Behavioral responses of streamer F mutants of *Dictyostelium discoideum*: effects of cyclic GMP on cell motility

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Summary

Streamer F (stmF) mutants have a prolonged increase in intracellular cGMP in response to addition of the chemoattractant cAMP. The speed of movement and area of stmF cells were quantitated as the cells were stimulated with a rapid, uniform increase in extracellular cAMP. The speed of stmF cells rapidly drops as does that of the wild-type, but then requires about 300 seconds to recover. In contrast, the speed of the parental strain, XP55, recovers within 60-70 seconds. This prolonged drop in speed correlates with the time during which intracellular cGMP remains high, suggesting that intracellular cGMP induces this prolonged reduction in speed. Mutants from other streamer complementation groups do not show this altered response. Area measurements indicate that stmF cells do not cringe or round up as XP55 does, but spread with the same kinetics as XP55. Chemotactic orientation of the stmF cells in stable spatial gradients is similar to or slightly greater than that of the wild-type. Tracking of cells moving during aggregation indicates that the stmF cells show large drops in speed between pulses, resulting in the banding pattern seen in streams. The cells can still respond to new pulses, resulting in an aggregation time that is similar to that of XP55.

Key words: cyclic GMP, chemotaxis, motility, *Dictyostelium*.

Introduction

*Dictyostelium discoideum* amoebae provide an important model system for the study of amoeboid chemotaxis and motility (for reviews, see Segall, 1990; van Haastert, 1991). In the growth phase, they grow and move as isolated amoebae, and are chemotactic to folate. Upon removal of food sources, the amoebae undergo a developmental cycle that involves the coordinated movement and morphogenesis of thousands of amoebae and ends in the formation of a fruiting body and spores (Loomis, 1975, 1982). The first stage in this cycle is the formation of an aggregate from which the future fruiting body will form. The aggregate is built by the oriented movement of the amoebae towards a common center. The orientation is imposed by waves of extracellular cAMP, initiated at the center and then relayed outwards by the amoebae as they move chemotactically towards the center (Alcantara and Monk, 1974; Tomchik and Devreotes, 1981). The chemotactic response to cAMP is initiated by cAMP binding to a cell-surface receptor (Klein et al., 1988) that is coupled to G proteins (Kumagai et al., 1989; Pupillo et al., 1989). Addition of cAMP to *Dictyostelium* amoebae generates a number of responses. These include increases in inositol phosphates (Europe-Finner and Newell, 1987; van Haastert et al., 1989) and intracellular cGMP (Mato et al., 1977; Wurster et al., 1977), changes in fluxes of ions such as calcium (Abe et al., 1988; Bumann et al., 1984), potassium (Aeckerle et al., 1985) and hydrogen (Aerts et al., 1987; Bumann et al., 1986), changes in the amount of filamentous actin (Hall et al., 1988; McRobbie and Newell, 1983), changes in the phosphorylation of myosin heavy and light chains (Berlot et al., 1985), and changes in cell movement and spreading (Segall, 1988; Varnum-Finney et al., 1988).

In order to understand the mechanisms underlying the process of aggregation, mutants have been isolated that are altered in aggregation properties (for review, see Kessin, 1988). Cells from several different complementation groups form long aggregation streams when grown as colonies on bacteria, and are termed streamer mutants (Ross and Newell, 1979). Cells of one complementation group of these streamer mutants, the streamer F (stmF) complementation group, are defective in a cGMP-specific phosphodiesterase (Coukell and Cameron, 1986; Ross and Newell, 1981; van Haastert et al., 1982). Normally, addition of extracellular cAMP leads to an increase in intracellular cGMP, which peaks 10 seconds after stimulation with cAMP and returns to prestimulus levels by 30 seconds. Mutants of the stmF complementation group show increases in cGMP that remain high for more than 60 seconds. The association of myosin II heavy chain with the Triton X-100
cytoskeleton is also prolonged in these cells (Liu and Newell, 1988), as are the influx of calcium ions (Menz et al., 1991) and changes in membrane potential (van Duijn and Wang, 1990).

To determine possible roles cGMP might play in modifying the movement of Dictyostelium amoebae, this paper describes experiments analyzing the behavior of the *stmf* mutants. It compares changes in cell speed and chemotaxis of *stmf* mutants and the parental strain in response to cAMP stimuli.

