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Regulation of myosin regulatory light chain phosphorylation via cyclic GMP during chemotaxis of *Dictyostelium*

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SUMMARY

Previous studies on the chemotactic movement of *Dictyostelium* have indicated a role for cyclic GMP in regulating the association of myosin II with the cytoskeleton. In this study we have examined the part played by phosphorylation of the 18 kDa myosin regulatory light chain in this event. Using streamer F mutant NP368 (which is deficient in the structural gene for cyclic GMP-specific phosphodiesterase) we find that, for the regulatory light chain kinase, the major peak of phosphorylation is delayed compared to the parental control strain XP55, occurring at 80 seconds rather than about 30 seconds in XP55. In two independently derived mutants that are unable to increase their cellular concentration of cyclic GMP (above basal levels) in response to a chemotactic stimulus of cyclic AMP (KI-10 and SA219), no increase in the phosphorylation of the light chain occurred, or movement of myosin II to the cytoskeleton. We also find a smaller peak of light chain phosphorylation that occurs within 10 seconds of cyclic AMP stimulation of the amoebae, and which is absent in the cyclic GMP-unresponsive strains. We conclude that cyclic GMP is involved in regulating light chain phosphorylation in this system. The possible significance of these findings is discussed and a model that relates these findings to published data on cytoskeletal myosin changes during chemotaxis is presented.

Key words: *Dictyostelium*, slime mould, myosin, cytoskeleton, cyclic GMP

INTRODUCTION

Recent studies have indicated that myosin II plays an important part in chemotactic cell movement of the amoebal organism *Dictyostelium*. It has been reported, for example, that myosin II translocates to the cortex in response to stimulation with the chemoattractant cyclic AMP (Yumura and Fukui, 1985; Nachmias et al., 1989; Yumura and Kitanishi-Yumura, 1992) and that this translocation is regulated by transient synthesis of cyclic GMP (Liu and Newell, 1988, 1991; Liu et al., 1993). This response is not concerned with the force generation involved in cell translocation, since null mutants of the myosin II heavy chain produced by gene disruption (De Lozanne and Spudich 1987; Wessels et al., 1988; Manstein et al., 1989) or by anti-sense RNA (Knecht and Loomis 1987, 1988) could move (in any random direction) with similar speed to wild-type cells. However, these mutants were less efficient at chemotactic movement and had a more rounded shape with random production of pseudopods, suggesting that the role of myosin II is connected with cell shape for efficient chemotaxis rather than being the chemotactic motor.

The evidence that cyclic GMP is involved in regulation of myosin translocation to the cytoskeleton came from work with the 'streamer F' mutants (Ross and Newell, 1979, 1981). In the wild-type strain, cyclic GMP is transiently formed in response to a cyclic AMP signal and shows a peak at 10 seconds. In streamer F mutants the cyclic GMP is not destroyed as rapidly as in the wild-type cells but persists for approximately fivefold longer due to a defect in the structural gene for the cyclic GMP-specific phosphodiesterase. (Ross and Newell, 1981; Van Haastert et al., 1982; Coukell and Cameron, 1986). The most obvious effect of this defective gene on the visible phenotype is that the amoebae remain in the elongated state during chemotaxis for approximately fivefold longer than the parental strain XP55. Examination of the cells' (Triton-insoluble) cytoskeleton revealed that, while the rapid accumulation of F-actin in the cytoskeleton (which peaks 5 seconds after a cyclic AMP stimulus) was unaffected in the streamer F mutants (McRobbie and Newell, 1984), these mutants showed a dramatically different pattern of association of myosin II heavy chain with the cytoskeleton (Liu and Newell, 1988). In the parental strain, association of myosin II heavy chain with the cytoskeleton was transient, with a peak at about 25-30 seconds. In the streamer mutants, however, this peak was persistent and only slowly declined to basal values.

