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Genes that control dorsoventral polarity affect gene expression along the anteroposterior axis of the *Drosophila* embryo

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Summary

At least 13 genes control the establishment of dorsoventral polarity in the *Drosophila* embryo and more than 30 genes control the anteroposterior pattern of body segments. Each group of genes is thought to control pattern formation along one body axis, independently of the other group. We have used the expression of the *fushi tarazu* (*ftz*) segmentation gene as a positional marker to investigate the relationship between the dorsoventral and anteroposterior axes. The *ftz* gene is normally expressed in seven transverse stripes. Changes in the striped pattern in embryos mutant for other genes (or progeny of females homozygous for maternal-effect mutations) can reveal alterations of cell fate resulting from such mutations. We show that in the absence of any of ten maternal-effect dorsoventral polarity gene functions, the characteristic stripes of *ftz* protein are altered. Normally there is a difference between *ftz* stripe spacing on the dorsal and ventral sides of the embryo; in dorsalized mutant embryos the *ftz* stripes appear to be altered so that dorsal-type spacing occurs on all sides of the embryo. These results indicate that cells respond to dorsoventral positional information in establishing early patterns of gene expression along the anteroposterior axis and that there may be more significant interactions between the different axes of positional information than previously determined.

Key words: positional information, maternal-effect genes, *Drosophila* development, dorsoventral polarity, gene expression, anteroposterior axis.

Introduction

Pattern formation in the *Drosophila* embryo requires the activities of several groups of genes that function in a region-specific manner during early development. The genes appear to act along either the shorter dorsoventral axis or the longer anteroposterior axis of the embryo. The absence of single gene products results in distinct alterations of cell fates and abnormal pattern formation. Among the genes that function along the dorsoventral axis, 11 loci have been identified thus far that are maternally expressed (Anderson & Nüsslein-Volhard, 1984; Anderson, Jürgens & Nüsslein-Volhard, 1984; T. Schüpbach, personal communication). The loss of any of ten of these gene products through mutation leads to 'dorsalization' of the embryo: cells at ventral and lateral positions assume dorsal fates and ventral structures fail to develop. The dorsoventral regulatory genes are thought to comprise an interacting network that establishes a 'gradient' of positional information along the dorsoventral axis (Nüsslein-Volhard, 1979; Anderson & Nüsslein-Volhard, 1984).

Anteroposterior pattern formation in the *Drosophila* embryo is best understood in terms of the genes that control the number and identity of body segments (Nüsslein-Volhard & Wieschaus, 1980). Segmentation and homeotic loci appear to function in determining the fates of groups of cells along this axis. For those segmentation genes whose pattern of expression is known, such as the *fushi tarazu* (*ftz*) and *engrailed* (*en*) genes, RNA transcripts (or proteins) are localized to distinct bands of cells encircling the embryo (Hafen, Kuroiwa & Gehring, 1984; Kornberg, Siden, O'Farrell & Simon, 1985; Carroll & Scott, 1985; DiNardo, Kuner, Theis & O'Farrell, 1985). Genes such as *ftz* and *en* are members of a hierarchy of interacting genes that control the formation of body segments and pattern formation within the segments (Howard & Ingham, 1986; Carroll & Scott, 1986; Carroll, Winslow, Schüpbach & Scott, 1986).

Genetic studies have suggested that pattern formation along each axis is controlled independently by two different sets of genes, since defects observed in single mutants appear to be restricted to one axis. The independence of the two sets of genes is difficult to assess definitively since abnormalities in embryonic development often lead to distorted structures that obscure normal pattern elements. For example, in dorsalized mutants that fail to gastrulate, segmentation is difficult to observe because the larva assumes a twisted form and most structures used to score anteroposterior development are ventral structures that are lacking in these mutants (Nüsslein-Volhard, 1979; Nüsslein-Volhard, Lohs-Schardin, Sander & Cremer, 1980). We have been able to more directly observe the influence of mutations by using antibodies to the *ftz* segmentation protein (Carroll & Scott, 1985) as a positional marker in the early embryo. We observe that disruption of dorsoventral polarity by mutations in dorsoventral regulatory loci results in positional shifts in *ftz* expression. We conclude that the axes of the embryo are not entirely independent. Certain aspects of anteroposterior gene expression respond to dorsoventral positional information.

