# Cloning and developmental expression of *Sna*, a murine homologue of the *Drosophila snail* gene

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## Summary

The genetic analysis of dorsoventral patterning in *Drosophila* has identified a zinc-finger gene, *snail*, that is required for mesoderm formation. The cloning and nuclease protection analysis of a *Xenopus* homologue of this gene has suggested a possible role in the mesoderm of vertebrates. Here, we describe the cloning of a murine homologue of *snail*, *Sna*, and in situ hybridisation studies of its developmental expression. Sequence analysis reveals substantial conservation of the second to fifth zinc fingers, but not of the first zinc finger in the *Sna* gene. Expression occurs in the ectoplacental cone, parietal endoderm, embryonic and extraembryonic meso-derm, in neural crest and in condensing precartilage. Based on the timing and spatial restriction of expression in embryonic mesoderm, we suggest that *Sna* might be required for the early development of this tissue, as is the case for its *Drosophila* counterpart. In addition, we propose that *Sna* might have an analogous role in the development of neural crest. The expression in condensing precartilage indicates that this gene also has a later function in chondrogenesis.

Key words: *snail*, mouse development, mesoderm formation, neural crest, chondrogenesis, *Drosophila*.

## Introduction

The formation of mesoderm and the early stages of its morphogenesis are critical events in the establishment of the vertebrate body plan. In the mouse, mesoderm forms by the delamination and migration of cells from the epithelial primitive ectoderm in a region known as the primitive streak (Tam and Beddington, 1987). These mesenchymal mesoderm cells become organised into several tissues, including the notochord and somites, which are the precursors to the trunk skeleton and musculature. The gross morphology of gastrulating embryos differs somewhat between vertebrate classes, but the cellular events of mesoderm migration and early morphogenesis are similar and thus it is likely that many of the underlying molecular mechanisms are conserved.

The induction, cell-type specification, migration and morphogenesis of mesoderm must occur through a series of interactions, involving both cell-cell signals and the regulation of gene expression. In order to understand the molecular basis of these events, it is necessary to identify gene products involved in the early development of mesoderm, and several approaches have been taken towards this end. Direct approaches in studies of the *Xenopus* embryo have shown that soluble factors, related to fibroblast growth factors (FGFs; Slack et al., 1987; Kimelman and Kirschner, 1987) and activin (Smith et al., 1990), induce mesoderm formation in tissue otherwise destined to form ectoderm. A

genetic approach has been taken to clone a gene, *Brachyury*, which is involved in the early morphogenesis of mesoderm in the mouse (Herrmann et al., 1990). Expression of this gene occurs in primitive ectoderm and mesoderm in the primitive streak, and then becomes restricted to the notochord, the tissue that is primarily affected in *Brachyury* mutants (Wilkinson et al., 1990). Studies of the *Xenopus Brachyury* gene have shown that, as in the mouse, it is expressed in prospective mesoderm cells prior to their migration, and have also found that expression is up-regulated as a primary response to mesoderm inducers (Smith et al., 1991). A number of other vertebrate genes are transiently expressed in early mesoderm, including *Xenopus* members of the TGFβ (reviewed by Whitman and Melton, 1989) and mouse members of the FGF (Wilkinson et al., 1988; Haub and Goldfarb, 1991; Hébert et al., 1991; Niswander and Martin, 1992) growth factor gene families, and mouse retinoic acid receptors and cellular retinoid binding proteins (Ruberté et al., 1990, 1991).

A further approach to identifying genes potentially involved in mesoderm formation in vertebrates has come from studies of the fruit fly *Drosophila melanogaster*. Genetic analysis has uncovered maternal and zygotically expressed genes that are involved in establishing polarity along the dorsoventral (D-V) axis of the fly embryo. A regulatory cascade of maternal genes leads to a gradient in the nuclear concentration of a transcription factor, *dorsal*, that determines pattern along the D-V axis (Roth et al., 1989,

Rushlow et al., 1989; Steward, 1989). At least two threshold concentrations of *dorsal* protein are detected along the D-V axis (Ray et al., 1991), with peak levels in ventral regions initiating the expression of two genes, *twist* and *snail*, that are required for the formation and morphogenesis of mesoderm. Initially the *twist* and *snail* genes act in parallel pathways, since they are activated independently of each other and both are necessary for mesoderm formation, but subsequently *snail* expression requires the *twist* gene product (Leptin, 1991; Ray et al., 1991). *twist* (Thisse et al., 1988) and *snail* (Boulay et al., 1987) encode potential transcription factors, with helix-loop-helix and zinc-finger DNA-binding domains, respectively, and thus it seems that they may regulate the expression of downstream target genes whose products are involved in the morphogenetic movements of gastrulation. Since different aspects of the cell shape changes and ingression of mesoderm cells are disrupted in *snail* mutants compared with *twist* mutants (Leptin and Grunewald, 1990), it has been suggested that these genes may regulate distinct targets, an idea supported by studies of gene expression in mutant embryos (Leptin, 1991).

A gene homologous to *twist* has been identified in *Xenopus* (*Xtwi*; Hopwood et al., 1989) and in mouse (*Mtwist*; Wolf et al., 1991) and in situ hybridisation studies have revealed that expression occurs in a subset of mesoderm and in neural crest in both of these species. The cloning of a *Xenopus* homologue of *snail*, *Xsna*, has shown that this gene, too, has been conserved between *Drosophila* and vertebrates (Sargent and Bennett, 1990). RNAase protection analysis revealed *Xsna* transcripts in the marginal zone of early gastrulae and in animal caps after treatment with activin or bFGF.

It is pertinent to analyse the in situ expression pattern of the vertebrate *snail* homologue, both to gain further clues as to its developmental role and to examine whether, by analogy with the situation in *Drosophila*, it might be involved in the control of morphogenesis during gastrulation. Here, we report the cloning of a mouse homologue of *snail*, *Sna*, and in situ hybridisation studies of its expression. We find that *Sna* is expressed in multiple lineages, including early mesoderm and neural crest. We discuss the implications of these findings for the role of *Sna* and its possible relationship with *Mtwist*, and the significance of the mesodermal expression of these genes in both vertebrates and *Drosophila*.

