T遺伝子はマウス胚の前後軸を決定するか

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Does the *T* gene determine the anteroposterior axis of a mouse embryo?

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ABSTRACT

The *t* complex in the mouse is essential to embryonic development. The semidominant mutation, *T*, is embryonic lethal in homozygotes. In *T/T* embryos, proper morphogenetic movement is hindered, causing the number of mesodermal cells to be reduced. The other effect of *T* mutation is shortening of the tail in heterozygotes, *T/+* and *T/t*. *t* is a series of recessive mutations in the *t* complex.

A detailed examination of tail shortening indicated the *T* and *t* mutations to exert various effects, such as the derangement of the pattern of necrosis, fusion or duplication of the neural tube and gut. The most severly affected structure is the notochord. The *t* mutation augments the effect of *T* mutation in the formation of normal notochord.

A gradient of phenotypic severity along anteroposterior axis of an embryo appeared to exist correlative to the dosage of the *T* gene.

Should the products of the *T* gene be responsible for inducing mesoderm and the establishment of the anteroposterior axis of an embryo, diverse effects of the *T* mutation on embryogenesis can be explained collectively.

1. INTRODUCTION

The *t* complex in the mouse covers the proximal one-third portion of chromosome 17 and includes the Brachyury locus (*T*) (Bennett, 1975; Silver, 1985). This locus was first identified by a dominant mutation, *T*, which causes a short-tailed phenotype in heterozygotes.

Homozygous embryos die at the 10th day *post coitum* (*p.c.*). In gastrulating *T/T* embryos, morphogenetic movement is abnormal and the number of mesodermal cells is reduced whereas that of ectoderm increases (Yanagisawa et al., 1981). These embryos show peculiar abnormalities in mesodermal derivatives such that the chordamesoderm is enlarged with poorly developed allantois, notochord, and somites. The poor development of allantois results in embryonic death at about the 10th day *p.c.* (Gluecksohn-Schoenheimer, 1944).

In the heterozygous condition, *T/+* mice have short tails. This defect is

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enhanced by "t complex tail interaction" (*tct*) mutations in the complex, so that *T/t* mice are tailless. *tct* maps very close to *T*, suggesting that it is an allele of *T* (Justice and Bode, 1988).

Recent progress in molecular biology has made possible the high resolution mapping of DNA markers in *t* haplotypes including *T* and *tct*. (Willison et al., 1986; Herrmann et al., 1986; Herrmann et al., 1987; Herrmann et al., 1990). Further information on the functions of the *T* gene should be obtained by studying the process of tail shortening in heterozygous embryos.

This article reports that the occurrence of a gradient of phenotypic severity is suggested in relation to gene dosage. It appears that the more posterior the region in an embryo and the later the stage of development, the greater is the requirement for high activity of the *T* gene. A hypothesis is proposed that products of the *T* gene are responsible for the induction of the mesoderm and for establishment of the anteroposterior axis of an embryo.

2. MATERIALS AND METHODS

Mice, $T/+$, $T^{Or1}/+$, T/t^{w32}, T/t^{1} and T/t^{w18} were originally supplied by Dr. D.

