Development 125, 3801-3808 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
DEV2308

A Serum Response Factor homolog is required for spore differentiation in *Dictyostelium*

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Accepted 28 July; published on WWW 7 September 1998

SUMMARY

A homolog of the Serum Response Factor (SRF) has been isolated from *Dictyostelium discoideum* and its function studied by analyzing the consequences of its gene disruption. The MADS-box region of *Dictyostelium* SRF (DdSRF) is highly conserved with those of the human, *Drosophila* and yeast homologs. *srfA* is a developmentally regulated gene expressed in prespore and spore cells. This gene plays an essential role in sporulation as its disruption leads to abnormal spore morphology and loss of viability. The mutant spores were round and cellulose deposition seemed to be partially affected. Initial prestalk and prespore cell differentiation did not seem to be compromised in the mutant since the expression of several cell-type-specific markers were found to be unaffected. However, the mRNA level of the spore marker *spiA* was greatly reduced. Activation of the cAMP-dependent protein kinase (PKA) by 8-Br-cAMP was not able to fully bypass the morphological defects of *srfA*$^-$ mutant spores, although this treatment induced *spiA* mRNA expression. Our results suggest that DdSRF is required for full maturation of spores and participates in the regulation of the expression of the spore-coat marker *spiA* and probably other maturation genes necessary for proper spore cell differentiation.

Key words: SRF, *Dictyostelium*, Sporulation, Differentiation, PKA

INTRODUCTION

Serum response factor (SRF) is a DNA-binding protein involved in the rapid and transient transcriptional activation of genes in response to serum in cultured mammalian cells (reviewed by Treisman and Ammerer, 1992; Johansen and Prywes, 1995; Cahill et al., 1996). SRF functions as a dimer, recognizing a DNA motif termed the Serum Response Element (SRE). A second component, the ternary complex factor (TCF) cooperates in the activation of transcription (Dalton and Treisman, 1992; Pellegrini et al., 1995). The SRF dimerization and DNA-binding domains are contained in a highly conserved 60 amino-acid region called the MADS-box (for the initials of the first identified members: MCM1 and ARG80 from yeast, Deficient from *Arabidopsis* and SRF from humans) (Shore and Sharrocks, 1995). The SRF-like group, the most evolutionary conserved subfamily of MADS-box transcription factors, includes the human, *Xenopus* and *Drosophila* SRF homologs and the yeast proteins MCM1 and ARG80 (Dubois et al., 1987; Norman et al., 1988; Passmore et al., 1988; Mohun et al., 1991; Affolter et al., 1994). The DNA consensus site recognized by the MADS-box family members is based on an A/T-rich sequence (Pollock and Treisman, 1990, 1991; Wynne and Treisman, 1992; Nurrish and Treisman, 1995). In *Drosophila*, SRF is required for the formation of the terminal branches of the tracheal system (Affolter et al., 1994; Guillemin et al., 1996) as well as in wing development where it regulates the differentiation of intervein cells (Montagne et al., 1996). In yeast, the SRF homolog MCM1 is involved in mating-type determination and is activated by the pheromone signal transduction pathway (reviewed by Herskowitz, 1989; Dolan and Fields, 1991).

In this paper, we describe the cloning, expression and function of a Serum Response Factor homolog from the social amoeba *Dictyostelium discoideum*. *srfA* is a developmentally regulated gene expressed in prespore and spore cells. Disruption of this gene leads to a specific defect in sporulation that affects spore viability.

Sporulation in *Dictyostelium* is a tightly regulated event that coordinates cell-type differentiation with morphogenesis (Loomis, 1996). Cells fated to become spores (prespore cells) appear early in development and progressively accumulate components of the spore coat until encapsulation takes place (Devine et al., 1983). An intercellular signal transduction pathway coordinating sporulation with fruiting body formation has recently been recognized. A peptide (SDF-2) is released by prestalk cells, signaling the underlying prespore cells to enter the final spore path (Anjard et al., 1998). The response to SDF-2 is very fast and does not require protein synthesis. Peptide secretion is depended on TagB/TagC, a putative ATP-driven membrane transporter (Shaulsky et al., 1995) and the signal is transduced by a two-component system in prespore cells. (Wang et al., 1996; Shaulsky et al., 1996). Genetic evidence suggested that the histidine kinase receptor DhkA could inhibit

the cAMP phosphodiesterase activity of the response regulator RegA leading to an increase of intracellular cAMP level that in turn activates PKA (Anjard et al., 1997; Shaulsky et al., 1998). However, recent biochemical data by Thomason et al. (1998) indicated that phosphorylation of RegA activates the phosphodiesterase domain, acting therefore as a pathway of PKA inhibition. Moreover, Chang et al. (1998) proposed that RdeA, a member of the H2 module family of phosphotransferases, functions immediately upstream of RegA. Another signaling peptide, SDF-1 (originally SDF) (Anjard et al., 1997) was shown to be phosphorylated by PKA and secreted by the cells acting as a spore differentiation factor in an unknown signal transduction pathway that requires protein synthesis (Anjard et al., 1997). A model of signal transduction pathways leading to sporulation has been recently proposed (Anjard et al., 1998) in which SDF-1 and SDF-2 eventually mediate their effects by activating PKA.

