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Transplantation of localized anterior determinants in *Chironomus* eggs by microinjection

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THE ROLE OF BIOASSAYS IN THE ANALYSIS OF LOCALIZED CYTOPLASMIC DETERMINANTS

Operational criteria for localized cytoplasmic determinants

In most animal embryos early determinative steps appear to be controlled by uneven distributions of cytoplasmic components (Davidson, 1976). The segregation of localized cytoplasmic components into different blastomeres seems to determine certain cell lineages, or to set up polarities, which in turn become the basis for cell interactions later. Early investigators were often guided by the localization of visible markers such as pigments or basophilic granules in animal eggs. Removal or local inactivation of cytoplasm containing such components was usually followed by embryonic defects. However, studies of this kind do not prove that the visibly localized components determine cells, or that the defects were caused by inactivation of localized components.

Localized determinants are best defined by operational criteria. First, the components under consideration must be localized. The localization may be exclusive or merely quantitative. Second, it must be shown that the localized components are necessary and/or sufficient for cell determination. The traditional tests under these criteria involve some means for translocating cytoplasmic components, and a bioassay for blastomere determination. This will be illustrated using as a paradigm the determination of pole cells which are the primordial germ cells in dipterans and other insects. As the pole cells are formed, they incorporate posterior pole plasm which contains basophilic and electron dense granules known as polar granules or the oosome. In order to determine whether the polar granules are associated with germ cell determinants, the following experiments have been carried out.

Heterotopic transplantation

Illmensee & Mahowald (1974) have microinjected posterior pole plasm from *Drosophila* eggs to anterior or lateral regions of recipient eggs. This was followed by the development, at the transplantation sites, of cells showing the morphology

Key words: *Chironomus*, microinjection, egg, determinants, anterior, transplantation.

of pole cells. In order to test whether these cells could also function as primordial germ cells, they were transplanted next to the normal pole cells of secondary host embryos. The offspring of some of the secondary hosts showed the genetic markers of both the secondary host and the primary recipient of the transplanted posterior pole plasm. This experiment proved that posterior pole plasm was sufficient for the determination of primordial germ cells. In order to demonstrate that the germ cell determinants were localized to the posterior pole, it would have been necessary to transplant cytoplasm from regions other than the posterior pole. This important control experiment was furnished, somewhat indirectly, in a subsequent study by Illmensee, Mahowald & Loomis (1976). The authors showed that posterior cytoplasm from unfertilized eggs and oocytes at stages 14 or 13 was sufficient to induce primordial germ cells ectopically. Posterior cytoplasm from younger oocytes was not effective. Thus, ectopic pole cell formation was not caused by injection of any cytoplasm.

Rescue experiments

It has long been known that u.v. irradiation of posterior pole plasm prevents pole cell formation and causes sterility (Geigy, 1931). Both pole cell development and fertility were restored to u.v.-irradiated embryos by microinjecting posterior pole plasm from unirradiated donors (Okada, Kleinman & Schneiderman, 1974; Warn, 1975). Only posterior, but not anterior, cytoplasm had the rescuing effect. These results demonstrate that u.v.-sensitive components localized in the posterior pole plasm are necessary for pole cell formation and gamete development. Since both the synthesis and the deployment of localized cytoplasmic determinants are controlled by the maternal genome, rescue experiments demonstrating the existence of localized cytoplasmic determinants can also be carried out using recipients with maternal effect mutations. Again, localization of the rescuing activity must be shown by testing cytoplasm taken from different regions of the donor.

Bioassays for subcellular and molecular fractions

Either ectopic transplantation or rescue experiments can be used as a bioassay to test subcellular or molecular fractions for determinant activity. Such fractions can be conveniently prepared from whole embryos once the localization of the active components *in vivo* has been established by cytoplasmic transplantation. Any fraction showing activity in the bioassay can be subdivided and the subfractions tested again. Under favourable circumstances, it might be possible to identify the molecular nature, size and other characteristics of the determinants under consideration.

Limitations to heterotopic transplantation and rescue bioassays

There are technical and principal limitations to what can be expected from heterotopic transplantation or rescue bioassays. The biological effect may be

obscured by loss of determinant activity in the fractionation process, the trauma and electrolytic imbalance caused by microinjection, improper integration of the transplanted material into the recipient, and other incidental problems. Principal limitations are encountered if more than one active component is required for the biological effect registered in the bioassay. This problem is unlikely to be encountered if a single mutation is rescued. However, the problem is more likely to occur in the rescue of an experimentally induced lesion or in a heterotopic transplantation assay. This is again illustrated by the case of germ cell determinants in *Drosophila*. Most of the activity rescuing pole cell formation was found in a ribonucleoprotein particle (RNP) fraction (Ueda & Okada, 1982). However, this fraction did not restore to the recipients the capability of forming gametes. Also, the fraction did not cause ectopic pole cell formation. The results suggest that (i) pole cell formation requires at least two factors – one u.v.-sensitive and one u.v.-resistant, (ii) both of these factors are localized at the posterior pole, (iii) the RNP fraction rescuing pole cell formation contains the u.v.-sensitive, but not the u.v.-resistant, factor, and (iv) the capability of forming gametes requires at least one more u.v.-sensitive factor in the posterior pole plasm. In a sense, Ueda & Okada (1982) were fortunate in that their bioassay detected a partial resuce, i.e. pole cell formation. Had the bioassay been strictly for fertility, they might have missed the activity of the RNP particle fraction.

