Isolation of Sna, a mouse gene homologous to the Drosophila genes snail and escargot: its expression pattern suggests multiple roles during postimplantation development

DAVID E. SMITH, FRANCISCO FRANCO DEL AMO and THOMAS GRIDLEY*

Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110, USA

*Author for correspondence

Summary

The Drosophila gene snail encodes a zinc-finger protein that is required zygotically for mesoderm formation. Snail acts as a transcriptional repressor during the period of mesoderm formation by preventing expression of mesectodermal and ectodermal genes in the mesoderm anlage. A Xenopus homolog (xsnail) of snail has been cloned and it too is expressed early in the mesodermal germ layer. We have isolated cDNA clones of a mouse gene (termed Sna) closely related to snail and xsnail and another Drosophila gene termed escargot that also encodes a zinc-finger protein. Sna encodes a 264 amino acid protein that contains four zinc fingers. Developmental RNA blot analysis showed that Sna transcripts are expressed throughout postimplantation development. Analysis of the spatial and temporal localization of Sna transcripts by in situ hybridization to both whole-mount and sectioned embryos revealed that, in the gastrulating embryo, Sna is expressed throughout the primitive streak and in the entire mesodermal germ layer. By 9.5 days post coitum (dpc) Sna is expressed at high levels in cephalic neural crest and limb bud mesenchyme. In fact, by 10.5 dpc Sna expression is observed in most mesenchymal cells, whether of neural crest or mesodermal origin. Later in gestation, high levels of Sna expression are observed in condensing cartilage and in the mesenchymal component of several tissues (lung, kidney, teeth and vibrissae) that undergo epithelial-mesenchymal inductive interactions during development. These results suggest multiple roles for the Sna gene in gastrulation and organogenesis during murine development.

Key words: gastrulation, mesenchyme, mesoderm formation, neural crest, snail, twist, escargot.

Introduction

Gastrulation in a wide variety of organisms involves the transformation of an embryo with one or two embryonic germ layers into an embryo with three embryonic germ layers (ectoderm, endoderm and mesoderm). Although the morphogenetic changes that result in mesoderm formation vary greatly among individuals of different species, recent work has shown that several genes involved in mesoderm formation have been conserved during evolution.

In Drosophila, classical genetic studies have identified the genes involved in determining cell fates along the dorsoventral axis of the embryo, including the most ventral region which forms mesoderm (for reviews, see Anderson, 1987; Rushlow and Arora, 1990; St. Johnston and Nüsslein-Volhard, 1992). The maternal morphogen dorsal, which is related to the rel family of vertebrate transcription factors (for review, see Rushlow and Warrior, 1992), acts in the ventral region of the embryo to activate the expression of two zygotic genes, twist and snail, which are known to be required for mesoderm formation. Embryos homozygous for null mutations of twist and snail fail to form a normal ventral furrow, form no mesodermal germ layer and die late in embryogenesis (Simpson, 1983; Grau et al., 1984; Leptin and Grunewald, 1990). Twist is a transcriptional activator of mesoderm-specific genes, while snail acts to maintain proper germ layer boundaries by-repressing the expression within the mesoderm of regulatory genes of the lateral mesectoderm and neuroectoderm (Kosman et al., 1991; Leptin, 1991; Nambu et al., 1990). Molecular analysis of the twist and snail genes indicates that they encode proteins of the basic helix-loop-helix and zinc-finger families, respectively (Boulay et al., 1987; Thissel et al., 1988).

In vertebrates, the molecular aspects of mesoderm formation have been studied most extensively in Xenopus (for reviews, see Hopwood, 1990; Jessell and Melton, 1992; Smith, 1989). These studies have suggested that diffusible peptide growth factors initiate mesoderm formation in vivo. These signaling molecules emanate from the endoderm of the vegetal hemisphere to induce mesoderm in the neighboring marginal zone. During in vitro animal cap explant studies, members of the fibroblast growth factor (FGF),
transforming growth factor β (TGFβ) and Wnt gene families have all been shown to induce mesoderm structures. Some of these signaling molecules have been shown to be present in the embryo at the time of mesoderm induction (Isaacs et al., 1992; Kimelman et al., 1988; Slack and Isaacs, 1989; Thomsen et al., 1990). In addition, studies with a dominant-negative FGF receptor provide strong evidence for a FGF-like factor being involved in mesoderm induction in vivo (Amaya et al., 1991).

