Development 128, 1081-1088 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
DEV3344

Inducible nuclear translocation of a STAT protein in *Dictyostelium* prespore cells: implications for morphogenesis and cell-type regulation

Dirk Dormann*, Tomoaki Abe*, Cornelis J. Weijer and Jeffrey Williams‡

School of Life Sciences, University of Dundee, Wellcome Trust Biocentre, Dow Street, Dundee DD1 5EH, UK
*These two authors contributed equally to this work
‡Author for correspondence (e-mail: j.g.williams@dundee.ac.uk)

Accepted 8 January; published on WWW 13 March 2001

SUMMARY

Dd-STATa, the *Dictyostelium* STAT (signal transducer and activator of transcription) protein, is selectively localised in the nuclei of a small subset of prestalk cells located in the slug tip. Injection of cAMP into the extracellular spaces in the rear of the slug induces rapid nuclear translocation of a Dd-GFP:STATa fusion protein in prespore cells surrounding the site of injection. This suggests that cAMP signals that emanate from the tip direct the localised nuclear accumulation of Dd-STATa. It also shows that prespore cells are competent to respond to cAMP, by Dd-STATa activation, and it implies that cAMP signalling is in some way limiting in the rear of the slug. Co-injection of a specific inhibitor of the cAR1 serpentine cAMP receptor almost completely prevents the cAMP-induced nuclear translocation, showing that most or all of the cAMP signal is transduced by cAR1. Dd-GFP:STATa also rapidly translocates into the nuclei of cells adjoining the front and back cut edges when a slug is bisected. Less severe mechanical disturbances, such as pricking the rear of a slug with an unfilled micropipette, also cause a more limited nuclear translocation of Dd-GFP:STATa. We propose that these signalling events form part of a repair mechanism that is activated when the migrating slug suffers mechanical damage.

Movies available on-line:
http://www.personal.dundee.ac.uk/~cjweijer/STATa_GFP.htm and
http://www.biologists.com/Development/movies/dev3344.html

Key words: *Dictyostelium*, cAMP, STAT, Damage, Repair

INTRODUCTION

Developing *Dictyostelium* amoebae gather together in response to pulsatile cAMP signals that emanate from the centre of an aggregation territory containing up to 100,000 cells. A hemispherical mound is formed and morphogenetic movement of the cells causes a nipple-shaped tip to emerge at its apex. The tipped mound then reshapes itself, to form a slug-like structure. The tip guides all morphogenetic movement, from the time of its formation at the tipped mound stage, and has also been shown to function as an organiser: when transplanted onto the side of a slug it brings the tissue more distal to the site of transplantation under its control and initiates the formation of a secondary axis (Raper, 1940; Rubin and Robertson, 1975). The tip also guides the photo- and thermotactic movement of the slug (Darcy et al., 1994).

Despite its central importance, the tip is poorly characterised. The front one-fifth of the slug is composed of prestalk cells and the prespore cells occupy the rear four-fifths. The tip is therefore composed of prestalk cells. However, there is continuous cell movement within the slug. Hence the tip could in principle be a constantly shifting prestalk cell subpopulation, definable only by transitory signalling properties. Alternatively, the tip could be composed of a discrete subpopulation of the prestalk cells, with a characteristic differentiated phenotype that endows them with their unique signalling properties. One way to distinguish these possibilities is to search for tip-specific markers. The prestalk region is made up of two parts. The rear two-thirds is composed of pstO cells and the front one-third is composed of pstA cells (Early et al., 1993). Within the pstA region there is a small subset of cells, the pstA* cells, defined by their ability to use a subregion of the promoter of the *cudA* gene (Fukuzawa and Williams, 2000). The pstA* cells are located at the tip of the slug and have at least one property expected of the tip cells: they regulate the extent of slug migration (Smith and Williams 1980; Fukuzawa and Williams, 2000).

Genetic and biochemical evidence (Fukuzawa and Williams, 2000) suggests that the transcription factor that activates *cudA* expression within the slug tip is Dd-STATa, a structural and functional homologue of the metazoan STAT (signal transducers and activators of transcription) proteins (Kawata et al., 1997). The STAT proteins migrate to the nucleus and regulate gene expression when activated by tyrosine phosphorylation (reviewed by Bromberg and Darnell, 2000). At the mound stage, Dd-STATa is nuclear localised in all cells but by the slug stage DdSTATa becomes nuclear enriched in a surface layer of pstA cells that is located in the tip (Araki et

al., 1998): the location expected for an activator of *cudA* gene transcription. Thus, the small group of anterior cells where *cudA* is expressed, and Dd-STATa is nuclear localised, are prime candidates to be the tip cells.

Tip function is not well understood but there is a body of evidence to suggest that it is a source of pulsatile cAMP signalling and that these signals control slug behaviour. Tips have been shown to release cAMP as detected by the attraction of aggregation competent cells (Bonner, 1949; Miura and Siegert, 2000). Optical density waves have been observed in the prespore zone of slugs and cell movement in the prespore zone has been shown to be periodic (Dormann et al., 1997; Weijer, 1999) and to be dependent on the presence of a tip (D. D. and C. J. W., unpublished). cAMP also acts as the extracellular signal that triggers tyrosine phosphorylation and nuclear translocation of Dd-STATa during aggregation (Araki, et al., 1998). Activation is very rapid and is mediated by cAR1, the major cAMP receptor that functions at that developmental stage. CAR1 is a serpentine receptor and such receptors normally couple to heterotrimeric G proteins (reviewed by Schnitzler et al., 1995). However, activation of Dd-STATa occurs by a G protein-independent pathway. As cAMP is the signal that directs nuclear translocation of Dd-STATa during aggregation we set out to determine whether cAMP plays the same role in the slug. We show that it does and we also demonstrate that mechanical disturbance of the slug causes nuclear translocation of Dd-STATa.