A wealth of information is available regarding the role of PKA in spore differentiation and gene expression: overexpression of the PKA catalytic subunit in prespore cells leads to precocious sporulation (Burki et al., 1991; Mann et al., 1991, 1994; Hopper et al., 1993, 1995) and similarly, activation of PKA by the membrane-permeable derivative of cAMP, 8-Br-cAMP, induces sporulation in submerge culture after cell disaggregation (Maeda, 1988; Kay, 1989). Moreover, the expression of a dominant inhibitor of the PKA in prespore cells blocks spore differentiation (Hooper et al., 1993). It has been suggested that activation of specific spore gene expression is mediated by PKA just prior to final encapsulation (Mann et al., 1994). Overexpression of the catalytic subunit of PKA in prespore cells leads to a rapid activation of *spiA* expression, a spore coat protein whose expression is concomitant with spore encapsulation (Anjard et al., 1992; Mann et al., 1994).

The results described in this paper suggest that the *Dictyostelium* SRF homolog (DdSRF) may play a role in the final steps of spore differentiation. *srfA*$^-$ cells express the prespore marker cotB but the expression of the spore marker *spiA* is greatly reduced. Activation of PKA by 8-Br-cAMP does re-establish the high levels of *spiA* expression suggesting that DdSRF plays a role either upstream or in parallel to PKA activity in the regulation of *spiA* gene expression during terminal differentiation. However, this treatment does not bypass the morphological defects of the mutant spores suggesting that DdSRF may also be functioning independently of PKA and this function would be essential for proper spore formation.

MATERIALS AND METHODS

Cells, growth, transformation and development

Dictyostelium cells were grown axenically in HL5 medium (Sussman, 1987). Transformations were carried out as described by Kuspa and Loomis (1992), and blasticidin selection according to Adachi et al. (1994). Transformants were plated in SM plates in association with *Klebsiella aerogenes* for clonal isolation (Sussman, 1987). Development on filters was performed on nitrocellulose as described by Shaulsky and Loomis (1993). Detergent and heat treatment of spores were carried out as described by Shaulsky and Loomis (1993) and Nuckolls et al. (1996). The viability of wild-type and mutant spores were determined by counting the plaques formed after plating on *Klebsiella aerogenes* SM plates.

Molecular cloning of *srfA* and knock-out construct

Degenerated oligonucleotides were designed from the conserved mammalian MADS-box region. The 5′ oligonucleotide was derived from the amino acid sequence: RVKIKMEF and the 3′ oligonucleotide from the amino acid sequence: ETGHVYT. The 160 pb fragment obtained after genomic PCR was cloned into pGEMt easy vector (PROMEGA) according to the manufacturer's instructions. This fragment was used to screen a cDNA library prepared from RNA of cells developed to the slug stage (Shaulsky et al., 1995).

The knock-out vector was obtained by inserting a blasticidin-resistance cassette into the *Cla*I site of a cDNA clone containing the complete *srfA* open reading frame. In order to flank the blasticidin cassette with restriction sites convenient for cloning into the *Cla*I of the cDNA clone, the following subcloning steps were performed: the *Eco*RI-*Hin*dIII fragment from pUCBsrBam (Adachi et al., 1994) containing a blasticidin-resistance cassette was cloned into pBluescript. The cassette was cut out again by *Kpn*I and cloned once more into pBluescript SK looking for the orientation that gives two *Cla*I sites at both ends of the cassette.

Nucleic acid manipulation and sequencing

Standard methods of nucleic acid manipulation were carried out according to Ausubel et al. (1992) and Sambrook et al. (1989).

RNA was isolated at different times of development on filters using Trizol reagent (Life Technologies).

Nucleotide sequences were determined by the dideoxy chain-termination method using the Taq Dye Deoxy Terminator Cycle sequencing kit and a 373A Sequencer from Applied Biosystems.

In situ hybridization, X-gal staining and calcofluor staining

The original PCR fragment containing the *Dictyostelium* MADS-box sequence (see Materials and methods: molecular cloning of *srfA*) was cloned into pBluescript plasmid and the antisense riboprobe was obtained by using the DIG RNA labeling Kit from Boehringer Mannheim according to manufacturer's instructions. Hybridizations to fixed structures were carried out as described by Escalante and Loomis (1995). For in situ hybridizations to the cell-type-specific markers (ecmA, ecmB and cotB), riboprobes and experimental conditions were as described by Wang et al. (1996).

Spores from strains carrying *lacZ* reporters were collected from the sorus and permeabilized in 20% DMSO, 0.1% NP40 in Z-buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$) for 30 minutes. Fixation with formaldehyde and staining was performed according to Wang et al. (1996).

