Development 113, 913–917 (1991)
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Expression pattern of the *Brachyury* gene in whole-mount T^{Wis}/T^{Wis} mutant embryos

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Summary

The murine *Brachyury (T)* gene is required in mesoderm formation. Mutants carrying different *T* alleles show a graded severity of defects correlated with gene dosage along the body axis. The phenotypes range from shortening of the tail to the malformation of sacral vertebrae in heterozygotes, and to disruption of trunk development and embryonic death in homozygotes. Defects include a severe disturbance of the primitive streak, an early cessation of mesoderm formation and absence of the allantois and notochord, the latter resulting in an abnormality of the neural tube and somites. The *T* gene is expressed in nascent mesoderm and in the notochord of wild-type embryos. Here the expression of *T* in whole-mount mutant embryos homozygous for the *T* allele T^{Wis} is described. The T^{Wis} gene product is altered, but the T^{Wis}/T^{Wis} phenotype is very similar to that of T/T embryos which lack *T*. In early T^{Wis}/T^{Wis} embryos *T* expression is normal, but ceases prematurely during early organogenesis coincident with a cessation of mesoderm formation. The archenteron/node region is disrupted and the extension of the notochord precursor comes to a halt, followed by a decrease and finally a complete loss of *T* gene expression in the primitive streak and the head process/notochord precursor. It appears that the primary defect of the mutant embryo is the disruption of the notochord precursor in the node region which is required for axis elongation. Thus the *T* gene product is directly or indirectly involved in the organization of axial development.

Key words: *Brachyury*, mouse embryogenesis, notochord, whole-mount *in situ* hybridization.

Introduction

The *Brachyury* (*T*) gene of the mouse is required in mesoderm formation. In T/T embryos, trunk development and mesoderm formation come to a standstill during early organogenesis and the head process/notochord precursor disintegrates. As a consequence of the defective notochord, the neural tube and somites are abnormal (Chesley, 1935; Glücksohn-Schönheimer, 1938; Grüneberg, 1958; Yanagisawa *et al.* 1981). The allantois is strongly reduced in homozygotes resulting in embryonic death (Glücksohn-Schönheimer, 1944). Heterozygous animals are short-tailed or tailless (Dobrovolskaia-Zavadskaia, 1927). The most severely affected structure in the mutants is the notochord (Chesley, 1935; Gluecksohn-Schoenheimer, 1938; Yanagisawa, 1990). A correlation between the severity of the phenotype and *T* gene dosage has been suggested (McMurray and Shin, 1988; Yanagisawa, 1990): the more posterior along the axis the higher is the requirement for a high dose of the *T* gene to allow normal axial development. Until the recent cloning of the *T* gene (Herrmann *et al.* 1990), an assessment of the role of the *T* gene in mesoderm formation had not been possible. Wilkinson *et al.* (1990) showed that the *T* gene is expressed in the nascent mesoderm of the primitive streak and in the notochord precursor and its derivatives, the tissues most affected by the *T* mutation, but *T* expression is down regulated or turned off in mesoderm cells migrating away from the streak. This finding allowed the conclusion that the *T* gene may play a direct role in mesoderm formation and in the morphogenesis of the notochord. Here an attempt is made to elucidate further the role of the *T* gene in axial development.

A protocol for whole-mount *in situ* hybridization of mouse embryos has been established that allows the analysis of gene expression in whole early embryos. It complements the presently available technology using sectioned embryos and radioactively labelled probes (Wilkinson *et al.* 1987), and provides a resolution comparable to immunochemical staining. The method is based on previously published protocols (Wilkinson *et al.* 1987; Tautz and Pfeifle, 1989; Hemmati-Brivanlou *et al.* 1990). The procedure was used for the analysis of *T* gene expression in mutant embryos carrying the *T* allele T^{Wis} (Shedlovsky *et al.* 1988), using 1.8 kb of the cDNA for the *T* gene as probe. The T^{Wis} mutation results from the insertion of a transposable element into the *T* gene (Herrmann *et al.* 1990). The T^{Wis} allele is transcribed and translated; the altered T^{Wis} transcripts