Bioassays based on inhibition of specific molecules

Assays based on inhibition should identify each factor required for a biological response independently of how many cofactors are required. Antibodies have been used to interfere with a variety of processes *in vivo* (Scheer, this volume; Strome, this volume). The injection of anti-sense globin mRNA has been shown to inhibit the translation of coinjected globin mRNA in *Xenopus* oocytes (Melton, 1985, and this volume). The expression of the *Krüppel* gene in *Drosophila* embryos has been suppressed, i.e. the *Krüppel* phenotype has been generated, in genetically wild-type embryos, by injecting anti-sense RNA transcribed *in vitro* from cloned *Krüppel* cDNA (Rosenberg *et al.* 1985; Jäckle, this volume). Bioassays based on *in vivo* inhibition may be complicated by intracellular breakdown of the inhibitor, limited access of the inhibitor to its target molecule, and other constraints imposed by the cellular environment. It would seem that these difficulties could be overcome by combining a rescue or heterotopic transplantation bioassay with a pretreatment *in vitro* to selectively remove individual components from a biologically active mixture. A selective loss of activity would conclusively demonstrate that the removed component is necessary or sufficient, respectively, for the determinative event under study. The localization of RNA sequences or proteins thus identified could be tested directly by *in situ* hybridization or immunohistological techniques.

ABNORMAL ANTEROPOSTERIOR BODY PATTERNS IN DIPTERAN EGGS

Double abdomens, double cephalons and inverted embryos

Eggs of chironomid midges are most favourable experimental systems for analysing the determination of anteroposterior polarity, which is easily perturbed by experimental interference. This was first observed by Yajima (1960, 1964) with eggs of *Chironomus dorsalis*. After centrifugation or u.v. irradiation of eggs or early embryos, he observed the development of 'double cephalon' and 'double abdomen' patterns. The latter showed a replacement of cephalic, thoracic and anterior abdominal segments by a mirror image of a set of posterior abdominal segments (Fig. 1A,B). The double cephalons showed a mirror-image duplication of head structures, including labrum, eyes and stomodaeum, whereas thorax and abdomen were missing. Similar phenotypes were obtained with another chironomid, *Smittia* sp. (Kalthoff & Sander, 1968; Kalthoff, Hanel & Zissler, 1977). Centrifugation, especially in conjunction with u.v. irradiation, was also found to cause the development of 'inverted embryos' (Yajima, 1978; Rau & Kalthoff, 1980). These look normal except that they develop in reverse anteroposterior orientation so that their pole cells become trapped in the head. The double cephalon and double abdomen patterns observed in chironomids resemble some of the embryonic phenotypes of the *Drosophila* mutants *dicephalic* (Lohs-Schardin, 1982) and *bicaudal* (Nüsslein-Volhard, 1977). Similarly abnormal body patterns have been observed in other insects (see Kalthoff, 1983).

Symmetrical and asymmetrical double abdomens

Yajima (1970) has studied the prevalent double cephalon and double abdomen phenotypes histologically. He found that they were symmetrical in their external and internal structures, except for the pole cells which developed only in the posterior egg half. Percy, Kuhn & Kalthoff (1986) have analysed double abdomen and other abnormal body patterns in *Chironomus samoensis* by scanning electron microscopy. Most double abdomens were symmetrical with a plane of polarity reversal in a midpiece that seems to arise by fusion at the level of the fourth to second (Fig. 1A,B) abdominal segment. However, some double abdomens showed distinctly asymmetrical patterns where the anterior abdomens were shorter, and comprised fewer segments, than their posterior counterparts. The asymmetries ranged from moderate to extreme. Moderately asymmetrical double abdomens showed, for instance, a set of five abdominal segments joined to a set of seven abdominal segments. An extremely asymmetrical double abdomen is illustrated in Fig. 1C,D. The posterior part comprised all abdominal and thoracic segments as well as a cranial structure, the hypostome. The anterior part of this double abdomen consisted merely of the posterior half of the tenth abdominal segment showing the typical set of terminal structures. Such asymmetrical double abdomens involve the juxtaposition, at the plane of polarity reversal, of widely disparate segments. Such juxtapositions seem relevant to 'gradient' models of pattern specification which ascribe the determination of segment characters to the

