Expression of inhibin subunits and follistatin during postimplantation mouse development: decidual expression of activin and expression of follistatin in primitive streak, somites and hindbrain

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SUMMARY

Members of the activin family are believed to act as mesoderm-inducing factors during early amphibian development. Little is known, however, about mesoderm formation in the mammalian embryo, and as one approach to investigating this we have studied activin and follistatin expression during early mouse development. Activins are homo- or heterodimers of the $\beta_A$ or $\beta_B$ subunits of inhibin, itself a heterodimer consisting of one of the $\beta$ subunits together with an $\alpha$ subunit. Follistatin is a single-chain polypeptide which inhibits activin function.

Expression of the inhibin $\alpha$ chain could not be detected in embryonic or extraembryonic tissues at any of the stages studied (5.5 to 8.5 days) and expression of the $\beta_A$ and $\beta_B$ subunits could only be observed in the deciduum in cells surrounding the embryo. Expression of follistatin could also be detected in the deciduum, but in a pattern complementary to that of the $\beta$ subunits. Embryonic expression of follistatin first occurred in the primitive streak, and at later stages transcripts were detectable in the somites and in rhombomeres 2, 4 and 6 of the hindbrain. These results are consistent with a role for activin in mesoderm formation in the mouse embryo, and suggest functions for follistatin in addition to its role as an inhibitor of activin.

Key words: mouse embryo, mesoderm induction, primitive streak, somites, hindbrain, activin, follistatin

INTRODUCTION

Mesoderm formation in amphibian embryos occurs as the result of an inductive interaction in which cells of the vegetal hemisphere of the embryo act on overlying equatorial cells (see reviews by Kimelman et al., 1992; Jessell and Melton, 1992; Sive, 1993; Beddington and Smith, 1993). Candidate ‘mesoderm-inducing factors’ include members of the fibroblast growth factor (FGF) and transforming growth factor type $\beta$ (TGF-$\beta$) families. Of the TGF-$\beta$ family, the activins are perhaps the most potent inducing agents (see above reviews), and evidence that they do play a role in mesoderm induction in $Xenopus$ comes from experiments in which a truncated form of a type IIB activin receptor is over-expressed; in many cases this abolishes mesoderm formation completely (Hemmati-Brivanlou and Melton, 1992).

The activins are homo- or heterodimers of the $\beta_A$ or $\beta_B$ subunits of inhibin, itself a heterodimer consisting of one of the $\beta$ subunits together with an $\alpha$ subunit (Ling et al., 1988). In studying the activins, it is therefore necessary to analyse the expression of all three subunits in order to know which of the dimers might be present.

Although much is known about mesoderm induction in amphibia, virtually nothing is known about how mesoderm arises in mammalian embryos. In a recent experiment, Blum et al. (1992) showed that activin treatment of early mouse embryos causes the homeobox-containing gene goosecoid to be expressed throughout the entire epiblast, rather than being restricted to the anterior end of the primitive streak. This is consistent with the idea that mesoderm in mammalian embryos arises through an inductive event involving an activin-like molecule, but the timing of induction, the location of the inducing cells and the identities of the natural inducing molecules are all unknown (see Beddington and Smith, 1993).

In this paper, we continue our investigation of the potential role of activin in mesoderm induction in the mouse embryo. In previous work (Albano et al., 1993), we studied pre-implantation embryos, and found that activin is present in all cells of the morula, that it becomes restricted to the inner cell mass of the blastocyst at 3.5 days, but that the situation is reversed at 4.5 days, with expression confined to the trophectoderm. Here, therefore, we study activin expression at 5.5 to 8.5 days of development. Our results indicate that activins are not expressed in the embryo proper during these stages, but that transcripts are present at
high levels in the deciduum. There is, however, no evidence for asymmetric localisation of activin expression in the deciduum, and this raised the possibility that the effects of activin might be modulated by activin-binding proteins such as follistatin (Nakamura et al., 1990), which inhibits the mesoderm-inducing activity of activin (Asashima et al., 1991). We find that follistatin is expressed in the deciduum in a complementary pattern to the inhibin

