The EMBO Journal Vol.2 No.10 pp.1695 – 1699, 1983

Partial rescue of *dorsal*, a maternal effect mutation affecting the dorso-ventral pattern of the *Drosophila* embryo, by the injection of wild-type cytoplasm

Pedro Santamaria and Christiane Nüsslein-Volhard*

Centre de génétique moleculaire du CNRS, 91190 Gif sur Yvette, France, and Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Spemannstr. 37-39, D-7400 Tübingen, FRG

Communicated by C. Nüsslein-Volhard
Received on 31 May 1983; revised on 12 July 1983

Mutant alleles at the maternal effect locus *dorsal* cause a dorsalization of the *Drosophila* embryo. In extreme mutants, the embryos develop exclusively structures which derive from the dorsal-most region in normal eggs, in less strong phenotypes in addition to dorsal structures, structures normally derived from a dorso-lateral to lateral egg region are formed. Injection of cytoplasm from wild-type embryos into mutant embryos partially restores the dorso-ventral pattern in that injected embryos develop additional structures never formed in uninjected control embryos or embryos injected with mutant cytoplasm. The phenotype of injected embryos resembles that of weaker alleles at the *dorsal* locus indicating that the wild-type cytoplasm partially rescues the mutant phenotype. The response of the mutant embryos is restricted to the site of injection and occurs only when cytoplasm is injected into the ventral and not into the dorsal side of mutant embryos. The rescuing activity appears to be equally distributed in cleavage stage wild-type embryos, whereas, in syncytial blastoderm embryos, cytoplasm from the ventral side is about twice as effective as that taken from the dorsal side.

Key words: pattern formation/*dorsal*/cytoplasmic rescue/maternal effect gene

Introduction

In *Drosophila*, the establishment of the anterior-posterior and dorso-ventral embryonic axes is initiated during oogenesis under the control of the maternal genome. Maternal effect mutants which abolish the polarity of the developing embryo indicate that morphogenetic substances necessary for normal embryonic development are placed into the egg before fertilization. Mutants at one such maternal effect locus, *dorsal*, show an extreme phenotype in which the embryo is completely dorsalized: at all positions along the dorso-ventral egg axis structures are formed which derive from the dorsal-most egg region in normal development. Weaker phenotypes, revealed by hypomorphic alleles, and the dominant temperature-sensitive phenotype, show partially dorsalized embryos in which the dorsal and lateral anlagen are extended towards ventral at the expense of ventral anlagen. These phenotypes suggest that the *dorsal* gene is involved in a system controlling the pattern of spatial differentiation along the entire dorso-ventral egg axis. The coordinated shift and expansion of dorsal and lateral anlagen on the fate map towards the ventral side of the egg observed in weak phenotypes suggest that a gradient mechanism is involved in which distinct morphogenetic fates are determined by the concentration rather than the quality of an effector molecule (Nüsslein-Volhard, 1979a, 1979b; Nüsslein-Volhard *et al.*, 1980).

*To whom reprint requests should be sent.

Recent genetic data (unpublished) indicate that *dorsal* is one of several maternal effect genes which participate in the process of determining position along the dorso-ventral egg axis. To distinguish between each of these genes and identify their functions, a thorough analysis of the genetics and the embryology of the mutants and finally the biochemistry of the gene products must be performed (for review, see Anderson and Nüsslein-Volhard, 1983). The *dorsal* gene is a strictly maternal gene and the *dorsal* phenotype is not influenced by the genotype of the fertilizing sperm. *Dorsal* does not affect traits of the fly other than embryonic pattern formation like viability, adult morphology, fecundity and male fertility and thus must be expressed specifically during oogenesis (Nüsslein-Volhard, 1979a). Temperature shift experiments with a temperature-sensitive *dorsal* phenotype indicate, on the other hand, that the phenotype can still be influenced after the egg is laid (unpublished observations). This means that the process in which the *dorsal* gene product is involved is not completed during oogenesis but extends through the first hours of embryogenesis. Here we show that the morphogenetic defects in *dorsal* embryos can be partially rescued by the injection of cytoplasm from wild-type embryos.

