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Original Article

Transient expression of SPARC in the dorsal axis of early *Xenopus* embryos: correlation with calcium-dependent adhesion and electrical coupling

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ABSTRACT Our comprehension of the molecular mechanisms underlying embryogenesis has been greatly enhanced by the identification and characterization of associated extracellular matrix macromolecules. Using *Xenopus laevis* as a model, we investigated the expression and distribution of SPARC (Secreted Protein, Acidic, Rich in Cysteine; also called osteonectin and BM-40) during early embryonic development. SPARC has been found to be enriched in tissues undergoing rapid morphological development, differentiation, and remodeling. In *Xenopus, SPARC* transcripts are first expressed by primordial cells which give rise to the first embryonic tissues, the notochord and somites. SPARC RNA levels remained high throughout the rapid morphological development and differentiation phase of these tissues, and then rapidly decreased. Of particular interest, *SPARC* protein began to accumulate within the intersomitic clefts at the onset of trunk myotome contraction. The intersomitic enrichment of *SPARC* remained high as long as the myotomes remained electrically coupled, principally by gap junctions. As myotomes became innervated, SPARC expression decreased dramatically within the somites. *SPARC* was also found to be enriched within other tissues, such as the neural tube and epidermis. In addition, the selective spatial-temporal enrichment of SPARC suggests it makes important calcium-dependent contributions to early morphological development.

KEY WORDS: *SPARC, osteonectin, BM-40, Xenopus development, notochord marker*

Introduction

Several calcium-binding transmembrane and extracellular matrix (ECM) glycoproteins have been identified which have important structural and morphoregulatory functions during embryonic development (Hay, 1991; Adams and Watt, 1993). For example, selective cell-cell aggregation and sorting is in part promoted by cadherins, a family of calcium-dependent transmembrane cell adhesion receptors (Takeichi, 1991). In addition to cadherins, laminin (LM), a major glycoprotein constituent of basement membranes, may also make use of calcium to promote cell-substratum adhesion. At low LM concentrations, binding to neural crest cells via a subset of integrin receptors is divalent cation independent. However at high LM concentrations, not only does the LM aggregate appear to bind to a different subset of integrin receptors, but binding requires divalent cations such as Ca^{2+} or Mn^{2+} (Lallier and Bronner-Fraser, 1991). Another calcium-binding ECM glycoprotein expressed at high levels during early embryonic development is SPARC — also called osteonectin, BM (Basement Membrane Protein)-40 (Termine *et al.*, 1981; Dziadek *et al.*, 1986; Mason *et al.*, 1986) — the focus of this investigation. SPARC is a 43 kDa calcium-binding glycoprotein which is evolutionarily conserved among mammals, amphibians, and nematodes (Termine *et al.*, 1981; Mason *et al.*, 1986; Swaroop *et al.*, 1988; Damjanovski *et al.*, 1992; Schwarzbauer and Spencer, 1993). Analysis of amino acid sequences derived from cDNA sequences demonstrates that amphibian and nematode *SPARC* share 80% and 38% sequence identity respectively to mammalian *SPARC*. *In situ* hybridization and immunocytochemical studies have shown that *SPARC* is expressed at high levels by embryonic tissues undergoing rapid morphological development, differentiation and remodeling, e.g. neurogenesis, somitogenesis, chondrogenesis, osteogenesis and angiogenesis (Sage *et al.*, 1989a; Ringuette *et al.*, 1992). Injection of anti-SPARC antibodies into developing embryos, or ectopic overexpression of SPARC, leads to severe developmental defects and/or developmental arrests, reflecting a critical requirement for SPARC during embryonic development (Purcell *et al.*, 1993;

Abbreviations used in this paper: BBBA, 2:1 benzyl benzoate and benzoic alcohol; BCIP, 5-bromo-4-chloro-3-indolylphosphate p-toludine salt; BM, basement membrane; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; ECM, extracellular matrix; EGTA, ethyleneglycol bis-(ß-amino ethyl ether) N-N'-tetra acetic acid; FN, fibronectin; LM, laminin; MOPS, 3-(N-morpholino) propanesulfonic acid; NBT, nitroblue tetrazolium chloride; PBS, phosphate buffered saline; PDGF, platelet derived growth factor; SEM, scanning electron microscope; SPARC, secreted protein acidic rich in cysteine; TGFß, transforming growth factor beta.

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Schwarzbauer and Spencer, 1993). As a rule, most ECM macromolecules have multiple morphogenetic roles during early embryonic development. Although the precise function(s) of SPARC during early embryonic development remains to be established, numerous *in vitro* binding and tissue culture studies indicate that it is also a multifunctional protein. SPARC has an affinity for collagens and hydroxyapatite and is a potent inhibitor of calcium-phosphate growth *in vitro* (Romberg *et al.*, 1986). This raises the possibility that SPARC may have an inhibitory role in modulating tissue mineralization; a role which is likely to be carried out by the glutamic acid-rich N-terminal domain which can bind 5-8 Ca^{2+} ions. SPARC binds to and inhibits the action of PDGF (AB and BB dimers) (Raines *et al.*, 1992). Even though SPARC has no affinity for TGFß, it blocks binding of TGFß to its receptors (Hasselaar *et al.*, 1991). Therefore, SPARC may have important functions in regulating cell proliferation by buffering the effects of these (and perhaps other) cytokines. Recent evidence also indicates that SPARC has the potential to regulate the expression of matrix-degrading proteases and cognate inhibitors (Tremble *et al.*, 1993). SPARC inhibits the spreading and focal contacts of endothelial, fibroblast and smooth muscle cells in culture, indicating that SPARC has anti-adhesive properties. Synthetic peptides indicate that the anti-adhesive ability of SPARC can be attributed to its N-terminal and C-terminal domains (Sage *et al.*, 1989b). Thus, SPARC can be envisioned as regulating cell shape and migration *in vivo* by modulating cell-matrix contacts.

