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cGMP potentiates receptor-stimulated Ca^{2+} influx in *Dictyostelium discoideum*

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Abstract

Binding of extracellular cAMP to surface receptors induces at least two responses in *Dictyostelium discoideum*, the G-protein-dependent activation of guanylyl cyclase, and the opening of a plasma membrane Ca^{2+} channel. Some experiments suggest that intracellular cGMP opens the Ca^{2+} channel, while others demonstrate that the channel can open in the absence of functional G-proteins (and thus in the absence of cGMP formation). We have analysed $^{45}Ca^{2+}$ uptake in three mutants with altered cGMP formation. Mutant *stm*F shows a prolonged cGMP response due to deletion of an intracellular phosphodiesterase. Uptake of receptor-stimulated $^{45}Ca^{2+}$ is enhanced about two-fold in this mutant if compared to wild-type cells, suggesting that cGMP regulates the opening of the channel. Mutant KI-7 has very low levels of surface cAMP receptors, but nevertheless an enhanced receptor-stimulated cGMP response due to a defect in the turn-off of guanylyl cyclase. This mutant shows poor receptor-stimulated $^{45}Ca^{2+}$ uptake, suggesting that cGMP alone is not sufficient to open the Ca^{2+} channel. Finally, mutant KI-8 has no cGMP due to the absence of nearly all guanylyl cyclase activity. The mutant shows significant but reduced $^{45}Ca^{2+}$ uptake (19% of wild-type; 60% if corrected for the reduced level of surface cAMP receptors), suggesting that the channel can open in the absence of cGMP. Taken together, the results demonstrate that receptor-stimulated Ca^{2+} influx is not directly induced by cGMP formation; it can occur in the absence of cGMP, but is potentiated two- to four-fold by cGMP. © 1998 Elsevier Science B.V.

Keywords: Calcium ion channel; cyclic GMP; Chemotaxis; Mutant; (*Dictyostelium*)

1. Introduction

Investigations on the transduction pathways of chemotactic signals in *Dictyostelium discoideum* have provided detailed information on the role of surface receptors, G-proteins, effector enzymes and second messengers for chemotaxis (reviewed in Ref. [1]). In this amoeboid microorganism, chemotactic movement is required for two important phenomenon, feeding and morphogenesis. In seeking for its bacterial food source, cells move to folic acid, a compound secreted by bacteria. Upon starvation, *Dictyostelium* cells start to secrete cAMP and switch their chemotactic sensitivity from folic acid to cAMP. Cells collect in aggregates that develops into a fruiting body which consists of spore and stalk cells.

Abbreviations: cAMP, cyclic adenosine 3,5-monophosphate; cGMP, cyclic guanosine 3,5-monophosphate; IP_3, D-myo inositol 1,4,5-trisphosphate; *stm*F, streamer F mutant

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Binding of cAMP to its specific cell surface receptors triggers the extension of pseudopodia in the direction of the chemoattractant. In amoeboid movement, the motive force is supposed to be generated by ATP-dependent dynamic sliding of conventional myosin filaments on plasma membrane-bound actin fibers [2]. Pseudopod extension takes place within 10 s after chemotactic stimulation. Therefore, the second messengers that regulate the actomyosin system should be rapidly formed after chemotactic stimulation.

The fast elevations of Ca^{2+} and cGMP in the cytosol upon cAMP-stimulation are identified as second messengers for chemotaxis. Cytosolic Ca^{2+} is thought to provide polymerization of actin and translocation of actin filaments to the plasma membrane [3]. On the other hand, cGMP was found to be important for the phosphorylation of three threonines in the tail region of conventional myosin [4,5]. This phosphorylation is important for the effective interaction with actin fibers because binding of myosin to actin fibers depends on the filamentous state of myosin [6], which is provided by self-assembly at its coiled-coil tail and inhibited by cGMP-mediated phosphorylation. The role of cGMP was identified with the help of chemotactic mutants. The non-chemotactic mutants KI-8 and KI-10 show no cAMP-mediated cGMP response [7] and exhibit no increase of the phosphorylation of conventional myosin ([8], Kuwayama and van Haastert, unpublished observation). Conversely, *stm*F mutants, which lack cGMP phosphodiesterase activity, show a prolonged myosin phosphorylation in accordance with the enhanced cGMP response upon cAMP stimulation [5].