Results

The dorsal phenotype: criteria for rescue of mutant embryos

Embryos produced by females homozygous for strong *dorsal* alleles (e.g., dl^1) develop a yolk-filled tube of hypoderm covered by the fine hairs characteristic for the dorsal side of wild-type larvae. Cuticular preparations show only the structures normally derived from a dorsal position in the embryonic fate map (Lohs-Schardin *et al.*, 1979). No ventral denticle bands are developed. Of the head structures, only the median tooth or labrum is frequently present, but other structures including the sense organs of the antenno-maxillary complex are always lacking. At the posterior end of the *dorsal* embryo, a set of rudimentary posterior spiracles with the characteristic crown of long hairs frequently develop (Figure 1d). These spiracles, however, always lack the tracheal endings, the Filzkörper, a structure which is characterized by its opaque, slightly fluorescent texture (Figure 1a). The tuft, a clump of denticles normally located posterior to the anal plates, is frequently found. Trachea are lacking altogether, and also the sense organs of the thorax, the Keilin's organs and the black sense organs, are never found.

In weak alleles at the same locus (e.g., dl^2) a progressive appearance of further cuticular structures is observed. These derive, in normal embryos, from a more lateral position of the fate map (Lohs-Schardin *et al.*, 1979). Useful markers are the Filzkörper in the posterior region, the antenno-maxillary sense organs of the head (Figure 1e) and the black sense organs of the thorax in the anterior region of the larva, as well as the tracheal branches throughout the larva. In even weaker phenotypes, in addition to these structures, ventral denticle bands appear which are, however, greatly reduced in width compared with the prominent denticle bands covering the ventral side of a normal larva. Genetic evidence based on gene dosage studies involving deficiencies of the *dorsal* gene

