© 2021. Published by The Company of Biologists Ltd | Journal of Cell Science (2021) 134, jcs258777. doi:10.1242/jcs.258777

SHORT REPORT

Cyclic AMP is dispensable for allorecognition in *Dictyostelium* cells overexpressing PKA-C

Shigenori Hirose[1,2], Mariko Katoh-Kurasawa[1] and Gad Shaulsky[1,*]

ABSTRACT

Allorecognition and tissue formation are interconnected processes that require signaling between matching pairs of the polymorphic transmembrane proteins TgrB1 and TgrC1 in *Dictyostelium*. Extracellular and intracellular cAMP signaling are essential to many developmental processes. The three adenylate cyclase genes, *acaA*, *acrA* and *acgA* are required for aggregation, culmination and spore dormancy, respectively, and some of their functions can be suppressed by activation of the cAMP-dependent protein kinase PKA. Previous studies have suggested that cAMP signaling might be dispensable for allorecognition and tissue formation, while others have argued that it is essential throughout development. Here, we show that allorecognition and tissue formation do not require cAMP production as long as PKA is active. We eliminated cAMP production by deleting the three adenylate cyclases and overexpressed PKA-C to enable aggregation. The cells exhibited cell polarization, tissue formation and cooperation with allotype-compatible wild-type cells, but not with incompatible cells. Therefore, TgrB1-TgrC1 signaling controls allorecognition and tissue formation, while cAMP is dispensable as long as PKA-C is overexpressed.

KEY WORDS: *Dictyostelium*, Cyclic AMP, Allorecognition, Tissue formation

INTRODUCTION

Cyclic adenosine monophosphate (cAMP) is a key regulator of *Dictyostelium discoideum* development (Loomis, 2014). The *D. discoideum* genome harbors three adenylate cyclase genes that encode structurally different enzymes, which are dispensable for growth but required at different developmental stages (Pitt et al., 1992; Soderbom et al., 1999; van Es et al., 1996). Deletion of *acaA*, which encodes the aggregation-stage enzyme, results in the absence of aggregation and subsequent development. Overexpression of the cAMP-dependent protein kinase catalytic subunit (PKA-C) in *acaA*$^-$ cells suppresses the aggregationless phenotype, leading to development with near-normal morphology and expression of developmental markers (Wang and Kuspa, 1997). The second gene, *acrA*, is dispensable for aggregation but required for terminal differentiation (Kim et al., 1998; Soderbom et al., 1999). Deletion of both *acaA* and *acrA* results in an aggregationless phenotype and absence of subsequent development. Overexpression of PKA-C suppresses the aggregationless phenotype and partially restores development, but not fruiting body formation or spore development (Anjard et al., 2001). Therefore, cAMP has an uncharacterized role in terminal differentiation in addition to activation of PKA-C. Moreover, cAMP signaling regulates developmental gene expression, but different combinations of *acaA* and *acrA* mutations indicate that intracellular and extracellular cAMP signaling regulate gene expression in different ways (Iranfar et al., 2003). The third adenylate cyclase gene, *acgA*, is expressed in prespore cells during terminal differentiation and is essential for spore dormancy under high osmolarity (van Es et al., 1996). Moreover, the combined activities of *acrA* and *acgA* are required for prespore differentiation (Alvarez-Curto et al., 2007). Addition of extracellular cAMP or ectopic expression of *acgA* in *acaA*$^-$ cells restores aggregation and subsequent development (Pitt et al., 1993, 1992), and ectopic expression of *acaA* in *acrA*$^-$ cells restores post-aggregative development (Anjard et al., 2001). Therefore, *D. discoideum* development involves a complex interplay between the three adenylate cyclase genes and their relationships with PKA-C and cAMP signaling.

cAMP waves propagate in exquisite patterns through the developing structures, starting at aggregation and continuing during slug migration and fruiting body formation (Singer et al., 2019). It is broadly accepted that cAMP signaling participates in almost every step in *D. discoideum* development, with a notable exception, which is the process of tissue formation and allorecognition that follows aggregation. After starvation, *D. discoideum* cells aggregate into mounds of ∼50,000 cells, using extracellular cAMP as a chemoattractant (Artemenko et al., 2014). Aggregation leads to streaming, in which polarized cells follow each other in a head-to-tail orientation using localized cAMP signals to coordinate motility (Kessin, 2001; Kriebel et al., 2003; Ross and Newell, 1981). Later, the cells form loose aggregates that rotate around themselves while beginning to express cell-type-specific genes and acquiring tissue properties (Kessin, 2001). During streaming and loose-aggregate formation, the cells begin to express the *tgrB1* and *tgrC1* genes that encode two transmembrane proteins (Benabentos et al., 2009). These proteins mediate cell–cell adhesion and are essential for morphogenesis beyond the loose aggregate stage and subsequent development and differentiation (Benabentos et al., 2009; Dynes et al., 1994; Kibler et al., 2003). These genes are highly polymorphic in natural populations of *D. discoideum* (Benabentos et al., 2009; Gruenheit et al., 2017; Ostrowski et al., 2015), and a matching pair of *tgrB1* and *tgrC1* alleles is necessary and sufficient for allorecognition (Benabentos et al., 2009; Hirose et al., 2011). Intriguingly, when a small proportions of cells with a given *tgrB1-tgrC1* allotype are developed in chimerae with a majority of cells of an incompatible allotype, the minority cells do not cooperate with the majority (Hirose et al., 2015). The minority cells fail to polarize and rotate in the same direction as the majority cells and they do not express cell-type-specific genes even though

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. [2]Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030, USA.