The rapid elevation of cytosolic Ca^{2+} can be promoted either by release from internal Ca^{2+} stores via IP_3 [9] or by influx of extracellular Ca^{2+} across the plasma membrane [10]. The release of Ca^{2+} by IP_3 is suggested not to be important for chemotaxis, because a mutant which lacks the IP_3-forming enzyme phospholipase C exhibits relatively normal chemotaxis to folic acid and cAMP [11]. Therefore, the influx of Ca^{2+} may play an important role in chemotactic signal transduction.

It is still controversial how activation of the cAMP receptor leads to opening of the Ca^{2+} channel. In one mechanism, it has been proposed that cGMP formation leads to opening of the channel, while other experiments suggest that receptor-stimulated cGMP formation is not required for Ca^{2+} influx. The role of cGMP was suggested by the observation that mutant *stm*F showed a prolonged depletion of the extracellular Ca^{2+} concentration [12]. However, the role of cGMP was disputed using mutants with a deletion of the $G\alpha 2$ subunit of heterotrimeric G-proteins; upon cAMP stimulation these mutants show no cGMP response but still significant influx of $^{45}Ca^{2+}$ [13,14].

To clarify whether and how cGMP activates the Ca^{2+} channel, we measured cAMP-stimulated $^{45}Ca^{2+}$ influx with a sensitive Ca^{2+} uptake assay using three cGMP mutants, *stm*F with normal levels of cAMP receptors and enhanced cGMP formation, mutant KI-7 with low levels of cAMP receptors and also enhanced cGMP formation, and mutant KI-8 which lacks nearly all guanylyl cyclase activity [15]. The results demonstrate that cGMP formation is neither sufficient nor essential, but that it potentiates two- to four-fold the receptor-stimulated influx of $^{45}Ca^{2+}$.

2. Materials and methods

2.1. Materials

$^{45}CaCl_2$ (10.9 mCi/mg Ca^{2+}; 1Ci = 37GBq) was obtained from Amersham. cAMP and cGMP were from Boehringer Manheim, Germany.

2.2. Strains and culture conditions

KI-7, KI-8 [15], *stm*F mutant NP368 [16] and their parental strain XP55 were grown on 1/3 SM plate (0.3% glucose, 0.3% bactopeptone, 1.5% agar and 40 mM KH_2PO_4/Na_2HPO_4, pH 6.0) with *Escherichia coli* B/r. Cells were harvested in the late logarithmic phase with 10 mM KH_2PO_4/Na_2HPO_4, pH 6.5 (PB). Bacteria were removed by three centrifugations at $300 \times g$ for 3 min. Cells were starved for 5 h by shaking in PB at a density of 10^7 cells/ml at 21°C.

2.3. Ca^{2+} uptake assay

The method for measuring Ca^{2+} uptake was based on the previously described assay with a few modifications [13,14]. In this assay, cells are incubated with radioactive $^{45}Ca^{2+}$ for a specified time, followed by

determination of the cell-associated radioactivity. Thus, in this assay, we measure the balance between influx of extracellular $^{45}Ca^{2+}$ and the efflux of some of the $^{45}Ca^{2+}$ that have entered the cell in the previous period. When the incubation period is very short, uptake is identical to influx. It should be mentioned that no information is obtained about the intracellular localization of the $^{45}Ca^{2+}$ taken up by the cells.

Cells harvested after starvation were washed and re-suspended in H buffer (20 mM Hepes/KOH, 5 mM KCl, pH 7.0) at a density of 10^8 cells/ml and aerated at 21°C for 10 min. The reactions were started by adding 100 μl of the cell suspension to the same volume of an uptake mixture (20 mM Hepes/KOH, 5 mM KCl, 10 μM $CaCl_2$ and 0.25 μCi $^{45}CaCl_2$, pH 7.0) with or without 10 μM cAMP. The reactions were terminated by adding 800 μl of ice-cold H buffer containing 12.5 mM $CaCl_2$. The cell suspensions were centrifuged immediately for 4 s at $16\,000 \times g$. The supernatants were aspirated and the pellets were re-suspended in 100 μl of H buffer. After addition of scintillator the radioactivity was determined. Nonspecific $^{45}Ca^{2+}$-binding was measured by adding cells to an uptake mixture containing 10 mM $CaCl_2$. Receptor-stimulated $^{45}Ca^{2+}$ uptake was calculated for each time point by subtracting the amount of $^{45}Ca^{2+}$ uptake in resting cells from the $^{45}Ca^{2+}$ uptake in cAMP-stimulated cells. The amount of $^{45}Ca^{2+}$ taken up by resting wild type and the three mutant cells was not significantly different from each other.

