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Short Report

The MADS-box transcription factor SRFA regulates different aspects of *Dictyostelium discoideum* development

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ABSTRACT The MADS-box transcription factor SRFA is expressed at several stages of *Dictyostelium discoideum* development. In early developmental stages is expressed in the prestalk region while it is expressed in the prespore region at culmination. The complex pattern of expression is obtained through the use of four different promoter regions that are active in different cell types and at different developmental stages. The analyses of *srfA* deficient strains has shown that this transcription factor is necessary for slug migration, morphogenesis and spore differentiation. Differential screening between Wild-type and *srfA*$^-$ strains has allowed the isolation of five cDNA clones whose expression is dependent on *srfA*. These genes are expressed at culmination in Wild-type strains but not in *srfA* $^-$ strains. The expression of only one or these genes can be induced in *srfA* $^-$ strains by treatment with the Protein Kinase A activator 8-Br-cAMP, indicating a complex interaction between Protein Kinase A and SRFA in the regulation of *Dictyostelium discoideum* development.

Objectives

The social amoeba *D. discoideum* is one of the simplest model systems utilized to study cell differentiation and development. *D. discoideum* usually grows as individual amoebae but under starvation the cells aggregate and develop into a fruiting body where spores are formed as resistance forms (Brown and Firtel, 1999). Formation of the fruiting body involves an initial step of cell aggregation and later processes of cell differentiation and morphogenesis, without cell proliferation. The fruiting body is composed of a limited number of cell types, mainly stalk cells, that form the basal disk and the stalk, and the spores, that are placed on top of the stalk. Besides, this organism can be easily manipulated and is amenable to genetic analyses (Escalante and Vicente, 2000). We had previously isolated a gene, *srfA*, coding for a protein homologous to animal Serum Response Factor, a transcription factor of the MADS-box family (Escalante and Sastre, 1998). *D. discoideum srfA* gene was interrupted by homologous recombination and showed to be necessary for spore terminal differentiation, slug migration and morphogenesis (Escalante and Sastre, 1998; Escalante *et al.,* 2001). Our laboratory is interested in a detailed study of the role played by *srfA* in *D. discoideum* development and the progresses made in the study of the regulation of *srfA* expression and in the isolation of genes regulated by *srfA* are reported.

Regulation of *srfA* gene expression

The regulation of *srfA* gene expression has been recently studied (Escalante *et al.,* 2001). A 4 kb long DNA fragment containing the promoter region of the *srfA* gene was cloned through several PCR and reverse-PCR reactions. The nucleotide sequence of this DNA fragment was compared to that of several cDNA clones containing 5'-untranslated regions (5'-UTR) of *srfA* mRNAs, obtained from the public Japanese cDNA data bank or by rapid amplification of cDNA ends (RACE) reactions. This analyses showed the existence of four alternative first exons coding for different 5'-UTRs of the mRNAs. All different mRNAs coded for the same protein since the initiation codon is located in exon 2, that is shared by all mRNAs. The existence of four different first exons is due to the presence of four different promoters, P1 to P4. The pattern of expression of the four promoters has been studied using different constructs where the LacZ reporter gene has been placed under the transcriptional control of each promoter. The results obtained are summarized in Figure 1. Each promoter regulates gene expression with a specific pattern. Early LacZ expression in prestalk cells is obtained under the control of promoters P1 and P2. Promoter P2 also induces strong LacZ expression in the prespore region under conditions that favor slug migration. Promoter P4 directs LacZ expression in few cells scattered along the structures with a pattern similar to the previously described Anterior Like Cells (ALC). Promoters P3 and/or P4 are responsible for high levels of LacZ expression in prespore and spore cells at culmination. The physiological importance of the specific patterns of *srfA* expression has been demonstrated because some of the phenotypic defects observed in *srfA*$^-$ strains can be specifically reverted by *srfA* re-expression under the control of some promoters. For example, *srfA* re-expression in prespore and spore cells under P3+P4 promoters specifically recovers spore differentiation. Similarly, *srfA* re-expression from promoter P2 rescues slug migration and fruiting body morphology, but not spore differentiation.

