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Serum response factor is essential for mesoderm formation during mouse embryogenesis

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The transcription factor serum response factor (SRF), a phylogenetically conserved nuclear protein, mediates the rapid transcriptional response to extracellular stimuli, e.g. growth and differentiation signals. DNA–protein complexes containing SRF or its homologues function as nuclear targets of the Ras/MAPK signalling network, thereby directing gene activities associated with processes as diverse as pheromone signalling, cell-cycle progression (transitions G_0–G_1 and G_2–M), neuronal synaptic transmission and muscle cell differentiation. So far, the activity of mammalian SRF has been studied exclusively in cultured cells. To study SRF function in a multicellular organism we generated an *Srf* null allele in mice. SRF-deficient embryos (*Srf*$^{-/-}$) have a severe gastrulation defect and do not develop to term. They consist of misfolded ectodermal and endodermal cell layers, do not form a primitive streak or any detectable mesodermal cells and fail to express the developmental marker genes *Bra (T)*, *Bmp-2/4* and *Shh*. Activation of the SRF-regulated immediate early genes *Egr-1* and c-*fos*, as well as the α-*Actin* gene, is severely impaired. Our study identifies SRF as a new and essential regulator of mammalian mesoderm formation. We therefore suggest that in mammals Ras/MAPK signalling contributes to mesoderm induction, as is the case in amphibia.

Keywords: gastrulation/immediate early genes/mesoderm induction/serum response element/serum response factor

Introduction

The transcription factor serum response factor (SRF) (Prywes and Roeder, 1987; Schröter *et al*., 1987; Treisman, 1987; Norman *et al*., 1988) and SRF-directed gene activity have become one of the best characterized model systems for understanding the molecular mechanisms underlying signal-dependent gene regulation (Johansen and Prywes, 1995; Treisman, 1995). SRF directs the signal-induced activity of 'immediate early' genes (IEGs) by binding to the serum response elements (SREs) of IEG promoters (Treisman, 1986, 1987, 1992, 1995; Herrera *et al*., 1989; Herschman, 1991). Functional SRF binding sites have been identified in the promoters of some 30 different genes so far (Cahill *et al*., 1995), including c-*fos*, *Egr-1* and various α-*Actin* genes.

SRF, a MADS-box-containing transcription factor (Schwarz-Sommer *et al*., 1990; Shore and Sharrocks, 1995), has been characterized extensively, both in structural (Pellegrini *et al*., 1995) and functional (Treisman, 1995) terms. DNA recognition by SRF is directed by $CC(A/T)_6GG$ sequences, called CArG-boxes, found within SREs. SRF is able to recruit additional proteins to SREs. Such accessory factors comprise the ternary complex factors (TCFs) (Shaw *et al*., 1989a; Treisman, 1994), which belong to the Ets family of transcription factors (Hipskind *et al*., 1991; Dalton and Treisman, 1992). TCFs include the proteins Elk-1 (Hipskind *et al*., 1991; Dalton and Treisman, 1992), Sap-1 (Dalton and Treisman, 1992) and Net/ERP/Sap-2 (Giovane *et al*., 1994; Lopez *et al*., 1994; Price *et al*., 1995). Other SRF-interacting proteins are the Ets protein Fli-1 (Magnaghi-Jaulin *et al*., 1996), the homeodomain-protein Phox-1 (Grueneberg *et al*., 1992, 1997), the HTLV-1 protein Tax1 (Fujii *et al*., 1992), the p65 subunit of NF-κB (Franzoso *et al*., 1996), the myogenic bHLH heterodimers myogenin–E12 and MyoD–E12 (Groisman *et al*., 1996), and the cardiogenic homeodomain protein Nkx-2.5 (Chen and Schwartz, 1996).

SRF-containing transcription factor complexes are nuclear targets of intracellular signalling cascades, primarily the cascades of the MAP kinase network (Treisman, 1996). The Ets/TCF proteins represent direct targets of the three best characterized types of MAP kinase, i.e ERKs, Jnk/SAPK2 and p38/SAPK1 (Gille *et al*., 1992, 1995; Janknecht *et al*., 1993; Marais *et al*., 1993; Hipskind *et al*., 1994; Hill and Treisman, 1995; Zinck *et al*., 1995; Cahill *et al*., 1996; Price *et al*., 1996; Raingeaud *et al*., 1996; Treisman, 1996). SRF, a phospho-protein itself, is targeted by direct (Janknecht *et al*., 1992; Marais *et al*., 1992; Rivera *et al*., 1993; Miranti *et al*., 1995) and, possibly, indirect signalling mechanisms (Hill and Treisman, 1995; Hill *et al*., 1995).

Given the transcriptional induction of SRF-regulated genes (i.e. IEGs) during the mitogen-induced G_0–G_1 transition (Herschman, 1991), an essential involvement of SRF has been assumed in the control of proliferation and cell-cycle progression (Johansen and Prywes, 1995). Additionally, SRE-regulated gene activity during the G_2–M transition of K562 human erythroleukaemia cells (Liu *et al*., 1994), in light of the essential function performed by the *Saccharomyces cerevisiae* SRF-homologue Mcm1 at the G_2–M transition (Althöfer *et al*., 1995; Maher *et al*., 1995), further suggested that SRF participates in cell-cycle control.

In addition to regulating genes during the cell cycle, SRF and related MADS-box factors have been demonstrated as

being essential for post-replicative cell-type-specific gene regulation, namely neuronal- (Ghosh and Greenberg, 1995) and muscle-specific gene expression (Vandromme *et al*., 1992; Buckingham, 1994; Soulez *et al*., 1996; Firulli and Olson, 1997). The α-*Actin* genes have served as paradigms for SRF-directed myocyte-specific gene expression (Mohun *et al*., 1989; Sartorelli *et al*., 1990; Moss *et al*., 1994; Chen and Schwartz, 1996; Sepulveda *et al*., 1998). This includes cardiac, skeletal and vascular muscle *Actin* genes. For example, the combinatorial action of SRF, Nkx-2 and GATA-4, as part of a multi-component transcriptional regulatory complex, was shown to regulate the cardiac α-*Actin* gene in early cardiac progenitor cells (Sepulveda *et al*., 1998).

SRF expression studies support the proposed role for SRF in post-replicative neuronal and muscle gene expression. In the adult rat nervous system, SRF immunoreactivity was present in the vast majority of neurons in the forebrain, cortex, striatum, amygdala and hippocampus, and in some scattered neurons in the medulla and spinal cord (Herdegen *et al*., 1997). In the chicken, SRF expression was found to be restricted to tissues of mesodermal and neuroectodermal origin (Croissant *et al*., 1996). During chicken embryogenesis and the progression of gastrulation, strongly localized *Srf* mRNA expression was observed in the primitive streak, the neural groove, lateral plate mesoderm, Hensen's node, the precardiac splanchnic mesoderm, the myocardium and the somites. Strong SRF protein expression was seen in the myocardium of the developing chicken heart and the myotomal portion of the somites (Croissant *et al*., 1996). In the mouse, highest *Srf* mRNA levels were seen in adult skeletal and cardiac muscle. During mouse embryonic development, *Srf* transcripts were found to be enriched in smooth muscle media of the vessels, the myocardium of the heart and myotomal portions of somites (Belaguli *et al*., 1997).

Further light was shed on the biological role of SRF in a living organism by the identification of the *Drosophila melanogaster* genes *pruned* (Guillemin *et al*., 1996) and *blistered* (Montagne *et al*., 1996) as two alleles of the *Drosophila* SRF homologue (DSRF) (Affolter *et al*., 1994). The corresponding mutant phenotypes revealed SRF functions in the development of the wing disc and the tracheal system.

We sought to expand the genetic analysis of SRF function into the vertebrate system and therefore generated, using homologous recombination, *Srf* null alleles in embryonal stem (ES) cells and in the mouse. Our analysis revealed an essential function of SRF for inductive gene regulatory events leading to mesoderm formation during gastrulation.

Results

Expression pattern of SRF during early mouse development

To guide our functional analysis of SRF we first investigated the expression pattern of the *Srf* gene, at both RNA and protein levels, during the early stages of mouse development. Staining of sectioned embryos with an SRF-specific antiserum revealed expression at E6.5 in ectoderm as well as endoderm, both embryonic and extra-embryonic (Figure 1A). At E7.5, SRF protein could be seen in all three germ layers of wild-type (wt) embryos (Figure 1B). Interestingly, at E8.5, this ubiquitous distribution became a regionally localized one and SRF protein expression was found to be high in the developing heart (Figure 1C–E), but barely detectable in other tissues. This expression was specific for the myocardium (Figure 1E). At E10.5 we also detected distinct SRF protein expression in the developing myotome (Figure 1F). Northern blotting of E8.5–E12.5 embryonal RNA preparations detected two mRNA species (Figure 2) which possibly represent two differently polyadenylated variants (Norman *et al*., 1988; Belaguli *et al*., 1997). Embryonic (E8.5) protein extracts also showed SRF-associated specific DNA-binding activity toward SRE sequences. These studies confirm and extend the analysis of Belaguli *et al*. (1997) and provide, for the first time, insight on SRF protein expression during mouse early embryogenesis.