are recognized by the *T* gene probe. Heterozygous $T^{Wis}/+$ mice in general are tailless, suggesting that the altered T^{Wis} product interferes with the activity of the normal gene product, resulting in a more severe phenotype in $T^{Wis}/+$ animals compared with the loss of one gene copy, such as in $T/+$ animals which have short tails. T^{Wis}/T^{Wis} embryos, however, closely resemble T/T embryos. In contrast to the original *T* deletion, the T^{Wis} allele, being expressed in the embryo, allows the analysis of the expression of the *T* gene in the mutant embryo by *in situ* hybridization.

Materials and methods

Embryos

All embryos have been derived from matings of $T^{Wis}/+\times T^{Wis}/+$ tailless heterozygous mice. Therefore, 25% of the embryos are expected to be homozygous for the mutant allele T^{Wis}. Plugs were checked for the morning after mating, noon was taken as 0.5 days of gestation. However, since a wide variation in developmental stages both between and within litters was observed, the stage of each embryo was determined again according to the appearance of the head process (7.3 days), amniotic cavity (7.5 days), neural plate (7.75 days), neural folds (8 days) and somites (8.12 days or older). Embryos were dissected in ice-cold DMEM containing 20 mM Hepes pH 7.4 The trophectoderm and parietal endoderm or most of the yolk sac and amnion were removed. Embryos were fixed overnight in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) at 4°C, washed twice in PBS, then in 50% PBS in ethanol (EtOH), 70% EtOH and stored in 70% EtOH in the coldroom or processed further.

Genotyping of stained embryos

After *in situ* hybridization, staining and photography, embryos of interest were genotyped: parts of the extraembryonic membranes were dissected and transferred to a tube with 50 μl phosphate-buffered saline/0.1% Tween-20. Protein was digested with proteinase K overnight at 55°C, after addition of 50 mM Tris–HCl pH 9.5 and 250 $\mu g\,ml^{-1}$ proteinase K. The enzyme was heat inactivated at 96°C for 15 min. Two μl of the solution were used for DNA fragment amplification by Taq polymerase chain reaction in a 20 μl reaction volume containing 0.2 mM each of dATP/dGTP/dCTP/dTTP, 100 $\mu g\,ml^{-1}$ BSA, 100 ng of each of the primers, 1 unit Taq polymerase and buffer according to the suppliers' (Pharmacia, Boehringer) recommendation. Forty-five cycles of the following steps were performed: 94°C for 30 s, 60°C for 1 min, 72°C for 30 s. 10 μl of the reaction mixture were separated on a 2% agarose gel containing ethidium bromide. The primers were chosen to produce fragments of approximately 85 base-pairs for T^{Wis} and 135 base-pairs for the wild-type (+) allele. The genotypes of embryos shown on Fig. 1 are as follows: Fig. 1A, +/+; 1C, +/+; 1E, +/+; 1F, T^{Wis}/T^{Wis}; 1G, T^{Wis}/T^{Wis}; 1K (left), T^{Wis}/T^{Wis}; 1L, T^{Wis}/T^{Wis} (data not shown).

Probe

The sequence from base 1 to base 1764 of the cDNA for the *T* gene, pme75 (Herrmann *et al.* 1990), was inserted into plasmid vectors suitable for RNA transcription *in vitro*. Antisense and sense RNA probes were generated by run-off transcription from the T7 promoter of plasmids containing the cDNA in either orientation. The pme75 probe is specific for the *T* gene; no other genes have been detected in hybridizations of cDNA or genomic DNA libraries under high or reduced stringency (Herrmann, unpublished).