In an attempt to identify which mesoderm-specific regulatory genes might respond to these signaling molecules, the cloning of Xenopus homologs to mesoderm regulatory genes of Drosophila has been undertaken. Homologs to twist and snail have been identified in Xenopus (Hopwood et al., 1989; Sargent and Bennett, 1990). Both genes are expressed in the marginal zone and respond to mesoderm inducing factors in the animal cap explant studies. The identification of such homologs in Xenopus suggest that there may be a conservation of required regulatory genes in evolutionarily divergent modes of mesoderm formation.

Less is known about the molecular aspects of mesoderm formation in chick and mouse embryos (reviewed by Stern, 1992). Recent work on the chick has shown that peptide growth factors, which induce mesoderm in the Xenopus animal cap explant studies, have some comparable effects on early chick embryos. For example, chick epiblasts cultured in the presence of conditioned medium from an activin-secreting tissue culture cell line develop axial structures including a notochord and bilaterally symmetric somites (Mitrani et al., 1990). This study also demonstrated that transcripts for activin βA, but not activin βB, were present at the stage when axial mesoderm induction takes place in the chick (Mitrani et al., 1990). Recently, it was shown that localized application of activin to the chick blastoderm can generate ectopic axes (Ziv et al., 1992). A number of recent experiments suggest that mesoderm induction and formation of the embryonic axis (i.e., generation of the primitive streak) are different and separable events in the chick, and may involve different inducing factors (reviewed by Stern, 1992).

In the mouse, studies to localize RNA and protein expression of several peptide growth factors have been performed. RNA localization studies have shown that three members of the mammalian FGF family, Fgf-3 (int-2), Fgf-4 and Fgf-5 are expressed in the primitive streak region of the gastrulating mouse embryo (Haub and Goldfarb, 1991; Hébert et al., 1991; Niswander and Martin, 1992; Wilkinson et al., 1988). Similar studies analyzing the localization of RNA transcripts of TGFβ1, TGFβ2, activin βA, activin βB and inhibin α were recently performed (Manova et al., 1992). Transcripts from three of these genes (TGFβ1, TGFβ2 and activin βA) were expressed at a time consistent with their products having a role in mesoderm formation. However, these genes were not expressed in the embryo but were expressed in the uterine decidua (Manova et al., 1992). These results, however, may be in contradiction with the finding of TGFβ2 protein in the gastrulating mouse embryo, particularly in the visceral endoderm (Slager et al., 1991).

The isolation of murine homologs of genes important for development in other organisms has been a valuable addition to the array of techniques that can be used to study mammalian development (for reviews, see Gridley, 1991; Kessel and Gruss, 1990; Rossant and Hopkins, 1992). As mentioned above, Xenopus homologs of both snail and twist have been reported (Hopwood et al., 1989; Sargent and Bennett, 1990), and a mouse homolog of twist (mtwist) has also recently been cloned (Wolf et al., 1991). However, the temporal and spatial distribution of mtwist in the early embryo suggests that it is involved in the specification of specific subpopulations of mesodermal cells and not in the initiation of mesoderm formation. We describe in this paper the cloning of Sna, a mouse homolog of the Drosophila genes snail and escargot and the Xenopus gene xsnail. An analysis by in situ hybridization of the spatial and temporal localization of Sna RNA expression suggests multiple roles for the Sna gene in gastrulation and organogenesis during postimplantation mouse development.

Materials and methods

Embryo isolation

Embryos were obtained from natural matings of C57Bl/6 mice. The day on which the vaginal plug was detected was designated 0.5 dpc.

