J. Embryol. exp. Morph. Vol. 57, pp. 233–240, 1980
Printed in Great Britain © Company of Biologists Limited 1980

Evidence for tip control of the 'slug/fruit' switch in slugs of *Dictyostelium discoideum*

By ELIZABETH SMITH[1] AND KEITH L. WILLIAMS[2]

From the Genetics Department, Research School of Biological Sciences, Australian National University, Canberra City

SUMMARY

Reciprocal transplants were performed between slugs of two strains of *Dictyostelium discoideum*. Slugs of one strain (NP84 'slugger') showed prolonged migration, while slugs of the other strain (AX3 'fruiter') migrated for a short period only. The transplant experiments showed that the 'slug/fruit' characteristic is tip dependent, since an NP84 tip induced an AX3 rear to migrate for a prolonged period, while an AX3 tip induced the rear of a NP84 slug to fruit without migration. These findings are not consistent with the hypothesis that tips of all stages release only one signal which is interpreted differently by the rest of the cells in the aggregate at different stages. Rather, we propose that the tip of a *D. discoideum* aggregate may release a number of signals, one of which is stage dependent, and triggers the 'slug/fruit' switch.

INTRODUCTION

The question of how morphogenesis occurs has been studied in many organisms yet little is known of its mechanism (MacWilliams & Bonner, 1979). The cellular slime mould, *Dictyostelium discoideum*, provides a particularly amenable system for investigating the problem because there are only three cell types in the fruiting body which is formed by aggregation of cells rather than heirarchical cell division from a zygote. One component of the morphogenetic apparatus is an organizer that, in *D. discoideum*, is the tip of the aggregate (Raper, 1940). It is established that the tip has a role in morphogenesis but the precise nature and complexity of this role is unknown. At the outset it should be made clear that the tip has not been adequately defined, although it is clear that it involves the anterior cells (possibly about 10%) (Raper, 1940). Four general properties have been attributed to the tip of the migrating slug. The slug tip is (i) the organizer region which acts at all stages from aggregation to fruiting body formation (Raper, 1940) and this function may involve continuous secretion of cAMP (Rubin, 1976); (ii) the source of inhibition of secondary tip

[1] *Author's address:* Biochemistry Department, Faculty of Science, Australian National University, P.O. Box 4, Canberra, A.C.T. 2601, Australia.

[2] *Author's address:* Max Planck Institut für Biochemie, 8033 Martinsried bei München, Fed. Rep. Germany.

formation (Durston, 1976); (iii) the region which responds to light stimuli (Francis, 1964; Poff & Loomis, 1973) and (iv) possibly involved in determination of cell pattern, since isolated tip cells of certain strains induce differentiation of other slug cells into stalk cells (Town & Stanford, 1977).

In this report a new tip property, which involves the decision by a slug to continue migrating or to construct a fruiting body (the 'slug/fruit' switch), has been elucidated using transplants between slugs of genetically different strains of *D. discoideum*. The nature of signals from the tip of the slime mould aggregate is re-evaluated.

MATERIALS AND METHODS

Strains

Two genetically different strains were used in this work. They are NP84, an axenic (*axe*-352), growth-temperature-sensitive (*tsgN*350) derivative of the V12 isolate (North & Williams, 1978) which was obtained after mutagenesis with *N*-methyl-*N'*nitro-*N*-nitrosoguanidine (NTG) and ultraviolet light (Williams, unpublished), and AX3, an axenic strain obtained from the NC4 isolate after repeated mutagenesis (Loomis, 1971). These strains were used because they have several different 'slugging' phenotypes which are examined in this and a subsequent paper (Smith & Williams, in preparation).

