Int. J. Dev. Biol. 45 (S1): S147-S148 (2001)

Short Report

Expression and role of adenylyl cyclases during late development in *Dictyostelium discoideum*

ELISA ALVAREZ-CURTO, MARCEL E. MEIMA and PAULINE SCHAAP*

Department of Biochemistry, Wellcome Trust Building, University of Dundee, Scotland

ABSTRACT Development in the social amoeba *Dictyostelium discoideum* is characterized by the formation of a multicellular structure with a well defined temporal and spatial pattern. Via the activation of cAMP receptors and protein kinase A, cAMP signalling pathways are responsible for many developmentally regulated gene expression events in the organism. cAMP acts as chemoattractant, as an extracellular morphogen and as intracellular messenger. Three adenylyl cyclases have been described so far, ACA, ACG and ACB (Pitt *et al.,* 1992; Meima and Schaap, 1999; Soderbom *et al.,* 1999). Null mutants of these enzymes show defects in aggregation, spore germination (Van Es *et al.,* 1996) and terminal differentiation respectively. To elucidate their role in pattern formation in more detail we use LacZ-promotor constructs and *in situ* hybridization to compare the wild type pattern with some of the adenylyl cyclase mutants.

Material and Methods

The expression pattern of different cell type specific genes was studied using *in situ* hybridization with nonradioactive-labeled mRNA probes in fixed whole mounts of wild type, *acb*$^-$, and *acg*$^-$ cells prepared at different stages of development. Cells were plated at 10^6 cells/cm^2 on dialysis membrane filters, which were placed on non-nutrient agar and allowed to develop to the slug and fruiting body stages. They were then collected and hybridized with the various anti-sense mRNA's: *ecmA*, which was used as prestalk marker, and *cotB,* which was used as prespore marker. We use this technique also to analyze the pattern of the adenylyl cyclase G. Sense mRNA was used as control.

ACA and *ACG* expression patterns were analyzed in another set of experiments using wild type and *acb*$^-$ cells transformed with fusions of the ACA (Verkerke-Van Wijk *et al.,* 2001) or ACG promoters to labile ß-galactosidase reporter (ACA-ile-gal and ACG-ile-gal respectively). Cells were developed on nitrocellulose filters and stained for ß-galactosidase activity at the slug and fruiting body stages.We used a peptide derived antiserum against ACG to visualize the pattern of ACG protein in whole mounts. Western blots were performed to immunodetect levels of ACG protein using the same peptide derived antiserum.

Results

Using labile ß-galactosidase promotor constructs (Fig. 1) we show that in both wild type and in *acb*$^-$, *ACA* is expressed in the tip of the migrating slug and the stalk of the fruiting body. On the other hand, *ACG* is strongly expressed in the spore head of the fruiting body and at a lower level in the prespore region of the slug. *In situ* hybridization with an ACG mRNA probe (Fig. 3A) and immunostaining using an anti-ACG antibody (Fig. 2B) showed that ACG is present and it has the same pattern in both wild type and *acb*$^-$ in the slug and fruiting body stages. The results from the Western blots (Fig. 2A) also indicate that ACG protein is normally expressed in the *acb*$^-$.We looked at cell type specific gene expression in the adenylyl cyclase mutants using the pattern of expression of *ecmA* and *cotB* (Fig. 3B). *ecmA* is expressed in prestalk cells and not prepore cells, starting at the mound stage and it encodes for a large extracellular matrix protein, ST430