In order to assess whether any of the above functions observed *in vitro* reflect *in vivo* SPARC functions during embryonic development, we have examined its precise spatiotemporal distribution in early *Xenopus* embryos. Previous studies in our laboratory had demonstrated that *SPARC* transcripts are first expressed by mid-gastrulation/early neurula embryos (Ringuette *et al.*, 1992). Both transcripts and protein were found to be particularly enriched in the dorsal axis. By whole-mount *in situ* hybridization and immunohistochemical analysis we now report that *SPARC* undergoes dramatic changes in expression during axial development and decreases significantly once axial structures have been formed. SPARC levels were also found to be significantly high during the initial phases of the morphogenetic development and differentiation of other tissues.

Results

Expression of SPARC transcripts during early notochord and somitic development

We previously demonstrated by Northern analysis that *SPARC* RNA were first detectable at stage 13, mid-gastrulation/early neurula (Ringuette *et al.*, 1992). Transcripts accumulated rapidly, reaching a plateau by mid-tailbud stage 30, when 24-25 paired somites had formed. To obtain a three dimensional view of the spatiotemporal expression of *SPARC* transcripts, digoxigenin-labeled cRNA probes, corresponding to N-terminal glutamic acid and cysteine-rich domains of *SPARC*, were used for whole-mount *in situ* hybridization. Staining was initially observed shortly after an epithelial notochord rod became morphologically distinct (data not shown). A stain gradient, decreasing in a cranial to caudal direction, was observed within the longitudinal ridges of the paraxial mesoderm by stage 18 (Fig. 1A). The staining intensity was greatest within the anterior trunk region where 2-3 paired somites had formed. As the wave of somitogenesis progressed towards the posterior end, a matching shift in the stain gradient was also observed. By stage 25 (pre-tailbud), when somite segmentation had reached the caudal region, the anterior to posterior SPARC stain gradient was no longer visible (Fig. 1B). Similar levels of staining were observed at the center of each segmented myotome, overlapping the nuclei (Fig. 1E). High levels of staining were visible within notochord and overlying neural floor plate. To confirm the distribution of *SPARC* transcripts, mid-trunk transverse sections of the stained embryos were performed. Analysis of sections confirmed that mRNAs were abundant within the notochord, somites and the floor plate of the neural tube (Fig. 3A). However, low level staining was also observed overlapping the endodermal region fated to form the sub-notochordal rod; a staining masked by the notochord staining when viewed in whole-mounts. The above data therefore indicated that by the pre-tailbud stage expression of *SPARC* transcripts by ectodermal and endodermal tissues was mainly restricted to tissues adjacent to the notochord.

Transient patterns of SPARC RNA expression in the tailbud embryo

SPARC transcripts continued to be expressed by notochordal cells until early tailbud stage 27. However by mid-tailbud stage 33, a period corresponding to the beginning of notochord cell vacuolation, mRNA expression by notochordal cells was significantly lower (Fig. 1C). In contrast, increased expression of *SPARC* transcripts by the neural floor plate was evident. The decreased staining within the notochord made the staining of the sub-notochordal rod much more visible. Indeed, by stage 33 staining levels appeared very similar for both the neural floor plate and sub-notochordal rod, giving a double track appearance when the embryos were viewed laterally (Fig. 1C). Transverse sections through the trunk of stained embryos confirmed high levels of *SPARC* mRNA expression by the above tissues and its virtual absence within the notochord (Fig. 3B). At higher magnifications, the non-uniform staining of somitic myotome cells of pre-tailbud stage 25 embryos was more apparent. The most intense staining within the myotomes forming the 15-16 paired somites was observed overlapping the centralized nuclei (Fig. 1E). As somitogenesis progressed and spontaneous contraction of primary myotomes began, the staining intensity was gradually shifted towards the rostral and caudal poles of the somites. Staining adjacent to the intersomitic cleft reached a maximum by stage 33, when 30-32 paired somites had formed (Fig. 1F) and the hatched embryos were swimming due to rhythmic contractions of segmented myotomes. At later stages, staining in the trunk of the embryo gradually diminished. Shortly after stage 37/38 (when migration of secondary myoblasts had begun to invade the intermyotomal septal of paraxial myotomes), staining was still visible within the lateral region of anterior somites but could not be detected along the remainder of the dorsal axis (Fig. 1D). In contrast, staining began to increase within developing ventral anterior structures, such as the pharyngeal folds, gills and region of the heart primordia. Staining of the surface ectoderm was also more apparent than at earlier stages. Control anti-sense *Xenopus* cytoskeletal actin cRNA probe revealed a distribution pattern distinct from that of *SPARC* (Fig. 1G). Furthermore, *SPARC* sense cRNA revealed no significant staining (Fig. 1H).

