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Antagonistic Effects of Signal Transduction by Intracellular and Extracellular cAMP on Gene Regulation in *Dictyostelium*

Ingrid Endl, Angelika Konzok, and Wolfgang Nellen*

Max-Planck-Institut f. Biochemie, D-82152 Martinsried, and University of Kassel, Department of Genetics, D-34109 Kassel, Germany

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In *Dictyostelium*, cAMP plays a role as an intracellular second messenger and in addition, as an extracellular first messenger. Both functions are thought to be tightly linked because adenylyl cyclase is coupled via G-proteins to the cell surface cAMP receptor cAR 1. Using the discoidin I gene family as a molecular marker for the first stages of development, we show here that induction of transcription requires the G-protein subunit $\alpha 2$ and thus an as yet unidentified surface receptor, CRAC (cytosolic regulator of adenylyl cyclase), and PKA. Induction can be conferred by an increase in intracellular cAMP. In contrast, transcriptional down-regulation occurs by stimulation of cAR 1 with extracellular cAMP and a subsequent, G-protein-independent Ca^{2+} influx. In a G$\alpha 2$ gene disruption mutant, discoidin I expression can be efficiently modulated by analogues simulating intracellular cAMP (discoidin induction) and extracellular cAMP (discoidin down-regulation). We thus demonstrate possible antagonistic functions of intra- and extracellular cAMP.

INTRODUCTION

In *Dictyostelium*, like in other eukaryotes, cAMP plays a central role as an intracellular second messenger that can activate PKA. In addition, extracellular cAMP serves a hormone-like function and stimulates cAMP cell surface receptors (cARs). By G-protein–mediated signal transduction, ligand binding activates various effector enzymes including PLC and adenylyl cyclase. Extracellular cAMP may thus increase the levels of intracellular cAMP. Some developmental processes are controlled by PKA, suggesting that regulation of intracellular cAMP concentration is a key factor in modulating gene expression (Harwood *et al.*, 1992). On the other hand, it has been shown that extracellular cAMP is sufficient to restore developmental gene expression in a mutant lacking the major adenylyl cyclase ACA (Pitt *et al.*, 1993). More recently, there have been reports of cAR-mediated, but G-protein–independent signaling pathways, especially Ca^{2+} influx (Milne and Devreotes, 1993; Schlatterer *et al.*, 1994).

The discoidins are cyptoplasmic proteins expressed several generations before the onset of starvation and are required for streaming of cells to aggregation centers (Alexander *et al.*, 1983; Crowley *et al.*, 1985). It has also been shown that a lack of discoidin I prevents cell elongation. This is consistent with the inability to form ordered streams and may indicate an involvement of the discoidins in the organization of the cytoskeleton (Alexander *et al.*, 1992).

Regulation of the discoidin I gene family is an excellent indicator for signal transduction events involved in very early gene expression (growth-differentiation transition, in contrast to the later event of aggregation; see also DISCUSSION). Several mutants with defects in discoidin regulation have been isolated and partially analyzed (Alexander *et al.*, 1983, 1986; Wetterauer *et al.*, 1993; Konzok, Zeng, and Nellen, unpublished data), but so far the mutations could not be attributed to specific components in regulatory pathways. We have previously dissected the promoter of the discoidin Iγ gene, identified some of the ele-

* Corresponding author: University of Kassel, Department of Genetics FB19, Heinrich-Plett-Str. 40, D-34109 Kassel, Germany.

ments involved in discoidin regulation, and correlated them with extracellular signals (Vauti *et al.*, 1990; Blusch *et al.*, 1992, 1995; Blusch and Nellen, 1994). Here we specifically investigate the pathways leading to developmental induction and cAMP mediated down-regulation of discoidin expression.

Discoidin is first induced at low levels (prestarvation response) by the extracellular "prestarvation factor" PSF (Clarke *et al.*, 1987); subsequently, a developmental signal, probably the secreted "conditioned media factor" CMF (Clarke *et al.*, 1992; Blusch *et al.*, 1995), mediates high level expression. Most likely, the low PSF-mediated induction is not required for the following, strong developmental induction: cells from PSF-prestimulated cultures reach the same levels of discoidin expression at the same time of development as cells from low density cultures that have not yet accumulated PSF (Huitl and Nellen, unpublished observations).