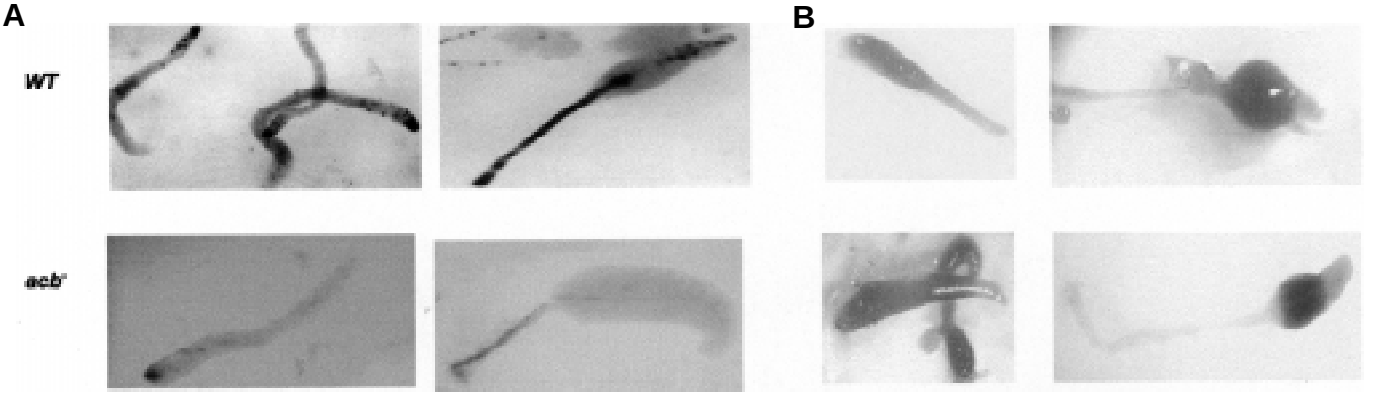

Fig.1. Pattern of expression of *ACA* and *ACG* using β-galactosidase promotor constructs. *ACA is observed in the tip of the slug and stalk of the fruiting body of both wild type and ACB null* **(A)**, *whereas ACG is expressed in the prespore region of the slug and strongly in the sporehead of the fruiting body of wild type and acb*$^-$ **(B)**.

***Address correspondence to:** Pauline Schaap. Department of Biochemistry, Wellcome Trust Building, University of Dundee, Dundee, DD1 5EH, Scotland. e-mail: p.schaap@dundee.ac.uk

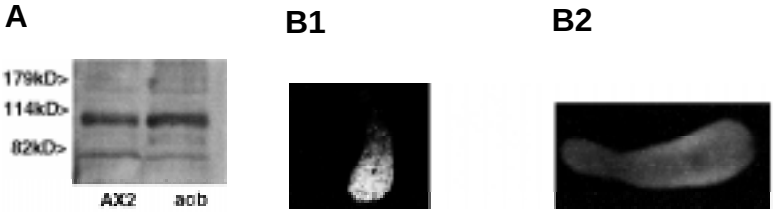

Fig. 2. ACG protein immunodotection and pattern in slugs. (A) *Western blots were performed with samples collected at the fruiting body stage and show that the 98 kDa ACG protein is present in both wild type and acb*$^-$ **(B1,B2)** *ACG immunostaining in wild type and acb*$^-$ *respectively.*

(McRobbie *et al.,* 1988). *cotB* is expressed in the prespore cells starting at the mound and it encodes the spore coat protein SP70 (Fosnaugh *et al.,* 1989). The *ecmA* and *cotB* patterns were analyzed in the wild type NC4, *acb*$^-$ and *acg*$^-$ mutants by *in situ* hybridization. In the *acb*$^-$ mutant we find that *ecmA* is expressed in the prestalk region and in the anterior-like cells scattered throughout the prespore region whereas *cotB* expression is detected in the prespore region of the slug. Both patterns are not significantly different from those in the wild type. The same results are found when performing *in situ* hybridizations with *ecmA* and *cotB* probes in the *acg*$^-$ cell line.