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(\text{\textit{M}} \quad \text{\textit{V}} \quad \text{\textit{C}} \quad \text{\textit{A}} \quad \text{\textit{R}} \quad \text{\textit{H}})

The follistatin probe was prepared as described by Albano et al. (1993). The follistatin probe was prepared by performing the polymerase chain reaction (PCR) on reverse transcribed rat ovary RNA using specific rat follistatin primers (Shimasaki et al., 1989). Approximately 80 hybridising plaques were obtained from the primary screen and the strongest positives were subjected to further rounds of screening. The purified plaques were rescued and analysed by restriction enzyme mapping and digestion sequencing. The longest cDNA obtained (fol 1) was approximately 0.8 kb. When compared with rat follistatin, fol 1 proved to contain most of the protein coding sequence, but it lacked 278 bases 3′ to the open reading frame of mouse follistatin followed by the 3′ untranslated region.

**MATERIALS AND METHODS**

**Mouse embryos**

Mouse embryos were obtained from matings of the outbred strain MF1 or from crosses between F1 CBA × C57BL10 females and MF1 or F1 CBA × C57BL10 studs. Noon on the day of appearance of the vaginal plug was taken as 0.5 days of development.

**cDNA cloning of follistatin**

1×10⁶ plaques of a Lambda Unizap (Stratagene) mouse ovary cDNA library were screened as previously described (Albano et al., 1993). A 440 base pair follistatin probe was obtained by performing the polymerase chain reaction (PCR) on reverse transcribed rat ovary RNA using specific rat follistatin primers (Shimasaki et al., 1989). Approximately 80 hybridising plaques were obtained from the primary screen and the strongest positives were subjected to further rounds of screening. The purified plaques were rescued and analysed by restriction enzyme mapping and digestion sequencing. The longest cDNA obtained (fol 1) was approximately 0.8 kb. When compared with rat follistatin, fol 1 proved to contain most of the protein coding sequence, but it lacked 278 bases 3′ to the open reading frame of mouse follistatin followed by the 3′ untranslated region.

**RNAase protection assays**

These were performed according to the method of Krieg and Melton (1987). DNA was obtained by the method of Chomczynski and Sacchi (1987) or by extraction with lithium chloride (Auffray and Rougeon, 1979). Inhibin α, βA, and βB probes, and a 4′-yactin loading-control probe, were as described by Albano et al. (1993). The follistatin probe was prepared by annealing 100 pmol of a 4′-yactin loading-control probe to 100 pmol of a 4′-yactin loading-control probe in the presence of 0.5 M LiCl.

**Fig. 1.** The sequence of the open reading frame and 3′ untranslated region of mouse follistatin. The HindII/EcoRI fragment used as RNAase protection and in situ hybridisation probes is underlined. Potential sites of asparagine-linked glycosylation are in bold type.
by subcloning a BamHI/HincII fragment of fol 1 into pBluescript KS+ (the BamHI site is derived from the polylinker of pBluescript SK+), digesting with EcoRV and transcribing with T3 RNA polymerase.

In situ hybridisation

Radioactive in situ hybridisations were carried out on 5 μm sections as described by Wilkinson and Green (1990). 35S-labelled probes specific for inhibin α, βA, and βB chains were as described by Albano et al. (1993), and the follistatin probe was as above. Whole-mount in situ hybridisations were carried out according to Wilkinson (1992), using the hybridisation conditions of Rosen and Beddington (1993). Probes were as above. 8 μm sections were prepared from whole-mount specimens following dehydration through an alcohol series, clearing in Histoclear (National Diagnostics) and embedding in paraffin wax (Histoplast, m.p. 56°C).

