segments lacking in the anterior abdomen should be expected after application of u.v. or RNase in mild doses at late stages. Such asymmetric patterns, however, were not observed under these conditions.

**Length of segments in the double abdomen germ band.** From his computer simulation and from the ligation experiments of Sander (1975), Meinhardt (1977) derives a correspondence between different inhibitor concentrations and the evoked segment characters (fig. 5A, B). Using this correspondence, he predicts the arrangement of segments in double abdomens. The symmetrical inhibitor profile resulting from his simulation shows a relatively sharp peak at each terminal end and a shallow minimum in between. The segments evoked according to Meinhardt’s prediction differ considerably in their longitudinal extent. If the average length of the distal segments on each side is taken as a unit, the element in the middle of the simulated double abdomen
is about 5 units long, and the segments on both sides of this central element are almost 2 units long (Meinhardt, fig. 5c). Meinhardt says that this prediction was 'in substantial agreement with the experimental results'. This is not exactly so. The length of the actual segments as they first become visible, is very uniform except for a larger element usually found in the middle of the germ band (Kalthoff, 1975, and unpublished observations). The segments proximal to each of the terminal segments fuse with the neighbouring anterior segment and therefore appear temporarily shorter (Kalthoff & Sander, 1968). The element in the middle of the double abdomen germ band is usually larger than the average segment, but does not extend beyond twice the normal segment length. Also, the segments on both sides of the central elements are as long as the average segment (Fig. 1; see also Kalthoff & Sander, 1968; Sander, 1975). These observations therefore do not support Meinhardt's (1977) model.

Production of 'double cephalons' by centrifugation

Eggs of chironomid midges, after centrifugation with their longitudinal axis parallel to the centrifugal force, may produce both double abdomens and double cephalons (Yajima, 1960; Gauss & Sander, 1966; Overton & Raab, 1967; Kalthoff et al. 1977). The double cephalons consist of mirror-image duplicated heads (and possibly adjacent parts of the thorax) while abdominal segments are completely lacking. A similar type of pattern aberration was also found in Drosophila (Lohs-Schardin & Sander, 1976). Although Meinhardt (1977) refers to the work of Yajima (1960) he makes no attempt to explain the generation of double cephalons in terms of his model, and I cannot see how this could be achieved.

The generation of double cephalons is also at variance with the view of determination associated with Meinhardt's (1977) model. In an attempt to account for certain results of the ligation experiments of Sander (1960, 1975) with Euscelis and Smittia eggs, Meinhardt (1977, p. 131) has proposed that 'all cells (or nuclei with their plasma environment) begin in a determination state corresponding to the most anterior structure formed, the extraembryonic membrane, and that the determination of any other structure involves passing irreversibly through all the lower (more anterior) levels of determination until the final level of determination controlled by the local morphogen concentration is reached'. This postulate helps to resolve difficulties in the interpretation of ligation experiments but also creates new problems. The rule states that cells cannot return to more anterior ('lower') states of determination once they have reached more posterior ('higher') levels. In contrast to this rule, posterior halves of eggs from chironomid midges can be reprogrammed, by centrifugation, to form head structures until shortly before blastoderm formation (Gauss & Sander, 1966; Overton & Raab, 1967; Kalthoff et al. 1977). This result does not support the idea that nuclei or cells in the egg proceed irreversibly and with a constant rate towards increasingly posterior levels of determination; such a process would have led to advanced posterior determination states in the posterior egg half at a time when this egg half can still be caused to produce head structures.
DISCUSSION

