Extrakaryotic determinants, a link between oogenesis and embryonic pattern formation in insects*

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In most insect species, the genome of the zygote nucleus is not the only store of information for early embryogenesis. Much indirect evidence and some hard data indicate that determinants or signals in the cytoplasm of the egg cell and perhaps even in the egg shell provide developmental information of paramount importance. This extrakaryotic information is arranged in a non-random spatial pattern which more or less stringently directs embryonic patterning. Embryonic pattern formation is defined as the sum of events establishing the basic organization of the future larval body, an organization which is first evident in the segmented germ band. As the extrakaryotic information is already present at the time of egg deposition, it must derive from oogenesis. Oogenesis thus cannot be viewed exclusively as a process whereby building material and energy sources for the construction of a new individual are accumulated – a view tacitly taken most by present-day investigators of oogenesis – but also as a process incorporating into the egg a kind of spatial or positional information (Wolpert 1969) which acts as a "construction blueprint". Naturally the character of this information in different species will depend on variable details of oogenesis, but in some form or the other it should be present in almost all pterygote insects. A notable exception where such information appears ruled out are parasitic hymenopterans with extensive polyembryony. Here the egg cell can be extremely small (down to 25 μm, see Ivanova-Kasas 1972), and this tiny egg cell proliferates in apparently random fashion within the host, producing hundreds of daughter cells each of which may give rise to an embryo. By their very mode of origin these daughter cells cannot possibly inherit extrakaryotic spatial information much more complex than simple cell polarity.

When considering the role of extrakaryotic information, a brief look at an array of various insect eggs used for research (Fig. 1) will be useful. Considerable differences exist between species not only in egg size but also with respect to the extent of the territory which is occupied by the germ anlage or embryonic rudiment within the egg (heavily stippled in Fig. 1). Even more striking differences emerge when the transformation of the germ anlage into the germ band is observed. These and other differences led Krause (1939) to establishing a series of "Insect Egg Types". This series ranges from the extreme short germ type to the extreme long germ type. The types are characterized at several levels starting from the cytoarchitecture of the egg cell, but in respect to embryonic patterning the main distinction is in the mode of segment formation. In the extreme short germ type, all or nearly all metameric segments of the germ band originate by proliferation near the posterior region of the germ anlage. Frequently the segments become visible in antero-posterior sequence as the germ anlage stretches and thereby approaches the proportions of the germ band; formally, this process may be compared to the transformation of an annelid trochophore into the segmented worm. In the extreme long

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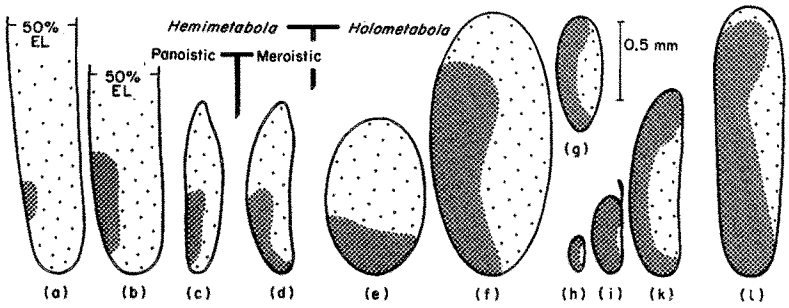

Fig. 1. Various insect eggs drawn to scale (from Sander 1976). The eggs are seen from the left hand side and the region of the germ anlage is heavily stippled. The germ anlage consists of about 1000 cells in *Oecanthus* (P. Baader, 1968) and more than 5000 cells in *Drosophila* (Turner & Mahowald 1976). Type of oogenesis and postembryonic development are indicated at the top. Represented are the crickets *Oecanthus pellucens* (a) and *Acheta domesticus* (b), the damsel fly *Platycnemis pennipes* (c), the leafhopper *Euscelis plebejus* (d), the beetles *Tenebrio molitor* (e), *Leptinotarsa decemlineata* (f) and *Bruchidius obtectus* (g), the dipterans *Smittia* spec. (h), *Drosophila melanogaster* (i) and *Calliphora erythrocephala* (k), and the hymenopteran *Apis mellifica* (l).