SPARC protein expression and distribution

Since SPARC is a secreted glycoprotein its tissue distribution could not be predicted based solely on its pattern of mRNA expression. Therefore we made use of anti-SPARC monoclonal

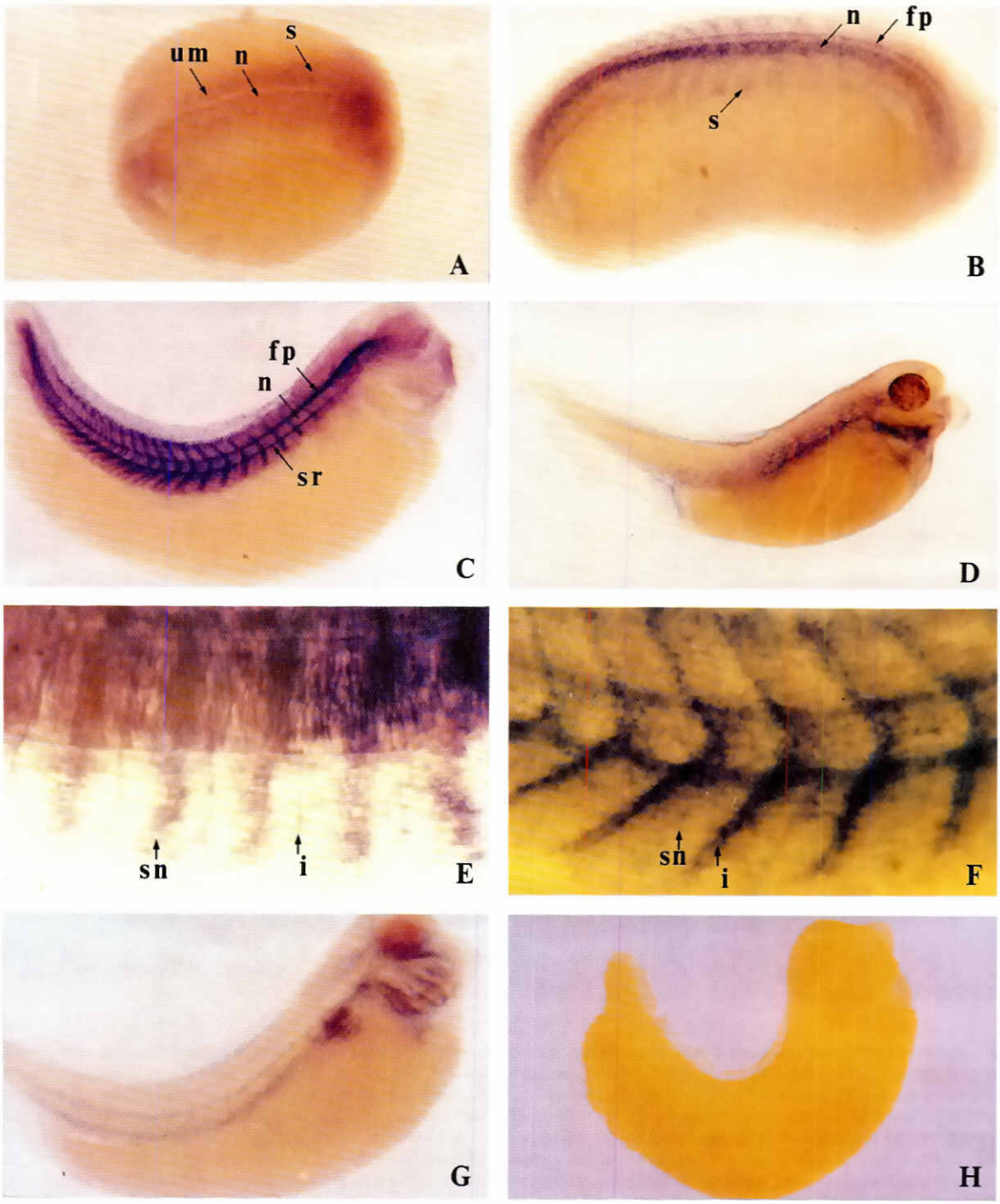

Fig. 1. Whole-mount *in situ* localization of *SPARC* RNA during early *Xenopus* development. *A 460 bp EcoRI fragment of the 5' region of Xenopus SPARC was subcloned into pGEM-4Z, and digoxigenin-labeled transcripts were generated using the flanking viral promoters and cognate RNA polymerases.* **(A)** *Shortly after neurulation (stage 18), SPARC RNA was expressed by the notochord (n), and in the segmented (s) and unsegmented (um) somitic mesoderm.* **(B)** *At stage 25, SPARC message was abundant in the notochord (n), the neural floor plate (fp), and in the somites (s).* **(C)** *By early tailbud (stage 33), SPARC messages were no longer evident in the notochord (n), but were abundant in the floor plate (fp) of the neural tube and sub-notochordal rod (sr) of the endoderm.* **(D)** *Towards the end of somitogenesis (stage 43) the presence of SPARC message in the dorsal axis was greatly diminished. Expression was now up-regulated in the anterior region of the embryo where organogenesis was progressing.* **(E)** *Closer examination of the distribution of SPARC RNA within maturing somites revealed that SPARC messages at stage 25 were localized primarily over the centrally located nuclei within each myotome (sn). Very little SPARC RNA was found near the anterior and posterior poles of the myotome adjacent to the intersomitic cleft (i).* **(F)** *As somites matured (stage 33), SPARC RNA was no longer centrally localized above the nuclei (sn), but was now abundant at the anterior and posterior poles of the somite, adjacent to the intersomitic cleft (i). As controls, Xenopus cytoskeletal actin cRNA* **(G)** *and SPARC sense cRNA probes* **(H)** *were used.*