Culture conditions

Approximately 10^5 *D. discoideum* amoebae were inoculated on SM agar plates (Sussman, 1966) in conjunction with *Klebsiella aerogenes*. After 48 h at $21{\cdot}5 \pm 1{\cdot}0$ °C most of the bacteria had been consumed so the amoebae were washed, put on to water agar plates and exposed to unilateral light as previously described (Smith & Williams, 1979) except that opaque PVC containers with two holes on one side (3 mm diameter, 4 mm apart) were used instead of aluminium foil to cover the plates. After 22–32 h, when the cells had aggregated, slugs had formed and migrated 1–2 cm towards the light (i.e. the holes), individual slugs were transferred to marked positions on fresh water agar plates. This was done by breaking the slime trail close to the rear of a slug and lifting the slug by its slime trail, using a fine platinum wire. After 0·5–3 h from the time of transfer, transplant operations were performed on the slugs which had resumed migration towards the light.

Transplant experiments

Reciprocal transplants between two slugs, which were matched as closely as possible with respect to size, were performed using a microspatula (Raper, 1940) by cutting each slug transversely 20–30 % of the length back from the tip and gently separating the two parts. The posterior portion (rear) of each slug in the pair was lifted and rejoined to the anterior portion of the other slug.

For convenience we refer to the anterior portion of the slug as the 'front' throughout the results. This term does not imply any special properties of the cells in the anterior portion and, if there is a particular group of organizer cells in the tip of the slug, we assume that we have transplanted all these and possibly also all the prestalk cells. During the transplant procedure slugs were exposed to uniform, relatively bright light and dry air for less than 10 min before being returned to the standard slugging conditions. Plates were examined either 14 or 24 h after transplants had been performed, when numbers and positions of slugs and fruiting bodies were recorded.

Genotypes of cells in chimeras

Spores from individual fruiting bodies were collected with a tungsten needle into 0·1 ml salt solution (SS) (Bonner, 1947) and left for 30 min before counting. Known numbers of spores were plated with *K. aerogenes* on SM agar plates. After 72 h at 21·5 ± 1·0 °C, colonies of NP84 were visible as very diffuse circles (5–8 mm diam.) on the lawn of bacteria. AX3 colonies appeared somewhat later (84–96 h) as very tight circles (1–3 mm diam.). The proportions of spores of each strain in a spore head could thus be determined (assuming equal plating efficiency for the two strains). Since NP84 colonies are very diffuse and fast-growing, in cases where NP84 cells were in excess, additional SM agar plates were incubated at 27 °C to allow AX3 colonies to grow without NP84, which is a growth-temperature-sensitive strain.

In order to determine the proportions of the two strains in the tips of chimeric slugs, the front 20–30% of the slug was removed into 25 μl SS in a 1·6 ml Eppendorf centrifuge tube and vibrated vigorously for 10 sec on a cross-bar rotated by an electric motor, to completely dissociate the cells. The dissociated cells in a 5 μl sample were then counted in a haemacytometer, and a further 5 μl sample was diluted appropriately before plating an aliquot on SM agar with *K. aerogenes*. Colonies of strains AX3 and NP84 were distinguished and counted as described above.

RESULTS

'Slugging/fruiting' phenotype of strains NP84 and AX3

The experiments reported here make use of the fact that strains AX3 and NP84 have markedly different slugging properties. Under our standard slugging conditions (Smith & Williams, 1979) most slugs of strain AX3 migrate for a short time (1–2 days) before forming a fruiting body. By contrast, under the same conditions, NP84 slugs migrate for an extended time of at least 5–6 days. For technical reasons we have not investigated the maximum time and distance which NP84 slugs will migrate, but slugs of NP84 have been observed to migrate for 11 days. Therefore NP84 shows almost no tendency to form fruiting bodies under our slugging conditions.

Table 1. *Outcome of (reciprocal) transplant experiments involving slugs of strains NP84 and AX3*

Front	Rear	Number of transplants	Number of successful transplants*	Nature of successful chimeras: Single slugs	Nature of successful chimeras: Single fruiting bodies
NP84	NP84	61	54 (90%)	53	1
AX3	AX3	58	22 (38%)	14	8
NP84	AX3	177	103 (58%)	102	1
AX3	NP84	185	70 (38%)	7†	63

* The criterion of success for a transplant was the formation of a single mass (a slug or a fruiting body) from the two parts. The results show that the success rate was not the same for each transplant. The success of these and other transplant experiments will be discussed elsewhere (Smith & Williams, In preparation).