**Materials and methods**

**Strains and cultures**

All strains described in this work were kindly provided by Peter Newell. Strains were kept on SM agar plates and passaged by streak cloning once per week. Once per month fresh strains were started from stocks kept as spores on silica gel. SM agar plates contained 15 g Bacto-agar (Difco), 10 g neutralized Bacto-heatmap (Oxoid), 1 g yeast extract, 10 g glucose, 1 g MgSO₄, 2.2 g KH₂PO₄, 1.3 g K₂HPO₄ in 1 liter of water. Amoebae were grown as cocultures with *Klebsiella aerogenes* bacteria on SM agar plates. For most experiments, 10⁵ amoebae were plated with bacteria on SM agar plates. The plates were then incubated at 22°C for 48 hours, until the bacterial lawn began to clear. The amoebae were harvested with 17 mM Sorensen’s phosphate buffer, pH 6.2 (termed phosphate buffer), and washed free of bacteria by 3 cycles of centrifugation at 300 g for 2 minutes followed by resuspension of the pellet in phosphate buffer. For starvation in suspension, the cells were shaken at a density of 1.5 × 10⁷ cells per ml at 175 revs/min for 8 hours. For studies of cells aggregating on plates, 0.4 ml of 1.5 × 10⁷ cells per ml were plated on a plate containing 2.5 mM MgCl₂ (or 1 mM MgCl₂, 1 mM CaCl₂), phosphate buffer, and 1% Bacto-agar, and the excess liquid was wicked off after allowing the cells to settle for 10 minutes. Liquid remaining on the surface was air dried in a laminar flow hood.

**Studies of cell movement and area in a flow cell**

Coverslips (22 mm × 40 mm) were dipped in 0.1 M HCl, rinsed in distilled water and allowed to dry. Just before cells were applied, the coverslips were coated with a 2 mg/ml BSA (Sigma) solution in Ca/Mg phosphate buffer (1 mM CaCl₂, 1 mM MgCl₂ in phosphate buffer). Cells starved in suspension for 8 hours were diluted to around 10⁵ per ml in Ca/Mg phosphate buffer, and 0.1 ml was placed on the coverslip. The coverslip was attached to the bottom of a flow cell (Segall, 1988), and buffer was pulled through the chamber at a rate of 3-5 μl/min using an LKB Varioperpex II peristaltic pump. A Rheodyne valve (no. 5302) was used to switch between solutions. A time lag between switching solutions and the arrival of the new solution at the flow chamber was subtracted from all data.

The cells were observed with a Nikon Diaphot inverted microscope with a x20 objective using bright-field optics and a stopped-down aperture diaphragm. The image was analyzed using a MotionAnalysis (Santa Rosa, CA) digitizing system and Expertvision and DMS (Soll, 1986) software. In brief, an NEC TI-23A CCD camera was attached to the side port of the microscope and its output fed to a Panasonic AG6720 time-lapse S-VHS VCR and the Motionanalysis VP110 digitizer. Typically, experiments were recorded at a time-lapse rate of 1:12.7. For analysis, the videotape was replayed at normal speed and the output fed to the VP110. The edges of the cells in the image were determined by the VP110 at a speed of 2 Hz (net frame rate 1 per 6.35 seconds) and stored in an Amdek 386 personal computer. This information was then used by the Expertvision software to determine the cell centroid, speed of centroid movement, and direction of movement. For experiments in which cell area was measured, the DMS software was used to determine cell area, and speed of centroid movement.

Preliminary studies with XP55 and NP368 indicated several differences from previous work with AX2. First, AX2 cells had displayed a brief increase in cell motility upon addition of folate or cAMP followed by a longer lasting decrease in motility (Segall, 1988). The increase in motility was not seen with XP55 cells or mutants derived from XP55 in this study. This appears to be due to the differences in image processing systems, since preliminary studies of folate-induced responses by NP368 and XP55 using the system described (Segall, 1988) had shown the increase in motility (data not shown). Second, the speed of movement of AX2 was unaffected by the continued presence of low concentrations (10⁻⁷ M) of cAMP in the flow cell. The speed of movement of XP55 and NP368 cells, on the other hand, was sometimes relatively low (3-5 μm/min) in the absence of cAMP in the flow cell. The continued presence of 2 × 10⁻⁹ M cAMP raised the average speed of movement to 7-10 μm/min. Higher concentrations had little further effect. Therefore, for the data reported in this paper, at least 2 × 10⁻⁹ M cAMP was present in all the solutions to reduce the variability in prestimulus speed of movement of cells. The only effect this appeared to have was on the amplitude of the speed of movement - the time course of the changes in speed seen in response to stimulation with higher concentrations of cAMP was unaffected.