This expression analysis shows that strong embryonic SRF expression occurs ubiquitously in all germ layers at times before and after the onset of mesoderm formation. Interestingly, subsequent to the onset of organogenesis, domains of localized, strong SRF protein expression are found in specific mesodermal tissues, namely the heart myocardium and the myotome. These SRF expression patterns in mouse embryos are congruent with those found in chicken embryos (Croissant *et al*., 1996).

Targeted disruption of Srf in ES cells by homologous recombination

In order to analyse the function of SRF in vertebrates by genetic means, we generated a null mutation of *Srf* by homologous recombination in embryonic stem (ES) cells. To construct the recombination vector we cloned and structurally characterized the genomic *Srf* locus (Figure 3 and data not shown), as performed in parallel by Belaguli *et al*. (1997). Our *Srf* targeting vector (for details of the construction see Materials and methods) was designed to delete sequences encoding essential functions of SRF, namely dimerization and DNA binding (Figure 3) (Pellegrini *et al*., 1995). Twenty-three independent ES cell clones were identified as having undergone correct recombination at one *Srf* allele, as determined by genomic PCR and Southern blotting (not shown). ES cells heterozygous for the mutated *Srf* allele showed no phenotypic abnormalities. Two independent ES cell clones were used for blastocyst injections to generate chimeric mice, followed by subsequent breeding to obtain germline transmission and establishment of heterozygous $Srf^{+/-}$ mouse strains.

Embryonal lethality of embryos lacking SRF

Like the genotypically identical ES cells, mice heterozygous for the mutated *Srf* allele showed no detectable phenotypic abnormalities. In contrast, upon breeding heterozygous $Srf^{+/-}$ animals no $Srf^{-/-}$ offspring were born, indicating that the *Srf* mutation was lethal during embryogenesis (Figure 4A and B). To determine the time of embryonic lethality, embryos at different stages were analysed by morphological criteria and by genotyping. For embryos up to E9.5 Mendelian distribution of the mutated alleles was still observed (Figure 4B); however, after E12.5 $Srf^{-/-}$ embryos could no longer be detected. Thus, the embryonally lethal phenotype observed here for $Srf^{-/-}$ mouse embryos clearly reveals an essential

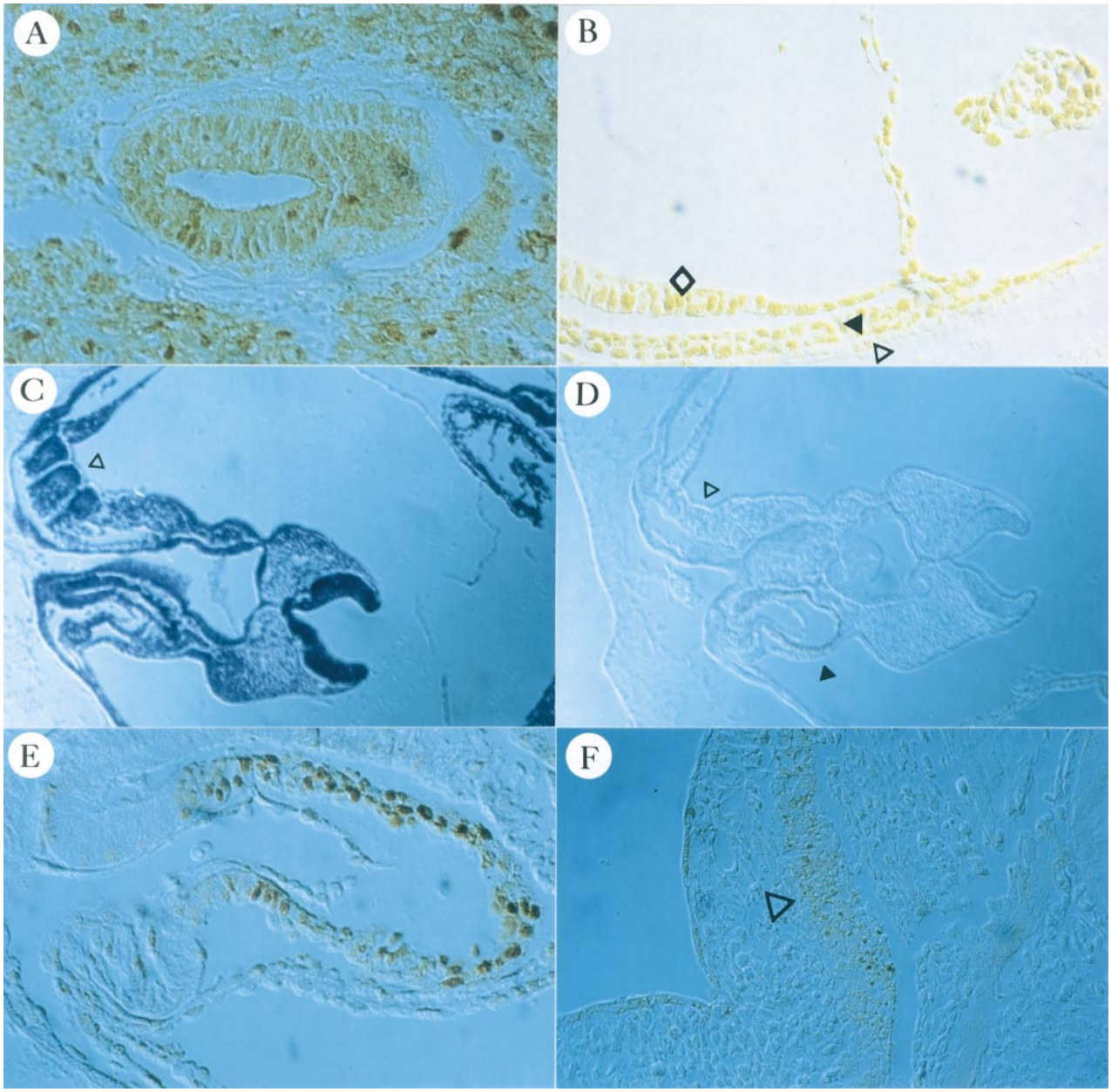


Fig. 1. SRF expression from E6.5 to E10.5, as detected by immunohistochemistry using an SRF-specific antiserum. (**A**) At E6.5 SRF was detectable in the embryonic as well as extra-embryonic ectoderm and endoderm. (**B**) At E7.5 SRF is expressed in all three germ layers (ectoderm: □; mesoderm: ▲; endoderm: △) in both the embryonic and extra-embryonic parts of the embryo. Instead, at E8.5 (**C** and **D**), SRF was found selectively and highly expressed in the developing heart (▲) and was hardly detectable in other regions of the embryo; e.g. no expression could be detected in the developing somites (△). Expression in the heart was specific for the myocardium (**E**). Expression of SRF in the myotome (△) was first detected at E10.5 (**F**). Panel (C) represents the histological stain of the identical embryo stained with anti-SRF in panel (D).

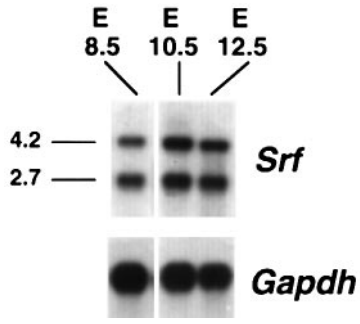


Fig. 2. Northern blot analysis of *Srf* expression during wt embryonal development.

requirement for SRF activity during the early stages of murine embryogenesis.

The mutated Srf allele represents a bona fide null allele

Homodimerization and specific DNA-binding to SRE sequences are essential to SRF's function as a transcription regulator (Johansen and Prywes, 1993; Sharrocks *et al*., 1993). The targeted *Srf* allele was intended to have deleted *Srf* coding sequences contributing to both these functions (Pellegrini *et al*., 1995). Indeed no SRF-derived SRE-binding activity (Figure 4C) or SRF protein (Figure 5E) was found in phenotypically or genotypically identified

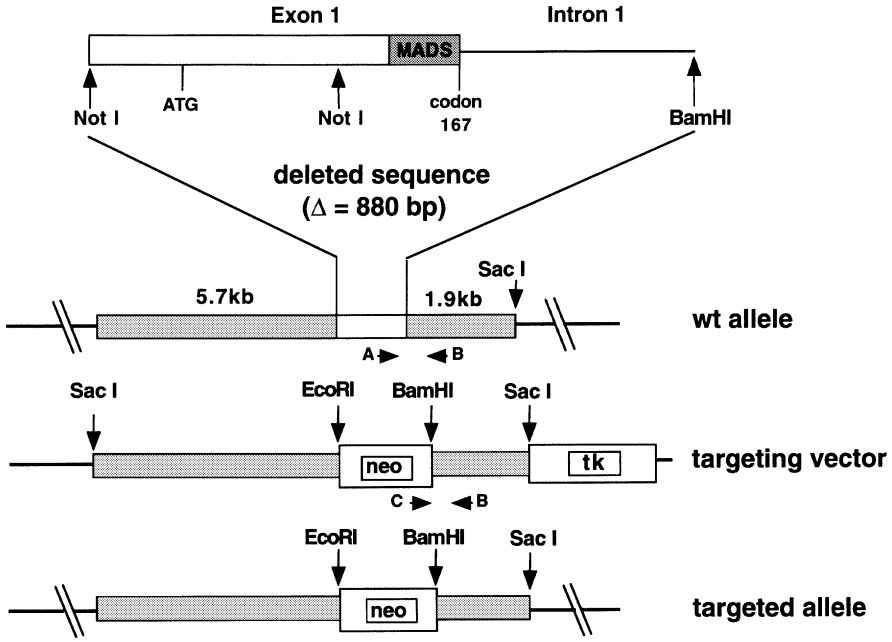
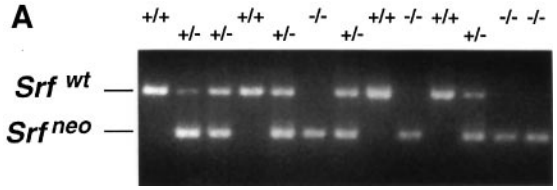


Fig. 3. Targeted disruption of the mouse *Srf* gene. Maps of part of the wild-type *Srf* locus, the targeting vector and the recombined allele. The genomic sequences cloned into the recombination vector, as well as their corresponding positions in the wt and recombined alleles, are marked as shaded bars (sizes indicated). An 880 bp segment is deleted in the targeted allele (top). This segment covered parts of exon 1 and intron 1, including SRF sequences encoding amino acid residues 1–167. Arrows (A, B and C) indicate primers used for embryo genotyping by PCR.