germ type, on the other hand, the germ anlage is proportioned similar to the germ band, and segmentation is a much more rapid process which without cell proliferation subdivides the prospective metameric part of the germ anlage *in situ* into the full number of segments. This process may also follow a certain spatial-temporal order, the first segment borders becoming visible in the gnathocephalon or the anterior thorax, but at most it takes a few hours while segment proliferation in the short germ type may take days or weeks. This difference is reflected by the fate maps of early stages like blastoderm or germ anlage. In the short germ type, the germ anlage essentially consists of a large procephalic territory and a small posterior region giving rise to most body segments and the telson. In the long germ type, all prospective segments even in the blastoderm appear to occupy territories proportionate to the segments visible in the germ band. The former fact becomes obvious when looking at a series of stages in short germ development (Fig. 2) while the latter was proven also by experimental fate mapping. In the *Drosophila* egg, which represents the extreme long germ type, small groups of blastoderm cells were irradiated with a UV laser microbeam and the site of irradiation was plotted against the location of cuticle defects in the larvae hatching from the irradiated eggs. Figure 3 shows that with this method a linear correlation was found between the site of irradiation and the location of larval defects, meaning that the progenitor cells for the different segments must be spaced in the blastoderm in the same proportions as in the larval body (Lohs-Schardin *et al.* 1979). Calculations based on several methods of fate mapping (see Schubiger and Newman 1982) indicate that the individual thoracic and abdominal segments occupy transverse stripes each about four blastoderm cells wide.

Thus, the essential difference between the extreme short germ and long germ types is not in the size of the germ anlage as compared to egg size – a criterion that might be suggested by Fig. 1 –, but in the prospective fates of the different regions of the germ anlage, and in the concomitant transformation of the germ anlage into the germ band by sequential segment proliferation or by *in situ* subdivision, respectively.

The types of early embryogenesis just outlined are extremes with many variants in between. By way of generalisation, the transition from one extreme to the other may be characterized as the extension of *in situ* subdivision towards the more posterior segments. In the example shown in Fig. 2, only two or three metameric segments can be recognized to begin with. In intermediary forms like house cricket, damsel fly, and leafhopper (Fig. 1 b–d), the germ band parts arising by *in situ* subdivision of the germ anlage blastema may include the

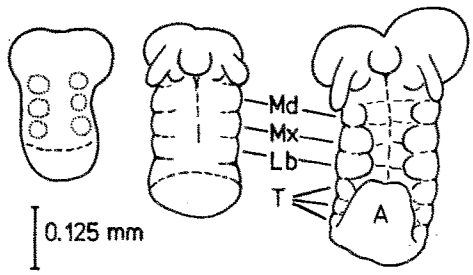

Fig. 2. Short germ development as seen in the bristle-tail *Petrobius brevistylis* (modified from Larink 1969, see also Machida 1981). Metamery is first indicated by 3 pairs of mesoderm blocks in the late germ anlage stage (left). The first appendages to appear are antennae, mandibles and first maxillae; segmentation becomes evident in the gnathocephalon earlier than in thorax and abdomen (middle). When the thoracic segments become apparent (right), the abdomen is still unsegmented and much shorter than in the fully segmented germ band. Developmental time is subject to considerable variation in this species; it was (from left to right) 22 days, 21 days, and 16 days, respectively.

entire head and thorax, with only the abdomen proliferating (for a discussion, see Sander 1976, 1981). Another important point is that during evolution the transition from short germ to long germ development probably was no unique event but took place several times. This is indicated by the fact that parallel transitions from short germ towards intermediate or long germ development can be noted among recent forms within several groups, e.g. the Orthoptera and Coleoptera. It would be very useful to have more detailed data on the mode of segment formation in a larger number of species from various systematic groups; this is a task waiting for the descriptive embryologist, a task I want to stress particularly in this country with its active group of scientists advancing our knowledge in this field.

As oogenesis provides the basis for embryogenesis, we may expect correlations between variants of both. Such correlations may indeed exist (see Fig. 1) and, again by way of generalization, one might state that extreme short germ development seems to occur only in panoistic forms, and extreme long germ development only in meroistic-polytrophic forms. More detailed correlations cannot be demonstrated at the moment, and once established, they may well turn out to be complex. Again, collecting descriptive data would be the first and indispensable step for analyzing this aspect of the amazing variability of insects.