Surprisingly, the developmental discoidin induction, which is independent of cAR 1, requires the G-protein $\alpha 2$ subunit (Blusch *et al.*, 1995) known to participate predominantly in signaling mediated by extracellular cAMP via cAR 1.

Several hours after the onset of development, transcription of the discoidin genes is down-regulated by cAMP via the cell surface receptor cAR 1. As we show here, down-regulation is independent of $G\alpha 2$. This results in the unexpected situation that the cAMP mediated down-regulation pathway does not require the $G\alpha 2$ subunit thought to be linked to extracellular cAMP signaling in early development, while $G\alpha 2$ is required in the induction pathway that is independent of cAR 1 and extracellular cAMP. Both pathways therefore deviate substantially from the classical view of signal transduction in *Dictyostelium*.

Using various mutant strains, cAMP analogues, and drugs, we show here that discoidin induction is mediated by intracellular cAMP and requires PKA. Down-regulation is conferred by stimulation of cAR 1 with extracellular cAMP, subsequent Ca^{2+} influx, and an additional, unknown signal.

MATERIALS AND METHODS

Dictyostelium cells were freshly inoculated from frozen stocks at least every 3 wk and were kept on plates on a lawn of *Klebsiella aerogenes*. For mutant strains, the aggregation minus phenotype was always monitored. For the experiments, cells were grown in suspension culture in association with *Klebsiella aerogenes* as described by Blusch *et al.* (1995). Cells were harvested at a maximum density of 2×10^6/ml. For development, cells were washed free of bacteria by differential centrifugation and resuspended in 17 mM potassium phosphate buffer (pH 6.9) at a density of 2×10^7 cells/ml. Development was done for 4.5 h (unless otherwise indicated) in shaking culture with the additions specified in the text. 2′ deoxy (2′H) cAMP, 8-bromo (8-Br) cAMP and A23187 were purchased from Sigma (St. Louis, MO).

RNA was prepared as described previously (Maniak *et al.*, 1989). Ten micrograms of total cellular RNA were separated on a denaturing gel containing formaldehyde, blotted to a nylon membrane, and hybridized with a discoidin-specific, ^{32}P-labeled in vitro transcript. Equal loading of the lanes was confirmed by the intensity of ethidium bromide staining of ribosomal RNA.

RESULTS

We (Blusch *et al.*, 1995) and others (Clarke *et al.*, 1992) have previously suggested that the conditioned media factor CMF may be the extracellular signal mediating developmental induction of discoidin expression. Because we have shown that $G\alpha 2$ is required for induction (Blusch *et al.*, 1995) and because both $G\alpha 2$ as well as CMF are implicated in activation of adenylyl cyclase (Yuen *et al.*, 1995), we tested the possible involvement of intracellular cAMP in signaling discoidin induction.

Intracellular cAMP Can Induce Discoidin Expression

We first stimulated wild-type cells with the cAMP analogues 2′H-cAMP (receptor agonist) and 8-Br cAMP (PKA agonist) during development. Figure 1 shows that 2′H-cAMP can substitute for cAMP in down-regulating discoidin expression. This confirms our previous data (Blusch *et al.*, 1995) in which a disruption of the gene for the cAR 1 receptor impaired down-regulation. In contrast, 8-Br cAMP had no effect. This clearly demonstrated that extracellular but not intracellular cAMP was required for cAMP-mediated discoidin down-regulation. In addition, the result showed that the high concentrations of 8-Br cAMP did not activate the receptor because we never observed a decrease in discoidin mRNA accumulation as would then be expected.

