SHORT COMMUNICATIONS

**Synergistic Fruiting Body Construction by
Polysphondylium violaceum Mutants**

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INTRODUCTION

In *Dictyostelium discoideum*, the most studied of the cellular slime moulds, aggregation of post-vegetative myxamoebae produces a sluglike pseudoplasmodium which is subsequently transformed into a sorocarp composed of a terminal cluster of spores supported by a column of stalk cells (Bonner, 1967). The emergence of the sorocarp from the population of myxamoebae provides a model developmental system for the study of the genetic and cellular activities involved in cytodifferentiation and morphogenesis. One approach to analysis of the developmental process in these organisms has been the study of mutants incapable of completing sorocarp formation. In *D. discoideum* and *D. purpureum*, some morphogenetically defective mutants can interact synergistically to construct sorocarps of wild-type morphology (Sussman, 1954; Ono, Yanagisawa & Yamada, 1968; Weber & Raper, 1971; Yamada *et al.*, 1973) and study of the nature of these interactions may provide valuable insights into normal morphogenesis. Synergistic fruiting body formation in *Polysphondylium violaceum*, which produces a branched sorocarp, appears to be far less common (Rafaeli, 1962) than it is in *D. discoideum* and *D. purpureum*. A low incidence of synergism among 40 developmental mutants of *P. violaceum* is reported here, and a reason for this lack of co-operation suggested.

METHODS

The 40 mutant stocks used in this study were obtained by nitrosoguanidine (Yanagisawa, Loomis & Sussman, 1962) or ultraviolet (Sussman & Sussman, 1953) mutagenesis from wild-type stocks PVD7201 to PVD7210 of *Polysphondylium violaceum*. The parent stocks are morphogenetically compatible. The mutants were grouped into four developmental classes based on their terminal phenotypes. Fifteen stocks were aggregateless (*agg*); six could aggregate, but failed to develop further (*ps*); nine arrested at the pseudoplasmodium stage (*fri*); and ten formed incomplete or otherwise aberrant fruiting bodies (*fab*). The mutants were maintained on 0.2 % Cerophyl agar (Cerophyl Laboratories, Kansas City, Missouri 64112, U.S.A.) with *Escherichia coli* as the bacterial associate.

The ability of the mutants to act synergistically in fruiting body formation was tested as follows: cell suspensions of 3-day plate cultures were harvested in cold Bonner's salt solution (Bonner, 1947). Suspensions of mutant pairs to be tested were inoculated as adjacent drops about 3 cm apart on Cerophyl agar plates previously spread with a lawn of *E. coli*. Cell proliferation was checked at 48 h and synergism at the intersect of the two mutant

Table 1. Synergistic combinations of *Polysphondylium* mutants

	<i>agg</i> ⁹⁶	<i>agg</i> ²¹⁰	<i>agg</i> ¹⁰²⁴	<i>frt</i> ^{uv13}	<i>frt</i> ^{uv21}	<i>frt</i> ²⁴	<i>frt</i> ²⁶	<i>fab</i> ⁶⁶
<i>agg</i> ⁹⁶	—	.	.	.	.	.	.	.
<i>agg</i> ²¹⁰	—	—	.	.	.	.	.	.
<i>agg</i> ¹⁰²⁴	—	—	—	.	.	.	.	.
<i>frt</i> ^{uv13}	—	—	—	—	.	.	.	.
<i>frt</i> ^{uv21}	±	—	—	—	—	.	.	.
<i>frt</i> ²⁴	+	—	—	—	—	—	.	.
<i>frt</i> ²⁶	+	—	—	±	—	—	—	.
<i>fab</i> ⁶⁶	±	—	±	—	±	±	+	—

+, Fruiting bodies with wild-type morphology produced; ±, morphogenesis more normal than with either parent, but fruiting bodies of wild-type morphology not produced; —, no complete synergism observed.

populations was assayed after one week of incubation at 25 °C. The ability of the mutants to form synergistic fruiting bodies was tested in duplicate for all possible pairs.