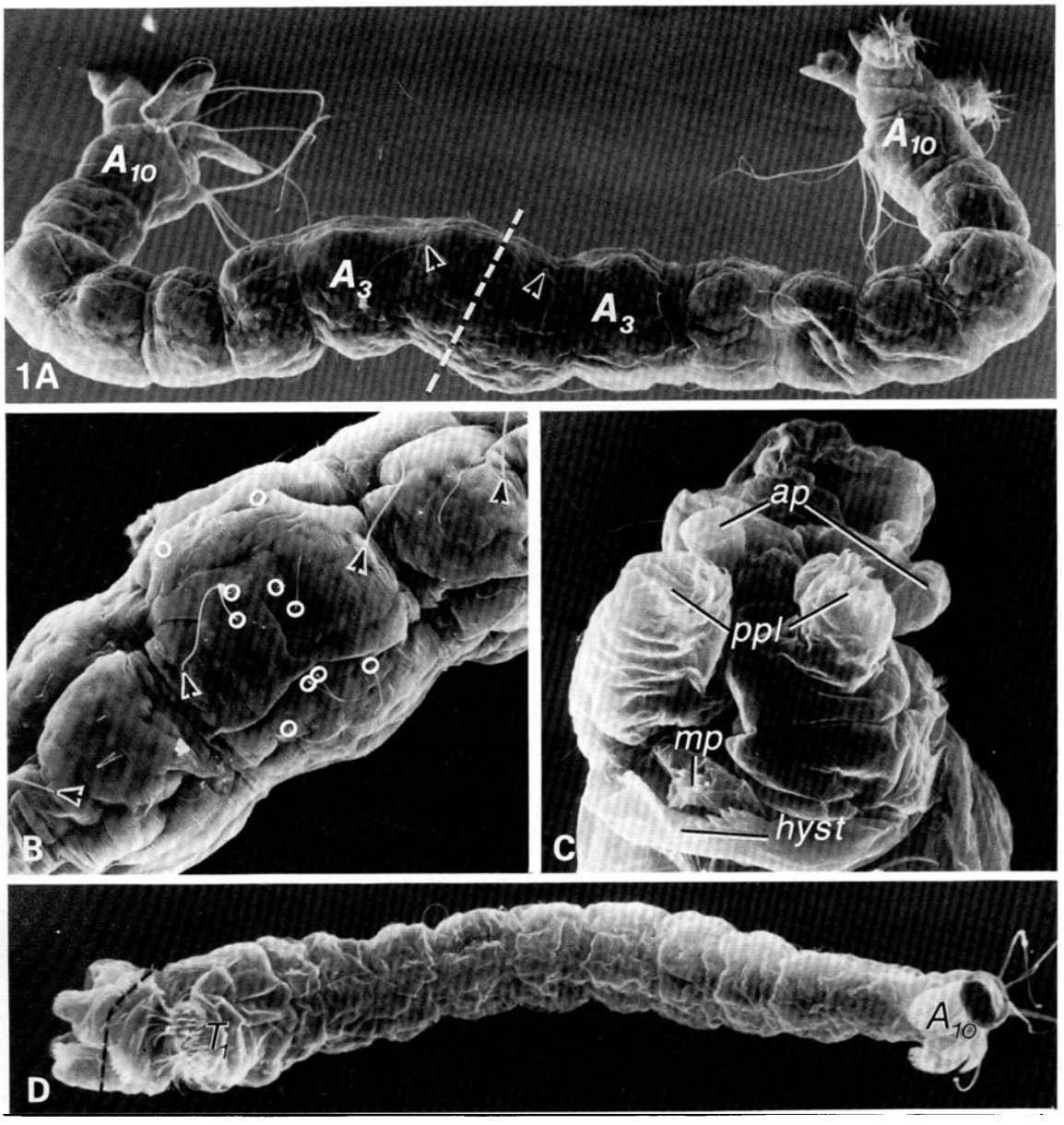


Fig. 1. Symmetrical and asymmetrical double abdomen larvae in *Chironomus samoensis*. Larvae were fixed for scanning electron microscopy with osmic acid (Percy *et al.* 1986). The specimen shown in panel A is symmetrical, with a plane of polarity reversal in a midpiece apparently formed by fusion at the level of abdominal segment A_2. Note long lateral setae at the posterior margins of segments A_3 to A_7, and at both margins of the midpiece. These are shown at higher magnification in panel B, which also illustrates, in a different specimen, the symmetrical origin of smaller setae relative to the plane of polarity reversal. An extremely asymmetrical double abdomen is shown in panels C and D. An apparently normal set of segments extends from the terminal abdominal segment (A_{10}) to the posterior part of the head including the hypostome (*hyst*) and a maxillary palp (*mp*). This set of segments is joined, at the plane of polarity reversal (hatched line), with the posterior half of an A_{10} segment. It carries the typical set of terminal abdominal stuctures including posterior prolegs (*ppl*), anal papillae, and rectum (above the anal papillae in panel C).

local concentration of a diffusible 'morphogen'. In terms of such models, the juxtaposition of widely disparate structures would translate into a steep discontinuity in the morphogen concentration which seems difficult to reconcile with diffusion.

Spontaneous double abdomen, a genetic variant of Chironomus samoensis

The symmetrical and asymmetrical double abdomen patterns described above have been obtained after u.v. irradiation of the anterior pole region of eggs and early embryos of various chironomids. We have also isolated what seems to be a mutant strain of *Chironomus samoensis* in which double abdomen patterns are formed spontaneously. We call this strain the *spontaneous double abdomen* (*sda*) strain as opposed to the normal (*N*) strain. Females of chironomid species deposit their eggs as clusters in a gelatinous matrix. A cluster contains a few dozen or several hundreds of eggs depending on species. A female seems to lay only one cluster in her lifetime. By inbreeding, the frequency of clusters with double abdomen embryos has increased to more than 60 % in the *sda* strain, whereas in the *N* strain such clusters are rare (less than 10 %). Also, the frequency of double abdomens embryos among the developing embryos of a given cluster ranges up to 100 % in the *sda* strain, but is usually very low in the *N* strain. By a reciprocal crossing experiment, we have determined that the *sda* trait in our *C. samoensis* strain is inherited maternally (Fig. 2). We assume that the *sda* trait is caused by a mutation in one or more genes expressed during oogenesis and involved in the synthesis or deployment of cytoplasmic components establishing the anteroposterior polarity of the egg. We consider it very unlikely that the *sda* trait is caused by environmental factors because the *sda* and *N* strains are kept side-by-side in the same room using the same culture conditions.

There is a striking similarity between the *sda* variant of *Chironomus samoensis* and the *bicaudal* mutant of *Drosophila melanogaster* (Nüsslein-Volhard, 1977). In both cases, the embryonic phenotype is controlled strictly by the maternal genotype. Also, the range of *bicaudal* phenotypes corresponds almost exactly to the symmetrical double abdomens, asymmetrical double abdomens and truncated embryos observed in *C. samoensis* (Percy *et al.* 1986). The extensive similarities suggest that the mutations in the two species interfere with very similar developmental programmes which may utilize homologous gene products.

