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Adenylyl Cyclase A Expression Is Tip-Specific in *Dictyostelium* Slugs and Directs StatA Nuclear Translocation and *CudA* Gene Expression

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cAMP oscillations, generated by adenylyl cyclase A (ACA), coordinate cell aggregation in *Dictyostelium* and have also been implicated in organizer function during multicellular development. We used a gene fusion of the *ACA* promoter with a labile *lacZ* derivative to study the expression pattern of *ACA*. During aggregation, most cells expressed *ACA*, but thereafter expression was lost in all cells except those of the anterior tip. Before aggregation, *ACA* transcription was strongly upregulated by nanomolar cAMP pulses. Postaggregative transcription was sustained by nanomolar cAMP pulses, but downregulated by a continuous micromolar cAMP stimulus and by the stalk-cell-inducing factor DIF. Earlier work showed that the transcription factor StatA displays tip-specific nuclear translocation and directs tip-specific expression of the nuclear protein CudA, which is essential for culmination. Both StatA and CudA were present in nuclei throughout the entire slug in an *aca* null mutant that expresses *ACA* from the constitutive actin15 promoter. This suggests that the tip-specific expression of *ACA* directs tip-specific nuclear translocation of StatA and tip-specific expression of *CudA*.

***Key Words: Dictyostelium discoideum;* adenylyl cyclase A; organizer; cAMP oscillations; STAT transcription factor.**

INTRODUCTION

Starving *Dictyostelium* amoebae secrete cAMP pulses that trigger chemotaxis and the expression of genes that are required for cell aggregation. Cells collect into mounds at the center of pulsatile signaling, and these mounds transform into migrating slugs and fruiting structures through a finely orchestrated program of cell movements. The slug tip functions as a classical embryological organizer to induce and coordinate morphogenetic movement, most likely by virtue of being a pacemaker for cAMP oscillations (Bretschneider *et al.,* 1995; Schaap, 1986).

Three adenylyl cyclases have been identified in *Dictyostelium*. The germination adenylyl cyclase (ACG) is expressed in prespore cells and spores (Pitt *et al.,* 1992; Meima and Schaap, unpublished results). Adenylyl cyclase B (ACB) is optimally expressed during culmination and is essential for the maturation of the spores (Kim *et al.,* 1998; Meima and Schaap, 1999; Soderbom *et al.,* 1999). The aggregation adenylyl cyclase (ACA) is homologous to the G-protein-regulated mammalian adenylyl cyclases. ACA is expressed at high levels during aggregation and reduced levels during multicellular development (Pitt *et al.,* 1992). Its activity is regulated by positive and negative feedback loops, which control its oscillatory activation pattern. Excitation occurs when cAMP binds to a surface cAMP receptor cAR1, which induces dissociation of the G-protein G2 into its α and $\beta\gamma$ subunits. The free $\beta\gamma$ subunits initiate a process that leads to recruitment of cytosolic factors to the plasma membrane, where they activate adenylyl cyclase. cAMP acting on cAR1 also induces adaptation, which terminates the response. After hydrolysis of cAMP by an extracellular phosphodiesterase (PDE) activity, the cells de-adapt and the

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next cAMP pulse can be initiated (Parent and Devreotes, 1996).

The role of ACA in postaggregative development is less well understood. Cell movement patterns suggestive of oscillatory cAMP signaling have been observed (Steinbock *et al.*, 1993), but components of the cAMP signaling system such as ACA, cAR1, and PDE are considerably downregulated in the slug (Franke and Kessin, 1992; Klein *et al.*, 1998; Pitt *et al.*, 1992). This downregulation is surprising in view of the fact that most postaggregative gene-induction events require much higher (micromolar) cAMP concentrations than the nanomolar concentrations that suffice before aggregation (Oyama and Blumberg, 1986; Schaap and Van Driel, 1985). It is not clear how accumulation of these high cAMP levels is achieved.

To obtain more information on these issues, we studied the expression pattern of ACA during multicellular morphogenesis, the regulation of ACA transcription by extracellular signal molecules and its possible function in the slug stage.

MATERIALS AND METHODS

Construction of ACA–lacZ Gene Fusions

A full genomic clone of ACA containing 0.6 kb of sequence upstream of the start codon (pGSP9) was generated previously (Pitt *et al.*, 1992). We determined the nucleotide sequence of the 5′ noncoding region of this clone on both strands by means of the dideoxynucleotide chain-termination method using an ALF automatic sequencer (Pharmacia LKB). All reactions were performed three to four times. The sequence is deposited with GenBank under Accession No. L05496.

The 5′ noncoding region was amplified by PCR, using a 5′-oligonucleotide (TAATCTAGATATCGAATTCAAATTTTT) that generates an *Xba*I restriction site, and a 3′-oligonucleotide (CATTGGTGAGCTGGATCCCAATTTTTAATAATTTT) that generates a *Bam*HI site. The *Xba*I/*Bam*HI-digested *ACA* promoter region was used to replace the *Xba*I/*Bgl*II PsA promoter fragment from the PsA-ubi-ala-gal and PsA-ubi-ile-gal plasmids (Detterbeck *et al.*, 1994), yielding the ACA-ubi-ala-gal and ACA-ubi-ile-gal plasmids. These gene fusions use the ATG from the ubiquitin gene as a start codon and the vectors contain the G418 selection cassette. The ACA-ubi-ala-gal vector was transformed into *Dictyostelium* AX3 cells and the ACA-ubi-ile-gal vector into AX3 and AX2 cells and into *statA* and *cudA* null mutants (Fukuzawa *et al.*, 1997; Mohanty *et al.*, 1999). The transformed cells were selected by culture on axenic medium that was supplemented with 20 μg/ml G418.