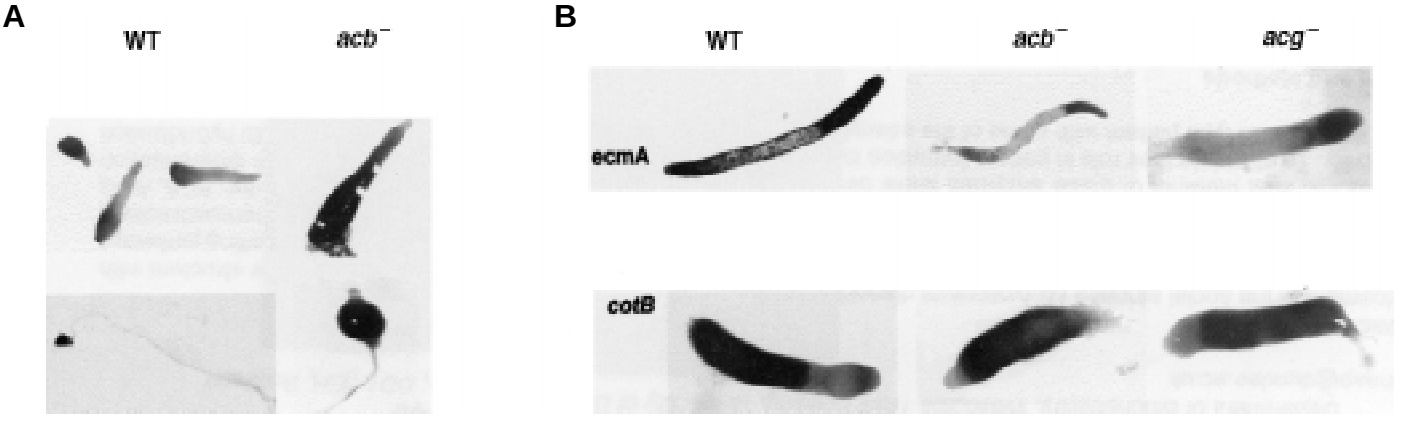

Fig. 3. *In situ* hybridization. *ACG mRNA probe* **(A)** *and ecmA and cotB cell type specific markers* **(B)** *in wild type and different adenylyl cyclase mutants (see text for details).*

Conclusions

Induction of prespore gene expression after the aggregative stages requires micromolar concentrations of extracellular cAMP. The expression of ACA strongly decreases after aggregation especially in the prespore region whereas it has been shown that ACB activity levels are strongly increased after slugs have formed (Meima and Schaap, 1999). This makes ACB the most likely candidate for this extracellular cAMP accumulation. However, since prespore expression in the ACB null is normal, and also stalk cell differentiation, which requires PKA activity occurs virtually normally in this cell line, there must be another source of cAMP. The expression pattern of *ACA* and *ACG* appears to be normal in the *acb*$^-$ indicating that the disruption of the *ACB* gene does not result in an ectopic expression of any of the other adenylyl cyclases. *ACB* disruption does not seem to have a significant effect on the pattern of the markers *ecmA* and *cotB*. Nevertheless we find two remarkable facts about the pattern of expression of *ACG*; first that it is normally expressed in acb$^-$ mutants when it has been reported that spore genes such as *spiA* are completely absent in this mutant cell line, and second that *ACG* expression seems to be mainly concentrated at the rear of the prespore zone of the slug. This indicates that the regulation of *ACG* expression might be distinct from that of other spore genes. The phenotype of the *acb*$^-$ could be still explained by a functional redundancy of ACA with ACB in stalk cell differentiation and /or ACG with ACB in prespore gene expression. Alternatively, a fourth adenylyl cyclase might be present and involved in gene expression events during late development.

References

FOSNAUGH, K.L. and LOOMIS, W.F. (1989) Spore coat genes SP60 and SP70 of *Dictyostelium discoideum. Mol. Cell Biol.* 5215-8.

MCROBBIE, S.J., JERMYN, K.A., DUFFY, K., BLIGHT, K. and WILLIAMS, J.G. (1988) Two DIF-inducible, prestalk-specific mRNAs of *Dictyostelium* encode extracellular matrix proteins of the slug. *Development* 104: 275-84.

MEIMA, M.E. and SCHAAP, P. (1999). Fingerprinting of adenylyl cyclase activities during *Dictyostelium* development indicates a dominant role for adenylyl cyclase B in terminal differentiation. *Dev. Biol.* 212:182-90.

PITT, G.S., MILONA, N., BORLEIS, J., REED, R.R. and DEVREOTES, P.N. (1992) Structurally distinct and stage-specific adenylyl cyclase genes play different roles in *Dictyostelium* development. *Cell* 69:305-15.

SODERBOM, F., ANJARD C., IRANFAR, N. and LOOMIS, W.F. (1999) *Development* 126: 5463-71.

VAN ES, S., VIRDY, K.J., PITT, G.S., MEIMA, M., SANDS, T.W., DEVREOTES, P.N., COTTER, D.A. and SCHAAP, P. (1996) Adenylyl cyclase G, an osmosensor controlling germination of *Dictyostelium* spores.*J. Biol. Chem.* 271: 23623-5.

VERKERKE-VAN WIJK, I., FUKUZAWA, M., DEVREOTES, P.N. and SCHAAP P. (2001) Adenylyl cyclase A expression is tip-specific in *Dictyostelium* slugs and directs StatA nuclear translocation and CudA gene expression.*Dev. Biol.* Jun;1:234(1):151-60.