*Author for correspondence (gadi@bcm.edu)

S.H., 0000-0001-6626-295X; M.K.-K., 0000-0002-6404-485X; G.S., 0000-0002-0532-0551

Handling Editor: John Heath
Received 13 April 2021; Accepted 16 June 2021

Journal of Cell Science (2021) 134, jcs258777. doi:10.1242/jcs.258777

they are exposed to the same extracellular cAMP signals as the majority cells. Moreover, the incompatible minority cells retain the ability to polarize and their response to cAMP *in vitro* is indistinguishable from the response of the majority cells, indicating that cAMP signaling alone is not sufficient for rotational movement and subsequent development (Hirose et al., 2015). It is possible that cAMP signaling is temporarily replaced by TgrB1-TgrC1 signaling during tissue formation and allorecognition because these proteins function as a receptor–ligand pair in a signaling pathway that mediates development and allorecognition (Hirose et al., 2017).

The relationship between cAMP signaling and TgrB1-TgrC1 signaling has been explored in several contexts. Early studies have shown that PKA-C activation does not suppress the developmental defects caused by deletion of *tgrC1* (Iranfar et al., 2006). Examination of a Gß-null mutation that abrogates cAMP signaling has shown that cAMP-chemotaxis is dispensable for post-aggregative morphogenesis and confirmed that *tgrB1* and *tgrC1* are required for developmental progression (Kida et al., 2019). Another study used cAMP-imaging and an *acaA*$^{-}$ strain that was pulsed with extracellular cAMP to show that cAMP signaling is dispensable for collective cell movement during slug development (Hashimura et al., 2019). Finally, a study that used microfluidics and microsphere-based manipulations has shown that developing cells prefer the cell–cell contact TgrB1-TgrC1 signal to the soluble cAMP signal and that developmental cell sorting may involve differential responses of prespore and prestalk cells to the two signals (Fujimori et al., 2019).

The complexity of cAMP production by three enzymes, and the intricate relationships between the TgrB1-TgrC1 and cAMP signaling mechanisms prompted us to test cooperative cell migration and allorecognition in the complete absence of cAMP production. We generated null mutations in the three adenylate cyclase genes of *D. discoideum* and overexpressed PKA-C to facilitate aggregation and some post-aggregative development in the absence of cAMP production. We found that the cells were able to polarize and aggregate into rotating mounds, showing that cAMP production is dispensable for cooperative cell migration and tissue formation. We also found that the cells cooperated with wild-type cells of matching allotypes, and segregated from cells of a non-matching allotype, showing that cAMP production is dispensable for allorecognition.

RESULTS AND DISCUSSION

Development without cAMP

We generated a *D. discoideum* strain that cannot synthesize cAMP by insertional mutagenesis of the three known adenylate cyclase genes using homologous recombination and selectable marker recycling (Faix et al., 2004). We first mutated *acrA*, screened for strains that exhibited frail stalks and glassy sori (Soderbom et al., 1999) and validated the mutation by PCR. We then mutated *acgA* and validated by PCR, as there is no overt morphological defect (van Es et al., 1996). Finally, we mutated *acaA*, screened for aggregationless strains and validated by PCR. We also generated the *acaA*$^{-}$ mutation alone in the laboratory wild-type AX4. To test the level of cAMP production, we developed cells for 10 h, lysed them and measured the cAMP concentration. Fig. 1 shows that the *acaA*$^{-}$ cells produced low but measurable levels of cAMP at ∼16% of the wild-type level. The *acrA*$^{-}$*acgA*$^{-}$ cells produced ∼86% of the wild-type cAMP level, but the *AC3*^{KO} cells did not produce measurable levels of cAMP. Upon starvation on black nitrocellulose filters, the *AC3*^{KO} cells were also unable to aggregate (Fig. 2). Next,

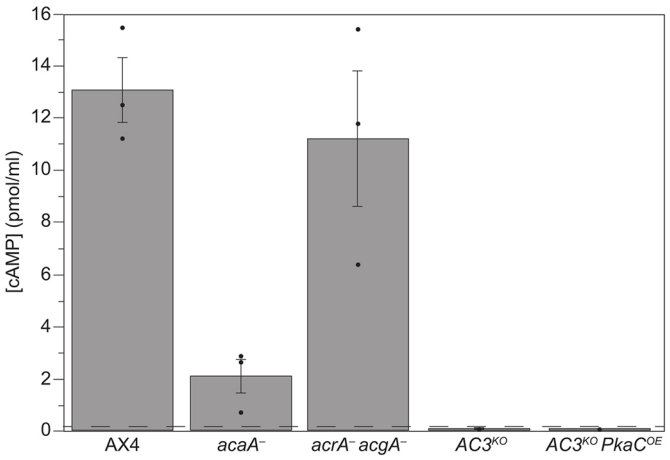

Fig. 1. cAMP production in adenylate cyclase mutant strains. We developed cells for 10 h on nitrocellulose filters, lysed them and measured the concentration of cAMP in the lysate with an ELISA assay (*y*-axis, pmol cAMP/ml). Bars represent the mean±s.e.m. of three independent replicates (except *AC3*^{KO}*PkaC*^{OE} that was measured in duplicates); dots represent the individual replicates. The horizontal dashed line represents the lowest limit of detection by the ELISA assay (0.078 pmol/ml) and the strain names are indicated below the *x*-axis.