**Measurement of intracellular cGMP**

Cells were starved for 8 hours, washed once in phosphate buffer and then resuspended at 2 × 10⁷ to 4 × 10⁷/ml. The suspension was aerated at 22°C for 10 minutes before stimulation. Samples (100 μl) were removed, added to 100 μl of 3.5% HClO₄, and frozen. For measuring cGMP content, the samples were neutralized with KHCO₃, centrifuged, and the supernatants assayed for cGMP using an Amersham radioimmunoassay kit.

**Chemotactic orientation in spatial gradients**

Cells starved for 8 hours in suspension were diluted to 10⁶ per ml and allowed to settle onto 22 mm × 40 mm coverslips precoated with 2 mg/ml BSA in Ca/Mg phosphate buffer. The coverslips were then placed on Zigmond chambers (Zigmond, 1977; Neuroprobe, Cabin John, MD) and the side troughs were filled with buffer. The chamber was placed on the stage of the inverted microscope, and evaporation from the troughs was reduced by enclosing the chamber with the inverted bottom of a 10 cm diameter Petri dish humidified by a moist filter. Cells were followed for half an hour under these conditions, and then one trough was refilled with buffer, while the other was refilled with buffer containing cAMP. Cell movement was tracked using the Motionanalysis system as described above using a digitizing rate of 1 Hz and analyzed using the Expertvision software. To reduce the effects of digitizing errors on directional measurements, every fifth frame was used for analysis, resulting in a net frame rate of 1 per 65 seconds. The gradient was aligned at 90° relative to the camera coordinate system. Orientation was calculated as the fraction of cell movement in the direction of the gradient (the mean sine of the direction of movement between successive frames). Turning rate was calculated as the rate of change of the direction of cell movement.
Analysis of movement during aggregation

For fluorescent labeling of cells, a method provided by K. Weijer was used. Cells were suspended at $6 \times 10^7$/ml in 10 mg/ml rhodamine-labelled dextran (Sigma, $M_r$ 10,000) in phosphate buffer. A 200 μl sample of this suspension was placed in a 1.5 ml Eppendorf tube and then on ice for 5 minutes. The cells were then sheared by drawing and expelling the solution using a 200 μl Gilson Pipetman. The shear force was increased by placing the tip of the Pipetman against the bottom of the tube during this procedure and repeating the process 40 times. After 5 minutes on ice, 0.1 M CaCl$_2$ was added, and after 5 more minutes on ice, the cells were washed 3 times with phosphate buffer and $6 \times 10^6$ cells were plated in the center of a Ca/Mg phosphate buffer, 1% Bacto-agar plate (final density $6 \times 10^5$ cells/cm$^2$). To make the plate thin enough for observation using an inverted microscope, 3 ml of the agar solution was poured into a 60 mm diameter Petri dish (Falcon 1007). At various times during aggregation, the Petri dishes were observed using a Nikon Diaphot inverted microscope equipped with a BioRad confocal scanning system. The movement of the rhodamine-labeled cells was recorded in time-lapse using the S-VHS recorder with the confocal microscope scanning the field once every 10 seconds using a x20 objective and internal magnification of x2. The tape was played back into the Motionanalysis system and speed of cell movement was calculated using the Expertvision software.

For hand-tracking of unlabeled cells, cells were starved on plates at either 22°C or 7°C. Cell movement was recorded in time-lapse with the S-VHS VCR using a long working distance x40 objective. The recording was played back using a ForA FA300 time base corrector into an Image 1/AT image processor installed in a Dell 310 computer with a Sony monitor. The nuclei of individual cells were tracked using a mouse and the track points feature of the 1/AT. The x,y and time points were stored and used to calculate the speed of movement of the nuclei (and thus the speed of movement of the cells) as a function of time.

For ensemble averaging the speed of nuclei of cells after starvation at 7°C, speed records of individual cells were aligned for averaging using a program written in BASIC as follows. First the optimum frequency and phase of oscillation of cell speed were determined by cross-correlating the speed as a function of time with sine waves of frequencies 150 to 500 Hz in steps of 10 Hz and determining the frequency and phase for which the cross-correlation was maximum. The phase information was used to objectively align all the speed traces to the first zero crossing with positive slope and then the traces were averaged.

