Genetic Analysis of a Gene Regulating the Timing of Developmental Events in *Dictyostelium discoideum*

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In *Dictyostelium discoideum*, there exist genes which regulate the timing of developmental events. In one class of these rapidly developing (*rde*) mutants, the rate of development is accelerated, resulting in the formation of spores and stalk cells in about 2/3 of the time required for the parent. Linkage analysis of one of the *rde* strains, HTY506, demonstrated that *rdeC1850*, carried by the mutant is located on linkage group III. Therefore HTY506 differs genetically from the strain FR17, the first *rde* mutant described which carries a mutation *rdeA1* on linkage group IV. While strain HTY506 has a pleiotropic phenotype, the altered characteristics of strain HTY506 appear to be caused by a single mutation, *rdeC1850*.

INTRODUCTION

During development, organisms undergo a systematic series of morphological and biochemical changes. However, little is known about the mechanisms which regulate the timing of developmental events. In *Dictyostelium discoideum*, this timing has been shown to be affected by certain mutations (Sonneborn *et al.*, 1963; Loomis, 1970; Atryzek, 1976; Kessin, 1977; Abe & Yanagisawa, 1983). For example, in a strain FR17 (Sonneborn *et al.*, 1963), all morphological and biochemical changes during development occur much earlier than in the wild-type parent without alteration in the normal order of these events. These mutants may be useful to study the temporal control of development.

Rapidly developing (*rde*) mutants have been isolated in several laboratories (Sonneborn *et al.*, 1963; Atryzek, 1976; Kessin, 1977), and Kessin (1977) has reported that FR17 carries a recessive mutation, *rdeA1*, located on linkage group IV.

To learn more about genetic control mechanisms of development, we have isolated a number of *rde* mutants and attempted to analyse these mutants genetically. We performed linkage analysis on one of the *rde* mutants, HTY506, and found that the mutation, *rdeC1850*, carried by HTY506 is located on linkage group III. This suggests that there exist at least two mechanisms involved in regulating the timing of development of *D. discoideum*.

METHODS

Strains. *Dictyostelium discoideum* strains used were originally derived from the wild-type strain NC4. Genotypes of haploid strains are given in Table 1. The rapidly developing mutant strain HTY506 was derived from XP55 by γ -ray irradiation (Abe & Yanagisawa, 1983). Strains NP2, XP55, XP95 and *Bacillus subtilis* 36.1 were kindly provided by Dr P. C. Newell (University of Oxford) and strain NP12 was a gift from Dr K. L. Williams (Max Planck Institute).

Growth and development. Amoebae were usually grown on nutrient agar plates in association with *Klebsiella aerogenes* at 22 °C (Sussman, 1966). For development of cells in liquid cultures, washed cells were suspended at a concentration of 1×10^7 cells ml⁻¹ in 20 mM-potassium phosphate buffer, pH 6.4 and shaken on a rotary shaker at 22 °C and 120 r.p.m.

Formation of heterozygous diploids. Heterozygous diploids were constructed according to the method of Newell *et al.* (1977). Diploids were usually recovered at a frequency of 1×10^{-5} . After two successive reclonings, diploid strains were identified by characteristic spore size, shape and genetic markers (Sussman & Sussman, 1962, 1963).

Table 1. *Genotypes of haploid strains of Dictyostelium discoideum used*

Strain	Genotype*									Reference†
	<i>cyc</i>	<i>whi</i>	<i>acr</i>	<i>axe</i>	<i>tsq</i>	<i>bsg</i>	<i>bwn</i>	<i>man</i>	<i>cob</i>	
XP55	A5					A5				a
XP95		A1	A1	A1	A1		A1	A2	A1	a
NP2				A1,B1	A1					b
NP12			A2	A1,B1	B3					b

* Genotypic symbols are as follows: *cyc*, cycloheximide resistance; *whi*, white sorocarp; *acr*, acriflavin and methanol resistance; *axe*, axenic growth; *tsq*, temperature-sensitive growth; *bsg*, lack of growth on *B. subtilis*; *bwn*, brown pigment; *man*, deficiency in α -mannosidase; *cob*, cobalt resistance.

† a, Ross & Newell (1979); b, Williams *et al.* (1974).

Haploidization. Haploid segregants were obtained from diploids plated clonally on nutrient agar plates containing the haploidizing agent, benlate at a concentration of 20–50 $\mu\text{g ml}^{-1}$ (Williams & Barrand, 1978). The plating efficiency of diploids is about 30% at 30 $\mu\text{g benlate ml}^{-1}$. Haploid segregants appearing on benlate plates after 4–5 d were tested for their phenotypes using standard methods (Williams *et al.*, 1974; Ratner & Newell, 1978).