we transformed the cells with a vector that encodes *pkaC* under the *act15* promoter (*pkaC*^{OE}). Fig. 2 shows that the *AC3*^{KO}*pkaC*^{OE} cells were capable of aggregation and mound formation, consistent with previous findings on *pkaC* overexpression in *acaA*$^{-}$ cells (Wang and Kuspa, 1997) and in *acaA*$^{-}$*acrA*$^{-}$ cells (Anjard et al., 2001). Comparison of the full developmental transcriptome of *AC3*^{KO}*pkaC*^{OE} to that of *acaA*$^{-}$*pkaC*^{OE} shows near identity (Katoh-Kurasawa et al., 2021), suggesting that the two strains exhibit similar developmental properties. Fig. S1 compares the morphological progression of *AC3*^{KO}*pkaC*^{OE} and AX4 cells that were grown and developed in association with bacteria. These data indicate that *AC3*^{KO}*pkaC*^{OE} cells are able to develop into tight aggregates like the wild type. Their development is attenuated after that stage, although a few of them form advanced structures, including fingers and fruiting bodies. We also compared the expression of *tgrB1* and *tgrC1* mRNA between *AC3*^{KO}*pkaC*^{OE} and AX4 using developmental transcriptome data (Katoh-Kurasawa et al., 2021). We found that the expression trajectory of the two genes was nearly identical in the two strains and the two genes remained co-regulated during development, although the mutant exhibited a twofold reduction in mRNA abundance at peak expression (Fig. S2). These phenotypes of *AC3*^{KO}*pkaC*^{OE} were not associated with restoration of cAMP production (Fig. 1). We conclude that *D. discoideum* cells are capable of aggregation and mound formation in the absence of cAMP production as long as *pkaC* is activated.

Tissue formation without cAMP

Rotational movement, cell polarization and head-to-tail organization are characteristics of the developmental transition from single-cell behavior to tissue formation in *D. discoideum* (Hirose et al., 2015). We generated tagged versions of *AC3*^{KO}*pkaC*^{OE} to measure these parameters. Fig. 3A and Movie 1 show rotational movement of *AC3*^{KO}*pkaC*^{OE} that express mCherry–H2B such that their nuclei are fluorescent. Not all the cells exhibit rotational movement, probably because aggregation of cells with little or no extracellular cAMP is more density dependent than the aggregation of wild-type cells (Wang and Kuspa, 1997), but the images are characteristic and

Journal of Cell Science (2021) 134, jcs258777. doi:10.1242/jcs.258777

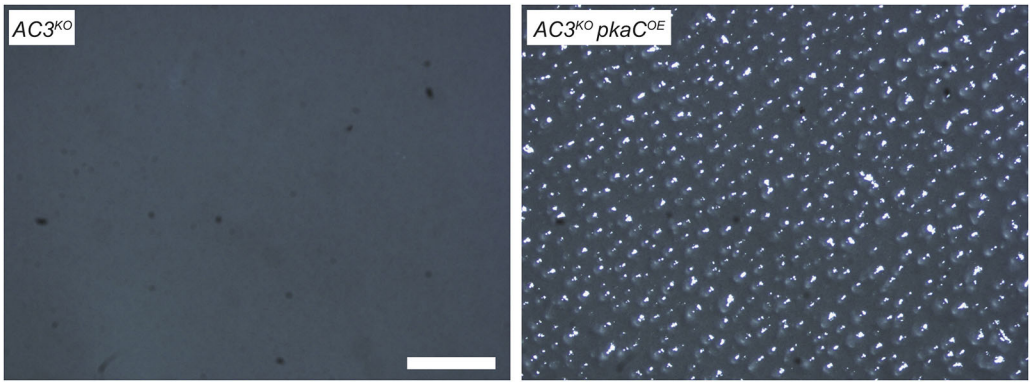

Fig. 2. PkaC overexpression modifies the developmental morphology of *AC3*^{KO}. We developed starving cells on black nitrocellulose filters for 10 h and photographed them with a dissecting microscope from above. Strain names are indicated in the panels. Scale bar: 10 mm.

many of the aggregates exhibit rotational movement. We also used two fluorescent markers that show polarization by labeling the leading edges of aggregating cells. Ras signaling in polarized cells was evident, in that raf_RBD–GFP, a marker of activated Ras (Sasaki et al., 2004), was localized to the leading edges of nearly all of the rotating cells (Fig. 3B; Movie 2). This finding also suggests that Ras localization to the leading edge is not necessarily induced by cAMP chemotaxis, as originally presumed (Sasaki et al., 2004), but rather a general property of polarized cells. Visualization of GFP–Lifeact, an F-actin marker (Lemieux et al., 2014), showed general cortical staining and strong polarization in the leading edges of nearly all of the rotating cells (Fig. 3C; Movie 3), as seen in the wild type during cAMP chemotaxis. Enhanced Ras activity and F-actin accumulation in the leading edges were evident in cells that were arranged in a head-to-tail fashion within the rotating mounds. These findings show that the *AC3*^{KO}*pkaC*^{OE} cells undergo rotational movement, polarization and head-to-tail organization without cAMP chemotaxis.