When now discussing some interlacings between oogenesis and embryonic pattern formation in a few well known forms, I first want to warn the reader that most conclusions are speculative rather than well proven.

To begin with polytrophic oogenesis, this type is characterized by nurse cells which under control of polyploid genomes synthesize various components such as RNA, ribosomes, mitochondria etc., and export these to the adjacent oocyte. The panoistic oocyte, on the other hand, has to rely for these functions on the essentially diploid genome of its own pro-meiotic nucleus. Both forms import yolk proteins from the hemolymph and therefore the time required for oocyte growth (vitellogenetic phase), which is also the time available for synthesizing the bulk of the components just mentioned, does not differ much (about 3 days in the house cricket, U. Baader 1969; 1 day in *Drosophila*, King 1970). Consequently, in polytrophic forms the egg cell embarks on embryogenesis with much larger stores of "ready-to-use" products than the panoistic oocyte.

It is this difference which led earlier authors to distinguish between cytoplasm-rich and yolk-rich insect eggs (Krause 1939, Bier 1970). As a consequence, the *Drosophila* egg can produce its 6000 blastoderm cells within less than 3 hours, while the cricket *Oecanthus* requires twice that time for a blastoderm of about 2000 cells (P. Baader 1968). The *Drosophila* egg takes one day from ovipositin to hatching at a temperature (23–25°C) where the domestic cricket takes one month and *Oecanthus* (Fig. 1a) even two months (P. Baader 1968). The gain in developmental speed linked to the transition from panoistic to meroistic oogenesis opens up entirely new ecological niches (e.g. decaying organic substrates as larval food in the case of higher dipterans).

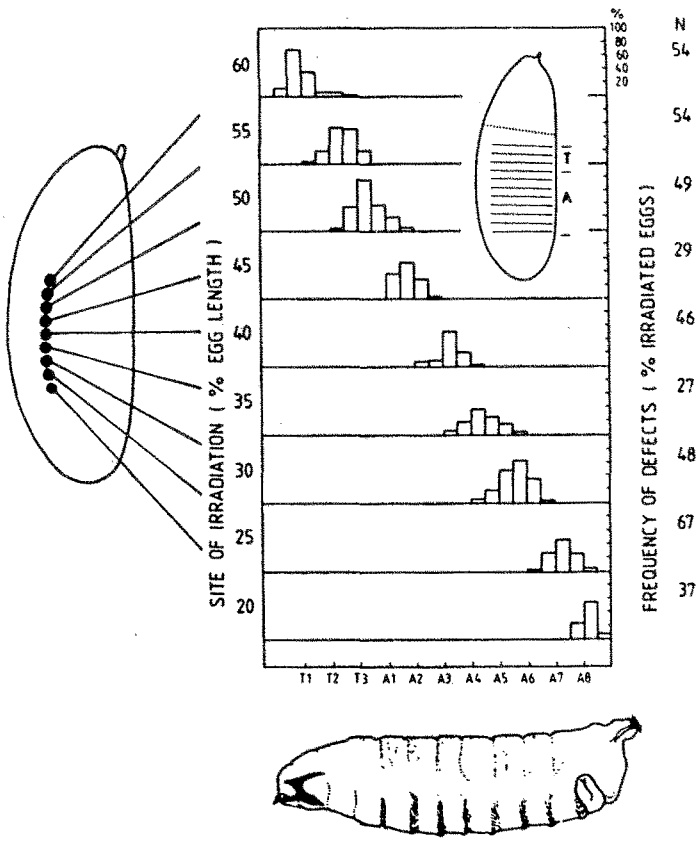

Fig. 3. Fate map of the *Drosophila* blastoderm (top right) reconstructed by UV laser defect mapping (from Lohs-Schardin *et al.* 1979, augmented by drawings of Ch. Nüsslein-Volhard). The dots in the blastoderm stage egg at the left represent the sites irradiated. The resulting defects were scored on the cuticle of the larva (bottom). The histograms show the distribution of cuticular defects after irradiation of different sites in the blastoderm. Note that all segments are represented in the blastoderm by nearly equal territories, quite in contrast to short germ development (Fig. 2).