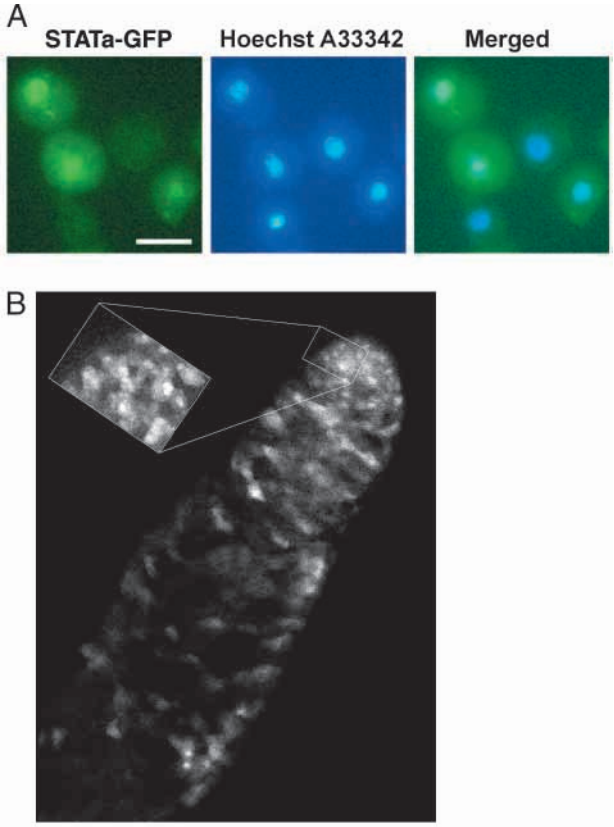

Fig. 1. (A) GFP:STATa translocation to the nucleus after cAMP stimulation. Cells expressing A15-GFP:STATa were washed once in KK2 buffer and resuspended to a density of 1×10^7 cells/ml. After 5 hours shaking in suspension, the cells were stimulated with 5 mM cAMP and methanol-fixed 5 minutes later. The DNA was subsequently detected by staining for 10 minutes with Hoechst 33342. (Left) GFP-fluorescence showing a number of brightly stained nuclei, indicating STATa-GFP accumulation; (middle) DNA staining of the same sample; (right) merged image showing the overlap in the nuclear staining patterns. Note that not all cells have responded to cAMP by nuclear translocation of GFP:STATa. (B) The nuclear accumulation pattern of GFP:STATa in the prestalk region of slugs transformed with A15-GFP:STATa. An A15-GFP:STATa transformed slug was viewed from below using a Leica DMRBE confocal microscope (TCS-NT). Nuclear localisation is almost entirely confined to the displayed region and the pattern resembles that of the endogenous Dd-STATa protein (see Fig. 8 and Araki et al., 1998). The image is a projection of a 24 section *z*-series. As the A15 promoter is constitutively active, this result shows that post-transcriptional regulation determines the nuclear localisation pattern. Scale bar in A: 10 μm for A.

MATERIALS AND METHODS

Construction of GFP:STATa

The Actin15-rsGFP/STATa fusion construct was created by inserting Red-shifted GFP (RSGFP), derived from pRSGFP-C1 (Clonetech), into an Actin15-STATa backbone as follows. RSGFP was amplified by PCR with one primer containing the 5′-*Bgl*II site adjacent to the 5′-end of the RSGFP-coding sequence, and the other containing the 3′-*Eco*RI site at the 3′-end of the RSGFP-coding sequence. The Actin15-STATa vector was cleaved with *Bgl*II/*Eco*RI and the RSGFP fragment was inserted. By this process, the N-terminal four amino acids of STATa (Met-Ser-Ser-Ala) and the C-terminal 26 amino acids of RSGFP were deleted. (The functionality of the fusion protein was tested by transforming STATa null mutant cells with this construct, and the construct successfully rescued the null mutant phenotype (data not shown).) The pspA-rsGFP/STATa construct was produced by excising the Actin15 promoter from Actin15-rsGFP/STATa, with *Xba*I/*Bgl*II, and replacing with the pspA promoter derived from pspA:NeoGal. (Traynor et al., 1992).

Preparation and manipulation of slugs

To induce development, cells were washed once in KK2 phosphate buffer (20 mM KH_2PO_4/K_2HPO_4, pH 6.8). After a wash in de-ionised water, the cell density was adjusted to ~2×10^8 cells/ml and small drops of 5 to 10 μl were deposited on 1% water agar plates (5 ml of 1% Difco-Bacto agar in deionised water in a petri dish with a diameter of 9 cm). After 30 minutes the supernatant was removed and the plates incubated in the dark at 22°C for 18 to 48 hours. For experiments on cAMP-containing agar, 300 μl drops of cell suspension were deposited on nitrocellulose filters (Millipore FHLC 02500, 2.5cm) laid on 1% agar plates and excess of supernatant was removed after 30 minutes. After 24 hours, the filters were transferred to agar containing 5 mM cAMP. After a further 5 minutes of incubation, the slugs were fixed in ice-cold 100% methanol. They were then passed through a rehydration series of 50% and 25% methanol in PBS, with 2 minutes incubation per step. The slugs, still attached to the filters, were then incubated with anti-STATa monoclonal antibody D4 at 4ºC for 10 hours (Araki, et al., 1998). After washing in PBS, they were incubated with pre-absorbed FITC-conjgated goat anti mouse IgG antibody (Sigma) at 22ºC for 4 hours. Finally, they were mounted in Citifluor AF1 (Citifluor, UK) and visualized in a LEICA DMRBE confocal microscope (model SP2). The images were processed using NIH-Image version 1.62.