Allorecognition without cAMP

To test whether cAMP was also dispensable for allorecognition, we compared the behavior of 10% *AC3*^{KO}*pkaC*^{OE} GFP–Lifeact cells when mixed with 90% compatible AX4 cells or with 90% incompatible *tgrBC*^{QS31} cells. In the compatible mix, the green fluorescent cells were evenly mixed with the unlabeled majority cells throughout the experiment (Fig. 4A–D). They also exhibited polarized staining (Movie 4), similar to the behavior of compatible wild-type cells under the same conditions (Hirose et al., 2015). The ability of the *AC3*^{KO}*pkaC*^{OE} to participate in cooperative movement suggests that cell-autonomous cAMP production is not essential for this developmental process.

Behavior of the same *AC3*^{KO}*pkaC*^{OE} GFP–Lifeact cells was quite different when mixed with 90% incompatible *tgrBC*^{QS31} cells (Fig. 4E–H; Movie 5). During early stages, as the streams coalesced into a rotating aggregate, the green fluorescent cells co-migrated with the unlabeled cells with similar speed and directionality (Fig. 4E; Movie 5, time 00:00–01:30). Later on, however, the labeled cells exhibited disoriented movement (Fig. 4F), occasional clumping (Fig. 4G) and subsequent exclusion from the rotating mound (Fig. 4H; Movie 5, time ≥02:00). We quantified the dispersion of the cells in the two experiments and found that the variance in the incompatible mix increased dramatically over time, significantly above the variance in the compatible mix, indicating clumping of the minority cells in the incompatible environment. This behavior is identical to the behavior of incompatible cells that have intact cAMP production (Hirose et al., 2015). These observations suggest that cell-autonomous cAMP production and soluble extracellular cAMP are dispensable for allorecognition and exclusion of incompatible cells from the aggregating tissue.

Conclusions

Our results show that cell-autonomous cAMP production and extracellular cAMP signaling are dispensable for tissue formation and allorecognition. These findings are consistent with previous observations (Fujimori et al., 2019; Hashimura et al., 2019; Hirose et al., 2015; Iranfar et al., 2006; Kida et al., 2019) and further support

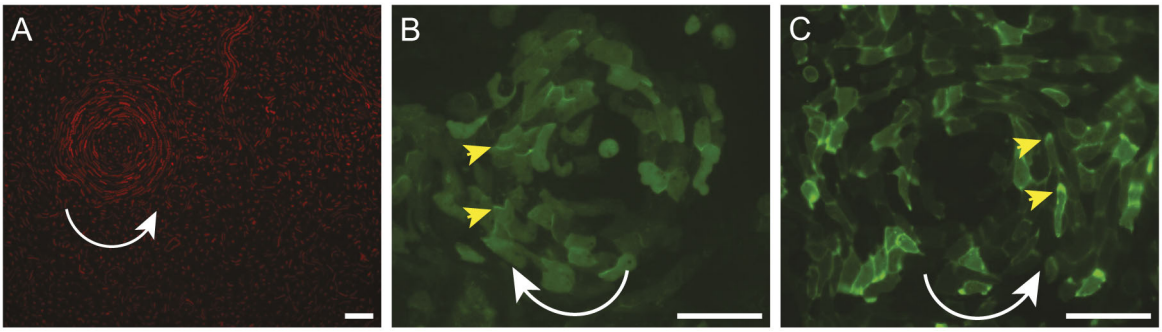

Fig. 3. Aggregate rotation and cell polarization in pure populations. We developed pure (unmixed) populations of *AC3*^{KO}*pkaC*^{OE} cells expressing various fluorescent protein conjugates under agar for 16 h and photographed them with fluorescent microscopy. (A) mCherry–H2B image showing the migratory trajectories of cells with 7 consecutive frames overlaid in a single image. (B) raf_RBD–GFP, single image. (C) GFP–Lifeact, single image. White arrows indicate the rotational direction. Yellow arrows indicate the front edges of selected polarized cells. Scale bars: 50 µm.