If a species is to take full advantage of the speed of early development made possible by the "invention" of nurse cells, it requires patterning mechanisms capable of completing their task within a short period rather than within the long time available in the embryogenesis of panoistic forms. This consideration may help to explain why the "spatial blueprint" of extrakaryotic patterning signals is apparently much more elaborate in long germ eggs than in short germ eggs. In the latter, extrakaryotic properties may perhaps convey only two informations, namely egg cell polarity and a localized signal indicating to the blastoderm cells where they should assemble when forming the germ anlage. This view is based on the normal course of development in several short germ species, which is fairly indeterminate or "floppy" in this respect (as noted first by Seidel 1924); experimental support comes from data of Miya and Kobayashi (1974) which seem to indicate that any group of blastoderm cells once assembled can proceed to form a germ band. Another potentially instructive instance is provided by old and new findings in phasmids. Cappe de Baillon (1940) noted many years ago that *Carausius* eggs lacking a micropyle fail to form an embryo, while two embryos may form in abnormal eggs carrying two micropyles (Fig. 4). Failure of an embryo to form in eggs without micropyle is easily explained by the finding of Pijnacker and Ferwerda (1976) that oxygen entering through the micropyle is required for removing the meiotic block of the oocyte. Cappe de Baillon (1940) found that in *Carausius* the headlobes always form

underneath the micropyle. One may therefore speculate that the specific physiological conditions created by the micropylar channel do not only serve for egg cell activation, but might also provide the signal attracting the blastoderm cells which then form the germ anlage. If so, this example would be useful in documenting that extrakaryotic spatial signals generated during oogenesis need not necessarily be located in the ooplasm; rather,

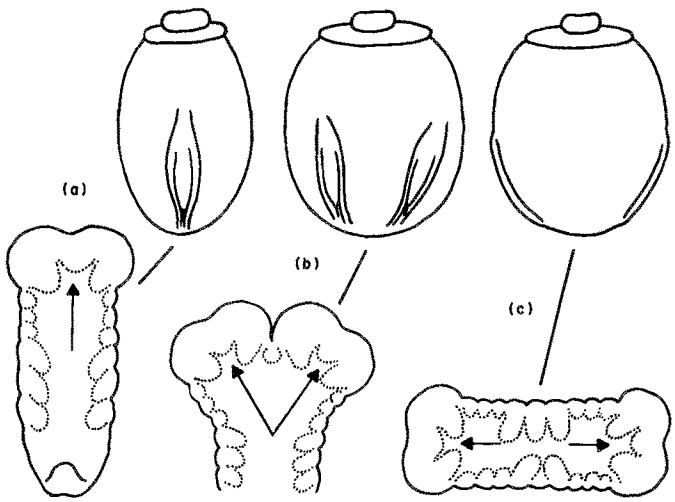

Fig. 4. Eggs and germ bands of the phasmid *Carausius morosus* (modified after Cappe de Baillon 1940). (a) normal egg seen from the ventral (= narrow) side carrying the rhomboid micropylar area, and normal germ band developing from such eggs. (b) egg with double micropylar apparatus, and germ band found in it. The angle between the two heads corresponds approximately to the angle of the micropyles on the egg circumference. (c) egg with two ventral sides (micropyles set at 180°) seen laterally, and "double cephalon" developing in it. Arrows indicate axial polarity. The germ bands in (b) and (c) are constructs made for illustrating the principle, but similar-looking real germ bands were published earlier by Cappe de Baillon (1927).

they might be encoded in the egg shell in some instances.

Another fact is of interest more from the evolutionary than from the developmental point of view. *Carausius* has no functional males and consequently no need for the micropyle as a means of sperm access. Nonetheless the micropyle has persisted and now serves for egg activation by permitting access of oxygen rather than sperm (and perhaps also for attracting the germ anlage cells, see above).