Materials and methods

Antibody staining

Embryos were collected, fixed, stained with anti-*ftz* antibody, staged and photographed as described in Carroll & Scott (1985).

Mutant embryos were obtained from females either homozygous or transheterozygous for maternal-effect mutations, or from crosses of heterozygotes in the cases of the *zen*, *twi*, and *sna* loci. Mutant stocks were kindly provided by Drs K. Anderson, C. Nüsslein-Volhard, T. Schüpbach and P. Simpson and by the Bowling Green Drosophila Stock Center.

Results and discussion

The pattern of ftz *expression in wild-type embryos*

We have previously described the details of *ftz* protein expression in the early *Drosophila* embryo (Carroll & Scott, 1985). In wild-type animals, the *ftz* protein is expressed in seven broad stripes of nuclei encircling the embryo. Initially the stripes and 'interstripes' are about the same width (Fig. 1A), but at the initiation of gastrulation the stripes are clearly narrower than the unstained regions that lack *ftz* protein (Fig. 1B). This transition represents a decrease in the number of cells expressing the *ftz* protein. From a lateral view (Fig. 1B) the spaces between the stripes appear to be even, but it is clear that the number of nuclei stained is fewer and the spacing of the stripes more compressed at the dorsal surface of the embryo (Fig. 1B). The dorsoventral difference in the expression of *ftz* in wild-type embryos is important to our interpretation of the *dorsal* class of mutants described below and merits further description.

During the early stages of development, there are more cells on the ventral surface of the embryo than on the dorsal surface. Between the anterior border of the first *ftz* stripe and the posterior border of the seventh stripe there are approximately 42–44 nuclei dorsally and 52–54 nuclei ventrally. This difference is not accommodated by a symmetrical reduction of each stripe and interstripe dorsally. Close inspection reveals that the periodicity of *ftz* stripes on the dorsal surface is uneven, with the first, second and seventh stripes being wider than the third through sixth (Fig. 1C). The second through fourth interstripes are reduced in width. In contrast, the stripes of the ventral surface of the embryo are spaced more evenly, approximately five nuclear diameters apart (Fig. 1D). Thus, some mechanism causes cells to activate *ftz* at different intervals depending upon dorsoventral position. The likely candidate is the group of genes that acts during oogenesis to control dorsoventral polarity.

Dorsoventral polarity loci and their effects on ftz *expression*

Eleven maternal-effect loci have been identified that affect the dorsoventral polarity of the developing embryo. Mutation of any one of ten of these genes leads to dorsalization of the embryo (Anderson & Nüsslein-Volhard, 1984). The dorsoventral polarity mutants form a normal cellular blastoderm and do not alter the egg shape (Nüsslein-Volhard, 1979). Therefore, gene expression can be examined in mutant embryos until the time when morphogenetic movements become severely defective at the initiation of gastrulation. Inspection of the *ftz* expression pattern in the early cellular blastoderm or just prior to the initiation of gastrulation in dorsoventral mutants reveals that dorsoventral polarity loci influence the pattern of *ftz* expression (Fig. 2). From the moment when the *ftz* stripes are first detectable, during cellularization of the blastoderm, the stripes encircling the embryo have an abnormal periodicity (Fig. 2A) and are formed in much more perpendicular orientation to the long axis of the animal in a *dorsal* embryo than those in the wild-type embryo. At the onset of gastrulation, the first two stripes of *ftz* protein are clearly wider than the next four stripes, and the second through fourth interstripes consist of fewer nuclei as well (Fig. 2B,C). This pattern is uniform around the entire circumference of the embryo and resembles the *ftz* pattern formed on the

dorsal surface of the wild-type embryo (compare Figs 1C and 2C). The abnormal pattern is not the result of specific cell movements. Rather, the *ftz* stripes have formed over a shorter interval of the egg length. Thus, in a *dorsal* mutant embryo, all cells assume a dorsal fate and the *ftz* gene is activated at a dorsal-type interval around the entire dorsoventral axis. Once gastrulation begins, the pattern of *ftz* expression becomes obscured by the abnormal furrows formed around the entire circumference of the embryo (Fig. 2D).