RESULTS

Mouse follistatin

Alternative splicing of pig, human and rat follistatin precursor RNA results in the formation of two precursor proteins of 344 or 317 amino acid residues (Esch et al., 1987; Shimasaki et al., 1988a,b, 1989). The smaller of the two, a carboxy-truncated form of the larger, lacks the C-terminal 27 amino acids encoded by the last exon. Both precursors contain an identical signal peptide sequence of 29 amino acids, so that the mature forms of the two precursors consist of 315 (FS-315) and 288 (FS-288) amino acids respectively. Most of the follistatin derived from pig ovaries comprises 303 amino acids, which is probably derived from FS-315 by proteolytic cleavage of the 12 carboxy-terminal residues. This form of follistatin is less potent than FS-288 in inhibiting the function of activin, but more potent than FS-315. Interestingly, these differences reflect the different abilities of the three forms of follistatin to bind to heparan sulphate proteoglycan, suggesting that cell-associated follistatin traps activin more tightly in the extracellular matrix (Inouye et al., 1991; Sugino et al., 1993).

The composite mouse follistatin cDNA shown in Fig. 1 is that of the larger form of follistatin. Its amino acid sequence is 99% identical to that of rat, but there are 343 amino acids rather than 344; the lysine at amino acid 241 in the rat sequence is absent in mouse. Potential sites of asparagine-linked glycosylation are in bold type. The HincII/EcoRV fragment used as RNAase protection and in situ hybridisation probes is underlined. RNA encoding the 343 amino acid precursor protects a 318 nucleotide fragment; assuming the structure of the mouse gene resembles that of the rat, RNA encoding the 317 amino acid precursor should protect fragments of 212 and 106 nucleotides.

Temporal expression patterns of inhibin subunit and follistatin mRNA

To study the temporal expression patterns of the inhibin chains and of follistatin during early mouse development, RNA was extracted from the embryonic constituent of the conceptus at 7.5 to 14.5 days of development and analysed by RNAase protection (Figs 2, 3). RNA was also isolated from extraembryonic regions of 7.5 day embryos, including Reichert’s membrane and adherent decidual cells. Significant inhibin subunit expression does not start until the onset of organogenesis: RNA encoding all three inhibin subunits is only clearly detectable from 10.5 days and persists at least until 13.5 days. When the extraembryonic regions of 7.5 day embryos were analysed, however, strong expression of the βA and βB subunits was observed (Fig. 2). No expression of the α subunit was detected.
RNAase protection showed that follistatin expression in the mouse embryo starts earlier than expression of the inhibin subunits; follistatin transcripts are present from 8.5 days until at least 14.5 days of development (Fig. 3). In two out of three experiments the probe protected exclusively a 318 nucleotide fragment, indicating that as in the rat (Michel et al., 1990), RNA encoding the larger form of follistatin (343 amino acids) is much more abundant than that encoding the smaller form. In the other experiment weak bands were also observed at 212 and 106 nucleotides, showing that RNA encoding the 317 amino acid precursor is, nevertheless, present. In situ hybridisation confirms the timing of follistatin expression, and indicates that it is under way as early as 6.5 days (see below).

Spatial expression patterns of the inhibin subunits

Implantation of the mouse embryo begins at 4.5 days of development and mesoderm formation at about 6.5 days. We therefore started our in situ hybridisation analysis of inhibin
Activin, follistatin and mouse embryos subunit expression at 5.0 to 5.5 days of development (Figs 4, 5). This stage precedes mesoderm formation and by this time the decidual reaction has begun and the implantation sites are easily identified as spaced bulges in the uterus. No inhibin α subunit expression was observed in any of the embryonic or extraembryonic tissues analysed at any stage. β subunit expression was not observed in embryonic tissues until at least 8.5 days, when whole-mount in situ hybridisation appeared to reveal weak expression of both βA and βB subunits in the heart (not shown).