Egg polarity in one form or the other must be responsible for the fact that the germ anlage in *Carausius* invariantly extends towards the posterior egg pole once it has formed in the "centre formateur" (Cappe de Baillon 1940). If two germ anlagen form, these extend both to the posterior pole and may either grow from there as a single posterior germ band (Fig. 4b) or form a double anterior mirror image (Fig. 4c) which seems formally comparable to the "double cephalon" aberration discussed below; the "compressed abdomen" reported by Cappe de Baillon (1927) as being attached to the middle of such a double cephalon may correspond to the "knobs" frequently emerging from the plane of polarity reversal in dipteran double monsters (e.g. *Sciara*, see Fig. 1 in Sander 1982).

In long germ development and in the intermediate forms investigated, some extrakaryotic spatial information can be characterized somewhat better, thanks to experimental results and the analysis of mutants. The best example is provided by the germ cell determinants in *Drosophila* (Illmensee 1976) but this example cannot serve as a model for the somatic regions of the egg cell (Sander 1975). In these it seems that certain peculiarities signal "anterior" and "posterior" to the embryonic cells in terminal locations, and that by some as yet unrecognized mechanism (see Sander 1981) the cells located in between "recognize" their position and

differentiate accordingly into the various parts of the body. One much-discussed means of conveying such positional information (Wolpert 1969) are morphogenetic gradients, which have been postulated on both experimental and genetic evidence; however, other possibilities exist and one will be discussed below. Evidence for a posterior signal or "posterior determinants" has been provided by experiments in the leafhopper *Euscelis* representing an intermediate egg type (see Sander 1976). Here material from the posterior egg pole can be shifted to other positions within the egg. Under appropriate experimental conditions, this translocated material will dramatically influence embryonic pattern formation, altering both local character and polarity of adjacent parts of the embryonic blastema from its new location. The fact that the effects are exerted over quite some distance (Fig. 5) was ascribed to some kind of morphogenetic gradient set up by the posterior pole material (Sander 1960), and elaborate computer simulations showed that indeed a gradient-type interpretation is possible for most results obtained in this and some other species (Meinhardt 1977). However, intercalation of positional values might well provide an alternative explanation even for a striking pattern previously considered the stronghold of gradient interpretations, namely the double abdomen (Fig. 5e) (Sander 1976, Nüsslein-Volhard

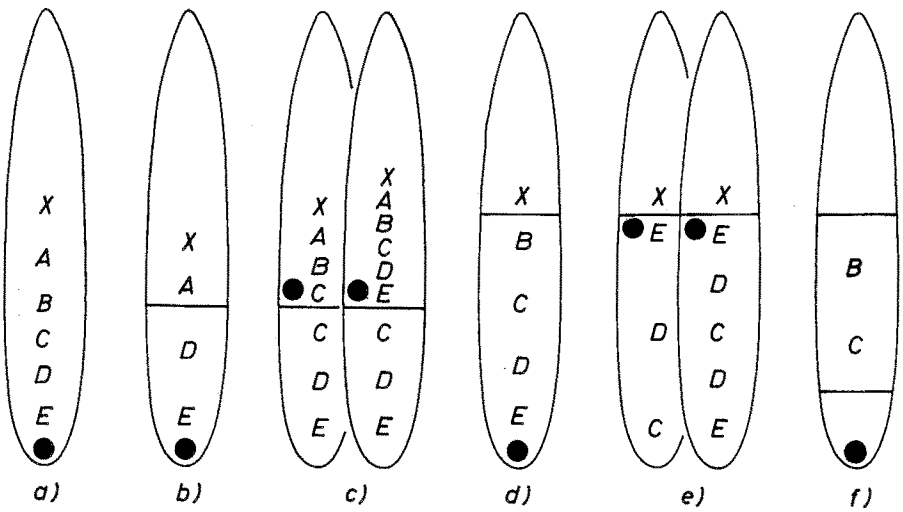

Fig. 5. Diagramm showing patterns formed in eggs of the leafhopper *Euscelis plebejus* (from Sander 1981). A–E = regions of the germ band (A procephalon, E end of abdomen), X = extraembryonic epithelia, black disk = posterior pole material incorporated during oogenesis. (a) normal pattern, (b) and (d) patterns found after constriction at the levels indicated by the bars, (c) posterior material induces posterior pattern elements in anterior fragments (compare with b), (e) posterior material at the anterior end of a fragment induces complete or partial pattern reversal (note "double abdomen" type in the right egg). (f) middle piece isolated soon after oviposition forms polarized middle pattern, thereby showing that some positional information must be encoded in the non-terminal regions, too, during oogenesis.