We have eliminated the possibility that genetic background effects of the dl^1 chromosome causes pattern alterations by demonstrating that the $dl^1/Df(2L)TW119$ progeny exhibit the same defect, as do all progeny of the other dorsalizing maternal-effect mutations. We have examined abnormal *ftz* gene expression caused by ten maternal-effect mutants for the dorsoventral polarity system. The loci and the alleles examined are listed in Table 1. All ten maternally active dorsalizing mutations affect the periodicity of *ftz* expression and cause the stripes to form at a dorsal-type periodicity over an interval of 42–44 cells. Additional examples of such mutant effects are shown for *spätzle* (Fig. 2E) and *gastrulation defective* (Fig. 2F). The patterns of *ftz* expression are indistinguishable in mutants for all ten maternal-effect dorsal–ventral loci.

Not all loci affecting dorsoventral polarity have effects on the *ftz* protein pattern. Embryos from homozygous mutant *cactus* mothers, which appear to be partially ventralized (T. Schüpbach & E. Wieschaus, personal communication), exhibit a completely normal *ftz* pattern. In addition, a few zygotic loci have been identified that appear to be components of the dorsoventral pattern formation system (Table 1). Mutations of the *zerknüllt* (Wakimoto, Turner & Kaufman, 1984) *twist* (Simpson, 1983), and *snail* (Grau, Carteret & Simpson, 1984) genes affect the formation of structures characteristic of the dorsal (*zen*) or ventral (*twi* and *sna*) surface. Partially dorsalizing alleles of the *twi* and *sna* loci (twi^{ey63} and sna^{ry1}) and strong *zen* mutants (zen^{w36}) do not have any effect on the periodicity of *ftz* expression (data not shown).

Interactions between the dorsoventral and anteroposterior axes pattern formation systems

It is improbable that any of the effects observed here represent a direct interaction between dorsoventral gene products and the *ftz* gene. It is more likely that the positions of the *ftz* stripes represent the outcome of a series of interactions that precede its expression in the early embryo. There are four principal reasons for this assertion. First, some of the maternally active loci have very early temperature-sensitive periods that end before *ftz* expression begins (Anderson & Nüsslein-Volhard, 1984). Second, the *ftz* patterns in all maternal-effect mutants are indistinguishable, suggesting that the maternal-effect genes act upon *ftz* through a common pathway an idea consistent with previous morphological studies of the dorsoventral genes (Anderson & Nüsslein-Volhard, 1984). Third, a large number of other maternally active and zygotically active genes have been shown to influence the initial pattern of *ftz* expression and some are probably affected by the dorsoventral genes and thus alter the *ftz* pattern (Carroll & Scott, 1986; Carroll *et al.* 1986). Fourth, the segmentation gene *hairy* which regulates the periodicity of *ftz* expression (Howard & Ingham, 1986; Carroll & Scott, 1986) has also been shown to respond to dorsoventral position in that its dorsal anterior stripe is duplicated ventrally in the absence

Table 1. *The effect of genes controlling dorsoventral polarity on* ftz *expression*