The application of the lateral inhibition model to insect embryogenesis by Meinhardt (1977) and our ensuing personal discussion have stimulated the design of new experiments and a reevaluation of previous results. It has turned out that several data are at variance with the model in its currently published version. The discrepancy that the effective targets for the u.v.-induction of double abdomens do not have the rapid turnover as postulated for the inhibitor in Meinhardt's model may be resolved if 'producers of the inhibitor' rather than the inhibitor itself are regarded as effective targets of u.v. and RNase (Meinhardt, personal communication). This revision would also reconcile the model with the presumed biochemical nature of the targets in Smittia. Other difficulties, however, will probably also persist in the unpublished version of the model. For instance, delayed photoreversal could still be explained only under special conditions if the autocatalytic activator increase is extremely time-consuming. The objections raised in connexion with the non-appearance of 2 abdomens in tandem arrangement, the non-existence of asymmetrical double abdomens lacking terminal segments and the discrepancy between the predicted and the actual length of segments in double abdomens probably apply to both the published and the unpublished version of Meinhardt's model. Finally, the double cephalons found after centrifugation at the preblastoderm stage do not support the view of determination associated with the model in either version.

Focussing the attention on those results which conflict with Meinhardt's model, I have neglected the results which the model can explain (see Meinhardt, 1977). It is also much easier to point out the shortcomings of a model than to construct it in such an explicit and quantitative manner as Meinhardt has done. Still I believe that the discrepancies discussed here are more than marginal. I therefore wish to propose, in an admittedly rudimentary form, an idea which may help to reconcile the data on double abdomen and double cephalon formation in dipterans with existing models.

I propose that the specification of the antero-posterior body pattern in dipteran eggs involves 2 separate, although not entirely independent processes termed 'metamerization' and 'antero-posterior decision'. Metamerization is visualized as a subdivision of the embryonic blastema into areas which eventually give rise to the body segments; this process is thought to proceed from a prospective terminal segment (not yet designated as anterior or posterior) towards the intervening body regions. The antero-posterior decision is whether a given terminal region builds the most anterior or the most posterior prospective segments. The metamerization process appears compatible with gradients of a diffusible substance as a carrier of positional information, and thus with an essential part of Meinhardt's (1977) model. I deviate from Meinhardt's view by considering the antero-posterior decision as basically distinct from the metamerization. I believe that the antero-posterior decision is a threshold phenomenon, triggered by whether the anterior determinants are active or inactive. In Smittia eggs, these determinants have been tentatively characterized as RNP particles stored near the anterior pole of the egg (see Results). These determinants are regarded as independent of the system specifying the distance of the cells
from the terminal ends. In other words, the anterior determinants are superimposed on a symmetrical concentration gradient or other carrier of positional information. While the formation of the normal segment pattern requires the activity of the anterior determinants during a critical period between nuclear migration and blastoderm formation, the aberrant pattern double abdomen is ascribed to the lack or inactivation below a threshold value of these components.

The morphogenetic programme then ‘falls’ into a state which leads to the formation of an abdomen with the terminal posterior segments in the position which is normally occupied by the terminal anterior segments. A speculative scheme to illustrate this idea in a very simple fashion has been outlined previously (Kalthoff, 1976).

Experimental interference causing double abdomen formation such as u.v. irradiation of, or RNase application to the anterior pole region is thought to affect the antero-posterior decision rather than the metamerization. This distinction readily explains the invariable symmetry of the double abdomens, and the fact that experimental parameters which strongly affect the yield of double abdomens (i.e. the antero-posterior decision) hardly affect the metameric pattern. The separation of the antero-posterior decision from the metamerization also accounts for the fact that photoreversal can be delayed, since the anterior determinants appear to be inactive until nuclear migration anyway (Kalthoff et al. 1975; Kandler-Singer & Kalthoff, 1976). The non-appearance of 2 abdomens in tandem arrangement, in contrast to Meinhardt’s (1977) prediction, is also in line with my interpretation which ascribes head formation to the activity of prelocalized determinants and not to the destruction of activator by a neighbouring inhibitor maximum. The length of segments cannot be deduced from my interpretation in its present form. The segment length as it becomes apparent in the germ band may also differ from the extent of the precursor cells in the germ Anlage. Notwithstanding this reservation, the variable size of the element found in the middle of a double abdomen can be interpreted as resulting from a mismatch of the metamerization processes in the 2 abdomens. This view is supported by the length of this element which ranges from less than normal segment length to twice that of a normal segment, while the segments adjacent to the central element do not differ from the average length. The double cephalons and double abdomens observed after transverse stratification of egg material can both be explained as a result of a faulty redistribution of shifted anterior determinants. The difficulties in Meinhardt’s interpretation are thus avoided.