U.v. irradiation and centrifugation of Chironomus samoensis *eggs*

Previous experiments with eggs of *Smittia* sp. and other chironomids had shown that u.v. irradiation of the anterior pole region caused the development of double abdomen embryos, whereas centrifugation led to the formation of double cephalons, double abdomens and inverted embryos (see Kalthoff, 1983). Experiments were carried out to test how these properties would be affected by the *sda* mutation of *Chironomus samoensis*. Anterior u.v. irradiation of eggs from the *sda*

strain induced double abdomen formation at much higher frequencies as compared to eggs from the *N* strain (Fig. 3). Centrifugation of eggs with zero spontaneous double abdomen frequency was followed by the development of normal embryos (65 %), double cephalons (20 %) and double abdomens (15 %). Eggs from clusters with an average spontaneous double abdomen frequency of 3 %, after centrifugation, developed mostly into double abdomens (more than 80 %) with only a few double cephalons. Virtually all double abdomens, and no double cephalons, were obtained with eggs from clusters with an average spontaneous double abdomen frequency of 20 % (data not shown). Taken together, the results indicate that the *sda* mutation causes a decrease in the ability to form double cephalons and a marked increase in the disposition to form double abdomens upon u.v. irradiation or centrifugation. The simplest interpretation would be the hypothesis that the *sda* strain is deficient in the same components that are inactivated during the u.v. induction of double abdomens and whose shift by centrifugation causes double cephalon formation. However, these components may also act in a synergistic fashion with the product(s) affected by the *sda* mutation.

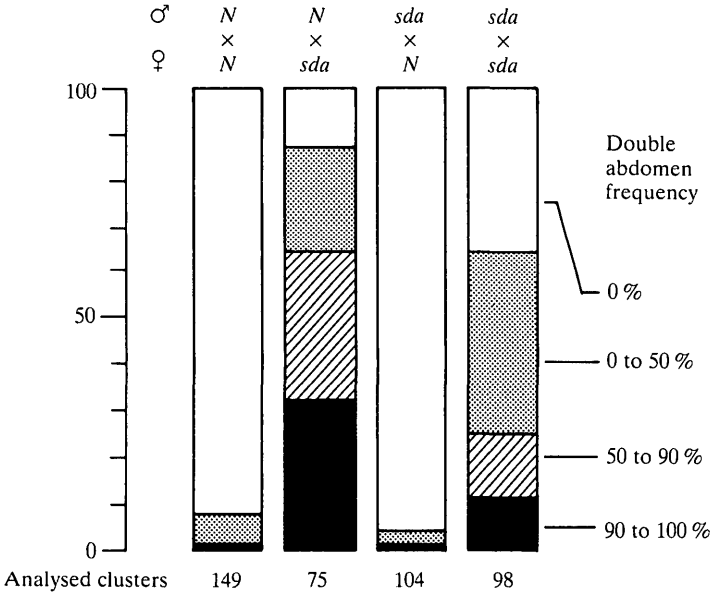


Fig. 2. Reciprocal crossing experiment showing the maternal inheritance of the *sda* (*spontaneous double abdomen*) trait in *Chironomus samoensis*. Males and females from our mutant (*sda*) and wild-type (*N*) strain were put together in mating cages. Egg clusters deposited in these cages were analysed and classified according to the percentage of developing embryos (i.e. embryos reaching the germ anlagen stage) and according to the double abdomen frequency (i.e. percentage of double abdomens among the developing embryos). Each cluster contains several hundred embryos; a female lays only one cluster. The data indicate very clearly that the double abdomen frequency is controlled by the female parent.

DEFINITION AND PROPERTIES OF ANTERIOR DETERMINANTS

Determination of anteroposterior polarity

The experimental procedures causing double abdomen formation include centrifugation, anterior u.v. irradiation, and injection of RNase at the anterior pole (see Kalthoff, 1983). The common effect of these treatments seems to be the inactivation, or displacement, of components required for the development of anterior structures. Since these components appear to be localized (see below), we have termed them anterior determinants. The egg components inactivated during u.v. induction of double abdomens are probably identical to those components whose shift by centrifugation causes double cephalon formation (Kalthoff, Rau & Edmont, 1982). It is apparent that anterior determinants are not part of a 'mosaic' of many qualitatively different components, each of which would specify the