In situ *hybridization of whole-mount embryos*

All steps were carried out in 1 ml for 5 min at room temperature, except where indicated. Embryos were rehydrated by passing through 50% EtOH, 30% EtOH and twice through PBS containing 0.1% Tween-20 (PBST). Postfixation was in 4% PFA in PBS for 20 min, followed by 2 washes in PBST. According to size, embryos were digested with proteinase K (20 $\mu g\,ml^{-1}$ in PBST) for 1 min (for 6–7 days *p.c.*), 3 min (for 7–7.5 days *p.c.*) or 5 min (for 7.75–8.75 days *p.c.*), rinsed briefly in PBST and washed again in PBST, and fixed in 4% PFA in PBS for 10 min. Embryos were rinsed in double-distilled water and acetylated by incubation for 10 min in 0.1 M triethanolamine pH 8 containing 2.5 μl acetic anhydride per ml, added just prior to use. After two washes in PBST, embryos were equilibrated in hybridisation (−) solution (hyb (−)) at 55°C containing 50% deionized formamide, 5×SSC, 20 mM Tris pH 8, 5 mM EDTA pH 8, 0.1% Tween-20. Most of the hyb (−) solution was discarded, except for approximately 20 μl containing the embryos, to which 20 μl of hyb (+) solution (hyb(−) containing 0.2% PVP, 0.2% Ficoll type 400, 4 $mg\,ml^{-1}$ yeast RNA, 2 $mg\,ml^{-1}$ heparin, 0.4 mM d-biotin) were added. Embryos were prehybridized at 55°C for 2–3 h and hybridized overnight (more than 12 h) at 55°C after addition of 2 μl biotin-labelled antisense RNA probe (1–5 μg RNA ml^{-1}) hydrolysed to an average of 150 base fragments and dissolved in hyb(−). The synthesis and hydrolysis of the RNA probe was essentially as described (Wilkinson *et al.* 1987; Cox *et al.* 1984), except that 2 μl of 4 mM bio-11-UTP (Sigma) was used instead of radiolabelled nucleotide analogs. The embryos were rinsed and then incubated in 50% formamide, 2×SSC for 20 min at 55°C, washed 3 times in 0.5 M NaCl, 10 mM Tris pH8, 5 mM EDTA, 0.1% Tween-20 for 10 min at 37°C, 30 min in the same solution containing 20 $\mu g\,ml^{-1}$ RNAase A and 100 i.u. ml^{-1} RNAase T1 at 37°C, washed in the same solution for 15 min at 37°C, washed 20 min in 50% formamide, 2×SSC at 55°C and 15 min each in 2×SSC, 0.1% Tween-20 and 0.2×SSC, 0.1% Tween-20 at 55°C. This solution was replaced by PBST at room temperature. The embryos were incubated in PBST containing 0.25 mi.u. ml^{-1} (1:2000 dilution) Streptavidin–betaGal complex (Boehringer) for 1 h with rotation, washed four times in PBST for 15 min under rotation, the solution was replaced with betaGal buffer (0.1 M NaPi pH 6.8–7.5, 2 mM $MgCl_2$, 0.02% NP40, 0.01% deoxycholate) and the embryos were stained in betaGal buffer containing 50 μl 2% X-Gal in DMF, 10 μl 0.5 M $K_3Fe(CN)_6$, 10 μl 0.5 M $K_4Fe(CN)_6$, 2 μl 0.5 M EGTA pH 8 per ml at 37°C. The required staining time was 12–36 h. Embryos were analysed and photographed in PBST and stored in 70% EtOH in the coldroom.