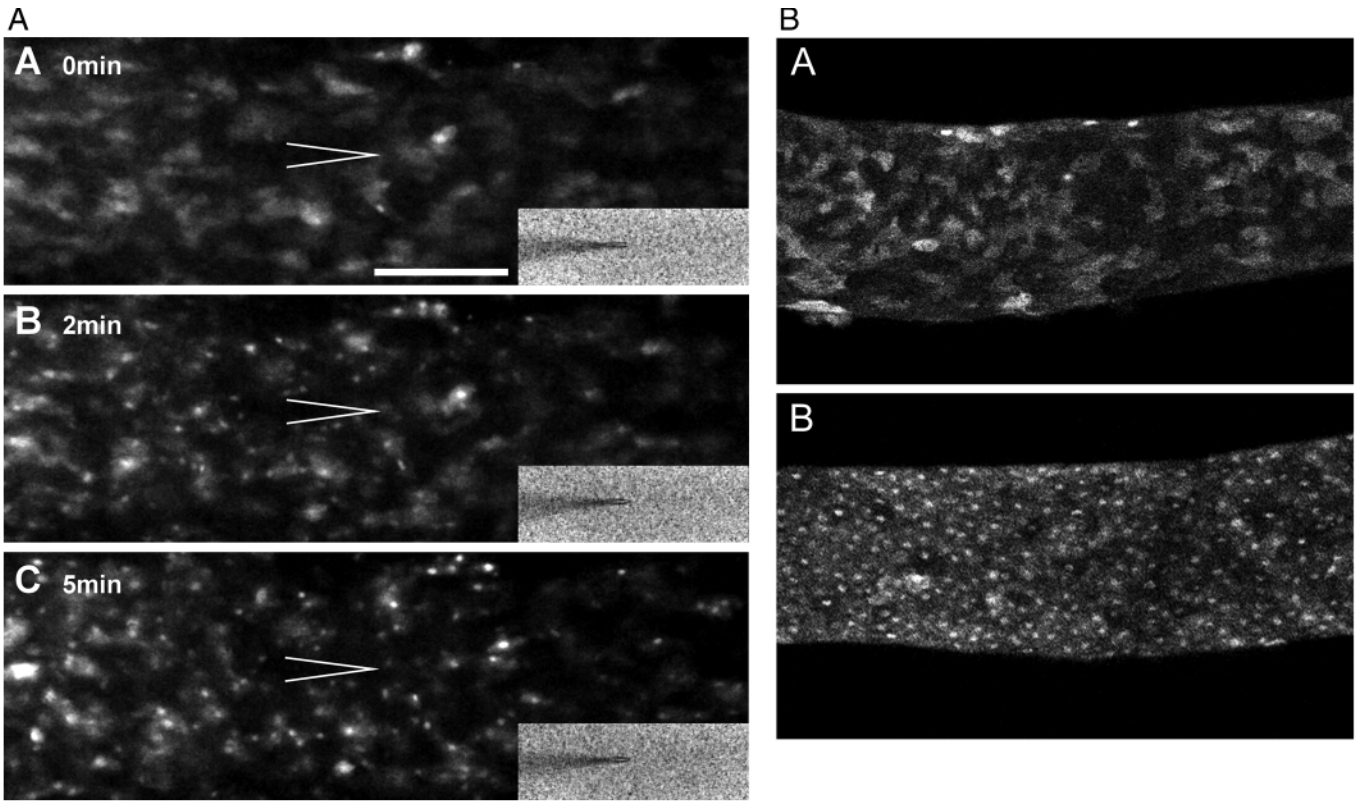

Fig. 2. (A) Induction of Dd-STATa nuclear translocation in the slug rear by injection of cAMP. The A15-GFP:STATa transformed slug was viewed from below through the agar. The micro-pipette containing 10^{-2}M cAMP was inserted from a dorsal position into the prespore region of the slug. The bright-field insets show the position of the pipette, the outline of the pipette tip is also indicated in the fluorescence images. cAMP was injected at time point 0 min (part A). After 2 minutes nuclear localization has markedly increased (part B), peaking about 5 minutes after the initial cAMP pulse (part C). A movie of this experiment can be seen at http://www.personal.dundee.ac.uk/~cjweijer/STATa_GFP.htm (B) Accumulation of nuclear Dd-STATa protein in the prespore zone of slugs transferred to cAMP-containing agar. Nitrocellulose filters bearing migrating slugs were transferred on to a water agar plate (part A), or an agar plate containing 5 mM cAMP (part B). After 5 minutes of incubation, the filters were fixed and the slugs stained as described in Materials and Methods. Both pictures shows the middle of the prespore zone and are the mid-sections of a confocal *z*-series. Scale bar in A: 50 μm.

Micro-injection

Microelectrodes were made from 1 mm Kwik-Fil glass capillaries (World Precision Instruments) using an electrode puller (Scientific & Research Instruments) and filled with a microliter syringe (Hamilton AG). Pressure injection was performed with a pneumatic pump (PV830, World Precision Instruments), which was computer controlled. The position of the micro-pipette was controlled with a mechanical joystick manipulator (Narishige, MN-151). The glass micropipette was also used in some experiments to cut the slug by making a rapid transverse cut across the width of the slug using the micromanipulator.

Videomicroscopy

Slugs were filmed through the agar on an inverted Axiovert 135 microscope (Zeiss; objective, F-32 LD 10×/NA 0.4) equipped with a cooled CCD camera (Hamamatsu, C4742-95). The slugs were submerged under silicon oil (BDH, Dow Corning 200/20cs) to reduce light scattering on their surfaces. The camera, a mechanical shutter for the brightfield illumination and a monochromator for the excitation of STATa-GFP fusion protein-labelled cells (TILL Photonics), were all controlled by Openlab software (version 2.3, Improvision) running on a Macintosh G3 PowerPC. Both fluorescence and brightfield images were taken every 10 seconds. At the end of the recording, images were saved in TIFF format and transferred to a PC for analysis with “Optimas” software (version 6.1, Media Cybernetics).

RESULTS

A Dd-GFP:STATa fusion protein is correctly nuclear localised

In order to monitor nuclear accumulation of Dd-STATa in vivo, the Dd-STATa protein was fused to GFP. An initial construct, where GFP was fused to the C terminus of Dd-STATa, was not nuclear enriched (T. Kawata and J. W., unpublished observations): probably because GFP sterically inhibits tyrosine phosphorylation, which occurs only 6 amino acids upstream of the C terminus. When GFP is fused to the N terminus of Dd-STATa (to yield GFP:STATa), and the fusion protein expressed under the control of the semi-constitutive Actin15 promoter (to yield A15-GFP:STATa), it displays nuclear enrichment (Fig. 1A). In this experiment we also confirmed that the GFP:STATa fusion protein mimics the behaviour of the endogenous protein, by showing that it migrates to the nucleus when cells developing in shaken suspension are treated with cAMP for 5 minutes (Fig. 1A). At the slug stage, the endogenous Dd-STATa protein is nuclear-enriched in a cortical layer of cells at the tip (Araki et al., 1998). The distribution is, however, somewhat variable from slug to slug. This probably reflects both the extensive cell movement within the prestalk region and the rapidity with which Dd-STATa moves in and out of the nucleus. Allowing for

this variability, GFP:STATa and the endogenous Dd-STATa protein show a similar spatial pattern of nuclear enrichment within the slug tip (Fig. 1B).