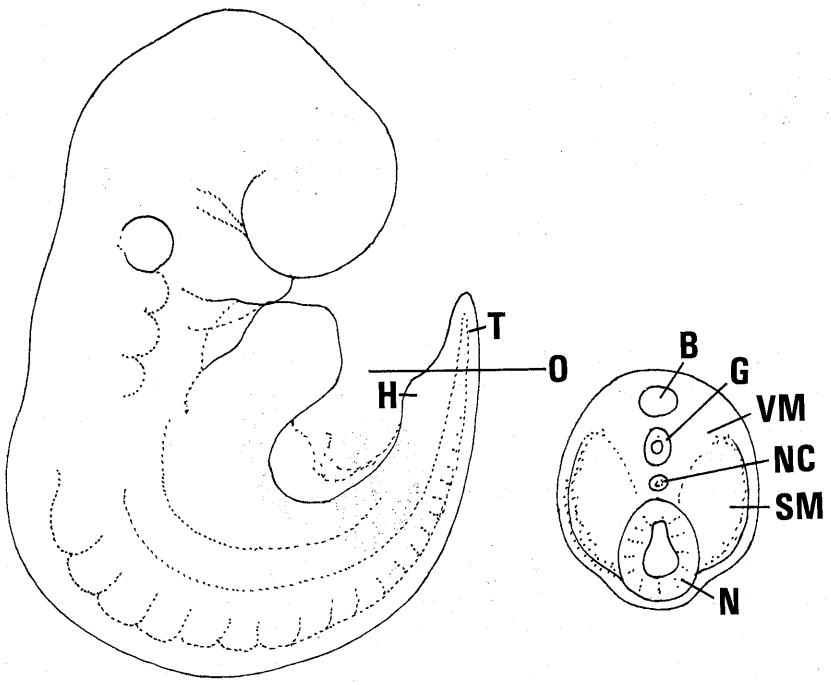

Fig. 1. Sectioning of the tail and a cross-section of the tail of normal mouse embryos. H, hind limb bud; T, tail; N, neural tube; NC, notochord; G, gut; SM, somitic mesoderm; B, blood vessel. Space is filled with cells of the ventral mesoderm (VM). The level of the hind limb bud was designated as section No. 0.

Bennett. The $T/+$ mice were in the 14th inbreeding generation at the time of present investigation. The T and T^{Or1} mutations include deletions and hence are null alleles (Silver et al., 1983; Herrmann et al., 1986; Herrmann et al., 1990).

t^M is a partial haplotype isolated from t^{w18} as a recombinant at the Mitsubishi Kasei Institute of Life Sciences (Yanagisawa and Koyama, 1982). Although the T/t^M mice are tailless, the t^M/t^M mice are viable and have tails of normal length.

Mice were maintained under an artificially controlled light cycle (4 AM-11 PM light) so that mating would occur between midnight and 2 AM.

The pregnant females were sacrificed at the 10th, 11th, 12th, and 13th day *p.c.* (day of plug=day 0).

The embryos were fixed in Bouin's fluid and embedded in paraplast (Sherwood Medical, U.S.A.). Cross sections, 7 μm in thickness, were cut as shown in Fig. 1, and stained with hematoxylin and eosin.

Each section was examined for regression of the tail gut, presence of notochord, neural tube, somitic mesoderm, and ventral mesoderm (Fig. 1). Examination was also made for the presence or absence of necrotic cells in each tissue and infiltration of blood corpuscles.

3. RESULTS

The tails of normal embryos begin to lengthen just before the 11th day *p.c.* (Fig. 2). In cross sections of the tail of 11 day $+/+$ embryos, the neural tube,

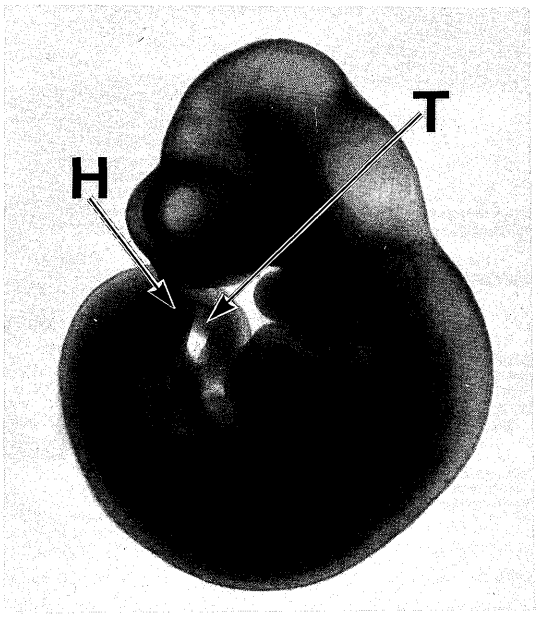

Fig. 2. Wild-type embryo at the 11th day *p.c.* (×16). T, tail; H, hind limb bud.

notochord, gut, somites and blood vessels were observed. Spaces around these tissues were filled with cells of ventral mesoderm (Fig. 1).