PCR amplification and cloning of zinc-finger domains from 8.5 dpc embryo cDNA

Total RNA was isolated from 8.5 dpc embryos by the acid guanidinium thiocyanate-phenol-chloroform technique (Chomczynski and Sacchi, 1987) using RNazol B (Biotex). First strand cDNA was synthesized from 10 µg total RNA using oligo (dT) priming and the Superscript cDNA synthesis kit (BRL) according to the manufacturer’s recommendations. Zinc-finger domain cDNAs were amplified using degenerate oligonucleotide primers corresponding to the amino acids TGEKFP (5′) and SRMSLL (3′), with additional 5′ nucleotides coding for the restriction sites EcoRI (5′ primer) and Sall (3′ primer). The primer sequences were:

5′ primer - GTGAATTCCA(GATC)GG(GATC)GA(AG)(CT)-(GATC)TT;
3′ primer - GTCGACCTGT(GATC)A(AG)(AG)(AG)-
(GATC)GACAT(GATC)CT(GT)(GATC)GA.

Standard PCR amplification was performed with 10% of the first strand cDNA using the following cycling parameters: denaturation, 95°C, 25 seconds; annealing, 58°C, 30 seconds; elongation, 72°C, 30 seconds for 40 cycles. The PCR product was gel-purified, extracted twice with phenol-chloroform, and ethanol precipitated with 20 µg of glycogen as carrier. The precipitate was then resuspended and digested with EcoRI and Sall and cloned into pGem7(zf+) (Promega). The inserts of the clones were sequenced by the dideoxy technique using T7 and SP6 primers.

cDNA library screening

The insert from a subclone of the amplified PCR product showing a high degree of homology to the Drosophila and Xenopus snail genes was purified from vector sequences and labelled with 32P using a random prime labelling kit (Pharmacia). Replica filter lifts of approximately 7.5×105 clones of an 8.5 dpc mouse embryo cDNA library (Farhner et al., 1987) were hybridized with 5×105 cts/minute/ml of the labelled insert. Hybridization was performed overnight at 65°C in 5× SSC, 5× Denhardt’s, 0.5% SDS and 100 µg/ml denatured salmon testes DNA. Filters were washed twice for 15 minutes at room temperature in 2× SSC, 0.5% SDS, and
twice for 30 minutes at 65°C in 0.2x SSC, 0.5% SDS. Individual hybridizing clones were plaque-purified and phage inserts were subcloned into pGEM7(zf+) (Promega). The longest clone was sequenced on both strands by the dideoxy technique using specific oligonucleotide primers.

In situ hybridization

In situ hybridization of embryo sections was performed as previously described (Franco del Amo et al., 1992; Smith and Gridley, 1992). The probe for Snail included nucleotides 730 to 1200 of the Sna cDNA. A probe for the Brachyury (T) gene was generously provided by Bernhard Herrmann (described by Herrmann, 1991). Plasmids were linearized with the appropriate enzymes, and 35S-labelled sense and antisense transcripts were generated by run-off transcription.

Whole-mount in situ hybridization was performed using procedures similar to those described for embryos of other organisms (Tautz and Pfeifle, 1989; Hemmati-Brivanlou et al., 1990), using a protocol kindly supplied by David Wilkinson (Wilkinson, 1992).

Results

PCR amplification of mouse zinc-finger sequences homologous to snail

A comparison of the published amino acid sequences of the Drosophila (Boulay et al., 1987) and Xenopus (Sargent and Bennett, 1990) snail genes (hereafter referred to as snail and xsnail, respectively) revealed extensive amino acid homology within the zinc-finger domains. To clone a mouse homolog of snail, we chose two of these conserved peptide regions for the creation of degenerate oligonucleotide primers for PCR amplification. The 5′ primer encompassed the peptide sequence TGKEKP, which is found in many zinc-finger proteins (Gibson et al., 1988; Nietfeld et al., 1989). The 3′ oligonucleotide primer encompassed the peptide sequence SRMSLL, which appears to be unique to the snail homologs. Both snail (Boulay et al., 1987) and xsnail (Sargent and Bennett, 1990) are expressed early in the mesoderm anlage. Therefore, assuming that the mouse homolog would also be expressed early in the mesoderm we used first strand cDNA made from 8.5 dpc embryo RNA as the template for the degenerate oligonucleotide-based PCR amplification (see Materials and methods). The PCR amplification products were cloned and 30 of these subclones were sequenced. Several of the sequenced clones were identical. Examination of this sequence indicated that it encoded a zinc-finger domain highly homologous to the zinc-finger domain of snail (77% amino acid identity) and xsnail (86% amino acid identity) (data not shown).