Results

Changes in speed in response to sudden increases in cAMP

Cells were starved in suspension for eight hours, and allowed to attach to a coverslip, which was then placed in a flow cell (Segall, 1988). When the solution flowing through the flow cell was switched to buffer containing $5 \times 10^{-5}$ M cAMP, the speed of the parental strain, XP55, dropped rapidly, reaching a minimum of 14 seconds after addition of cAMP (Fig. 1A). The speed then recovered within one minute in the continued presence of cAMP. When NP368 cells containing the $s t m F406$ mutation were exposed to the same stimulus, the speed dropped rapidly, but then required four to five minutes before returning to prestimulus values (Fig. 2A).

The altered kinetics of adaptation of the speed to the addition of cAMP were similar to the changes in intracellular cGMP induced by cAMP (Ross and Newell, 1981). In XP55, intracellular cGMP peaked about 10 seconds after addition of cAMP and had returned to near baseline by 30 seconds (Fig. 1B). In NP368, intracellular cGMP increased to a higher level and remained above baseline for three to four minutes (Fig. 2B). The prolonged presence of increased intracellular cGMP appears to be due to the loss of a cGMP-specific phosphodiesterase (Coukell and Cameron, 1986; van Haastert et al., 1982).
Fig. 2. Responses of starved NP368 cells to 50 nM cAMP. (A) Changes in cell speed. Cells crawling on a coverslip in a flow cell were tracked as described in Materials and methods. At 1 minute, the concentration of cAMP in the buffer flowing through the flow cell was switched from 2 nM to 50 nM. The data are the averaged speeds of 43 cells in 8 upshifts performed on 2 different preparations. (B) Changes in intracellular cGMP. Cells were suspended at $2 \times 10^7$ to $4 \times 10^7$ cells/ml and aerated for 10 minutes. A sample was taken 1 minute before stimulation (and plotted at 0 and 1 minute). The cells were stimulated with 50 nM cAMP at 1 minute and samples were taken at various times after stimulation. The data are the average of 4 experiments performed on 4 different days.

To confirm that the alteration in cell speed in response to the addition of cAMP was due to the decreased cGMP phosphodiesterase activity characteristic of $stmF$ mutations, four other mutants with a streamer phenotype were tested (Fig. 3). Only the $stmF$ allele NP377 showed a prolonged decrease in speed similar to that seen in the $stmF$ mutant NP368. Streamer mutants from other complementation groups that show normal cGMP responses, including NP371 ($stmB$), NP370 ($stmD$) and NP387($stmE$), did not show this alteration.

Experiments using folate to stimulate growth-phase cells were complicated by a strong orientation of both XP55 and NP368 growth-phase cells by the buffer flow (data not shown). Both strains showed clear movement against the direction of flow. Such orientation was not seen for cells starved for eight hours. The sensitivity of both strains to folate was relatively low, requiring 100 nM for a clear drop in speed. High concentrations of folate produced a reduction in speed that lasted 60 to 100 seconds for each strain. However, NP368 cells also show a prolonged increase in intracellular cGMP in response to folate (van Haastert et al., 1982; and data not shown). It is possible that the orientation induced by the flow suppresses the prolongation of the speed drop. Alternatively, the effect on motility induced by the streamer F mutation may be specific for amoebae that have been starved. Possibly the effect is mediated by a developmentally regulated protein.

Fig. 3. Responses of starved amoebae to 50 nM cAMP. Cells crawling on a coverslip in a flow cell were tracked as described in Materials and methods. At the time indicated on each graph, the cAMP concentration of the buffer flowing through the flow cell was switched from 2 nM to 50 nM cAMP. Data are the averaged speeds of 19 to 40 cells per strain.
Fig. 4. Changes in area in response to 1 μM cAMP. The average area of cells moving on a coverslip in the flow chamber was measured as a function of time. The cAMP concentration in the buffer flowing through the flow chamber was switched from 2 nM to 1 μM at 1 minute. (A) XP55: mean and standard error of the mean of the area of 119 cells from 14 flows on 3 different days. (B) NP368 and NP377: mean and standard error of the mean of the area of 140 cells from 15 flows on 5 different days.