B

	E3.5		E7.5		E8.5		E9.5		neo-nates	
	#	%	#	%	#	%	#	%	#	%
total	28	100	30	100	67	100	22	100	149	100
Srf (+/+)	7	25	8	26.6	19	28.3	5	22.7	47	31.5
Srf (+/-)	15	53	15	50	31	46.3	12	54.5	102	68.5
Srf (-/-)	6	22	7	23.3	17	25.3	5	22.7	0	0

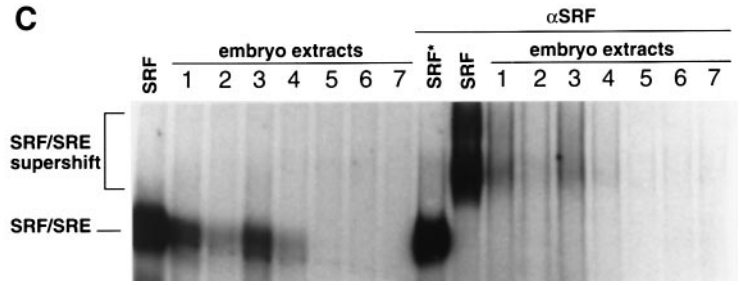


Fig. 4. (**A**) Genotyping of E9.5 embryos by PCR. Primers A to C (see Figure 3) were used to amplify DNA segments from embryo genomic DNAs. Allele-specific PCR fragments (wt allele, Srf_{wt}; mutated allele, Srf_{neo}) were separated by agarose gel electrophoresis. (**B**) Ratios of genotypes found with new-born animals and pre- and post-implantation embryos. (**C**) Electrophoretic mobility shift assays using an SRE probe and extracts from E8.5 embryos derived from $Srf^{+/-}$ matings. Embryos 1–4 differed somewhat in size but all showed primitive streak and head fold formation. Embryos 5–7 all lacked primitive streak and head fold formation and, therefore, displayed $Srf^{-/-}$ phenotypic appearance. SRF indicates the use of partially purified SRF protein, whereas SRF* indicates the additional presence of antibody blocking peptide. α-SRF indicates the use of a specific anti-SRF antiserum. No corresponding inter-embryonal differences in band-shift activity were displayed by these extracts with a DNA probe containing an Ets protein binding site (not shown), which served as a control for equal protein recovery.

$Srf^{-/-}$ embryos. RT–PCR studies also failed to detect any normal or aberrant *Srf* transcripts in these embryos.

Being unable to detect either wt SRF protein activity or any aberrant gene product derived from the targeted *Srf* locus in $Srf^{-/-}$ embryos, we conclude that our targeting strategy achieved the generation of an *Srf* null allele, and that the observed phenotype in $Srf^{-/-}$ embryos is a direct consequence of the lack of SRF.

Impaired gastrulation, lack of primitive streak formation, and absence of mesodermal cells in $Srf^{-/-}$ embryos

No phenotypic abnormalities were apparent upon comparing wt with mutated embryos at E6.5 (compare Figure 1A with Figure 5E or F). However, as early as E7.5 $Srf^{-/-}$ embryos could be distinguished from heterozygous or wt embryos by their reduced size. At E7.5, $Srf^{-/-}$ embryos

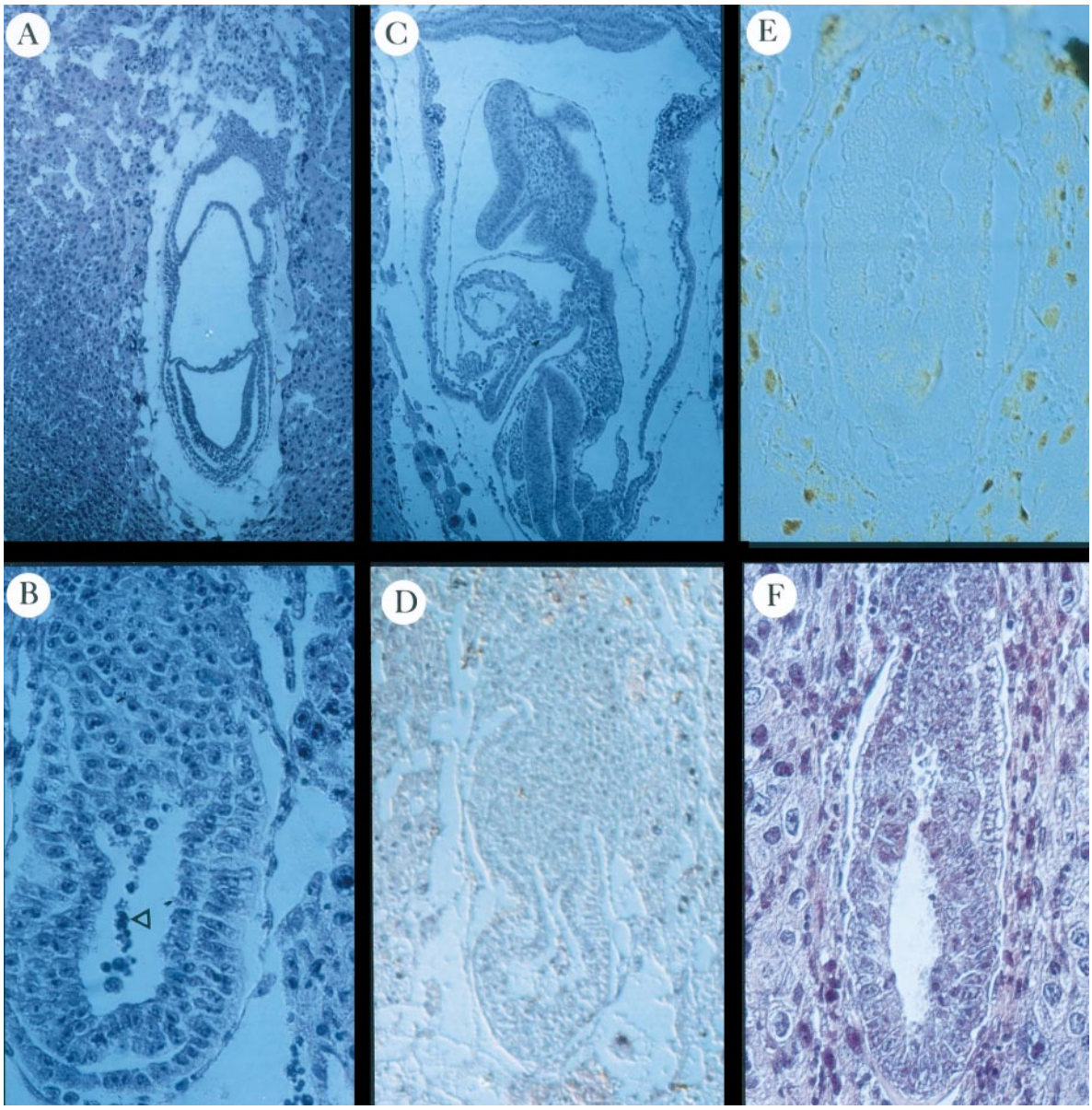


Fig. 5. Histology of paraffin sections from wt and *Srf*$^{-/-}$ embryos, including anti-SRF staining patterns. Wt embryos of E7.5 (**A**) and E8.5 (**C**) are compared with *Srf*$^{-/-}$ embryos of E7.5 (**B**) and E8.5 (**D**). Additionally, an E6.5 *Srf*$^{-/-}$ embryo is shown, displaying normal histological appearance (**F**) while revealing the complete lack of anti-SRF antibody reactivity (**E**). Sections (A) and (C) were photographed as 100× views of the microscopic fields, (D) as 200× view, and the other sections were photographed as 400× enlargements. Note the pyknotic cells in (B), marked by the open triangle.

showed delayed development and displayed late egg cylinder stage morphology (compare Figure 5A and B). They did not form a primitive streak and, histologically, no mesodermal cells were apparent. Instead, pyknotic cells could be detected in the embryonic cavity. At E7.5 some mutant embryos already displayed aberrant folding of both embryonic ectoderm and endoderm, which was seen more strongly at E8.5 in all *Srf*$^{-/-}$ embryos (compare Figure 5C and D). Disintegrating *Srf*$^{-/-}$ embryos started to appear from E8.5 and after E12.5 no such embryos could be detected.