## Materials and methods

### Screening of mouse embryo cDNA library

An 8.5-day mouse embryo cDNA library in λgt10 (kindly provided by Dr B. Hogan) was screened with a probe made from an *Eco*RI-*Pst*I fragment (bases 1-695) of *Xenopus snail* cDNA (Sargent and Bennett, 1990) which includes part of the zinc finger region. Hybridisation was performed at moderate stringency (5 × SSPE, 1% SDS, 55°C) followed by high stringency washes (0.2% SSC, 0.1% SDS, 60°C). A single clone with an insert of 1.4 kb was isolated and subcloned in pBluescript KS⁻. This clone was subsequently used to screen the same library to obtain longer clones. Sequence data from the clone with the longest insert (2

kb) indicated that it contained a sequence homologous to the N terminus of *Xsna*.

### DNA sequencing

Restriction fragments of the 1.4 kb and 2.0 kb clones were subcloned in pSP72 (Kreig and Melton, 1987) and sequenced on both strands by the dideoxy chain termination method (Sanger et al., 1977).

### In situ hybridisation

In situ hybridisation with ³⁵S-labelled antisense RNA probe followed by high stringency washing was carried out exactly as described by Wilkinson and Green (1990). An *Ava*II-*Hin*dIII fragment corresponding to residues 946-1050 in the 3′ untranslated region of the *Sna* cDNA was used for the synthesis of probe.

## Results

### Isolation and sequence analysis of Sna *cDNA clones*

In order to identify potential homologues of the *snail* gene, an 8.5-day mouse embryo cDNA library was screened at moderate stringency with a probe containing most of the coding region of the *Xsna* gene. A single 1.4 kb clone was identified in this initial screen that cross-hybridised at high stringency with the *Xsna* probe. Sequence analysis indicated that this clone encodes a mouse homologue, designated *Sna*, of the *Xsna* gene, but does not include N-terminal coding regions. Therefore, further screening of the mouse embryo library with the 1.4 kb *Sna* clone as a probe was carried out to obtain a longer, 2.0 kb, clone. The nucleotide sequence confirmed that this latter cDNA clone was a longer version of *Sna* and contains an open reading frame encoding a protein of 264 amino acids (Fig.1). The first ATG is preceded by one stop codon in the same open reading frame, and the first seven amino acids are identical in the *Xsna* gene (Fig.2A), suggesting that this ATG is likely to be the initiation codon for translation of *Sna*. The amino acid sequences for *Sna* and *Xsna* can be aligned throughout their length (Fig.2A), showing an overall sequence identity of 57%. Both proteins contain five conserved basic amino acids in the first 16 amino acids and an enrichment for serine and proline in the amino-terminal region. However, whereas *Xsna* and *snail* both encode a protein with five zinc fingers, *Sna* encodes four fingers that have 88% and 71% identity to fingers 2-5 of the *Xenopus* and *Drosophila* genes, respectively (Fig.2B). Recently, a *snail*-related gene in *Drosophila*, *escargot* (*esg*), has been described that has fingers with more similarity to *Xsna* than *snail* does (Whiteley et al., 1992). *escargot* has 78% amino acid sequence identity with fingers 2-5 of the *Sna* gene (Fig.2B).

### Developmental expression of Sna

Northern blot analysis of RNA from 8.5- to 17.5-day mouse embryos revealed a single *Sna* transcript, 2 kb in length, with no significant quantitative differences in level of expression, and analysis of RNA from 11 adult tissues revealed substantial expression in lung and at much lower levels in kidney and heart (data not shown). In order to

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                                        .50
                         MetProArgSerPheLeuValArgLysPro
            CCTAGGTCGCTCTGGCCAACATGCCGCGCTCCTTCCTGGTGAGGAAGCCG

                                        .110
         SerAspProArgArgLysProAsnTyrSerGluLeuGlnAspAlaCysValGluPheThr
         TCCGACCCCCGCCGGAAGCCCAACTATAGCGAGCTGCAGGACGCGTGTGTGGAGTTCACC

                                        .170
         PheGlnGlnProTyrAspGlnAlaHisLeuLeuAlaAlaIleProProProGluValLeu
         TTCCAGCAGCCCTACGACCAGGCCCACCTGCTGGCCGCCATCCCTCCGCCCGAGGTCCTC

                                        .230
         AsnProAlaAlaSerLeuProThrLeuIleTrpAspSerLeuLeuValProGlnValArg
         AACCCCGCCGCTTCGCTGCCCACCCTCATCTGGGACTCTCTCCTGGTACCCCAAGTGCGG

                                        .290
         ProValAlaTrpAlaThrLeuProLeuArgGluSerProLysAlaValGluLeuThrSer
         CCGGTTGCCTGGGCCACCCTCCCGCTGCGGGAGAGCCCCAAGGCCGTAGAGCTGACCTCG

                                        .350
         LeuSerAspGluAspSerGlyLysSerSerGlnProProSerProProSerProAlaPro
         CTGTCCGATGAGGACAGTGGCAAAAGCTCCCAGCCGCCCAGCCCGCCCTCGCCGGCGCCG

                                        .410
         SerSerPheSerSerThrSerAlaSerSerLeuGluAlaGluAlaPheIleAlaPheHis
         TCGTCCTTCTCGTCCACCTCGGCCTCCTCCCTGGAGGCCGAGGCCTTCATCGCCTTCCCT

                                        .470
         GlyLeuGlyGlnLeuProLysGlnLeuAlaArgLeuSerValAlaLysAspProGlnSer
         GGCTTGGGCCAACTTCCCAAGCAGCTGGCCAGGCTCTCGGTGGCCAAGGACCCCCAGTCG

                             .↓                .530
         ArgLysIlePheAsnCysLysTyrCysAsnLysGluTyrLeuSerLeuGlyAlaLeuLys
         CGGAAGATCTTCAACTGCAAATATTGTAACAAGGAGTACCTCAGCCTGGGCGCTCTGAAG

                                     .↓.     .590
         MetHisIleArgSerHisThrLeuProCysValCysThrThrCysGlyLysAlaPheSer
         ATGCACATCCGAAGCCACACGCTGCCTTGTGTCTGCACGACCTGTGGAAAGGCCTTCTCT

                                          .↓   .650
         ArgProTrpLeuLeuGlnGlyHisValArgThrHisThrGlyGluLysProPheSerCys
         AGGCCCTGGCTGCTTCAGGGCCACGTCCGCACCCACACTGGTGAGAAGCCATTCTCCTGC

                                        .710
         SerHisCysAsnArgAlaPheAlaValArgSerAsnLeuArgAlaHisLeuGlnThrHis
         TCCCACTGCAACCGTGCTTTTGCTGTCCGCTCCAACCTGCGTGCCCACCTCCAAACCCAC