Cellulose in spore coats was detected by staining with 1 μg/ml calcofluor (SIGMA) in spore buffer (10 mM MES pH 6.2, 20 mM KCl, 20 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$).

8-Br-cAMP treatment

Induction of sporulation was performed according to Richardson et al. (1991). Cells at the Mexican hat stage were collected and disaggregated in spore buffer with or without 20 mM 8-Br-cAMP. After 3½ hours of shaking at 200 revs/minute, the presence of spores were determined by phase-contrast microscopy.

RESULTS

Identification of the Serum Response Factor homolog in *Dictyostelium*

We used degenerated oligonucleotides derived from the *Drosophila* MADS-box sequence (see Materials and methods) to amplify a 162 bp PCR product from *Dictyostelium* genomic DNA. This fragment was subsequently cloned and sequenced. The deduced amino acid sequence showed a high level of

identity with known SRF proteins. This PCR fragment was used to isolate several cDNA clones from a *Dictyostelium* cDNA library constructed from RNA isolated at the slug stage (kindly provided by Dr W.F. Loomis).

The sequence of the cDNA clones contained a long open reading frame whose deduced amino acid sequence is shown in Fig. 1A. The conserved MADS-box is indicated by a thick underlining. Other amino acids outside the MADS-box are also conserved with the human SRF and have been indicated by a thin line. Following the conserved region, there are homopolymer runs of glutamine and asparagine, common occurrences in *Dictyostelium* proteins (Shaw et al., 1989). The level of similarity between DdSRF and the human, *Drosophila* and yeast SRF homologs is very high along the MADS box as shown in Fig. 1C. The MADS box contains the DNA-binding and the dimerization domains that have been shown to be essential for transcriptional regulation (Pellegrini et al., 1995).

As expected by recent phylogenetic studies based on protein sequences (Loomis and Smith, 1995) *Dictyostelium* SRF is more closely related to the mammalian and *Drosophila* homologs than to those of its yeast counterparts, MCM1 and ARG80, as shown in the phylogenetic tree of Fig. 1B.

srfA is a developmentally regulated gene expressed in prespore and spore cells

We studied the temporal pattern of expression of *srfA* during development by probing northern blots of RNA isolated at different times of development. As shown in Fig. 2, a single band was first detected at 8 hours of development when the cells had formed loose mounds. The level remained constant until the onset of culmination (20 hours) when a significant increase was observed.

We also determined the cell-type pattern of expression by in situ hybridization. Fig. 3 shows structures at different stages of development hybridized with a *srfA* riboprobe constructed from the original PCR fragment (see Materials and methods). In order to measure the extent of the background, we used as negative control a strain in which *srfA* has been disrupted (see below). This strain does not contain the mRNA sequence recognized by the riboprobe and was hybridized under the same experimental conditions and developed for the same time as wild type. As can be seen in Fig. 3A-C, prespore cells and spores stained strongly. The level of staining observed in the prestalk region was similar to that of the controls (Fig. 3D-F) and has been, therefore, considered as background.

Disruption of *srfA* leads to a specific defect in sporulation

The function of the gene was initially addressed by the study of the consequences of its disruption by homologous recombination.

A blasticidin-resistance cassette (BSR) was inserted between amino acids 77 and 78 in a *srfA* cDNA clone as described in Materials and methods. The insertion interrupts the gene just prior to the conserved MADS-box. This construct was used to transform wild-type strain AX4. Blasticidin-resistance transformants were plated in association with *Klebsiella aerogenes* for phenotype observation. PCR, Southern and northern analysis were carried out in order to confirm that the gene was disrupted. A schematic