Sectioning of embryos

Stained embryos were dehydrated through an ethanol series (70%, 90%, 95%, 100%, 100% ethanol), ethanol was replaced by acetone, finally the embryos were incubated overnight at room temperature in a 1:1 mixture of acetone and Araldite. Araldite was Durcupan ACM from Fluka and was prepared by mixing 54.33 gram of reagent A with 47.41 gram of reagent B, then adding 3.5 ml of reagent D and finally 2 ml of reagent C. The mixture was stored frozen (−20°C) and allowed to warm to room temperature before use. Block formers were filled with the Araldite mixture, the embryos transferred to the block formers and kept at room tempera-

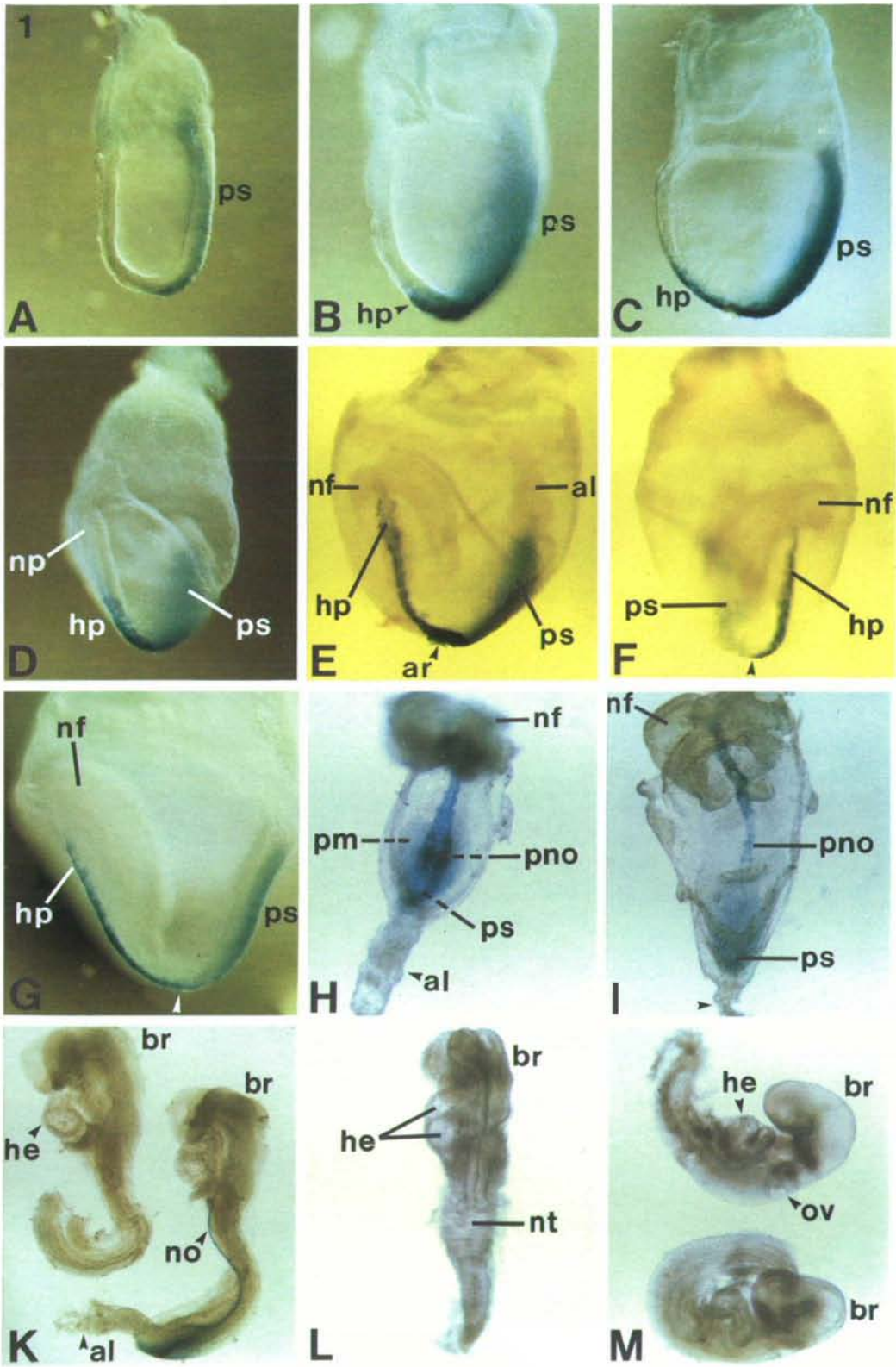

Fig. 1. Localisation of *T* gene transcripts in whole-mount normal and mutant T^{Wis}/T^{Wis} embryos. Embryos were hybridized *in situ* with biotin-labelled antisense RNA prepared from 1.8 kb of the *T* gene cDNA pme75 (Herrmann *et al.* 1990). The blue stain, resulting from beta-galactosidase activity, indicates *T* transcripts. All embryos have been derived from $T^{Wis}/+ \times T^{Wis}/+$ matings. 60 embryos of stages 7–7.75 days did not reveal visible differences between normal and mutant T^{Wis}/T^{Wis} embryos. (A, B, C, D) 7, 7.3, 7.5 and 7.75 day embryos, respectively; (E, H) normal 8 or 8.25 day embryos, respectively; (F, G) 8 day, (I) 8.25 day mutant embryos, note abnormalities of the archenteron/node and the allantois compared to normal embryos; (K) comparison of 8.5 day mutant (left) and normal (right) embryos; (L) 9 day mutant embryo; (M) 9.5 day mutant (upper) and normal (lower) embryos. Embryos in L and M have not been hybridized. (A, B, C, G, K, M) lateral view; (D, E, F) half frontal view; (H, L) dorsal, (I) ventral