Cell Culture and Incubation Conditions

AX3, AX2, and *aca*$^-$ (Pitt *et al.*, 1992) cells were grown in axenic medium, which was supplemented with 20 μg/ml G418 for all ACA-ubi-gal transformants and for *aca*$^-$/*A15ACA* and *aca*$^-$/*A15PKA* cells (Pitt *et al.*, 1992; Wang and Kuspa, 1997).

For developmental time courses, exponentially growing cells were harvested in 10 mM NaH_2PO_4/K_2HPO_4 buffer, pH 6.5 (PB) and incubated at 3×10^6 cells/cm^2 and 22°C, either directly on 1.5% agar in PB or on nitrocellulose filters (Protran; Schleicher and Schüell, Dassel, Germany) supported by PB agar. For gene-induction experiments, exponentially growing cells were washed and either resuspended directly in PB to 5×10^6 cells/ml or distributed on PB agar at a density of 3×10^6 cells/cm^2 and incubated at 22°C until tight aggregates had formed. Aggregates were dissociated by forcing them five times through a 19-gauge needle. Cells were resuspended in PB to 5×10^6 cells/ml and incubated at 150 rpm at 22°C in the presence of various stimuli, as indicated in the figure legends.

RNA Isolation and Analysis

Total RNA was isolated from 2×10^7 cells, size fractionated on 1.5% agarose gels containing 2.2 M formaldehyde (Nellen *et al.*, 1987), and transferred to Gene Screen membranes. Membranes were hybridized to [^{32}P]dATP-labeled *ACA* or *lacZ* DNA probes according to standard procedures.

Biochemical and Histochemical β-Galactosidase Assays

For biochemical assay of β-galactosidase activity, cells were lysed by freeze/thawing and 100-μl aliquots of lysate were incubated in microtiter-plate wells with 30 μl of 2.5× Z-buffer (Dingermann *et al.*, 1989) and 20 μl of 10 mg/ml ONPG at 22°C, until sufficient yellow color had developed. The OD_{415} was measured using a Bio-Rad microtiter-plate reader. All activities were standardized for the protein content of the cell lysates.

For histochemical staining, nitrocellulose filters supporting developing *Dictyostelium* cells were frozen at −20°C. Frozen structures were fixed in 0.25% glutaraldehyde and 2% Tween 20 in Z-buffer, washed with Z-buffer, and stained with X-gal for 1–4 h (Dingermann *et al.*, 1989).

Immunofluorescence

Single cells or cells from dissociated mounds or slugs were resuspended in PB to 10^7 cells/ml and placed as 10-μl aliquots in the wells of eight-well multitest slides (ICN Biomedicals, Irvine, CA). Cells were left to adhere for 10 min, fixed for 5 min in methanol, washed with PBS (0.8% NaCl in 10 mM phosphate, pH 7.4), and incubated overnight with 1:10 diluted antibody raised against the last 15 amino acids of ACA (Parent and Devreotes, 1995). Slides were washed twice by submersion in PBS, incubated for 3 h with 1:200 diluted FITC-conjugated goat anti-rabbit antibody (GARFITC), and washed with PBS twice more.

Intact slugs were gently floated from an inverted slice of supporting agar to 10 μl PB deposited in the wells of polylysine-coated eight-well multitest slides. The fluid was aspirated and the structures were fixed for 5 min in methanol, washed with PBS, incubated overnight with 10 μl of 1:1000 diluted monoclonal antibodies raised against StatA (Araki *et al.*, 1998) or CudA peptides (Fukuzawa *et al.*, 1997), and subsequently incubated for 3 h with 1:200 diluted FITC-conjugated goat anti-mouse antibody (GAMFITC). Structures were mounted and photographed using a Leica fluorescence microscope or a Leica TCS SP2 confocal laser-scanning microscope.

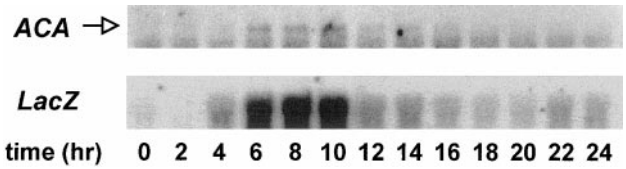

FIG. 1. Developmental regulation of *ACA* mRNA and *lacZ* mRNA in ACA-ubi-ile-gal transformants. ACA-ubi-ile-gal cells were incubated on PB agar at 22°C for 24 h. Samples for isolation of total RNA were taken at 2-h intervals. Northern blots were probed with ^{32}P-labeled *ACA* and *lacZ* cDNAs.

RESULTS

ACA Expression during Development

The genomic clone pGSP9 containing the entire *ACA* coding sequence and 0.6 kb of the 5′ noncoding region was used earlier to restore aggregation and development of *aca*$^-$ mutants and may be expected to contain the functional promoter (Pitt *et al.*, 1992). Preliminary studies with a fusion of the *ACA* 5′ noncoding region to the stable ubi-ala-gal derivative of the *LacZ* reporter gene showed sustained β-galactosidase activity in all cells after aggregation, even though *lacZ* mRNA had strongly declined (data not shown). We therefore fused the ACA promoter to the unstable ubi-ile-gal derivative, which has a protein half-life of about 1 h (Detterbeck *et al.*, 1994). To test whether *LacZ* expression from the ACA promoter was regulated similarly to the endogenous ACA mRNA, we compared the developmental regulation of *ACA* mRNA with that of *LacZ* mRNA. The endogenous 5.8-kb *ACA* mRNA, visible above the 26S rRNA band, is much less abundant than *lacZ* mRNA, which is transcribed from multiple copies of the ACA-ubi-ile-gal vector in transformed cells (Fig. 1). However, their developmental regulation is the same; *ACA* and *lacZ* mRNAs were absent from growing cells (T = 0 h) and started to increase after 6 h of starvation. Both mRNAs reached maximal levels at 10 h and thereafter decreased progressively.