Isolation of a cDNA clone encoding a homolog of snail

The PCR clone that was highly homologous to the snail genes was used to screen an 8.5 dpc cDNA library (Fahrner et al., 1987). Of 7.5×105 phage clones screened, 8 positive clones were identified, plaque purified and subcloned. Inserts from these clones ranged in size from 700 bp to 1400 bp. Restriction enzyme mapping and partial nucleotide sequence analysis indicated that these inserts formed a set of overlapping cDNA clones. The nucleotide and predicted amino acid sequence of a composite of two of the clones is presented in Fig. 1. A region of high amino acid homology with the amino terminus of the xsnail gene (Sargent and Bennett, 1990) was found in an open reading frame that began at the first Met codon (Fig. 2). The nucleotide sequence around this first Met codon also conforms well with the consensus sequence for eukaryotic translation initiation sites (Kozak, 1987). This suggests that this methionine codon represents the translation initiation codon for this cDNA clone. This open reading frame encoded a protein of 264 amino acids, which is similar in size to the 259 amino acid open reading frame encoding xsnail. Both of these predicted polypeptides are considerably smaller than the 390 amino acid polypeptide encoded by snail. Comparison of the amino acid sequence encoded by this mouse gene with the amino acid sequence of xsnail (Fig. 2) revealed two main areas of sequence conservation. The region of strongest sequence identity is the zinc-finger domain at the carboxy terminus of the protein. Another region of strong sequence conservation exists at the amino terminus of the protein. Other more limited regions of homology are scattered throughout the remaining areas of the protein. Because of the extensive amino acid conservation with xsnail and the observed expression pattern of the gene (discussed below), we have termed this mouse gene Sna.

Comparison of the amino acid sequence of the zinc-finger region of Sna with the zinc-finger regions encoded by the genes snail, xsnail and escargot (a recently identified Drosophila gene closely related to snail; Whiteley et al., 1992) reveals that the zinc-finger domain encoded by xsnail, escargot and Sna are more closely related to each other than they are to snail (Fig. 3). In the region of these proteins upstream (amino-terminal) of the zinc-finger domain, amino acid sequence homologies can be observed between snail and escargot (Whiteley et al., 1992) and between Sna and xsnail (Fig. 2), but no significant homologies in this region are observed when comparing the Drosophila proteins with the vertebrate proteins. The comparison of the zinc-finger domains (Fig. 3) also reveals that the protein encoded by the Sna gene only has four zinc fingers, instead of the five zinc fingers of snail, xsnail and escargot. The region of the first zinc finger has lost the conserved cysteine and histidine residues characteristic of zinc fingers of the Cys2/His2 class, although a few of the non-canonical amino acid residues in the first zinc finger are conserved with xsnail.

Spatial and temporal localization of Sna expression

We analyzed by northern blotting the steady state expression level of Sna during postimplantation mouse development, and detected a single Sna transcript of approximately 1.7 kb (data not shown). Levels of Sna RNA remained relatively constant from 7.5 dpc through 14.5 dpc. To analyze further the embryonic expression pattern of Sna, and in particular to address the question of a possible role for Sna during mesoderm formation in the mouse, we analyzed, by in situ hybridization, the spatial and temporal localization of Sna transcripts during postimplantation development.