Locus and allele(s) studied		Time synthesized	Phenotype	Effect on *ftz* periodicity
dorsal	(dl^1,dl^2,$Df(2L)TW119$)	maternal	dorsalized	dorsalized*
gastrulation defective	(gd^7)	maternal	dorsalized	dorsalized*
pipe	(pip^{286}/pip^{664})	maternal	dorsalized	dorsalized*
nudel	(ndl^{046}/ndl^{169})	maternal	dorsalized	dorsalized*
tube	(tub^{118}/tub^{238})	maternal	dorsalized	dorsalized*
snake	(snk^{073}/snk^{229})	maternal	dorsalized	dorsalized*
easter	(ea^2/ea^5)	maternal	dorsalized	dorsalized*
Toll	(Tl^{r632})	maternal	dorsalized	dorsalized*
pelle	(pll^{385})	maternal	dorsalized	dorsalized*
spätzle	(spz^{197})	maternal	dorsalized	dorsalized*
cactus	(cac^{PD74})	maternal	partially ventralized	no effect
zerknüllt	(zen^{w36})	zygotic	dorsal defective	no effect
snail	(sna^{ry1})	zygotic	partially dorsalized	no effect
twist	(twi^{ey63})	zygotic	partially dorsalized	no effect

**ftz* stripes shifted to periodicity of that found on wild-type dorsal surface.

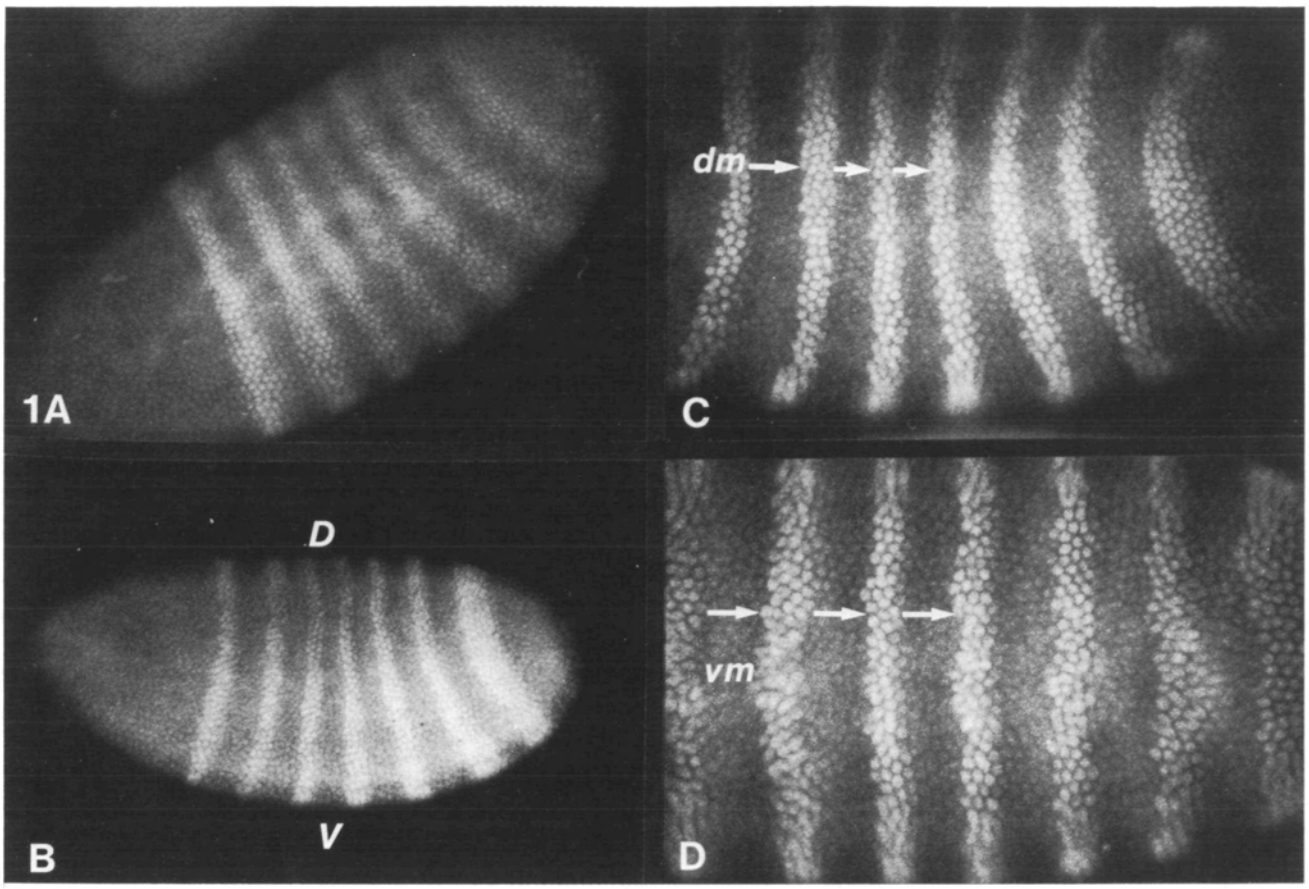

Fig. 1. Dorsal–ventral asymmetry in the periodicity of *ftz* protein stripes in the wild-type embryo. ×50. (A) Lateral view of embryo undergoing cellularization. The *ftz* protein stripes are first detectable as roughly evenly spaced stripes encircling the embryo. (B) Lateral view of embryo beginning gastrulation. The stripes have sharpened to be about three nuclei in width laterally and are separated by about five unstained nuclei. Note the difference in distance between the borders of the stripes dorsally (*D*) and ventrally (*V*). ×50. (C) High-magnification view of dorsal surface of embryo undergoing gastrulation. Note the width, shape and spacing of the stripes. The first two anterior stripes are wider (long arrow) and farther apart