These observations demonstrate that the lack of SRF causes an early embryonic phenotype at E7.0–E7.5, shortly after the normal onset of gastrulation. The apparently normal development up to E6.5 reveals that SRF is not essential for the proliferation of embryonal cells, at least up to E6.5. However, the observed phenotype of *Srf*$^{-/-}$ embryos implies an essential requirement for SRF during the process of mesoderm formation, possibly for mesoderm induction itself.

Absence of mesodermal marker gene expression in Srf$^{-/-}$ *embryos*

Next, we characterized in more detail the types of cell present and absent in *Srf*$^{-/-}$ embryos. Immunohistological stainings on paraffin-embedded sections of E7.5 embryos were used to identify cellular marker proteins of ectoderm (Oct-6 and cytoplasmic LEF-1) (Behrens *et al*., 1996; Huber *et al*., 1996; Zwart *et al*., 1996), mesoderm (Brachyury and nuclear LEF-1) (Wilkinson *et al*., 1990) and endoderm, which was specifically stained by an anti-HNF3β antiserum (Sasaki and Hogan, 1994). In wt

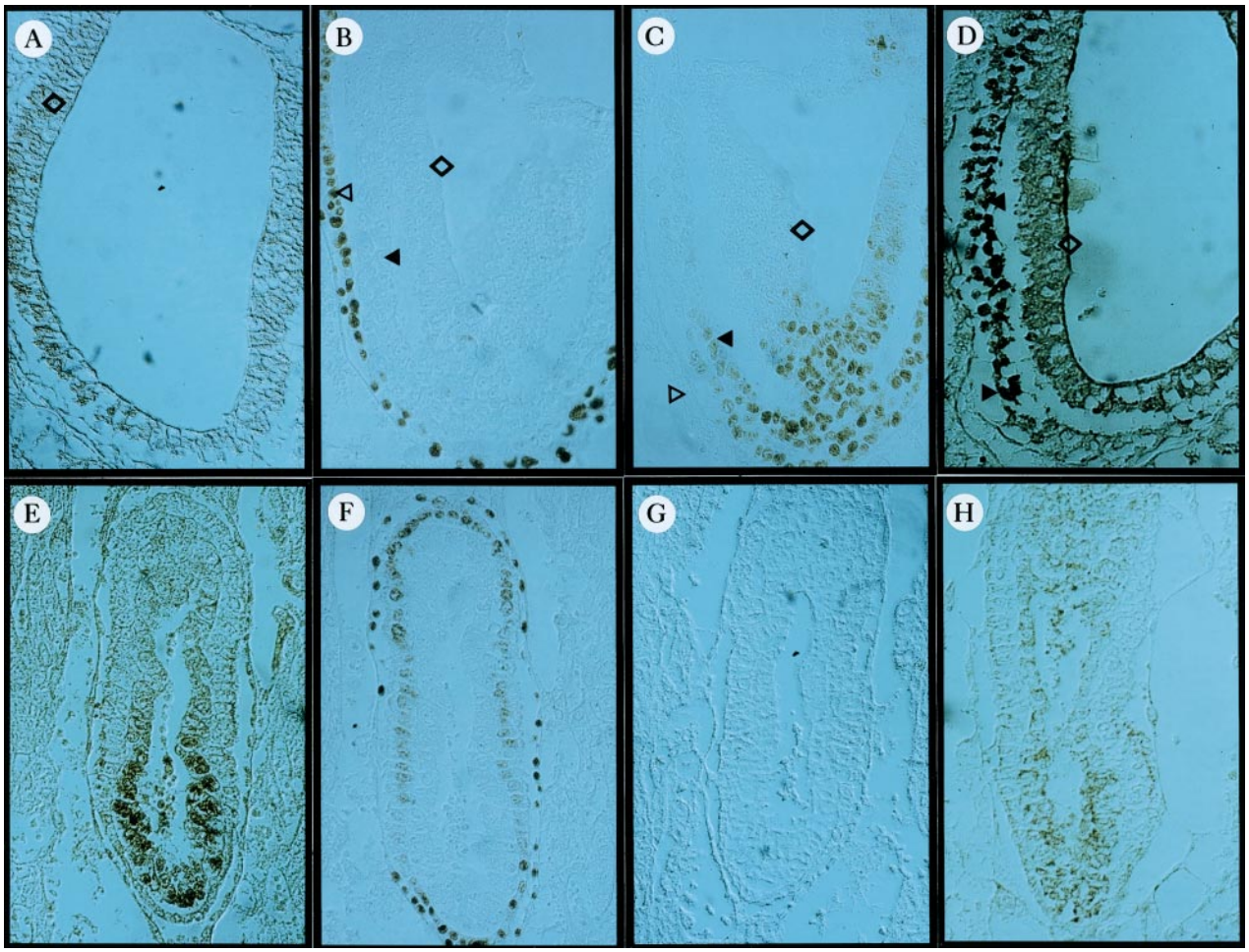


Fig. 6. Antibody staining of developmental marker proteins in E7.5 wt and $Srf^{-/-}$ embryos. Wt (**A**–**D**) and $Srf^{-/-}$ embryo stainings (**E**–**H**) were performed with antisera directed against Oct-6 (A and E), HNF-3β (B and F), Brachyury (C and G) or LEF-1 (D and H). Sections from identical embryos are each represented in (A and D), (B and C) and (E–H), respectively. All sections were photographed as 400× views of the microscopic fields. In wt embryos, the three germ layers are marked (ectoderm, □; mesoderm, ▲; endoderm, △).

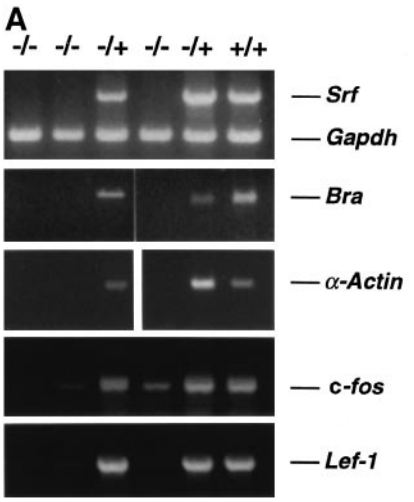
embryos, primitive ectoderm specifically expressed the transcription factor Oct-6 (Figure 6A), as did anterior definitive ectoderm and the chorion after closure of the proamniotic channel (Zwart *et al*., 1996). Staining for the transcription factor HNF-3β was specific for parietal and visceral endoderm of wt embryos (Figure 6B; see Materials and methods). In these wt embryos, *Brachyury* was highly expressed in the ectoderm surrounding the node and in the migrating mesoderm (Figure 6C). LEF-1 staining was found in the cytoplasm of ectodermal and, in contrast, in the nuclei of mesodermal wt cells (Figure 6D). This differential intracellular localization of LEF-1 possibly reflects nuclear translocation of LEF-1/β-catenin complexes after mesoderm formation (Behrens *et al*., 1996; Huber *et al*., 1996). In $Srf^{-/-}$ embryos strong staining for Oct-6 and HNF-3β was seen (Figure 6E and F), whereas, significantly, no Brachyury or nuclear LEF-1 could be detected at all (Figure 6G and H, respectively). Since the stainings were performed on successive sections of the same homozygous embryos we conclude that $Srf^{-/-}$ embryos consist of primitive ectoderm and endoderm, while clearly lacking mesoderm.

Expression of developmental marker genes in $Srf^{-/-}$ embryos

The lack of mesoderm in $Srf^{-/-}$ embryos was substantiated by RT–PCR studies that revealed absent or strongly impaired expression of developmental marker genes. In $Srf^{-/-}$ embryos ranging from E7.5 to E9.5, no transcripts could be detected for *Bra*, *Shh* and the TGFβ-related genes *Bmp2* and *Bmp4* (Figure 7A and B, and data not shown) (Wilkinson *et al*., 1990; Chiang *et al*., 1996; Zhang and Bradley, 1996). Significantly reduced mRNA levels were measured for *Gsc*, *Fgf-5* and *Nodal* (Figure 7B and data not shown) (Lemaire and Kodjabachian, 1996). This expression pattern of developmental marker genes correlates with an arrest in development of $Srf^{-/-}$ embryos during gastrulation, at the stage when mesoderm is being formed.