Journal of Cell Science (2021) 134, jcs258777. doi:10.1242/jcs.258777

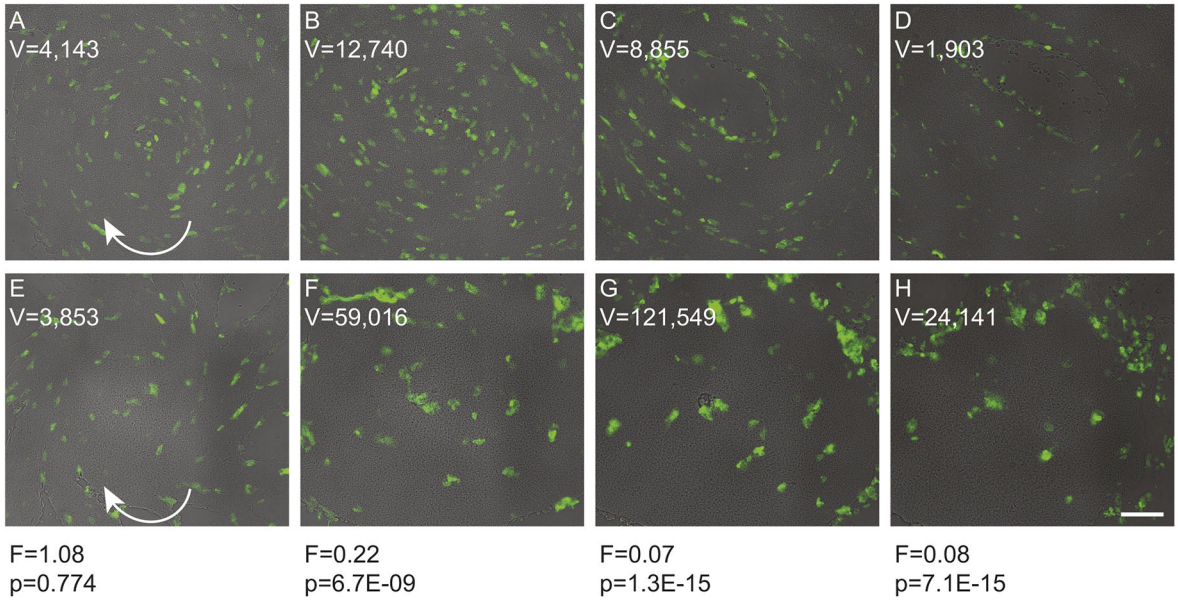

Fig. 4. Allorecognition in chimerae. We mixed 10% *AC3^{KO}pkaC^{OE}* GFP-Lifeact cells with 90% compatible AX4 cells (A–D) or 90% incompatible *tgrBC^{QS31}* cells (E–H), and developed them under agar for 16 h before photographing them. Images (bright field overlaid with fluorescence) are shown at 2-min intervals (A,E, 0 min; B,F, 2 min; C,G, 4 min; D,H, 6 min). White arrows indicate the rotational direction of the majority cells (bright field). The spatial distribution variance values (V) are shown inside each frame. The F-tests (F) and significance (*P*) values comparing the variances of each two time-matched samples (A and E; B and F; C and G; D and H) are shown below each pair. Scale bars: 100 µm.

the hypothesis that TgrB1-TgrC1 signaling replaces cAMP signaling during tissue formation and allorecognition (Hirose et al., 2017, 2015). These findings do not mean that cAMP signaling has no role in these processes, they only mean that it is not essential. Therefore, we are not challenging the observation of persistent oscillatory cAMP signals throughout *D. discoideum* development or the interpretation that these signals are functional during other stages of multicellular development (Singer et al., 2019). It is quite possible that cAMP signals continue to play a role in the process of tissue formation as well, even though this role is dispensable as long as PKA is active.

It is also important to note that aggregative cAMP chemotaxis is not replaced by TgrB1-TgrC1 signaling. These adhesion/signaling molecules are not expressed during chemotactic aggregation (Benabentos et al., 2009), and they are not sufficient to overcome the need for high cell density in the absence of cAMP chemotaxis or the need for PKA-C activation by cAMP (Anjard et al., 2001; Wang and Kuspa, 1997). In fact, cells that lack either *tgrB1* or *tgrC1* or both are capable of chemotactic aggregation (Benabentos et al., 2009). We also did not examine the roles of cAMP in development after tissue formation, but the strains we constructed could help in testing these roles (Fujimori et al., 2019; Singer et al., 2019).

Our results also indicate that TgrB1-TgrC1 signaling is not related to the unresolved question about the relationship between *acaA* and *acrA* activities. The absence of *acaA* function can be suppressed by activation of *pkaC*, although aggregation requires higher cell density because it is achieved by accretion rather than chemotaxis (Wang and Kuspa, 1997). The lack of *acrA* function can be suppressed by activation of *pkaC* or by ectopic expression of active *acaA*, indicating that *acrA* does not have a function other than cAMP synthesis, despite its composite structure (Anjard et al., 2001; Soderbom et al., 1999). Moreover, *acrA*$^{-}$ cells express active adenylate cyclase throughout development via continued expression of *acaA* (Soderbom et al., 1999). Nevertheless, *pkaC* overexpression is not sufficient to suppress the morphological defects of the *acaA*$^{-}$*acrA*$^{-}$ double mutant (Anjard et al., 2001), let alone the *AC3^{KO}* defect as shown here. Our findings are based on overexpression of the *pkaC* gene from the *actin15* promoter, which is not likely to restore wild-type levels of PKA-C activity. Nevertheless, these results suggest that cAMP has a role in post-aggregative development that is independent of PKA-C activation. Indeed, extracellular cAMP is essential for spore formation in isolated cells *in vitro* (Kay, 1982). Since we have shown that TgrB1-TgrC1 signaling is intact in these cells, the PKA-C-independent function of cAMP must also be unrelated to TgrB-TgrC1 signaling.