The distinction between metamerization and the antero-posterior decision proposed here is not only compatible with our own results but also with the ‘priority of the terminal characters’ as demonstrated by Yajima’s (1960) combined centrifugation and puncturing experiments with Chironomus eggs. Puncturing of the eggs resulted in progressive loss of intermediate but not the terminal segments in the double abdomens. This view is supported by the length of this element which ranges from less than normal segment length to twice that of a normal segment, while the segments adjacent to the central element do not differ from the average length. The double cephalons and double abdomens observed after transverse stratification of egg material can both be explained as a result of a faulty redistribution of shifted anterior determinants. The difficulties in Meinhardt’s interpretation are thus avoided.

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tial specification of pattern elements, starting from both terminal regions...'. Asymmetrical double abdomens including the terminal segments, and other patterns with gaps that are hard to explain on the basis of a continuous gradient, were also observed in the bicaudal mutant syndrome of Drosophila melanogaster (Bull, 1966, see below).

A problem with my interpretation is how, during normal development, the anterior and the posterior series of segments are joined together without gaps or repetitive segments. Gaps in the segment pattern indeed occur after fragmentation of unirradiated eggs before the blastoderm stage. The number of missing segments decreases from about 6 upon ligation during intravitelline cleavage to about 2 upon ligation after blastoderm formation (Sander, 1975; Ritter & Sander, personal communication). Throughout this period, double abdomens can be produced by u.v. irradiation. Likewise, photoreversal is possible until shortly before blastoderm formation (Kalthoff et al. 1975). This means that – in terms of the concept proposed – the metamerization in unirradiated eggs can proceed towards subdivision into an increasing number of metameric areas, while the antero-posterior decision is still open. In contrast to this apparent disjunction, a partial dependence of the metamerization on the antero-posterior decision was recently demonstrated by combined fragmentation and u.v. irradiation experiments (Ritter & Sander, personal communication). After transverse fragmentation during intravitelline cleavage, and subsequent u.v. irradiation of the anterior pole region, the anterior fragment forms an abdomen instead of head and thoracic segments. This result, in the first place, proves that an abdomen can be formed in the anterior fragment under conditions which virtually exclude interactions with the posterior fragment. With respect to the point to be made here, it is important that after combined fragmentation and u.v. irradiation the 2 abdomens together comprise about 18 segments as opposed to the 16 or 14 segments normally found in the double abdomens resulting after u.v. irradiation of non-fragmented eggs. This formation of additional segments in the separated abdomens contrasts with the gap in the pattern of anterior and posterior segment found after fragmentation of non-irradiated eggs. More data are obviously needed before the concept of longitudinal pattern formation as briefly laid out here and elsewhere (Kalthoff, 1976) can be elaborated in more detail and possibly extended to other insects. As it stands now it is admittedly far from matching the accurate performance of Meinhardt's (1977) current application of the lateral inhibition model. On the other hand, I believe that the distinction between metamerization and antero-posterior decision is more in line with aspects of homeotic transformations in insects which appear relevant to the topic discussed here.

Homoeotic mutations (see Ouweneel, 1976) and transdeterminations (Hadorn, 1966) are the result of gross changes in morphogenetic pathways in the imaginal disks of Drosophila. It is important that in antennapedia wild type and mutant mosaics specific leg parts always replace specific ('homologous') antennal parts (Postlethwait & Schneiderman, 1971). Thus transdetermined cells and their normal counterparts apparently respond differently to the same pattern-specifying signals. A concept which emerges from the phenomenon briefly summarized here is that cells in different imaginal disks differ from each other in the activity of a small number of controlling
genes, and that these differences cause different responses to similar or identical systems of positional information (see Postlethwait & Schneiderman, 1973; Bryant, 1974; Ouweneel, 1976). A related idea was also proposed by Wolpert (1969) who distinguished between the determination of boundary values and the positional information dependent on them.