Changes in area in response to sudden increases in cAMP
Changes in area were not detectable in response to 5 × 10^-8 M cAMP. However, consistent changes were seen in response to 10^-6 M cAMP. Around 27 seconds after addition of cAMP, XP55 cells showed a minimum in area, followed by an increase that was maximum at around 74 seconds (Fig. 4A). The changes in cell speed (Fig. 5A) were similar to those produced by 5 × 10^-6 M cAMP. Surprisingly, stmF cells did not decrease in area, showing only an increase in area peaking at about 54 seconds (Fig. 4B). The changes in cell speed were similar to those produced by 5 × 10^-8 M cAMP (Fig. 5B). Time-lapse recordings at high magnification confirmed that while XP55 cells tended to stop and decrease slightly in area after addition of cAMP, NP368 cells tended simply to stop and spread slightly (data not shown).

Fig. 5. Changes in speed in response to 1 μM cAMP. The speeds of movement of the cells used for Fig. 4 were calculated. The cAMP concentration in the buffer flowing through the flow cell was switched from 2 nM to 1 μM at 1 minute. (A) XP55; (B) NP368.

Chemotactic orientation in spatial gradients
Starved cells were placed in a Zigmond chemotaxis chamber and speed of movement, orientation and turning were measured before and after application of a gradient of 5 × 10^-8 M cAMP per mm (Table 1). Before application of the gradient, there were no significant differences between NP368 and XP55 cell parameters. In the presence of the gradient, there were only subtle differences: XP55 cells showed a 30% increase in speed, which was not seen with NP368 (P=0.001). Chemotactic orientation (the fraction of cell movement in the direction of the gradient) of NP368 was 20% higher than that of XP55 (P=0.05). Turning rates for both strains showed a slight decrease in the presence of the gradient, reflecting the orientation of cell movement.

Movement during aggregation
Since there was a prolonged decrease in cell speed after addition of cAMP in the flow cell it was possible that during aggregation stmF mutants would move more slowly towards the aggregation center. The average
speed of movement of cells in streams was measured for XP55 and NP368 in several ways. A small fraction of the cells was labeled with rhodamine dextran and then the movement of these cells in streams was measured during aggregation using the time-lapse scanning function of a confocal microscope (Fig. 6A). Alternatively, cells were not labeled but were tracked by hand using a long working distance ×40 objective (Fig. 6B). The results were similar in both cases. There were no consistent differences in the average speeds of XP55 and NP368 cells. The speed of movement in streams did vary with time of starvation for both strains, increasing as aggregation proceeded. The precise dependence of cell speed with aggregation time varied by one to two hours between experiments (data not shown).

Although the average speed of movement was quite similar for XP55 and NP368, the movement appeared more pulsatile for NP368. This was quantitated using cell populations synchronized by starvation at 7°C for 18 to 24 hours, followed by shifting to 22°C to allow aggregation to proceed (Ross and Newell, 1981). The speed of individual cells as a function of time was aligned and averaged to reveal periodic movement, as described in Materials and methods. For the data in Fig. 7, the average period was 220 seconds for XP55 cells and 230 seconds for NP368 cells. XP55 cells showed relatively continuous movement towards the aggregation centers (Fig. 7, open triangles), while NP368 showed dramatic periodicities (Fig. 7, filled circles), with cells taking clear synchronized steps followed by a period of little net motion. For NP368 the maximum speed was 3.3 times the minimum, compared to 1.5 for XP55.

Discussion

This paper reports measurements of the behavior of streamer F mutants under a variety of stimulation conditions. Streamer F mutants, and not other streamer complementation groups, show a prolonged increase in intracellular cGMP and a prolonged drop in cell speed upon stimulation with a step increase in extracellular cAMP. Area changes in response to large stimuli indicate that streamer F cells do not round up, although they still show the increase in area that normally follows the rounding up phase in the parental strain. Chemotac-