view. Abbreviations: ps, primitive streak; hp, head process; np, neural plate; nf, neural fold; ar, archenteron/node; al, allantois; pm, presomitic mesoderm; pno, notochord precursor in I, node region with emerging notochord precursor in H; he, heart; br, brain; no, notochord; nt, neural tube of extremely wavy appearance; ov, otic vesicle; arrowheads in F, G and I indicate abnormal archenteron/node or allantois, respectively. Cell types located underneath the dorsal primitive ectoderm/neurectoderm in H, are indicated by broken lines.

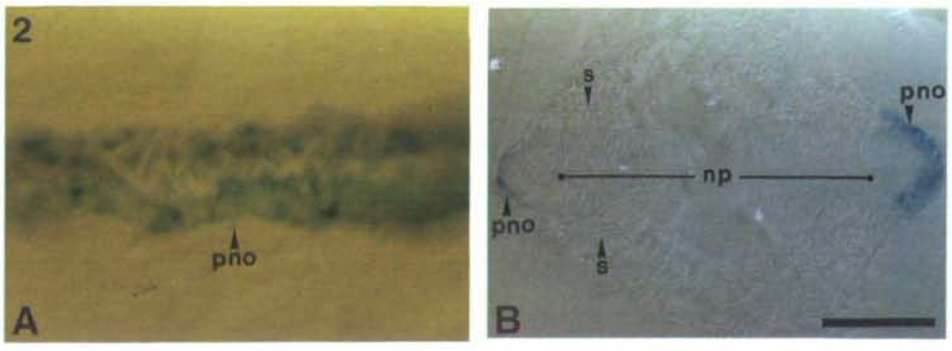

Fig. 2. High magnification photographs of an 8.25 day embryo showing the expression of the *T* gene by *in situ* hybridization (same embryo as in Fig. 1H). Fig. 2A shows a ventral view of part of the notochord precursor of the whole-mount embryo; due to the curvature of the embryo, only a subregion is in focus. Fig. 2B shows a transverse section of the same embryo. This section is from the distal half of the curved embryo. The anterior part of the embryo is to the left. Note that posteriorly (on the right) the notochord precursor is wider than anteriorly, demonstrating its wedge-shaped structure. In addition, the anterior neural plate has almost formed a tube and is flanked by two somites, while the younger posterior neural tube and flanking presomitic mesoderm are less developed. Abbreviations: np, neural plate; pno, notochord precursor; s, somite. Scale bar: 100 micrometer.

ture for 2 h. The embryos were oriented for sectioning, blocks were polymerized at 60°C for 1.5 days and sectioned (7 micrometer) on a Reichert-Jung Supercut 2050 microtome. The sections were mounted in Araldite and photographed on a Zeiss Axiophot with Nomarski optics.