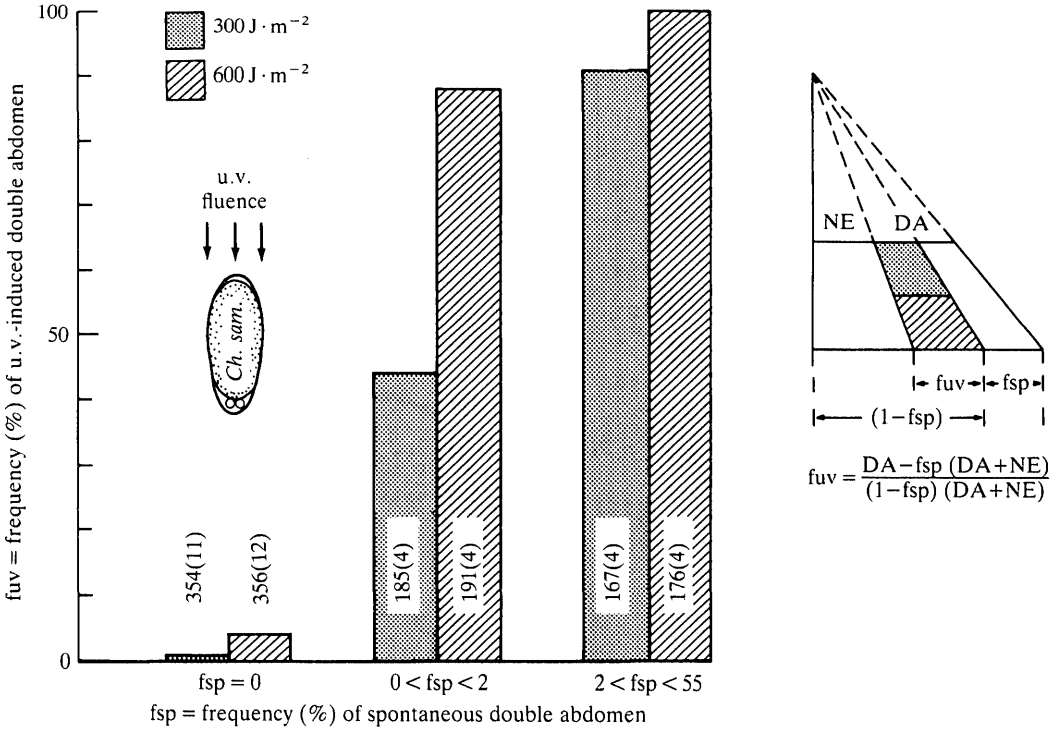


Fig. 3. Effect of spontaneous double abdomen frequency (fsp) on the frequency of u.v.-induced double abdomens (fuv) in *Chironomus samoensis*. fsp is the frequency of spontaneous double abdomens among the developing embryos of an egg cluster. fuv is the frequency of u.v.-induced double abdomens surviving after u.v. irradiation. This value is corrected for the contribution of fsp as indicated. Egg clusters were collected from the *N* strain or *sda* strain, and a sample of at least 100 eggs was set aside as an untreated control to determine fsp. If clusters with fsp values between 2% and 55% were used, u.v. irradiation with doses of 300 or 600 J m^{-2} was sufficient to cause double abdomen formation in virtually all embryos. The same treatment caused virtually no double abdomen formation when applied to eggs from clusters without any spontaneous double abdomens(fsp = 0). U.v. irradiation of clusters with very low spontaneous double abdomen frequencies ($0 < fsp < 2\%$) still boosted the fuv values to 45% (300 J m^{-2}) and 90% (600 J m^{-2}).

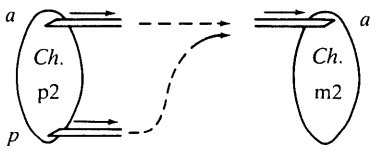
character of one segment. Rather, anterior determinants seem to be involved in a more general 'decision' between the development of anterior *versus* posterior elements of the body pattern. There is also no compelling evidence that anterior determinants would form a 'gradient' which determines different elements of the body pattern according to its local concentration. Rather, anterior (and possibly posterior) determinants may set up an initial polarity, causing later interactions between nuclei and cells to produce eventually the longitudinal body pattern.

Localization of anterior determinants

Results from previous experiments have suggested that anterior determinants have a degree of localization to the anterior pole. The efficiency of u.v. induction of double abdomen, as measured by the dose per target area required to produce a given yield of double abdomens, generally decreased from anterior to posterior. Microbeam experiments have indicated that, in newly laid *Smittia* eggs, a major fraction of anterior determinants is located in a target area of about 20 μm diameter right behind the anterior pole (Ripley & Kalthoff, 1983). This area is occupied by a cone of cytoplasm (Zissler & Sander, 1973) which appears to be derived from nurse cell cytoplasm transferred to the oocyte during oogenesis. Moreover, the ability of embryos to form double cephalons upon centrifugation is severely diminished after anterior u.v. irradiation but not after posterior irradiation (Kalthoff *et al.* 1982).

A bioassay developed recently in our laboratory has enabled us to test directly the localization of anterior determinants. Eggs from the *sda* strain of *Chironomus samoensis* were programmed for double abdomen development by anterior irradiation with a low u.v. dose. These embryos were subsequently microinjected with cytoplasm from unirradiated donors derived from the *N* strain. The transplanted cytoplasm was taken from either the anterior or the posterior pole region of the donor embryos and was delivered to the anterior pole region of the recipient embryos. The frequency of normal embryos developing in surviving recipients depended strongly on the donor site from which the transplanted cytoplasm was taken (Fig. 4). Anterior cytoplasm had a strong 'rescue' effect, i.e. it caused a significant increase in the frequency of normal embryos as compared to the uninjected control group. By contrast, the injection of posterior donor cytoplasm further enhanced the development of double abdomen at the expense of normal embryos. The latter effect was also observed after injection with water or buffer (Figs 5, 6); it appears to result from the trauma of the microinjection which may release endogenous RNases (Schmidt, Zissler, Sander & Kalthoff, 1975).