Uterine expression of the βA subunit is first detectable at 5.0-5.5 days in a few cells of the primary decidua near the distal tip of the embryo (Fig. 4A,B) while βB subunit expression starts in two patches of decidual cells in the inner zone around the ectoplacental cone (Fig. 5A,B). By 6.5 days

Fig. 4. Expression of the inhibin βA chain during early postimplantation mouse development studied by in situ hybridisation. (A,C,E,G) Bright-field images; (B,D,F,H) the corresponding dark-field images. (A,B) Longitudinal section at 5.5 days. Low levels of expression are visible in a few cells of the primary decidual zone near the distal tip of the embryo. (C,D) Longitudinal section at 6.5 days. Expression is detectable around the entire embryo and ectoplacental cone and in cells of the mesenchyme underlying the uterine epithelium above the ectoplacental cone. (E,F) Transverse section at 6.5 days shows expression surrounding the entire embryo. (G-J) Longitudinal sections at 7.5 (G,H) and 8.5 (I,J) days. βA subunit expression has declined and is no longer observed around the embryo but it is confined to cells underneath the luminal uterine epithelium and around the ectoplacental cone. Scale bar, 100 μm (B); 200 μm (D,H,I) and 100 μm (F).

Fig. 5. Expression of the inhibin βB chain during early postimplantation mouse development studied by in situ hybridisation. (A,C,E,G) Bright-field images; (B,D,F,H) the corresponding dark-field images. (A,B) Longitudinal section at 5.5 days. Expression is visible in two patches of decidual cells in the inner zone around the ectoplacental cone (C-F). Longitudinal sections at 6.5 (C,D) and 7.5 (E,F) days. At 6.5 days βB transcripts are present around the embryo and in cells surrounding the ectoplacental cone. At 7.5 days transcripts are only present around the ectoplacental cone. Apparent expression in cells around the entire embryo in (E,F) is due to blood cells. (G,H) Control longitudinal section at 6.5 days probed with a βB sense transcript. Scale bar, 200 μm for all panels.
βA subunit expression surrounds the whole embryo. Strong expression is also observed around the ectoplacental cone and in cells of the mesenchyme underlying the uterine epithelium above the ectoplacental cone (Fig. 4C-F). A cross section of the 6.5 day embryo shows that there is no asymmetric expression of the βA subunit (Fig. 4E,F). Decidual expression of the βB subunit occurs in cells surrounding the ectoplacental cone (Fig. 5C-F).

At 7.5-8.5 days, βA subunit expression has decreased and is no longer observed around the embryo but it is confined to cells underneath the luminal uterine epithelium and around the ectoplacental cone (Fig. 4G-J). βB subunit expression was also greatly reduced, with weak expression only detectable around the ectoplacental cone (Fig. 5E,F).

The results described in this part of the paper confirm those of Manova et al. (1992) for the inhibin βA chain, and extend their observations to include the βB chain.

**Decidual expression pattern of follistatin**

Expression of follistatin in decidual tissue occurs in a pattern complementary to that of the inhibin βA chain. At 5.5 and 6.5 days, follistatin expression is low in regions immediately surrounding the embryo, where βA subunit expression is high (see Fig. 4), but much stronger in cells nearer the periphery of the deciduum (Fig. 6). Decidual follistatin expression is significantly reduced by 7.5 days, and is now only detected in the outer cells (Fig. 7). Follistatin transcripts are now present, however, in parietal endoderm cells and in the primitive streak of the embryo (Fig. 7). To examine this embryonic expression of follistatin in more detail we turned to whole-mount in situ hybridisation.

**Embryonic expression pattern of follistatin**

Follistatin transcripts were observed in early streak-stage embryos at 6.5 days and were localised to the region of the primitive streak (Fig. 8A) and to parietal endoderm (see Fig. 7). A day later, high levels of follistatin mRNA persisted in the primitive streak (Fig. 8B) and in a spur of paraxial mesoderm underlying the cranial neural folds (Fig. 8C). Sections of 7.5 day embryos (Fig. 9A) showed hybridisation in both the ectodermal and mesodermal germ layers in the vicinity of the streak; this expression extended laterally to approximately half-way round the cylinder. Expression in the streak persisted at the headfold-stage but the ventral population of cells in the node did not appear to contain follistatin mRNA (Fig. 9B).

Transcripts declined in the primitive streak around the 4-5-somite stage, and by the 8- to 10-somite stage no signal could be detected in this region (Fig. 8D). High transcript levels were, however, evident in the somites and paraxial mesoderm.