                    .↓             .770
         SerAspValLysArgTyrGlnCysGlnAlaCysAlaArgThrPheSerArgMetSerLeu
         TCGGATGTGAAGAGATACCAGTGCCAGGCCTGTGCCCGAACCTTCTCCCGCATGTCCTTG

                         .↓     .830
         LeuHisLysHisGlnGluSerGlySerGlyGlyProArg      .
         CTCCACAAGCACCAAGAGTCTGGCTGCTCCGGAGGCCCTCGCTGACCCAGCTACCTCCCC
                                                      .890
         ATCCTCGCTGGCATCTTCCCGGAGCTCACCCTCCTCCTCACTGCCAGACTCCTTCCAGCC
                                                      .950
         TTGTCCGGGACCTGTGGCGACCATGTCTGGACCTGGTTCCTGCTTGGCTCTCTTGGAGGC
                                                      .1010
         CTTTGCCGCAGGAGGCTGATGGAGTGCCTTTGTACCCGCCCAGAGCCTCCGACGCCTCAG
         .
         TATTCATGAGGTGTA
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**Fig. 1.** Nucleotide and predicted amino acid sequence of *Sna* cDNA. The position of the four zinc finger regions is indicated with arrows.

examine the spatial distribution of transcripts we carried out in situ hybridisation analysis of embryos from early gastrulation to organogenesis stages of development.

Gastrulation in the mouse starts at about 6.25-6.5 days, when a primitive streak forms in which primitive ectoderm cells delaminate and migrate to form a mesenchymal mesoderm cell population. At this early streak stage, expression of *Sna* occurs both in mesoderm (Fig.3A-F) and, at lower levels, in primitive ectoderm (Fig.3A,B) in the primitive streak, but not in other regions of primitive ectoderm. A

similar pattern in mesoderm and primitive ectoderm is also seen in 7.5-day embryos (Fig.3G-N). Thus, at early gastrulation stages, *Sna* expression occurs both in mesoderm and in its epithelial progenitor in the primitive streak. In addition, expression occurs in two extraembryonic tissues of distinct lineages: the ectoplacental cone (Fig.3E-H), derived from trophectoderm, and the parietal endoderm (Fig.3A,B,E,F,I-N), derived from primitive endoderm.

At 8.5 days of development a similar expression of *Sna* in trophectoderm and parietal endoderm is seen, and expression in mesoderm occurs both in an extraembryonic derivative, the allantois (Fig.4A,B), and in the embryo proper (Fig.4C-F). In the embryo, morphogenesis of mesoderm is occurring in an anterior-to-posterior direction, such that more anterior regions consist of more mature derivatives. We observe that *Sna* expression occurs in the posterior of the embryo in presomitic mesoderm and persists in more anterior regions in paraxial and lateral mesoderm (Fig.4E,F). The analysis of serial transverse sections has not detected *Sna* expression in primitive ectoderm in the primitive streak at this stage of development (Fig.4C,D). A similar expression pattern is also seen at 9.5 days of development in caudal regions of the embryo (Fig.5E,F and data not shown). However, by 10.5 days a restriction of *Sna* expression is observed in the decondensing somites present in anterior regions of the trunk: *Sna* transcripts are not detected in the dermatome (Fig.5K,L,O,P), although expression does occur in the sclerotome (Fig.5K,L) and myotome (Fig.5O,P).

In addition to expression in mesoderm, *Sna* transcripts are detected at 8.5 days at the edges of the neural plate in presumptive premigratory neural crest, and expression is maintained in migrating neural crest cells (Fig.4E-H). A similar expression in migrating neural crest cells is observed in the head at 9.5 days (Fig.5C,D), and *Sna* transcripts persist in these cells as they migrate into the branchial arches (Fig.5A,B). The lower level of transcripts in the centre of the branchial arches (Fig.5A,B) correlates with the location of mesoderm destined to form the branchial arch muscles (for example, see Noden, 1975). In the 10.5-day embryo *Sna* expression occurs in a lateral-medial gradient in the branchial arches (Fig.5I,J) and, as at 9.5 days, is absent from the area of muscle formation (Fig.5M,N). We do not know whether *Sna* expression occurs in migrating crest cells in the trunk since these cells are intermingled with mesoderm which is expressing *Sna*. However, it is clear that expression is not found in all neural crest derivatives in the head and trunk, since transcripts are not detected in cranial (not shown) or spinal ganglia (Fig.5G,H).

At 12.5 days of development, expression is detected in the mesenchyme of several organs including the lung (Fig.6A,B,G,H), gut and kidney (not shown), and, as at 10.5 days, is not detected in developing muscle. However, the most striking and predominant site of expression at this stage is precartilage throughout the foetus, including the tail sclerotome (Fig.6A-D), prevertebrae (Fig.6A,B,E-H), ribs (Fig.6G,H), limbs (Fig.6I,J) and face (Fig.6A,B). In contrast, by 14.5 days of development expression is not detected in developing cartilage at any of these sites, except for the distal phalanges of the hindlimbs (Fig.6K-L) which

**(A)**

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Sna    MPRSFLVRKPSDPRRKPNYSELQDACVEFTFQQPYDQAHLLAAIPPPEVLNPAASLPTLI
Xsna   MPRSFLVKKHFSASKKPNYSELESQTVYISP FIYDKFPV    IPQPEILSTGAYYTPLV

Sna    WDSLLVPQVRPVAWATLPLRESPKAV        ELTSLSDEDS GKSSQPPSPPSPAPSS
Xsna   WDTGLLTTFFTSESDYKKSPISPSSSDDSSKPLDLTSFSSEDEGGKTSDPPSPASSA

Sna    FSSTSASSLEAEAFIA            FPGLGQLPKQLARLSVAKDPQSRKIFNCKYCNKEYL
Xsna         TEAEKFQCNLCSKSYSTFAGLSKH KQLHC    DSQTRKSFSCKYCEKEYV

Sna    SLGALKMHIRSHTLPCVCTTCGKAFSRPWLLQGHVRTHTGEKPFSCSHCNRAFAVRSNLR
Xsna   SLGALKMHIRSHTLPCVCKICGKAFSRPWLLQGHIRTHTGEKPFSCTHCNRAFADRSNLR

Sna    AHLQTHSDVKRYQCQACARTFSRMSLLHKHQESGCSGGPR
Xsna   AHLQTHSDVKKYQCKSCSRTFSRMSLLHKHEETGC
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**(B)**