Injection of cAMP into the rear of the slug induces localised nuclear translocation of GFP:STATa

We determined the effect of injecting cAMP into the rear part of the slug, a region where there is normally very little nuclear enrichment of GFP:STATa (Fig. 2A, part A – this and all the other time-lapse film data can be downloaded from the site indicated in the appropriate figure legend). Injection was performed using a micropipette filled with cAMP dissolved in water. Concentrations of cAMP ranging from 10^{-4} M to 10^{-2} M were tested and the optimal response was obtained with cAMP at 5×10^{-3} to 10^{-2} M. Many cells in the vicinity of the micro-pipette display a transitory nuclear accumulation of GFP:STATa. Accumulation occurs over a similar time scale to that of aggregation competent cells (Araki et al., 1998). There is a detectable response within 2 minutes of cAMP addition (Fig. 2A, part B) and the response is quantitatively maximal 5 minutes after the initiation of cAMP signalling (Fig. 2A, part C). Later, about 5-10 minutes after the cAMP-containing micropipette is retracted, nuclear translocation levels revert to the pre-injection state (data not shown).

Dd-STATa is essential for normal gene expression at the slug stage and for correct culmination (Mohanty et al., 1999; Fukuzawa and Williams, 2000). It seemed possible, therefore, that overexpression of Dd-STAT could affect the results described above. Therefore, we performed a conceptually similar experiment, except that we used non-transformed cells. Slugs were exposed to cAMP by transferring them to agar that contained cAMP and the Dd-STATa protein was detected immunohistochemically. The fact that Dd-STATa protein translocates to the nuclei of cells in the prespore region in the presence of cAMP (Fig. 2B) shows that, in this respect at least, cells transformed with the pspA-GFP:STATa fusion gene behave identically to untransformed cells.

Nuclear translocation of Dd-STATa in response to cAMP occurs in prespore cells

The prespore region is composed of prespore cells, intermixed with a smaller number of anterior-like cells (ALC); scattered cells that show many of the properties of prestalk cells (Sternfeld and David, 1982). In order to determine whether prespore cells display nuclear enrichment after injection of cAMP, we fused the GFP:STATa gene to the *pspA* (Early and Williams 1989), prespore-specific promoter (to yield pspA-GFP:STATa). When cAMP is injected into a slug expressing pspA-GFP:STATa, nuclear translocation occurs in a large area surrounding the needle (Fig. 3). The response is significantly stronger and more uniform than with A15-GFP:STATa. This presumably reflects the fact that expression directed by the A15 promoter decreases during the later stages of development; i.e. there is more reporter protein per cell when the pspA promoter is used. Because translocation is more easily detected in pspA-GFP:STATa cells, we used this strain for all subsequent experiments.

In order to monitor the nuclear translocation at the single cell level, slugs expressing GF:STATa were transferred to a caffeine-containing substratum before injection. Caffeine acts to immobilise the cells and this facilitates the quantitation (it is very difficult to identify the weakly labeled nuclei in fast-moving slug cells, especially before cAMP stimulation). The images in Fig. 4A, and the fluorescence intensity plots in Fig. 4B, confirm that nuclear accumulation occurs over a time scale of about 5 minutes but there is a detectable rise in fluorescence just 1 to 2 minutes after the addition of cAMP. Comparison of

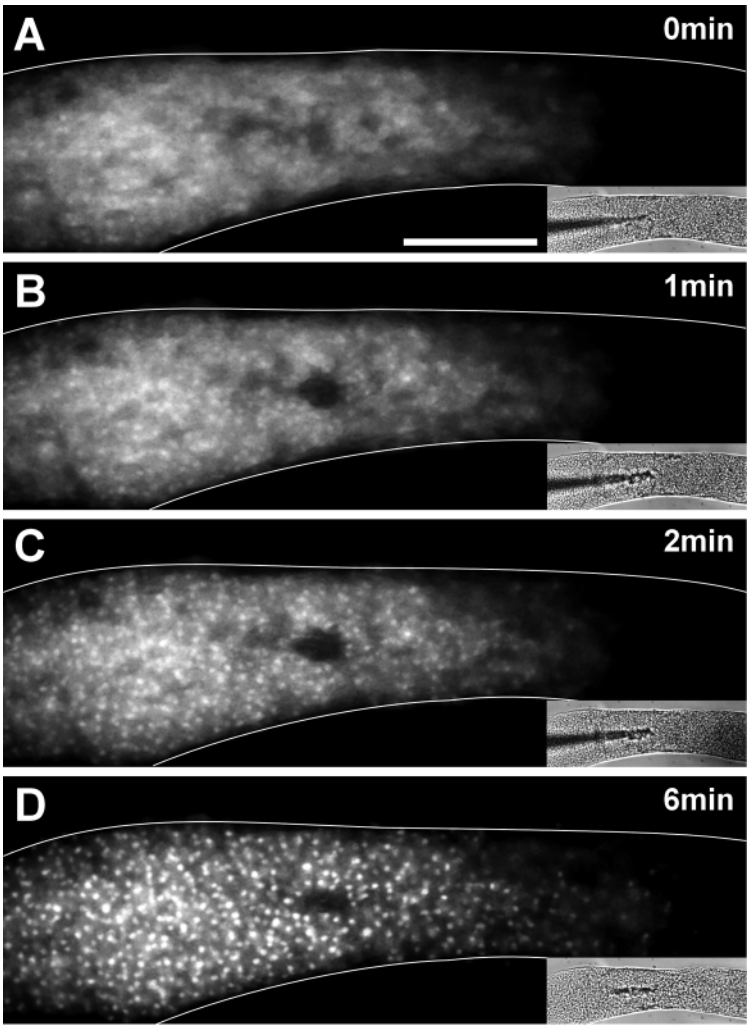

Fig. 3. Induction of Dd-STATa nuclear translocation by injection of cAMP in slugs expressing GFP:STATa under the control of the pspA promoter The outline of the slug in this sequence is indicated by the white lines. The slug is moving from left to right, the prestalk/prespore boundary is visible at the right-hand side of the fluorescence images, as only prespore cells express the GFP fusion protein. The insets show the corresponding bright-field images and the position of the micropipette. At the 0 min time point (A) a single cAMP pulse was applied (concentration in the micro-electrode: 10^{-2} M cAMP). After 1 minute the first signs of nuclear localization are visible (B), the brightness of the nuclei increases further (C) and peaks after about 6 minutes (D). The micropipette was removed after 4.5 minutes. Scale bar: 50 μm. A movie of this experiment can be seen at http://www.personal.dundee.ac.uk/~cjweijer/STATa_GFP.htm