MATERIALS AND METHODS

Expression vectors and strain construction

The *D. discoideum* strains were derivatives of AX4 (Knecht et al., 1986) as detailed in Table S1. The three adenylate cyclase genes were knocked out sequentially in this order: *acrA*, *acgA* and *acaA*. AcrA was knocked out by homologous recombination using a published *acrA* knockout vector (Chen et al., 2010). After confirmation by developmental morphology and PCR analysis, the Blasticidin S selection cassette was removed by Cre-LoxP recombination (Faix et al., 2004). The *acgA* knockout vector was generated using *acgA* 5′ (nucleotides 29–922) and *acgA* 3′ (nucleotides 995–2033) sequences as homologous recombination arms. The 5′ and 3′ arms were cloned between the KpnI-HindIII and the BamHI-NotI sites of pLPBLP (Faix et al., 2004), respectively. The *acgA* gene was knocked out with the *acgA* knockout vector in the *acrA*$^{-}$ strain and the Blasticidin S selection cassette was removed again by Cre-LoxP recombination after confirmation by PCR analysis. The *acaA* knockout vector was generated using *acaA* 5′ (nucleotides 120–1045) and *acaA* 3′ (nucleotides 2217–3069) sequences as homologous recombination arms. The 5′ and 3′ arms were cloned between the KpnI-HindIII and the BamHI-NotI sites of pLPBLP, respectively. The *acaA* gene was knocked out with the *acaA* knockout vector in AX4 and in the *acrA*$^{-}$*acgA*$^{-}$ double null strain. The desired mutants were screened for an aggregationless phenotype and confirmed by PCR analysis. The *pkaC* gene was amplified by PCR with a reverse primer that introduced a HA-tag sequence (underlined) at the 3′ end of the coding sequence: 5′-TTACTAGTTTAAGCATAATCTGGAACATCATATGGATA AAAA-TCCTTGAAAAGATGTGCA-3′. The PCR product was cloned between the BamHI and XbaI sites of pDXA-HC (Manstein et al., 1995).

The expression vector was introduced into the $AC3^{KO}$ strain and clones were screened for loose aggregate morphology. To generate strains with a nuclear fluorescent protein tag, we constructed the vector pDM304 pkaC-HA mCherry-H2B, in which the HA-tagged *pkaC* gene was cloned between the BglII and SpeI sites and another expression cassette, carrying the mCherry coding sequence fused to the histone H2B gene at its C-terminus and driven by *act15* promoter, was cloned into the NgoMIV site of the pDM304 vector, between the pUC ori and the Ddp1 ori segments (Veltman et al., 2009). We also transformed the $AC3^{KO}pkaC^{OE}$ strain with pDM1066 GFP-Lifeact (Lemieux et al., 2014) and separately with pDM115 raf_RBD-GFP (Sasaki et al., 2004), both with hygromycin selection.

Cell culture, strain maintenance, development and imaging

We cultured cells at 22°C in HL5 medium with the necessary supplements and antibiotics as described previously (Hirose et al., 2011). To induce development, we washed exponentially growing cells twice with KK2 buffer (20 mM potassium phosphate, pH6.4) to remove nutrients and deposited them at a density of 2.5×10^6 cells/cm^2 on black nitrocellulose filters on top of a paper pad soaked with PDF buffer (20 mM KCl, 9.2 mM K_2HPO_4, 13.2 mM KH_2PO_4, 5.3 mM $MgCl_2$ and 1 mM $CaCl_2$, pH6.4) and incubated in the dark at 22°C to record morphological progression as described previously (Hirose et al., 2015). Rotational cell movement and segregation during development were tested by developing the cells between glass and agar. Development in pure populations and in 10:90 mixes, were performed as described previously (Hirose et al., 2015). All the images shown are characteristic of at least three replicates. Image analysis and cell dispersion were performed according to Hirose et al. (2017) as follows: we converted the 8-bit monochromatic images into binary images in the green channel. To quantify cell distribution, we divided each image into an 8×8 grid, where each of the equal 64 squares was defined as a region of interest (ROI). We counted the number of green pixels in each ROI using ImageJ 1.53a (Schneider et al., 2012) and computed the variance between these values across the entire image. The statistical significance of the difference between variances was calculated by an *F*-test.

Growth and development in association with bacteria were performed as described previously (Loomis and Ashworth, 1968) except that *Klebsiella pneumonia* bacteria were used and the amoebae were plated at a density of 20–40 cells per plate. The plates were incubated at 22°C until plaques were formed in the bacterial lawn that contained fully developed structures. Images were captured with a camera mounted on a dissecting microscope such that the bacterial lawn, as well as the various developmental stages, within the plaque were included in a single image.

cAMP measurement

We starved 5×10^7 cells on black nitrocellulose filters as above. We collected the cells, washed with PDF and lysed them with 0.1 M HCl to an equivalent of 10^5 cells/µl. We measured cAMP concentration with the Cyclic AMP ELISA kit (Item No. 581001 Cayman Chemical, 0.078-10 pmol/ml assay range) according to the manufacturer's recommended protocol. Each strain was tested three independent times except for $AC3^{KO}PkaC^{OE}$, which was measured in duplicates.

Acknowledgements

We thank Adam Kuspa and Peter Lehman for helpful discussions.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.H., G.S.; Methodology: S.H., G.S.; Validation: S.H., M.K.-K., G.S.; Formal analysis: S.H., M.K.-K., G.S.; Investigation: S.H., G.S.; Resources: S.H.; Data curation: S.H., G.S.; Writing - original draft: G.S.; Writing - review & editing: S.H., M.K.-K., G.S.; Visualization: S.H., M.K.-K., G.S.; Supervision: G.S.; Project administration: G.S.; Funding acquisition: G.S.

Funding

This work was supported by grant R35 GM118016 from the National Institutes of Health/National Institute of General Medical Sciences. Deposited in PMC for release after 12 months.