Reduced expression levels in $Srf^{-/-}$ embryos of the SRE-regulated genes c-fos, Egr-1 and α-Actin

In tissue culture cells SRF contributes significantly to the transient induction of immediate early genes, such as *Egr-1* and c-*fos*. This gene activation during the cellular G_0–G_1 transition is directed by SRE sequences and occurs efficiently upon activation of the MAP kinase signalling network (Herschman, 1991; Cahill *et al*., 1996; Treisman, 1996). Figure 7A and B (and data not shown) show that in $Srf^{-/-}$ embryos both the c-*fos* and *Egr-1* genes are drastically reduced in their expression levels. It is important to note, however, that the activity of both genes was not abolished completely in the $Srf^{-/-}$ embryos, suggesting



B

		Srf	Bra	Shh	Bmp2	α-actin	Gsc	Nodal	c-fos	Egr-1	Gapdh
Srf (+/+)	E7.5	+	+	-	+	+	+	+	(+)	(+)	+
	E8.5	+	+	+	+	+	+	+	+	+	+
	E9.5	+	+	+	+	+	+	+	+	+	+
Srf (+/-)	E7.5	+	+	-	+	+	+	+	(+)	(+)	+
	E8.5	+	+	+	+	+	+	+	+	+	+
	E9.5	+	+	+	+	+	+	+	+	+	+
Srf (-/-)	E7.5	-	-	-	-	nd	(+)	(+)	(+)	(+)	+
	E8.5	-	-	-	-	-	(+)	(+)	(+)	(+)	+
	E9.5	-	-	-	-	-	(+)	(+)	(+)	(+)	+

Fig. 7. Expression analysis by RT–PCR of embryonal marker genes in wt and mutated *Srf* embryos. (**A**) RT–PCR patterns representing marker gene expression in E9.5 embryos of the $Srf^{+/+}$, $Srf^{+/-}$ and $Srf^{-/-}$ genotypes. (**B**) Summary representation of developmental marker gene expression levels determined by RT–PCR. Not shown are expression patterns of *Bmp4* and *Fgf-5* which displayed the same patterns as the ones of *Bmp2* and *Nodal*, respectively. Semi-quantitative expression levels are indicated by the symbols +, – and (+). nd, not determined.

that basal expression levels of these genes are modulated additionally by factors other than SRF.

The α-*Actin* genes, i.e. those encoding the skeletal, cardiac and vascular smooth muscle α-actins, also represent well-characterized direct SRF target genes (Muscat *et al*., 1988; Taylor *et al*., 1989; Treisman, 1992; Moss *et al*., 1994). In wt embryos, α-*Actin* expression was already observed at times before (E7.5) and concomitant with (E8.5–E9.5) the onset of myogenesis (Figure 7A and B). Heterozygous $Srf^{+/-}$ embryos displayed *Actin* expression levels comparable with wt. However, no α-*Actin* gene expression could be detected by our RT–PCR analysis in $Srf^{-/-}$ embryos. This applied equally to skeletal, cardiac and smooth muscle α-*Actin* genes (Figure 7A and B, and data not shown).

This analysis demonstrates that, in mouse embryos, SRE-regulated genes are severely impaired in their expression in the absence of SRF. Furthermore, this expression analysis confirms that SRF is essential for the transcriptional activation of these genes *in vivo*. This indicates that SRE control sequences are indeed the DNA-binding sites through which SRF exerts its transcriptional regulatory function in living organisms (Treisman, 1987; Herrera *et al*., 1989).

Discussion

By generating a null allele of the *Srf* gene we identified a new function for the transcription factor SRF in mouse embryogenesis. SRF is essential for mesoderm formation during gastrulation. Mouse embryos lacking SRF develop normally until day 6.5 of embryogenesis. However, formation of the mesodermal germ layer does not occur and, consequently, SRF-negative embryos die *in utero*. This represents the first genetic analysis of SRF function within vertebrates.

SRF is not essential for cellular proliferation

SRE/SRF-directed gene activation has been observed at different stages of the cell cycle, i.e. at the G_0–G_1 transition (IEG activation; Gauthier-Rouvière *et al*., 1991a; Herschman, 1991), during G_1 (Gauthier-Rouvière *et al*., 1991b), and at the G_2–M transition (Liu *et al*., 1994). Accordingly, an essential role for SRF in the regulation of cell-cycle progression has been assumed. In support of this notion, the *S.cerevisiae* homologue of SRF, Mcm1, was shown to be essential for the G_2–M transition in yeast cells (Althöfer *et al*., 1995; Maher *et al*., 1995). In contrast, the phenotype of the $Srf^{-/-}$ embryos revealed that the lack of mammalian SRF did not prevent cell proliferation *per se*, since the $Srf^{-/-}$ embryos developed normally up to E6.5 (Figure 5E) and continued to grow even in the absence of mesoderm (Figure 5B and D). Therefore it is unlikely that general proliferative defects formed the basis of the defective mesoderm formation in $Srf^{-/-}$ embryos. Similarly, preliminary studies with ES cells homozygous for the mutated *Srf* allele failed to detect any severe consequences on cell proliferation (B.Weinhold, S.Arsenian, A.Nordheim and U.Rüther, unpublished observations). Interestingly, Roch *et al*. (1998) have shown that cells homozygous for the *blistered* mutation, representing an allele encoding a defective DSRF, are also not affected in their proliferation but rather in their capacity to differentiate into vein or intervein tissue in the developing *Drosophila* wing. Taken together, it can be concluded that SRF is not essential for normal progression of the cell cycle, nor are the products of the SRF-regulated genes c-*fos* (Wang *et al*., 1992) and *egr-1* (Lee *et al*., 1996), the absence of which did not reveal any general proliferative defects (Field *et al*., 1992).

Impaired expression of SRE-regulated genes in $Srf^{-/-}$ embryos

SREs have been strongly implicated in directing the transient, growth-factor-induced transcriptional activation of the immediate early genes (IEGs) c-*fos* and *Egr-1*, and the muscle-specific regulation of α- and γ-*Actin* genes. Whereas these genes are expressed efficiently at E7.5–E8.5 in wt embryos, we see a drastic impairment of their activation in $Srf^{-/-}$ embryos. Thus, our data provide strong evidence that these SRE-containing genes are indeed regulated by SRF in the living organism, at least during mouse embryogenesis at E7.5–E8.5.

The observed phenotype of $Srf^{-/-}$ embryos is not likely to be due to impaired expression of c-*fos* and *Egr-1*, since individually, none of these display severe gastrulation defects when mutated (Wang *et al*., 1992; Lee *et al*., 1996); a corresponding double null mutation has yet to

be generated. Impaired *Actin* gene expression may well contribute to the phenotype (see below). Although the expression of c-*fos* and *Egr-1* was found to be drastically impaired, it was not prevented fully. This may reflect the contribution of other promoter elements to the expression of these genes. Alternatively, it may hint at a dual role of SRF *in vivo*, in that SRF may contribute to the basal repression of target genes (Shaw *et al*., 1989b), in addition to mediating their transient transcriptional induction.

SRF is essential for mesoderm formation in the mouse embryo

Although molecular events of gastrulation, specifically mesoderm induction and patterning, are much better characterized in amphibians than in mice (Smith, 1995), gene 'knockout' strategies have provided important insights into vertebrate early embryogenesis (St-Jacques and McMahon, 1996; Tam and Behringer, 1997). Mesoderm formation involves inductive signalling events that are activated by mesoderm inducing factors (MIFs), such as activin, BMPs and FGFs, leading to the conversion of ectodermal cells into migrating mesodermal cells (Beddington and Smith, 1993). The accompanying changes in gene expression resemble in part a classical 'immediate early' response (Herschman, 1991) whose maintenance in *Xenopus laevis* mesoderm induction requires Ras/Raf/MAP kinase signalling (LaBonne and Whitman, 1994).

Up to E6.5, the $Srf^{-/-}$ mouse embryos were phenotypically indistinguishable from wt embryos; however, after the onset of gastrulation it was evident that the embryos lacking SRF did not form mesodermal cells. They did not express the *Brachyury (T)* gene, which encodes a tissue-specific transcription factor called T protein (Kispert *et al*., 1995). The T protein is required for differentiation of the notochord and formation of mesoderm during posterior development. Furthermore, $Srf^{-/-}$ embryos did not express the bone morphogenetic proteins BMP2 and BMP4. Lack of mesoderm formation was observed previously in embryos carrying a null allele of the BMP receptor gene *Bmpr* (Mishina *et al*., 1995). We conclude that SRF activity is required early during the execution of the genetic programme that establishes the third germ layer formation. Our study therefore identifies SRF as a new and essential contributor to mammalian mesoderm formation.

Since we have found SRF expression in both embryonic and extra-embryonic cells of wt embryos (Figure 1), participation of SRF-mediated signals derived from extra-embryonic cells cannot be excluded.

SRF activity and FGF signalling during development of vertebrates and invertebrates

Since SRF is a well-characterized transcriptional mediator of the Ras/Raf/MAPK signalling network (Cahill *et al*., 1996; Treisman, 1996), this may imply that, in congruency with amphibian mesoderm induction (Smith, 1995), mammalian mesoderm induction also involves MAPK signalling. SRF is a potent mediator of FGF signalling (Treisman, 1996). FGF molecules have been shown to be important components in the regulation of gastrulation in vertebrates (Smith, 1995; Tam and Behringer, 1997). However, separate inactivation of individual FGFs (i.e. FGF3, 4, 5 or 8) still allowed the formation of mesodermal cells in homozogous mouse embryos. Recently a nuclear mediator of BMP signalling, the Tlx-2 transcription factor, has been inactivated and the homozygous embryos displayed a phenotype similar to the $Bmp4^{-/-}$ embryos, while exhibiting a penetrance of the early embronic lethal phenotype which was higher than that observed with $Bmp4^{-/-}$ embryos (Tang *et al*., 1998). The authors attribute this to the transcription factor being positioned at the bottom of converging signalling pathways activated by different BMPs, thereby preventing compensation by separate members of the BMP family. By analogy it could be speculated that SRF represented an essential mediator of converging FGF signals in gastrulation, whereby inactivation of SRF would lead to a strong phenotype by preventing compensation by separate FGF pathways. This would then contrast with the weaker phenotypes obtained upon inactivation of individual FGF molecules of partially overlapping expression patterns. This implies that SRF is an essential component of signalling by several FGF molecules involved in vertebrate gastrulation, thereby acting as a point of convergence for different FGF signals. Furthermore, it will be of great interest to determine the nature of the SRF accessory proteins that may aid SRF in perceiving the different signals and converting them into a specific transcriptional response at the onset of gastrulation.