To visualize *ACA* promoter activity in developing structures, ACA-ubi-ile-gal transformants were starved on nitrocellulose filters, fixed, and stained with X-gal. In newly formed aggregates most cells initially stained intensely (Fig. 2A). Tipped aggregates and first fingers still showed staining in cells throughout the structures (Fig. 2B), although during early slug migration, expression started to decrease in all cells that are posterior from the tip, until expression became almost tip-specific in slugs that had migrated for a few hours, with only a few scattered cells staining in the prespore region (Fig. 2C). At the onset of culmination, expression in the tip of the slug started to expand into the newly formed stalk (Fig. 2D), until in mid- and late culminants, the entire stalk was stained (Figs. 2E and 2F).

Distribution of ACA Protein in Slugs

Loss of ACA mRNA from cells distal to the tip need not necessarily involve loss of ACA function in these cells, when the ACA protein is stable. To test whether tip-specific expression of ACA is also reflected at the protein level, we visualized ACA protein with an ACA antibody. This antibody was raised earlier against the C-terminal 15 amino acids of ACA and used to identify ACA protein on Western blots (Parent and Devreotes, 1995). To test the specificity of the antibody for immunocytology we first compared staining in growing cells of an *aca*$^-$ mutant and an *aca*$^-$ mutant that expresses ACA from the constitutive actin15 promoter (Pitt *et al.*, 1992). The *aca*$^-$ cells showed some nonspecific reactivity with the ACA antibody in the cytosol (Fig. 3B), whereas the *aca*$^-$*/A15ACA* cells additionally showed intense specific staining at the cell periphery, where ACA is localized (Fig. 3A). Peripheral staining was also observed in wild-type cells during aggregation, when ACA is optimally expressed (Fig. 3C). In addition, there appeared to be reactivity to vesicular compartments. This may represent the rapid recycling of the plasma membrane through endosomal compartments, which is characteristic of *Dictyostelium* amoebae (Aguado-Velasco and Bretscher, 1999; Thilo and Vogel, 1980).

The nonspecific reactivity in the cytosol prevented us from getting meaningful results with whole-mount staining of aggregates and slugs. We therefore dissected slugs into tips (about 5% of the whole slug) and a region taken from the middle of the posterior 75%. The tissue was dissociated into single cells and the cells were stained with ACA antibody. The tip cells showed the same pattern of peripheral staining as the *aca*$^-$*/A15ACA* cells (Fig. 3D). The posterior cells showed diffuse staining in the cytosol as well as some punctuate staining, which may be associated with the prespore vesicles. There was, however, no detectable staining at the periphery of the cells (Fig. 3E). This suggests that only the tip cells express ACA protein.

Signals Controlling ACA Promoter Activity

Expression of the majority of the aggregative genes is strongly enhanced by cAMP pulses, with the exception of the aggregative promoter of the extracellular phosphodiesterase (PDE) gene, which can also be activated by a continuous cAMP stimulus (Franke and Kessin, 1992; Noegel *et al.*, 1986). We first measured which cAMP stimulation regimen most effectively activates the *ACA* promoter. Vegetative ACA-ubi-ile-gal cells were stimulated with either 30 nM cAMP pulses, 1 μM Sp-cAMPS, or 100 μM cAMP. A 1 μM Sp-cAMPS treatment mimics a 20–50 nM continuous cAMP signal, because Sp-cAMPS is not hydrolyzed by PDE and has a 15- to 50-fold lower affinity for cARs than for cAMP (Johnson *et al.*, 1992). Figure 4A shows that cAMP pulses activated the *ACA* promoter most effectively. Stimulation with 100 μM cAMP yielded 20% of the levels induced by pulses, while 1 μM Sp-cAMPS had no effect on *ACA* activity.