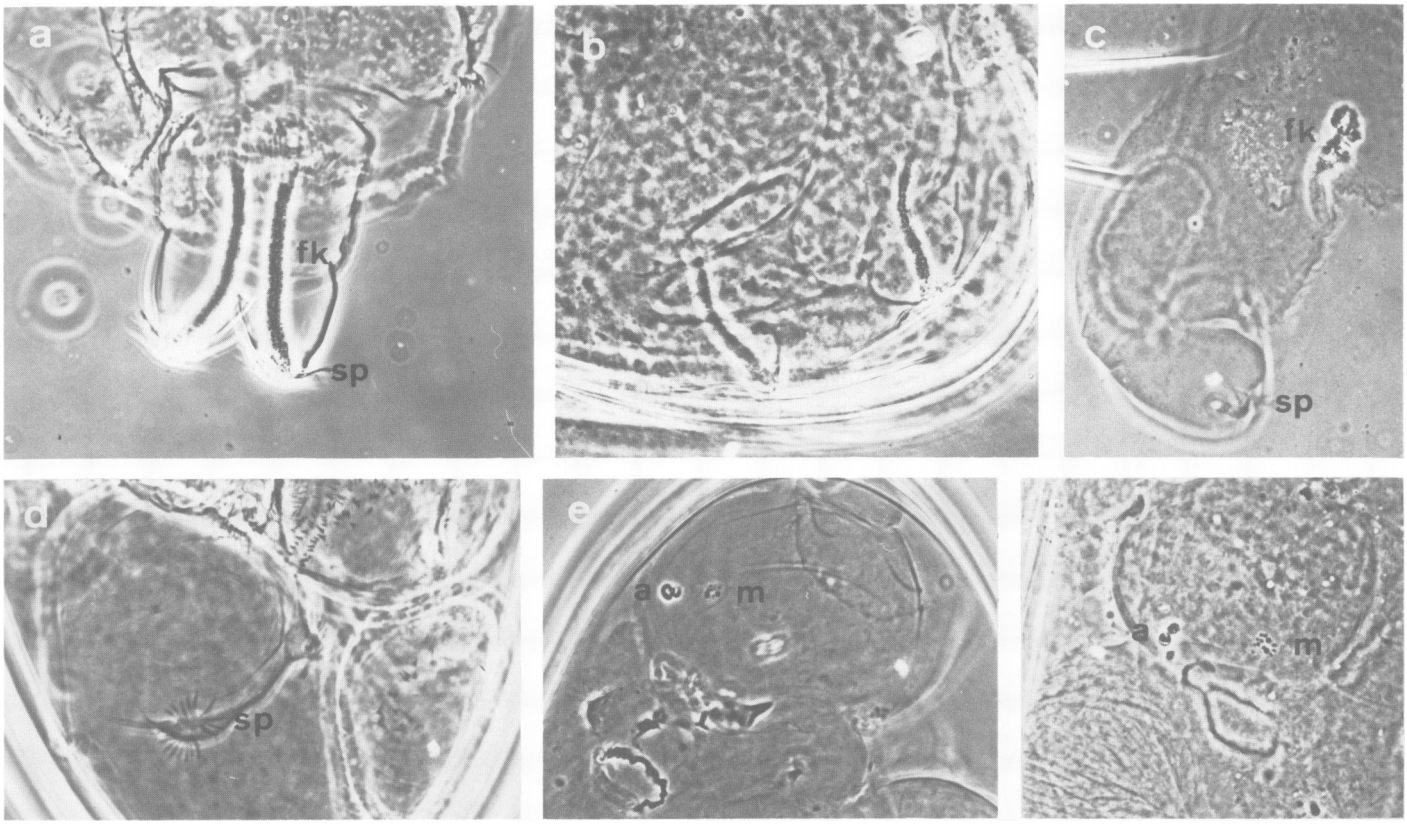

Fig. 1. Details from the cuticle of *dorsal* embryos injected with wild-type cytoplasm. **(a)** Posterior spiracles including the tracheal endings, the Filzkörper, of a wild-type larva. **(b)** Filzkörper connected to posterior spiracles in a dorsal embryo injected into the posterior ventral side with 200 pl of wild-type cytoplasm. **(c)** Small patch of Filzkörper material not connected with spiracle in an embryo injected posterior ventrally. **(d)** Uninjected dorsal embryo showing rudimentary spiracles but no Filzkörper. **(e)** Head sense organs in a *dorsal*^{2} embryo. **(f)** Head sense organs in an embryo injected mid-ventrally with wild-type cytoplasm. a = antennal organ, fk = Filzkörper, m = maxillary organ, s = spiracle.

indicate that the strong phenotype is caused by lack of function at the *dorsal* locus (null- or amorphic phenotype), while in the weak alleles some residual gene activity remains (Nüsslein-Volhard, 1979b).

The injection procedure

Cytoplasm was taken from donor embryos laid by females homozygous for the wild-type copy of the *dorsal* gene (+/+) and injected into embryos derived from dl^1/dl^1 homozygous females. Since we expected the dorsal gene to exert its function very early during embryogenesis, both donor and recipient embryos were chosen in the early cleavage stage, before pole cell formation, except when otherwise stated. Cytoplasm was taken from a cortical egg region (see Figure 2) which in cleavage stage embryos does not yet contain nuclei. Similar results were obtained if cytoplasm was taken from unfertilized eggs (data not shown). Injected embryos were allowed to develop for 2 days, fixed and cleared and scored for the appearance of cuticular structures never seen in uninjected control embryos. Such structures were indeed found in a fraction of the injected embryos. Figure 1b shows details from the cuticle of a *dorsal* embryo injected at the posterior ventral side with wild-type cytoplasm: a pair of well developed Filzkörper and adjacent tracheal branches have formed. In Figure 1f sense organs resembling those of the antennomaxillary complex are shown, which developed in an embryo injected in the ventral side in the middle egg region. Since Filzkörper material could also be unequivocally identified in very small patches (Figure 1c), this structure was found to be