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**Fig. 6.** Expression of follistatin during early postimplantation mouse development studied by in situ hybridisation. (A,C,E,G) Bright-field images. (B,D,F,H) are the corresponding dark-field images. (A-D) Longitudinal sections at 5.5 (A,B) and 6.5 (C, D) days. Expression of follistatin is low in regions immediately surrounding the embryo but stronger in cells nearer the periphery of the deciduum. (E,F) Transverse section at 6.5 days shows low levels of follistatin transcripts in cells immediately surrounding the embryo, but higher levels towards the periphery. (G,H) Control transverse section at 6.5 days probed with a follistatin sense transcript. Scale bar, 200 μm, for all panels.
of the cranial region (Figs 8D, 9C) and low levels of mRNA could be detected in the presomatic mesoderm. In addition, by the 5-somite stage two prominent stripes of expression could be distinguished in the developing hindbrain (Fig. 8D). By 9.5 days it was clear that neurulated expression was limited to rhombomeres 2, 4 and 6 (Fig. 8E,G), although transcripts were absent in the ventral midline of the hindbrain (data not shown). Expression was still evident in the cranial paraxial mesoderm at 9.5 days up to the forebrain/midbrain junction as well as in all mature somites (Fig. 8E-G). Highest levels were present in the dermamyotome (not shown). Caudally, a graded hybridisation signal was apparent in the presomatic mesoderm, the levels of mRNA increasing towards its rostral aspect (Fig. 8F). However, follistatin transcripts were undetectable in the most rostral presomatic cells (those about to undergo segmentation) and in the most recently formed somite (Fig. 8E,F), although there is a narrow trail of positively stained cells, of unknown character, immediately ventrolateral to the somites in this region (Fig. 8E,F).

Whole-mount in situ hybridisation using probes specific for inhibin α, βA and βB subunits did not detect expression of these genes in primitive streak, somites or hindbrain at any of these stages (not shown).

**DISCUSSION**

The mesoderm of amphibian embryos such as *Xenopus* is formed through an inductive interaction in which signals from the vegetal hemisphere of the embryo, one of which is believed to be activin, act on overlying animal pole cells (see Introduction). Little is known about mesoderm formation in the mammalian embryo, however, and to investigate this we have examined the expression patterns of the inhibin α, βA, and βB chains, and of follistatin, during post-implantation mouse development. Our results, like those of Manova et al. (1992), show that activin is not expressed in the embryo during mesoderm formation, but that βA and βB transcripts are present in decidual tissue surrounding the embryo at the appropriate stages. Transcripts encoding the inhibin α chain are not detectable, indicating that activin, rather than inhibin, is likely to be present. Our data also show that expression of follistatin occurs in the deciduum, but in a pattern complementary to that of activin, such that it might not be expected to inhibit the function of decidual activin. The temporal and spatial expression patterns of the inhibin β subunits and of follistatin are summarised in Fig. 10.

Might activin derived from the deciduum act as an embryonic mesoderm-inducing factor, as suggested, for example, by Manova et al. (1992)? If so, the most likely candidate is activin A, a homodimer of the βA subunit, because Vassalli et al. (1994) have recently shown that mice deficient in the inhibin βB subunit are viable and capable of carrying pregnancies to the end of gestation. The inhibin βA subunit is indeed expressed at high levels around the embryo at the time of mesoderm formation, and follistatin, which inhibits the mesoderm-inducing action of activin, is excluded from this region. Since follistatin is also expressed in parietal endoderm, it is not clear whether activin derived from decidual tissue could reach the embryo proper to act as a mesoderm-inducing factor. However, it is noteworthy that mice homozygous for a mutated TGF-β1 allele show variable ability to survive to birth, and it has been suggested that the survival of some homozygous animals is due to provision of TGF-β1 by the deciduum (Shull et al., 1992; see also Kulkarni et al., 1993). Further experiments are in progress to resolve this issue.