FGF-signalling and SRF function could also be correlated functionally in another developmental process, namely formation of the tracheal system of *D.melanogaster*. Tracheal cell migration and the branching pattern of tracheae are dependent on the functioning of the FGF homologue *branchless* (Sutherland *et al*., 1996) and DSRF (Affolter *et al*., 1994; Guillemin *et al*., 1996). Specifically, terminal branching and the shape of terminal cells are under the control of DSRF or its mutated allele *pruned*.

The defect in α-*Actin* expression observed here in the $Srf^{-/-}$ background, together with our finding of early embryonic α-*Actin* gene expression in the mouse (Figure 7B), invites the speculation that SRF-directed α-*Actin* synthesis may be essential for the generation and the proper functioning of mesodermal cells. Specific cytoskeletal requirements for α-*Actin* expression might be associated with controlled signal transduction in mesoderm-committed ectodermal cells or, alternatively, with the migration of newly formed mesodermal cells. Interestingly, lack of DSRF in the *D.melanogaster* mutant *pruned* was also interpreted to result in a defect in cytoskeletal architecture (Guillemin *et al*., 1996). Recently, we have also shown in *X.laevis* early embryos that SRF-containing ternary complexes are involved in the mesoderm-specific expression of the *Xegr-1* gene (Panitz *et al*., 1998). Here again, we found that SRF-containing regulatory complexes mediate FGF-induced expression of *Xegr-1*. An involvement of MAP kinase signalling was also demonstrated.

The present work identifies SRF as an essential nuclear component of the regulatory network controlling murine mesoderm formation. The further characterization of MIF-induced signalling steps targeting SRF-containing complexes, as well as the identification of new SRF target genes regulated thereby, promises a deeper understanding of the molecular mechanisms regulating germ layer formation in mammalian embryogenesis. Studies directed

towards that aim will now be aided greatly by the availability of murine stem cells and embryos which lack SRF.

Materials and methods

Targeting of the murine Srf locus

The *Srf* locus was cloned from a strain 129/SV genomic library (Lambda Fix II, Stratagene). In the recombination vector we replaced a 0.88 kb *Not*I–*Bam*HI fragment, containing SRF coding sequences for amino acid residues 1–167, with the PGK-promoter-driven neomycin phosphotransferase gene (Soriano *et al*., 1991) (Figure 3). Sequences 5.7 kb upstream and 1.9 kb downstream of this segment were placed into the pBS-SK vector (Stratagene). Additional introduction of the HSV *tk* gene, regulated by its own promoter, allowed positive/negative selection (350 μg/ml G418, 2 μg/ml gancyclovir) (Mansour *et al*., 1988) of ES14 cells electroporated with *Not*I-linearized target vector DNA. Selected ES cell clones were used directly for PCR genotyping, expansion and confirmatory Southern blotting. Two independent ES clones with single integration events were injected into C57Bl/6 blastocysts to obtain germ-line transmitting chimeric mice and, subsequently, $Srf^{+/-}$ mice for breeding.

Histology and immunohistochemistry

Embryos were fixed for at least 4 h in 4% paraformaldehyde or in an aqueous solution containing 35% methanol, 35% acetone and 5% acetic acid (Zwart *et al*., 1996). They were subsequently dehydrated, embedded in paraffin and sectioned at 7 μm. The sections were then either stained with haematoxylin and eosin or used for immunostaining. Immunostains were performed using the vectastain kit (vector labs) as described by the manufacturer. The anti-SRF antiserum (Santa Cruz) was further characterized by ourselves with regard to its SRF specificity using Western blotting, immunohistochemistry, band-shift studies and peptide blocking experiments. Surprisingly, the antibody specific for HNF-3β stained exclusively parietal and visceral endoderm in wt as well as mutant embryos and was therefore used as a marker for the presence and integrity of endodermal cell layers only.

Genotyping of ES cells, embryos and sectioned embryos by PCR

All experimental details for genotyping, i.e. primer sequences, PCR reaction conditions, etc. are available upon request. Electroporated ES cells were genotyped by PCR. Of 500 selected ES colonies a total of 23 revealed the correct diagnostic 2.1 kb PCR fragment. For PCR genotyping of embryos and tail DNA, using primers A, B and C (Figure 3), genomic DNA and total RNA were isolated simultaneously using the PANext Combi DNA/RNA extraction kit (PAN Systems GmbH). Genotyping of embryos after paraffin sectioning required demounting in xylene, followed by soaking in 100% ethanol and air drying. Embryonic tissues were scraped from prewetted sections, transferred into lysis buffer and processed as described previously (Imamoto and Soriano, 1993).

Expression analysis by RT–PCR

Detailed protocols for RT–PCR measurements are available upon request. A quantity (20 ng) of total embryonal RNA was used for reverse transcription using Superscript reverse transcriptase II (Gibco-BRL). One-twentieth of this reaction was used for PCR amplification with specific primers. For mRNA input controls, RT–PCR reactions were carried out in the presence of the additional *Gapdh* primers.

Northern blotting

For Northern analysis, RNA from E8.5 to E12.5 embryos was isolated using the Ultraspec RNA isolation kit (Biotecx Lab.). After isolation of poly(A)$^+$ RNA (Oligotex mRNA kit, Qiagen), 2–4 μg of RNA were electrophoresed and transferred to GeneScreen Plus nylon membrane (NEN, Boston). Filters were hybridized with [^{32}P]dATP labelled mouse *Srf* cDNA or *Gapdh* cDNA probes under standard conditions.

Electrophoretic mobility shift assay

Whole-cell extracts were prepared from E8.5 embryos in lysis buffer and 2 μg of protein were used for mobility shift assays as described (Hipskind *et al*., 1994). SRF-specific antibody [SRF(G20), Santa Cruz] was incubated with binding buffer for 10 min before the cell extracts were added.

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References

Affolter,M., Montagne,J., Walldorf,U., Groppe,J., Kloter,U. and LaRosa,M. (1994) The *Drosophila* SRF homolog is expressed in a subset of tracheal cells and maps within a genomic region required for tracheal development. *Development*, **120**, 743–753.

Althöfer,H., Schleiffer,A., Wassmann,K., Nordheim,A. and Ammerer,G. (1995) Mcm1 is required to coordinate G_2-specific transcription in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **15**, 5917–5928.

Beddington,R.S.P. and Smith,J.C. (1993) Control of vertebrate gastrulation: inducing signals and responding genes. *Curr. Opin. Genet. Dev.*, **3**, 655–661.

Behrens,J., von Kries,J.P., Kühl,M., Bruhn,L., Wedlich,D., Grosschedl,R. and Birchmeier,W. (1996) Functional interaction of β-catenin with the transcription factor LEF-1. *Nature*, **382**, 638–642.

Belaguli,N.S., Schildmeyer,L.A. and Schwartz,R.J. (1997) Organization and myogenic restricted expression of the murine serum response factor gene. *J. Biol. Chem.*, **272**, 18222–18231.

Buckingham,M. (1994) Molecular biology of muscle development. *Cell*, **78**, 15–21.

Cahill,M.A., Janknecht,R. and Nordheim,A. (1995) Signal uptake by the c-fos serum response element. *Inducible Gene Expression*, **2**, 39–72.

Cahill,M.A., Janknecht,R. and Nordheim,A. (1996) Signalling pathways: Jack of all cascades. *Curr. Biol.*, **6**, 16–19.

Chen,C.Y. and Schwartz,R.J. (1996) Recruitment of the tinman homolog Nkx-2.5 by serum response factor activates cardiac α-*Actin* gene transcription. *Mol. Cell. Biol.*, **16**, 6372–6384.

Chiang,C., Litingtung,Y., Lee,E., Young,K.E., Corden,J.L., Westphal,H. and Beachy,P.A. (1996) Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature*, **383**, 407–413.

Croissant,J.D., Kim,J.-H., Eichele,G., Goering,L., Lough,J., Prywes,R. and Schwartz,R.J. (1996) Avian serum response factor expression restricted primarily to muscle cell lineages is required for α-*Actin* gene transcription. *Dev. Biol.*, 250–264.

Dalton,S. and Treisman,R. (1992) Characterization of SAP-1, a protein recruited by serum response factor to the c-fos serum response element. *Cell*, **68**, 597–612.

Field,S.J., Johnson,R.S., Mortensen,R.M., Papaioannou,V.E., Spiegelman,B.M. and Greenberg,M.E. (1992) Growth and differentiation of embryonic stem cells that lack an intact c-*fos* gene. *Proc. Natl Acad. Sci. USA*, **89**, 9306–9310.

Firulli,A.B. and Olson,E.N. (1997) Modular regulation of muscle gene transcription: a mechanism for muscle cell diversity. *Trends Genet.*, **13**, 364–369.