† See Table 2B in which it is shown that at least four of these slugs had NP84 tips as a result of movement of NP84 cells into the tip. The remaining three slugs were not analysed.

Transplant experiments

To test whether the 'slugging' phenotype of NP84 and the 'fruiting' phenotype of AX3 were localized within a particular region of the slug or were cell autonomous properties, transplants were made between the front and rear of AX3 and NP84 slugs, as described in the Methods. For controls transplants were made between fronts and rears of slugs of the same strain. The results of successful transplants are summarized in Table 1. Almost all (53 out of 54) successful transplants involving only NP84 continued migrating as slugs, while about 30% (8 out of 22) of successful AX3–AX3 transplants fruited immediately; the remainder continued migrating for 1–2 days before fruiting. Thus homologous transplants did not greatly affect the 'slugging/fruiting' phenotype, except that some AX3–AX3 chimeras fruited immediately.

Transplants between NP84 and AX3 gave a clear-cut result. Of 103 successful transplants with an NP84 front and AX3 rear, all but one continued migrating for a sustained period. The single exception formed a fruiting body at the site of transplantation. On the other hand, most of the successful transplants with an AX3 front and NP84 rear (63 out of 70) formed a fruiting body at the origin rather than a migrating slug. The other seven transplants resulted in slugs which showed sustained migration characteristics of NP84. With the exception of these seven slugs which will be discussed in the next section, the clear conclusion from these experiments is that the front portion of the slugs of strains NP84 and AX3 have a qualitatively different organizing role. NP84 cells at the front cause an NP84/AX3 chimera to form a migrating slug, while AX3 cells at the front of an AX3/NP84 chimera induce immediate fruiting body formation.

Table 2. *Proportions of NP84 and AX3 cells in the front 25 % of chimeric slugs after 14–24 h migration*

(A) NP84 front/AX3 rear			(B) AX3 front/NP84 rear		
Chimeric slug	NP84 (%)	AX3 (%)	Chimeric slug	NP84 (%)	AX3 (%)
1	100	0	1	83	17
2	92	8	2	98	2
3	63	37	3	88	12
4	98	2	4	85	15
5	94	6			
6	97	3			
7	99	1			
8	97	3			
9	88	12			
10	91	9			
11	89	11			
12	59	41			

The proportions of NP84 and AX3 were determined as described in the Methods.

Absence of sorting out in chimeras formed from reciprocal transplants between AX3 and NP84

The conclusions drawn from the results of these transplant experiments would not be valid if significant sorting out of cells between the front and back of cell masses was occurring, and this was checked as described in the Methods. As shown in Table 2A for chimeras comprising an NP84 front and AX3 rear portion, there was always a high percentage (~90 % or greater in 10 out of 12 slugs examined) of NP84 cells in the front portion after 14 or 24 h migration. Since the relative sizes of the NP84 front portion transplanted and the front portion taken for analysis cannot be known exactly, this result is consistent with the maintenance of relative positions of NP84 and AX3 cells within the chimera. As shown in Table 2B, analysis of four of the seven exceptional AX3 front, NP84 rear chimeras which resulted in slugs which migrated for a sustained period, showed that the proportion of NP84 cells in the front portion of these slugs was in the same range (> 80 % NP84) as that for the transplants involving NP84 front and AX3 rear. We conclude that in these exceptional cases NP84 cells moved into the front of the slug and took over the tip function, so that a migrating slug rather than a fruiting body was formed. NP84 cells were found to be 'tip-loving' when vegetative amoebae of the two strains AX3 and NP84 were mixed and allowed to form slugs (data not shown).

Analysis of spores from fruiting bodies formed at the site of transplant from AX3 front and NP84 rear showed that the spore head contained virtually 100 % NP84 spores, indicating that the AX3 cells had retained their position in the tip (in contrast to the 7 that slugged) and become stalk cells.