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Sna    A              FPGLGQLPKQLARLSVAKD    PQSRKI
Xsna   CNLCSKSYSTFAGLSKH KQL      HCD    SQTRKS
esg    CPDCQKSYSTFSGLTKH QQF      HCPAAEGNQVKKS
snail  CDECQKMYSTSMGLSKH RQF      HCPAAECNQEKKT
        ↓    ↓    ↓      ↓   ↓    ↓

Sna    FNCKYCNKEYLSLGALKMHIRSHTLP
Xsna   FNCKYCEKEYVSLGALKMHIRSHTLP
esg    FSCKDCDKTYVSLGALKMHIRTHTLP
snail  HSCEECGKLYTTIGALKMHIRTHTLP
        ↓    ↓    ↓      ↓   ↓    ↓

Sna    CVCTTCGKAFSRPWLLQGHVRTHTGEKP
Xsna   CVCKICGKAFSRPWLLQGHIRTHTGEKP
esg    CKCNLCGKAFSRPWLLQGHIRTHTGEKP
snail  CKCPICGKAFSRPWLLQGHVRTHTGEKP
        ↓    ↓    ↓      ↓   ↓    ↓

Sna    FSCSHCNRAFAVRSNLRAHLQTHSDVKR
Xsna   FSCTHCNRAFADRSNLRAHLQTHSDVKK
esg    FSCQHCHRAFADRSNLRAHLQTHSDIKK
snail  FQCPDCPRSFADRSNLRAHQQTHVDVKK
        ↓    ↓    ↓      ↓   ↓    ↓

Sna    YQCQACARTFSRMSLLHKHQESGC
Xsna   YQCKSCSRTFSRMSLLHKHEETGC
esg    YSCTSCSKTFSRMSLLTKHSEGGC
snail  YACQVCHKSFSRMSLLNKHSSSNC
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**Fig. 2.** (A) Comparison of the predicted protein sequences of *Xenopus Xsna* and mouse *Sna*. (B) Alignment of the zinc-finger region of the *Drosophila*, *Xenopus* and *Mus snail* and *Drosophila escargot* (*esg*) proteins. Shading indicates amino acid identity compared with *Sna*. Gaps have been introduced in order to maximise the similarity of the aligned sequences. Arrows indicate cysteine, histidine, leucine and phenylalanine (or tyrosine) residues conserved in many zinc-finger sequences. *Sna* lacks the key residues of the first zinc finger found in *Xsna*, *sna* and *esg* so the alignment shown for this region may not indicate any significant conservation.

are the only sites of precartilage in the limbs at this stage (Martin, 1990). These observations indicate that expression is correlated with early stages of cartilage differentiation. Since *Sna* expression occurs in precartilage regardless of its embryonic origin, from neural crest in the head and from mesoderm in the trunk, it seems that there is continuity between *Sna* expression in these latter tissues and in precartilage.

## Discussion

### Structure of Sna protein

The *Sna* clones that we have isolated were obtained by screening at high stringency with *Xsna* sequences. The similarity between *Sna* and *Xsna* at the nucleotide and amino acid levels extends throughout the coding region, including zinc fingers 2-5 of *Xsna*, where all of the amino acid residues characteristic of zinc fingers are conserved. However, *Sna* encodes a protein lacking the first zinc finger found in *Xsna* protein. The observation that the zebrafish *snail* homologue also lacks the first zinc finger (M. Hammerschmidt, personal communication) suggests that this finger may not be critical for protein function. Nevertheless, since the zinc fingers are likely to be involved in binding to DNA, it is pertinent to examine whether this difference between *Sna* and *Xsna* protein has any consequence for the DNA sequence specificity of binding.

The N terminus of *Sna* is highly enriched for basic amino acids, which are conserved in the *Xsna* and *snail*. This may act as a nuclear translocation signal or be involved in transcriptional activation. The conservation of serine/proline rich sequences in N-terminal regions of the protein has
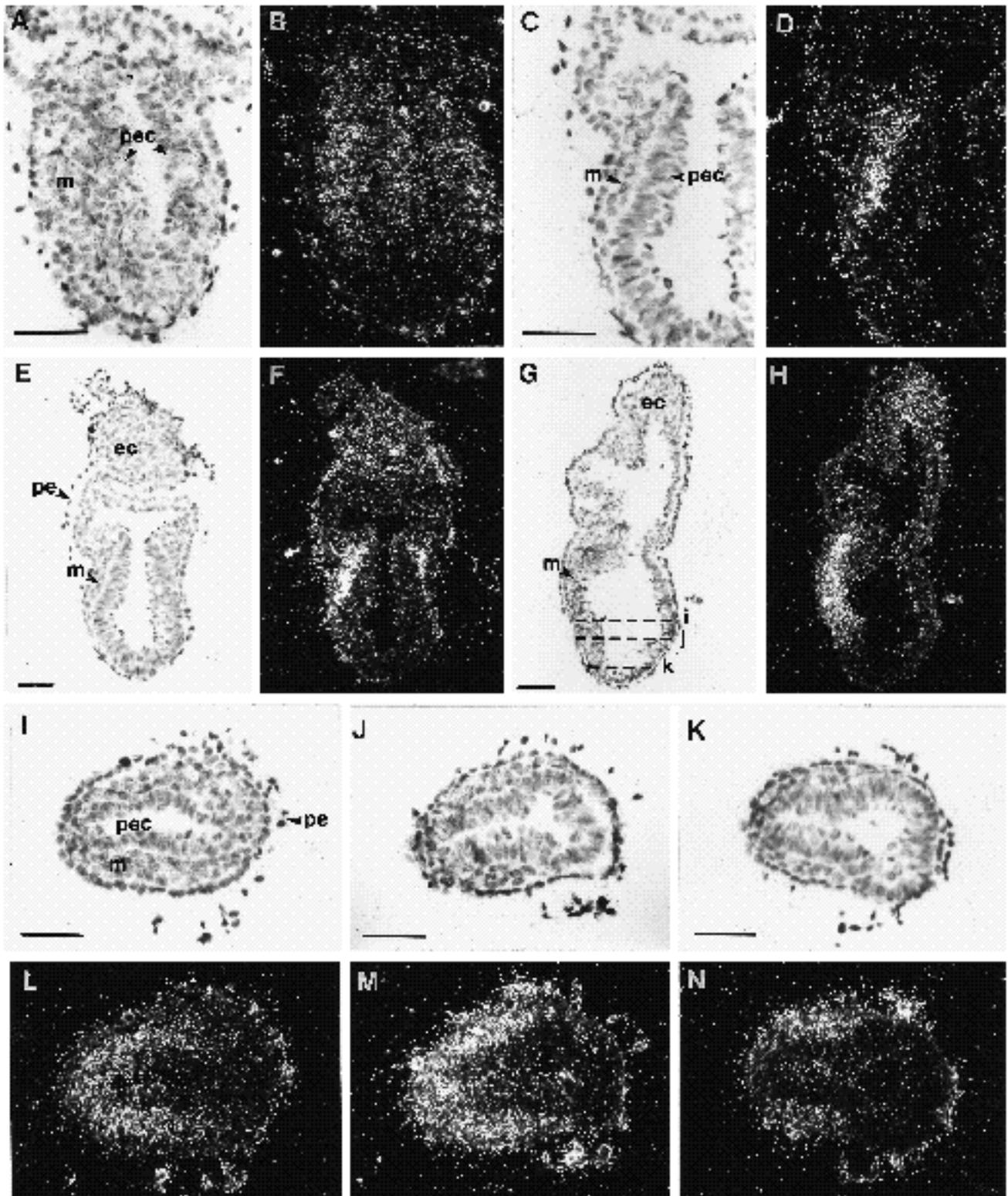
**Fig. 3.** Spatial localisation of *Sna* transcripts in gastrulation-stage embryos. Sections obtained from 6.5 (A-F) and 7.5-day mouse embryos (G-N) were hybridised with antisense *Sna* probe. Bright-field (A,C,E,G,I-K) or dark-field (B,D,F,H,L-N) photomicrographs are shown of longitudinal (A-H) and transverse sections (I-N). The section shown in (A,B) passes in a frontal plane through the primitive streak, whereas that shown in (C-F) passes through more lateral mesoderm and not through the streak. C,D are higher magnification photographs of the embryo shown in E,F. The dashed lines in G indicate the approximate positions of the transverse sections shown in I-N. ec, ectoplacental cone; m, mesoderm; pe, parietal endoderm; pec, primitive ectoderm. The bar indicates 50 μm.