RESULTS AND DISCUSSION

Of the 40 mutants tested, only three pairs produced fruiting bodies of wild-type morphology, although partial synergism was seen in six other combinations (Table 1). Five of the cases of complete or partial synergism involved *fab*⁶⁶, a mutant that produces twisted stalks and few sori, and four involved the aggregateless mutant *agg*⁹⁶. Of the three fully synergistic combinations, two paired an aggregateless mutant (*agg*⁹⁶) with a slug-forming partner, and one paired a slug-forming mutant with the abnormally fruiting mutant *fab*⁶⁶. No complete synergism was observed between mutants of similar terminal phenotypes.

The synergistic reticence displayed by *P. violaceum* is in marked contrast to the situation in *D. purpureum* and *D. discoideum*. In appropriate pairings, *D. purpureum* synergizes readily (Yamada *et al.*, 1973). Synergism in *D. discoideum* is somewhat less frequently observed, but is not uncommon (Sussman, 1954; Ono *et al.*, 1968; Weber & Raper, 1971). The only previously reported study on synergism in *P. violaceum* is that of Rafaeli (1962), who noted only one case of synergistic fruiting body formation in pairwise combinations of seven morphogenetic mutants. The work reported here indicates that the poor synergism observed by Rafaeli is a characteristic of the species rather than due to the small number of mutants she tested.

The ability of mutants to enter into synergistic combinations is believed to be related to the normal cell interactions involved in morphogenesis, as synergism rarely occurs between mutants of the same morphogenetic class (Sussman, 1954; Ono *et al.*, 1968; Weber & Raper, 1971; Yamada *et al.*, 1973). The low number of synergistic associations noted to date in *P. violaceum* has generated no comparable pattern of cell interactions. However, the low incidence of synergism itself implicates another aspect of this type of intercellular complementation. Synergism may be referable to the prestalk–prespore polarization seen in *D. discoideum* and *D. purpureum*, but not in *P. violaceum*. Prestalk and prespore cells can be demonstrated in migrating slugs of the two *Dictyostelium* species by regional differences in their reaction to vital dyes and histochemical stains (Bonner, 1967). The prestalk cells occupy the anterior part of the slug and the prespore cells occupy the posterior part, with a fairly sharp dividing line separating the two regions. Ultrastructural differences distinguishing the two cell types have also been described (Hohl & Hamamoto, 1969; Maeda &

Takeuchi, 1969). The regional polarization of *D. discoideum* is paralleled by gradients in cyclic AMP (c-AMP) and Ca^{2+} concentration, with higher concentrations of both substances in the prestalk region of the slug (Maeda & Maeda, 1973; Pan *et al.*, 1974). In *D. discoideum* the pre-aggregative population shows no cytological characteristics of prespore or prestalk differentiation, but is heterogeneous with regard to c-AMP and Ca^{2+} content (Maeda & Maeda, 1974). The cells of the fraction high in both c-AMP and Ca^{2+} were found in the prestalk region of the slug after aggregation, indicating that this heterogeneity is functionally significant. *Polysphondylium violaceum* demonstrates no prestalk–prespore divergence by any of the above indicators. In constructing the branched Polysphondylium fruiting body, prestalk–prespore differentiation occurs either as a very transient phase immediately before final stalk–spore differentiation or not at all, as attempts to catch the intermediate stage have been unsuccessful (Bonner, 1967). It is proposed that the ability of *D. discoideum* and *D. purpureum* to form synergistic fruiting bodies in appropriate combinations is directly related to the early divergence of prespore and prestalk populations in these species, and that *P. violaceum* fails to synergize effectively because this early differentiation does not occur. However, the restricted synergism observed in this species may be of value in analysing synergism, as characteristics of successful pairings may reveal the essential parameters of the process. Further work is being directed toward isolation and identification of effective synergists.

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