Further studies have suggested that the connection between cyclic GMP formation and movement of the myosin to the cytoskeleton may involve phosphorylation of the myosin heavy chain. Earlier studies by Berlot et al. (1985, 1987) indicated that myosin II heavy chain is phosphorylated (mainly on threonine residues) in response to a cyclic AMP stimulus. More recent studies by Liu and Newell (1991) have provided evidence that phosphorylation of the myosin heavy chain is abnormal in the streamer F mutants and is considerably

delayed compared to the parental strain, with a peak at about 60-80 seconds rather than 25-30 seconds. It was suggested that phosphorylation of the myosin heavy chain removes it from the cytoskeleton. In support of this it was found that little of the myosin that was on the cytoskeleton was phosphorylated compared to that in the soluble cell fraction. On the basis of these findings a model for the control of myosin heavy chain by cyclic GMP has been proposed (Liu and Newell, 1991), and recently data has been published confirming part of this model (Yumura and Kitanishi-Yumura, 1993).

Besides the heavy chains, myosin II is also composed of two types of 'light' chain. In other non-muscle cells, the light chains are thought to play an active part in the control of myosin's cellular actions (Spudich, 1989; Tan et al., 1992) and the studies described below were undertaken to examine their role in *Dictyostelium.* This organism has been found to possess a phosphorylatable 18 kDa 'regulatory' light chain and a 16 kDa 'essential' non-phosphorylated myosin light chain (Clarke and Spudich, 1974; Kuczmarski and Spudich, 1980). That the essential light chain is indeed essential for chemotaxis was recently shown by Pollenz et al. (1992) using an antisense RNA mutant strain expressing less than 0.5% of the wild-type levels of this subunit. This mutant was similar in its phenotype to the myosin II heavy chain null mutants with very poor chemotactic movement. The involvement of the 'regulatory' light chain in chemotaxis was first indicated by the work of Berlot et al. (1985, 1987), who observed transient increases in the level of myosin regulatory light chain phosphorylation after cyclic AMP stimulation, and evidence was presented that this resulted from changes in light chain kinase activity rather than in the activity of the light chain phosphorylase. Further evidence for this involvement was reported by Griffith et al. (1987), who found that the actin-activated Mg^{2+}-ATPase activity of the myosin was directly related to the extent of phosphorylation of the regulatory light chain. The recent work of Uyeda and Spudich (1993) with a strain that possesses a mutant myosin heavy chain lacking the binding site for the regulatory light chain suggests that the latter is not essential for cell viability. Of particular interest is their finding that the regulatory light chain actually inhibits actin-activated Mg^{2+}-ATPase and it is the phosphorylation of the light chain that relieves this inhibition. The work described below was aimed at exploring the role of phosphorylation of the regulatory light chain of myosin II during chemotaxis using the streamer mutants, and mutants lacking the normal cyclic GMP response. The results suggest that cyclic GMP plays a role in regulating the regulatory light chain kinase, an action that may be involved in movement of myosin II on the cytoskeleton via its actin-activated Mg^{2+}-ATPase.

MATERIALS AND METHODS

Materials

RNase A (from bovine pancreas) was obtained from Boehringer-Mannheim. Phenol-indo-2,6,-dichlo-phenol (PIDCP), Triton X-100, acrylamide and bis acrylamide were from BDH. Immuno-Precipitin (formalin-fixed *Staphylococcus* A cells) was from Bethesda Research Laboratories. Goat anti-mouse IgG (H+L) alkaline phosphatase conjugate and ammonium persulphate were from Bio-Rad. X-ray film was supplied by Fuji. Coomassie Brilliant Blue R, Trizma base, phenylmethylsulphonyl fluoride (PMSF), iodoacetamide, TEMED, ATP, 5-bromo-4-chloro-3-indolyl phosphate (BCIP), nitro blue tetrazolium (NBT), albumin (BSA, essentially globulin free), cyclic AMP, dithiothreitol (DTT), EDTA, EGTA, TPCK, TLCK and MES (2-[*N*-morpholino]ethanesulphonic acid) were obtained from Sigma.

Buffers

Lysis buffer, pH 7.5 (×2 strength) contained: Tris-HCl 80 mM, NP-40 0.4% (v/v), DTT 4 mM, EDTA 20 mM, potassium phosphate 400 mM, sodium azide 8 mM, PMSF 4 mM, TPCK 2 mM, TLCK 2 mM, NaF 20 mM, sodium pyrophosphate 50 mM, ATP 10 mM, RNase A 100 μg ml^{-1}.

MES buffer, pH 6.15, contained: MES 20 mM, $MgSO_4$ 2 mM, $CaCl_2$ 0.2 mM.