T/+ or *T*/*t* embryos at the 11th day *p.c.* could be distinguished grossly from normal embryos by the configuration of the tail. At the 11th day, the tail length of heterozygotes was still comparable to that of the wild type. In heterozygous embryos, the posterior portion of the tail showed a diameter less than that of a normal tail at the corresponding stage.

In 11 day +/+ embryos, the gut could be seen in the tail, but was resorbed gradually after 12 days. At the 11th day, there were necrotic cells in that region of the gut just posterior to the cloaca.

At the 12th day, cell death could be seen to proceed posteriorly to the region observed at the 11th day, and the gut in the region anterior had regressed to the necrotic zone. At the 13th day, necrotic wave was seen to traverse more to the posterior region, and the region without the gut expanded (Fig. 3).

Although necrosis was observed only in the gut at the 11th day, at the 12th day, necrotic cells could also be seen in the neural tube, somitic mesoderm and ventral mesoderm. The distribution of necrotic cells was tissue- and stage-specific and the necrotic wave traversed anterior to posterior (Figs. 4, 5, and 6).

In the mutants, the pattern of necrosis was aberrant, and there was no

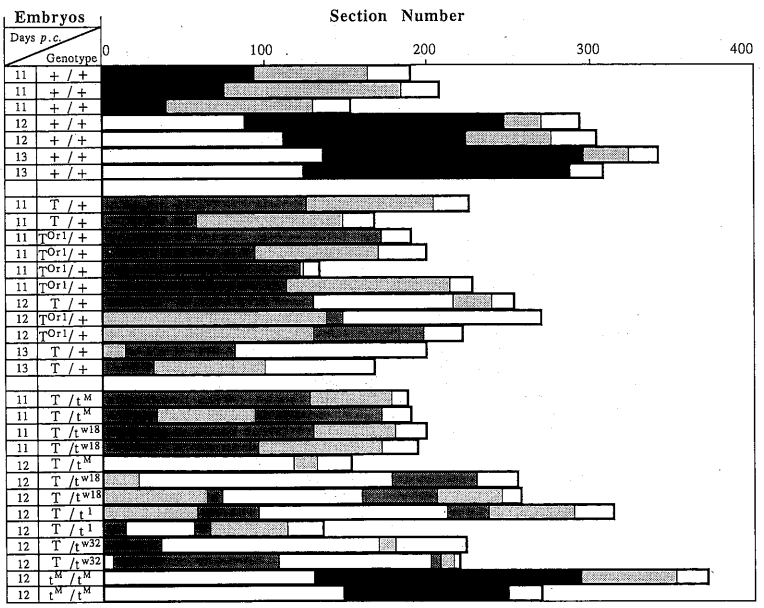

Fig. 3. Regression of the gut in the posterior region of +/+, *T*/+, *T*/*t*, and t^M/t^M embryos. Ordinate: Section number. The level of the hind limb-bud was desigated as section No. 0. The sections were numbered consecutively, proceeding toward the tip of the tail. ▒, region where the gut is present; ═, region where the gut is not present; ■, extensive necrosis; ▓, slight necrosis.