It is also possible that activin A performs a function in early mouse development distinct from mesoderm induction. One possibility, mentioned below, is that it is involved in mesodermal patterning. In addition, activin is known to have potent mitogenic activity for embryonal carcinoma cells (Hashimoto et al., 1990; Schubert and Kimura, 1991). EC cells share properties with cells of the epiblast, which undergo rapid growth during postimplantation development (Snow, 1977), and it may be that their rapid rate of cell division is due in part to activin derived from the deciduum.

**Follistatin and early mouse development**

The inhibin βA subunit is expressed around the entire circumference of the embryo (Fig. 4E,H), so if it acts as a decidually derived mesoderm-inducing factor, it remains unclear how polarity of the egg cylinder is established so that mesoderm formation is localised. One naïve view would be that polarity is established in the embryo, rather than in the deciduum, by localising expression of an inhibitor of activin function to the anterior region of the embryonic egg cylinder. If this were the case, activin would only be able to induce a primitive streak in the posterior region. To our surprise, for at least one inhibitor of activin function, the opposite was true: follistatin is expressed at high levels in the primitive streak itself and in epiblast cells destined to ingress through the streak (Figs 8A,B, 9A; see Lawson et al., 1991). At first sight, this observation might argue against a role for activin in mesoderm induction.

![Fig. 7. Expression of follistatin at 7.5 days of mouse development. (A) Bright-field, (B) dark-field views. Decidual expression of follistatin has declined, and persists only in the extreme periphery of the deciduum (arrow). Transcripts are visible, however, in the primitive streak of the embryo proper and in parietal endoderm cells. Bright patches are blood cells. Scale bar, 350 µm.](image-url)
Fig. 8. Whole-mount in situ hybridisation of follistatin expression in early mouse development. (A) Posterior view of a 6.5-day early primitive streak stage embryo. Expression is restricted to the primitive streak region. (B) Lateral view of 7.5-day embryos. Follistatin transcripts are localised to the primitive streak and adjacent mesoderm and ectoderm. (C) Lateral view of headfold-stage embryos. Expression persists in the primitive streak and follistatin mRNA is also detected in paraxial mesoderm (arrowhead). (D) Dorsal view of 8.5 day early somite-stage embryos; the youngest embryo is on the right and the oldest on the left. Transcript levels decline in the primitive streak region, and cannot be detected by the 10-somite stage. Strong expression is evident in the paraxial mesoderm and two stripes are present in rhombomeres 1 and 2, and 4 of the hindbrain neurectoderm. (E) Lateral view of 9.5 day embryos. Three stripes of expression are apparent in the hindbrain neurectoderm corresponding to rhombomeres 1 and 2, 4 and 6. Transcripts are evident in all mature somites and in the cranial paraxial mesoderm extending rostrally to the midbrain/forebrain junction (embryo on the left). Expression is detectable in presomitic mesoderm but is absent in the most recently formed somite and in the most rostral aspect of the presomitic mesoderm. There is a narrow trail of ventrolateral cells expressing follistatin in this region (arrowhead). (F) Expression in the paraxial mesoderm of an 18-somite stage embryo. Transcript levels increase in a graded manner from caudal to rostral in the presomitic mesoderm and, except for the most recently formed somite and for mesoderm in the process of segmenting (arrowhead), they remain high in the somites. (G) Dorsal view of the hindbrain region of an 18-somite stage embryo, showing the alternating pattern of rhombomeric expression and the presence of transcripts in the cranial paraxial mesoderm (arrowhead). Scale bar, 250 μm (A); 200 μm (B,C,F); 300 μm (D,G), and 400 μm in (E).
in the mouse. However, mesoderm induction may occur before the onset of zygotic expression of follistatin, as it does in Xenopus (Tashiro et al., 1991). In this case, follistatin expression might represent a rapid consequence of mesoderm induction and could serve to limit the spread of the inducing signal. If the function of activin is to pattern mesoderm rather than to induce it, the presence of follistatin in posterior regions might inhibit its function here while allowing it to remain active in anterior tissue.