precedent in other putative transcription factors and may indicate functional constraint, perhaps in the transactivation of target genes. Clearly, a critical step for the further analy-



**Fig. 4.** Spatial localisation of *Sna* transcripts in 8.5-day mouse embryos. Sections were used for in situ hybridisation with antisense *Sna* RNA probe. Bright-field (left) and dark-field (right) photomicrographs are shown of sagittal (A,B) or transverse sections (C-H). The dashed lines in A indicate the approximate positions of the transverse sections shown in C,D and E,F. G,H are higher magnification photographs of E,F. al, allantois; ec, ectoplacental cone; h, heart; nc, neural crest; m, mesoderm; np, neural plate; pe, parietal endoderm; pec, primitive ectoderm. The bar indicates 100 μm.

sis of the function of *Sna* as a transcription factor will be the identification of its in vivo targets.

There is substantial sequence identity between the zinc fingers of *Sna* and the *Drosophila snail* gene, indicating that they are closely related. However, there is a stronger identity between *Sna* (and *Xsna*) and *escargot*, a *snail*-related zinc-finger gene cloned through an enhancer trap screen in *Drosophila* (Whiteley et al., 1992). Although these data could indicate that *Sna* and *Xsna* are, in fact, true homologues of *escargot* rather than *snail*, we have been unable to identify any other *snail*-related genes by low stringency screening in the mouse and *Xenopus*. Moreover, whereas *Sna* and *snail* are both expressed in mesoderm, there is no obvious similarity with the expression of *escargot* which occurs in a highly complex and dynamic pattern, principally in ectoderm and its neural derivatives (Whiteley et al., 1992). There is, at present, no simple and convincing explanation of the evolutionary relationship between the structure and developmental function of these *snail*-related genes. Clarification may come through their cloning and sequencing from further vertebrate and insect species, and other animal phyla, and comparative studies of their expression patterns.