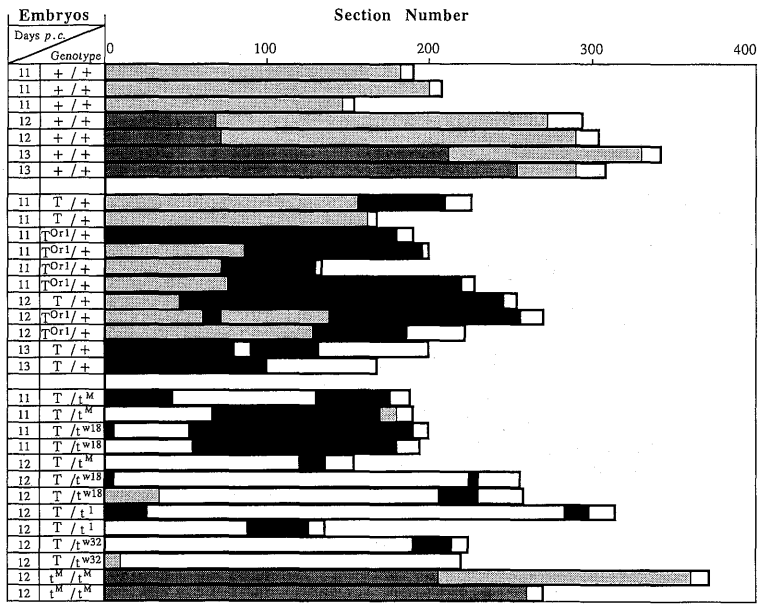

Fig. 4. Necrosis in the neural tube in the posterior region of +/+, *T*/+, *T*/*t*, and t^M/t^M embryos. Ordinate: Section number. The sections were numbered as described in Fig. 3. ▒, region where the neural tube is present; ═, region where the neural tube is not present; ■, extensive necrosis; ▓, slight necrosis.

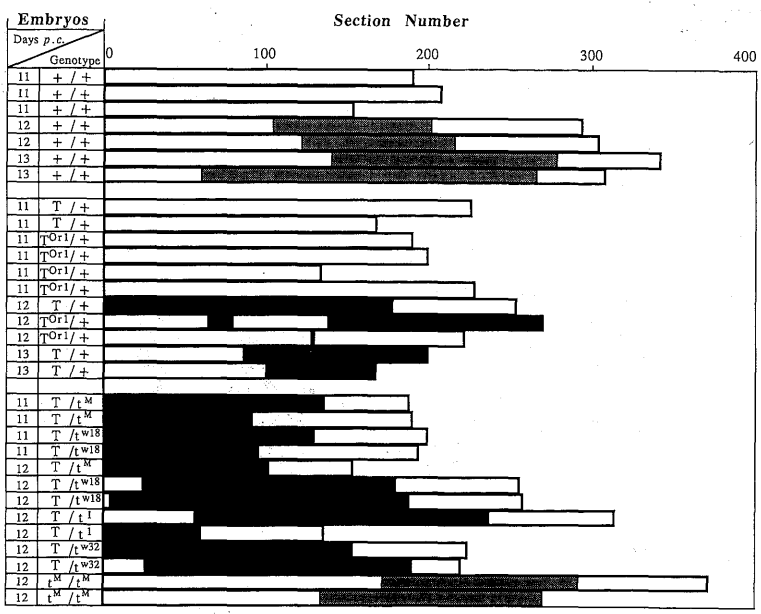

Fig. 5. Necrosis in the somitic mesoderm in the posterior region of +/+, *T*/+, *T*/*t* and t^M/t^M embryos. The somitic mesoderm is present in all sections except those at the posterior end of the tail. Ordinate: Section number. The sections were numbered as described in Fig. 3. ■, extensive necrosis; ▓, slight necrosis.