Results and discussion

Fig. 1A–K shows the expression of the *T* gene in whole-mount embryos at early primitive streak (7 days) to early somitogenesis stage (8.5 days). Mesoderm formation begins at about 6.5 days in the primitive streak which is formed at the posterior end of the embryonic axis of the egg cylinder. *T* gene expression is first observed in the early primitive streak (not shown). In 7 day embryos, *T* gene transcripts are also detected in cells along the midline of the posterior half to the distal tip of the egg cylinder (Fig. 1A), which might indicate an anterior extension of the primitive streak. The expression appears to increase at least from the early (Fig. 1A) to the mid primitive streak stage (Fig. 1B). At 7.3 days post coitum (*p.c.*), cells expressing *T* form the head process (Fig. 1B), the anterior end of which comes to lie at the forebrain-midbrain boundary (not shown). The head process is contiguous with the notochord precursor (Fig. 1H); it differentiates into the notochord (Fig. 1K). The neural plate is visible at about 7.75 days *p.c.* (Fig. 1D). In cells of the head process/notochord precursor, *T* appears to be expressed more strongly than in cells in the primitive streak (not shown); it is down-regulated to undetectable levels in mesoderm cells migrating away from the streak (Fig. 1A–E, H) (Wilkinson *et al.* 1990). Mutant (T^{Wis}/T^{Wis}) embryos of the stages 7–7.75 days could not be identified and distinguished from normal ($T^{Wis}/+$ or $+/+$) embryos, either by the level of transcription or the tissue distribution of the transcripts. The archenteron/node of the normal 8 day embryo is a plate with a large number of *T*-expressing cells. It marks the posterior extreme of the head process/notochord precursor (Fig. 1E) which is wedge shaped, widening towards the node region (Fig. 1H and 2). However, in 8 day T^{Wis}/T^{Wis} embryos the archenteron/node is disrupted (Fig. 1F, G), the first visible difference between normal and mutant embryos. Defects in the archenteron region of T/T embryos have been reported previously (Fujimoto and Yanagisawa, 1983).

In contrast to the normal embryo (Fig. 1E, H, K), the expression of *T* in the homozygous mutant ceases between 8 and 8.5 days *p.c.* (8 somite stage) in the primitive streak and the head process/notochord precursor (Fig. 1F, G, I, K). The extension of the notochord precursor comes to a halt at around 8 days of gestation. Thereafter, the mutant embryos develop a kink in the streak posterior to the end of the notochord precursor (Fig. 1I, K), and the expression of *T* decreases over several hours in the primitive streak and the head process/notochord precursor. The posterior extreme of the primitive streak is the last structure where transcripts of the *T* gene are detectable (not shown). In contrast, *T* gene expression persists in the normal embryo in the primitive streak, and in the notochord and its derivative (Fig. 1H, K) (Wilkinson *et al.* 1990). The disappearance of transcripts in the head process/notochord precursor correlates with its inability to differentiate into notochord (Chesley, 1935), a consequence of the lack of functional *T* product (Rashbass *et al.* 1991). Axial elongation is disrupted (Fig. 1L, M), the neural tube posterior to the hind brain develops abnormally (Fig. 1L) and somites are not formed (not shown), a result very similar to what is found in T/T embryos, which show a kinked neural tube and form several abnormal somites (Chesley, 1935).