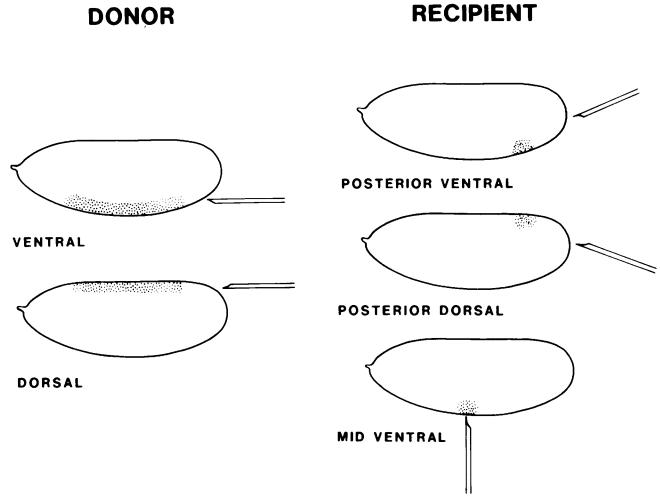

Fig. 2. Position of transplanted cytoplasm in donor and recipient embryos. The figure also indicates the orientation and point of entry of the needle

the most useful and reliable indicator for rescuing activity. Except for one case, ventral denticle bands were never found in injected embryos, but frequently tracheae and sometimes black sense organs of the thorax.

Reproducibility of assay

In the injection experiments, cytoplasm from a large region of one wild-type donor embryo was injected into several reci-

pient embryos. The amount of injected material was not controlled in most of the experiments, and depended somewhat on the state of dryness of the injected embryos. Nevertheless, we found that the frequency of rescued embryos for a particular donor-host combination was fairly reproducible from experiment to experiment. In four separate series, performed within a period of more than 1 year, cytoplasm was taken from the ventral region of early cleavage wild-type embryos and injected into the posterior ventral side of *dorsal* embryos (see Figure 2). A total of 304 injected embryos developed of which 27% had developed Filzkörper material. The success rates in the individual experiments were 27% (36), 37% (86), 24% (125) and 19% (57).

Dosage dependence of rescue

The partial rescue of injected mutant embryos depends on the wild-type activity of the *dorsal* gene in the maternal genotype of donor eggs. If embryos from *dorsal* females are taken as donors, no rescue is observed (Table I) compared with the 24% rescued embryos injected with eggs from wild-type females. Further, the degree of rescue, as measured by the frequency of injected embryos developing Filzkörper material, depends on the amount of injected material. In most of the experiments described in this paper, the exact amount of injected material was not measured, nor was an attempt made to always inject the same amount. We feel confident, however, that on average this amount was reproduced from experiment to experiment. A set of eggs were injected with as much cytoplasm as possible and significantly more embryos showed signs of rescues than with the normal amount injected. In a further series of experiments, the amount of injected cytoplasm was controlled and calibrated by measuring the diameter of an equal drop injected into the oil. Injection of 200 pl (corresponding to ~2% of the egg volume (10 nl) led to the rescue of more than twice as many embryos than injection of a fifth of that volume (Table I).

It is difficult to quantify the degree of rescue in individual embryos, whereas the frequency of rescued embryos remains a reliable criterion. We noted, however, that in embryos injected with the larger volume of cytoplasm the rescued structures frequently approached a normal morphology (Figure 1b). In these series, embryos with two separated well organized tracheal endings were common, while in the experiments with the normal (smaller) amount of cytoplasm, the Filzkörper material often was distributed in a rather disorganized patch, often not even connected to spiracles (see Figure 1c).