DISCUSSION

In the experiments reported here we have shown that the decision of slugs of *D. discoideum* to continue migrating or to form a fruiting body is predominantly a tip-controlled function. Cells of strain NP84 in the rear of a slug can be induced to follow an unnatural developmental pathway, i.e. form a fruiting body, under conditions in which an NP84 slug would continue to migrate, by the replacement of the NP84 tip by an AX3 tip. Conversely, where an NP84 tip is transplanted on to the rear position of an AX3 slug, the resulting chimera migrates for an extended period instead of migrating for only a short period or fruiting immediately, as an AX3 slug would.

These results have implications for the role of the tip (organizer) in the multicellular stages of *D. discoideum*. A number of properties have already been attributed to the slug tip, whose function as an organiser must be complex, involving several molecular signals which affect different aspects of morphogenesis. While this requires a new attitude towards the role and complexity of the tip, there are some indications that such a view is warranted. Three, possibly distinct, general properties can be proposed: those involved with holding the aggregate together and guiding morphogenetic movement, those involved with cell patterning, and those controlling the transitions between different morphogenetic stages. It is important to consider that there may also be properties of aggregates that are cell autonomous and not tip controlled. We have discovered such a cell autonomous property in slugs which involves the dropping of large numbers of cells in the slime trail (Smith & Williams, In preparation).

The first of these tip properties, involving maintenance of the integrity of the aggregate and morphogenetic movement, may be the same throughout morphogenesis. Rubin & Robertson (1975) have described such a tip role which may be mediated by cAMP signalling (Rubin, 1976). There is at present little evidence for tip involvement in cell patterning (Durston, 1977) although the low MW factor DIF (Town & Stanford, 1977; Kay, Town & Gross, 1979) is a candidate for a molecular signal with this role. Our experiments provide the first evidence for the third proposed tip function, involving transition between different morphogenetic stages. They show that a stage-dependent decision by the slug to either continue migrating or to stop and construct a fruiting body, is controlled by a signal(s) from the tip, i.e. the tip has a 'leader' role at least in the 'slug/fruit' decision. We also show that there is a cell autonomous component to the response, since cells of NP84 interpret a signal from an AX3 tip as a 'fruit immediately' command, whereas AX3 cells respond to the signal from an AX3 tip as a 'migrate for a short time longer' command (Table 1).

The decision to migrate or form a fruiting body is a major developmental switch for the *D. discoideum* aggregate and is triggered by a number of environmental factors (Newell, Telser & Sussman, 1969). Our results with these AX3, NP84 transplants do not allow us to distinguish whether the tip is the source

of only one signal, which is quantitatively different in the two strains, or whether there are two or more signals (e.g. high levels of 'fruiting-inducer' in AX3 and high levels of 'slugging-inducer' in NP84) controlling the decision to form a fruiting body or to continue migrating. Sussman and co-workers have accumulated considerable biochemical evidence concerning the 'slug/fruit' decision, and have evidence for both diffusible 'fruiting-inducers' [substance(s) associated with purine metabolism (Brackenbury, Schindler, Alexander & Sussman, 1974; Cohen & Sussman, 1975), and a small anionic compound (Sussman, Schindler & Kim, 1978)] and 'slugging inducer' [for which they have evidence for the involvement of ammonia (Schindler & Sussman, 1977)]. They have not attempted to determine the localisation of these factors within the aggregate. Recently Sussman, Schindler & Kim (1978) have isolated a series of 'slugger' mutants which, like strain NP84, continue to migrate under conditions in which wild-type aggregates form fruiting bodies. Analysis of these and other mutants in transplant experiments should help to elucidate the complexities of tip-dependent and tip-independent components of this interesting developmental switch.

We thank Ailsa Chambers for technical assistance and Drs J. Gross and R. Kay and the referees for helpful comments on the manuscript.

REFERENCES

BONNER, J. T. (1947). Evidence for the formation of cell aggregates by chemotaxis in the development of the slime mold, *Dictyostelium discoideum*. *J. exp. Zool.* **106**, 1–26.