The expression of the *T* gene in normal embryos has two distinguishable patterns. In notochord cells it persists throughout embryogenesis at least until 17.5 days of gestation, whereas in the mesoderm generated in the primitive streak it is down regulated to undetectable levels soon after ingression (Wilkinson *et al.* 1990). The analysis of T/T embryonic stem cell chimeras demonstrated that the maintenance and differentiation of the notochord requires the cell-autonomous action of the *T* gene (Rashbass *et al.* 1991). The notochord of such chimeras was either discontinuous or contained pycnotic cells. Dead cells were also seen amongst some nascent mesoderm cells emerging from the primitive streak (Rashbass *et al.* 1991). Thus the T gene may have different functions in different cells, which might correspond to the level of its expression and/or the interaction of its product with other gene products expressed differentially in the embryo. The phenotype of T^{Wis}/T^{Wis} embryos seems to be the sum of two defects, the disruption of the notochord precursor in the node and the standstill of mesoderm formation in the primitive streak.

A number of mesoderm abnormalities of T/T embryos have been reported, including a strongly reduced allantois (Glücksohn-Schönheimer, 1944), reduced mesoderm cell motility (Hashimoto *et al.* 1987) and galactosyltransferase activity (Shur, 1982), abnormalities of the extracellular matrix (Jacobs-Cohen *et al.* 1983) and a reduced mesoderm/ectoderm ratio (Yanagisawa *et al.* 1981). These abnormalities occur in mesoderm generated in the primitive streak. In 8 day T/T embryos an abrupt increase of mesodermal cells in the posterior end of the primitive streak, combined with a defiency of mesodermal cells in the more anterior region of the streak, and a dense appearance of the streak, indicating a high cell density in the primitive ectoderm, have been described (Yanagisawa *et al.* 1981). Since the proliferation rate of T/T cells is unchanged, it was assumed that the mesodermal cells of the posterior end of the streak were abnormal in their ability to migrate away from the streak (Yanagisawa *et al.* 1981). It is intriguing that this anomaly occurs fairly abruptly at around 8 days *p.c.*, when the disruption of the node becomes apparent, whereas younger T/T embryos showed only slightly reduced mesoderm/ectoderm ratios compared to normal embryos.

T/T embryonic stem cell chimeras develop a reduced allantois due to spreading of allantoic cells onto the

amnion. The degree of disruption of the allantois correlates with the percentage of chimerism. However, there is no apparent selection against T/T cells contributing to the allantois, which does not express the T gene. Apparently, affected cells are not unable to form mesoderm or to migrate, but possibly are inappropriately specified (Rashbass *et al.* 1991). Similarly, the somites and neural tube of homozygous mutant embryos are abnormal (Chesley, 1935; Grüneberg, 1958). The abrupt changes in the primitive streak, and the abnormalities of prospective allantoic and somitic cells and the neural tube might be due to inappropriate axis organizing activity. This interpretation emphasizes the role of the notochord precursor in the node in organizing axial development, in particular the patterning of the mesoderm generated in the primitive streak, comprising the paraxial to ventral and extraembryonic mesoderm.

The Hensen's node of the chick embryo has been analyzed in some detail. It is generally considered to be the 'organizer' of the amniote embryo since it can differentiate into a number of tissues and can induce a second embryonic axis when grafted into a host embryo (for review see Leikola, 1976). Spratt (1955) has subdivided this structure into the central chorda center and two lateral somite centers. In excision experiments, removal of the entire node area lead to a cessation of the formation of mesodermal axial organs by the remainder of the blastoderm. Segmental plate and neural tissue sometimes, however, developed posterior to the hole. This phenotype resembles that of T/T or T^{Wis}/T^{Wis} embryos, with the notable difference that in Spratt's experiment the node was physically removed rather than disrupted due to loss of a particular gene product.