The rescue experiments were hampered by the variable response of *Chironomus* clusters to u.v. irradiation (Fig. 3). In Fig. 4, we have broken down the results from our rescue experiments according to the frequency of double abdomen embryos in the irradiated but uninjected control group. When this group showed double abdomen frequencies between 20 % and 80 %, the results were the clearest. With zero to 20 % double abdomens in the control, the margin for rescue by anterior cytoplasm was necessarily small, but the effect of injecting posterior



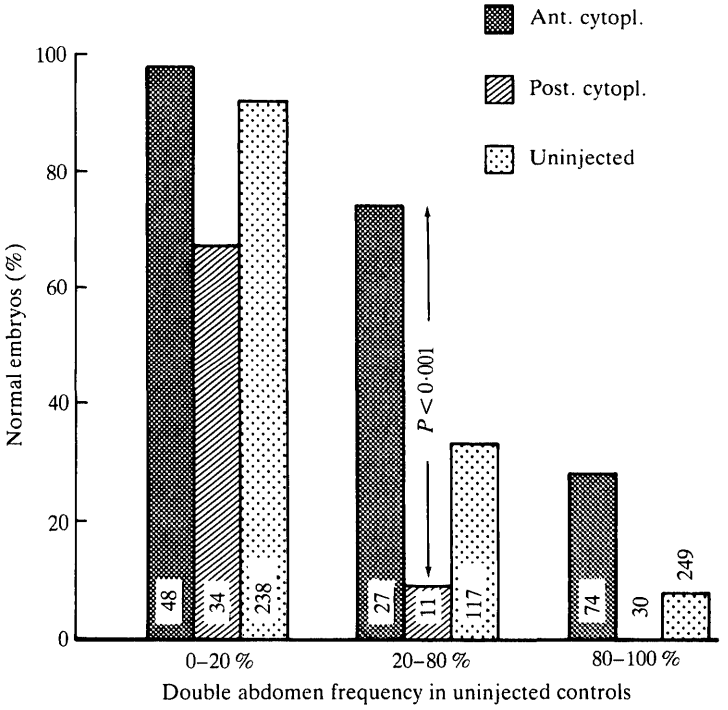


Fig. 4. Transplantation of cytoplasm from anterior or posterior pole regions of *Chironomus samoensis* embryos to the anterior pole region of embryos from the same species. Recipient and donor embryos were lined up on a cover slip in a drop of 0·3% agarose and 0·2% gelatine. The medium was allowed to dry so that the embryos became stuck to the glass. Embryos were then kept under halocarbon oil for the duration of the injection. The donor embryos were at the two-pole-cell stage (p2), or slightly beyond, when their cytoplasm was removed. The recipients, which had been irradiated anteriorly after pole cell formation with u.v. doses between 300 and 900 J m^{-2}, were at a late nuclear migration (m2) stage at the time of transplantation. Cytoplasm was transplanted using bevelled glass needles of about 3 μm outer diameter mounted on a micromanipulator. The volume of the transplanted material was estimated to be 1–2% of the egg volume. After injection, embryos were kept in a salt solution (0·7% NaCl, 0·3% KCl, 0·02% $CaCl_2$) and allowed to develop at room temperature in light-proof boxes. The body pattern of the recipient embryos was determined at the germband stage when the head capsule and anal papillae are clearly visible under the stereomicroscope. These structures, along with the overall morphology of the embryos, allowed us to distinguish unequivocally between normal embryos and double abdomens. In fact, many of the 'rescued' normal embryos hatched and seemed normal in every respect. Within the double abdomen class, we found a higher proportion of asymmetrical specimens among the injected embryos (an average of 20%) than among the uninjected controls (less than 5%). The numbers at the base of each column in this diagram indicate the numbers of surviving embryos. The percentage of identifiable double abdomens and normal embryos from all injected embryos in an experiment is referred to as the average survival rate. The average survival rate in this experiment was 58%. Since the frequency of double abdomens in the uninjected controls differed widely, the results are shown in three subsets (see text). The injection of anterior cytoplasm caused a significant increase in the frequency of normal embryos at the expense of double abdomens, as compared to results after injection of posterior cytoplasm and as compared to the uninjected control embryos.

cytoplasm was showing quite clearly. With 80 % to 100 % double abdomens in the control group, the rescue effect was clear but the effect of posterior injection was necessarily limited.

The rescue experiments described here show clearly that anterior determinants in *Chironomus samoensis* eggs are localized anteriorly. The results therefore indicate that anterior determinants are specific components and not ubiquitous ones such as ribosomes. We wish to point out that the injected volume of cytoplasm was small, probably between 1 % and 2 % of the egg volume. This shows again how easily the 'decision' between forming the anterior *versus* the posterior elements of the body pattern can be 'tipped' in the chironomid embryos, especially in the mutant and u.v.-irradiated embryos used as recipients in these experiments.

Molecular nature of anterior determinants

We have used the 'rescue' bioassay to determine whether anterior determinants in *Chironomus* eggs contain RNA moieties. Injection of total egg RNA caused a significant 'rescue' effect over the level of the uninjected control embryos and, even more so, over the level of recipients injected with buffer (Fig. 5). Total RNA showed clearly more rescuing activity than poly(A)$^-$RNA prepared from the same batch and injected at the same concentration. Poly(A)$^+$RNA prepared from the same batch, although injected at a tenfold lower concentration, showed the highest rescuing activity. These results indicate that all or most of the rescuing activity is associated with the poly(A)$^+$ fraction. The low activity still found in the poly(A)$^-$ fraction may result from incomplete poly(A)$^+$ selection. Alternatively, some poly(A)$^-$RNA may be able to substitute for poly(A)$^+$RNA.

The rescuing activity of egg RNA confirms the conclusion drawn from earlier experiments. The photoreversibility of the u.v. induction of double abdomens by light of longer wavelengths has been ascribed to light-dependent repair of u.v. damage to nucleic acids (Kalthoff, 1971). The action spectrum for u.v. induction of double abdomens in *Smittia* suggested that a nucleic acid–protein complex acts as the effective target of u.v. (Kalthoff, 1973). It has also been shown that application of RNase, or reconstituted RNase fragments, to the anterior pole of *Smittia* causes double abdomen formation (Kandler-Singer & Kalthoff, 1976). However, none of our experiments excludes the possibility that, in addition to RNA-containing components, other localized, but u.v.-insensitive, components are required to determine the anterior pole. The association of most of the rescuing activity with poly(A)$^+$RNA suggests, although it does not prove, the involvement of mRNA. This hypothesis is compatible with the light-dependent repair of mRNA extracted from u.v.-irradiated *Smittia* eggs and translated *in vitro* (Jäckle & Kalthoff, 1980).