than the third through sixth stripes (short arrows). The sixth stripe is spaced well apart from the seventh stripe. *dm*, dorsal midline. ×75. (D) Ventral surface of embryo at the same stage as (C). The stripes are evenly spaced, about five nuclei apart (arrows), in contrast to the dorsal surface (C). *vm*, ventral midline. ×75.

of the *dl* product (Ingham, Howard & Ish-Horowicz, 1985). We also note that the periodicity of the *hairy* striped pattern appears to be affected in *dl* embryos (see fig. 6 in Ingham *et al.* 1985). An alteration in *hairy* expression alone would be expected to lead to an alteration in *ftz* expression.

The dorsalizing series of mutants are believed to represent a group of genes that function to establish a graded continuum of positional information across the dorsoventral axis (Anderson & Nüsslein-Volhard, 1984). Mutations that eliminate a single gene's function in the system appear to reduce all information to a dorsal ground state. Since this dorsal ground state impacts upon the normal anteroposterior system, one must expect that there is a specific mechanistic link between components of the two patterning systems used in the two axes. The most likely explanation for the positional changes in anteroposterior gene expression observed here is that regulatory components (or signals) of the anteroposterior system are normally asymmetrically organized by action of the dorsoventral polarity system. We suspect that the anteroposterior regulatory components involved are those that regulate the pattern of segmentation gene activity, perhaps maternally expressed products such as those of the *bicaudal*-type loci (Mohler & Wieschaus, 1985) or the maternal segmentation loci (Schüpbach & Wieschaus, 1986).