For measuring the $^{45}Ca^{2+}$ uptake rate, 100 μl of the cells were stimulated with 10 μM cAMP at $t = 0$ s. The $^{45}Ca^{2+}$ uptake mixture was added at $t = x$ s, and the reaction was terminated at $t = x + 10$ s. Thus, at different times after cAMP stimulation we measured the amount of $^{45}Ca^{2+}$ influx during 10 s.

2.4. Assays for cGMP response and surface cAMP receptors

cAMP-induced cGMP accumulation and cell surface cAMP receptor were assayed with the isotope dilution assay and the ammonium sulfate assay, respectively, as previously described [17,18]. In the ammonium sulfate assay, all the cell surface cAMP-receptors are stabilized, thereby total cAMP-binding activity is measured.

3. Results

3.1. Ca^{2+} uptake in a stmF mutant by cAMP stimulation

*Stm*F mutants were isolated as strains exhibiting long streams during aggregation due to a prolonged chemotactic elongation of the amoeboid cells [16]. Detailed studies revealed an enhanced and prolonged cGMP formation upon cAMP stimulation in this mutant, due to the reduced activity of a cGMP-specific phosphodiesterase (Fig. 1). In previous experiments, Ca^{2+} transport was measured in *stm*F mutants by monitoring the extracellular free Ca^{2+} concentration with a Ca^{2+} sensitive electrode demonstrating an enhanced and prolonged depletion of extracellular Ca^{2+} uptake. The kinetics of this depletion coordinated with the prolonged cGMP response in *stm*F mutants, leading to the hypothesis that cGMP opens the Ca^{2+} channel [12].

We determined Ca^{2+} uptake in *stm*F mutant NP368 by adding $^{45}Ca^{2+}$ to the cell suspension and measuring the radioactivity taken up by cells (see Section 2). In wild-type XP55 cells, stimulation of $^{45}Ca^{2+}$ influx by cAMP started within 5 s and lasted for 30 s. Then the level of receptor-stimulated $^{45}Ca^{2+}$ taken up by the cells gradually decreased due to its release to the medium. In *stm*F mutant NP368, more

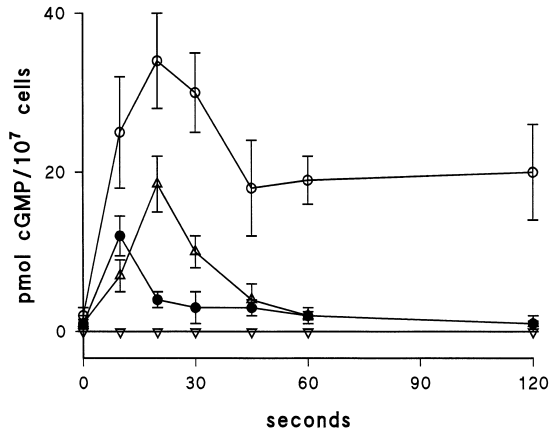

Fig. 1. Time course of cAMP-stimulated cGMP accumulation of wild-type XP55 (●), *stm*F NP368 (○), KI-7 (△) and KI-8 (▽) cells. Starved cells were stimulated with 10 μM cAMP and assayed for cGMP accumulation as described in Section 2. Error bars represent standard deviations of two independent experiments with triplicate determinations.

$^{45}Ca^{2+}$ was taken up than in XP55; at 30 s after receptor stimulation, uptake was about 2.4-fold higher in *stm*F. As the increased uptake is not simply due to an increase in the number of cAMP receptors (Table 1), and because deletion of cGMP phosphodiesterase is the only identified mutation in *stm*F, the results suggest that the increased $^{45}Ca^{2+}$ uptake is mediated by the enhanced cGMP formation. This conclusion is consistent with the observed enhanced depletion of extracellular Ca^{2+} upon stimulation of *stm*F cells with cAMP [12].