However, the results did not indicate the predicted participation of intracellular cAMP in discoidin induction because we never observed any significant increase in RNA levels upon addition of 8-Br-cAMP. A possible explanation could be that expression was al-

Figure 1. A Northern blot shows the effect of 8-Br cAMP and 2′H-cAMP on discoidin mRNA expression in developing AX2 cells. Cells were cultured in bacterial suspension, then washed and set up for 4.5 h of development in suspension without or with the addition of 7 mM 8-Br cAMP and 0.1 mM 2′H-cAMP as indicated. RNA was prepared, separated on a denaturing gel, transferred to a membrane, and probed for discoidin mRNA. Although there was almost complete down-regulation in response to 2′H-cAMP, application of 8-Br cAMP had no significant effect on discoidin gene expression.

ready at the maximal level and a further induction was not possible.

When the Gα2$^-$ mutant JH104 is starved, a slight induction of discoidin expression, due to PSF, is usually observed but the subsequent strong developmental induction is lost (Blusch *et al.*, 1995).

Gα2 is known to be required for the activation of adenylyl cyclase and thus for the production of intracellular as well as extracellular cAMP. As explained above, extracellular cAMP could not be involved in discoidin induction because it mediated down-regulation. The Gα2 gene disruption strain allowed us to examine whether the defect in the G-protein could be bypassed by intracellular cAMP. Figure 2 shows that discoidin was induced in JH104 by the PKA agonist 8-Br cAMP but not by the receptor agonist 2'H-cAMP (our unpublished observations). The amount of discoidin mRNA was substantially increased compared with development without additions or with addition of cAMP, and reached almost wild-type levels. There was a dose-dependent response to 8-Br cAMP with requirement for high concentrations (3 mM or more) to obtain the maximal response. These concentrations are similar to those used by others (Kay, 1989; Riley *et al.*, 1989; Maeda, 1992) to induce spore differentiation in late development.

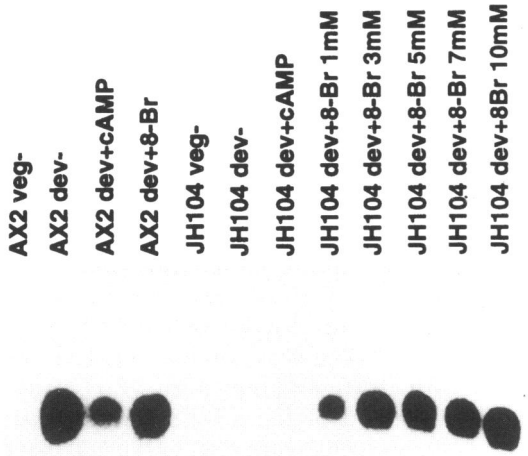

Figure 2. JH104 cells and, for comparison, wild-type AX2 cells were grown in bacterial suspension (veg-) and then developed shaking in phosphate buffer for 4.5 h (dev-). cAMP (0.1 mM) and 8-Br cAMP at the concentrations indicated were added respectively to the cultures at the onset of development. RNA was isolated and probed on a Northern blot as in Figure 1. Induction of discoidin by 8-Br cAMP in JH104 is slightly lower than the wild-type levels obtained in developing AX2 cells. The very low Gα2-independent, PSF-mediated induction of discoidin in developing cells without any additions (Blusch *et al.*, 1995) cannot be seen in this exposure.

Components of the Pathway to PKA Are Involved in Discoidin Induction

The data suggest that an extracellular signal triggers a Gα2-mediated increase in intracellular cAMP and induces discoidin expression. The signal is not known but CMF, which functions via a cell surface receptor (Jain and Gomer, 1994) and is involved in the activation of adenylyl-cyclase and guanylyl-cyclase (Yuen *et al.*, 1995), is a likely candidate. Adenylyl cyclase activation via receptors is mediated by the cytosolic factor CRAC (Insall *et al.*, 1994), which apparently couples the G-protein βγ subunits after their dissociation from Gα2. Yuen *et al.* (1995) have recently shown that CMF can induce this pathway.

A disruption of CRAC should therefore generate a similar phenotype as the Gα2 mutant in that discoidin would not be expressed above basal levels but should be inducible by 8-Br cAMP. We therefore tested discoidin expression in the CRAC$^-$ mutant dagA$^-$ (generously provided by R. Insall and P. Devreotes; Insall *et al.*, 1994). In accordance with our predictions, the mutant was very similar to the Gα2$^-$ strain (Figure 3): it expressed very low levels of discoidin and could be stimulated by 8-Br cAMP.