Cell culture

All cultures used NC4-derived *Dictyostelium discoideum* strains grown in association with *Klebsiella aerogenes*, strain OXF1, on SM nutrient agar (Sussman, 1966). The streamer mutant NP368 was derived from strain XP55 (Ross and Newell, 1979, 1981). Strain KI-10, which lacked the cyclic GMP response to pulses of cyclic AMP (Kuwayama et al., 1993; Liu et al., 1993), was derived from XP55 and was kindly provided by Dr Hidekazu Kuwayama. Strains SA31 and SA219 were discoidin minus mutants both derived from strain HJR-1 (Alexander et al., 1983). Although not the parental strain, strain SA31 was used as the control strain for strain SA219 (which lacked the cyclic GMP response to cyclic AMP) because it was the most closely related discoidin minus strain available and was wild type in its cyclic GMP response.

Harvesting and ^{32}P-labelling of amoebae

Amoebae were prepared by growth as lawns on SM agar under conditions permitting uniform clearing of the bacteria by the feeding amoebae. Amoebae were harvested from the bacterial plates in MES buffer and washed free of bacteria by centrifugation at 190 ***g*** for 2 minutes. After three washes, the cells were resuspended in MES buffer at 2×10^7 ml^{-1} and shaken at 170 revs min^{-1} at 22°C in an orbital shaker for 7 hours. The developed cells were then resuspended in MES at 5×10^7 cells ml^{-1} and labelled with [^{32}P]orthophosphate, 0.1 mCi ml^{-1} for 30 minutes. After three washes with MES, the labelled cells were resuspended at 10^8 cells ml^{-1} for immunoprecipitation of myosin.

Purification of JIG-3 anti-MHC antibody, immunoprecipitation of myosin II, electrophoresis, densitometry and immunoblotting

These techniques have been described (Liu and Newell, 1991).

Measurement of intracellular cyclic GMP accumulation

Cyclic GMP assays were carried out according to the method of Van Haastert et al. (1981) using a radioimmunoassay kit supplied by Amersham International Plc.

RESULTS

Isolation of myosin light chains

Myosin II was isolated from $^{32}PO_4$-labelled whole cells using immunoprecipitation by the anti-myosin II heavy chain monoclonal antibody JIG-3 (Liu and Newell, 1991). The component light chains that were co-precipitated with the heavy chain by the antibody were then separated by SDS-polyacrylamide gel electrophoresis.

Evidence is provided in Fig. 1 for the separation of the myosin II from a high background of other proteins remaining