Dependence of rescued structures on site of injection

When cytoplasm was injected into the posterior ventral region of *dorsal* embryos, 28% of the embryos developed Filzkörper, but in none of 176 developed embryos were antenno-maxillary sense organs found. Conversely, mid-ventral injection leads to the appearance of antenno-maxillary sense organs, black organs of the thorax as well as trachea, but Filzkörper were not seen in these embryos (Table II). Thus, it appears that the nature of the rescued structures depends rather strictly on the site of injection with respect to the position along the anterior-posterior egg axis, in that posterior injections lead to the appearance of posterior, and anterior injection to anterior structures in the recipient embryo. This result indicates that the rescuing activity in the wild-type cytoplasm does not spread immediately upon injection but exerts a rather localized effect on the area close to the site of injection.

Injection into the posterior dorsal (in contrast to the posterior ventral) side of mutant embryos does not lead to a significant fraction of rescued embryos (Table II). Thus, although *dorsal* embryos show no signs of dorso-ventral polarity in both the differentiated embryo and during early stages of gastrulation, the dorsal and ventral sides of the embryos clearly behave differently with respect to their response upon injection of wild-type cytoplasm.

Spatial distribution of rescuing activity in donor eggs

Since *dorsal* embryos show no signs of dorso-ventral polarity, it was of interest to see whether the rescuing activity was unequally distributed in wild-type embryos. Cytoplasm was taken from either the dorsal or ventral side of early cleavage wild-type embryos and injected into the posterior ventral region of *dorsal* embryos of the same age. Table III shows that there is no difference in rescuing activity sug-

Table I. Dosage-dependence of rescue

Maternal genotype of donor[a]	Amount injected[a]	Number of recipient embryos			Rescue %
		Total	Developed	Rescued[c]	
1 *dl/dl*	Normal	210	133	0	0
2 +/+	Normal	176	125	30	24
3 +/+	Above normal	108	59	25	42
4 +/+	40 pl	109	60	9	15
5 +/+	200 pl	131	46	19	41

[a]The genotype of *dl/dl* ♀ ♀ was *dl cn sca/dl cn sca*, and of +/+ ♀ ♀, *y f*^{36a}; *mwh jv*. Cytoplasm was taken from the ventral side of cleavage stage embryos.
[b]In experiments 2 and 3 of this series the exact amount was not measured but corresponded approximately to that of exp. 4 (normal) and 5 (above normal) respectively. 40 pl corresponds to ~0.4% of the total egg volume (10 nl).
[c]Recipient embryos were injected in the posterior ventral side in all experiments. The criterion for rescue was the development of Filzkörper.

Table II. Dependence of rescued structures on site of injection

Site of injection in recipient embryos[a]	Number of recipient embryos			% rescued	Number of embryos which developed			
	Total	Developed	Rescued		Filzkörper	Trachea	Head sense organs	Black sense organs
–	280	267	0	0	0	0	0	0
Posterior ventral	214	176	50	28	50	13	0	0
Mid-ventral	198	153	32	26	0	21	12	16
Posterior dorsal	174	134	1	1	1	0	0	0

[a]Donor eggs were from *y, f*^{36a}; *mwh jv* ♀ ♀; the cytoplasm was taken from the ventral side of cleavage stage embryos.

Table III. Spatial distribution of rescuing activity in donor eggs

Donor			Number of recipient embryos[a]			% rescued
Mat genotype	Origin of cytoplasm	Age	Injected	Developed	Rescued	
+/+	Ventral	Cleavage	176	125	30	24
+/+	Dorsal		158	126	34	27
+/+	Ventral	Syncytial	276	165	35	21
+/+	Dorsal	Blastoderm	371	223	24	11
dl/dl	Ventral	Cleavage	210	133	0	0
dl/dl	Dorsal		119	102	0	0

[a]Cleavage stage *dorsal* embryos were injected into the posterior ventral side in all experiments. The criterion for rescue was the production of Filzkörper material.

gesting that, in early embryos, the activity is equally distributed along the dorsal-ventral egg axis. If, however, cytoplasm is taken from syncytial blastoderm embryos (2–3 h old), a distinct difference in rescuing activity of dorsal and ventral cytoplasm is observed. While the rescuing activity of the cytoplasm taken from the ventral side remains approximately constant, that of the cytoplasm from the dorsal side has dropped to about half compared with younger embryos. The difference is statistically significant (χ^2 test, $p<0.01$).