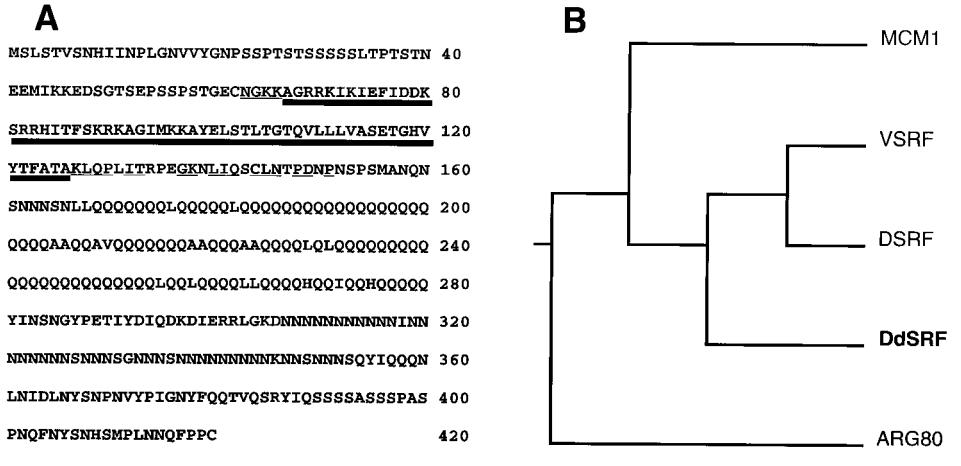

Fig. 1. Predicted amino acid sequence of DdSRF, phylogenetic analysis and alignment. (A) Deduced amino acid sequence from DdSRF cDNA clones. The conserved MADS-box region is indicated by a thick underlining. Additional sequence conservation with the human SRF is indicated by a thin line. (B) Phylogenetic tree constructed by the PHYLIP software available via 'ftp' from 'ftp.genetics.washington.edu'. The MADS-box from *Arabidopsis thaliana* protein AP1 was used as an outgroup. (C) The MADS box of the yeast proteins MCM1 and ARG80, the human SRF (VSRF), the *Drosophila* SRF (DSRF) and the *Dictyostelium discoideum* SRF (DdSRF) were aligned using the Clustal W program. Identical amino acids are indicated by black boxes; closely related by grey boxes. The nucleotide sequence reported in this paper has been submitted to the EMBL Data Bank with accession number AJ224893.

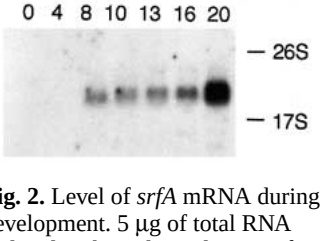

Fig. 2. Level of *srfA* mRNA during development. 5 μg of total RNA isolated at the indicated times of development (hours) were electrophoretically separated, transferred to a filter and hybridized with a *srfA* probe containing the MADS box.

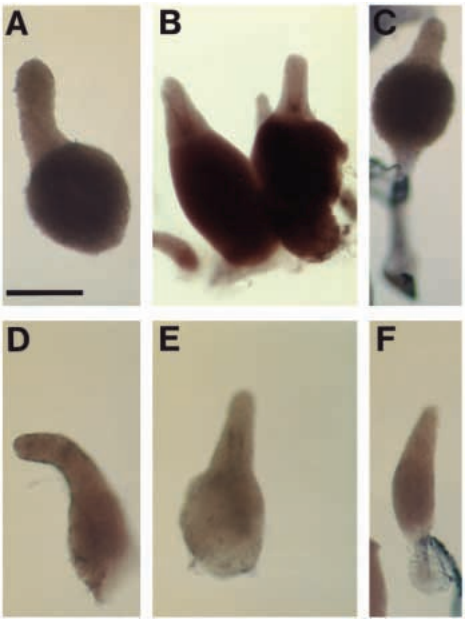

Fig. 3. In situ localization of *srfA* mRNA. (A-C) Wild-type and (D-F) *srfA*⁻ structures at the finger stage (A,D) and early culminants (B,C,E,F) were fixed and hybridized with a riboprobe containing the MADS-box region of DdSRF (bar, 0.2 mm).

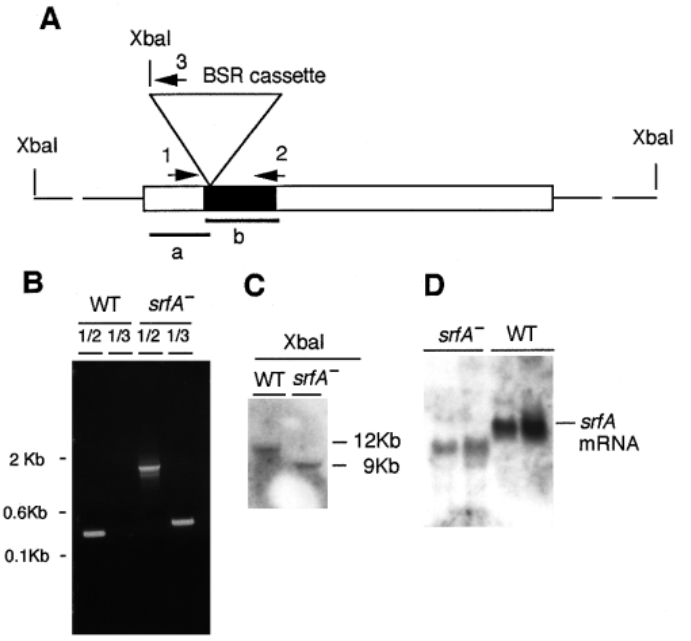

Fig. 4. Analysis of homologous recombination and loss of expression of *srfA*. (A) Schematic representation of *srfA* locus and BSR cassette insertion. The MADS region is indicated by a black box over the coding sequence of *srfA* (open box). The position of the oligos is shown by arrows (1,2,3). Probes 'a' and 'b' used for hybridization are indicated by underlining. The position of the restriction enzyme *Xba*I is indicated over the BSR cassette. Two other *Xba*I sites located in the genomic region that surround *srfA-coding* sequence are also indicated. (B) PCR analysis of genomic DNA from wild type or from *srfA* mutant were performed with the indicated pairs of oligos. (C) Southern analysis of DNA from wild type and mutant cut with the restriction enzyme *Xba*I. Hybridizations were carried out with the probe 'a'. (D) Northern analysis of RNA isolated at 16 or 18 hours of development from *srfA* mutant and wild type and hybridized with probe 'a'. No signal was obtained in the mutant RNA when probe 'b' was used (data not shown).