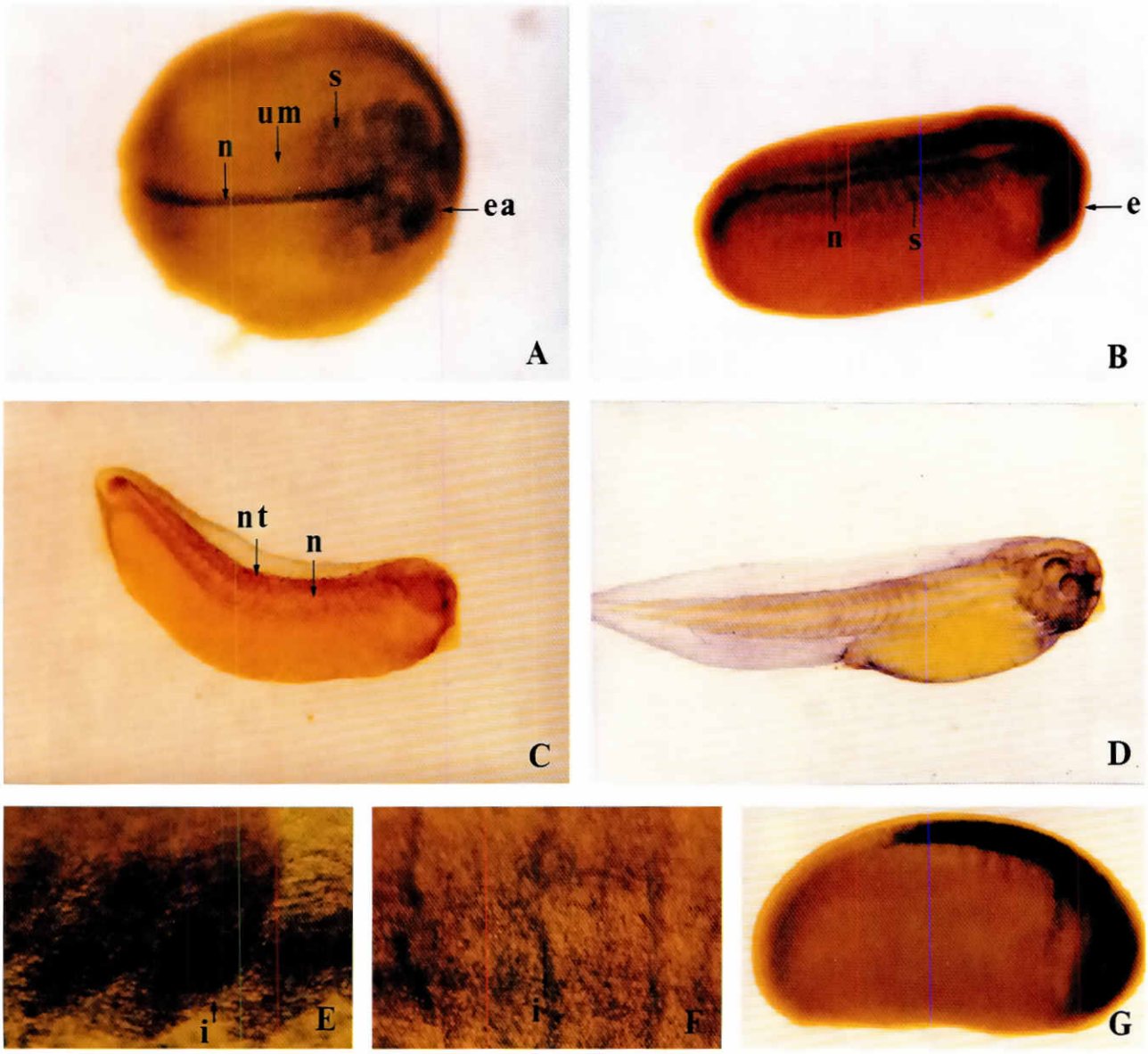

Fig. 2. Whole-mount immunolocalization of SPARC during early *Xenopus* embryogenesis. *An anti-SPARC monoclonal anti-body (MAb-ON3), which cross-reacted with Xenopus SPARC, was used to localize SPARC.* **(A)** *During early somitogenesis (stage 18), SPARC was detected within the notochord (n), the unsegmented dorsal mesoderm (um), segmented somites (s), and eye anlage (ea).* **(B)** *By stage 25 the notochord tube (n), eyes (e) and somites (s) all expressed high levels of SPARC. At higher magnification* **(E)** *SPARC was found within the somites, and was becoming detectable within the intersomitic cleft (i).* **(C)** *By stage 33 SPARC levels decreased in the notochord (n), but remain elevated within the neural tube (nt). The protein exhibited a striated chevron distribution along the somites. At higher magnification* **(F)** *this striation could be seen as a result of SPARC accumulation within the intersomitic clefts (i).* **(D)** *The stage-45 embryo showed decreased levels of SPARC in the dorsal axis, but increased anterior accumulation. As a control, a neural-specific antibody* **(G)** *was used.*