We then investigated the next step in this pathway by examining a disruption mutant of the adenylyl cyclase A gene (ACA$^-$, generously provided by P. Devreotes; Pitt *et al.*, 1992, 1993). We expected to find a similar phenotype as seen in the Gα2 and CRAC$^-$ mutants. Surprisingly, ACA$^-$ cells showed discoidin expression similar to wild-type cells (Figure 4): neither induction in development nor down-regulation by 2'H-cAMP was significantly impaired.

The data obtained with 8-Br cAMP in the Gα2 and CRAC$^-$ mutants clearly indicated an induction path-

Figure 3. Discoidin mRNA expression in the CRAC$^-$ mutant dagA$^-$. Cells were grown and developed as described in Figure 1 with or without the addition of 7 mM 8-Br cAMP. The autoradiogram can be directly compared with the AX2 RNA shown in Figure 1 because both sets of samples have been analyzed on the same gel. Similar to the data in Figure 2, induction by 8-Br cAMP is slightly lower than that seen in developing AX2 cells. The weak signal seen with RNA from developing dagA$^-$ cells is due to the Gα2-independent induction by PSF (Blusch *et al.*, 1995).

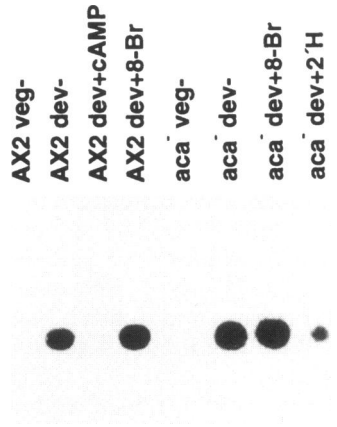

Figure 4. Discoidin gene expression in ACA$^-$ cells. Cells of the ACA$^-$ strain and, for comparison, of the wild-type AX2 strain were grown and developed as in the previous experiments. For AX2, 0.1 mM cAMP and 7 mM 8-Br cAMP, respectively, were added at the onset of development. For ACA$^-$ cells, 0.1 mM 2'H-cAMP and 7 mM 8-Br cAMP were tested. In both strains, the extracellular signal molecules efficiently down-regulated discoidin expression while 8-Br cAMP had no significant effect. Induction of discoidin mRNA accumulation in the ACA$^-$ cells was indistinguishable from wild-type levels.

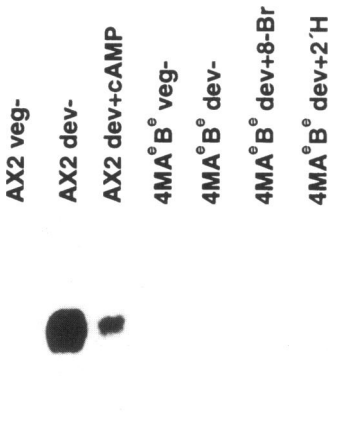

Figure 5. AX2 cells and the mutant strain 4 MA^{E}B^E were grown and developed as for the other experiments. Addition of 8-Br cAMP (7 mM) and 2'H-cAMP (0.1 mM) had no effect on the lack of discoidin expression in the mutant.

way via intracellular cAMP but the ACA$^-$ mutant failed to confirm this.

A second adenylyl-cyclase, ACG, has been cloned from *Dictyostelium* but this enzyme is only expressed in germinating spores and is most likely independent of G-proteins (Pitt *et al.*, 1992). ACG was thus unlikely to be involved in discoidin regulation. A possible explanation for an intracellular cAMP effect that does not require ACA would be the presence of an as yet undetected adenylyl-cyclase (ACX in Figure 8).