### Table 1. Comparison of XP55 and NP368 in Zigmond chambers

<table>
<thead>
<tr>
<th>Conditions</th>
<th>n</th>
<th>Speed (μm/min)</th>
<th>Orientation</th>
<th>Turning (deg./min)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± s.e.</td>
<td>Mean ± s.e.</td>
<td>Mean ± s.e.</td>
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<td>XP55</td>
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</tr>
<tr>
<td>Buffer</td>
<td>39</td>
<td>6.7 ± 0.42</td>
<td>0.040 ± 0.045</td>
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<td>0.485 ± 0.039</td>
<td>28.5 ± 1.4</td>
</tr>
<tr>
<td>NP368</td>
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</tr>
<tr>
<td>Buffer</td>
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<td>0.078 ± 0.046</td>
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</tr>
<tr>
<td>cAMP</td>
<td>54</td>
<td>7.1 ± 0.30</td>
<td>0.594 ± 0.036</td>
<td>29.4 ± 2.0</td>
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</table>

Cells were placed in a Zigmond chamber with buffer in both wells and after 20 minutes cells were tracked for at least 10 minutes (buffer condition). Then one well was flushed with buffer, and the other with buffer containing 5 × 10⁻⁸ M cAMP. After waiting 10 minutes to allow the gradient to form, cells were tracked for at least 10 minutes (cAMP condition). The orientation was calculated as the average fraction of cell movement in the direction of the gradient, with straight movement up the gradient corresponding to 1.0, random movement corresponding to 0.0 and straight movement down the gradient corresponding to −1.0. Data are the average of 3 experiments for XP55 and NP368. n, total number of cells; s.e., standard error of the mean. Two tailed t-tests for: the speed increase for XP55 compared to NP368 in the gradient (P<0.001); the increase in orientation for NP368 compared to XP55 in the gradient (P<0.05).
tic orientation in stable spatial gradients appears to be slightly improved, although the speed is slightly decreased. Surprisingly, the average speed of movement of cells in streams towards aggregation centers is not altered. There is, however, a higher amplitude of oscillation in cell speed during aggregation.

A simple explanation for the streamer phenotype, based on the observation that increases in cAMP lead to prolonged decreases in cell speed, would be that the reduced cell speed leads to a prolonged aggregation phase, resulting in long streams of cells moving slowly towards the aggregation center. Consistent with this hypothesis is the observation that streamer F cells plated at low density show reduced speeds during aggregation as single cells (McNamara, 1987). However, during later aggregation in streams this hypothesis is untenable for two reasons. First, as described above, since the rate of cell movement in streams towards the aggregation center is similar for \textit{stmF} cells and the parental strain, the streamer phenotype cannot be due to a reduced rate of cell movement in streams. Second, when cells are washed and starved on phosphate agar plates, the time required for aggregation is similar for \textit{stmF} and parental cells (Ross and Newell, 1981), again indicating that the rate of cell movement is not a limiting factor for aggregation.

The apparent difference between aggregating single cells and cells moving in streams may in part be due to the continued ability of \textit{stmF} cells to respond to new stimuli. Experiments with repeated, brief increases in cAMP with \textit{stmF} cells indicate that although the average cell speed is reduced for extended periods of time, the cells can still undergo changes in area corresponding to the addition of cAMP (data not shown). Since cells in streams are exposed to strong spatial gradients as a wave of cAMP is relayed outward from the aggregation center, the resultant changes in area might be directional and result in pseudopod extensions in the direction of the aggregation center. This could result in net movement of the cell towards the center. The strong oscillations in cell speed that are seen as \textit{stmF} cells are moving in streams are consistent with the temporal increases in cAMP still generating the same sort of long-lasting suppression of cell speed as was seen in the flow cell, upon which are superimposed these hypothetical pseudopod extensions that occur due to the spatial gradient, and which result in net cell movement towards the aggregation center. During early aggregation, waves of cAMP occur every eight to ten minutes, while during late aggregation they occur more rapidly, every four to five minutes (Durston, 1974; Gerisch, 1971). The higher frequency of cAMP pulsing combined with the ability to respond to new stimuli may minimize differences in responses between \textit{stmF} cells and the parental strain during later aggregation.

The correlation between the time course of intracellular cGMP concentration and cell speed suggests that increases in intracellular cGMP can lead to a drop in cell speed. The biochemical targets of cGMP in \textit{Dictyostelium} are, as yet, unclear. Intracellular cGMP could play a role in the control of gene transcription or translation as well as affecting cell motility. Several cGMP binding proteins have been described (Mato et al., 1978; Parissenti and Coukell, 1990), as well as a kinase (Wanner and Wurster, 1990). \textit{stmF} mutants show prolonged influxes of extracellular calcium, with kinetics similar to those of the intracellular cGMP content (Menz et al., 1991), concomitant with prolonged hyperpolarization (van Duijn and Wang, 1990). It is possible that intracellular cGMP opens potassium channels and calcium channels (Mueller et al., 1986), causing hyperpolarization via potassium efflux, but also allowing extracellular calcium to flow in. Either the increased intracellular calcium or the hyperpolarization (or both) could have an effect on the cytoskeleton.