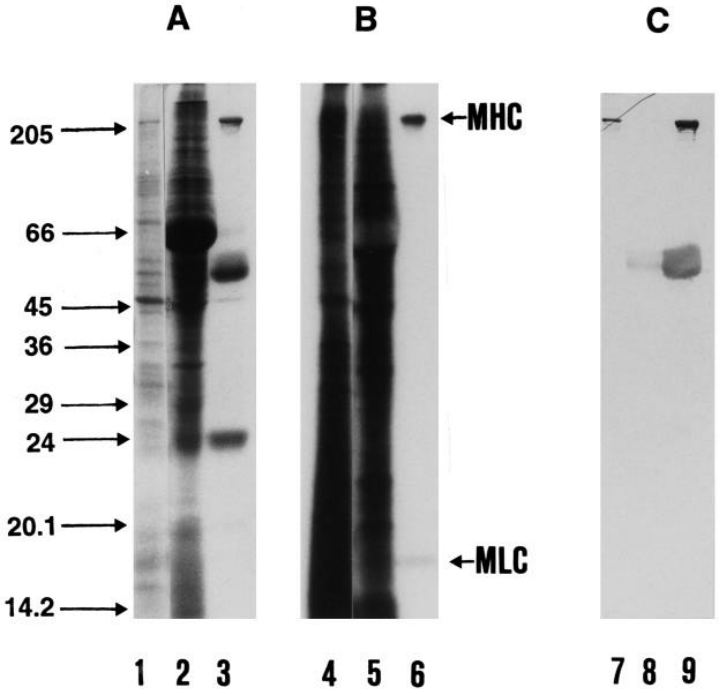

Fig. 1. Isolation of myosin II by immunoprecipitation using JIG-3 anti-myosin II monoclonal antibody from amoebae labelled in vivo with $^{32}PO_4$ and gel separation into heavy (MHC) and light (MLC) chains. (A) Coomassie Blue-stained SDS-PAGE gel (12%); (B) autoradiograph of A; (C) western blots of A using JIG-3 (anti-myosin heavy chain) monoclonal antibody; lanes 1, 4, 7, whole cell protein (a small amount of sample was loaded for clarity); lanes 2, 5, 8, supernatant remaining after immunoprecipitation of myosin (the amount loaded was high, to test the lack of reaction with the western blot in lane 8); lanes 3, 6, 9, immunoprecipitated myosin II (M_r = 210,000). The lower molecular mass bands in lane 3 are the two components of the JIG-3 antibody used for precipitation, the heavy chain of which reacts with the alkaline phosphatase-conjugated anti-mouse IgG antiserum in lane 9. (For routine assay of the light chain, even higher loading of the gels was used, which optimized the gel for the 18 kDa light chain band and gave a more distinct, quantifiable band.) Note that the JIG-3 antibody could not react with the light chains (lane 9) but the light chains were co-precipitated with the heavy chain. Positions of molecular mass markers (kDa) are shown in the left-hand margin.

in the supernatant seen using Coomassie Blue staining (lane 3), autoradiography (lane 6) and western blotting with JIG-3 (lane 9). The essential and regulatory light chains were distinguished by their characteristic molecular mass values (16 and 18 kDa, respectively; Clarke and Spudich, 1974). The light chains were authenticated and shown to be precipitated along with the myosin heavy chain, by immunoblotting with polyclonal anti-myosin antibodies, from Dr Rex Chisholm (Fig. 2).

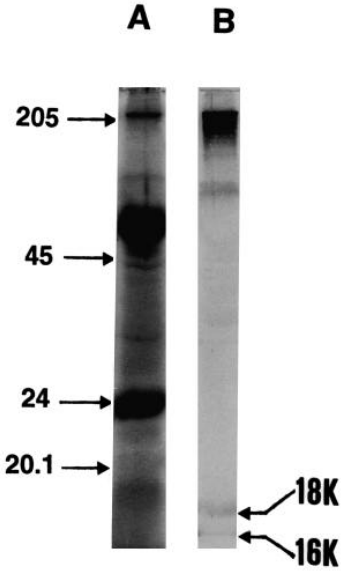

Fig. 2. Authentication of myosin light chains. (A) Coomassie Blue-stained SDS-PAGE gel (12%) of whole cell protein; (B) western blot of myosin heavy and light chains immunoprecipitated from whole cell extracts with JIG-3 anti-myosin II heavy chain monoclonal antibody and visualized using (as the first antibody) rabbit anti-myosin polyclonal antiserum, and (as second antibody) an alkaline phosphatase conjugated anti-rabbit IgG antiserum from Bio-Rad. K=kDa.

Changes in phosphorylation of the myosin regulatory light chain during chemotaxis of parental strain XP55 and streamer F mutant NP368

Developing XP55 cells that had been labelled in vivo with $^{32}PO_4$ for 30 minutes, were stimulated with 200 nM cyclic AMP, and the reaction was stopped by an equal volume of lysis buffer at preset times. Myosin II was then immunoprecipitated using JIG-3 monoclonal antibody and the regulatory light chain was analysed by gel electrophoresis and autoradiography. It was found that incorporation of radioactivity into the light chain was rapid, with a small peak at 5-10 seconds after cyclic AMP stimulation, followed by the main peak at approximately 30 seconds (Fig. 3). From the study of Griffith et al. (1987), who showed that unstimulated amoebae have approximately 30% of their regulatory light chains phosphorylated (0.3 mol P_i/mol light chain with a maximum phosphorylation by myosin light chain kinase of 1 mol P_i/mol light chain), the 1.5-fold increase in phosphorylation of the light chain at peak in Fig. 3 would represent approximately 45% phosphorylation.

In the streamer F mutant, stimulation by 200 nM cyclic AMP produced a similar small phosphorylation peak at 5-10 seconds, which was followed, as in XP55, by a much larger peak. However, this peak of phosphorylation occurred much later in the mutant with a maximum at about 80 seconds, slowly declining to basal values at 150 seconds (Fig. 3). Because the primary defect in the streamer F mutant is in the cyclic GMP-specific phosphodiesterase structural gene, the finding of a delay in the phosphorylation of the light chain in the mutant compared to the parental strain leads us to conclude that cyclic GMP is (directly or indirectly) involved in regulating this phosphorylation.