1979). If Cappe de Baillon's double cephalons (Fig. 4c) originate from two formative centres as he inferred, the proliferation of both germ anlagen must come to a stop when they meet without continuing in a common thorax and abdomen. The morphological symmetry could then be the result of intercalation between any juxtaposed segments or regions of discrepant positional information (see Sander 1981). The same principle might be responsible for the strict symmetry observed in most double abdomens. This interpretation moreover would be superior to the gradient models in coping with oblique planes of symmetry, a situation found in double abdomens of the leafhopper and of the beetle *Callosobruchus* (Fig. 6) which would call for very skewed gradients while local cell-cell interaction (Sander and Nübler-Jung 1981) could easily achieve this.

The double abdomen malformation first induced by Yajima (1960) has since been obtained in several insect forms including other chironomids (see Kalthoff 1979), a leafhopper (Fig. 5), a sciarid (Perondini *et al.* 1982), further dipterans (H. Yajima, personal communication), and beetles (Schnetter 1965, van der Meer 1984) (Fig. 6). Except in the leafhopper, the double abdomens were induced by destructive interventions like centrifugation, temporary fragmentation, UV irradiation, or injection of RNase. The effects of UV irradiation of the anterior egg region seemed to suggest that the formation of posterior structures essentially follows from the absence of a functional signal for "anterior". However, Yajima's data (1960, 1964) and recent evidence from *Smittia* suggest that in chironomids both anterior and posterior determinants exist (as claimed for the leaf hopper, see Sander 1960), and that it is their relative strength at each egg pole which decides the pathway (anterior or posterior) that is followed there (Kalthoff *et al.* 1982).

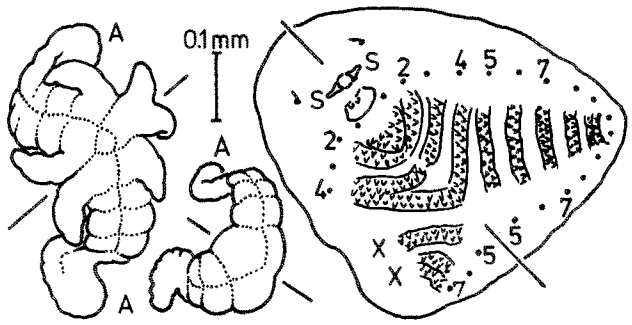

Fig. 6. Double abdomen type embryos with oblique level of mirroring symmetry. Left and middle: germ bands from posterior fragments of leafhopper eggs ligated after translocation of posterior pole material (see Fig. 5e) (modified from Sander 1963 and 1961, respectively). The symmetry plane (bars) in the left germ band runs from the anterior border of the metathorax (left hand side, note leg buds of opposite polarity) to the middle of the mesothorax (right hand side, note leg branches of opposite polarity wedged in between the metathoracic legs). In the younger germ band (middle), the border probably runs from the middle of the metathorax at the left to near its anterior border at the right. The right figure, drawn after a photograph of van der Meer (1984), represents the larval cuticle of a partial double abdomen from the beetle *Callosobruchus maculatus*. S = lateral spine of the first abdominal segment, 2–7 = serial numbers of segments (the adjacent dots represent segmental cuticle markers). The mirroring plane (bars) cuts through segments 1–4 of the "normal" abdomen (right) at an angle of about 45° while segments 5–11 are unaffected. The left abdomen is mirroring the right abdomen in segments 1–4 while lacking one lateral half in segments 5–7 (X); all structures posterior to segment 7 are missing, too.