We performed in situ hybridization analysis on both whole-mount embryos from 7.5 dpc to 9.5 dpc and to sections of embryos from 7.5 dpc through 10.5 dpc and at 13.5
The antisense riboprobe used in these studies was generated from nucleotides 730 to 1200 of the Sna cDNA clone. This region includes the last zinc finger as well as approximately 400 nucleotides of 3′ untranslated sequence. Identical hybridization results were obtained using antisense riboprobes from the 5′ end of the cDNA, as well as an antisense riboprobe made from the clone of the initial PCR product (not shown). Control hybridization of embryo sections with sense-strand riboprobes did not show any signal (not shown).

Hybridization of the antisense Sna riboprobe to transverse and sagittal sections of 7.5 dpc embryos revealed high levels of expression in parietal endoderm, in the primitive streak and in mesoderm migrating away from the primitive streak (Fig. 4B,C). At this stage all mesoderm appeared to express high levels of Sna. This was confirmed by examination of whole-mount embryos hybridized with a Sna antisense riboprobe, which revealed Sna expression throughout the primitive streak, in the migrating mesodermal wings and in part of the ectoplacental cone (Fig. 4A). By comparison, hybridization of transverse sections of 7.5 dpc embryos with an antisense riboprobe for the Brachyury (T) gene revealed high levels of expression in the primitive streak and in mesodermal medial to the streak, but T gene expression was not observed in more lateral mesoderm (Fig. 4D and Herrmann, 1991; Wilkinson et al., 1990).

At 8.5 dpc, high levels of Sna expression were observed in the allantois, in cephalic mesenchyme and in both presomitic and somitic mesoderm (Fig. 5). To assess whether Sna transcripts were expressed in cephalic mesenchyme of mesodermal and neural crest derivation, sections of a 7-somite, 8.5 dpc embryo were hybridized with antisense riboprobes for Sna and for cellular retinoic acid binding protein I (CRABP I). CRABP I has been shown to be a marker for both cranial and trunk neural crest in mice and chicks (Maden and Holder, 1992; Ruberte et al., 1991). In mouse embryos, prior to the 4-somite stage CRABP I is expressed in cranial mesenchyme of mesodermal origin, but by the 7-somite stage mesodermal expression has diminished and CRABP I expression appears to be specific for
Expression of a mouse homolog of snail (Ruberte et al., 1991). Hybridization of serial, near-adjacent sections with both riboprobes demonstrated that Sna is expressed in migrating cranial neural crest (Fig. 6).

Examination of whole-mount embryos at 9.5 dpc hybridized with the Sna antisense riboprobe (Fig. 7A) revealed high levels of expression in regions colonized by cephalic neural crest, in somites, in the forelimb bud and in tail bud mesenchyme. In the forelimb bud Sna was expressed at high levels throughout the mesenchyme but was absent from the apical ectodermal ridge (Fig. 7B). In somites Sna expression was not present in dermatome, but was present in myotome (Fig. 7C,D and data not shown) and was expressed at higher levels in sclerotome (Fig. 7D). By 10.5 dpc, a majority of mesenchymal cells, whether of neural crest or of mesodermal origin, expressed high levels of Sna RNA (Fig. 7E). Neuroepithelium in the brain and spinal cord did not express Sna (Fig. 7E), nor did neuronal derivatives of neural crest such as trigeminal or dorsal root ganglia (Fig. 7E).

Later in gestation, the overall level of Sna expression diminished but high levels of expression were maintained in condensing cartilage, in the choroid plexus and in the mesenchymal component of several tissues that undergo reciprocal epithelial-mesenchymal inductive interactions during their development (Fig. 8). Sna expression was observed both in cells undergoing intramembranous bone formation, characteristic of the skull (Fig. 8A), and in cells undergoing endochondral bone formation, characteristic of the axial and appendicular skeleton (Fig. 8E,G). At 13.5 dpc, another site of Sna expression was the choroid plexus (Fig. 8A,B), which serves to secrete cerebrospinal fluid into the ventricles of the brain. Sna expression was observed in the mesenchymal layer of the choroid plexus and in mesenchyme that appeared to be poised to migrate into the involuting choroidal epithelium (Fig. 8B).