*Potential roles of* Sna

We find that, like the *Drosophila snail* gene, *Sna* is expressed in mesoderm cells during early stages of their morphogenetic movements. During early-mid gastrulation stages (6.5-7.5 days of development), *Sna* expression is found in primitive ectoderm in the primitive streak and throughout migrating mesoderm cells. At later stages of gastrulation (8.5-9.5 days), however, expression is not found in primitive ectoderm, but does occur in early mesoderm and persists in several of its derivatives: the allantois, lateral mesoderm and somites. These data argue that *Sna* has a role in mesoderm development, presumably by regulating the transcription of specific target genes, but it is not possible to deduce when *Sna* function is required on the basis of these expression patterns. It is tempting to suggest that *Sna* might have an early role by extrapolating from the requirement for *snail* function for the ingression of mesoderm in *Drosophila* (Leptin, 1991; Alberga et al., 1991). Indeed, the expression of *Sna* in primitive ectoderm at early gastrulation stages presages the delamination of these cells to form mesoderm. *snail* appears to act by repressing ectodermal genes whose expression may be inconsistent with mesoderm morphogenesis (Leptin, 1991), so it will be interesting to determine whether *Sna* serves an analogous role. However, it is not clear why a different pattern of *Sna* expression is seen at late stages of gastrulation (8.5 days onwards), when expression occurs upon, rather than prior to, ingression. One possibility is that this correlates with the progressive changes in the fate of mesodermal cells that occur during the period of gastrulation, for example the formation of extraembryonic mesoderm only at early stages (Tam and Beddington, 1987). However, *Sna* expression does not correlate solely with precursors of extraembryonic mesoderm, since transcripts are detected in primitive ectoderm cells in the anterior streak at 7.5 days, cells which are destined to form embryonic mesoderm (Tam and Beddington, 1987). In addition, whereas primitive ecto-
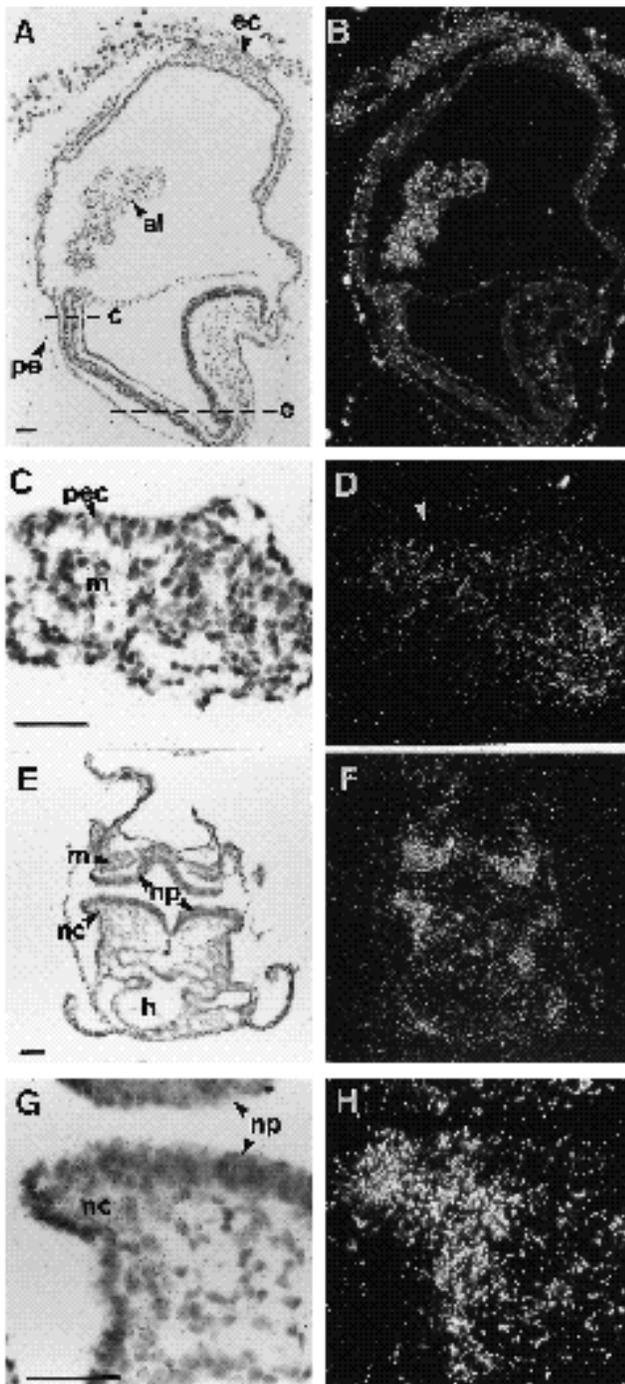
derm is initially the only source of paraxial (somitic) meso-derm, from 8.5 days onwards mesenchyme of the tail bud also contributes to this derivative (Tam and Tan, 1992).

However, it is difficult to correlate this quantitative shift in the relative contribution of primitive ectoderm with the qualitative change in *Sna* expression in this tissue. Never-
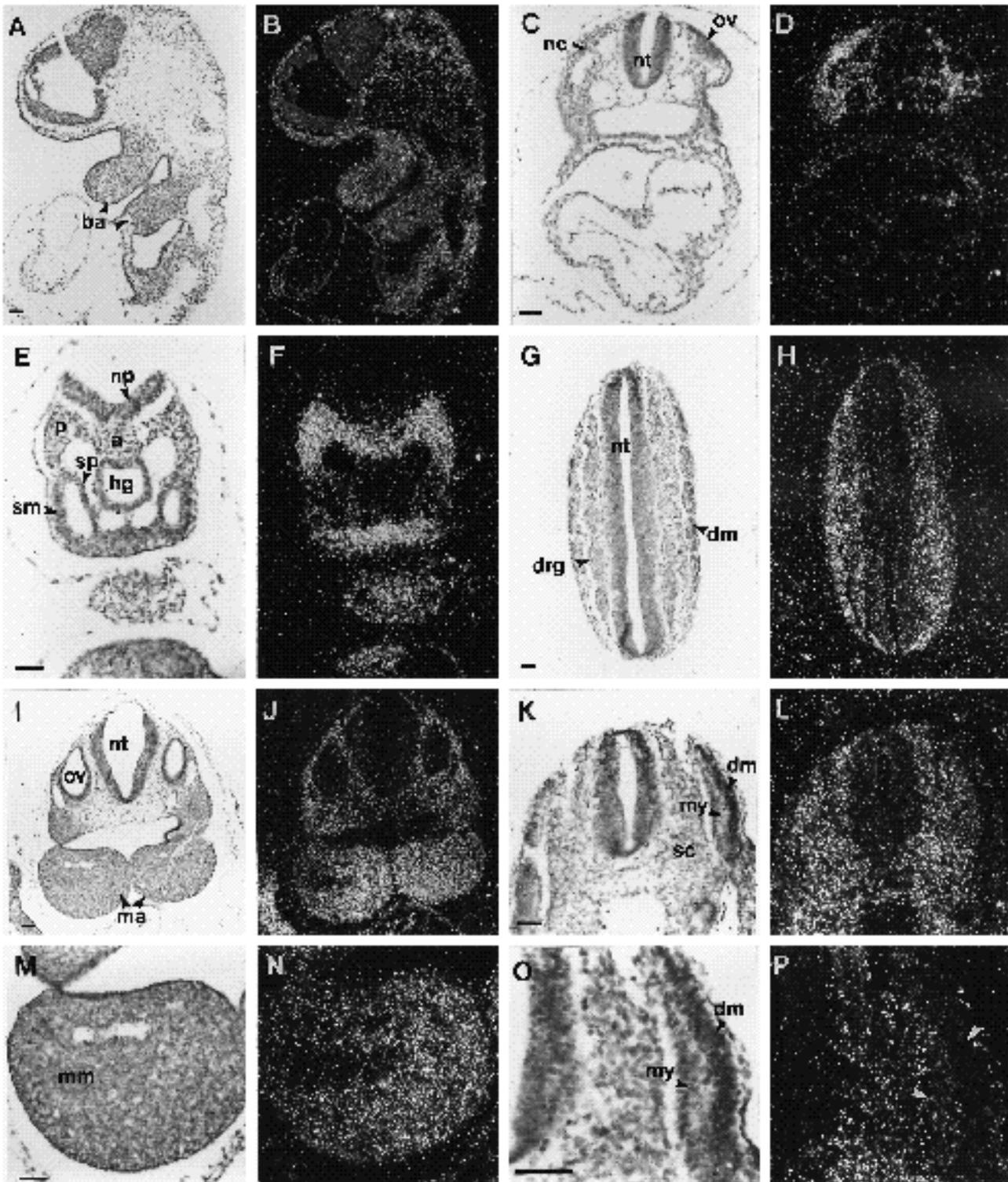