RESULTS

Rapidly developing mutants have been classified into two complementation groups, A and C (Abe & Yanagisawa, 1983), the former corresponding to Kessin's *rdeA* mutants (Kessin, 1977), and the latter representing previously unreported groups of mutants. In this report, linkage analysis of the group C mutation in strain HTY506 is presented. Strain HTY506 was derived from XP55 by γ -ray irradiation resulting in approximately 10% survival. HTY506 formed heat-resistant spore and stalk cells 16 h after the initiation of development, while the parental strain XP55 did not fully differentiate until 24 h. This mutant also showed an abnormal final morphology, and was able to differentiate under conditions in which normal development was arrested without the addition of exogenous cAMP. For example, cells of HTY506 could form spore and stalk cells in liquid media (60–70% of spores and 10–20% of stalk cells to total cells) and were able to form stalk cells without cell-to-cell contact. More detailed characteristics of group C mutants have been described (Abe *et al.*, 1981; Abe & Yanagisawa, 1983).

In order to assign *rdeC*1850, carried by HTY506 to a linkage group, the diploid strain DTY2 was constructed by parasexual fusion of HTY506 with a multiply marked tester strain, XP95. By plating spores of DTY2 on to benlate plates, we recovered several hundred haploid segregants whose phenotypes are shown in Table 2. The most easily scored phenotype caused by *rdeC* is aberrant final morphology. However, as the tester strain itself often shows abnormal fruiting structures, we also checked where necessary either, (1) the time required for spore formation or (2) the ability to differentiate into spore and stalk cells in liquid media.

The results demonstrated that the mutation *rdeC*1850 segregated independently from the markers on linkage group I, VI and VII. Although a non-random segregation pattern was observed relative to linkage group II and IV, the presence of recombinant phenotypes demonstrated that the *rdeC* locus is not on linkage group II and IV. In contrast, the marker *bsgA*5 on linkage group III always co-segregated with *rdeC*1850. Thus all the *RdeC*⁺ segregants possess *tsqA* and all the *RdeC* segregants are *bsgA*. Based on these results, we conclude that the mutation is located on linkage group III.

Two comments regarding these data must be made. First, the number of clones with the *RdeC* phenotype is several-fold higher than that of *RdeC*⁺. This is thought to be caused by a difference in growth rate on benlate plates between *RdeC* segregants and *RdeC*⁺ segregants.

As shown in Fig. 1(a), while the growth rate of HTY506 was lower than the wild-type strain XP95, it still showed substantial growth on benlate plates, though strain XP95 barely grew on benlate. Only tiny colonies were produced even after 10 d growth. Furthermore, *RdeC*⁺

Fig. 1. Effect of benlate on growth rate of parental haploid strains and segregants. Cells of each strain were inoculated with toothpicks on to lawns of *K. aerogenes* growing on nutrient agar plates, with or without benlate ($30 \mu\text{g ml}^{-1}$). Growth was measured by marking the boundaries of colonies at intervals. (a) Control, HTY506 (○), XP95 (□); benlate, HTY506 (●), XP95 (■). (b) Control, RdeC⁺ segregants (△), RdeC⁺ segregants (▽); benlate, RdeC segregants (▲), RdeC⁺ segregants (▼).

segregants also grew poorly on benlate (Fig. 1b). Therefore, we assume that the relatively poor growth of RdeC⁺ segregants on benlate results in a greater number of RdeC segregants than RdeC⁺.

The second point concerns the apparent non-random segregation pattern observed relative to the markers on linkage group II and IV. For example, only three classes of haploid progeny involved with linkage group II were recovered. The RdeC⁺ AcrA⁺ WhiA⁺ class of segregant from DTY2 was completely absent. Ross & Newell (1979) reported similar results in the cross of XP55-derived mutants with XP95. They postulate that this is caused by unidentified mutations on chromosome II of XP95 and chromosome III of XP55 which individually are not harmful, but in combination are deleterious. Alternatively, since strains carrying an *acrA* mutation show cross-resistance to benlate (Williams & Barrand, 1978), it is expected that the segregants carrying *acrA* should grow rapidly on benlate. In addition, RdeC⁺ segregants also grew poorly on benlate. Therefore, apparently RdeC⁺ AcrA⁺ WhiA⁺ segregants did not appear on benlate. Nevertheless, the presence of the RdeC⁺ AcrA⁺ WhiA and RdeC AcrA WhiA class of segregants clearly shows the absence of linkage of *rdeC* with chromosome II.