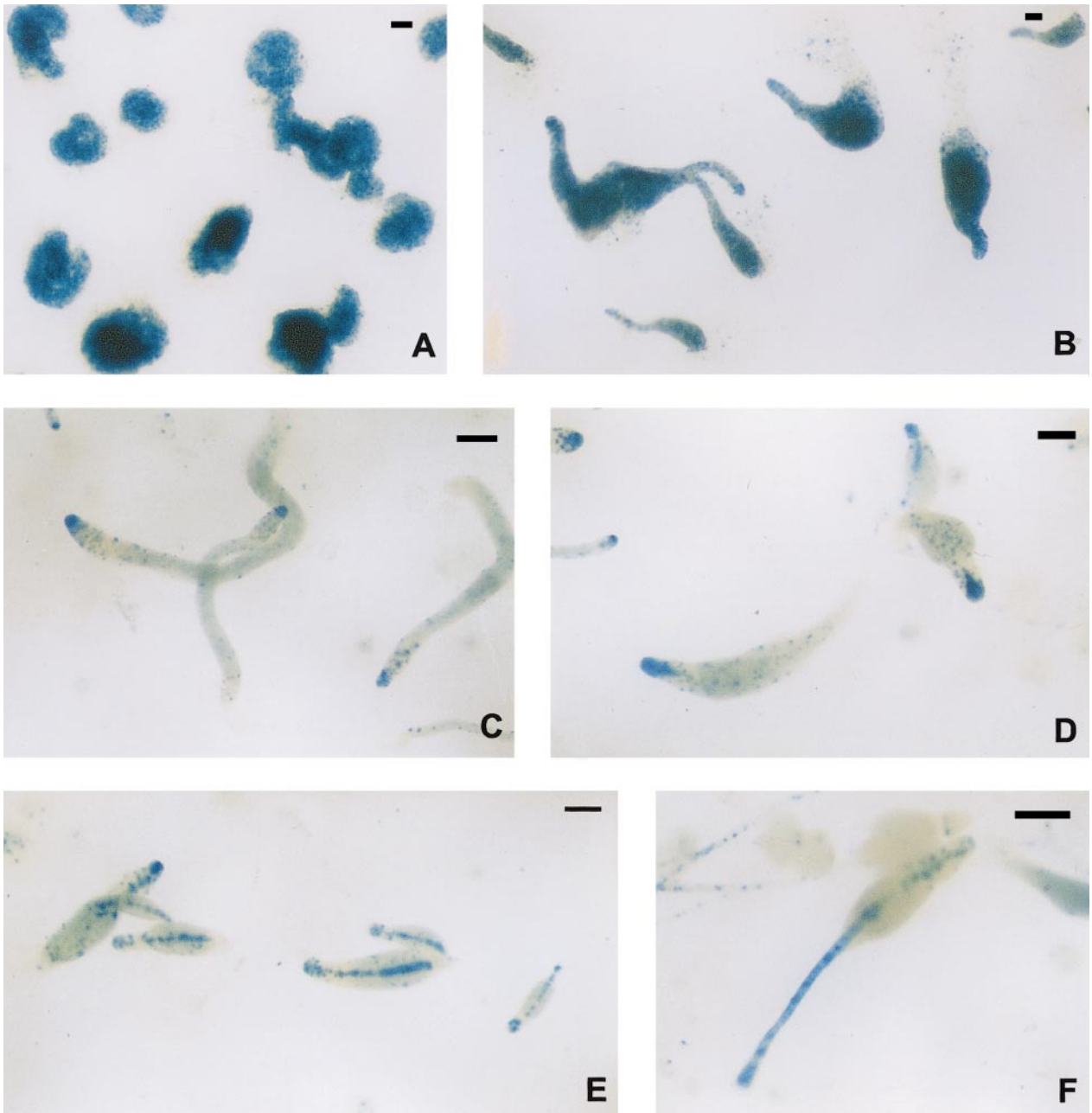

FIG. 2. *In situ* detection of *ACA* promoter activity. ACA-ubi-ile-gal cells were incubated at 22°C on nitrocellulose filters and fixed in glutaraldehyde when the developmental stages of interest had been reached. The structures were incubated with X-gal to visualize β-galactosidase activity: (A) early aggregates; (B) tipped aggregates/first fingers; (C) slugs; (D) early culminants; (E) mid culminants; (F) late culminant. Scale bars: 100 μm.

After aggregation, the predominant known signals for gene regulation are either micromolar cAMP, which induces prespore gene expression, or DIF, which induces prestalk gene expression (Firtel, 1995). We added DIF to the repertoire of stimuli used to study regulation of ACA during postaggregative development. Tight aggregates were dissociated into single cells and incubated for 8 h with 30 nM cAMP pulses, 1 μM Sp-cAMPS, 100 μM cAMP, 100 nM DIF, and the combination of 100 μM cAMP and 100 nM DIF. Figure 4B shows that in untreated cells, *ACA* promoter activity showed a small increase followed by a slow decrease to about 75% of maximal