Franzoso,G., Carlson,L., Brown,K., Doucher,M.B., Bressler,P. and Siebenlist,U. (1996) Activation of the serum response factor by p65/NF-κB. *EMBO J.*, **15**, 3403–3412.

Fujii,M., Tsuchiya,H., Chuhjo,T., Akizawa,T. and Seiki,M. (1992) Interaction of HTLV-1 Tax1 with p67SRF causes the aberrant induction of cellular immediate early genes through CArG boxes. *Genes Dev.*, **6**, 2066–2076.

Gauthier-Rouvière,C., Basset,M., Blanchard,J.-M., Cavadore,J.-C., Fernandez,A. and Lamb,N.J.C. (1991a) Casein kinase II induces c-fos expression via the serum response element pathway and p67SRF phosphorylation in living fibroblasts. *EMBO J.*, **10**, 2921–2930.

Gauthier-Rouvière,C., Cavadore,J.-C., Blanchard,J.-M., Lamb,N.J.C. and Fernandez,A. (1991b) p67SRF is a constitutive nuclear protein implicated in the modulation of genes required throughout the G_1 period. *Cell Regulation*, **2**, 575–588.

Ghosh,A. and Greenberg,M.E. (1995) Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science*, **268**, 239–247.

Gille,H., Sharrocks,A.D. and Shaw,P.E. (1992) Phosphorylation of transcription factor p62TCF by MAP kinase stimulates ternary complex formation at c-fos promoter. *Nature*, **358**, 414–417.

Gille,H., Strahl,T. and Shaw,P.E. (1995) Activation of ternary complex factor Elk-1 by stress-activated protein kinases. *Curr. Biol.*, **5**, 1191–1200.

Giovane,A., Pintzas,A., Maira,S.-M., Sobieszczuk,P. and Wasylyk,B. (1994) Net, a new Ets transcription factor that is activated by Ras. *Genes Dev.*, **8**, 1502–1513.

Groisman,R., Masutani,H., Leibovitch,M.-P., Robin,P., Soudant,I., Trouche,D. and Harel-Bellan,A. (1996) Physical interaction between the mitogen-responsive serum response factor and myogenic basic-helix–loop–helix proteins. *J. Biol. Chem.*, **271**, 5258–5264.

Grueneberg,D.A., Natesan,S., Alexandre,C. and Gilman,M.Z. (1992) Human and *Drosophila* homeodomain, proteins that enhance the DNA-binding activity of serum response factor. *Science*, **257**, 1089–1095.

Grueneberg,D.A., Henry,R.W., Brauer,A., Novina,C.D., Cheriyath,V., Roy,A.L. and Gilman,M. (1997) A multifunctional DNA-binding protein that promotes the formation of serum response factor/homeodomain complexes: identity to TFII-I. *Genes Dev.*, **11**, 2482–2493.

Guillemin,K., Groppe,J., Dücker,K., Treisman,R., Hafen,E., Affolter,M. and Krasnow,M.A. (1996) The *pruned* gene encodes the *Drosophila* serum response factor and regulates cytoplasmic outgrowth during terminal branching of the tracheal system. *Development*, **122**, 1353–1362.

Herdegen,T., Blume,A., Buschmann,T., Georgakopoulos,E., Winter,C., Schmid,W., Hsieh,T.F., Zimmermann,M. and Gass,P. (1997) Expression of activating transcription factor-2, serum response factor and cAMP/Ca response element binding protein in the adult rat brain following generalized seizures, nerve fibre lesion and ultraviolet irradiation. *Neuroscience*, **81**, 199–212.

Herrera,R.E., Shaw,P.E. and Nordheim,A. (1989) Occupation of the c-fos serum response element *in vivo* by a multi-protein complex is unaltered by growth factor induction. *Nature*, **340**, 68–70.

Herschman,H.R. (1991) Primary response genes induced by growth factors and tumor promoters. *Annu. Rev. Biochem.*, **60**, 281–319.

Hill,C.S. and Treisman,R. (1995) Differential activation of c-fos promoter elements by serum, lysophosphatic acid, G proteins and polypeptide growth factors. *EMBO J.*, **14**, 5037–5047.

Hill,C.S., Wynne,J. and Treisman,R. (1995) The Rho family GTPases RhoA, Rac1 and CDC42Hs regulate transcriptional activation by SRF. *Cell*, **81**, 1159–1170.

Hipskind,R.A., Rao,V.N., Mueller,C.G.F., Reddy,E.S.P. and Nordheim,A. (1991) Ets-related protein Elk-1 is homologous to the c-fos regulatory factor p62TCF. *Nature*, **354**, 531–534.

Hipskind,R.A., Büscher,D., Nordheim,A. and Baccarini,M. (1994) Ras/MAP kinase-dependent and independent signaling pathways target distinct ternary complex factors. *Genes Dev.*, **8**, 1803–1814.

Huber,O., Korn,R., McLaughlin,J., Ohsugi,M., Herrmann,B.G. and Kemler,R. (1996) Nuclear localization of β-catenin by interaction with transcription factor LEF-1. *Mech. Dev.*, **59**, 3–10.

Imamoto,A. and Soriano,P. (1993) Disruption of *csk* gene, encoding a negative regulator of Src family tyrosine kinases, leads to neural tube defects and embryonic lethality in mice. *Cell*, **73**, 1117–1124.

Janknecht,R., Hipskind,R.A., Houthaeve,T., Nordheim,A. and Stunnenberg,H.G. (1992) Identification of multiple SRF N-terminal phosphorylation sites affecting DNA binding properties. *EMBO J.*, **11**, 1045–1054.

Janknecht,R., Ernst,W.H., Pingoud,V. and Nordheim,A. (1993) Activation of ternary complex factor Elk-1 by MAP kinases. *EMBO J.*, **12**, 5097–5104.

Johansen,F.-E. and Prywes,R. (1993) Identification of transcriptional activation and inhibitory domains in serum response factor (SRF) by using GALA-SRF constructs. *Mol. Cell. Biol.*, **13**, 4640–4647.

Johansen,F.-E. and Prywes,R. (1995) Serum response factor: transcriptional regulation of genes induced by growth factors and differentiation. *Biochim. Biophys. Acta*, **1242**, 1–10.

Kispert,A., Koschorz,B. and Hermann,B.G. (1995) The T protein encoded by Brachyury is a tissue-specific transcription factor. *EMBO J.*, **14**, 4763–4772.

LaBonne,C. and Whitman,M. (1994) Mesoderm induction by activin requires FGF-mediated intracellular signals. *Development*, **120**, 463–472.

Lee,S.L., Sadovsky,Y., Swirnoff,A.H., Polish,J.A., Goda,P., Gavrilina,G. and Milbrandt,J. (1996) Luteinizing hormone deficiency and female infertility in mice lacking the transcription factor NGF1-A (Egr-1). *Science*, **273**, 1219–1221.

Lemaire,P. and Kodjabachian,L. (1996) The vertebrate organizer: structure and molecules. *Trends Genet.*, **12**, 525–531.

Liu,S.-H., Lee,H.-H., Chen,J.-J., Chuang,C.-F. and Ng,S.-Y. (1994) Serum response element-regulated transcription in the cell cycle: possible correlation with microtubule reorganization. *Cell Growth Differ.*, **5**, 447–455.

Lopez,M., Oettgen,P., Akbarali,Y., Dendorfer,U. and Liebermann,T. (1994) ERP, a new member of the ets transcription factor/oncoprotein family: cloning, characterization and differential expression during B-lymphocyte development. *Mol. Cell. Biol.*, **14**, 3292–3309.

Magnaghi-Jaulin,L., Masutani,H., Robin,P., Lipinski,M. and Harel-Bellan,A. (1996) SRE elements are binding sites for the fusion protein EWS-FLI-1. *Nucleic Acids Res.*, **24**, 1052–1058.

Maher,M., Cong,F., Kindelberger,D., Nasmyth,K. and Dalton,S. (1995) Cell cycle-regulated transcription of the *CLB2* gene is dependent on Mcm1 and a ternary complex factor. *Mol. Cell. Biol.*, **15**, 3129–3137.

Mansour,S.L., Thomas,K.R. and Capecchi,M.R. (1988) Disruption of the proto-oncogene *int2* in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. *Nature*, **336**, 348–352.

Marais,R.M., Hsuan,J.J., McGuigan,C., Wynne,J. and Treisman,R. (1992) Casein kinase II phosphorylation increases the rate of serum response factor-binding site exchange. *EMBO J.*, **11**, 97–105.

Marais,R., Wynne,J. and Treisman,R. (1993) The SRF accessory protein Elk-1 contains a growth factor-regulated trascriptional activation domain. *Cell*, **73**, 381–393.

Miranti,C.K., Ginty,D.D., Huang,G., Chatila,T. and Greenberg,M.E. (1995) Calcium activates serum response factor-dependent transcription by a Ras- and Elk-1-independent mechanism that involves a Ca^{2+}/calmodulin-dependent kinase. *Mol. Cell. Biol.*, **15**, 3672–3684.

Mishina,Y., Suzuki,A., Ueno,N. and Behringer,R.R. (1995) Bmpr encodes a type I bone morphogenic protein receptor that is essential for gastriculation during mouse embryogenesis. *Genes Dev.*, **9**, 3027–3037.