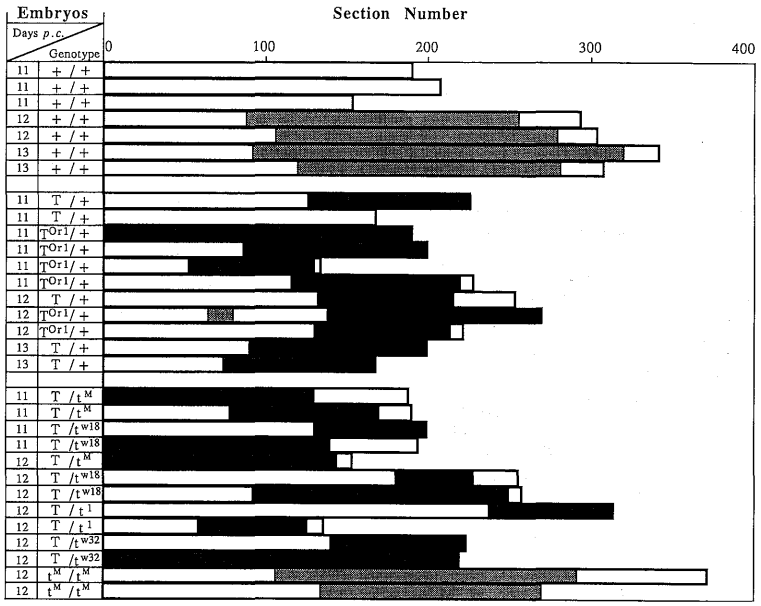

Fig. 6. Necrosis in the ventral mesoderm of the posterior region of +/+, *T*/+, *T*/*t*, and t^M/t^M embryos. Cells of the ventral mesoderm are present in all sections. Ordinate: Section number. The sections were numbered as described in Fig. 3. ■, extensive necrosis; ▒, slight necrosis.

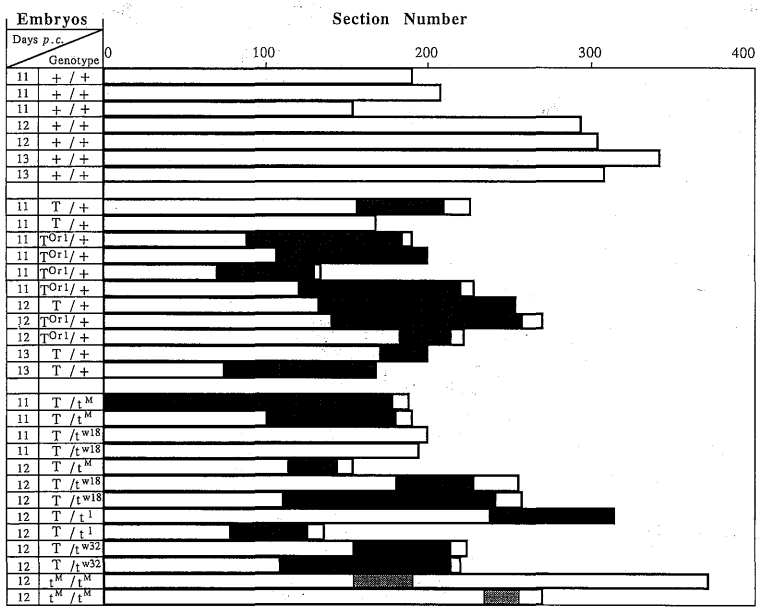

Fig. 7. Infiltration of the blood corpuscles in the posterior region of +/+, *T*/+, *T*/*t*, and t^M/t^M embryos. Ordinate: Section number. The sections were numbered as described in Fig. 3. ■, extensive infiltration of blood corpuscles; ▒, slight infiltration of blood corpuscles.

regression of the gut. Although there were some necrotic cells in the mutant gut, their number was much less than that noted in the wild type. The cells susceptible to necrosis may not have been of sufficient number to bring about the regression of the gut in the mutants.

A greater number of cells was susceptible to necrosis in the neural tube and mesoderm of the mutant (Figs. 4, 5, and 6). Extensive necrosis in the ventral mesoderm appeared to cause the blood vessels to expand, infiltration of blood corpuscles and eventually the loss of that region of the tail (compare Figs. 6 and 7).

In *T/t* embryos at the 13th day, the infiltration of blood corpuscles was extensive and most tissues in the tail region had been disrupted.

No abnormality in total cell number in the tail region was observed which might be brought about by abnormal cell migration or mitosis.

In *T*/+, the notochord was normal at the 10th day in the tail region, but at the 11th day, it was frequently integrated in the neural tube or gut, in agreement with Chesley (1935). On some occasions, notochord was seen to be abnormally located. In *T/t* embryos, no abnormality could be found at the 9th day. Whereas at the 10th day, no notochordal structure was observable as an isolated entity in the tail region. In t^M/t^M and $t^M/+$ embryos, morphologically normal notochord was present (Fig. 8).