The claim for anterior determinants is as yet lacking confirmation by transplantation experiments of the type positively demonstrating posterior determinants in the leafhopper egg (Fig. 5), but the amount of mutually supporting experimental and molecular data obtained in *Smittia* (Kalthoff *et al.* 1982, reviewed in Kalthoff 1979) leaves little doubt as to their existence; the "double cephalon" aberration first produced by Yajima (1960, 1964) in chironomids also provides strong evidence in favour of anterior determinants. In our context the most important conclusion is that the anterior determinants of *Smittia* become localized in the anterior cytoplasm of the oocyte *before* the onset of embryogenesis while their function is required only around the blastoderm stage (Kalthoff 1979), when the embryo starts using its own genes (review see Berry 1982). It is then that specific "indicator proteins" for anterior and posterior development are first synthesized by the embryo (Jäckle and Kalthoff 1981).

Maternal effect mutants altering embryonic pattern formation provide an alternative to experimental analysis and a complementary way of studying extrakaryotic determinants. Such mutants are affecting the female insect not in its external phenotype but in its capacity to incorporate one or the other extrakaryotic signal in the appropriate region of (some) eggs which it produces. The first case of a heritable anomaly probably

representing this type was described in a mosquito (Price 1958) but the best studied instances come from *Drosophila* with its immense potential for genetic analysis. *Drosophila* females homozygous for *bicaudal* (Bull 1966, Nüsslein-Volhard 1979) produce eggs which develop double abdomens instead of normal larvae. Some eggs of *dicephalic* females, on the other hand, carry anterior markers at both poles and, if embryogenesis ensues (which is rare), mostly produce "double anterior" embryos and occasionally a perfect double cephalon (Lohs-Schardin 1982) (Fig. 7b). *Dicephalic* is unique among *Drosophila* mutants in showing visible anomalies of oogenesis which can be linked to the embryonic patterning defect. Some follicles from homozygous *dic* females carry nurse cells at both poles of the oocyte instead of only the anterior pole (Fig. 7a). This anomaly apparently results from aberrant arrangement of the normal number of nurse cells (Lohs-Schardin 1982), and it is invariably followed by formation of an anterior structure (the mycropylar cone) at both poles of the vitelline envelope (Bohrmann 1981). However, the location of the respiratory appendages of the chorion (Fig. 7b), another marker for "anterior", seems to be determined by the location of the oocyte nucleus rather than the nurse cells (J. Bohrmann, unpublished results). A few of the viable *dic* eggs instead of yielding double anterior larvae produce defective larvae of uniform polarity, or double abdomen type embryos (Lohs-Schardin 1982). The simplest explanation for this finding is to assume that the abnormally located nurse cells sometimes fail to provide a sufficient amount of anterior determinants to the adjacent part of oocyte, whereupon an abdomen is formed there instead of a head – as in *Smittia* after UV inactivation of the anterior determinants. This interpretation is compatible with the frequencies of the three types of embryo in the *dic* egg (Table 1).

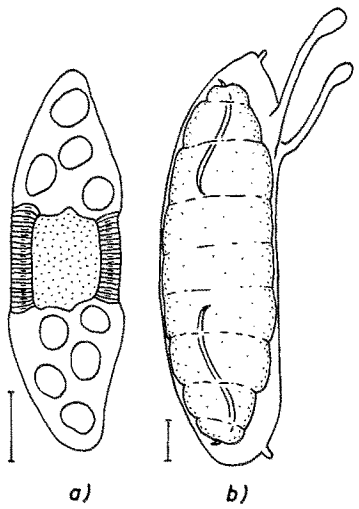

Fig. 7. The maternal effect mutant *dicephalic* of *Drosophila melanogaster* (from Sander and Nübler-Jung 1981). (a) *dic* follicle with split nurse cell group (some nuclei shown in outline) and oocyte in between (stippled). (b) *dic* egg carrying micropylar cones at either end and containing a "double cephalon" larva with plane of symmetry in the first abdominal segment.

Before leaving the topic of maternal effect mutants, mention should be made of the mutant *dorsal* in *Drosophila*, where the mother apparently fails to build determinants signalling "ventral" into the appropriate region of the oocyte (Nüsslein-Volhard 1979). This was concluded from UV laser microbeam irradiations (for technique see Fig. 3) which showed that in *dorsal* eggs the ventral blastoderm cells give rise to rather dorsal structures instead of the mesoderm which they should form (Nüsslein-Volhard *et al.* 1980).

