PstO cells and the ALC, and it is not DIF inducible. We show their ability to utilize different regions of the ecmA promoter and can also be distinguished using noncompound promoters (5). PstO differentiation is induced by DIF-1, a polyketide synthesized by the prespore cells, but the pstA inducer is unknown (4, 6). There are anterior-like cells (ALC) in the prespore region; at culmination, some of the ALC and some of the pstO cells differentiate further to form the lower and upper cups, ancillary stalk cell structures that cradle the developing spore head (3).

MybE is a single Myb domain protein of a kind found in plants but not animals (2). It binds to a 22-nucleotide (nt) region of the ecmA promoter that directs prestalk-specific, DIF-inducible gene expression when multimerized (2). In a mybE null (mybE−) strain the ecmA promoter is inactive in the pstO cells and the ALC, and it is not DIF inducible. We show that mrrA, a novel gene of unknown function, and ecmB, a gene encoding an extracellular matrix protein, are both spatially regulated by MybE during culmination.

The mrrA gene is strongly expressed in upper and lower cup cells at culmination. MrrA (dictyBase reference no. DDB0168555) was identified in a small-scale microarray screen searching for genes that are aberrantly expressed in the MybE null strain. It was somewhat underexpressed in the null strain. It is predicted to be a single-pass, integral membrane protein (with transmembrane helices at amino acids 4 to 22, presumably a signal peptide, and 196 to 218). In the predicted extracytoplasmic domain it shows homology to the repeats that make up most of the extracytoplasmic domain of the cation-independent mannose 6-phosphate receptor (pfam00878). Hence, we term it MrrA for Mannose 6-phosphate receptor-repeat A.

The complete mrrA upstream promoter region (see Fig. 1A legend) was fused to lacZ, and the construct, mrrA:lacZ, was introduced into both Ax2 cells and mybE− cells. In parental structures during slug formation, staining cells are concentrated at the rear and there is a weak band of staining in the pstO region. At the equivalent stage, the rear region of the mybE− structures is also stained but there is less pstO staining. Thus, just as for ecmA, expression in pstO cells requires MybE (2). The posterior staining of both strains and the pstO staining in the parental strain disappear after a period of slug migration, probably through deposition into the slime trail, where large clumps of stained material are visible (Fig. 1A). During culmination of the parental strain the upper cup and lower cup become strongly stained. The fact that mrrA is activated only in stalk ancillary structures at culmination distinguishes it from ecmB, the paradigmatic marker of stalk cell differentiation, which is activated in ancillary structures and at the entrance to the stalk tube (3) (Fig. 1B).

In the mybE− strain mrrA:lacZ expression in the upper cup appears to be normal but there are no expressing cells in the position of the lower cup (Fig. 1A). Because there is a rearward loss of mrrA:lacZ-expressing cells during slug migration, it was important to check whether there is a physical structure corresponding to the lower cup in the mybE− strain. When mutant cells are labeled with neutral red, a vital dye that stains all prestalk cells, a red-stained lower cup is observable (Fig. 1A). Thus, the mrrA reporter construct requires MybE for its expression in lower cup cells but not in upper cup cells.

In a previous study analyzing ecmB expression in the mybE null strain, we quoted unpublished data that showed stained cells in the position of the lower cup (2). The mrrA results described above caused us to revisit this issue. We now find that during culmination of the mybE− strain ecmB:lacZ is expressed normally in the upper cup but is not expressed in the lower cup (Fig. 1). We are unsure of the reason for this disparity, but the MybE null strain is developmentally highly aberrant. Hence, rearward accumulation of cells in “stalked” migrating slugs may have been mistaken for a lower cup.

The mrrA promoter contains a functional MybE binding site. Just over 250 nt upstream of the mrrA initiation codon there is a 9-nt identity with the region of the ecmO promoter
element that contains a Myb dyad (2). When the region −292 to −252 is used in a band-shift assay with recombinant MybE protein a retarded band is observed (Fig. 2A). A multiply point-mutated form was created by replacing the Myb dyad with randomly selected nucleotides (Fig. 2A). This is a significantly less effective competitor. Thus, MybE binds specifically to the Myb dyad.

A lacZ fusion, containing two tandemly arrayed copies of region −292 to −252 of the mrrA promoter upstream of basal promoter elements, was constructed (Fig. 2B). When transformed into Ax2 cells, expression of lacZ is first detectable during culmination in scattered cells in the positions of the upper and lower cups (Fig. 2B). The mutations in the MybE site that prevent MybE binding in vitro were inserted into a tandemly duplicated form of the −292 to −252 region. Again this sequence was cloned upstream of basal promoter elements and transformed into parental cells. The mutant construct shows no detectable level of transcription. There is also a perfect Myb dyad (AACTGTT) at nt −362 in the ecmB promoter, but the ecmB promoter is relatively complex, with multiple activator domains, and we have not analyzed it further.

Thus, the Myb dyad within the mrrA promoter binds MybE in vitro and, when dimerized, a region containing the dyad directs upper and lower cup-specific expression. The expression is weak, but it is spatially specific, and point mutations that ablate Myb binding in vitro prevent expression. Therefore, a Myb site is necessary for upper cup expression but, as we have shown, MybE itself is not necessary. Presumably, one of the
other Dictyostelium Myb family proteins serves this function. In conjunction, these results imply a major role for Myb family proteins in ancillary stalk cell differentiation.

REFERENCES