Assuming that PKA is the target for cAMP, we tested a strain overexpressing a mutant regulatory (R) subunit of PKA (4 MA^{E}B^E, generously provided by M. Veron; Harwood *et al.*, 1992). This R-subunit still binds to the catalytic subunit but not to cAMP and thus renders PKA constitutively inactive. As shown in Figure 5, the mutant cells do not express discoidin at all and, as expected, cannot be activated by 8-Br cAMP. We also observed that, in contrast to CRAC$^-$ and Gα2$^-$ mutants, overexpressors of PKA-R-A^{E}B^E did not, even in long exposures of the autoradiogram, show the low levels of discoidin expression due to PSF induction (see e.g. Figure 3 and Blusch *et al.*, 1995). This result is supported by data from Clarke's group who found that PKA is also required for PSF-mediated induction (Burdine and Clarke, 1995). As explained in the introduction, it is unlikely that the low, PSF-induced discoidin levels are a requirement for full developmental discoidin induction. We therefore believe that the failure of the PKA mutant strain to express discoidin is not a secondary effect due to the lack of induction by PSF but rather that PKA is a common component of both pathways.

Down-regulation of Discoidin Is Independent of Gα2

With most of the known signal transduction components involved in the induction pathway, it was unclear how the antagonistic pathway, down-regulation by cAMP, was mediated.

Down-regulation clearly required cAR 1 (Figure 1; Blusch *et al.*, 1995). cAR 1 is usually implicated in Gα2-mediated signal transduction; however, our previous data and the results presented here made it difficult to explain how the same G-protein could be simultaneously involved in both the positive and the negative regulation of discoidin expression. The Gα2$^-$ mutant allowed us to determine whether Gα2 was necessary for discoidin down-regulation.

We induced JH104 cells at the onset of development with 7 mM 8-Br cAMP and then added 2'H-cAMP 1 or 2 h later for the rest of the 4.5-h period of development. As controls, cells were also harvested after 1 or 2 h of development in the presence of 8-Br cAMP alone. The data in Figure 6 show that after 1 and 2 h of induction with 8-Br cAMP, substantial amounts of discoidin mRNA have accumulated. Subsequent treatment with 2'H-cAMP reduced this mRNA to undetectable levels. This clearly demonstrated that discoidin down-regulation by extracellular cAMP did not require the G-protein α2. Although it has previously been shown that down-regulation of discoidin occurs on the transcriptional level (Devine *et al.*, 1982), this has not been confirmed for this specific experimental setup, and we cannot exclude a reduction in mRNA stability as reported for gp80 by Mendez Souza *et al.* (1994).

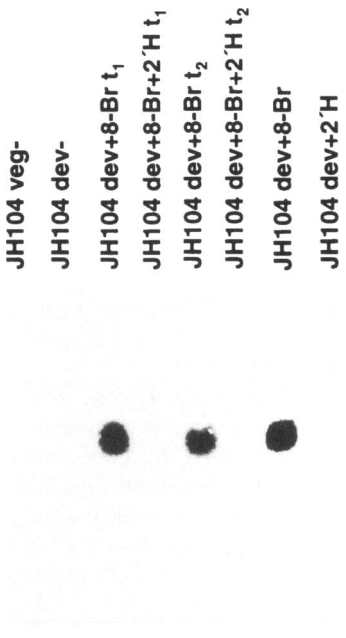

Figure 6. Induction and down-regulation in JH104 by 8-Br cAMP and 2'H-cAMP. Cells were grown and developed as described for the other experiments. 8-Br cAMP was added at a concentration of 7 mM at the onset of development. For lanes 2'H t1 and 2'H t2, 2'H cAMP was added at 0.1 mM, 1 or 2 h, respectively, after the beginning of starvation. Except for lanes 8-Br t1 (harvest 1 h after the onset of development) and 8-Br t2 (harvest 2 h after the onset of development), cells were shaken for a total of 4.5 h in starvation buffer. Lanes labeled 8-Br and 2'H show RNA from JH104 cells developed in the presence of the respective analogue alone for 4.5 h. The very low Gα2-independent, PSF-mediated induction of discoidin in developing cells without any additions (Blusch *et al.*, 1995) cannot be seen in this exposure.