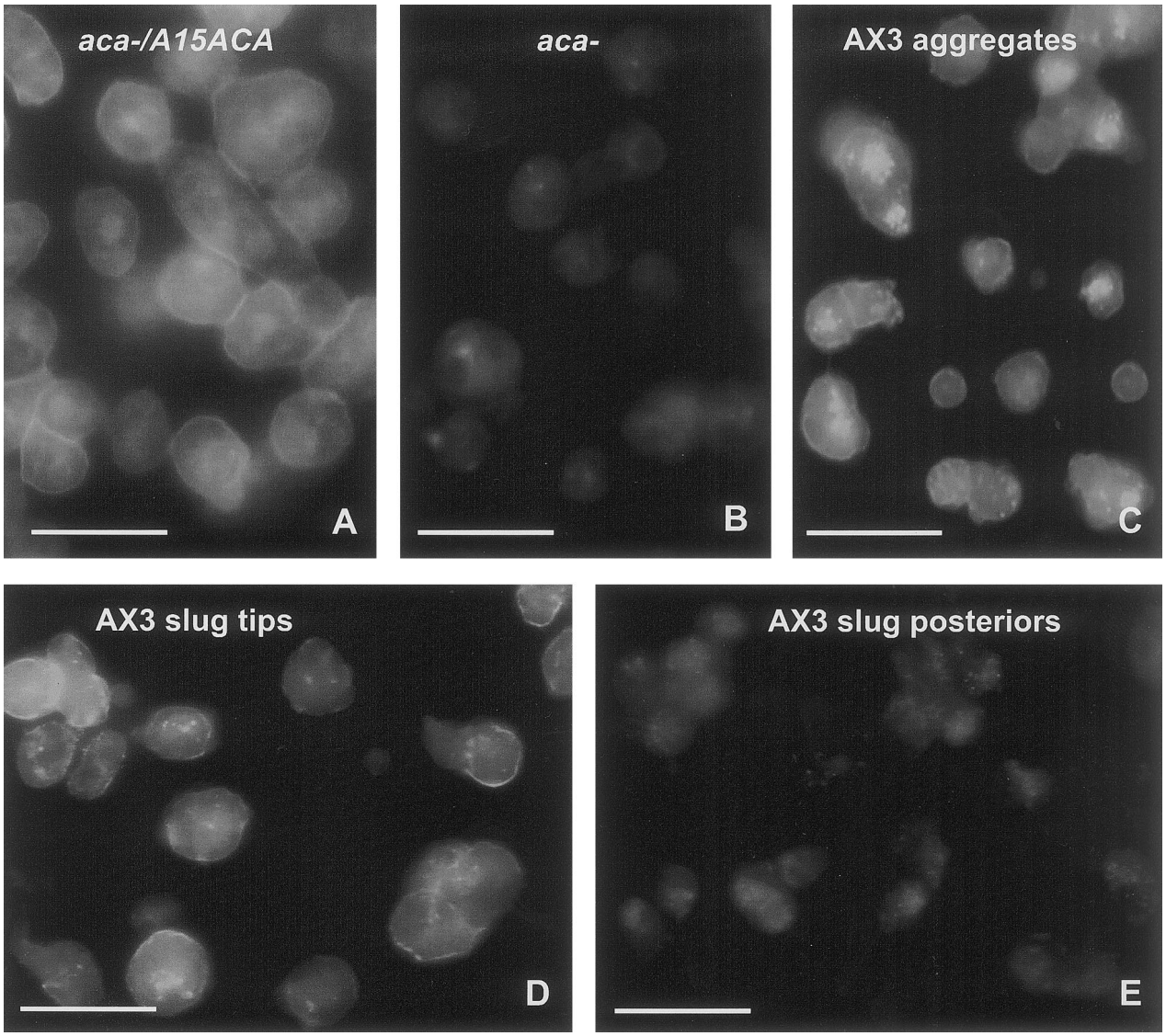

FIG. 3. Localization of ACA protein. *aca*$^-$*/A15ACA* and *aca*$^-$ cells were harvested from growth culture, resuspended in PB at 10^7 cells/ml, and placed as 10-μl aliquots on microscope slides (A, B). AX3 cells were incubated at 22°C until loose aggregates or migrating slugs had formed. Loose aggregates were dissociated and placed on slides (C). Slugs were dissected and batches of 10 tips (most anterior 5%) or three midposterior regions were placed in 10 μl PB, dissociated by vigorous pipetting, and deposited on microscope slides (D, E). All preparations were stained with ACA antibody and GARFITC.

expression. This decrease was more pronounced in cells treated with 1 μM Sp-cAMPS or 100 mM DIF, and most severe in cells incubated with 100 μM cAMP or 100 μM cAMP in combination with 100 nM DIF. Here *ACA* expression decreased for more than 70%. cAMP pulses of 30 nM induced a small increase of *ACA* promoter activity, which suggests that the same signal that upregulates ACA expression before aggregation, acts to maintain expression once aggregates have formed.

A Possible Function for Tip-Specific Expression of ACA

The developmental regulation of ACA expression is correlated with the nuclear translocation of the *Dictyostelium* transcription factor StatA. StatA is expressed throughout development; it is present in the cytosol during growth and the first few hours of development, but moves to the nucleus when cells are collecting into aggregates. This

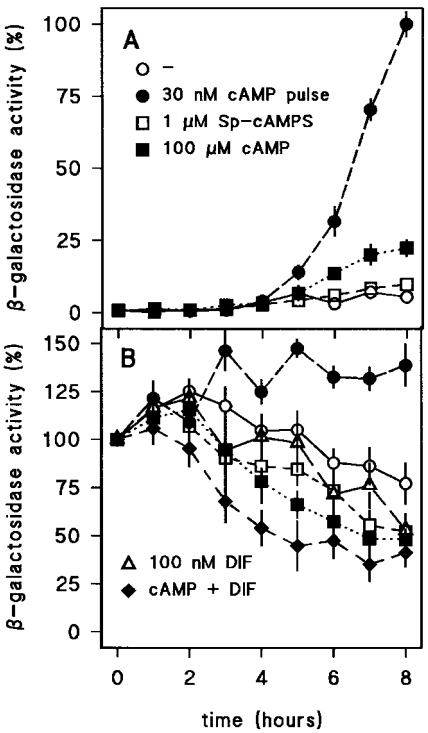

FIG. 4. Regulation of *ACA* gene expression by various stimuli. (A) Vegetative ACA-ubi-ile-gal cells were incubated for 8 h in PB without additives, with 30 nM cAMP pulses at 6-min intervals, 1 μM of Sp-cAMPS, added once, or with 100 μM cAMP added at 60-min intervals. Cell lysates were assayed for β-galactosidase activity at 1-h intervals. Data are expressed as the percentage of β-galactosidase activity after 8 h of stimulation with 30 nM cAMP pulses. (B) ACA-ubi-ile-gal cells were developed to tight aggregates, resuspended in PB, and incubated with the same stimuli as in (A) and additionally with 100 nM DIF and 100 nM DIF plus 100 μM cAMP. Data are expressed as the percentage of the β-galactosidase activity at $T = 0$ h. Both panels represent the means $\pm$ SEM of four experiments performed in triplicate.

nuclear translocation can be induced by cAMP and is accompanied by phosphorylation of a conserved tyrosine residue. In aggregates, all cells display StatA in the nucleus, but when slugs are formed all cells posterior to the tip relocate StatA back to the cytosol (Araki *et al.,* 1998; Kawata *et al.,* 1996). Nuclear StatA induces expression of the nuclear factor CudA at the slug tip. The *CudA* gene shows a rather unique expression pattern. It is transcribed in the posterior prespore region from a distal promoter region and in the tip of slugs from a proximal region. In *statA* null mutants tip-specific expression of *CudA* is absent, while *CudA* expression in the prespore region is normal (Fukuzawa *et al.,* 1997; Fukuzawa and Williams, 2000).