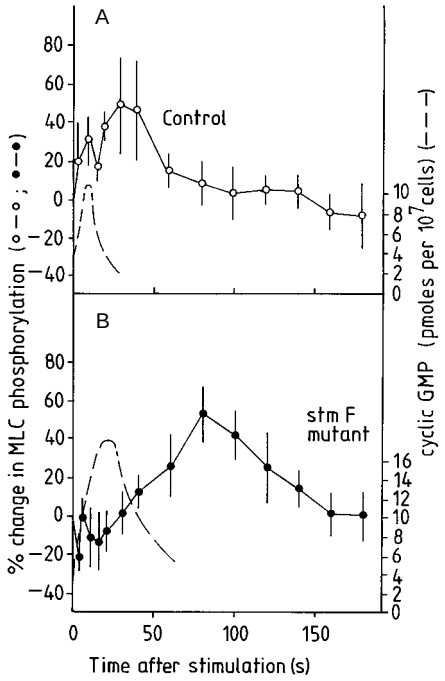

Fig. 3. Time course of changes in myosin light chain phosphorylation after cyclic AMP stimulation of parental strain XP55 and streamer F mutant NP368. Results are expressed as percentage change over prestimulus value. The broken lines indicate the cellular accumulation levels of cyclic GMP after cyclic AMP stimulation (pmoles per 10^7 cells). (A) XP55 (○–○); (B) streamer F mutant (●–●). Error bars represent the s.e.m. from three experiments.

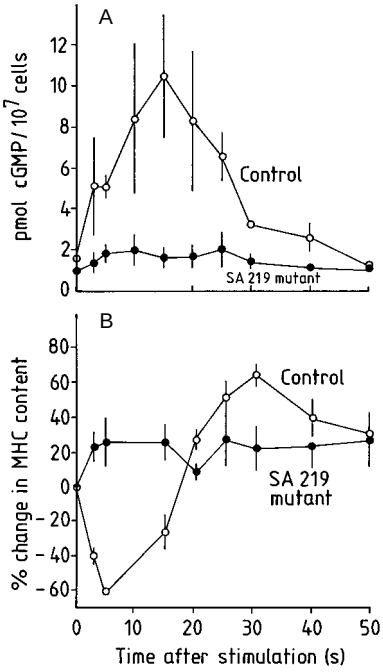

Fig. 4. Cyclic GMP and cytoskeletal myosin II responses to cyclic AMP stimulation in cells of mutant SA219 (●–●) and control strain SA31 (○–○). Cyclic GMP (A) was assayed in perchloric acid cell extracts made between 0 and 50 seconds after stimulation of the amoebae with 100 nM cyclic AMP. (B) Time course of changes in myosin heavy chain associated with the cytoskeletons of 8 hour developing cells following stimulation with 100 nM cyclic AMP. The prestimulus values for cytoskeletal myosin heavy chain content were similar in mutant and control strains. Error bars represent s.e.m. from three experiments.

Myosin light chain phosphorylation in cyclic GMP-deficient strains

To confirm the connection between cyclic GMP accumulation and light chain phosphorylation, two independently derived mutants were tested that fail to increase their cyclic GMP concentration above the basal level in response to stimulation of the cells with cyclic AMP. The first mutant, KI-10, has recently been described (Kuwayama et al., 1993; Liu et al., 1993). It lacks a cyclic GMP response due to a dominant mutation on linkage group I, and fails to increase the association of myosin with the cytoskeleton or to phosphorylate myosin heavy chain in response to cyclic AMP, although its actin response is normal. The second mutant, SA219, in a different genetic background (Alexander et al., 1983) was found to have a similar phenotype to KI-10 in failing to increase cyclic GMP in response to cyclic AMP (Fig. 4 top panel). It also showed no cytoskeletal myosin accumulation (Fig. 4, lower panel) and did not phosphorylate myosin heavy chain in response to cyclic AMP although, like KI-10, its actin response was normal (data not shown). The control strain used was SA31 (Alexander et al., 1983), which was derived from the same parental strain, HJR-1, but showed a normal cyclic GMP response.