representation of *srfA* locus and the BSR cassette insertion with MADS-box indicated by a black box is shown in Fig. 4A. Oligos, probes and restriction sites used for the analysis are also indicated. The different pattern of PCR fragments (Fig. 4B) and the shift in the size of the hybridizing band in the Southern (Fig. 4C) indicate that the transformants carry the BSR cassette at the expected position of the *srfA* genomic locus. This insertion might result in the formation of a truncated mRNA that could code for the first 77 amino acids of DdSRF. This truncated mRNA was detected when the probe 'a' was used but no hybridization signal was obtained when the 3′-probe 'b' was used (data not shown), indicating that no mRNA containing the majority of DdSRF-coding sequence is produced (Fig. 4D). Since the functional domains located in the MADS box lie downstream of the insertion point, the resulting genotype is that of a null mutant.

No alterations were found initially in the morphology of the transformants that showed normal and well-proportioned fruiting bodies (Fig. 5). However, closer inspection of the spores revealed abnormal spore morphology. While normal wild-type spores were ellipsoid and phase bright, the mutant spores were round and not as phase bright (Fig. 5). A large number of independent transformants showing the same phenotype were obtained for several strains (see below). A more detailed study of the phenotype was performed by observing synchronized development on buffered filters. The only defect was a reproducible 3-4 hours delay in culmination. Spore viability was determined after 40 hours of filter development. Only 0.5 % of the mutant spores were viable after detergent treatment when compared with wild type. An additional heat treatment, which does not affect wild-type viability, reduced mutant spore viability to less than 0.1%.

In order to study the formation of the spore coat, cellulose was detected in wild-type and mutant cells by calcofluor staining (Fig. 6A). To sporulate, the prespore cells release the prespore vesicles and these components assemble two proteinaceus layers. Cellulose is synthesized and deposited between these layers (West and Erdos, 1990). While all the normal spores showed strong fluorescent staining, a lower proportion of mutant cells were stained and the intensity was less. The variable amount of cellulose produced by the mutant is, nevertheless, indicative of partial formation of the spore coats.

Gene expression in *srfA*⁻ null mutant: the mRNA level of the spore marker *spiA* is greatly reduced

To study spore gene expression during development, the *srfA* gene was disrupted in strains carrying the *lacZ* gene under the control of the *cotB* prespore promoter and the *spiA* spore promoter (kindly provided by Dr W. F. Loomis). Spores from *cotB::lacZ* and *spiA::lacZ* original strains and those with the disrupted *srfA* gene (*srfA*⁻ *cotB::lacZ* and *srfA*⁻ *spiA::lacZ*) were collected and stained for β-galactosidase (β-gal) activity. As shown in Fig. 6B, the same level of blue staining was observed in mutant cells carrying the *cotB* prespore marker

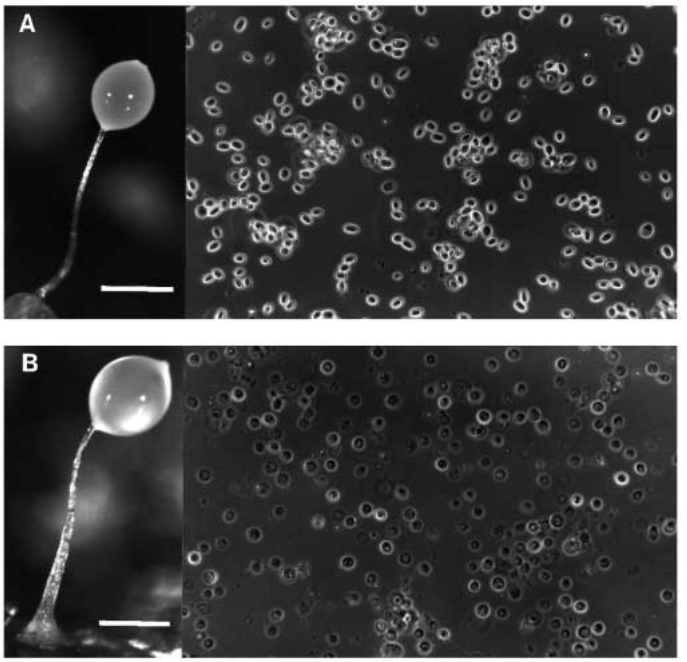

Fig. 5. Phenotype of *srfA*⁻. Fruiting bodies developed on SM plates in association with *Klebsiella aerogenes* from wild type (A) and *srfA*⁻ mutant (B) are shown in the right panels (bar, 0.2 mm). Spores contained in the sorus were photographed under a phase-contrast microscope (magnification, ×400).