BRACKENBURY, R. W., SCHINDLER, J., ALEXANDER, S. & SUSSMAN, M. (1974). A choice of morphogenetic pathways in *Dictyostelium discoideum* induced by the adenosine analog Formycin B. *J. molec. Biol.* **90**, 529–539.

COHEN, A. & SUSSMAN, M. (1975). Guanosine metabolism and regulation of fruiting body construction in *Dictyostelium discoideum*. *Proc. natn. Acad. Sci., U.S.A.* **72**, 4479–4482.

DURSTON, A. J. (1976). Tip formation is regulated by an inhibitory gradient in the *Dictyostelium discoideum* slug. *Nature* **263**, 126–129.

DURSTON, A. J. & VORK, F. (1977). The control of morphogenesis and pattern in the *Dictyostelium discoideum* slug. In *Development and Differentiation in Cellular Slime Moulds* (ed. P. Cappuccinelli and J. M. Ashworth), pp. 17–26, Elsevier/North Holland.

FRANCIS, D. W. (1964). Some studies on phototaxis of *Dictyostelium*. *J. cell. comp. Physiol.* **64**, 131–138.

KAY, R. R., TOWN, C. D. & GROSS, J. D. (1979). Cell differentiation in *Dictyostelium discoideum*. *Differentiation* **13**, 7–14.

LOOMIS, W. F. (1971). Sensitivity of *Dictyostelium discoideum* to nucleic acid analogues. *Expl Cell Res.* **64**, 484–486.

MACWILLIAMS, H. K. & BONNER, J. T. (1979). The prestalk-prespore pattern in cellular slime molds. *Differentiation* **14**, 1–22.

NEWELL, P. C., TELSER, A. & SUSSMAN, M. (1969). Alternative developmental pathways determined by environmental conditions in the cellular slime mold, *Dictyostelium discoideum*. *J. Bact.* **100**, 763–768.

NORTH, M. J. & WILLIAMS, K. L. (1978). Relationship between the axenic phenotype and sensitivity to ω-aminocarboxylic acids in *Dictyostelium discoideum*. *J. gen. Microbiol.* **107**, 223–230.

POFF, K. L. & LOOMIS, W. F. (1973). Control of phototactic migration in *Dictyostelium discoideum*. *Expl Cell Res.* **82**, 236–240.

RAPER, K. B. (1940). Pseudoplasmodium formation and organisation in *Dictyostelium discoideum*. *J. Elisha Mitchell Scient. Soc.* **56**, 241–282.

RUBIN, J. (1976). The signal from fruiting body and conus tips of *Dictyostelium discoideum*. *J. Embryol. exp. Morph.* **36**, 261–271.

RUBIN, J. & ROBERTSON, A. (1975). The tip of *Dictyostelium discoideum* pseudoplasmodium as an organiser. *J. Embryol. exp. Morph.* **33**, 227–241.

SCHINDLER, J. & SUSSMAN, M. (1977). Ammonia determines the choice of morphogenetic pathways in *Dictyostelium discoideum*. *J. molec. Biol.* **116**, 161–169.

SMITH, E. & WILLIAMS, K. L. (1979). Preparation of slime sheath from *Dictyostelium discoideum*. *FEMS Microbiology Letters* **6**, 119–122.

SUSSMAN, M. (1966). Biochemical and genetic methods in the study of cellular slime mold development. In *Methods in Cell Physiology*, vol. 2 (ed. D. Prescott), pp. 397–409. Academic Press.

SUSSMAN, M., SCHINDLER, J. & KIM, H. (1978). 'Sluggers', a new class of morphogenetic mutants of *D. discoideum*. *Expl Cell Res.* **116**, 217–227.

TOWN, C. D. & STANFORD, E. (1977). Stalk cell differentiation by cells from migrating slugs of *Dictyostelium discoideum*: special properties of tip cells. *J. Embryol. exp. Morph.* **42**, 105–113.

(Received 15 November 1979, revised 21 January 1980)