When the KI-10 and SA219 mutants were tested for their ability to phosphorylate the myosin regulatory light chain in response to cyclic AMP, neither showed any positive response (Fig. 5). A slight negative response was sometimes observable, as seen in Fig. 5, although the mean values of the data are not significantly different from zero. In both of the control strains XP55 and SA31, a peak of phosphorylation was seen at 5-10 seconds, which was followed by the major phosphorylation peak at 30 seconds. It is noteworthy (see below) that in strain SA31 the two peaks were more clearly distinct and separated than in XP55.

DISCUSSION

The data presented, which show a lack of phosphorylation of myosin II regulatory light chain in response to chemotactic stimulation in the cyclic GMP-deficient mutants, and an altered pattern in the streamer F mutant, clearly indicate a role for cyclic GMP in the regulation of this molecule. A comparison of the data for phosphorylation of the light chain with those previously published for phosphorylation of the myosin heavy chain (Liu and Newell, 1991) reveals a similar pattern. For the heavy chain, a small peak of phosphorylation was seen at 5-10 seconds in the parental strain (XP55) followed by a large peak at 30-40 seconds, which was delayed in the streamer mutants to 60-80 seconds. In the present study, a similar small peak of phosphorylation of the light chain at 5-10 seconds was followed by a larger peak at about 30 seconds and this was also delayed in the streamer mutants to about 80 seconds.

In an earlier study, Berlot et al. (1985, 1987) reported an approximately 3- to 10-fold increase in the light chain phosphorylation using the axenic mutant strain AX3. Such an increase, which is apparently larger than that reported here,

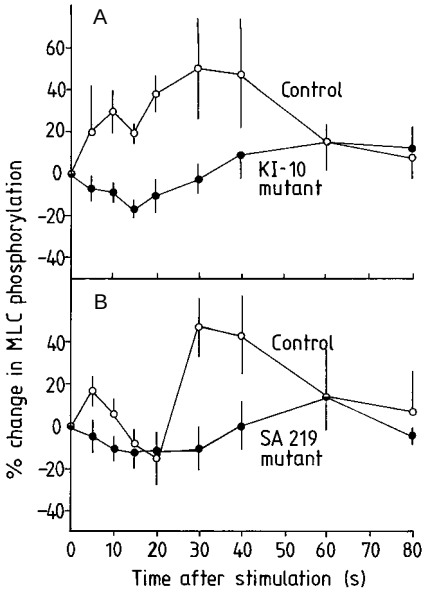

Fig. 5. Time course of changes in phosphorylation of myosin regulatory light chain after cyclic AMP stimulation of 8 hour developing cells. Results are expressed as percentage change over the prestimulus value. (A) XP55 control (○–○); mutant KI-10 (●–●). (B) SA31 control (○–○); mutant SA219 (●–●). The prestimulus values for phosphorylation of myosin light chain content were similar in mutant and control strains. Error bars represent s.e.m. from four experiments.

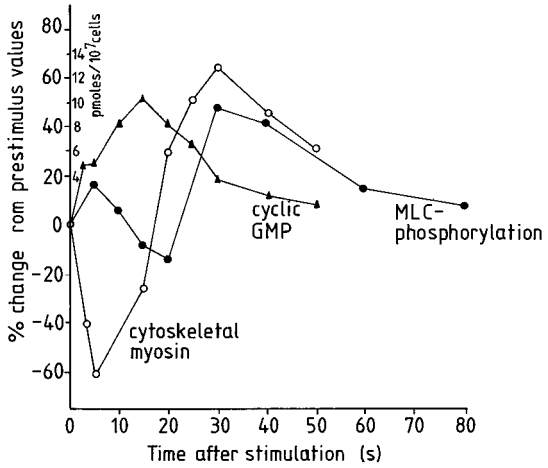

Fig. 6. Superimposed time courses of changes in cyclic GMP (▲–▲), cytoskeletal myosin (○–○), and myosin light chain phosphorylation (●–●) in control strain SA31 after stimulation of 8 hour developing cells with cyclic AMP. The data (taken from Figs 4 and 5) are plotted as percentage change over the prestimulus values (myosin, light chain phosphorylation) and pmoles per 10^7 cells (cyclic GMP). The data for strain XP55 (not shown) are qualitatively similar, although the first and second peaks are less clearly defined than for SA31.

was probably due, however, to smaller starting basal values that (in the case of the 10-fold increases) were artificially reduced by pretreatment of the amoebae with 5 mM caffeine for 30 minutes. Another difference between the present report and that of Berlot et al., is that they did not observe the rapidly formed small peak. This difference is due, presumably, either to their use of caffeine or, for cells not treated with caffeine, to their lack of early (0-10 second) time points.