3.2. Ca^{2+} influx rate in a stmF mutant

To investigate further how cGMP regulates Ca^{2+} uptake after cAMP stimulation, we measured the rate of Ca^{2+} influx in wild-type XP55 and NP368 cells. Uptake of $^{45}Ca^{2+}$ is the difference between influx and efflux. When the incubation with $^{45}Ca^{2+}$ is very short, not enough time and intracellular $^{45}Ca^{2+}$ are available to provide substantial efflux; thus, $^{45}Ca^{2+}$ uptake is nearly identical to $^{45}Ca^{2+}$ influx. Cells were stimulated with cAMP and incubated with $^{45}Ca^{2+}$ for only 10 s; the incubation with $^{45}Ca^{2+}$ started at different times before or after cAMP stimulation. Thus, the Ca^{2+} influx rate was determined during cAMP stimulation of the cells. The results (Fig. 3) reveal that cAMP stimulates the Ca^{2+} influx rate to a maximum obtained at 10–20 s after stimulation; the influx rate recovers basal levels at about 60 s. In *stm*F mutant NP368 the maximal influx rate is about 2.8-fold higher than in wild-type cells. However, the kinetics of the response is not different from that of wild-type cells, since the maximal Ca^{2+} influx rate is still reached at 10–20 s and basal levels are recovered in 60 s (Fig. 3). This observation is not consistent with previous experiments showing that cAMP induces a persistent depletion of the extracellular Ca^{2+} concentration in *stm*F mutant cells [12]. However, it could be consistent if intracellular cGMP inhibits the efflux of Ca^{2+}.

By integration of the $^{45}Ca^{2+}$ influx rate from Fig. 3 over time, it is possible to calculate for each time point the amount of $^{45}Ca^{2+}$ that has entered the cell when $^{45}Ca^{2+}$ would have been present continuously. This calculated $^{45}Ca^{2+}$ influx is then compared with the actual measured $^{45}Ca^{2+}$ uptake in Fig. 2, the difference being the $^{45}Ca^{2+}$ efflux. The results of this calculation is shown in Fig. 4, revealing that during the first 60 s after cAMP stimulation, the calculated efflux is essentially identical for wild-type and *stm*F. Afterwards, the calculated efflux in *stm*F is about 1.7-fold higher than that of wild-type cells. This enhanced efflux is probably related to the higher intracellular level of $^{45}Ca^{2+}$ in *stm*F. This calculation does not provide any evidence that intracellular cGMP inhibits the efflux of Ca^{2+}.

3.3. Ca^{2+} uptake in mutant KI-7

The observation in Fig. 3 that at 60 s after stimulation, high concentration of cGMP still exists in mutant *stm*F while the Ca^{2+} influx rate returned to basal levels suggests that either cGMP alone cannot open the Ca^{2+} channel or that the channel is actively closed by a mechanism independent of cGMP. We have selected mutant KI-7 for further investigations on the role of cGMP for opening the plasma membrane Ca^{2+} channel. KI-7 is a non-chemotactic mu-

Table 1
cGMP formation, cAMP binding and Ca^{2+} uptake in cGMP mutants

Strain	cGMP response % to XP55	cAMP binding % to XP55	Ca^{2+} uptake		Ca^{2+} uptake/cAMP binding	
			pmol/10^7 cells	(% to XP55)	Ratio	(% to XP55)
XP55	100 ± 18	100 ± 7.6	69.2 ± 7.7	(100 ± 11)	0.69 ± 0.09	(100 ± 13)
NP368	283 ± 50	93 ± 11	103.2 ± 9.9	(149 ± 15)	1.11 ± 0.14	(161 ± 20)
KI-7	154 ± 29	14 ± 5.0	1.8 ± 0.8	(2.6 ± 1.2)	0.13 ± 0.07	(18 ± 11)
KI-8	0	32 ± 8.4	13.3 ± 3.0	(19.2 ± 4.0)	0.42 ± 0.15	(61 ± 23)

The data for the cGMP response were obtained from Fig. 1; Surface cAMP binding sites were determined as described in Section 2; cAMP-stimulated Ca^{2+} uptake was measured for 15 s after stimulation with cAMP as shown in Fig. 2. The values of 100% in cGMP response and cAMP binding are 12.5 pmol/10^7 cells and 50 pmol/10^7 cells, respectively. All data represent two independent experiments with triplicate determinations.