compared with wild-type spores. However, the level of staining for the *spiA* spore marker was greatly reduced in the mutant spores. We next studied the temporal pattern of gene expression by northern blots as shown in Fig. 7A. RNA from wild-type and *srfA*⁻ mutant were isolated at different times of development and probed for the indicated genes. Except for a 3-4 hours delay, the level and timing of expression of the prestalk markers *ecmA* and *ecmB* and the prespore marker *cotB* was found to be unaffected in the mutant. On the contrary, the level of the spore marker *spiA* was greatly affected. In concordance with the β-gal staining shown above, a low expression of *spiA* was also detected at 30 hours. The spatial pattern of expression of the cell-type markers ecmA, ecmB and cotB was also studied in the mutant by in situ hybridization at different times of development and we found no differences with wild type (data not shown).

Activation of PKA is sufficient for sporulation in wild-type and many mutant backgrounds (Loomis, 1996; Anjard et al., 1998). PKA is also a positive regulator of prespore and spore gene expression (Mann, 1994; Hooper, 1995) and it has been suggested that *spiA* might be regulated by this enzyme directly at the level of gene expression (Mann et al., 1994). We found that the pattern and the level of mRNA expression of the catalytic subunit of PKA was unaffected in the mutant as shown in Fig. 7A, suggesting that the defects in spore differentiation observed in the mutant were not directly caused by the absence of PKA gene expression during terminal differentiation.

8-Br-cAMP activation of PKA in *srfA*⁻ cells

We wanted to investigate the consequences of activating PKA in *srfA*⁻ mutant cells. The permeable cAMP derivative 8-Br-cAMP is believed to enter the cells and bind to the regulatory subunit of the PKA, activating the catalytic subunit. When wild-type cells were disaggregated from early culminants and exposed to 8-Br-cAMP, sporulation was induced very efficiently. About 50% of the cells differentiated into ellipsoid, phase-bright spores (Fig. 7B). However, the majority of the mutant cells only reached a rounded morphology after the treatment with 8-Br-cAMP. A few ellipsoid cells could be seen but they were larger and less shiny than wild-type spores (Fig. 7B). SpiA expression was determined by northern blot and was found to be induced by 8-Br-cAMP (Fig. 7C) suggesting that DdSRF may not be directly regulating *spiA* gene expression. On the contrary, the inability of *srfA*⁻ cells to form ellipsoid spores even when PKA is activated suggests that DdSRF is also functioning independently to PKA in a pathway essential for full spore maturation.

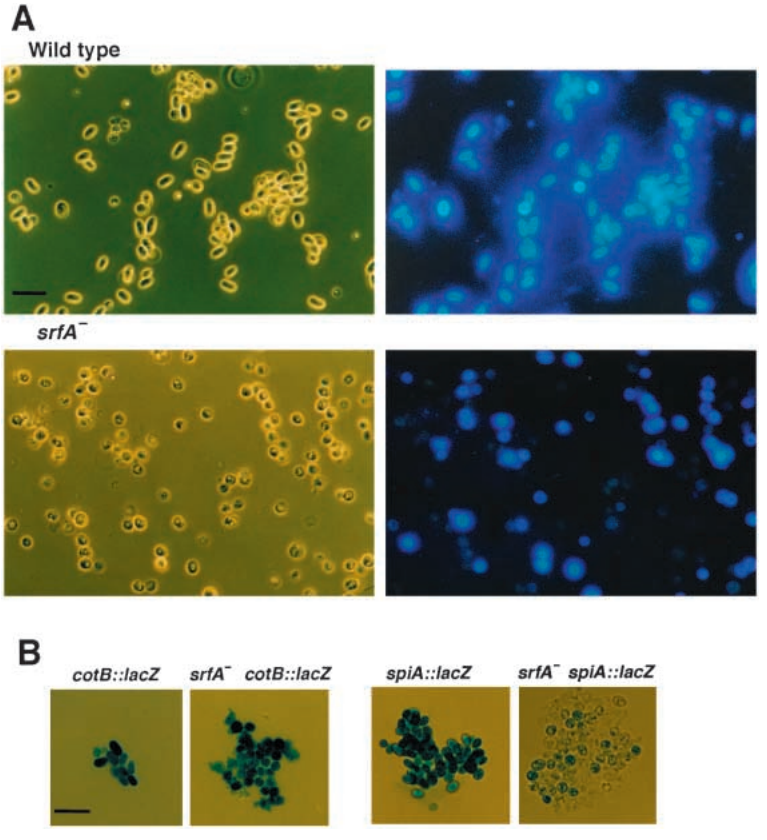

Fig. 6. Cellulose detection by calcofluor staining and expression of prespore and spore markers. (A) Spores from wild type and *srfA*⁻ were collected from the sorus and stained with the fluorescent dye calcofluor. The left panels show the phase-contrast microscopic images and the right panels the fluorescent view from the same field (bar, 10 μm). (B) *srfA*⁻ was knock out in strains expressing *lacZ* under the control of prespore and spore markers: *cotB::lacZ* (strain TL1, kindly provided by Dr William F Loomis) and *spiA::lacZ* (strain TL10, also provided by Dr Loomis). Spores from parental and mutant strains were collected and stained for β-gal detection for the same time and under the same conditions. Bar, 10 μm.