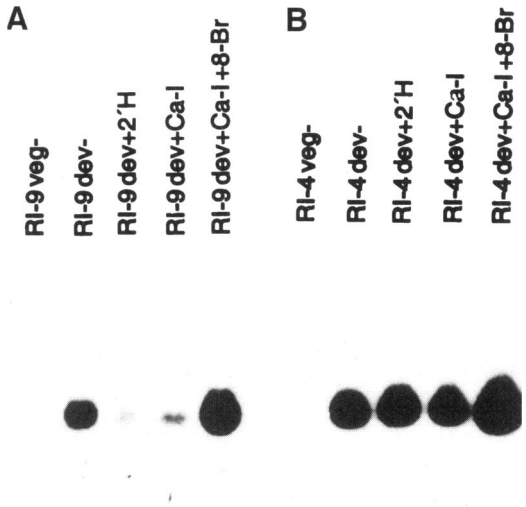

Figure 7. (A) Ca^{2+} influx mediates discoidin down-regulation in RI-9 cells supplemented with the cAR 1 gene. The RI-9 cells were generated by subsequent disruption of the cAR 1 and cAR 3 genes. The RI-9 rescue strain used here has the cAR 1 gene re-introduced and is only deficient for cAR 3 (Insall, personal communication). Cells were grown and developed as for the previous experiments. In development, cells were treated with 0.1 mM 2'H cAMP, 5 μM A23187 plus 1 mM Ca^{2+}, or 7 mM 8-Br cAMP as indicated. (B) Ca^{2+} influx is not sufficient to mediate discoidin down-regulation in RI-4 cells (cAR 1^-/cAR 3^-). The experiment was performed in the same way as the one shown in Figure 7A.

Ca^{2+} Can Bypass cAR 1

Repression of discoidin is mediated by high concentrations of extracellular cAMP (Vauti *et al.*, 1990), requires cAR 1, and is Gα2 independent. It is known that high concentrations of cAMP mediate a G-protein–independent Ca^{2+} influx via cAR 1 (Milne and Devreotes, 1993; Schlatterer *et al.*, 1994). We therefore tested whether Ca^{2+} could bypass the function of cAMP in repression of discoidin mRNA accumulation. Indeed, application of Ca^{2+} and the ionophore A23187 on wild-type cells resulted in a substantial repression of discoidin mRNA accumulation similar to that obtained with 2'H-cAMP (our unpublished data). The same was true for RI-9 cells (carrying gene disruptions for cAR 1 and cAR 3) that had been partially rescued by reintroducing the cAR 1 gene (Figure 7A). The results demonstrated that a Ca^{2+} influx was sufficient for discoidin down-regulation.

We then tried the same experiment on a mutant defective in the receptors cAR 1 and cAR 3 (RI-4, kindly provided by R. Insall and P. Devreotes). As expected, induction of discoidin expression was normal and there was no down-regulation by 2'H-cAMP. Surprisingly, however, Ca^{2+} together with the ionophore had no effect, indicating that the Ca^{2+} influx was not sufficient for the negative pathway in this strain (Figure 7B). We therefore assume that an additional component, generated by cAR 1, is required in conjunction with the Ca^{2+} influx to mediate down-regulation of discoidin.

A summary of the data is shown in the model in Figure 8.

DISCUSSION

Our data demonstrate an unexpected differential gene regulation by intracellular and extracellular cAMP and thus provide the first evidence for antagonistic effects of 1st and 2nd messenger cAMP in *Dictyostelium*. These results refer to pre-aggregation development, i.e., the growth-development transition. This is different from what is usually termed "early development," which refers to aggregating or at least aggregation-competent cells. Proteins that have been implicated to be absolutely necessary for early development because cells fail to aggregate when their genes have been disrupted (e.g. Gα2, cAR 1, ACA, Gβ etc.) are not