To test whether ACA at slug tips induces nuclear translocation of StatA and, in turn, tip-specific expression of *CudA* at this position, we compared localization of StatA and CudA protein in wild-type slugs to that in *aca*$^-$/*A15ACA* slugs, which express ACA constitutively. If ACA regulates the localization, the prediction would be that *aca*$^-$/*A15ACA* slugs show nuclear localization of StatA and CudA over their entire length. StatA and CudA localization were visualized with monoclonal antibodies against the two proteins. Figure 5A shows the characteristic tip-specific nuclear localization of StatA in a wild-type slug. The *aca*$^-$/*A15ACA* slugs showed nuclear staining of StatA throughout the entire slug (Fig. 5B). Figure 5C shows the typical tip- and prespore-specific localization of CudA. As was reported earlier, CudA nuclear staining is usually more pronounced in tip cells than in prespore cells and extends into the central core of the tip (Fukuzawa *et al.,* 1997; Fukuzawa and Williams, 2000). In *aca*$^-$/*A15ACA* slugs, cells throughout the slug showed nuclear staining with the CudA antibody (Fig. 5D). The observation that both StatA nuclear translocation and CudA expression closely follow the pattern of ACA expression suggests that ACA activity induces these events.

The *aca*$^-$ cells cannot aggregate, but aggregation and fruiting body formation can be partially restored by overexpression of the PKA catalytic subunit from the actin15 promoter (Wang and Kuspa, 1997). To test whether ACA was essential for StatA nuclear translocation, we stained *aca*$^-$/*A15PKA* slugs with StatA antibody. Of 23 stained slugs, 12 slugs showed no nuclear StatA localization at all (Fig. 6A), while in the remaining 11 a thin rim of stained nuclei was present at the outer periphery of the tip (Fig. 6B). In wild-type slugs, the anterior one-fifth to one-fourth of all slugs was stained (Fig. 5A) and (Araki *et al.,* 1998).

In theory, the presence of StatA and CudA at the tip could also be responsible for the continued expression of ACA. To test this possibility we transformed *statA* and *cudA* null mutants with the ACA-ubi-ile-gal construct. Tip-specific expression of ACA in the *cudA*$^-$ cells (Fig. 7C) was almost indistinguishable from that in the parent strain AX2 (Fig. 7A). In *statA*$^-$ slugs, slug morphology is abnormal because these cells also carry a chemotactic defect (Mohanty *et al.,* 1999). However, ACA expression was still enriched at the slug tip (Fig. 7B). StatA and CudA are apparently not required for tip-specific expression of ACA.

DISCUSSION

Tip-Specific ACA Expression Is Not Essential for Organizer Function

ACA mRNA was shown earlier to reach high levels during aggregation and to decrease to barely detectable levels later during development (Pitt *et al.,* 1992). We show here that this decrease is primarily due to the fact that after slug formation ACA promoter activity is lost from almost all cells, except those at the tip. The slug tip can induce a secondary patterning axis when transplanted to a host slug (Raper, 1940). This property has been attributed to the fact that the tip generates autonomous oscillations of cAMP