DISCUSSION

The MADS-box family of transcription factors displays an extraordinary range of biological roles. They are involved in functions as diverse as the rapid response to growth factors in mammalian cells, muscle development in vertebrates, arginine metabolism and mating-type determination in yeast, trachea and wing development in *Drosophila* and flower development in higher plants (reviewed in Shore and Sharrocks, 1995; Theißen et al., 1996). We have identified a *Dictyostelium* gene that encodes a protein highly similar to those encoding the Serum Response Factor, the most evolutionary conserved subfamily of MADS-box genes. Only 4 out of 58 amino acids in the DNA-binding and dimerization domain (MADS box) have diverged between *Dictyostelium* and human. The sequence surrounding the MADS box is also well conserved (21 identical amino acids out of 29). Based on this sequence homology it is very likely that the DdSRF functions as a transcription factor.

srfA is expressed in prespore and spore cells as shown by in situ hybridization and no specific signal could be seen in the anterior prestalk region. Its mRNA expression, as determined by Northern analysis, begins at 8 hours of development, the time of loose mound formation when the initial divergence of prespore and prestalk cells takes place. Its levels remain low until 20 hours, by the time of the onset of culmination, when a very strong induction could be observed. This pattern of expression is different to that of a typical prespore marker as the one shown by *cotB* or to that of the spore marker *spiA* (compare Figs 2 and 7).

The function of DdSRF in *Dictyostelium* development was analyzed by gene targeting. Disruption of the gene by the BSR cassette was expected to produce a null mutant as the functional MADS box was located downstream of the insertion and was therefore not transcribed, as determined by northern analysis with a 3′ probe. Disruption of *srfA* leads to abnormal spore morphology and greatly reduces spore viability. Except for a delay of 3-4 hours in culmination, no defects in the overall morphology of the structures were observed during development of the mutant strains. Prestalk gene expression, investigated by the temporal and spatial pattern of expression of the prestalk markers *ecmA* and *ecmB* was found to be normal. Therefore, it seems that DdSRF plays little or no role until culmination.

The expression of the prespore marker *cotB* was also found to be unaffected in *srfA*$^-$ mutant suggesting that basic prespore differentiation is not regulated by DdSRF. However, spore gene expression of *spiA* was greatly reduced. SpiA is a structural protein located in the inner face of the spore coats (Richardson and Loomis, 1992). Disruption of this gene leads to spores that are indistinguishable from those of wild type but show a fast decrease of viability when submerged in buffer. The reduced level of *spiA* expression can not obviously account for the much stronger phenotype of *srfA*$^-$ mutant: *srfA*$^-$ spores only reached a rounded morphology and partial cellulose assembling. DdSRF must be affecting other components besides *spiA* that are essential in spore differentiation. Moreover, *Dictyostelium* mutants in the dual-specificity kinase SplA shows an overall phenotype very similar to that of the *srfA*$^-$ mutants (Nuckolls et al., 1996); however, the level of expression of the spore marker *spiA* was shown to be normal (Nuckolls et al., 1996). It has been previously shown that the spore coat proteins are initially accumulated in vesicles and secreted later to assemble the spore coats (Hohl and Hamamoto, 1969; Devine et al., 1983). Since cellulose deposition in *srfA*$^-$ spores is partially affected, it is conceivable that DdSRF might be essential in regulating the expression of genes necessary for this final process of exocytosis.