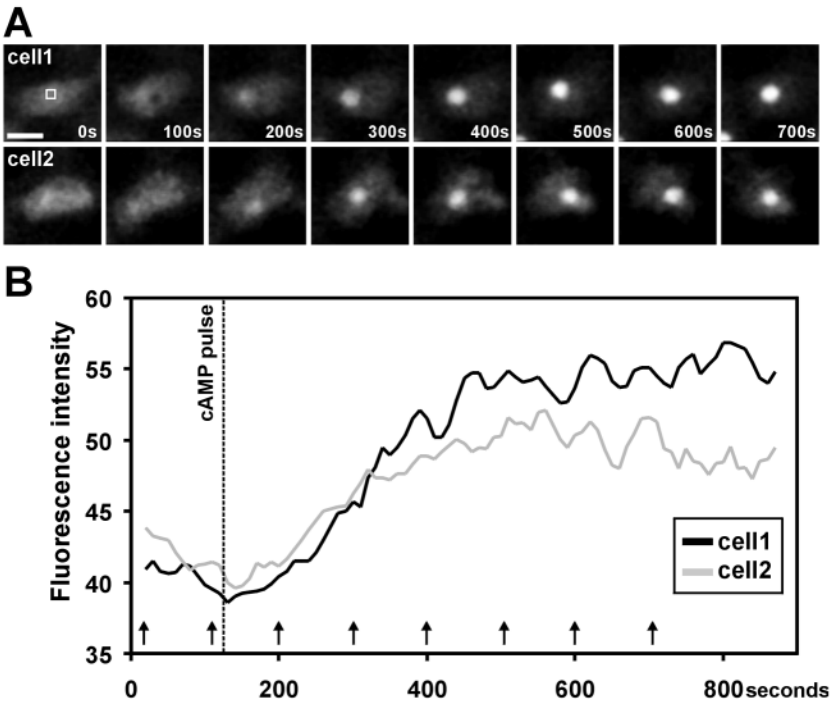

Fig. 4. Quantitative analysis of Dd-STATa nuclear translocation in prespore cells. A slug with cells expressing pspA-GFP:STATa was transferred to a water agar plate containing 5 mM caffeine using a fine syringe needle. Recording started 7 minutes after the transfer – this corresponds to the 0s time point in (A). After 120 seconds the micropipette was inserted and cAMP (10^{-2} M cAMP) was injected once. (A) Images of two cells at different time points. The white square over the nucleus of cell 1 indicates the 5×5 pixel measuring window, which was used to determine the average brightness of the nucleus. Following cAMP stimulation, GFP:STATa translocates rapidly to the nucleus depleting the cytoplasm of the fusion protein. (B) The change of brightness of the nuclei from the two cells in A over time. The curves were smoothed using a running average over three data points. The broken line marks the time of the cAMP injection, the arrows refer to the time points of the images shown in A. Scale bar in A: 5 μm. A movie of this experiment can be seen at http://www.personal.dundee.ac.uk/~cjweijer/STATa_GFP.htm

cells at different points in this time course (Fig. 4A) suggests there to be a simple re-distribution of the protein from cytoplasm to nucleus – i.e. the loss of cytoplasmic fluorescence seems to be matched by an approximately equivalent gain in nuclear signal. Thus, prespore cells are competent to respond to cAMP by the rapid translocation of GFP:STATa from the cytoplasm to the nucleus.

STATa nuclear translocation in prespore cells is mainly mediated by cAR1

cAR1 is the major cAMP receptor that is active during early development and there is no nuclear translocation of Dd-STATa in response to cAMP in a cAR1 null strain. The cAR1 null strain arrests development prior to aggregation so, in order to study cAR1 during multicellular development, we used the pharmacological agent IPA (2′,3′-*o*-isopropylidene-adenosine): an adenosine analogue, which specifically inhibits binding of cAMP to cAR1. There are three other cAMP receptors, which function later during development than cAR1, but they are insensitive to IPA. When cAMP alone is injected there is the expected widespread nuclear translocation of GFP:STATa (Fig. 5A). When IPA is co-injected with cAMP there is a large reduction

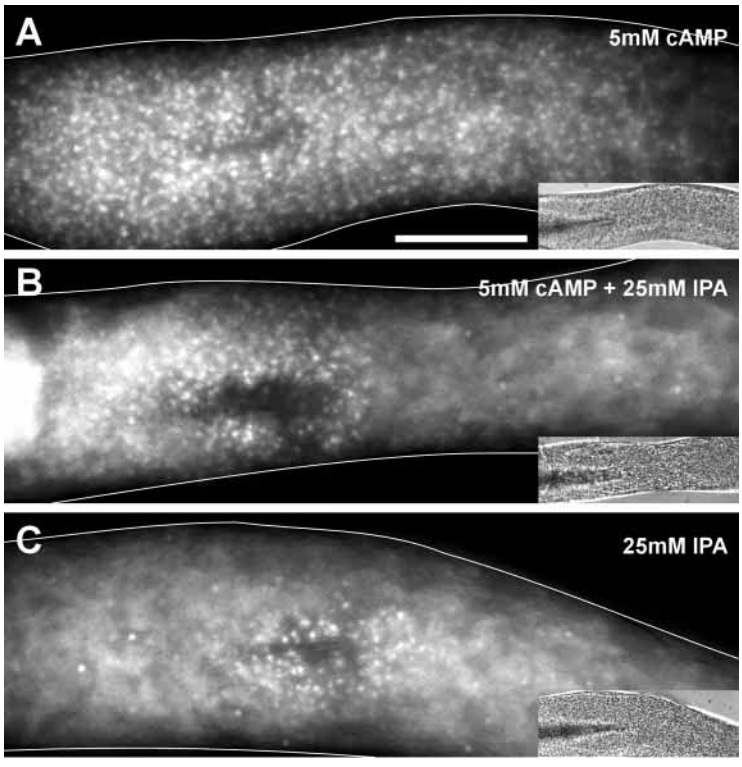

Fig. 5. Effect of the cAR1 inhibitor IPA on the cAMP-induced accumulation of GFP:STATa. GFP:STATa was expressed under the prespore specific pspA promotor. In all experiments shown the concentration of DMSO, which served as solvent for IPA, was kept at 25% (v/v), even when cAMP was injected without IPA. The images were taken four minutes after pulsing with the different compounds. (A) Injection of 5×10^{-3} M cAMP (concentration in micropipette) leads to a dramatic increase of nuclear localization in large parts of the prespore region. (B) Co-injection of the same amount of cAMP with a fivefold excess (over cAMP) of the cAR1 inhibitor IPA reduces the number of cells showing a positive response, and nuclear localization remains restricted to the cells directly surrounding the injection site. (C) Injection of the inhibitor IPA alone shows a very localized nuclear translocation similar to the effects induced by mechanical disturbance. Scale bar: 50 μm. A movie of this experiment can be seen at http://www.personal.dundee.ac.uk/~cjweijer/STATa_GFP.htm

in the extent of nuclear translocation (Fig. 5B). Thus, cAR1 is partially or wholly responsible for mediating the cAMP-induced translocation of GFP:STATa. We cannot determine whether cAR1 is entirely responsible for signal perception, because we found that insertion of a needle emitting IPA alone, or even of an unfilled micropipette, causes a limited GFP:STATa nuclear translocation (Fig. 6A,B). Thus, translocation of Dd-STATa can be triggered by mechanical disturbance.