Regarding the cytoskeleton, actin filaments are probably not a major target for cGMP, since the kinetics of actin filament formation appear normal (Liu and Newell, 1988), and cAMP-stimulated increases in cell area, which involve actin filament formation in pseudopods (Hall et al., 1988), are also fairly normal. A number of observations suggest that, in affecting cell motility, cGMP may be affecting myosin II. Compared to the parental strain, stimulation of streamer F mutants with cAMP results in a prolonged association of myosin II heavy chain with the cytoskeleton (Liu and Newell, 1988) as well as a 30 second delay in phosphorylation of the myosin II heavy chain (Liu and Newell, 1991). In addition, streamer F cells do not show the decrease in area that normally follows the drop in speed. Mutants lacking myosin II heavy chains also show no decrease in area in response to addition of cAMP, only an increase in area (Fukui et al., 1990). Thus, cGMP might be suppressing contractions mediated by myosin II. If myosin-dependent contraction produced the rounding up (and concomitant decrease in

\begin{figure}
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\includegraphics[width=\textwidth]{fig7.png}
\caption{Periodicity of movement during aggregation. Cells were starved on agar overnight at 7°C, and then shifted to 22°C. Video recordings from 3 dishes for each strain were made as the cells began synchronized movement towards aggregation centers. The movement of the cells was digitized using the Image 1/AT system. Oscillations in cell speed were aligned as described in Materials and methods and the average speed during the oscillation is plotted. Open triangles, XP55, 29 cells; filled circles, NP368, 32 cells.}
\end{figure}
area), a suppression of this by prolonged intracellular cGMP might lead to the observed effect. Myosin II mutants also show a decreased average speed (Wessels et al., 1988), as do stmF mutants, while intracellular cGMP is elevated. These observations are all consistent with the possibility that myosin II-induced functions are inhibited while intracellular cGMP concentrations are elevated. In smooth muscle, increases in intracellular cGMP lead to relaxation and inhibition of myosin II (Waldman and Murad, 1987; but see Sarcevic et al., 1990). None of the myosin II heavy and light chain kinases that have been isolated from Dictyostelium discoideum are affected by cGMP in vitro (Cote and Bukiejko, 1987; Ravid and Spudich, 1989; Tan and Spudich, 1990). Although there is autophosphorylation of these kinases in vitro, in vivo changes in their phosphorylation level in response to cAMP have not yet been determined. As mentioned above, compared to XP55, myosin II heavy chain phosphorylation is delayed 30 to 40 seconds in the streamer F mutants (Liu and Newell, 1991). Since heavy chain phosphorylation antagonizes thick filament formation in vitro (Kuczmarski and Spudich, 1980; Kuczmarski et al., 1987; Pasternak et al., 1989), a delay in heavy chain phosphorylation could contribute to the prolonged association of myosin with the cytoskeleton. If re-arrangement of the myosin on the cytoskeleton is necessary for cells to move most efficiently, the extended association of myosin with the cytoskeleton (reflecting an inability to rearrange) might in turn lead to reduced cell speed. However, how this would block the contraction of streamer F cells (an event that occurs right after association of myosin with the cytoskeleton in XP55) is unclear.

In conclusion, the effects of prolonged intracellular cGMP reported in this paper provide an indication of what the normal role of cGMP may be in chemotactic responses. Measurements of chemotaxis in spatial gradients of cAMP suggest that streamer F mutants may have slightly improved chemotactic responses compared to the parental strain. In the flow cell, in which cAMP concentration is suddenly increased with very little spatial gradient, random cell movement is inhibited in streamer F mutants. In a spatial gradient, chemoattractant binding at the front of the cell as it moves up the gradient could generate production of cGMP, which could then diffuse throughout the cell to suppress random steps. Suppression of random cell movement could improve chemotactic responses by reducing incorrect steps, and thus allowing steps up the gradient to form a greater fraction of total cell movement. The net result would be more efficient movement up the gradient.

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