Recently Selleck and Stern (1991) showed that the somitic precursors in the node contribute only to the medial halves of the somites, and that single cells in regions between the prospective notochord and somite areas of the node contribute progeny to both the notochord and the somites. Progeny cells that contribute to the somites always come to lie rostral to their sister cells that become part of the notochord. This may explain why heterozygous $T^c/+$ (Searle, 1966) and $T^{Wis}/+$ (unpublished observations) mice frequently show vertebral abnormalities rostral to the tail, missing due to the lack of a notochord in the caudal region (Gluecksohn-Schoenheimer, 1938; Yanagisawa, 1990). If the notochord/somite progenitor cells express the T gene, the skeletal malformations in the mutants might stem from insufficient amounts of T product in the affected somitic cells. If T expression is confined to notochord precursor cells after separation into the notochord and somite lineages, inappropriate specification of the somitic cells by a signal from the notochord precursor would be a more likely explanation for the skeletal defects in the mutants. In any case the T gene would be strongly implicated.

In the amphibian embryo mesoderm is formed by the inductive interaction between endoderm and ectoderm (Nieuwkoop, 1979). Peptide growth factors are implicated in this induction process (Slack *et al.* 1987; Kimelman and Kirschner, 1987; Smith *et al.* 1988). However, patterning of the mesoderm and ectoderm is believed to be achieved by another signal coming from the 'organizer' in the dorsal blastopore lip (Smith and Slack, 1983). Removal of the dorsal blastopore lip from midgastrulation embryos leads to mesoderm defects in the trunk, removal from late gastrulae to mesoderm defects in the tail (Lehmann, 1926). These defects are reminiscent of the phenotypes of the T^{Wis}/T^{Wis} and T/T, or $T/+$ embryos, respectively, supporting the idea that the T mutant phenotype primarily results from the disintegration of the notochord precursor in the node.

In the mouse, the notochord precursor and the node of 8–8.25 day embryos, as visualized by *in situ* hybridization with the T gene probe, appear as a wedge-shaped structure ending in a broad plate. Either the wedge-shaped structure is extending posteriorly, or part of the cells switch off the T gene, eventually forming a narrow line of cells, or both. The definitive gut endoderm and the notochord precursor of the mouse emerge from the node together, whereby the notochord precursor initially forms a plate in the roof of the gut before it separates from it to form a rod-like structure (Jurand, 1974; Poelmann, 1981). Some of the T-expressing cells of the wedge-shaped notochord precursor might switch off the T gene and contribute to the formation of the gut. The experiments of Selleck and Stern (1991) on the chick suggest that extension of the notochord precursor relative to the somites also plays a role in notochord development.

In T mutants a correlation between gene dosage and severity of the phenotype has been observed (McMurray and Shin, 1988; Yanagisawa, 1990). Posteriorly, notochord formation has a higher requirement for functional T gene product than anteriorly. The early head process/notochord precursor formation is achieved even in the absence of the T product. Differentiation of the head process/notochord precursor into the notochord, however, requires a functional T gene product. There is genetic evidence for a mutation linked to T which can at least partially complement the T phenotype (Lyon and Meredith, 1964). A simple assumption would be that this other gene product may resemble that of the T gene. Anterior notochord precursor formation might require a minimal amount of the T or T-like products, whereas completion of the tail might need the concerted activity of both genes.

Recent work has shown that the T gene of *X. laevis* is expressed by competent cells in response to the mesoderm-inducing peptide growth factors XTC-MIF and bFGF, even when protein synthesis is blocked (Smith *et al.* 1991). Prospective notochord cells in the mouse might respond in a similar way to a signal involved in notochord cell recruitment. Since the T gene appears to be, directly or indirectly, involved in axis organization, a positive feed-back interaction between T gene expression and the axis organizing and/or notochord recruiting signal(s) seems possible.

Consequently the T gene might provide access to the

identification and cloning of factors required for mesoderm formation and axial organisation of the mouse embryo.

I thank U. Schwarz for support, D. Weigel and A. Authaler for technical assistance, U. Schwarz, R. Beddington, D. Stott and A. Kispert for discussions and critical comments on the manuscript, W. Dove and J.-L. Guenet for mice carrying the T^{Wis} allele, H. Sinner for maintenance of mouse stocks and R. Schnabel, R. Grömke, W. Herdt and H. Schwarz for help with microscopy and photography.

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(Accepted 18 August 1991)