Mechanical disturbance of the slug induces localised nuclear translocation of Dd-STATa

The nuclear translocation of GFP:STATa that occurs when an empty micropipette is inserted is tightly localised around the injection point (Fig. 6A,B), and the fluorescence over the nuclei is relatively weak, but the effect can be amplified if a bubble of nitrogen is injected (Fig. 6C,D). The bubble exerts hydrostatic pressure on the cells and, consistent with this causing the effect, the cells displaying nuclear translocation encircle the drop. In nature, the mechanical damage most likely to occur is to slug integrity (see Discussion). We therefore determined the effect of the complete bisection of the slug. This results in strong nuclear accumulation of GFP:STATa in cells that border the front and back cut edges (Fig. 7). Nuclear translocation is detectable within two just minutes of bisection.

Nuclear localisation of Dd-STATa in response to mechanical damage was a novel and unexpected finding and we felt it important to confirm that the endogenous Dd-STATa protein behaves similarly to GFP:STATa. This was of course only possible by fixation and staining of different slugs, one bisected and the other left unmanipulated, except for transfer to fixative and staining. There is very strong nuclear staining in slug halves fixed and stained for Dd-STATa just 3 minutes after bisection (Fig. 8), confirming the results obtained with GFP:STATa. (The area over which translocation of the endogenous Dd-STATa protein occurs was, reproducibly, larger than in the GFP:STATa bisection experiments, which may reflect differences in the protocol used for cutting – the fact that the GFP:STATa experiments were performed under oil and that the GFP:STATa samples could be directly observed, without the need for further manipulation.)

DISCUSSION

The Dd-STATa protein is strongly nuclear localised in cells in the slug tip while prespore cells show only a low level of immunostaining, which is approximately equivalent in the cytoplasm and the nucleus. Similarly, when the GFP:STATa protein is ectopically expressed, from either the actin15 or the pspA promoter, only a very few cells within the prespore region show any sign of nuclear enrichment. Earlier, at the tight aggregate stage however, Dd-STATa is strongly nuclear enriched in all cells. Two alternative explanations for these observations are: (1) those cells that become prespore cells lose the signalling competence needed for translocation of Dd-STATa, or (2) extracellular signalling changes during slug formation, such that only cells within the slug tip perceive the signal. As cAMP is the signalling molecule that induces nuclear translocation during aggregation, and because of the evidence that the slug tip is likely to be a source of cAMP signalling, we determined the effect of exposing prespore cells to cAMP. The result was a rapid and robust translocation of Dd-STATa to the nucleus. Thus, prespore cells definitely retain

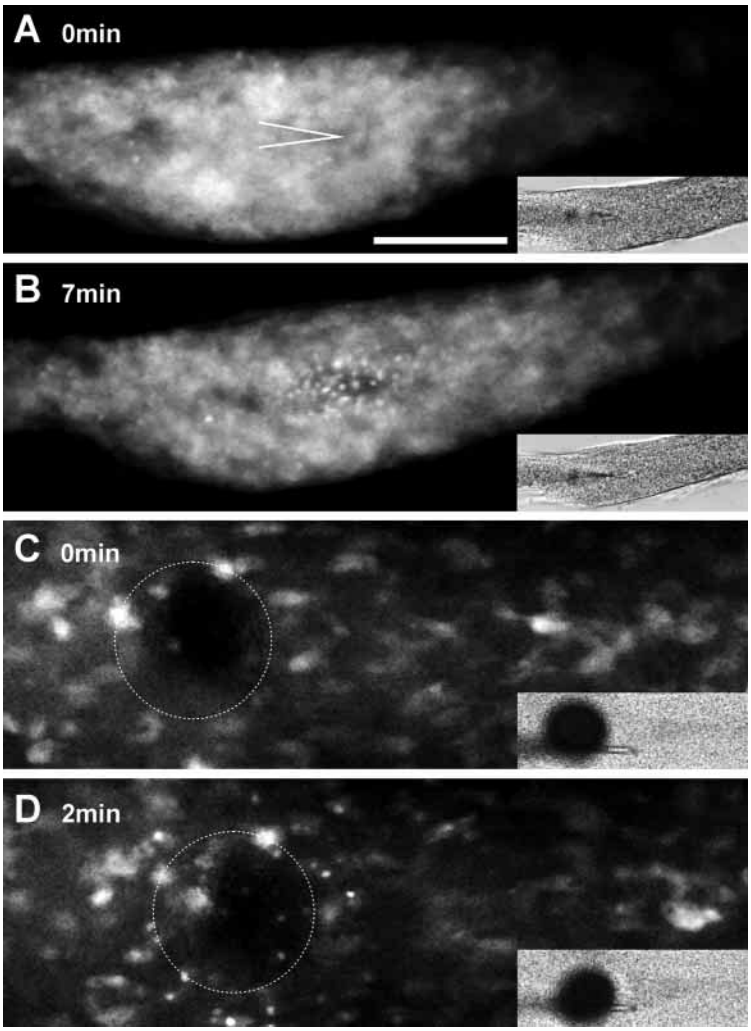

Fig. 6. Accumulation of nuclear GFP:STATa protein in response to mechanical stimulation. (A,B) Accumulation of GFP:STATa in nuclei of cells surrounding an empty micropipette. The expression of GFP:STATa is driven by the pspA promoter. The position of the pipette tip is indicated by the white arrow head in the fluorescence image. (A) Image taken seconds after insertion of the empty micropipette. (B) After a few minutes, there is a clear nuclear translocation in cells surrounding the tip. (C,D) Accumulation of nuclear A15-GFP:STATa protein in slugs injected with a bubble of nitrogen. An empty glass pipette was inserted into the slug and N_2 gas, which was used to drive the pressure injection, was directly injected into the slug. (C) Prespore zone of the slug seconds after the pulse, the bubble is visible as a black circle in the brightfield image (inset in bottom right corner). The outline of the N_2 bubble is indicated by a broken white line in the fluorescence image. (D) Two minutes after injection the nuclear translocation of GFP:STATa is clearly visible in cells surrounding the bubble. Scale bar: 50 μm. A movie of this experiment can be seen at http://www.personal.dundee.ac.uk/~cjweijer/STATa_GFP.htm