Peer review history

The peer review history is available online at https://journals.biologists.com/jcs/article-lookup/doi/10.1242/jcs.258777

References

Alvarez-Curto, E., Saran, S., Meima, M., Zobel, J., Scott, C. and Schaap, P. (2007). cAMP production by adenylyl cyclase G induces prespore differentiation in Dictyostelium slugs. *Development* **134**, 959-966. doi:10.1242/dev.02775

Anjard, C., Söderbom, F. and Loomis, W. F. (2001). Requirements for the adenylyl cyclases in the development of Dictyostelium. *Development* **128**, 3649-3654. doi:10.1242/dev.128.18.3649

Artemenko, Y., Lampert, T. J. and Devreotes, P. N. (2014). Moving towards a paradigm: common mechanisms of chemotactic signaling in Dictyostelium and mammalian leukocytes. *Cell. Mol. Life Sci.* **71**, 3711-3747. doi:10.1007/s00018-014-1638-8

Benabentos, R., Hirose, S., Sucgang, R., Curk, T., Katoh, M., Ostrowski, E. A., Strassmann, J. E., Queller, D. C., Zupan, B., Shaulsky, G. et al. (2009). Polymorphic members of the lag gene family mediate kin discrimination in Dictyostelium. *Curr. Biol.* **19**, 567-572. doi:10.1016/j.cub.2009.02.037

Chen, Z.-H., Schilde, C. and Schaap, P. (2010). Functional dissection of adenylate cyclase R, an inducer of spore encapsulation. *J. Biol. Chem.* **285**, 41724-41731. doi:10.1074/jbc.M110.156380

Dynes, J. L., Clark, A. M., Shaulsky, G., Kuspa, A., Loomis, W. F. and Firtel, R. A. (1994). LagC is required for cell-cell interactions that are essential for cell-type differentiation in Dictyostelium. *Genes Dev.* **8**, 948-958. doi:10.1101/gad.8.8.948

Faix, J., Kreppel, L., Shaulsky, G., Schleicher, M. and Kimmel, A. R. (2004). A rapid and efficient method to generate multiple gene disruptions in Dictyostelium discoideum using a single selectable marker and the Cre-loxP system. *Nucleic Acids Res.* **32**, e143. doi:10.1093/nar/gnh136

Fujimori, T., Nakajima, A., Shimada, N. and Sawai, S. (2019). Tissue self-organization based on collective cell migration by contact activation of locomotion and chemotaxis. *Proc. Natl. Acad. Sci. USA* **116**, 4291-4296. doi:10.1073/pnas.1815063116

Gruenheit, N., Parkinson, K., Stewart, B., Howie, J. A., Wolf, J. B. and Thompson, C. R. L. (2017). A polychromatic 'greenbeard' locus determines patterns of cooperation in a social amoeba. *Nat. Commun.* **8**, 14171. doi:10.1038/ncomms14171

Hashimura, H., Morimoto, Y. V., Yasui, M. and Ueda, M. (2019). Collective cell migration of Dictyostelium without cAMP oscillations at multicellular stages. *Commun. Biol.* **2**, 34. doi:10.1038/s42003-018-0273-6

Hirose, S., Benabentos, R., Ho, H.-I., Kuspa, A. and Shaulsky, G. (2011). Self-recognition in social amoebae is mediated by allelic pairs of tiger genes. *Science* **333**, 467-470. doi:10.1126/science.1203903

Hirose, S., Santhanam, B., Katoh-Kurosawa, M., Shaulsky, G. and Kuspa, A. (2015). Allorecognition, via TgrB1 and TgrC1, mediates the transition from unicellularity to multicellularity in the social amoeba Dictyostelium discoideum. *Development* **142**, 3561-3570. doi:10.1242/dev.123281

Hirose, S., Chen, G., Kuspa, A. and Shaulsky, G. (2017). The polymorphic proteins TgrB1 and TgrC1 function as a ligand-receptor pair in Dictyostelium allorecognition. *J. Cell Sci.* **130**, 4002-4012. doi:10.1242/jcs.208975

Iranfar, N., Fuller, D. and Loomis, W. F. (2003). Genome-wide expression analyses of gene regulation during early development of Dictyostelium discoideum. *Eukaryot. Cell* **2**, 664-670. doi:10.1128/EC.2.4.664-670.2003

Iranfar, N., Fuller, D. and Loomis, W. F. (2006). Transcriptional regulation of post-aggregation genes in Dictyostelium by a feed-forward loop involving GBF and LagC. *Dev. Biol.* **290**, 460-469. doi:10.1016/j.ydbio.2005.11.035

Katoh-Kurasawa, M., Hrovatin, K., Hirose, S., Webb, A., Ho, H.-I., Zupan, B. and Shaulsky, G. (2021). Transcriptional milestones in Dictyostelium development. *Genome Res.* [in press]. doi:10.1101/gr.275496.121

Kay, R. R. (1982). cAMP and spore differentiation in Dictyostelium discoideum. *Proc. Natl. Acad. Sci. USA* **79**, 3228-3231. doi:10.1073/pnas.79.10.3228

Kessin, R. H. (2001). *Dictyostelium - Evolution, Cell Biology, and the Development of Multicellularity*. Cambridge, UK: Cambridge Univ. Press.