anti-body (MAb-ON3), which cross-reacted with *Xenopus* SPARC, to localize by whole-mount immunohistochemistry the distribution of SPARC relative to its mRNA distribution. Because of the auto-fluorescence of yolk protein-enriched cells, alkaline phosphatase-tagged secondary antibodies were used to visualize ON-3 binding.

SPARC localization during early notochord and somite morphogenesis and differentiation

SPARC immunoreactivity was visible on the dorsal aspect of neurula embryos at stage 18 (Fig. 2A) in a pattern similar to its mRNA distribution (Fig. 1A). The medial dorsal notochord, which was still undergoing antero-posterior elongation and secreting an ECM sheath, showed pronounced staining. Similarly, significant levels of staining were evident within the pre-somitic and somitic mesoderm. Higher levels of staining were observed in the central regions of the segmented myotomes overlapping the nuclei, with no significant accumulation at somite boundaries. The staining highlighted segmented somites as the wave of segmentation

progressed towards the rostral end of the embryos. Anterior staining of the region corresponding to the eye anlage was more evident than observed by whole-mount *in situ* hybridization (Fig. 2A). Relative to neurula embryos (stages 14-20), significantly higher dorsal axis staining was observed during early tailbud (stages 20-30). Notochord rod staining remained high throughout its elongation phase. When stage-25 embryos were viewed dorsally (Fig. 2B), no significant staining of the neural tube was evident, making the underlying stained notochordal rod clearly visible. The 16 paired somites of this stage also expressed high levels of SPARC protein. As observed with its cognate mRNA distribution, high levels of SPARC protein were observed along the entire dorsal axis, highlighting individual somites. The protein also appeared evenly distributed along the entire length of the elongated myotomes (Fig. 2E). Anterior mediolateral staining overlapping the developing eye was also pronounced, and punctate staining of the surface ectoderm was now evident (Fig. 2B). When a neural-specific antibody was used (Fig. 2G), a distribution pattern distinct from that of SPARC was observed.

SPARC protein distribution in tailbud and tailed embryos

Notochordal rod staining, which began to decrease at stage 30 (early tailbud), was no longer visible by stage 33/34 (mid-tailbud) (Fig. 2C). However at stage 33, significant staining was observed throughout the neural tube, with the exception of the floor plate (Fig. 3C). The absence of SPARC protein from the floor plate was surprising, in light of the fact that its mRNA expression in the neural tube was restricted to this region. By this stage, staining within the intersomitic cleft was higher than within myotomes, accentuating the chevron topography of the segmented somites (Fig. 2F). The accumulation of SPARC within the intersomitic cleft coincided with the translocation of its cognate mRNA to the cranial and caudal poles of the elongated myotomes. The intersomitic enrichment of SPARC confirmed previous immunofluorescent studies (Ringuette *et al.*, 1992). Staining of the surface ectoderm observed in stage-25 embryos was now much more intense and could be localized to the sensorial layer of the surface ectoderm (Fig. 3C). Immunostaining within the intersomitic clefts was still visible at stage 40/41, though *SPARC* RNA transcripts were absent from the myotomes approximately 24 h earlier (Fig. 2D). Within the rostral region, the pattern of *SPARC* expression coincided with tissues undergoing rapid development, e.g., laryngeal visceral pouches, gills, cardiac primordia, and tracheal cavity. Even though albino embryos were used, periodic albinism resulted in the pigmentation of the retinal pigmented epithelia, masking, to a degree, immunostaining in the eye. Significant immunostaining continued to be visible within the surface ectoderm, which by this stage of development was now undergoing rapid morphogenetic development and differentiation.

Discussion

Previous Northern blot studies demonstrated that embryonic expression of *SPARC* began at the mid-gastrula/early neural stage (Ringuette *et al.*, 1992). *SPARC* transcripts levels increased rapidly until early organogenesis. Whole-mount analysis has now demonstrated that the first significant levels of SPARC transcript and protein were found associated with the notochord shortly after it became morphologically distinct. Shortly thereafter, SPARC was also observed within the longitudinal ridges of the paraxial mesoderm, just prior to the appearance of the first somites. Increases in *SPARC* levels were then observed as notochord elongation and