Fig. 2. Alignment and comparison of the predicted amino acid sequences of Sna and xsnail. The sequences were aligned with the GAP program of the GCG Sequence Analysis Software Package (Devereaux et al., 1984). Gaps inserted into the sequences are indicated by a dot (.). Amino acid residues identical between the two sequences are shown on the consensus sequence on the bottom line. The xsnail sequence was obtained from Sargent and Bennett, 1990.

Fig. 3. Comparison of zinc-finger domains of snail-related genes. The amino acid sequence of the zinc-finger region of snail (Boulay et al., 1987) is compared to the zinc-finger regions of the related genes escargot (Whiteley et al., 1992), xsnail (Sargent and Bennett, 1990) and Sna. Zinc fingers are boxed in grey. Identity to the snail sequence is indicated by a dash (−). Gaps inserted into the sequences to maximize homology are indicated by a dot (.). Amino acid residues that differ from the snail sequence but are common to escargot, xsnail, and Sna are boxed in white. Note that the Sna gene has lost the first of the five zinc fingers conserved in the other family members. The translation stop signal at the carboxy terminus of these proteins is indicated with an asterisk.
At 13.5 dpc, Sna was expressed at high levels in metanephrogenic mesenchyme of the kidney (Fig. 8D), in lung mesenchyme (Fig. 8E) and in condensing mesenchyme beneath the first molar tooth bud (Fig. 8C). At 16.5 dpc Sna was expressed at high levels in mesenchyme in the snout, particularly mesenchymal condensations around the developing whisker follicles (Fig. 8F). These mesenchymal condensations will go on to form the dermal papillae. The development of all these tissues has been shown to involve a series of reciprocal inductive interactions between the epithelial and mesenchymal cell layers (Hardy, 1992; Lumsden, 1988; Saxén, 1987; Thesleff et al., 1989). The maintenance of Sna expression in these tissues suggests that Sna might be involved in signals emanating from mesenchyme during these reciprocal inductive interactions.

Discussion

In this report we have presented the cloning and postimplantation expression pattern of a mouse gene (Sna) homologous to the Drosophila genes snail and escargot and the Xenopus gene xsnail. After submission of this report, we discovered that Nieto et al. (1992) had also submitted a manuscript describing the cloning and expression pattern of this gene. The results they report are very similar to our results.

A mouse homolog of snail or escargot?

While analysis of the amino acid sequences in the zinc-finger region of snail, escargot, xsnail and Sna clearly indicates that escargot, xsnail and Sna form a group more closely related to each other than they are to snail (Fig. 3), the expression patterns support the notion that both xsnail and Sna are functionally more closely related to snail. Snail is expressed early in Drosophila embryogenesis throughout the mesoderm anlage (Alberga et al., 1991; Leptin, 1991), while escargot is expressed in a complex and rapidly changing pattern, primarily in cells of ectodermal origin (Whiteley et al., 1992). While we cannot exclude the possibility that a gene with greater homology to snail exists in the mouse genome, neither the PCR amplification reaction with degenerate oligonucleotides nor our screen of the 8.5 dpc cDNA library with the probe cloned from the PCR amplification identified such a gene.

RNA blot analysis demonstrated that Sna is expressed throughout postimplantation development. This maintenance of Sna expression after initial formation of the mesoderm is similar to that observed for xsnail. Both our results with Sna and those of xsnail would indicate a role for vertebrate snail genes well after the initial specification of the mesodermal lineage. Such a role is supported by an analysis of the spatial and temporal localization of Sna transcripts during development.

Expression pattern of Sna in the gastrulating embryo suggests a role in mesoderm formation

Sna is expressed throughout the primitive streak and the entire mesodermal germ layer at 7.5 dpc. This expression pattern suggests that Sna may be required both for the initial formation of the mesodermal germ layer as well as its maintenance. This pattern of expression can be contrasted to that of Brachyury (T) at the same stage, which is expressed in the primitive streak and in mesoderm medial to the streak, but is not expressed in more lateral mesoderm (Fig. 4D, and Hermann, 1991; Wilkinson et al., 1990). Mutant alleles of T suggest that it is required for proper notochord formation and axial development (Hermann, 1991; Yanagisawa et al., 1981). Examination of Sna expression in T mutants will help determine if Sna acts upstream or downstream of T during mesoderm formation in the mouse.