In this cross, we also found that the appearance of segregants was highly skewed against the *bwnA* marker. To re-examine the relationship of *rdeC* locus to group IV, HTY506 was crossed to NP12 (which carries a marker *tsgB3* mutation on linkage group IV) to form strain DTY9, from which haploid segregants were isolated using benlate. As may be seen in Table 3, the *rdeC*1850 mutation segregated independently from a marker *tsgB3*, while segregating with *bsgA5*. These experiments confirmed earlier results that the *rdeC*1850 mutation is located on linkage group III.

As mentioned above, *rdeC* mutants were scored on the basis of their abnormal final morphology. In order to test the possibility that other characteristics of these mutants such as the capacity to differentiate in liquid media and rapid development are caused by the same mutation, we examined the characteristics of about 50 independently isolated haploid segregants from diploid DTY6 (Table 4). DTY6 was produced by the fusion of HTY506 (which carries *bsgA* on linkage group III) with NP2 bearing *tsgA* on group III. In all cases, segregants carrying chromosome III of HTY506 formed spores and stalk cells by 18 h on a solid substratum. The fruiting structure had an aberrant morphology. All of the segregants could differentiate into spore and stalk cells in liquid culture. In contrast, segregants carrying chromosome III of NP2 produced normal fruiting-bodies with normal timing, and did not form spores and stalk cells in a shaken liquid suspension. These results suggest that the pleiotropic phenotype of group C mutants is caused by the same single mutation, and that if multiple mutations are involved, they must be linked.

Table 3. *Pattern of phenotypes of haploid segregants from DTY9*

Diploid	Parental haploid	Linkage group	III	IV		
DTY9	<div> <div>NP12</div> <div>HTY506</div> </div>		+	<i>tsgB</i>		
			<i>bsgA</i>	+		
			Bsg	Bsg ⁺	Tsg	Tsg ⁺
	RdeC ⁺		0	17	13	4
	RdeC		89	0	15	74

Table 4. *Characteristics of haploid segregants from DTY6*

	Morphology		Appearance of spores at:		Differentiation* in liquid	
	Normal	Aberrant	18 h	24 h	+	—
Bsg	0	24	24	0	24	0
Bsg ⁺	25	0	0	25	0	25
Tsg	25	0	0	25	0	25
Tsg ⁺	0	24	24	0	24	0

* + indicates that cells differentiate into considerable number of spores (about 60–70% of total cells) in a liquid suspension culture by 20 h after the initiation of development; — indicates no spores appeared in liquid culture.

DISCUSSION

The pleiotropic nature of the group C mutant phenotype could indicate multiple mutations occurring within the same linkage group. However, the characteristics of group C mutants always co-segregated and independent group C mutants could be isolated frequently, even after a relatively mild mutagenic treatment (about 10% survival). For example, of the 16 *rde* mutants isolated, 10 were group C mutants, while only two were from group A, which is believed to carry a single mutation (Kessin, 1977). These results suggest that the pleiotropic phenotype of group C mutants is caused by a single mutation.

Several research groups have isolated mutants showing phenotypes similar to our *rde* mutants (Sonneborn *et al.*, 1963; Atryzek, 1976; Kessin, 1977). The assignment of *rdeC*1850 to linkage group III confirmed our previous conclusion that group C mutants differ genetically from group A mutants carrying *rdeA* on linkage group IV. While another group of *rde* mutants, group B (i.e. *rdeB*), has been described (Kessin, 1977), these strains have been lost, and were therefore not available for inclusion in our genetic analysis. However, several non-genetic characteristics suggest that our group C mutants differ from these group B mutants, as well as group A mutants. In group A and group B mutants, cell aggregation occurs several hours earlier than in their parental strains, while group C mutants aggregate with normal timing. In group C mutants, it is mainly the time required for spore and stalk cell differentiation following the completion of aggregation that is reduced (Abe & Yanagisawa, 1983). This suggests that there are at least two mechanisms involved in regulating the timing of development of *D. discoideum*.

The phenotypes of sporogenous mutants, described by Gross's group (Town *et al.*, 1976; Gross *et al.*, 1981), share some characteristics in common with our *rde* mutants, e.g. abnormal final morphology and differentiation under unusual conditions. An analysis between the sporogenous mutants and our *rde* mutants would be interesting.

To date, the genes affected by the *rde* mutations are not known, however, cAMP metabolism is significantly altered in both group A and group C mutants (Coukell & Chan, 1980; Abe & Yanagisawa, 1983). Characteristics similar to those seen in the *rde* mutants, can be induced by the addition of cAMP to wild-type cells (Gerisch *et al.*, 1975; Town *et al.*, 1976; Feit *et al.*, 1978; Nestle & Sussman, 1972). Hence, the phenotypes of *rde* mutants may be caused by their altered cAMP metabolism.

Further genetic and biochemical studies on *rde* mutants will provide clues to identity of the genes which regulate the timing of development.

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