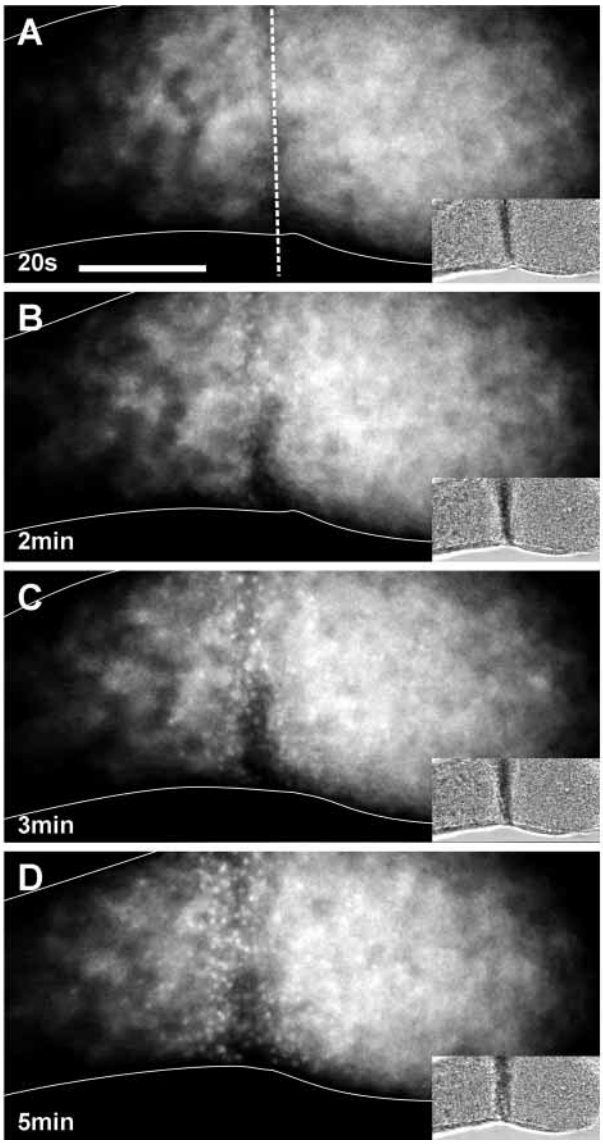

Fig. 7. Accumulation of nuclear GFP:STATa protein in bisected slugs. A cut was made through the prespore region of the slug with the tip of an empty glass pipette using the micromanipulator. The broken line in A indicates the position of the cut, which is also recognizable in the brightfield insets. The outline of the slug is marked by white lines; the slug moves from right to left. After 2 minutes (B) the first nuclei become discernible around the cutting site, translocation increases further (C) and peaks at about 5 minutes (D). The nuclear translocation is selectively localised to the cells surrounding the cutting site. Scale bar: 50 μm. A movie of this experiment can be seen at http://www.personal.dundee.ac.uk/~cjweijer/STATa_GFP.htm

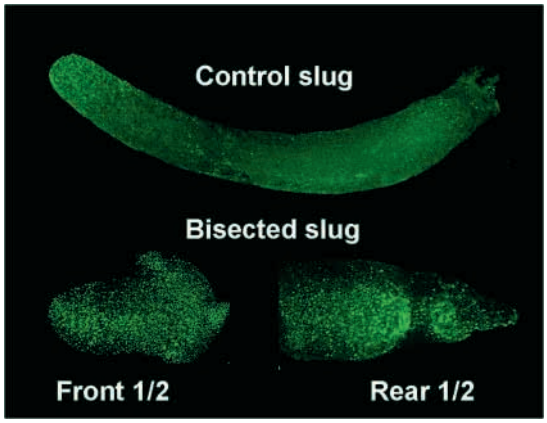

Fig. 8. Accumulation of nuclear Dd-STATa protein in bisected slugs. A slug migrating on water agar was bisected, the halves were pushed apart and three minutes later the separated parts were picked up, fixed and stained for Dd-STATa, exactly as described previously (Araki et al., 1998). The control slug was treated similarly, except that it was not bissected. The samples were visualised by confocal microscopy as described in the legend to Fig. 1. The image is a projection of a 24 section *z*-series.

competence to translocate Dd-STATa to the nucleus in response to cAMP.

The fact that prespore cells are competent to respond to cAMP but only do so when cAMP is micro-injected is surprising, because prespore-specific gene expression requires extracellular cAMP signalling, both when assayed using cells in suspension (Mehdy et al., 1983; Schaap and van Driel, 1985; Gomer, et al., 1986) and when cAMP levels are enzymatically manipulated in the intact slug (Wang, et al., 1988). The simplest explanations for this apparent paradox are that

(1) cAMP levels in the prespore region are below the crucical threshold needed for translocation of Dd-STATa. If Dd-STATa is indeed nuclear enriched within the slug tip because extracellular cAMP levels are high there, then GFP:STATa would seem to be acting as a sensor, monitoring the perceived extracellular cAMP levels in different parts of the slug.

(2) The translocation process adapts to the cAMP level in the prespore region. One reason for this could be that cAMP is only relayed by the anterior like cells in the prespore zone (Bretschneider et al, 1995), as this would be expected to result in a large reduction of the amplitude of the cAMP waves.

(3) There are quantitative differences in sensitivity to cAMP. We cannot readily control the perceived cAMP concentration in our experiments, hence we cannot exclude the possibility that the prespore cells have a lower sensitivity to cAMP than the prestalk cells (i.e. we may be over-coming a lowered sensitivity of the prespore cells by exposing them to a very high concentration of cAMP). The important point remains, however, that the prespore cells do fully retain the capacity to undertake nuclear translocation of Dd-STATa when exposed to high cAMP levels.

GFP:STATa also rapidly translocates to the nucleus when the integrity of the slug is disrupted: by insertion of a needle or, more traumatically, by the surgical bisection of the entire slug. Translocation is centred around the point of damage. The area displaying translocation increases with the extent of the injury but is not completely inhibited by injecting IPA, a cAR1 inhibitor. This suggests either that mechanical damage works in part by a cAR1-independent mechanism or that cAR1 is used, but in a ligand-independent manner. It could also be that some part of the signal is transduced by another cAMP receptor, such as cAR3.