Regulation of the small peak and the later major peak of both the light chain and (from previous data of Liu and Newell, 1991) the heavy chain phosphorylation are clearly different. Although the small peak is sometimes too small to be well defined (as, for example, for the streamer mutant in Fig. 3) and the later peak can appear to be partially superimposed on the small peak (see, for example, the control peak in Fig. 5), there is no doubt about the small peak's separate identity if the data for all of the strains studied are taken together. In the case of strain SA31 (the control in the lower panel of Fig. 5) it may be noticed that there is greater separation of the peaks than in other strains studied. To examine the relative timing of the myosin peaks with the peaks for cyclic GMP formation and light chain phosphorylation in greater detail, the early responses induced in strain SA31 by cyclic AMP are superimposed (Fig. 6). This reveals that the small early peak of light chain phosphorylation from 0 to 5 seconds is correlated in its timing with a small burst of dissociation of myosin from the cytoskeleton (an event also seen in earlier studies with other strains including the wild-type NC4 strain). Although the involvement of cyclic GMP in regulating this rapid response must be regarded as tentative, it is noticeable that this peak is absent in both of the mutants lacking the cyclic GMP response (Fig. 5). Interestingly, such an effect would have to be brought about by a *positive* action of cyclic GMP in *stimulating* myosin light chain phosphorylation in an entirely distinct fashion from the inhibitory effects of cyclic GMP on the later peak, which is discussed below. However, such a stimulatory effect would be consistent with the finding by Tan and Spudich (1990, 1991), that the cloned light chain kinase gene possesses cyclic GMP-dependent protein kinase phosphorylation sites. A positive role for cyclic GMP in the regulation of myosin light chain phosphorylation is also suggested by the very recent unpublished finding of Linda Silveira, John Tan and James Spudich (personal communication) that (while cyclic GMP alone has no direct effect on the kinase enzyme) it can stimulate the activity of one form of myosin light chain kinase when added to a fresh lysate of cells in vitro. The significance of such a rapid action of cyclic GMP for chemotaxis could be that it results in localized breakdown of the cortical shell of myosin, an event that is postulated by Spudich (1989) to be required for the protrusion of pseudopodia in the direction of the signal source, one of the early events following stimulation by cyclic AMP.

The later peak of light chain phosphorylation is correlated with a decrease in the level of cyclic GMP (Figs 3 and 6). The phosphorylation of the light chain (and heavy chain as shown previously) is also correlated with the dissociation of the myosin II from thick filaments on the cytoskeleton during this time. The role of heavy chain phosphorylation is thought to be due to bending of the myosin tail, as such bent myosin molecules cannot participate readily in thick filaments (Kuczmarski and Spudich, 1980; Kuczmarski et al., 1987; Côté and McCrea, 1987; Pasternak et al., 1989; Egelhoff et al., 1993). A corresponding role for phosphorylation of the regulatory light chain is, however, unlikely, as Griffith et al. (1987) found that phosphorylation of the light chain had no effect on the ability of the myosin to form thick filaments in vitro. We