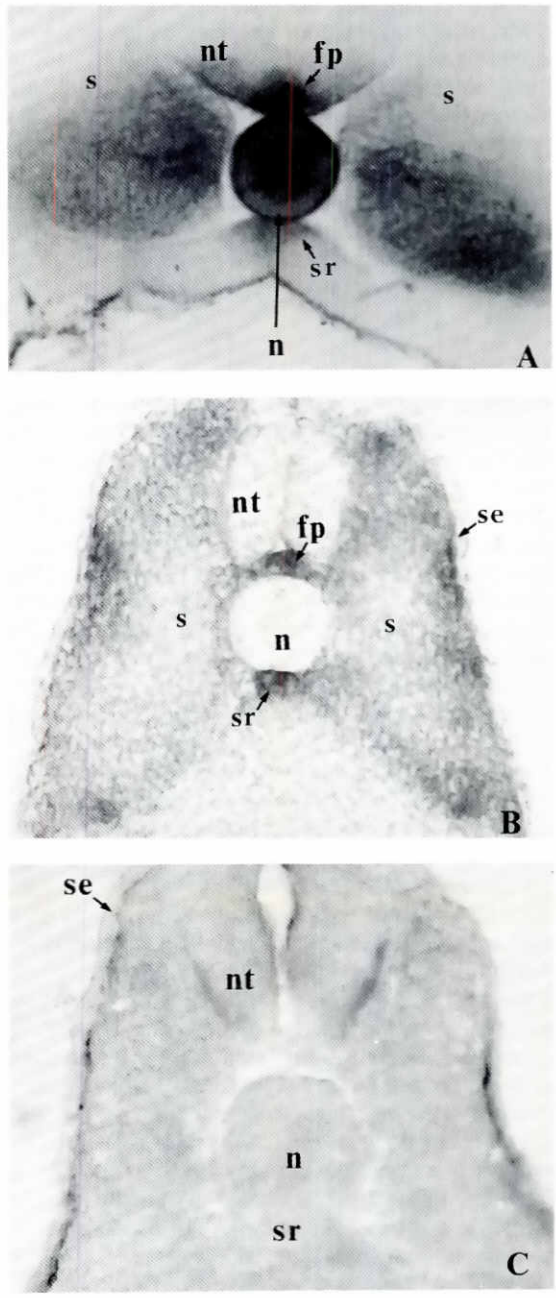

Fig. 3. Mid-trunk cross-sectional analysis of *SPARC* RNA and protein localization. *To more closely examine the distribution of SPARC RNA and protein within the embryo, mid-trunk cross section were performed.* **(A)** *At stage 18 SPARC message was found to be abundant in the notochord (n), flanking somites (s), floor plate (fp) of the neural tube (nt), and the endodermal cells fated to become the sub-notochordal rod (sr).* **(B)** *By the early tailbud stage (33), the highly vacuolated notochord (n) contained few SPARC messages. The somites (s), neural tube (nt), particularly its floor plate (fp) and the sub-notochordal rod (sr) all expressed high levels of SPARC message. SPARC RNA was also detected in the surface ectoderm (se). To compare the distribution of SPARC protein to its cognate mRNA, immunohistochemistry was performed using ON3.* **(C)** *At stage 33 SPARC protein was no longer present in the notochord (n) and sub-notochordal rod (sr). SPARC staining was still visible in the neural tube (nt), and the sensorial layer of the surface ectoderm (se).*

somitogenesis progressed. The data therefore indicated that *SPARC* was involved in mesoderm development only after mesoderm induction had occurred. Extensive studies have demonstrated that notochord and somite formation have similarities in their developmental strategies (Keller, 1991). However, they form radically different structures with different developmental fates, which also reflect mechanistic differences. Since SPARC has been assumed to have multiple functions, it is likely to have both common and distinct functions in these tissues. In light of this potential complexity, the expression and potential role(s) of SPARC in the formation of notochord, somites and other tissues will be discussed separately.

Notochord morphogenesis

Very shortly after the involution of axial mesoderm in the blastoporal region (stage 11), prospective notochordal cells begin to undergo convergent extension movements to form an axial epithelial-like rod (Keller, 1991). By scanning electron microscopy (SEM), the notochordal cells can be distinguished from adjacent primordial somitic cells by their flatter appearance and tighter associations. Their homotypic associations and rearrangements are in part dependent on cadherin and integrin receptors, which promote cell-cell and cell-matrix adhesion respectively. Adhesion by cadherins, and a subset of the integrin family, has been found to be calcium-dependent (Takeichi, 1991; Bronner-Fraser, 1993). Several integrins have been found to be expressed by presumptive notochordal cells by mid-gastrulation (Wittaker and DeSimone, 1993). Increases in EP-cadherins expression are also detectable in the notochord at this stage (Levi *et al.*, 1991). In addition to these receptors, SEM and immunohistochemical studies have shown that notochord cells secrete large amounts of ECM macromolecules. A basement membrane (BM)-like structure composed of fibronectin, laminin and glycosaminoglycans surrounds the extending notochord by stage 14/15 (Hay, 1991; Perris *et al.*, 1991; Welsch *et al.*, 1991; Ueda *et al.*, 1992). By the time the notochord induces the overlying ectoderm at the beginning of neurulation, collagen types IV and VI are also detectable in the BM. An ECM sheath composed of collagen types II, X, and proteoglycans are then laid down around the BM (Perris *et al.*, 1991; Welsch *et al.*, 1991). Both the BM and the perinotochordal sheath alter their molecular composition as development progresses. Our data now demonstrates that SPARC is also a major ECM glycoprotein secreted by the developing notochord. Its widespread distribution may reflect a role in modulating the adhesive ability of calcium-dependent cadherin and integrin receptors. The N-terminal domain of SPARC has been demonstrated to bind up to 8 calcium ions with a dissociation constant of 10^{-3}-10^{-5} M (Maurer *et al.*, 1992), approximately equal to ECM calcium levels (Romberg *et al.*, 1986). Hence, one of SPARC's functions may be to modulate the binding affinities of these receptors by acting as a local calcium-reservoir and chelator.