Mohun,T.G., Taylor,M.V., Garrett,N. and Gurdon,G.B. (1989) The CArG promoter sequence is necessary for muscle-specific transcription of the cardiac actin gene in *Xenopus* embryos. *EMBO J.*, **8**, 1153–1161.

Montagne,J., Groppe,J., Guillemin,K., Krasnow,M.A., Gehring,W.J. and Affolter,M. (1996) The *Drosophila* serum response factor gene is required for the formation of intervein tissue of the wing and allelic to blistered. *Development*, **122**, 2589–2597.

Moss,J.B., McQuinn,T.C. and Schwartz,R.J. (1994) The avian cardiac α-Actin promoter is regulated through a pair of complex elements composed of E boxes and serum response elements that bind both positive- and negative-acting factors. *Am. Soc. Biochem. Mol. Biol.*, **269**, 12731–12740.

Muscat,G.E.O., Gufstafson,T.A. and Kedes,L. (1988) A common factor regulates skeletal and cardiac α-*Actin* gene transcription in muscle. *Mol. Cell. Biol.*, **8**, 4120–4133.

Norman,C., Runswick,M., Pollock,R. and Treisman,R. (1988) Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-fos serum response element. *Cell*, **55**, 989–1003.

Panitz,F., Krain,B., Hollemann,T., Nordheim,A. and Pieler,T. (1998) The Spemann Organizer-expressed zinc finger gene *Xegr-1* responds to the MAP kinase/Ets-SRF signal transduction pathway. *EMBO J.*, **17**, 4414–4425.

Pellegrini,L., Tan,S. and Richmond,T.J. (1995) Structure of serum response factor core bound to DNA. *Nature*, **376**, 490–498.

Price,M.A., Rogers,A.E. and Treisman,R. (1995) Comparative analysis of the ternary complex factors Elk-1, SAP-1a and SAP-2 (ERP/NET). *EMBO J.*, **14**, 2589–2601.

Price,M.A., Cruzalegui,F.H. and Treisman,R. (1996) The p38 and ERK MAP kinase pathways cooperate to activate ternary complex factors and c-fos transcription in response to UV light. *EMBO J.*, **15**, 6552–6563.

Prywes,R. and Roeder,R.G. (1987) Purification of the c-fos enhancer-binding protein. *Mol. Cell. Biol.*, **7**, 3482–3489.

Raingeaud,J., Whitmarsh,A.J., Barrett,T., Dérijard,B. and Davis,R.J. (1996) MKK3- and MKK6-regulated gene expression is mediated by p38 mitogen-activated protein kinase signal transduction pathway. *Mol. Cell. Biol.*, **16**, 1247–1255.

Rivera,V.M., Miranti,C.K., Misra,R.P., Ginty,D.D., Chen,R.-H., Blenis,J. and Greenberg,M.E. (1993) A growth factor-induced kinase phosphorylates the serum response factor at a site that regulates its DNA-binding activity. *Mol. Cell. Biol.*, **13**, 6260–6273.

Roch,R., Baonza,A., Marin-Blanco,E. and Garcia-Bellido,A. (1998) Genetic interactions and cell behaviour in blistered mutants during proliferation and differentiation of the *Drosophila* wing. *Development*, **125**, 1823–1832.

Sartorelli,V., Webster,K.A. and Kedes,L. (1990) Muscle-specific expression of the cardiac α-*Actin* gene requires MyoD1, CArG-box binding factor and Sp1. *Genes Dev.*, **4**, 1811–1822.

Sasaki,H. and Hogan,B.L.M. (1994) HNF-3b as a regulator of floor plate development. *Cell*, **76**, 103–115.

Schröter,H., Shaw,P.E. and Nordheim,A. (1987) Purification of intercalator-released p67, a polypeptide that interacts specifically with the c-fos serum response element. *Nucleic Acids Res.*, **14**, 10145–10158.

Schwarz-Sommer,Z., Huijser,P., Nacken,W., Saedler,H. and Sommer,H. (1990) Genetic control of flower development by homeotic genes in *Antirrhinum majus*. *Science*, **250**, 931–936.

Sepulveda,J.L., Belaguli,N., Nigam,V., Chen,C.-Y., Nemer,M. and Schwartz,R.J. (1998) GATA-4 and Nkx-2.5 coactivate Nkx-2 DNA binding targets: role for regulating early cardiac gene expression. *Mol. Cell. Biol.*, **18**, 3405–3415.

Sharrocks,A.D., Gille,H. and Shaw,P.E. (1993) Identification of amino acids essential for DNA binding and dimerization in p67SRF: implications for a novel DNA-binding motif. *Mol. Cell. Biol.*, **13**, 123–132.

Shaw,P.E., Schröter,H. and Nordheim,A. (1989a) The ability of a ternary complex to form over the serum response element correlates with serum inducibility of the human c-fos promoter. *Cell*, **56**, 563–572.

Shaw,P.E., Frasch,S. and Nordheim,A. (1989b) Repression of c-fos transcription is mediated through p67SRF bound to the SRE. *EMBO J.*, **8**, 2567–2574.

Shore,P. and Sharrocks,A.D. (1995) The MADS-box family of transcription factors. *Eur. J. Biochem.*, **229**, 1–13.

Smith,J.C. (1995) Mesoderm-inducing factors and mesodermal patterning. *Curr. Opin. Cell Biol.*, **7**, 856–861.

Soriano,P., Montgomery,C., Geske,R. and Bradley,A. (1991) Targeted disruption of the c-*src* proto-oncogene leads to osteopetrosis in mice. *Cell*, **64**, 693–702.

Soulez,M., Rouviere,C.G., Chafey,P., Hentzen,D., Vandromme,M., Lautredou,N., Lamb,N., Kahn,A. and Tuil,D. (1996) Growth and differentiation of C2 myogenic cells are dependent on serum response factor. *Mol. Cell. Biol.*, **16**, 6065–6074.

St-Jacques,B. and McMahon,A.P. (1996) Early mouse development: lessons from gene targeting. *Curr. Opin. Genet. Dev.*, **6**, 439–444.

Sutherland,D., Samakovlis,C. and Krasnow,M.A. (1996) *branchless* encodes a *Drosophila* FGF homolog that controls tracheal cell migration and the pattern of branching. *Cell*, **87**, 1091–1101.

Tam,P.P.L. and Behringer,R.R. (1997) Mouse gastrulation: the formation of a mammalian body plan. *Mech. Dev.*, **68**, 3–25.

Tang,S.J., Hoodless,P.A., Lu,Z., Breitman,M.L., McInnes,R.R., Wrana,J.L. and Buchwald,M. (1998) The *Tlx-2* homeobox gene is a downstream target of BMP signalling and is required for mouse mesoderm development. *Development*, **125**, 1877–1887.

Taylor,M., Treisman,R., Garrett,N. and Mohun,T. (1989) Muscle-specific (CArG) and serum-response (SRE) promoter elements are functionally interchangeable in *Xenopus* embryos and mouse fibroblasts. *Development*, **106**, 67–78.

Treisman,R. (1986) Identification of a protein-binding site that mediates transcriptional response of the c-*fos* gene to serum factors. *Cell*, **46**, 567–574.

Treisman,R. (1987) Identification and purification of a polypeptide that binds to the c-fos serum response element. *EMBO J.*, **6**, 2711–2717.

Treisman,R. (1992) The serum response element. *Trends Biochem. Sci.*, **17**, 423–426.

Treisman,R. (1994) Ternary complex factors: growth factor regulated transcriptional activators. *Curr. Opin. Genet. Dev.*, **4**, 96–101.

Treisman,R. (1995) Journey to the surface of the cell: Fos regulation and the SRE. *EMBO J.*, **14**, 4905–4913.

Treisman,R. (1996) Regulation of transcription by MAP kinase cascades. *Curr. Opin. Cell Biol.*, **8**, 205–215.

Vandromme,M., Gauthier-Rouvière,C., Carnac,G., Lamb,N. and Fernandez,A. (1992) Serum response factor p67SRF is expressed and required during myogenic differentiation of both mouse C2 and rat L6 muscle cell lines. *J. Cell Biol.*, **118**, 1489–1500.

Wang,Z.-Q., Ovitt,C., Grigoriadis,A.E., Möhle-Steinlein,U., Rüther,U. and Wagner,E.F. (1992) Bone and haematopoietic defects in mice lacking c-*fos*. *Nature*, **360**, 741–744.

Wilkinson,D.G., Bhatt,S. and Herrmann,B.G. (1990) Expression pattern of the mouse *T* gene and its role in mesoderm formation. *Nature*, **343**, 657–659.

Zhang,H. and Bradley,A. (1996) Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. *Development*, **122**, 2977–2986.

Zinck,R., Cahill,M.A., Kracht,M., Sachsenmaier,C., Hipskind,R.A. and Nordheim,A. (1995) Protein synthesis inhibitors reveal differential regulation of mitogen-activated protein kinase and stress-activated protein kinase pathways that converge on Elk-1. *Mol. Cell. Biol.*, **15**, 4930–4938.

Zwart,R., Broos,L., Grosveld,G. and Meijer,D. (1996) The restricted expression pattern of the POU factor Oct-6 during early development of the mouse nervous system. *Mech. Dev.*, **54**, 185–194.

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