Transdetermination and homoeotic mutations apparently represent basic switches, at different regulatory levels, of the same morphogenetic programme. To emphasize its sharply distinct alternate stationary states, Kauffman (1973) has coined the term 'bistable control circuit'. Such circuits can be constructed, with feedback loops of regulatory genes as elements (Davidson & Britten, 1971). The homoeotic mutation engrailed in Drosophila which transforms the posterior ‘compartments’ of the thoracic segments more or less completely into anterior compartments apparently causes, in the posterior compartment, the disfunction of a ‘selector gene’ (Garcia-Bellido, 1975). This gene is thought to be active in the posterior and inactive in the anterior wing compartment during normal development so that a defective condition of this gene would lead to anterior instead of posterior wing structures (Lawrence & Morata, 1976). The failure of the normal gene function in the posterior compartment is apparently caused by a defective condition in the selector gene itself, since its expression is cell autonomous (Garcia-Bellido & Santamaria, 1972).

Another type of homoeotic transformation, at least in theory, might be caused by a failure in the regional activation of selector genes. In normal development, selector genes have to be switched on in certain compartments but not in others. This may be achieved, at least in early embryogenesis, by prelocalized cytoplasmic determinants. A regional gene activation of this type is apparently defective in the mutant bicaudal (Bull, 1966) of Drosophila melanogaster. The phenotypes include longitudinal duplications of the posterior abdominal segments just like the epigenetically produced double abdomens in Smittia and other chironomid eggs. The bicaudal mutation is maternally inherited. The production of abdominal segments in the anterior egg half may therefore be ascribed to the lack or the defective state of cytoplasmic determinants which become localized in the anterior egg half during oogenesis and serve to activate selector genes required for head and thorax formation in normal development. The anterior cytoplasmic determinants, in this interpretation of the bicaudal case, would exactly correspond to the anterior determinants in Smittia eggs which are thought to switch, by activity or non-activity, the morphogenetic programme from normal segment pattern to double abdomen. Consequently, I propose that the bicaudal mutation interferes with the antero-posterior decision in the Drosophila egg. The metamerization which seems also affected in the bicaudal phenotypes, still appears as a separate process. This is indicated by such bicaudal phenotypes which exhibit anterior structures in the ventral and posterior structures in the dorsal parts of their anterior ends (Nüsslein, personal communication). A similar type of split segment pattern was observed in eggs of the pea beetle, Callosobruchus maculatus. Upon temporary ligation of these eggs, more than 20% of the developing embryos displayed the normal segment pattern in one lateral half and a double abdomen pattern in the contralateral half (Van der Meer, personal communication).
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It seems hard to conceive how such discontinuous patterns could be explained by a model based upon a rapidly diffusible morphogen as the only control of embryonic differentiation (Meinhardt, 1977). However, the separation of the antero-posterior decision from the metamerization as proposed here facilitates the explanation of such patterns. As the antero-posterior decision is ascribed to the above or below threshold activity of a localized determinant, a defective condition or localization of this determinant may render it just insufficient within subregions of the anterior egg half. According to the resulting activity or inactivity of the selector gene(s), identical concentrations of inhibitors or other carriers of positional information could be interpreted differently so that both anterior and posterior structures are formed within the anterior egg half.

The spatial differentiation of a developing insect embryo apparently involves the regional activation of selector genes under the control of localized cytoplasmic determinants. The activity of such genes seems to determine, in a combinatory way, the gross character of compartments i.e. regions within the developing system. Within such compartments, gradients of diffusible molecules or other carriers of positional information may release superimposed differential gene activities which in turn may define subregions and so forth. On this background, the difference between Meinhardt's (1977) model and my proposition may be stated as follows. In Meinhardt's model, the entire insect embryo is metamerized under control of the local concentration of a diffusible morphogen. In contrast, I propose that the longitudinal segment pattern is composed from 2 separately specified sets of segments. This is ascribed to a strictly alternative antero-posterior decision superimposed on a symmetrical mode of metamerization within the anterior and posterior halves of the developing embryo.

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