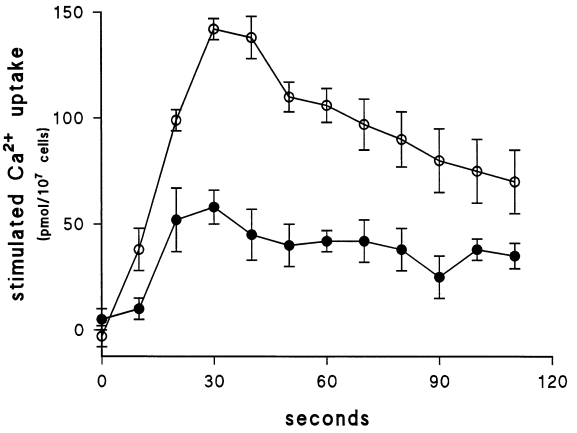

Fig. 2. Time course of cAMP-stimulated Ca^{2+} uptake by wild-type XP55 (●) and *stm*F NP368 (○) cells. Starved cells were assayed for $^{45}Ca^{2+}$ uptake using standard conditions as described in Section 2. Ca^{2+} uptake was measured in nonstimulated and cAMP-stimulated cells; the data shown were obtained by subtraction of basal Ca^{2+} uptake from receptor-stimulated Ca^{2+} uptake at each time point. Error bars represent standard deviations of two independent experiments with triplicate determinations. Basal Ca^{2+} uptake was about 6 pmol/10^7 cells 10 s for XP55 and NP368.

tant which has low levels of cAMP receptors (Table 1). Nevertheless, it accumulates more cGMP than wild-type cells (Fig. 1), because the receptor-stimu-

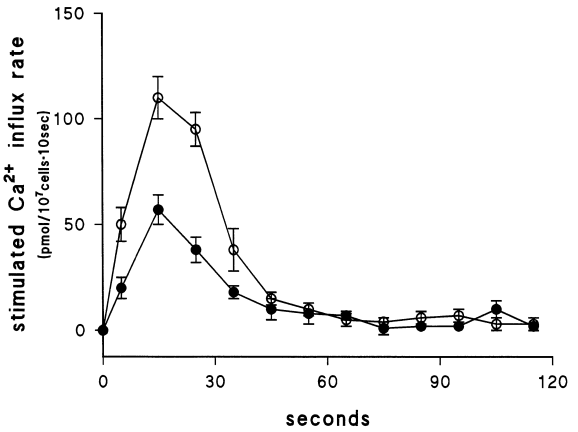

Fig. 3. Time course of the cAMP-stimulated Ca^{2+} influx rate in XP55 (●) and NP368 (○) cells. Starved cells were assayed for $^{45}Ca^{2+}$ uptake during 10 s; the uptake started at different times after cAMP stimulation. Each value represents the Ca^{2+} influx/10 s from 5 s before till 5 s after each time point, where the time represents the period after cAMP stimulation. The influx rate of non-stimulated cells was subtracted from all data. Error bars represent standard deviations of two independent experiments with triplicate determinations.

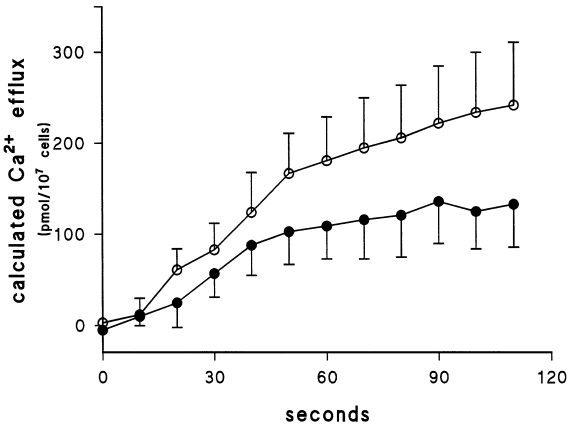

Fig. 4. Calculated efflux after cAMP stimulation of XP55 (●) and NP368 cells (○).The influx was calculated by integration of the data from Fig. 3 over time. The data shown are the difference between the calculated influx and the measured uptake from Fig. 2, being the calculated efflux.

lated guanylyl cyclase is not turned-off at 10 s after stimulation [19,7]. The uptake of $^{45}Ca^{2+}$ was measured in mutant KI-7 (Table 1), showing very little influx of $^{45}Ca^{2+}$. Even when expressed as Ca^{2+} uptake per cAMP binding sites, mutant KI-7 shows poor uptake. This observation provides additional evidence that cGMP alone is not sufficient to trigger the activation of Ca^{2+} influx.