Activity of anterior determinants after heterospecific transplantation

The determinations of anteroposterior polarity in chironomid and in *Drosophila* eggs have many similarities and may rely on the utilization of homologous

genes. In fact, the observation of double abdomen 'monsters' in other species suggests that the underlying morphogenetic programme may be common to the dipterans and possibly other insect orders. However, the failure to phenocopy the *Drosophila bicaudal* mutant with u.v. (Bownes & Kalthoff, 1974) or RNase

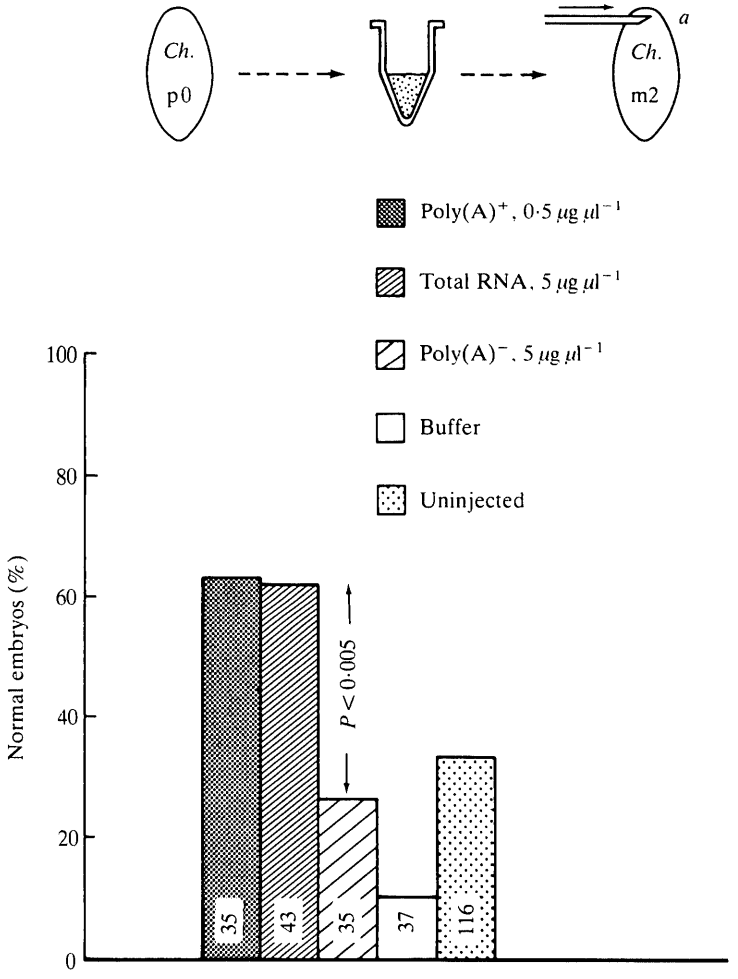


Fig. 5. Microinjection of total RNA and RNA fractions from unirradiated *Chironomus samoensis* eggs into the anterior pole region of *C. samoensis* embryos. The general procedures were as described in the legend to Fig. 4. Total RNA was extracted from newly laid eggs with SDS/proteinase K. From part of this batch, poly(A)$^+$RNA and poly(A)$^-$RNA were prepared by oligo(dT) affinity chromatography. The fractions were injected, at the concentrations indicated, in a buffer containing 25 mM-KCl, 2·5 mM-Pipes, 0·5 mM-EDTA, 10 mM-DDT and 2 i.u. μl^{-1} RNasin. Injection of either total RNA or poly(A)$^+$RNA caused a significant increase in the frequency of normal embryos at the expense of double abdomens. Injection of poly(A)$^-$RNA had a small 'rescuing' effect (26% *versus* 11% normal embryos) as compared to the embryos injected with buffer. Injection of either poly(A)$^-$RNA or buffer increased the frequency of double abdomens, at the expense of normal embryos, in comparison to the uninjected control embryos. The average survival rate of injected embryos in this experiment was 54%.

(Zalokar, 1982), as well as other observations, suggests that these programmes may have become modified during evolution.

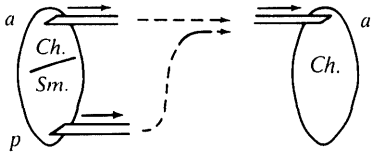
The availability of a bioassay for anterior determinants should allow us to test directly the anterior determinant activity of cytoplasm or RNA obtained from other species at different stages of development. So far, we have tested only cytoplasm obtained from eggs of *Smittia* sp., a representative of the subfamily Orthocladiinae within the family Chironomidae. The cytoplasm was injected into eggs of *Chironomus samoensis*, a representative of the subfamily Chironominae. Anterior cytoplasm from both *C. samoensis* and *Smittia* sp. clearly had a rescuing effect (Fig. 6). After injection of posterior cytoplasm from either species, the frequency of normal embryos was close to the level observed in uninjected control embryos. This is in contrast to the results of the experiments shown in Fig. 4, in which injection of posterior cytoplasm caused the formation of fewer normal embryos, and more double abdomens, than observed in the uninjected controls. Injection of buffer led to an increase in the frequency of double abdomens, at the expense of normal embryos, in comparison to the uninjected controls. This is in accord with the control experiments carried out as part of the test of RNA fractions (Fig. 5). Taken together, the results suggest that the puncturing associated with the injection procedure enhances double abdomen formation. The enhancement appears to be offset, to a variable extent, by anterior determinants at subcritical levels of activity or by other components present in posterior cytoplasm.