Somitogenesis

Similar levels of *SPARC* were detected within the axial and paraxial mesoderm during the early stage of neurulation. It is therefore conceivable that SPARC carries out similar functions during the early phases of notochord and somitic formation. However, when 20-22 paired somites had formed, dramatic changes in the spatial distribution of *SPARC* mRNA began to occur. The biological significance of these changes may be linked to the unique features of segmentation in *Xenopus*. Relative to most vertebrates, *Xenopus* somite segmentation and differentiation occurs rapidly (Hamilton, 1969; Radice *et al.*, 1989). The first segmented somites are visible in stage-16 embryos and segmentation progresses in a posterior direction at a rate of one pair of somites per 20 minutes. By stage 26, the segmented myotomes are sufficiently differentiated to undergo contractions (Radice *et al.*, 1989). Consequently only 40 h after fertilization, a swimming tadpole is hatched — which can escape from predators and search for food. Rhythmic contraction of the early embryos is triggered by neural impulses to unsegmented head somites. The depolarization signals are then propagated, in part, through the segment trunk somites via gap junctions (Blackshaw and Warner, 1975; Kordylewski, 1978; Radice *et al.*, 1989). In other model systems, the formation and stability of these gap junctions have been shown to be promoted by (calcium dependent) cadherins (Jongen *et al.*, 1991). Furthermore gating of gap junctions has also been demonstrated to be controlled by intracellular calcium ions (Bennet and Verselis, 1992). It was therefore of special interest that the commencement of trunk myotome contractions coincided with the appearance of SPARC within the intersomitic clefts. This accumulation was preceded by the translocation of *SPARC* mRNA to the apices of the myotomes — indicating that SPARC protein which accumulated within the clefts was biosynthesized adjacent to the cleft. Since SPARC can bind calcium ion with dissociation constants similar to the ECM calcium levels, it is conceivable that it acts as an extracellular calcium reservoir to affect: (1) the stability of gap junctions; and (2) the gating required for the depolarization wave to be propagated down the embryonic axis. Consistent with this hypothesis is the observation that once trunk somites become innervated later in development, both gap junctions (Radice *et al.*, 1989) and *SPARC* levels decrease dramatically within the segmented mesoderm. The widespread distribution of *SPARC* within the unsegmented mesoderm suggests that it also has roles independent of modulating myotome contractions. Previous work in our laboratory has demonstrated that when anti-SPARC antibodies were microinjected into blastula embryos, somite segmentation was severely disrupted (Purcell *et al.*, 1993). Myotomes were poorly stacked and embryos demonstrated no coordinated swimming motion. Thus SPARC may also have important functions in somite segmentation and differentiation.

Ectoderm tissues

Unlike the co-localization of *SPARC* message and protein in notochord and somites, the pattern of SPARC protein distribution in the neural tube did not match the spatial expression of its mRNA. Shortly after neural induction, *SPARC* transcript began to accumulate within the floor plate of the neural tube; however little or no protein could be detected. In contrast, little *SPARC* mRNA could be detected in the remainder of the neural tube, but it became enriched in protein. It is therefore conceivable that protein synthesized by the floor plate is recruited by the neural tube. Since the notochord and somites are both enclosed within ECM sheaths by this stage, it is unlikely that they contribute to the accumulation of SPARC within the neural tube. Since during early neural tube development cells are extensively interconnected by gap junctions (Minkoff *et al.*, 1991; Ruangvoravat and Lo, 1992), SPARC may have similar functions in the neural tube and somites in tailbud embryos. However caution must be applied since SPARC has been demonstrated to modulate cell migration *in vitro*, and therefore like other multifunctional ECM proteins, SPARC is likely to play other roles. Punctate staining of the surface ectoderm was consist-