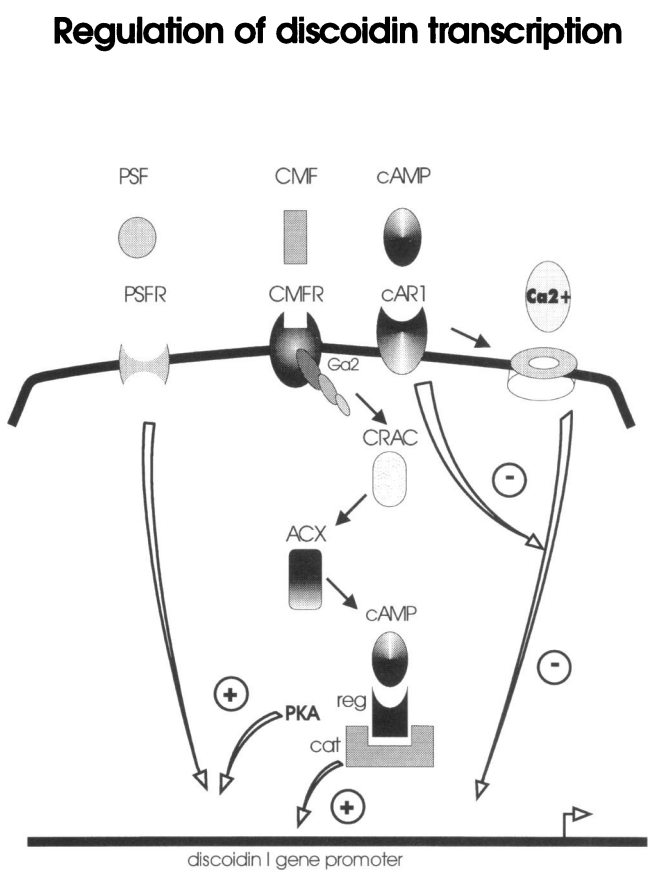

Figure 8. The model shows the four signal molecules PSF, CMF, cAMP, and Ca^{2+} discussed in this paper. The PSF receptor is speculative, Burdine and Clarke (1995) have so far only shown that G-proteins are not involved in transmission of the signal. The same paper describes a participation of PKA in discoidin induction by PSF. Developmental induction that is most likely initiated by CMF depends on a G-protein coupled receptor and requires Gα2, CRAC, and PKA. We failed to demonstrate the participation of an adenylyl cyclase even though our data suggest some activity generating intracellular cAMP. Therefore we included ACX as a yet unidentified adenylyl cyclase. PKA is required for discoidin induction. Repression of discoidin expression is conferred via cAR 1 and independent of Gα2. cAR 1-mediated Ca^{2+} influx in conjunction with an additional, unknown receptor-elicited signal, down-regulates discoidin. CRAC and ACX are drawn inside the cell only for clearer presentation.

strictly required for the growth-development transition, e.g. the regulation of the discoidin I gene family: cAR 1^- cells show normal discoidin induction, Gα2^- cells respond to intra- and extracellular cAMP, and ACA^- cells are normal in discoidin induction and down-regulation. It is therefore important to distinguish between early development and the growth-differentiation transition.

Our experiments were initiated by the previous finding that the G-protein α2 subunit, which was believed to predominantly couple to the cell surface receptor cAR 1 (reviewed in Firtel, 1991), was not involved in cAMP-mediated down-regulation of discoidin expression but rather in its up-regulation, and required a different signal and receptor (Blusch *et al.*, 1995). On the other hand we had shown that cAR 1 was required for down-regulation of discoidin expression. We therefore set up a regime (using the Gα2^- mutant) that demonstrated that intracellular cAMP could induce and extracellular cAMP could repress discoidin expression.

Further experiments shed some light on the signal transduction pathways involved in positive and negative discoidin regulation.

Disruption of the gene for CRAC, the cytosolic regulator of adenylyl cyclase, resulted in a phenotype similar to that seen in the Gα2 gene disruption strain. Matching results have been obtained previously by Drummond and Chisholm (1990), who showed that discoidin induction (except for low, probably PSF-induced levels) was impaired in the *synag 7* strain that fails to produce functional CRAC. CRAC has been suggested to activate adenylyl cyclase by coupling to the βγ subunits when they are released from the Gα2βγ complex (Insall *et al.*, 1994).