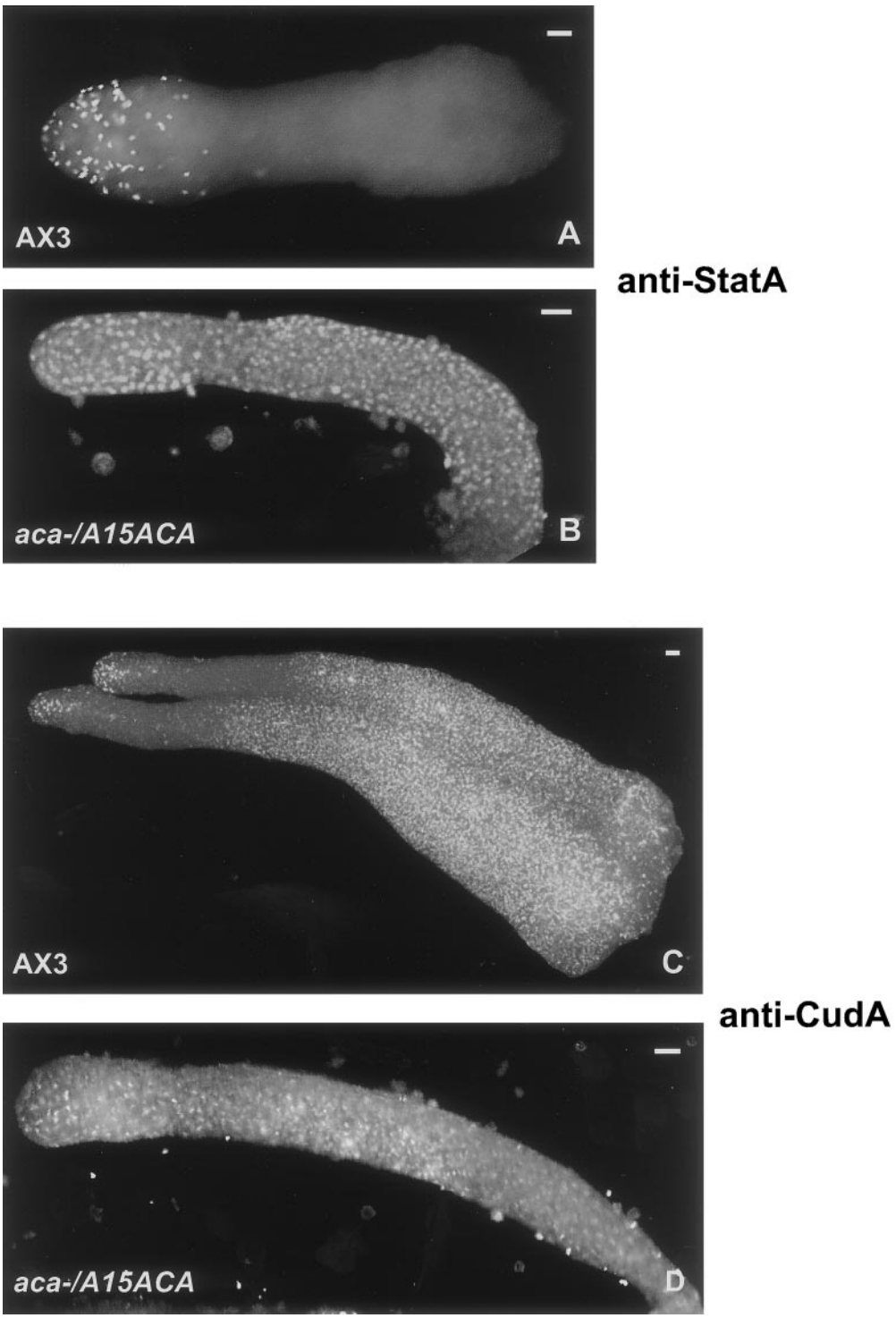

FIG. 5. StatA and CudA localization in AX3 cells and *aca*$^{-}$*/A15ACA* mutants. Wild-type AX3 cells (A, C) and *aca*$^{-}$*/A15ACA* mutants (B, D) were incubated on PB agar at 22°C until migrating slugs had formed. Intact slugs were transferred to microscope slides and stained with either StatA (A, B) or CudA (C, D) specific monoclonal antibodies and GAMFITC. Scale bars: 10 μm.

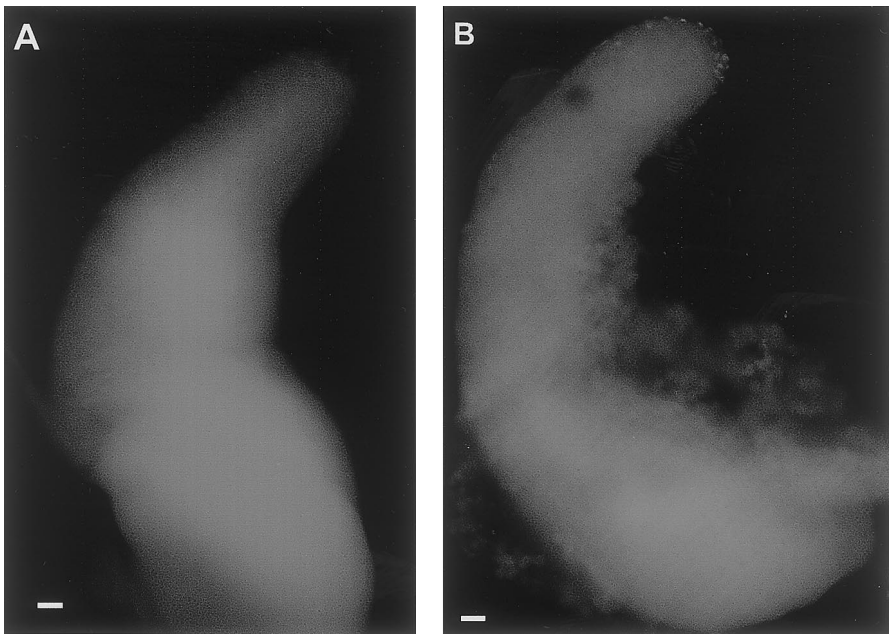

FIG. 6. StatA localization in *aca*$^-$*/A15PKA* slugs. *aca*$^-$*/A15PKA* cells were incubated on PB agar at 22°C until slugs had formed, which occurred from about 5% of the aggregates. Individual slugs were transferred to microscope slides and stained with StatA-specific monoclonal antibodies and GAMFITC. (A) and (B) represent the two patterns of StatA staining that were observed. Scale bars: 10 μm.

(Bretschneider *et al.*, 1995; Schaap, 1986). The present finding of preferential ACA expression in the slug tip would agree well with such a hypothesis. However, three lines of evidence suggest that morphogenesis cannot be dependent on tip-specific expression of ACA. *aca*$^-$ mutants, although incapable of going through development unassisted, can form small slugs and fruiting bodies when prestimulated with extracellular cAMP stimuli (Pitt *et al.*, 1993). *aca*$^-$ cells that overexpress PKA constitutively can form aggregates and slugs at high cell density, although slug formation is not very efficient (Wang and Kuspa, 1997). *aca*$^-$ cells that express ACA in all cells from the constitutive actin15 promoter show virtually normal aggregation and multicellular development (Pitt *et al.*, 1992).

It seems more likely that tip-specific *ACA* expression is simply a consequence of the fact that only at the tip the conditions are conducive for continued *ACA* transcription. During preaggregative development, *ACA* transcription is upregulated by nanomolar cAMP pulses. This is also the case for other genes involved in cell aggregation, such as *cAR1*, *Gα2*, and *csA* (Firtel, 1995), and represents the extensive positive feedback that the cAMP signaling system exerts on its own expression. Once the *ACA* gene is optimally expressed in aggregates, none of the signaling regimes that we used caused a pronounced upregulation of expression. Most signals, such as a continuous cAMP stimulus and DIF, the signals that induce prespore and prestalk gene expression, respectively (Firtel, 1995), downregulated *ACA* transcription, especially when they were combined. Nanomolar cAMP pulses induced a moderate upregulation of expression. Extracellular PDE, another essential component of pulsatile cAMP signaling, is also almost exclusively expressed at the anterior region of the slug (Hall *et al.*, 1993). This could mean that only here are optimal conditions for pulsatile signaling and continued *ACA* transcription maintained. Alternatively, it remains possible that *ACA* transcription at the tip is induced by some unknown signal that we did not test.

Which Adenylyl Cyclase Produces cAMP for Prespore Gene Induction?