ently observed by whole-mount *in situ* hybridization. Sectional analysis revealed that punctate staining was generated by a subset of cells associated with the sensorial layer. However, the staining pattern observed by whole-mount immunohistochemistry was in part dependent on the fixative used. The DMSO/methanol fixative used in this study gave the best view of internal tissues. However, while comparable staining of internal tissues was obtained with paraformaldehyde fixation, a much stronger staining of the surface ectoderm was observed, in particular the surface ciliated cells (data not shown). These ciliated cells are a subset of ectodermal cells which migrate from the deep ectoderm to the surface of the embryo at about the hatching stage (Drysdale and Elinson, 1992). SPARC could potentially play a role in this migration, as it is hypothesized to have anti-adhesive properties. It is also recognized that coordinated beating of ciliated epithelial cells relies, in part, on the gap junctions which connect them within the epithelial sheet (Willecke *et al.*, 1991; Hennemann *et al.*, 1992). Thus electrical coupling could still play a role in cilia action, and SPARC's presence could modulate this action. It is also important to note that Rohon-Beard sensorial neurons, which are extending within the sensorial layer, are also connected via gap junctions at this stage (Roberts, 1969; Roberts and Taylor, 1982). Several *in vitro* studies of SPARC's functions have demonstrated that SPARC inhibited cell migration, leading investigators to compare SPARC to other glycoproteins with anti-adhesive abilities, such as thrombospondin and tenascin. It is important to note that structurally SPARC bears strong similarities to other calcium-binding ECM glycoproteins that make use of acidic amino acid residue sequence motifs to bind high levels of calcium. For example, bone sialoprotein — BSP, (Butler, 1991) — shares a number of characteristics with SPARC: they are both small (32 and 33 kDa core peptide); each is enriched in bone; and both have glu/asp-rich N-terminal domains which bind to calcium with disassociation constants similar to that of ECM calcium levels. However they differ in a number of important ways, principal among which is that BSP promotes ECM mineralization, while SPARC inhibits it. Furthermore, SPARC expression is not restricted to mineralized tissues like BSP. Therefore, though SPARC may share functional traits and sequence motifs with other ECM proteins, its precise role within a tissue can only be deduced by understanding its complex regulation and interactions within its microenvironment. It was to some degree surprising how rapidly *SPARC* levels dropped once a tissue became morphologically distinct. This observation fostered the comparison of *SPARC* expression to the distribution of calcium-dependent cell receptors and ECM macromolecules. While some overlap was apparent, the distribution pattern of *SPARC* was unique. Therefore, our analysis that SPARC is intrinsically involved with calcium-dependent cell adhesion events is likely to be too simple. Several *in vitro* studies have demonstrated that the non-calcium binding regions of SPARC have important biological activities, such as inhibiting cell migration and cell-cycle arrest (Lane and Sage, 1990; Funk and Sage, 1991). Consequently it is likely that SPARC has both calcium-dependent and -independent functions which are likely to differ in different tissues. Deciphering the precise morphological contributions of any ECM macromolecules within a complex ECM network is very difficult for many reasons, e.g. due to functional redundancies and changes in activity caused by subtle changes in ECM and cell microenvironments. The high levels and changes in *SPARC* expression during somitogenesis may offer the best opportunity to explore some of the functions of SPARC during early embryogenesis. Not only do somites represent an abundant embryonic tissue, but they can easily be cultured *in vitro* and micro-manipulated. Moreover the cellular and molecular events underlying somite formation, differentiation and functions have been extensively studies in *Xenopus*. Hence the role of SPARC during somitogenesis represents a logical starting point for future studies.

Materials and Methods

Albino *Xenopus* embryos were fertilized and grown according to standard procedures. Staging of embryos was according to Nieuwkoop and Faber (1956).

Whole-mount in situ hybridization

A 460 bp EcoRI fragment of the 5' region of *Xenopus SPARC* (Damjanovski *et al.*, 1992) was subcloned into pGEM-4Z. Digoxigenin-labeled sense and anti-sense transcripts were generated using SP6 and T7 viral promoters. *In situ* hybridizations was performed as per Harland (1991) with the following modification. Embryos were fixed in MEMPFA (0.1 M MOPS, 2 mM EGTA, 1 mM $MgSO_4$, 3.7% paraformaldehyde), for 2 h at room temperature. A control type 8 cytoskeletal actin anti-sense cRNA probe (Mohun and Garrett, 1987) was used. For sectioning, *in situ* stained, methanol-stored embryos were embedded in molten Paraplast, and 40-50 μm sections were taken.

Whole-mount immunohistochemistry

Embryos were fixed at room temperature for 2 h using 25% DMSO in methanol. Embryos were washed and blocked as follows: 2x 30 min PBS, 0.1% triton-X100, 2x 1 h PBS, 0.1% triton-X100, 0.1% BSA, 0.1% dry milk powder at room temperature. All subsequent washes and incubations are at 4°C. Anti-bovine SPARC monoclonal anti-body ON3 (Malaval *et al.*, 1991), which cross-reacts with *Xenopus* SPARC (Ringuette *et al.*, 1992) was diluted 1:50 in PBS, 0.1% triton-X100, 0.1% BSA, 0.1% dry milk powder. The embryos were incubated with the diluted antibody, rocking gently overnight. Embryos were then washed 5x 1 h, PBS, 0.1% triton-X100, and 1X overnight in PBS, 0.1% triton-X100, 0.1% BSA, 0.1% dry milk powder. An alkaline phosphatase-tagged goat anti-mouse secondary antibody (Bohringer-Mannheim) was diluted 1:200 in PBS, 0.1% triton-X100, 0.1% BSA, 0.1% dry milk powder and incubated overnight with the embryos. Embryos were subsequently washed 5x 1 h, PBS, 0.1% triton-X100, and 1x rocking overnight. Embryos were incubated 2x 10 min in alkaline phosphatase buffer (100 mM Tris pH 9.5, 50 mM $MgCl_2$, 100 mM NaCl, 0.1% Tween 20, 5 mM levamisol). The colormetric reaction was achieved using NBT and BCIP at room temperature. Embryos were post-fixed in Bouin's fixative for 2 h as per Harland (1991). Embryos were then dehydrated in methanol and cleared with BBBA for photography (Harland, 1991). For sectioning embryos were taken from methanol, embedded in molten Paraplast, and 40-50 μm sections were taken. As a control, a neural specific antibody (2G9, Jones and Woodland, 1989) was used.

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