Sna expression during organogenesis

By 10.5 dpc, most mesenchymal cells in the embryo, whether of mesodermal or neural crest derivation, express Sna. Cranial neural crest contributes to structures in the head, such as connective tissue and skeletal elements, that are exclusively of mesodermal origin in the trunk (Noden, 1988). Thus, expression of Sna in mesenchymal cells of both mesodermal and neural crest origin may reflect the similar fates that will be adopted by these cells. At 8.5 dpc expression levels appear to be highest in cephalic mesenchyme and in presomitic mesoderm. Sna expression is maintained as somites condense, but expression becomes progressively confined to the scleratome, which adopts a mesenchymal configuration, and is lost from the dermatome, which maintains an epithelial configuration.

It is interesting to compare the RNA expression pattern we have observed for Sna and the pattern reported for mtwist (Wolf et al., 1991). Mtwist was not detected in mesoderm or the primitive streak at 7.5 dpc, but was first detected in somites at around 8.0 dpc. Subsequently, mtwist and Sna were both expressed in most mesenchymal cells, particularly those in the branchial arches, limb buds and scleratome. This is reminiscent of the common expression domain of snail and twist in the prospective mesoderm of the cellular blastoderm-stage Drosophila embryo (Leptin, 1991), and may indicate that the Sna and mtwist genes share regulatory elements responsible for expression after initial mesoderm specification.

Later in gestation, Sna expression becomes progressively confined to cartilage and the mesenchymal component of several tissues that undergo reciprocal epithelial-mesenchymal inductive interactions during their development. This localization of Sna transcripts suggests that Sna may have multiple roles during postimplantation development, and may be involved in chondrogenesis and in epithelial-mesenchymal inductive interactions.

Does Sna encode a transcription factor?

Snail has been shown to function as a transcriptional repressor during early Drosophila embryogenesis (Kosman et al., 1991; Leptin, 1991; Nambu et al., 1990). One of the genes whose transcription is negatively regulated by snail protein is single-minded (sim; Leptin, 1991; Nambu et al., 1990). Recently, binding of recombinant snail protein to the promoter region of the sim gene was demonstrated (Kasai et al., 1992). DNAase I footprint analysis showed that the recombinant snail protein protected a 14-bp consensus sequence that was present nine times in the regulatory region of the sim gene (Kasai et al., 1992).
Sna protein acts as a transcriptional repressor during mouse embryogenesis. However, given that there is essentially no amino acid homology between snail and Sna upstream of the zinc-finger domain, it is possible that Sna might function just as well as a transcriptional activator. We expect that the Sna protein could bind DNA, although the fact that the Sna protein only has four zinc fingers, rather than the five zinc fingers of the snail protein, may affect its binding specificity and/or affinity. It will be interesting to determine if recombiant Sna protein can bind and protect the same sites as snail protein in the sim regulatory region. Experiments are in progress to answer some of these questions.

We thank Dave Wilkinson for exchanging data and providing the whole-mount in situ hybridization protocol before publication, Bernhard Herrmann for the Brachury probe and Chris Rushlow and Andy McMahon for reading the manuscript. F.F. del Amo was supported by a fellowship from the Ministerio de Educación y Ciencia of Spain (no. FP90-32450447).