3.4. Receptor-stimulated Ca^{2+} uptake in cGMP null mutant KI-8

To investigate whether intracellular cGMP is essential for the activation of Ca^{2+} influx, we measured $^{45}Ca^{2+}$ uptake in the non-chemotactic mutant KI-8 which has no detectable intracellular cGMP due to very low levels of guanylyl cyclase activity [15]. We observed that cAMP induces a significant increase of $^{45}Ca^{2+}$ uptake in comparison to uptake of nonstimulated cells (Table 1). In comparison to its parental strain XP55, cAMP-simulated $^{45}Ca^{2+}$ uptake is reduced; when corrected for the reduced level of cAMP surface receptors in KI-8, the receptor-stimulated uptake increases from $19 \pm 4.0\%$ to $61 \pm 23\%$ relative to wild-type cells (Table 1). These results indicate that cAMP can open the Ca^{2+} channel in the absence of cGMP formation, even in the absence of basal cGMP. However, the Ca^{2+} influx is significantly less than in wild-type cells; take together with the results

from mutant *stm*F, and KI-7 we conclude that cGMP does not directly activate the Ca^{2+} channel but that it potentiates the receptor-stimulated Ca^{2+} influx in *D. discoideum*.

4. Discussion

A sensitive $^{45}Ca^{2+}$ uptake assay was used in this study to investigate the role of cGMP on trans-plasma membrane Ca^{2+} uptake in *D. discoideum*. Previously this assay was used with a Gα2-null strain that over-expresses cAMP surface receptors showing that this strain possessed significant receptor-stimulated Ca^{2+} uptake ([14]). This observation indicates that Ca^{2+} uptake is not activated via the Gα2 subunit. Since Gα2-null cells show no cGMP response, this implies that Ca^{2+} uptake is not stimulated via cGMP accumulation. On the other hand, mutant *stm*F with an enhanced cGMP response shows an increased depletion of extracellular Ca^{2+} upon receptor stimulation, suggesting that cGMP regulates the Ca^{2+} channel. We reproduced this observation using the sensitive $^{45}Ca^{2+}$ uptake assay. These two seemingly contradictory observations could be explained if cGMP would inhibit the efflux of Ca^{2+}. Therefore, we carefully analysed the rate of $^{45}Ca^{2+}$ influx after cAMP stimulation, integrated the influx rate over time and compared this influx with the actually measured uptake. The difference between uptake and calculated influx is the efflux of $^{45}Ca^{2+}$. The data do not provide evidence that the efflux is inhibited in *stm*F.

The experiments measuring the $^{45}Ca^{2+}$ influx rate in *stm*F reveal that cGMP levels are still high at 60 s after cAMP stimulation, but that the influx rate has already returned to basal levels as in wild-type cells. The conclusion that cGMP alone cannot open the Ca^{2+} channel is supported by observations with mutant KI-7. Considering the low levels of surface cAMP receptors, this mutant shows a very large cGMP response, but a very small receptor-stimulated Ca^{2+} uptake. Finally, we used mutant KI-8 to see if cGMP is essential at all for opening of the Ca^{2+} channel; this mutant has very low guanylyl cyclase activity, and therefore no basal or receptor-stimulated cGMP. We observed significant receptor-stimulated Ca^{2+} uptake in KI-8, demonstrating that cGMP is not essential. Quantitative analysis of the data reveal $19 \pm 4.0\%$ receptor-stimulated $^{45}Ca^{2+}$ uptake relative to wild-type cells. It has been demonstrated for Gα2-null cells that the amount of $^{45}Ca^{2+}$ uptake is proportional to the level of surface cAMP receptors ([14]). Thus, over-expression of the cAMP receptor CAR1 in Gα2-null cells leads to an increase of the receptor-stimulate Ca^{2+} influx. The low levels of receptors may at least partially explain the reduced Ca^{2+} uptake in KI-8. The data show that cAMP induces $61 \pm 23\%$ Ca^{2+} uptake per cAMP receptor if compared to wild-type cells. The previous study with Gα2-null/CAR1 over-expressing strain also reveal a Ca^{2+} uptake per cAMP receptor that was about half as much as in wild-type cells [14]. Both observation suggest that cGMP is not essential but potentiates receptor-stimulated Ca^{2+} uptake. This is supported by the observation that in *stm*F, the amount of receptor-stimulated Ca^{2+} uptake is enhanced without a change of kinetics. In summary, cGMP alone cannot induce Ca^{2+} influx (KI-7 and *stm*F at 1 min after stimulation), cGMP is not essential (KI-8 and Gα2-null cells), but Ca^{2+} uptake is reduced two-fold when cGMP is absent (KI-8) and enhanced two-fold when large a cGMP formation is induced (*stm*F). Thus, the cAMP receptor opens the Ca^{2+} channel in a G-protein-independent way; the Ca^{2+} influx is potentiated by receptor-stimulated cGMP formation, which requires an active G-protein (Fig. 5). Several mechanisms could explain how cGMP may stimulate Ca^{2+} influx; it could prolong the mean opening time of the channel or it may stimulate the current.

