Identification of Genes Dependent on the MADS Box Transcription Factor SrfA in Dictyostelium discoideum Development†

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Analysis of microarrays containing 6,345 Dictyostelium discoideum genes has identified 21 whose expression is dependent on the MADS box transcription factor SrfA. In wild-type cells, all of these genes are induced late in development. At least four of them are necessary for proper spore differentiation, stability, and/or germination.

Dictyostelium discoideum strains carrying null mutations in the srfA gene form abnormal spores that do not resist adverse conditions (4). srfA codes for a protein homologue to serum response factor transcription factors that bind to the minor groove of DNA through a conserved domain, the MADS box (13, 17). Ultrastructural analyses of srfA spores have shown that actin rods are initiated but do not elongate as in wild-type spores and subsequently disassemble. The spore coats of srfA spores are initially indistinguishable from those of wild-type spores but become shredded with time (7). These structural changes are accompanied by reduced expression of spore-specific genes, such as spiA (3, 4). Altogether, these data suggest that srfA is necessary for the late steps of spore differentiation.

Microarrays containing 6,345 D. discoideum cDNA clones (9), including 690 previously characterized developmental genes and 5,655 cDNA clones from the Japanese EST Project (12), were used to identify genes dependent on SrfA for their expression. With Trizol reagent (Gibco BRL), RNAs were isolated from wild-type (AX4) and srfA-null (IIB100, derived from AX4) strains at 2-h intervals throughout development on filters (16) and compared to time-averaged RNA from wild-type cells, as previously described (9). Temporal changes for each gene were analyzed in an Axon Genepix 4000B scanner with GeneSpring software from Silicon Genetics. The total Cy3 signal was normalized to the total Cy5 signal after background subtraction to allow independent slides to be compared. The Cy3/Cy5 ratios of individual genes were then calculated. Each sample was hybridized to two or more microarrays, and each developmental time course was repeated at least twice. A list of the genes and mean values used for subsequent analyses are available at http://www.biology.ucsd.edu/loomis-cgi/microarray/paper2.html (see Tables S1 and S2 in the supplemental material).

Genes that were expressed at lower levels in the mutant cells than in wild-type cells were all induced after 20 h of development. Therefore, RNAs from the two last developmental stages of each strain (22 and 24 h for wild-type cells and 26 and 28 h for srfA cells, due to a slight delay in culmination observed in the mutant strain [6]) were directly compared to each other. Microarrays were simultaneously hybridized with Cy3 and Cy5 probes generated from wild-type and srfA strains, respectively. Thirty genes showed a more-than-threefold higher signal level with wild-type samples than with mutant samples and were considered candidates for SrfA-dependent genes. Microarray data are publicly available at http://www.biology.ucsd.edu/loomis-cgi/microarray/srfA_paper.html (see Table S3 in the supplemental material).

The pattern of expression of the potential SrfA-dependent...
genes was further analyzed by Northern blot hybridization as previously described (5). Twenty-two genes were confirmed to be SrfA dependent (Fig. 1 and 2). The expression of the first group of 11 genes, shown in Fig. 1 and Table 1, is detected exclusively during culmination in wild-type cells. The expression of the second group of 11 genes, shown in Fig. 2 and Table 1, is detected at low levels at earlier stages and induced at high levels during culmination. This late induction was not observed in srfA/H11002 strains. As a control, we analyzed several genes that are expressed late in development but did not give higher signals for wild-type samples than for srfA/H11002 samples on the microarrays, including the well-characterized prespore-specific gene pspA (1, 2). Northern blot analyses confirmed these results (data not shown). We also determined the cell type specificity of these genes by interrogating the published microarray data for separated prestalk and prespore cells (10, 11). Ten of the SrfA-dependent genes are preferentially enriched in prespore cells and spores, while three are preferentially expressed in prestalk and stalk cells (Table 1).

Nine of the SrfA-dependent genes encode known Dictyostelium proteins (present in the Preliminary Directory of Dictyostelium Genes [http://dicty.sdsc.edu/annot-020303.html]), and the others show significant similarity to known proteins in other organisms (Table 1). Two of the cDNA clones, SSK268 and SLJ453, coded for nonoverlapping regions of the same gene. Three other genes (sigB, sigC, and sigD), previously recognized from a subtractive library to be SrfA dependent (3) but not represented on the microarrays, were included in Table 1 for completeness. Mutational analyses have shown that catB, plcD, cofB, and spiA are each necessary for normal spore maturation or germination. catB codes for catalase B and mutant spores have been shown to be abnormally sensitive to H2O2 (8). plcD codes for phospholipase C, which is required for regulation of spore germination (18). cofB codes for coflin B, which associates with the actin rods in mature spores (15).