Isolation of *srfA* -dependent genes

The comparison of mRNA expression patterns between Wild-type and *srfA* $^-$ strains could allow the identification of genes whose expression is dependent on *srfA*. A differential screening of mRNAs expressed at late culmination stages between Wild-type and *srfA* $^-$ strains has been performed using the PCR-Select cDNA Subtraction Kit from Clontech. Five cDNA clones have been isolated that are differentially expressed. The five clones correspond to developmentally regulated genes whose expression is induced at culmination in the spore region of the fruiting body. The expression or these five genes was nearly undetectable in *srfA* $^-$ strains. These results indicate that the expression or these genes is dependent on *srfA*. The dependence might be direct since SRFA could be a transcription

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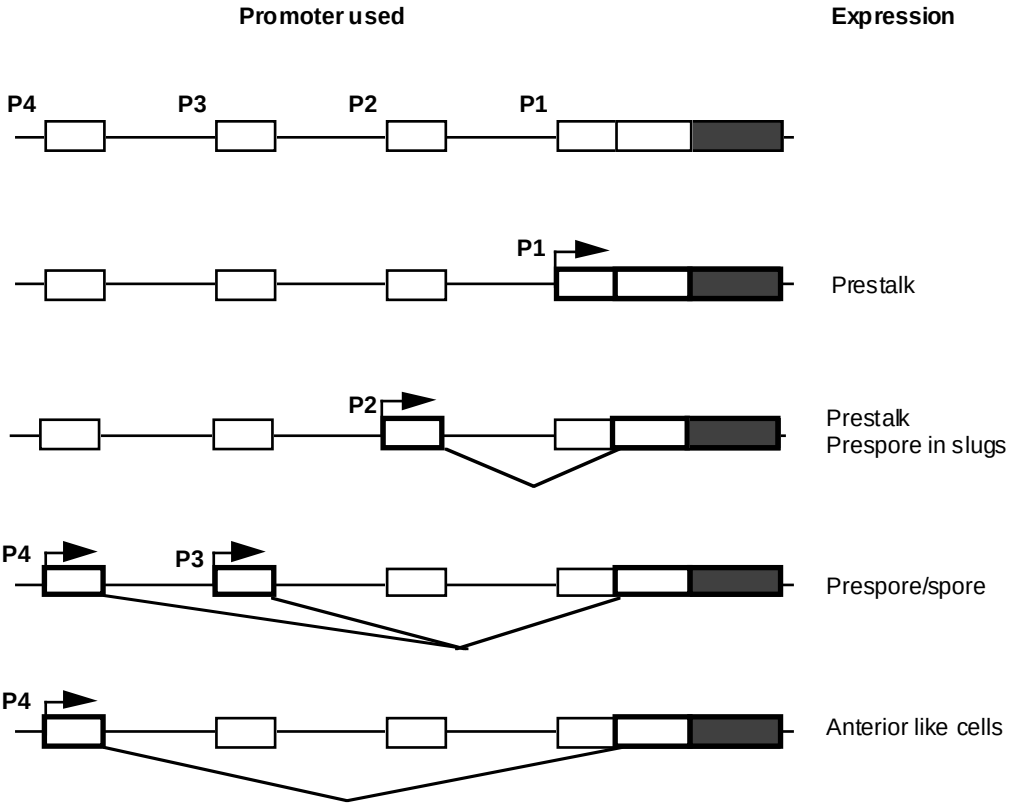

Fig. 1. Schematic representation of the structure of the *srfA* gene promoter. *The upper diagram indicates the position of the four different promoter regions (P1 to P4). Exons are indicated as boxes behind each promoter. The protein coding region, placed in exon 2, is indicated as a dashed box. Lower diagrams show the splicing events that are associated with the use of each promoter. Transcription start sites are indicated by arrows. Exons contained in each mRNA are shown in bold. The column to the right indicates the region of the developing structure where each promoter is active.*

factor that activates the expression of these genes through binding to their promoters. Alternatively, SRFA could indirectly regulate the expression of these genes by inducing other transcription factors that bind to their promoters. A detailed study of the expression of these genes and of their promoter regions will be necessary to discern between these possibilities. One of these genes, and the spore marker gene *SpiA*, can be induced by activation of the Protein Kinase A (PKA) after treatment of dissociated cells with 8-Br-cAMP, even in *srfA*$^-$ strains. These data indicated the concerted regulation of some of these genes, but not all, by SRFA and PKA-dependent pathways. It is well established that the PKA pathway plays a pivotal role in the regulation of *D. discoideum* development and, in particular, in spore differentiation (Harwood *et al.,* 1992). Further characterization of the genes whose expression is dependent on SRFA and PKA could provide valuable information to understand the interaction between these regulatory pathways in *D. discoideum*.

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