In every mutant studied, including *T*/+ and t^M/t^M (normal tail), extensive

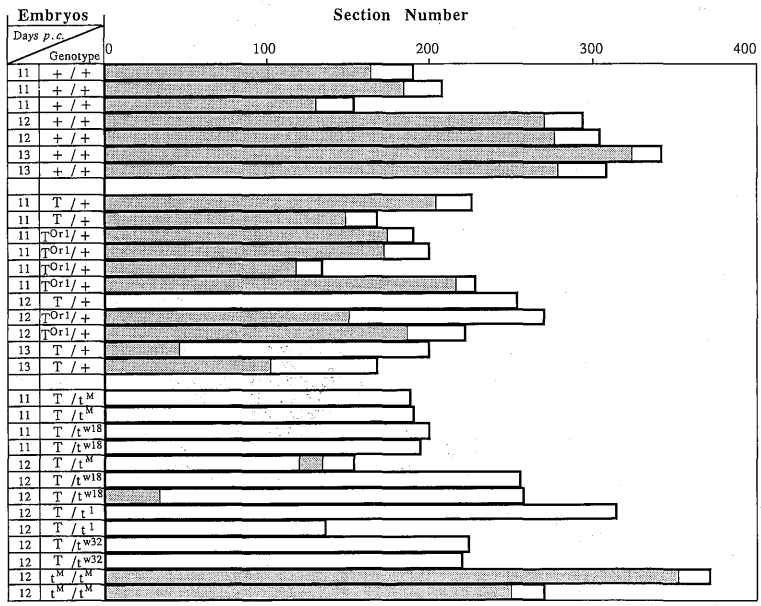

Fig. 8. The notochord in the posterior region of +/+, *T*/+, *T/t*, and t^M/t^M embryos. Ordinate: Section number. The sections were numbered as described in Fig. 3. ▒, region where the notochord is present; —, region where the notochord is not present.

duplication of the neural tube and gut was noted, and fusion of the neural tube and gut also had taken place. Abnormality was always severer in the posterior region of an embryo. The $t^M/+$ embryos did not show such abnormality.

The effects of mutation were more pronounced in the order, $T/t > T/+ > t^M/t^M$, but no abnormality could be found in $t/+$.

The absence of the notochord was not always accompanied by the morphological abnormality. In t^M/t^M, this structure was present but abnormal infiltration of the blood corpuscles, though to a slight extent, could be seen.

In $T/+$ embryos, there was no gut regression in the region where the notochord was present. In regions where the notochord could not be detected, necrotic cells in the somitic mesoderm were abundant (compare Figs. 5 and 8). With dislocation of the notochord in $T/+$ embryos, extensive cell death frequently occurred only in somitic mesoderm distantly located from the notochord.

4. DISCUSSION

In the tail region of $+/+$ embryos, necrosis traversed posteriorly, starting from the region just posterior to the cloaca, causing the tail gut that had formed in this region to be resorbed.

In $T/+$ or T/t embryos, the pattern of the necrosis was deranged and there was no gut resorption. Instead, other tissues had become targets of necrosis to cause tail shortening.

The mechanism for cell-killing in a developing thail is not known. During the destruction of the mammary rudiments of 14 day mouse fetuses, the mesenchyme, activated by the action of androgen, condenses about the epithelial gland buds, causing necrosis (Dürnberger and Kratochwill, 1980). Cell-killing appears to be a complicated process that includes tissue interaction.

No notochord could be observed in this study as an isolated entity in the posterior region of T/t embryos from the 10th day on. This is in agreement with Gluecksohn-Waelsch (1938). Grüneberg (1958) claimed the notochord to be present throughout the length of the body in T/t embryos although incorporated in the neural tube.