**Fig. 5.** In situ hybridisation analysis of *Sna* transcripts in 9.5-day and 10.5-day mouse embryos. Bright-field (A,C,E,G,I,K,M,O) and dark-field (B,D,F,H,J,L,N,P) photomicrographs are shown of 9.5-day (A-F) and 10.5-day (G-P) mouse embryos in parasagittal (A,B), transverse (C-F,I-P) and frontal (G,H) sections. a, axial mesoderm; ba, branchial arches; dm, dermatome; drg, dorsal root ganglion; hg, hindgut; ma, mandibular arch; mm, mandibular muscle; my, myotome; nc, neural crest; nt, neural tube; ov, otic vesicle; p, paraxial mesoderm; sc, sclerotome; sm, somatic mesoderm; sp, splanchnic mesoderm. The bar indicates 100 μm.
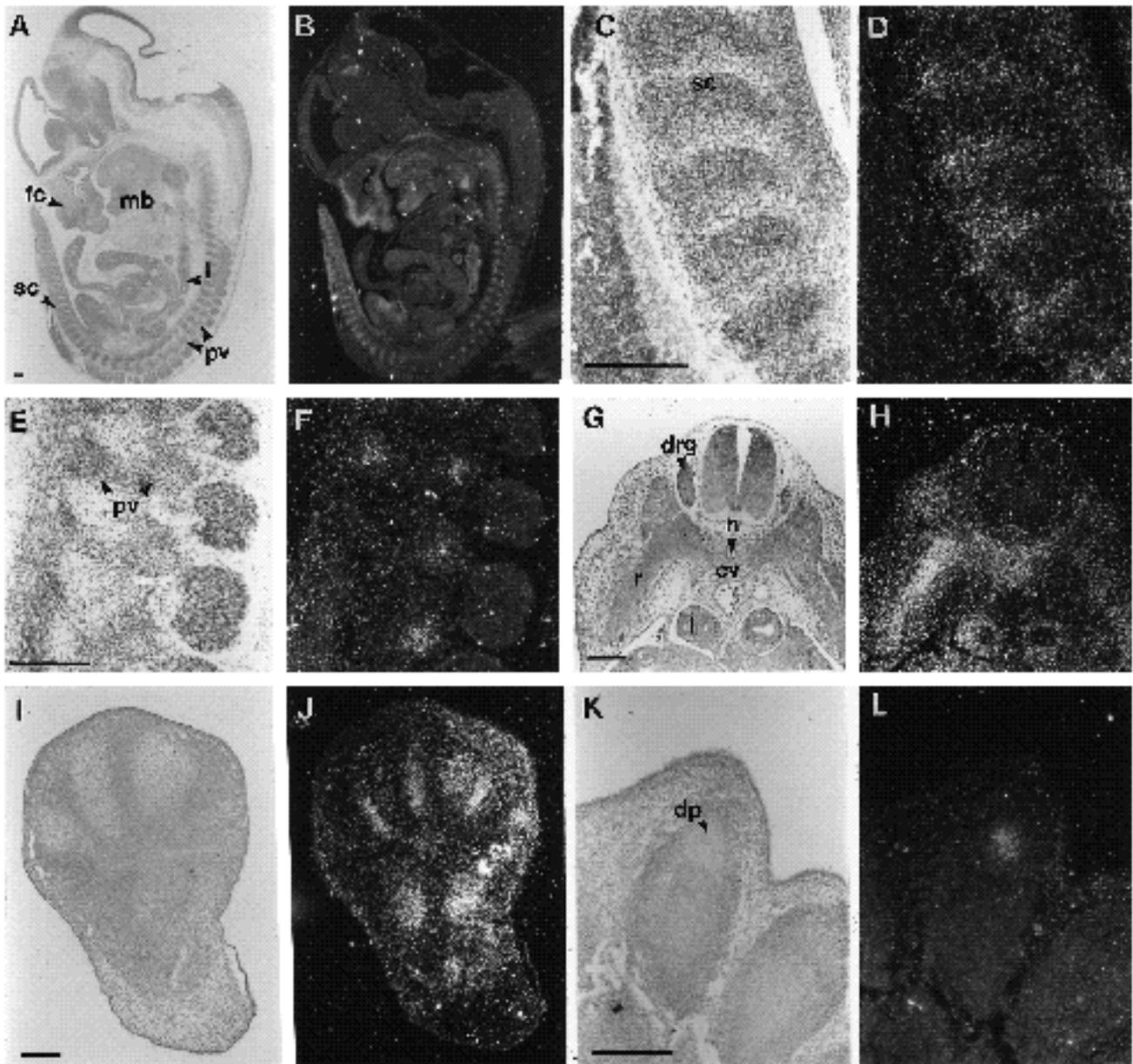
**Fig. 6.** In situ hybridisation analysis of *Sna* transcripts in 12.5-day and 14.5-day mouse embryos. Bright-field (A,C,E,G,I,K) and dark-field (B,D,F,H,J,L) photomicrographs are shown of parasagittal (A-F) and transverse midtrunk sections (G,H) of 12.5-day mouse embryos and longitudinal sections of 12.5-day (I,J) and 14.5-day (K,L) hindlimb buds. C-F are higher magnification photographs of A,B. cv, centrum of vertebrae; dp, distal phalange; drg, dorsal root ganglion; l, lung; mb, mandible; fc, facial cartilage; n, notochord; pv, prevertebrae; r, rib; sc, sclerotome. The bar indicates 200 μm.

theless, it remains possible that the change in *Sna* expression may reflect some other difference in the specification state of primitive ectoderm cells at early and late phases of mesoderm formation.

It is particularly intriguing that *Sna* is also expressed in early neural crest because, like mesoderm, this tissue forms by the delamination and migration of cells from an epithelium. The observation of *Sna* transcripts in the lateral neural plate, but not later in the neural tube, suggests that this expression is initiated in neural crest cells prior to the onset of their migration, analogous to the situation in the early primitive streak. The expression of *Sna* in myotomal cells

also correlates with cell movement since it occurs during the migration of muscle precursor cells from the dermamyotome (Kaehn et al., 1988), but there is no expression in muscle later in development. On the other hand, transcripts are not seen in the dermatome, but weak expression can be observed at 14.5 days of embryogenesis in the forming dermis (unpublished observations), perhaps related to the condensation of the dermis precursor cells; we cannot assess whether *Sna* expression also occurs during earlier stages of dermatomal cell migration since these cells cannot be distinguished from other mesenchymal cells.