Kibler, K., Svetz, J., Nguyen, T.-L., Shaw, C. and Shaulsky, G. (2003). A cell-adhesion pathway regulates intercellular communication during Dictyostelium development. *Dev. Biol.* **264**, 506-521. doi:10.1016/j.ydbio.2003.08.025

Kida, Y., Pan, K. and Kuwayama, H. (2019). Some chemotactic mutants can be progress through development in chimeric populations. *Differentiation* **105**, 71-79. doi:10.1016/j.diff.2019.02.001

Kim, H.-J., Chang, W.-T., Meima, M., Gross, J. D. and Schaap, P. (1998). A novel adenylyl cyclase detected in rapidly developing mutants of Dictyostelium. *J. Biol. Chem.* **273**, 30859-30862. doi:10.1074/jbc.273.47.30859

Knecht, D. A., Cohen, S. M., Loomis, W. F. and Lodish, H. F. (1986). Developmental regulation of Dictyostelium discoideum actin gene fusions carried on low-copy and high-copy transformation vectors. *Mol. Cell Biol.* **6**, 3973-3983. doi:10.1128/mcb.6.11.3973-3983.1986

Kriebel, P. W., Barr, V. A. and Parent, C. A. (2003). Adenylyl cyclase localization regulates streaming during chemotaxis. *Cell* **112**, 549-560. doi:10.1016/S0092-8674(03)00081-3

Lemieux, M. G., Janzen, D., Hwang, R., Roldan, J., Jarchum, I. and Knecht, D. A. (2014). Visualization of the actin cytoskeleton: different F-actin-binding probes tell different stories. *Cytoskeleton* **71**, 157-169. doi:10.1002/cm.21160

Loomis, W. F. (2014). Cell signaling during development of Dictyostelium. *Dev. Biol.* **391**, 1-16. doi:10.1016/j.ydbio.2014.04.001

Loomis, W. F. and Ashworth, J. M. (1968). Plaque-size mutants of the cellular slime mould Dictyostelium discoideum. *J. Gen. Microbiol.* **53**, 181-186. doi:10.1099/00221287-53-2-181

Manstein, D. J., Schuster, H.-P., Morandini, P. and Hunt, D. M. (1995). Cloning vectors for the production of proteins in Dictyostelium discoideum. *Gene* **162**, 129-134. doi:10.1016/0378-1119(95)00351-6

Ostrowski, E. A., Shen, Y., Tian, X., Sucgang, R., Jiang, H., Qu, J., Katoh-Kurasawa, M., Brock, D. A., Dinh, C., Lara-Garduno, F. et al. (2015). Genomic signatures of cooperation and conflict in the social amoeba. *Curr. Biol.* **25**, 1661-1665. doi:10.1016/j.cub.2015.04.059

Pitt, G. S., Milona, N., Borleis, J., Lin, K. C., Reed, R. R. and Devreotes, P. N. (1992). Structurally distinct and stage-specific adenylyl cyclase genes play different roles in Dictyostelium development. *Cell* **69**, 305-315. doi:10.1016/0092-8674(92)90411-5

Pitt, G. S., Brandt, R., Lin, K. C., Devreotes, P. N. and Schaap, P. (1993). Extracellular cAMP is sufficient to restore developmental gene expression and morphogenesis in Dictyostelium cells lacking the aggregation adenylyl cyclase (ACA). *Genes Dev.* **7**, 2172-2180. doi:10.1101/gad.7.11.2172

Ross, F. M. and Newell, P. C. (1981). Streamers: Chemotactic mutants of Dictyostelium discoideum with altered cyclic GMP metabolism. *J. Gen. Microbiol.* **127**, 339-350. doi:10.1099/00221287-127-2-339

Sasaki, A. T., Chun, C., Takeda, K. and Firtel, R. A. (2004). Localized Ras signaling at the leading edge regulates PI3K, cell polarity, and directional cell movement. *J. Cell Biol.* **167**, 505-518. doi:10.1083/jcb.200406177

Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671-675. doi:10.1038/nmeth.2089

Singer, G., Araki, T. and Weijer, C. J. (2019). Oscillatory cAMP cell-cell signalling persists during multicellular Dictyostelium development. *Commun. Biol.* **2**, 139. doi:10.1038/s42003-019-0371-0

Soderbom, F., Anjard, C., Iranfar, N., Fuller, D. and Loomis, W. F. (1999). An adenylyl cyclase that functions during late development of Dictyostelium. *Development* **126**, 5463-5471. doi:10.1242/dev.126.23.5463

van Es, S., Virdy, K. J., Pitt, G. S., Meima, M., Sands, T. W., Devreotes, P. N., Cotter, D. A. and Schaap, P. (1996). Adenylyl cyclase G, an osmosensor controlling germination of Dictyostelium spores. *J. Biol. Chem.* **271**, 23623-23625. doi:10.1074/jbc.271.39.23623

Veltman, D. M., Akar, G., Bosgraaf, L. and Van Haastert, P. J. M. (2009). A new set of small, extrachromosomal expression vectors for Dictyostelium discoideum. *Plasmid* **61**, 110-118. doi:10.1016/j.plasmid.2008.11.003

Wang, B. and Kuspa, A. (1997). Dictyostelium development in the absence of cAMP. *Science* **277**, 251-254. doi:10.1126/science.277.5323.251