References


Fgf-4


(Accepted 1 September 1992)
Fig. 4. Expression of the *Sna* and *T* genes at 7.5 dpc. (A) Hybridization of a whole-mount embryo with an antisense *Sna* riboprobe. The purple color indicates detection of *Sna* RNA transcripts. Expression is observed throughout the primitive streak, in the advancing mesodermal wings and in part of the ectoplacental cone. The arrowhead indicates the anterior limit of migration of the mesoderm. (B,C) Transverse (B) and slightly oblique sagittal (C) sections hybridized with an antisense *Sna* riboprobe. High levels of *Sna* expression are observed throughout the mesoderm, in the primitive streak and in parietal endoderm cells (arrowheads). (D) Transverse section of an embryo similar to that in B hybridized with an antisense *T* riboprobe. High levels of *T* gene expression are observed in the primitive streak and in mesoderm adjacent to the streak, but not in more lateral mesoderm. Abbreviations: ec, ectoplacental cone; ee, embryonic ectoderm; m, mesoderm; ps, primitive streak.

Fig. 5. Expression of *Sna* at 8.5 dpc. (A,D) Hybridization of whole-mount embryos. High levels of expression are observed in cephalic mesenchyme in the headfold region, in somites (arrowheads), in presomitic mesoderm and in the allantois. (B,C,E) Transverse (B,C) and sagittal (E) sections hybridized with an antisense *Sna* riboprobe. High levels of expression are observed in cephalic mesenchyme and in presomitic mesoderm. No expression is observed in neuroepithelium. Abbreviations: al, allantois; cm, cephalic mesenchyme; hf, headfold; pm, presomitic mesoderm.

Fig. 6. Expression of *Sna* and CRABP I in cranial neural crest. Near-adjacent serial transverse sections of a 7-somite, 8.5 dpc embryo were hybridized with antisense riboprobes for *Sna* (A,C) or CRABP I (B,D). (A,B) *Sna* transcripts are observed in all cephalic mesenchyme, while CRABP I transcripts are not observed in cephalic mesenchyme of mesodermal origin (closed arrow in B). (C,D) Higher magnification view of sections shown in A and B. Neural crest delaminating from the lateral edge of the hindbrain neuroepithelium (open arrows) express both *Sna* and CRABP I transcripts. As previously observed (Ruberte et al., 1991) CRABP I is expressed at very high levels in the hindbrain, with strong labelling both apically and basally in the neuroepithelium and in migrating neural crest. Magnifications: (A,B) 200×; (C,D) 400×. Abbreviations: g, foregut; h, neural epithelium of the caudal hindbrain.

Fig. 7. Expression of *Sna* at 9.5 and 10.5 dpc. (A) Hybridization of a 9.5 dpc whole-mount embryo. High levels of expression are observed in tail bud mesenchyme, in somites (open arrowheads) and in the forelimb bud. Expression is also observed in areas colonized by cephalic neural crest, particularly the branchial arches (closed arrowhead). (B-D) Hybridization of 9.5 dpc embryo sections with an antisense *Sna* riboprobe. (B) Transverse section through the region of the forelimb buds. Very high levels of *Sna* expression are observed throughout the limb bud mesenchyme, but no expression is observed in the apical ectodermal ridge. Expression is also observed in paraxial and lateral mesoderm. Expression is not observed in the spinal cord and in the gut endoderm. (C) Parasagittal section. *Sna* expression is observed in most mesenchymal cells, whether of neural crest or of mesodermal origin. Expression is entirely absent from neuroepithelium. The white arrow indicates the region enlarged in D. (D) In the somites, *Sna* is expressed at highest levels in the scleratome, at lower levels in the myotome and appears to be largely absent from the dermatome. (E,F) Hybridization of 10.5 dpc embryo sections with an antisense *Sna* riboprobe. (E) Parasagittal section. The expression is similar to that observed at 9.5 dpc. Note that neuronal derivatives of both cranial and trunk neural crest, such as trigeminal and dorsal root ganglia, do not express *Sna* at this stage. The white arrow indicates the region enlarged in F. (F) High levels of expression are observed in mesenchyme surrounding the epithelial layer of a developing lung bud. Magnifications: (C,E) 25×; (B) 100×; (D) 200×; (F) 400×. Abbreviations: aer, apical ectodermal ridge; d, dermatome; fb, forelimb bud; m, myotome; sc, scleratome.