### Table 1. Possible functions of the proteins encoded by SrfA-dependent genes

| cDNA (gene)       | GenBank accession no. | Product                          | Closest homologa | Gene expression patternb |
|-------------------|------------------------|----------------------------------|------------------|-------------------------|
| SLK452            | AY386221               | Catalase B                       |                  | 1, spores               |
| SSF584            | AY392429               | Peroxinectin                     |                  | 1                       |
| SLA632            | AY392430               | Heat shock protein 88            |                  | 2                       |
| SSB695            | AU037272               | Unknown                          | Low-temperature  | 1                       |
| SSG695            | AY392431               | Unknown                          | DNA ligase (29)  | 1                       |
| SSE445            | AY392432               | Unknown                          | DNA helicase (39) | 1                       |
| SLF664            | AY392438               | Unknown                          | DNA repair protein RAD50 (19) | 1, spores |
| Cell signaling    | SSK576                 | AY392433                         | Phospholipase C  | 2, spores               |
| SJE895            | AY392434               | Unknown                          | 5’ AMP-activated gamma subunit (27) | 2 |
| Cytoskeleton and spore coat | SSK455     | D37981                           | Cofilin B        | 2, spores               |
| SLB816            | X54452                 | SpiAa                           |                  | 1, spores               |
| SSK208/SLJ453     | AY392441               | Unknown                          | Blackjack protein, microtubule associated (19) | 2, spores |
| sigD              | AY387647               | Unknownc                         | Spore coat proteins (28) | 1, spores |
| Vesicle trafficking| SSM796                | AY392436                         | Unknown          | Synaptobrevin (40)      | 2 |
| SSB611            | AY392437               | Unknown                          | Mitochondrial carrier protein RIM (31) | 1 |
| Cell adhesion     | SSJ826                 | AY392438                         | Unknown          | Tenascin X (31)         | 1, stalks |
| SSJ726            | AY392439               | Unknown                          | P-selectin (24)  | 1, stalks               |
| Metabolism        | SLE765                 | AY387644                         | SigA malic enzymec | 2, spores |
| SSA535            | AY392440               | 3-Oxoaetyl-acyl carrier         |                  | 2, stalks               |
| SSJ666            | AY392442               | Unknown                          | Alkaline dihydroceramidase (22) | 2 |
| Other proteins    | SLA429                 | AY392443                         | Unknown          | endotoxin (23)          | 2 |
| SSB579            | AY392444               | Unknownc                         | RNA-binding proteins (33) | 1, spores |
| sigB              | AY387645               | Unknownc                         | GP63 metalloproteinase (27) | 1, spores |
| sigC              | AY387646               | Phgl1b                           |                  | 2                       |

a The percentage of amino acid identity is given in parentheses.
b Expression pattern 1 indicates genes that are detected exclusively at late developmental stages while expression pattern 2 indicates genes also detected at earlier stages.
c Genes previously identified as SrfA dependent (3). The genes coding for SigD, SigC (phg1b), and SigB were not present on the microarrays.
Absence of actin rods results in round spores with very low viability. Spores deficient in SpiA show decreased viability under submerged conditions (14). Null mutations in malA, sigB, sigC, or sigD cause no apparent defects in spores (3). The other Srfa-dependent genes can be clustered on the basis of putative function of their closest homologs (Table 1). Possible functions include stress responses, actin cytoskeleton organization, metabolic regulation, prespore vesicle fusion, and spore coat stability.

In summary, a total of 24 Srfa-dependent genes have been identified, all of which are expressed late in development. No genes differentially expressed in slug or mid-culmination structures were found, even if srfa is expressed at these developmental stages (6). In addition, these studies have uncovered a novel program of gene expression that is activated late in Dictyostelium development. Thirty-nine genes were found that increased their expression at least threefold between 22 and 24 h of development. A significant proportion of these genes are dependent on Srfa for their expression and might be involved in many of the processes required for terminal spore differentiation.

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