Early development is largely under the influence of extrakaryotic determinants as shown by these examples, but subsequent steps of embryonic pattern formation must progressively rely on the embryo's own

genetic information. Such steps can be analyzed using "zygotic" patterning mutants in which the developmental anomaly is due to defects in the embryo's genome (derived from the zygote) rather than the maternal genome. In order to complete the generalized picture drawn in this essay I shall briefly mention two classes of zygotic patterning mutants in *Drosophila*. The first class is represented by the "segmentation mutants" discovered recently (Nüsslein-Volhard and Wieschaus 1980, Sander *et al.* 1980, 1981) which apparently affect functions involved in establishing metameric subunits of blastoderm or germ anlage. The amazing effects of these mutants are as yet little understood and we therefore refrain from discussing them here. The other class of zygotic mutants maps in the *bithorax* complex analyzed in great depth by E. B. Lewis (for a review see Lewis 1978). Like the *E*-alleles in *Bombyx*, the *bithorax* alleles affect functions involved in assigning specific characters to

Table 1. Embryonic phenotypes in the maternal effect mutant *dicephalic* of *Drosophila melanogaster* (data from Lohs-Schardin 1982). The expected number of cases (bottom line) was calculated on the assumption that the decision "anterior or posterior" is taken independently at each pole.

Phenotype	Double anterior	Uniform polarity	Double posterior
Number of embryos found	68	15	3
Number of cases expected	67	18	1

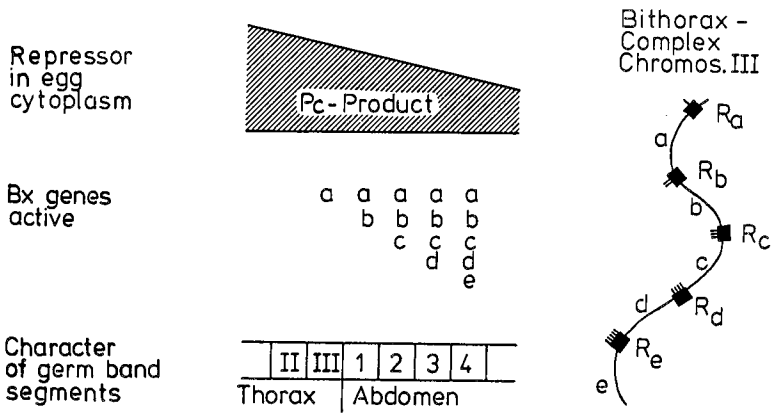

Fig. 8. Generalized diagram of functions in the *bithorax* complex of *Drosophila melanogaster*, based on data and interpretations of Lewis (1978). The individual character of the germ band segments named at the bottom is due to the activity of the various *bithorax* genes a–e; the more posterior the segment character, the more gene functions are needed (middle). The spatial coordination of different functions along the egg axis is warranted by a gradient of repressor in the egg cytoplasm (top) and by the linear arrangement of the *bithorax* genes in the DNA molecule (at the right). Each gene is controlled by a regulatory region (R_a . . . R_e). The affinity of these regions to the repressor molecule increases from gene to gene (see number of bars on black squares) so that blocking R_a needs much higher concentrations of the repressor than blocking R_e. The repressor molecule may be controlled by the *Polycomb* locus (*Pc*).

the individual body segments. The general finding most important for embryonic pattern formation is the fact that the loci specifying different segments in the posterior body half are arranged on the DNA of the chromosome in the same spatial sequence as the corresponding segments in the germ band. There are some exceptions

and a lot of complications to this principle, but in order to convey its basic structure I have designed Fig. 8 from the data and interpretations of Lewis (1978). This figure leads us back to the topic of extrakaryotic determinants because the spatial coordination of the different *bithorax* functions is provided in Lewis' interpretation by an extrakaryotic repressor, the *Pc* gene product. The polarized, gradient type distribution postulated for this hypothetical repressor in the ooplasm must be due to extrakaryotic signals built into the egg during oogenesis.

In closing I would like to stress again the fact that, in the interest of providing a comprehensible picture, I have proposed many generalizations which may not stand the test of time. However, I feel that scientific progress depends on testable generalizations based on present knowledge, however scanty, and this may excuse if not justify the approach chosen in this essay.

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