Discussion

Mutations affecting morphogenetic processes provide a powerful tool to dissect the complex process of pattern formation into individual components. A mutation affects primarily only a single component of the system, the gene product. For the molecular analysis of the genetic lesions, the early embryo provides a most suitable system, since substances could, in principle, be added back to the system by injection into the egg and thus restore, in part, the normal function of the gene. Such an approach was first described by Briggs and Cassens (1966) who showed that the developmental block occurring before gastrulation in eggs from females homozygous for the *o* mutation in *Ambystoma mexicanum* can be surpassed if cytoplasm from wild-type eggs is injected into mutant eggs. In *Drosophila*, the maternal effect exerted by the mutations *rudimentary* (*r*) and *deep orange (dor)* has been shown to be rescuable by the injection of wild-type cytoplasm (Garen and Gehring, 1972; Okada *et al.*, 1974). The genes *r* and *dor* probably affect rather general metabolic functions which may be required in particularly large amounts in the rapidly developing early embryo. Both mutations have a zygotic as well as a maternal inheritance, indicating that the genes are required throughout development. *r* codes for enzymes of the pyrimidine biosynthetic pathway and mutant embryos can also be rescued by a pyrimidine-rich diet for the females or the injection of RNA or pyrimidines into the egg. In both *dor* and *r*, the introduction of a wild-type gene copy via the male sperm can also rescue the maternal phenotype, indicating that the gene product can be supplied following either maternal or zygotic transcription.

Here the partial rescue of the phenotype of the maternal effect mutant *dorsal* is described. *Dorsal* differs from the above-mentioned mutants in that it clearly has a morphogenetic function. It is one of several genes that are involved in the establishment of the dorso-ventral embryonic pattern (see Anderson and Nüsslein-Volhard, 1983). Embryos from *dorsal* homozygous females develop a very limited set of dorsal cuticular structures at all positions along the dorso-ventral egg axis. The *dorsal* phenotype is not rescuable by the paternal genotype. The partial rescuability of the phenotype by the injection of wild-type cytoplasm indicates that the gene, although specifically transcribed during oogenesis, exerts its function later, during early embryogenesis. The rescuing effect depends on the amount of wild-type cytoplasm injected and is not observed with cytoplasm taken from mutant embryos (Table I). Thus, the rescuing activity in the cytoplasm depends on the presence of a functional *dorsal*$^+$ gene in the mothers of the donor embryos.

The rescued embryos are still far from being normal. Considering that the injected amount of wild-type cytoplasm does not exceed ~2% of the total egg volume, this is not surprising. As expected, the rescued phenotype resembles closely that of weak mutations at the *dorsal* locus which still have some residual gene activity. The allele *dl*^{2}, for example, produces, in addition to dorsal cuticle tracheae, Filzkörper and head sense organs, structures also found in rescued *dorsal*^{1} embryos.