In $T/+$ embryos, the notochord could be seen but was frequently fused to the neural tube or gut or was abnornally located.

Abnormality in the notochord was severer in T/t embryos than in $T/+$. Mutation in the *tct* gene enhances defects in notochord development in *T* mutation. This structure is the most severely affected tissue in T/t embryos.

Wilkinson et al. (1990) reported *T* gene expression to be down regulated to undetectable levels in paraxial and lateral mesoderm. Expression of the *T* gene, however, persists in the notochord of 9.5 day embryos. With completion of gastrulation, the only site of *T* gene expression detected is the notochord.

One of the possibilities may be that the notochord controls the pattern of

necrosis in the tail region.

A normal notochord may not necessarily be present for cell survival in general, since gut cells were found not to incur necrosis and to survive in the mutants. It would appear from the present study, however, that the notochord must be present for the viability of somitic mesoderm.

In chick, somites rapidly lose their structural integrity following separation from the neural tube and notochord (Teillet and Le Douarin, 1983; Stern and Bellairs, 1984).

The *tct* gene in t^{M} haplotype, and hence that of the t^{w18}, must have lesser activity than normal, but more than that of the null allele *T*, in the formation of normal notochord.

A gradient of phenotypic severity was noted to be present in the decreasing order of *T*/*t*, *T*/+, t^{M}/t^{M}, and *t*/+ (+/+). If *tct* is an allele of *T*, a correlation seems to exist between the dosage of the *T* gene and development along the anteroposterior axis of the body. A similar notion has been proposed by MacMurray and Shin (1988).

If defects in the *T*/*T* embryos are taken into consideration, the situation becomes clearer. In 7 day *T*/*T* embryos, appearance of the first mesodermal cell from the primitive streak delays by 2–3 hrs compared to normal embryos (Yanagisawa et al., 1981), and hence the number of mesodermal cells is less.

At the early 8th day, when the total cell number of embryos is about 5.0×10^{4}, the effects of *T*/*T* mutation on morphogenetic movement suddenly become quite evident. From this stage on, cells derived from the epiblast become stuck in the primitive streak and thus hindering their morphogenetic movement to anterior region. This leads to anteroposterior gradient of abnormal morphology (Yanagisawa et al., 1981).

In *T*/*t* embryos, defects which cause taillessness are first evident at the 10th day, and at the 11th day in *T*/+.

The *T* gene is apparently active from the beginning to the end of anteroposterior body axis formation. The later the stage and the more posterior the region, the greater seems to be the requirement for a high dose of the *T* gene.

The *Xenopus* homeobox gene *xhox*3 is one of the first genes to be transcribed in the early embryos, and is expressed in a graded fashion along the anteroposterior axis in the axial mesoderm (Ruiz i Altaba and Melton, 1989a). High levels of *xhox*3 expression is correlated with posterior determination and low levels, with anterior determination. These findings led the authors to conceive that *xhox*3 is involved in interpreting positional information in the axial mesoderm along the anteroposterior axis.

In the course of research to investigate what caused the graded level of *xhox*3 expression, Ruiz i Altaba and Melton (1989b) found that basic fibroblast growth factor (bFGF) induced higher levels of *xhox*3 mRNA than did XTC-MIF. XTC-MIF is a *Xenopus* peptide growth factor-like mesoderm inducing factor of the

tumor growth factor-β family. As high and low levels of *xhox3* expression are correlated with posterior and anterior determination, respectively, this suggests that high doses of bFGF induce dorsal/posterior mesoderm, whereas high doses of XTC-MIF induce dorsal/anterior mesoderm.

Product of the *T* gene cloned by Herrmann et al. (1990) seems to be intracellular protein. They claimed that the *T* gene product had no significant base sequence homology with any known protein. Functionally, however, a close similarity seems to exist between bFGF and the *T* gene product. If product of this gene induce mesoderm formation and bring about the establishment of the anteroposterior axis, this then would collectively explain the various effects of the *T* and *tct* genes in embryogenesis.

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