In smooth muscle cells, evidence was obtained that cGMP may lower cytosolic Ca^{2+} concentration either by stimulation of Ca^{2+}-ATPase [20] or by inhibition of IP_3-mediated Ca^{2+} release from internal stores [21] leading to relaxation of muscle cells (reviewed in Ref. [22]). Regardless of the mechanism, the effect of cGMP on lowering cytosolic Ca^{2+} is mediated by cGMP-dependent protein kinase. In vertebrate photoreceptor rod outer segments, light-stimulated hydrolysis of cGMP leads to closing of the Ca^{2+} ion channel. This reaction is independent of cGMP-dependent protein kinase activity. cGMP binds directly to the ion channel at the carboxyl-terminal region leading to opening of the channel [23]. In *Dictyostelium*, a similar mechanism may exist as in photoreceptors, except that the channel is opened by

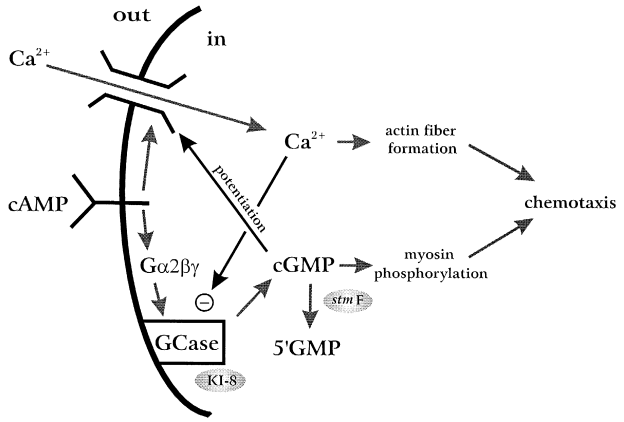

Fig. 5. Model of the regulation of Ca^{2+} uptake. opening of the Ca^{2+} channel is activated by the surface cAMP receptor via a G-protein-independent pathway. Guanylyl cyclase (GCase) is activated via the G-protein Gα2. Produced cGMP potentiates Ca^{2+} influx through the receptor-operated Ca^{2+} channel. Increased level of intracellular Ca^{2+} inhibits the activity of guanylyl cyclase. For the interaction of cytoskeletal protein, Ca^{2+} and cGMP trigger polymerization of actin and phosphorylation of conventional myosin tail, respectively, thought to lead to the formation of local pseudopodia.

the surface receptor and that cGMP only potentiates. Interestingly, in both vertebrate photoreceptor cells and *Dictyostelium*, Ca^{2+} inhibits guanylyl cyclase activity at physiological concentration in vitro [24]. This dual regulatory loop (Fig. 5) composed of cGMP-potentiated Ca^{2+} influx and Ca^{2+}-mediated inhibition of cGMP synthesis may allow the fast increase and rapid decrease of intracellular cGMP and Ca^{2+} levels. This fine-tuned regulation may be instrumental for the local fluctuations of Ca^{2+} and cGMP, and thereby participate in the regulation of directed pseudopod extensions.

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