The response of the mutant embryos depends strongly on the site of injection: only injection into the ventral but not into the dorsal side rescues mutant embryos. Further, the nature of the structures formed following ventral injection, depends upon the antero-posterior position within the recipient embryo (Table II, Figure 1). From these data a number of conclusions may be drawn. (i) The local response of the recipient embryo indicates that the active substance transferred in the wild-type cytoplasm does not spread very far from the site of injection and suggests slow diffusion of the rescuing principle. (ii) The nature of structures formed in recipient embryos is determined by a combination of influences from both the recipient and the donor embryo. The structures observed in rescued embryos – Filzkörper after posterior-ventral injection, head sense organs after mid-ventral injection – in normal embryos derive from 20% and 65% egg length (0% = posterior pole), respectively (Lohs-Schardin *et al.*, 1979; Underwood *et al.*, 1980), positions which correspond to the site of deposition of the donor cytoplasm in the recipient embryo. The antero-posterior coordinate of the structures, therefore, seems to be determined by the recipient embryo. With respect to the dorso-ventral egg axis, both Filzkörper and head sense organs derive from a dorso-lateral position (~15% egg half-circumference off the dorsal midline) in normal embryos, and in injected rescued embryos

close to the injection site, the ventral midline. Thus, the effect of the injected cytoplasm may best be described as a slight ventralization of the *dorsal* embryo at the site of injection. (iii) Despite the fact that *dorsal* embryos show no visible signs of dorso-ventral asymmetry, they must have an intrinsic dorso-ventral polarity. This is indicated by the finding that ventral, but not dorsal injections lead to a rescued phenotype. The polarity of the embryo may be established by the normal function of other genes involved in the process, and the lack of functional *dorsal* gene product may prevent its expression in a visible form. (iv) Since injection into the dorsal side of mutant embryos is without effect, the *dorsal*$^+$ gene function is specifically required at the ventral side of the embryo.

When cytoplasm is injected into the posterior ventral side of the recipient embryo, Filzkörper is formed regardless of whether the cytoplasm is taken from the dorsal or ventral side of the donor embryos (Table III). This indicates that the nature of the rescued structures does not depend on the origin of the donor cytoplasm. Quantitatively, no significant difference is observed between dorsal and ventral cytoplasm from cleavage stage embryos, suggesting equal distribution of the rescuing activity in young embryos. However, if taken from embryos shortly before cell formation, cytoplasm from the ventral side is twice as effective as that taken from the dorsal side. The difference observed is not as much as might be anticipated for a graded morphogen. However, since taking the cytoplasm from the donor embryo results in considerable streaming and mixing, our data are probably far from representing the true concentration differences. An unequal distribution of the rescuing activity is supported by the finding that the *dorsal*$^+$ function is required only at the ventral and not at the dorsal side of the embryo. A working hypothesis suggested by our data is as follows. The rescuing activity is initially equally distributed in the embryo. During early embryogenesis a depletion of the rescuing activity at the dorsal side of the egg takes place. This depletion is dependent on the normal activity of other genes involved in the process of establishing the dorso-ventral pattern and leads to a concentration difference of the rescuing principle on the dorsal and ventral egg side which controls the dorso-ventral polarity of the embryo.

At present, we do not know in which way the rescuing activity is related to the *dorsal* gene. It may be the gene product itself or a precursor-like stored mRNA; on the other hand, it may also be a substance the synthesis of which is dependent on the *dorsal* gene product. Preliminary data (Anderson and Nüsslein-Volhard, unpublished) indicate that rescuing activity is also detectable in partially purified homogenates from wild-type embryos. Thus, it seems feasible to isolate and characterize a substance with morphogenetic activity using partial rescue of mutant embryos as biological assay.

Materials and methods

Genetic strains

Wild-type donor eggs were obtained from a *y f*^{36a}; *mwh jv* stock. The *dorsal* allele used throughout these experiments was *dorsal*^{1}. The *dorsal* chromosome was further marked with *cn* and *sca*. Homozygous *dl*^{1} *cn sca* females were obtained from a *dl*^{1} *cn sca/CyO DTS100* stock. *DTS100* is a dominant temperature-sensitive lethal mutation induced in the *CyO* chromosome by Falke and Wright (1974). The stock was kept at 18°C and bottles after removal of the parents transferred to 25°C. Under these conditions, 70–90% of the emerging flies were *dl*-homozygotes and the few CyO-Durchbrenner were removed by sorting. The description of the markers used may be found in Lindsley and Grell (1968).