Although these data are compatible with *Sna* having a

role in regulating the development of mesoderm and neural crest, it is likely that this gene also has other roles. Expression occurs in the sclerotome of somites and later in precartilage, a derivative of mesoderm and neural crest (in the trunk and head, respectively; Le Douarin, 1982), suggesting that this gene has a distinct role in chondrogenesis. In addition, it seems that *Sna* may contribute to the development of the ectoplacental cone and parietal endoderm.

### Relationship with twist expression

It is intriguing that, like *Sna*, *twist* is expressed in both mesoderm and neural crest in the mouse (Wolf et al., 1991) and *Xenopus* (Hopwood et al., 1989) since the *Drosophila* homologues of these genes have similar expression patterns and probably regulate different sets of genes (Leptin, 1991). The expression of *Mtwist* appears to overlap with *Sna* in lateral mesoderm, the sclerotome component of somites, in cranial neural crest, and subsequently in developing cartilage and skin. However, *Sna* is expressed at the primitive streak stage and is therefore expressed before *Mtwist* (Wolf et al., 1991). In *Drosophila*, *snail* expression is initially activated independently of *twist*, but subsequently becomes dependent on *twist* (Leptin, 1991; Ray et al., 1991), so it is pertinent to examine whether an analogous regulatory interaction exists in vertebrates.

### Relationship with other genes implicated in mesoderm formation

Growth factors related to FGF and TGFβ (activin) have been implicated in inducing the formation of mesoderm in *Xenopus* (see Smith, 1989 for a review), and the expression of the *Brachyury* homologue is a primary response to this induction (Smith et al., 1991). In the mouse, *Brachyury* is expressed in the primitive ectoderm and mesoderm of the primitive streak (Wilkinson et al., 1990), and therefore the detection of *Sna* transcripts in these tissues at 6.5-7.5 days suggests that the activation of this gene is an early response to mesoderm induction. However, unlike *Sna*, *Brachyury* continues to be expressed in primitive ectoderm later during gastrulation, so at these stages the activation of *Sna* in developing mesoderm occurs subsequent to *Brachyury*. A similar overlap during early gastrulation occurs between *Sna* and the *Evx-1* homeobox gene which is expressed both in primitive ectoderm and mesoderm in the primitive streak (Dush and Martin, 1992). In this case, too, the expression of these genes subsequently diverges, since *Evx-1* continues to be expressed in primitive ectoderm at a stage when *Sna* expression is not detected in this tissue. In addition, *Evx-1* is down-regulated during the later morphogenesis of embryonic and extraembryonic mesoderm, while *Sna* expression persists in, for example, paraxial mesoderm and the allantois. It will be important to analyse whether the *Brachyury*, *Evx-1* and *Sna* genes are linked in a regulatory hierarchy and whether this changes between early and late stages of mesoderm formation.

Studies of several members of the FGF-related gene family have shown expression in distinct patterns during gastrulation in the mouse suggestive of stage-specific roles in mesoderm development. The *Fgf-5* gene is expressed throughout primitive ectoderm, and at early streak stages in anterior mesoderm, though not in posterior embryonic mesoderm or extraembryonic mesoderm (Hébert et al., 1991). In contrast, expression of the *Fgf-4* gene in gastrulating embryos is restricted to the primitive ectoderm in the streak and to anterior mesoderm (Niswander and Martin, 1992). Finally, the *int-2* (*Fgf-3*) gene is expressed in mesoderm adjacent to the primitive streak, and is down-regulated during the later morphogenesis of these cells, but is not expressed in primitive ectoderm (Wilkinson et al., 1988). None of these expression patterns correlates in a simple manner with *Sna* expression: at 6.5-7.5 days, *Sna* expression is coincident with the domain of the *Fgf-4* plus the *int-2* gene, but at later stages does not overlap with the former, and only overlaps with *int-2* in posterior, newly formed, embryonic mesoderm (and in parietal endoderm).

There is an overlap between the expression of *Sna* and the TGFβ family member BMP-4 (Jones et al., 1991) which is expressed in presomitic mesoderm, but not in the primitive ectoderm, in the mouse. In addition, it is possible that there may be a relationship between members of this family and *Sna* expression in precartilage, since TGF-β1, TGF-β2 and TGF-β3, and BMP-2a have all been implicated in the formation of cartilage and bone by expression studies and their effects on cartilage formation (Heine et al., 1987; Lehnert and Akhurst, 1988; Schmid et al.,1991; Millan et al., 1991; Pelton et al., 1991; Lyons et al., 1989; Wozney et al., 1988; Seyedin et al., 1986, 1987).

Finally, members of the retinoic acid receptor (RAR) family and cellular retinoid binding proteins are also expressed in early mesoderm (Ruberté et al., 1991). It is particularly striking that there is a strong similarity between the spatial distribution of transcripts for RAR-γ and *Sna*. Both genes are expressed in presomitic mesoderm, in the neural-crest-derived cells of the branchial arches and in all sites of precartilage condensation (Ruberté et al., 1990). However, unlike *Sna*, RAR-γ transcripts persist in more mature cartilage.

### Evolutionary significance

There are likely to be significant differences between the cascade of interactions along the dorsoventral axis that precede the specification of mesoderm in the syncitial *Drosophila* embryo and in vertebrates. Nevertheless, the expression of *snail*-related genes in the mesoderm of both mouse and *Drosophila* suggests that it has an ancient role conserved from the common ancestor. A function in regulating early aspects of mesoderm development would be consistent with this proposal, since gastrulation is believed to have evolved prior to the divergence of these phyla. The expression patterns of *twist* homologues also provide further support for such a conservation of molecular mechanisms of gastrulation.

There are, however, a number of differences in the expression of *snail* in *Drosophila* and *Sna* in the mouse that suggest that during evolution this gene has also been recruited to serve new functions in other tissues during development. Notably, expression occurs in the central and peripheral nervous system of *Drosophila* (Alberga et al., 1991) but not of the mouse. Moreover, *Sna* is expressed in neural crest, and this may be of some evolutionary significance, since this tissue is believed to have arisen in the chordate lineage and to have been crucial for cephalisation

and the formation of a sophisticated sensory system in vertebrates (Gans and Northcutt, 1983).

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*Note added in proof*

The sequence of *Sna* described in this paper has been assigned the accession number X67253 in the EMBL Data Library.