CONCLUDING REMARKS

In dipteran eggs, there is evidence for three sets of cytoplasmic determinants. One of these sets comprises the pole cell and germ cell determinants used in this review to illustrate the use of heterotopic transplantation and rescue bioassays. Another set of cytoplasmic components is involved in the determination of the anteroposterior polarity as outlined above. A third set of cytoplasmic factors is involved in setting up the dorsoventral body pattern of *Drosophila* embryos. These components are affected by maternal effect mutations in at least 10 loci (Nüsslein-Volhard, 1979; Anderson, Bokla & Nüsslein-Volhard, 1985). Most of the mutations have a dorsalizing effect; the progeny lack structures normally derived from ventral blastoderm cells. Seven of the mutants can be rescued by microinjection of cytoplasm, and six of these by injecting RNA (Anderson & Nüsslein-Volhard, 1984). However, only some of the gene products identified by these mutations seem to be localized.

Most of the maternal effect mutations or experimental treatments reported interfered with one set of determinants, but not with the others. For instance, double abdomen embryos have pole cells only in the posterior abdomen, although otherwise the anterior abdomen seems to be a perfect mirror image of its posterior partner (Fig. 1). Conversely, we have observed the formation of cells with distinct pole cell morphology at both the anterior and the posterior pole of *Chironomus*

embryos which otherwise developed a normal body pattern (Elbetieha & Kalthoff, unpublished data). For the most part, therefore, the cytoplasmic determinants of pole cells, of anteroposterior polarity, and of dorsoventral polarity seem to be synthesized and deployed independently of each other. However, there are several *Drosophila* mutants in which both pole cell formation and the morphology



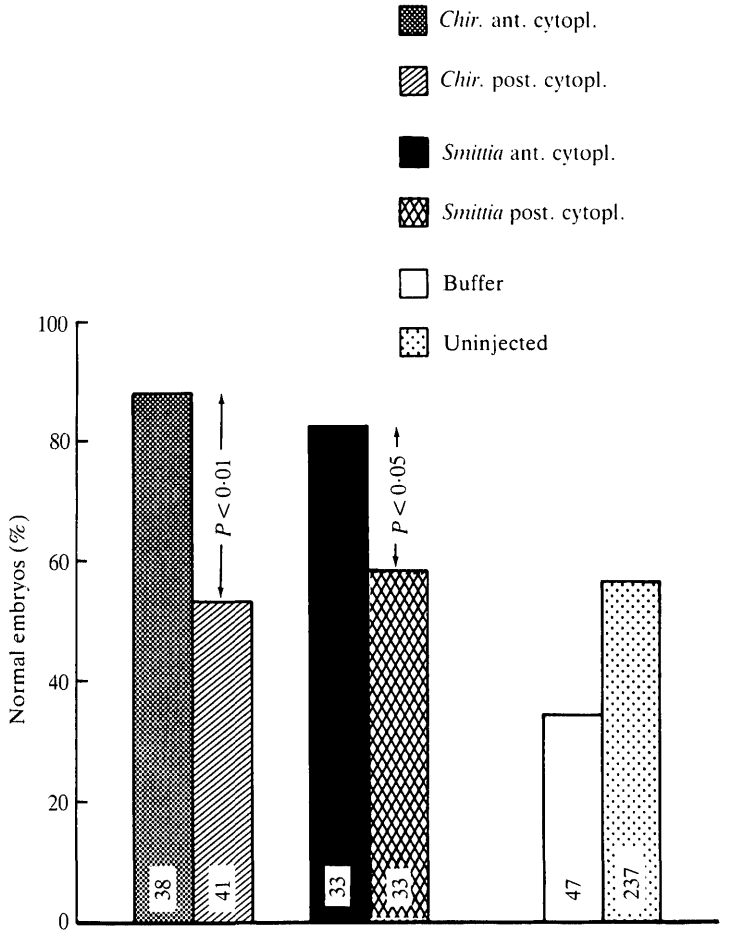


Fig. 6. Heterospecific transplantation of anterior or posterior cytoplasm from embryos of *Smittia* sp. to the anterior pole of embryos from *Chironomus samoensis*. For comparison, homospecific transplantation of cytoplasm from *C. samoensis* embryos was carried out as described in the legend to Fig. 4. Injection of anterior cytoplasm from either species had a rescuing effect which was significantly stronger than observed after injection of posterior cytoplasm. The average survival rate of injected embryos in this experiment was 68%.

of abdominal segments are affected (Boswell & Mahowald, 1985; Nüsslein-Volhard, this volume). In these cases, it seems possible that the gene products may be involved in the synthesis and/or deployment of both pole cell and posterior somatic determinants.

The ways in which determinants exert their effects on pattern formation appear to be quite diverse. While the pole cell determinants seem to act in a strictly local fashion, determinants of anteroposterior and dorsoventral polarity have wide-ranging effects. Some of these may be described in terms of 'gradients' of diffusible morphogens or sequential inductive interactions between nuclei or cells (Meinhardt & Gierer, 1980). However, it is becoming clear that the cellular and molecular mechanisms underlying the specification of the body pattern are very complex. It is hoped that the similarities between species observed so far will be indicative of a larger extent of homologies between the gene products involved. This should allow us to combine the insight from genetic studies in *Drosophila* with the conclusions drawn from experimental results obtained with other species.

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