Injection procedure

Eggs were collected in 1 h periods on Gif-egg medium (Zalokar *et al.*, 1975) at room temperature (22°C). They were dechorionated manually, mounted in a row in suitable orientation (see below) on a thin spread of glue. They were dried on silica gel until the surface appeared slightly wrinkled. Eggs to be injected with larger than normal amounts of cytoplasm were dried for somewhat longer periods. They were covered with Voltalef 15S oil and cytoplasm was transferred between donor and recipient embryos using the injection routine described by Zalokar (1981). The diameter of the needle was 15–18 μm. Cytoplasm was taken from the donor eggs from a rather large cortical region, usually from the ventral side, and distributed among 5–8 recipient embryos. The orientation of the embryos and the position of the transferred cytoplasm in donor and recipient embryos is shown in Figure 2.

In experiments involving cleavage stage embryos, eggs which had already started pole cell formation were discarded. Given the time it took to mount and dry the embryos and the time of pole cell formation, which takes place at 1 h 20 min at 22°C, the youngest eggs were ~20 min, the oldest 1 h 20 min old. In experiments involving syncytial blastoderm eggs as donors, the ones which had started cell formation (3 h 10 min) were not used as donors, and the youngest eggs were 2.5 h old. When the cytoplasm was taken from syncytial blastoderm embryos, care was taken to avoid contamination with nuclei. On the other hand, occasionally included nuclei should not influence the results since the *dorsal* phenotype is independent of the embryonic genotype.

After injection, the recipient eggs were placed into a humid chamber supplied with oxygen and left to develop for 2 days at room temperature. They were removed from the slides with heptane to remove the oil and fixed in glycerol-acetic acid (1:4) for 15 min at 60°C. After mounting and clearing in Hoyer's medium (Van der Meer, 1977), they were scored for the development of cuticular structures using phase contrast optics (Zeiss Neofluar x 40). Eggs which did not show any cuticular differentiation were scored as undeveloped. The data given in the tables are pooled from 1–3 experimental series. In each series, both the controls and the experiments were done with the same batch of flies and within a couple of days.

References

Anderson,K.V. and Nüsslein-Volhard,C. (1983) in Malacinski,G. (ed.), *Primers in Developmental Biology*, McMillan, NY, in press.

Briggs,R. and Cassens,G. (1966) *Proc. Natl. Acad. Sci. USA*, **55**, 1103-1109.

Falke,E.V. and Wright,T.R.F. (1972) *Drosophila Inf. Serv.*, **48**, 89-91.

Garen,A. and Gehring,W. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 2982-2985.

Lindsley,D.L. and Grell,E.H. (1967) *Genetic Variations of Drosophila melanogaster*, published by Carnegie Institute, Washington.

Lohs-Schardin,M., Cremer,C. and Nüsslein-Volhard,C. (1979) *Dev. Biol.*, **73**, 239-255.

Nüsslein-Volhard,C. (1979a) *Symp. Soc. Dev. Biol.*, **37**, 195-211.

Nüsslein-Volhard,C. (1979b), in Le Douarin,N. (ed.), *Cell Lineage, Stem Cells and Cell Determination*, pp. 69-81.

Nüsslein-Volhard,C., Lohs-Schardin,M., Sander,K. and Cremer,C. (1980) *Nature*, **283**, 474-476.

Okada,M., Kleinman,I.A. and Schneiderman,H.A. (1974) *Dev. Biol.*, **37**, 55-62.

Underwood,E.M., Turner,F.R. and Mahowald,A.P. (1980) *Dev. Biol.*, **74**, 286-301.

Van der Meer,J. (1977) *Drosophila Inf. Serv.*, **52**, 160.

Zalokar,M., Audit,C. and Erk,I. (1975) *Dev. Biol.*, **47**, 419-432.

Zalokar,M. (1981) *Microsc. Acta.*, **403**, 52-60.