Why should a second mechanism exist? In the conditions used to study *Dictyostelium* development in the laboratory

bisection is not likely to occur. However, time lapse films of slugs migrating through soil show that the bridging of soil particles often leads to slug breakage. Given sufficient time for re-proportioning, each of the two fragments generated goes on to form a normally proportioned fruiting body. In nature, therefore, some mechanism of damage repair seems likely to be essential. Damage-induced Dd-STATa translocation may form part of this response. Dd-STATa acts as a repressor for ecmB transcription and as an activator for cudA transcription (Mohanty et al., 1999; Fukuzawa and Williams, 2000) and it could also re-program gene expression in cells around the cut point. However, it is not essential for cell type regulation, because bisection of a Dd-STATa null slug yields two normally proportioned fruiting bodies over an approximately normal time scale (Abe, T. and Williams, J. G., unpublished observations). Perhaps there are other transcriptional regulators, redundant with Dd-STATa, that respond to the same damage pathway.

This work was supported by Wellcome Trust Program Grants to J. G. W. and C. J. W.

REFERENCES

Araki, T., Gamper, M., Early, A., Fukuzawa, M., Abe, T., Kawata, T., Kim, E., Firtel, R. A. and Williams, J. G. (1998). Developmentally and spatially regulated activation of a Dictyostelium STAT protein by a serpentine receptor. *EMBO J.* **17**, 4018-4028.

Bonner, J. T. (1949). The demonstration of acrasin in the later stages of the development of the slime mold Dictyostelium discoideum. *J. Exp. Zool.* **110**, 259-271.

Bretschneider, T., Siegert, F. and Weijer, C. J. (1995). Three-dimensional scroll waves of cAMP could direct cell movement and gene expression in Dictyostelium slugs. *Proc. Natl. Acad. Sci. USA* **92**, 4387-4391.

Bromberg, J. and Darnell, J. E., Jr (2000). The role of STATs in transcriptional control and their impact on cellular function. *Oncogene* **19**, 2468-2473.

Darcy, P. K., Wilczynska, Z. and Fisher, P. R. (1994). Genetic analysis of Dictyostelium slug phototaxis mutants. *Genetics* **137**, 977-985.

Dormann, D., Weijer, C. and Siegert, F. (1997). Twisted scroll waves organize Dictyostelium mucoroides slugs. *J. Cell Sci.* **110**, 1831-1837.

Early, A. E., Gaskell, M. J., Traynor, D. and Williams, J. G. (1993). Two distinct populations of prestalk cells within the tip of the migratory Dictyostelium slug with differing fates at culmination. *Development* **118**, 353-362.

Early, A. E. and Williams, J. G. (1989). Identification of sequences regulating the transcription of a Dictyostelium gene selectively expressed in prespore cells. *Nucleic Acids Res.* **17**, 6473-6484.

Fukuzawa, M. and Williams, J. G. (2000) Analysis of the promoter of the cudA gene reveals novel mechanisms of Dictyostelium cell type differentiation. *Development* **127**, 2705-2713

Gomer, R. H., Armstrong, D., Leichtling, B. H. and Firtel, R. A. (1986). cAMP induction of prespore and prestalk gene expression in Dictyostelium is mediated by the cell-surface cAMP receptor. *Proc. Natl. Acad. Sci. USA* **83**, 8624-8628.

Kawata, T., Shevchenko, A., Fukuzawa, M., Jermyn, K. A., Totty, N. F., Zhukovskaya, N. V., Sterling, A. E., Mann, M. and Williams, J. G. (1997). SH2 signaling in a lower eukaryote: A STAT protein that regulates stalk cell differentiation in Dictyostelium. *Cell* **89**, 909-916.

Mehdy, M. C., Ratner, D. and Firtel, R. A. (1983). Induction and modulation of cell-type specific gene expression in Dictyostelium. *Cell* **32**, 763-771.

Miura, K. and Siegert, F. (2000). Light affects cAMP signaling and cell movement activity in Dictyostelium discoideum. *Proc. Natl. Acad. Sci. USA* **97**, 2111-2116.

Mohanty, S., Jermyn, K. A., Early, A., Kawata, T., Aubry, L., Ceccarelli, A., Schaap, P., Williams, J. G. and Firtel, R. A. (1999) Evidence that the Dictyostelium Dd-STATa protein is a repressor that regulates commitment to stalk cell differentiation. *Development* **126**, 3391-3405.

Raper, K. B. (1940). Pseudoplasmodium formation and organization in Dictyostelium discoideum. *J. Elisha Mitchell Sci. Soc.* **56**, 241-282.

Rubin, J. and Robertson, A. (1975). The tip of Dictyostelium discoideum pseudoplasmodium as an organizer. *J. Embryol. Exp. Morphol.* **33**, 227-241.

Schaap, P. and van Driel, R. (1985). Induction of post-aggregative differentiation in Dictyostelium discoideum by cAMP. Evidence for the involvement of the cell surface cAMP receptor. *Exp. Cell Res.* **159**, 388-398.

Schnitzler, G. Briscoe, R., C., Brown, J. M. and Firtel, R. A. (1995). Serpentine cAMP receptors may act through a G protein-independent pathway to induce postaggregative development in Dictyostelium. *Cell* **81**, 737-745.

Smith, E. and Williams, K. (1980). Evidence for tip control of the “slug/fruit” switch in slugs of Dictyostelium discoideum. *J. Embryol. Exp. Morphol.* **57**, 233-240.

Sternfeld, J. and David, C. N. (1982). Fate and regulation of anterior-like cells in Dictyostelium slugs. *Dev. Biol.* **93**, 111-118.

Wang, M.,Van Driel, R. and Schaap, P. (1988). Cyclic AMP-phosphodiesterase induces dedifferentiation of prespore cells in Dictyostelium discoideum slugs: evidence that cyclic AMP is the morphogenetic signal for prespore differentiation. *Development* **103**, 611-618.

Traynor, D., Kessin, R. and Williams, J. G. (1992) Chemotactic sorting to cAMP in the multicellular stages of *Dictyostelium* development. *Proc. Natl. Acad. Sci. USA* **89**, 8303-8307.

Weijer, C. J. (1999). Morphogenetic cell movement in Dictyostelium. *Semin. Cell Dev. Biol.* **10**, 609-619.