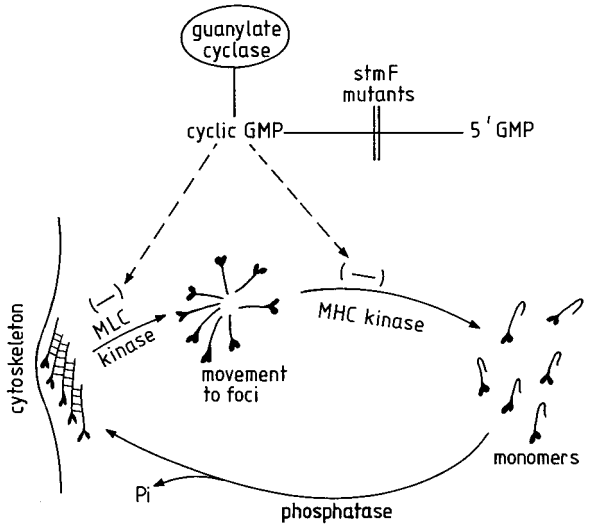

Fig. 7. Model of regulation by cyclic GMP of the dissociation of myosin from the cytoskeleton. The phosphorylated myosin II molecules are shown as bent monomers while the dephosphorylated molecules are in the form of parallel fibres involved in the formation of thick filaments on the cytoskeleton. The model proposes that cyclic GMP (directly or indirectly) inhibits myosin light chain and heavy chain phosphorylation, thereby inducing a shift in the equilibrium in favour of association of myosin on the cytoskeleton. In the absence of cyclic GMP, the action of myosin light chain kinase promotes the actin-activated Mg^{2+}-ATPase by phosphorylation of the myosin regulatory light chain and hence stimulates the movement of the myosin II molecules on cytoskeletal actin molecules to foci on the cytoskeleton where myosin heavy chain kinase is present, the phosphorylation then bringing about myosin dissociation. During the cyclic GMP peak induced by chemotactic cyclic AMP stimulation, the rate of myosin dissociation is decreased. Following the peak of cyclic GMP there is a transient increase in the rate of phosphorylation of light and heavy chains that had accumulated on the cytoskeleton, and the normal equilibrium is re-established. In streamer F (stmF) mutants, the period of cyclic GMP formation in response to chemotactic cyclic AMP stimulation is prolonged (due to failure to hydrolyse the cyclic GMP), leading to a prolonged association of myosin II with the cytoskeleton and a delay in the transient increase in rate of phosphorylation of the myosin light and heavy chains.

suggest that a more likely role is connected with the actin-activated Mg^{2+}-ATPase, as this is activated five- to sixfold by phosphorylation of the light chain (Griffith et al., 1987). In an in vitro (*Nitella*) motility assay, Griffith et al. also found that myosin that had been phosphorylated by myosin light chain kinase moved rapidly (at about 1.4 μm s^{-1}) compared to phosphatase-treated myosin, which moved slowly or not at all. The significance of this effect may be seen in conjunction with the results of the immunoelectron microscopic study of Yumura and Kitanishi-Yumura (1992), who showed that the mechanism of dissociation of myosin from the cytoskeleton is more complex than that of simply induced tail bending. They found that addition of ATP to membrane-cytoskeleton preparations of *Dictyostelium* caused contraction of the cytoskeleton with aggregation of part of the actin into foci within the actin network and movement of the myosin towards these foci before it dissociated. Their model postulated that myosin II filaments slide on actin filaments in the direction of the foci where the myosin heavy chain kinase is localized and, after addition of phosphates to the heavy chain, the molecules leave the cytoskeleton. Our model (Fig. 7) incorporates their immunocytochemical findings and the results of Griffith et al. (1987) on activation of actin-activated Mg^{2+}-ATPase, and suggests that the phosphorylation of the light chain stimulates this movement of the myosin over the actin network towards the foci. The myosin heavy chain kinase at the foci then brings about dissociation of the myosin as previously postulated. Inhibition by cyclic GMP of the light chain phosphorylation (seen for an extended period in the streamer mutants) inhibits the movement towards the foci and causes an accumulation of myosin II on the cytoskeleton as dissociation slows but association continues. The absence of any peaks of heavy or light chain phosphorylation in the cyclic GMP unresponsive mutants KI-10 or SA219 would be expected from this model, because in these mutants no build up of myosin on the cytoskeleton would occur and hence the subsequent peak of myosin phosphorylation that is normally seen in the wild-type strains during myosin dissociation would also be absent.

How inhibition of the light chain phosphorylation is achieved is unknown. Griffith et al. (1987) found that the purified light chain kinase was unaffected by cyclic nucleotides, although it was inhibited by high concentrations (1 mM) of Ca^{2+}. It is also apparently unaffected by calmodulin in vitro and, while it has some sequence homology to the calcium/calmodulin-binding domain of other MLCK enzymes, it is thought that this binding region does not satisfy the criteria of forming the basic amphiphilic alpha-helical structure that is essential for calcium/calmodulin-binding (Tan and Spudich, 1990). It differs, therefore, from the light chain kinase from some other systems such as smooth muscle (Nishikawa et al., 1984, 1985), and further studies are needed to establish the connection between cyclic GMP and the regulatory light chain kinase in vivo in this system.

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