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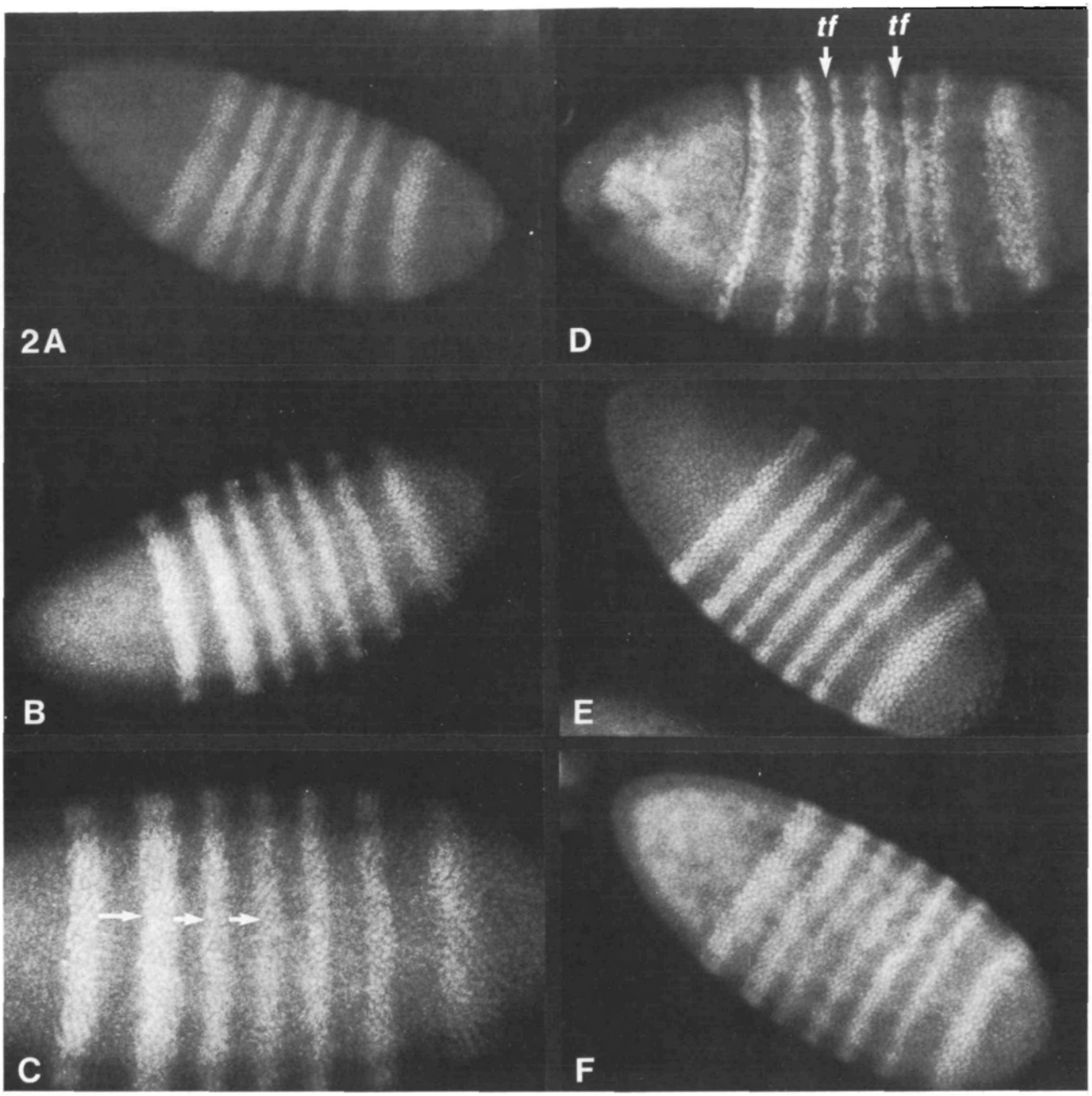

Fig. 2. The periodicity of *ftz* protein stripes in embryos from females homozygous for mutations in maternal-effect loci controlling dorsal–ventral polarity. (A) Cellular blastoderm-stage embryo from dl^1/dl^1 female. The initial stripe spacing is uneven (compare to Fig. 1A). ×50. (B) Early gastrulation embryo from dl^1/dl^1 female. The first two anterior *ftz* stripes are wider and farther apart than the next four stripes and appear to be more perpendicular to the long axis of the embryo than occurs in the wild-type embryo (compare to Fig. 1B). ×50. (C) Higher magnification view of embryo in (B). Note the width of the first interstripe (long arrow) compared to the second and third interstripes (short arrows). ×75. (D) Abnormal gastrulation in *dorsal* mutant embryo. The transverse folds (*tf*) encircle the embryo. ×50. (E) Embryo from female homozygous for spz^{197} allele. The stripes are spaced unevenly, as in *dorsal* mutants. ×50. (F) Embryo from female homozygous for the gd^7 allele. The pattern is identical to that of the other maternal dorsalizing mutants. ×50.

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