Induction of discoidin expression by the PKA agonist 8-Br cAMP implied a pathway mediated by PKA. Inactivation of the enzyme by overexpression of a mutant catalytic subunit that cannot bind cAMP, confirmed this assumption. Surprisingly, a disruption of the ACA gene, encoding the only or at least predominant adenylyl cyclase in growth and development, failed to show any effect on discoidin expression. To our knowledge, this is the first case where different phenotypes have been seen in ACA^- and in $CRAC^-$ cells. This suggests that CRAC may participate in additional pathways besides the activation of ACA.

Pitt *et al.* (1993) concluded from their experiments that intracellular cAMP produced by ACA was not required for developmental gene expression. Our data support this finding in that ACA^- cells are normal in respect to discoidin expression. On the other hand, we demonstrate that disruption of the pathway to adenylyl cyclase impairs discoidin regulation and that the intracellular cAMP analogue 8-Br cAMP can bypass these disruptions. Because ACA is not essential and because expression of ACG, the only other known adenylyl cyclase, is restricted to germinating spores (Pitt *et al.*, 1993), we have to postulate an additional and so far undetected adenylyl cyclase (ACX in Figure 8) to account for the function of intracellular cAMP in the regulation of discoidin expression. ACX would be induced by a similar pathway as ACA (including Gα2 and CRAC), but not necessarily by cAMP as ACA is. It may generate fluctuations in intracellular cAMP levels too low to be detected above the relatively high background intrinsic to cAMP measurements. In addition, ACX should be capable of increasing intracellular

cAMP levels without simultaneously increasing extracellular cAMP. As suggested by the experiment shown in Figure 6, extracellular cAMP would override the inducing effect of intracellular cAMP.

Another explanation for the failure of the ACA gene disruption strain to display the expected effect on discoidin regulation could be the ability of PKA to act independently of cAMP as proposed by Pitt *et al.* (1993), Mann and Firtel (1993), Insall *et al.* (1994), and Wu *et al.* (1995). However, this would not explain the lack of induction in strains defficient in signal transduction components leading to adenylyl cyclase or the specific stimulation of discoidin expression in response to 8-Br cAMP.

The possibility that 8-Br cAMP, when applied at high levels, might activate cGMP dependent pathways, can be ruled out; the $CRAC^-$ mutant that only induced discoidin expression upon 8-Br cAMP addition displays a normal cGMP response (Insall *et al.*, 1994).

It is not yet clear which signal and receptor mediates discoidin gene induction. There is accumulating evidence that it may be CMF and the CMF receptor. Our data demonstrate an involvement of Gα2 in discoidin induction and Gomer's group (Yuen *et al.*, 1995) has shown that adenylyl cyclase activation (dependent on Gα2 and mediated by CRAC) is impaired in a CMF antisense cell line. Unfortunately, we could not carry out experiments to prove the involvement of CMF in discoidin induction; in the available cell line, CMF antisense RNA is not expressed during growth on bacteria, and the cells therefore secrete wild-type levels of CMF upon starvation. It was therefore not possible to see if discoidin induction was lost in the mutant.

It has previously been shown that high levels of extracellular cAMP result in a receptor-mediated but G-protein–independent Ca^{2+} influx (Milne and Devreotes, 1993; Schlatterer *et al.*, 1994). Mendes Souza *et al.* (1995) demonstrated that this can be mimicked by Ca^{2+}/A23187. This could explain the divergence of pathways mediated by intra- and extracellular cAMP: high levels of cAMP cause Ca^{2+} influx, which represses discoidin. On the other hand, nanomolar pulses of cAMP, in the case of gp80/csA (Gerisch *et al.*, 1975), or possibly CMF signals in the case of discoidin, stimulate a G-protein–mediated pathway that results in gene induction.

There are some similarities between the signal transduction system described here and the one presented by the groups of Podgorski and Kessin (Wu *et al.*, 1995) for the regulation of the phosphodiesterase inhibitor gene. Like us, they find a requirement for PKA in phosphodiesterase inhibitor regulation but no requirement for ACA-mediated cAMP production.

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