It has been argued that neural differentiation in Xenopus occurs in cells that do not receive an activin signal (see Hemmati-Brivanlou and Melton, 1992), and this might suggest that follistatin, which blocks activin signalling, acts as a neural-inducing factor in the mouse embryo. However, if this were the case, one might expect follistatin to be expressed in an anterior rather than a posterior domain in the embryonic egg cylinder.

**Additional functions for follistatin?**

Examination of the expression pattern of follistatin suggests that it may have functions unrelated to inhibition of activin. This is particularly true at 8.5-9.5 days, when activin expression has declined in the deciduum and not yet begun in the embryo proper. But even at earlier stages, it is possible that follistatin plays a role in inhibiting the functions of other members of the TGFβ family such as nodal, which is expressed at early streak stages and is subsequently localised to the node (Zhou et al., 1993), and BMP-4, which is expressed in the deciduum and then in posterior mesoderm (Jones et al., 1991). This is under investigation.

Follistatin expression shows some unusual features at streak stages. For many genes expressed in the primitive streak, such as T (Wilkinson et al., 1990), Evx-1 (Dush and Martin, 1992), Wnt-5a and Wnt-3a (Takada et al., 1994) expression continues in the tailbud (Rashbass, Wilson, Rosen and Beddington, unpublished data). In contrast, follistatin expression declines in the streak at the early somite stage. Analysis of gene expression in homozygous T/T mutant embryos shows that expression of Evx-1, Wnt-5a and Wnt-3a is independent of wild-type T protein up to the early somite stage, but that it declines rapidly in mutant embryos thereafter (Rashbass, Wilson, Rosen and Beddington, unpublished data). Thus, the early somite stage may mark the transition from a stage when gene expression in the streak is maintained by extrinsic inducers to a time when cross-regulatory circuits within the streak/tailbud take over. The rapid disappearance of follistatin mRNA in the streak from the 4-somite stage onwards

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**Fig. 9.** Sections of embryos prepared from whole-mount in situ hybridisation specimens using a follistatin probe. (A) Transverse section of embryo at 7.5 days as in Fig. 8B. Transcripts are visible in both the ectodermal and mesodermal germ layers in the vicinity of the primitive streak. (B) Sagittal section of a headfold-stage embryo. Expression in the streak persists but the ventral part of the node (arrowhead) does not contain follistatin transcripts. (C) Transverse section of a 9.5 day embryo, showing follistatin expression in the somites. Scale bar, 35 µm (A); 50 µm (B); and 35 µm (C).
is consistent with follistatin being a component of the inducing pathway.

At later stages, follistatin may play a role in segmentation of the hindbrain and in somite formation. Activin is not expressed in either structure at this time, although it is noteworthy that inhibin βA transcripts are present in all rhombomeres of the Xenopus hindbrain at equivalent developmental stages (Dohrmann et al., 1993) and we are now investigating follistatin expression in this species.

One clue to the function of follistatin in the mouse embryo comes from comparison with the expression pattern of the receptor tyrosine kinase gene Sek (Nieto et al., 1992). Sek is expressed at high levels in rhombomeres 3 and 5 of the hindbrain and, in presomitic mesoderm, transcripts are restricted to the next two somites to condense. These patterns are virtually the complement of follistatin, where expression is observed in rhombomeres 2, 4 and 6 and is excluded from the most recently formed somite and from the next one to condense (Fig. 8E,F). It is possible, therefore, that follistatin binds the Sek ligand so that the ligand is only active in defined regions; that is, in rhombomeres 3 and 5 and in segmenting paraxial mesoderm.

The above possibilities are now under investigation. Meanwhile, the primary structure of follistatin provides few clues as to any additional function. Both forms contain four contiguous domains with sequence similarities to pancreatic secretory trypsin inhibitor and epidermal growth factor (Shimasaki et al., 1988b); such domains are also present in secretory trypsin inhibitor and epidermal growth factor. Meanwhile, the primary structure of follistatin provides few clues as to any additional function. Both forms contain four contiguous domains with sequence similarities to pancreatic secretory trypsin inhibitor and epidermal growth factor (Shimasaki et al., 1988b); such domains are also present in secretory trypsin inhibitor and epidermal growth factor.

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