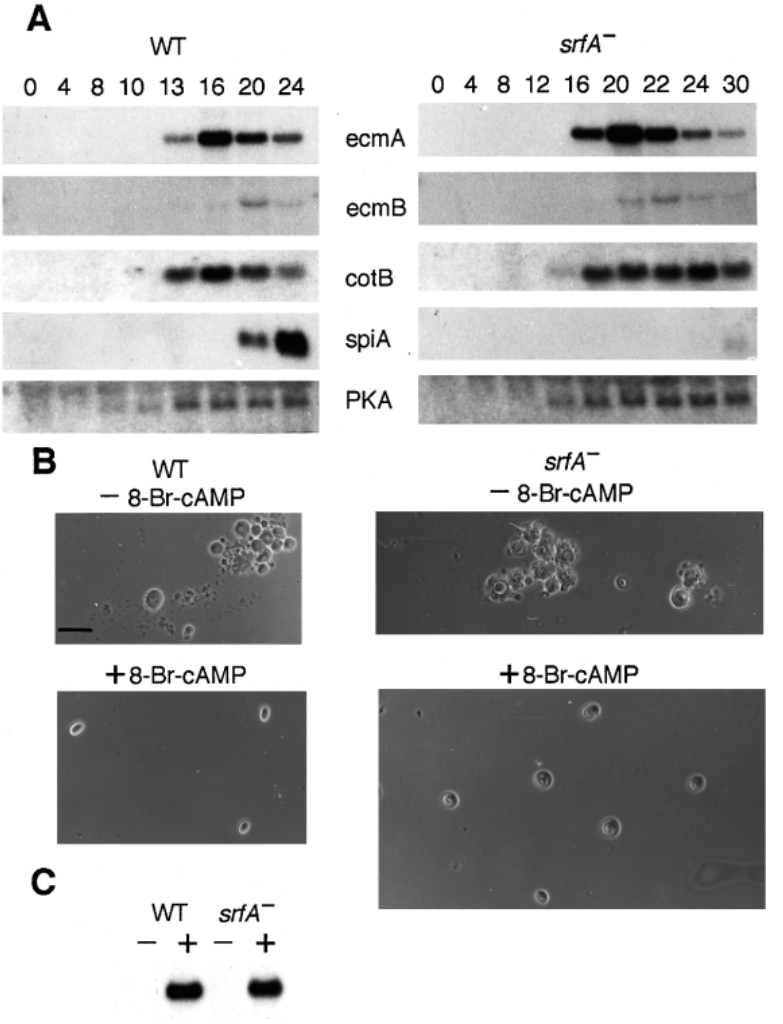

Fig. 7. Gene expression in srfA$^-$ mutant and 8-Br-cAMP treatment. (A) 5 μg of total RNA at different times of development (hours) from wild type and *srfA*$^-$ mutant were probed for the prestalk markers *ecmA* and *ecmB*, the prespore marker *cotB*, the spore marker *spiA* and the PKA catalytic subunit. (B) Cells disaggregated at the mexican hat stage (19 hours of development for WT and 22 hours for *srfA*$^-$) were resuspended in spore buffer and shaken in the presence (+) or absence (–) of 8-Br-cAMP. After the treatment the cells were photographed in a phase-contrast microscope. Bar, 10 μm. (C) RNAs isolated after the treatment with (+) or without 8-Br-cAMP (–) were electrophoretically separated and probed for the spore marker spiA.

The data described above raise the question of the physiological role of DdSRF in sporulation. By analogy to other SRF genes, our hypothesis is that *srfA* codes for a transcription factor that regulates the expression of other structural or regulatory genes required for spore differentiation. Since vertebrate SRF activity is regulated through several signal transduction pathways, it is also possible that DdSRF activity could be regulated by some of the signal pathways that control spore differentiation in *Dictyostelium* as described in the Introduction.

Although sporulation in *Dictyostelium* is very different from that in yeast and does not involve cell mating and meiosis, it is interesting to consider the possibility of an evolutionarily conserved mechanism in these two systems. One of the two yeast homologs of SRF, MCM1, is involved in the mating pheromone pathway that eventually leads to sporulation (Schultz et al., 1995). Moreover, a member of the yeast MAP kinase cascade that activates MCM1, STE20, has a putative *Dictyostelium* homolog, *mckA* (Shaulsky et al., 1996). Interestingly, *mckA* gene was isolated as a partial suppresser of the sporulation defect of *tagB* mutants.

We directly tested by northern the possibility that DdSRF could be regulating the level of expression of PKA during culmination. However, no differences were found in the mRNA levels of PKA in the mutant. Activation of PKA by 8-Br-cAMP is able to induce *spiA* gene expression in *srfA*$^-$ cells but not the formation of wild-type-looking spores. DdSRF may either function upstream or parallel to PKA in the activation of spiA gene expression. In the first case, DdSRF could be necessary for the expression or activity of some of the components of the regulatory pathway that activates PKA in the course of the spore differentiation program; activation of PKA with 8-Br-cAMP would bypass the blockage and activate *spiA* gene expression. In the second possibility, DdSRF and PKA pathways might function simultaneously in the activation of spiA promoter; the high-level artificial activation of PKA could override the requirement of the DdSRF activity. In addition, the fact that activation of PKA is not able to fully bypass the morphological defects of *srfA*$^-$ spores suggests that DdSRF, functioning independently of PKA, might also be essential for the expression of genes required for proper spore coat assembling or integrity.

It is likely that the activity of DdSRF is modulated by the newly emerging signal transduction pathways leading to spore formation. The establishment of the specific role that DdSRF plays in this regulatory process will require additional studies.

We thank Dr William F. Loomis for invaluable discussions and critical comments on the manuscript and also for providing strains, plasmids and cDNA library. We also thank Antonio Fernández and Ricardo Uña for the preparation of the figures and Gemma Rodríguez for sequencing. This work has been supported by a grant from Direccion General de Enseñanza Superior (grant number: PB95-0096).

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