The distribution of *ACA* in the slug presents us with an interesting conundrum. Induction and maintenance of prespore gene expression require micromolar cAMP concentrations (Oyama and Blumberg, 1986; Schaap and Van Driel, 1985; Wang *et al.*, 1988). It has been assumed that these concentrations can accumulate as a result of ACA-mediated oscillatory cAMP signaling, once the cells are closely packed in aggregates. Although this may be true for the mound stage where prespore genes are first expressed, it is less likely to occur in slug posteriors where *ACA* expression is almost completely downregulated. Two other adenylyl cyclases, ACB and ACG, are expressed in the slug stage (Meima and Schaap, 1999; Soderbom *et al.*, 1999; Meima and Schaap, unpublished results). ACG expression is restricted to the prespore cells, whereas the localization of ACB has not yet been reported. However, both the *acrA*$^-$

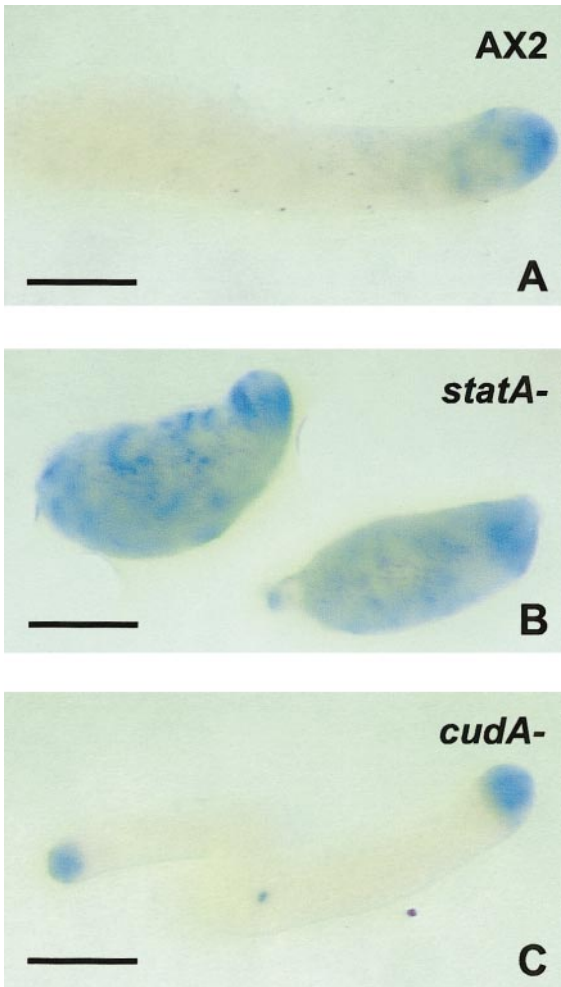

FIG. 7. ACA expression in *statA* and *cudA* null mutants. AX2 (A), *statA*$^-$ (B), and *cudA*$^-$ (C) cells were transformed with the ACA-ubi-ile-gal construct and incubated on filters at 22°C until migrating slugs had formed. The structures were fixed in glutaraldehyde and stained with X-gal to visualize β-galactosidase activity. Scale bars: 100 μm.

(*acb*$^-$) and the *acgA*$^-$ mutants show normal expression of prespore genes (Soderbom *et al.*, 1999; Meima and Schaap, unpublished results), which indicates that neither of the enzymes is solely responsible for prespore gene induction. ACB and ACG may be functionally redundant, but an *acrA*$^-$/*acgA*$^-$ mutant will be required to confirm this.

Tip-Specific ACA Expression Regulates StatA Nuclear Translocation and CudA Gene Expression

The nuclear factors StatA and CudA play crucial roles in *Dictyostelium* development. The *CudA* gene was isolated from a culmination-deficient mutant and is essential for both stalk and spore maturation (Fukuzawa *et al.*, 1997). StatA functions both as a repressor of precocious stalk cell differentiation and as an activator of *CudA* expression at the slug tip (Fukuzawa and Williams, 2000; Mohanty *et al.*, 1999). *CudA* is also expressed in the prespore region, but this requires a separate region of its promoter and is independent of StatA (Fukuzawa and Williams, 2000). StatA is translocated from the cytosol to the nucleus in response to extracellular cAMP. During normal development this occurs in all cells, when cells are aggregating. After aggregation StatA leaves the nucleus again in most cells, except those at the slug tip (Araki *et al.*, 1998). We show here that this is a consequence of the loss of ACA from all cells posterior to the tip, in that the *aca*$^-$*/A15ACA* mutant that expresses ACA throughout the slug also displays ubiquitous StatA nuclear translocation and *CudA* expression. In the *aca*$^-$*/A15PKA* mutant, StatA nuclear translocation is either absent or strongly reduced, which supports the notion that ACA is required for StatA nuclear translocation. However, the residual nuclear translocation is rather intriguing: are there other signals to induce StatA nuclear translocation, or does the *aca*$^-$*/A15PKA* mutant display another local source of cAMP? As mentioned above, the adenylyl cyclases ACB and ACG are also expressed in the slug stage and the constitutive expression of PKA could upregulate their expression in cell types, where they are not normally expressed.

It was recently found that StatA nuclear translocation can be induced in slug posteriors by microinjection of cAMP (Dormann *et al.*, 2001). In combination with the observed correlation between ACA expression and StatA/CudA localization, this presents us with a straightforward model for StatA and *CudA* regulation: Extracellular cAMP produced by ACA first induces nuclear translocation of StatA in all cells during aggregation. When the cells that are distal from the tip start to loose ACA expression, StatA relocates to the cytosol. StatA nuclear localization is retained only at the tip and only here can StatA bind to the tip-specific promoter region of *CudA* and activate its transcription.

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