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Mutations Affecting a Surface Glycoprotein, gp80, of *Dictyostelium discoideum*

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We isolated two independent mutations in *Dictyostelium discoideum* that result in the absence of the antigenic determinant recognized by monoclonal antibody E28D8. This antibody reacts with a post-translational modification on the surface glycoprotein gp80 and several other proteins. Both of the mutations occur in the same locus, *modB*, which was mapped to linkage group VI. The *modB* mutations result in sufficient alteration of gp80 that it is absent or unrecognizable by two-dimensional gel electrophoresis. Strains carrying *modB* mutations exhibit "contact sites A"-mediated cell-cell adhesion although more weakly than do wild-type strains and develop to fruiting bodies carrying viable spores. Although gp80 has been implicated in the mechanism of cell-cell adhesion in *D. discoideum*, it is clear from the behavior of these mutant strains that the determinant on gp80 recognized by E28D8 is not necessary for either morphogenesis or reduced EDTA-resistant adhesion.

Cells of *Dictyostelium discoideum* undergo a process of development during which they become able to form strong, EDTA-resistant intercellular adhesions (mediated by "contact sites A") (4). This EDTA-resistant adhesion can be blocked by Fab fragments of antibodies from sera prepared against developed cells. Although these polyclonal antibodies react with many proteins of developed cells, the adhesion-blocking activity can be effectively neutralized by a surface glycoprotein of ca. 80,000 daltons (gp80) (9, 10). Likewise, rabbit antibodies prepared against purified gp80 have been found to block EDTA-resistant adhesion (12). gp80 is undetectable during the first few hours of development and is synthesized and accumulates between 8 and 14 h of development coordinately with the acquisition of EDTA-resistant adhesion (12, 14). For these reasons it is thought that gp80 may play a direct role in the mechanism of cell-cell adhesion in developed *D. discoideum*.

Rabbit serum prepared against purified gp80 reacts not only with gp80 but with several other proteins, apparently due to the presence of shared antigenic determinants (11, 12). A few of these antigenic proteins are present in vegetative cells and exhaustive absorption of this serum with vegetative cells removes some but not all immunological reactivity to gp80 (11). However, the exhaustively absorbed serum is no longer found to block EDTA-resistant intercellular adhesion (11). These results raised the possibility that some determinants on gp80 might not be critical to the mechanism of adhesion, whereas others are clearly crucial as shown by the ability of purified gp80 alone to neutralize the adhesion-blocking activity of the antiserum.

Monoclonal antibodies have been raised against gp80 (11, 14, 15). At least one of these, E28D8, appears to react with a post-translational glycosyl modification present on gp80 as well as several other proteins (11). At the developmental stage when EDTA-resistant adhesion is first observed (late aggregation), monoclonal antibody E28D8 only reacts with two proteins, gp80 and p95 (11). Since rare mutations affecting specific genes can be isolated from the haploid cells of *D. discoideum*, we have analyzed the role in cell-cell adhesion of the antigenic determinant recognized by E28D8 by isolating mutants which fail to react with this monoclonal antibody. Two independent mutations were recovered that abolish reactivity of vegetative or developed *D. discoideum* cells with the monoclonal antibody. Cells carrying either of these mutations lack detectable amounts of gp80 but develop measurable EDTA-resistant adhesion and give rise to fruiting bodies containing viable spores.

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MATERIALS AND METHODS

Mutant screening. *D. discoideum* strain AX3 amoebae were grown axenically in HL5 medium as previously described (11). Amoebae were mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine to 10^{-3} to 10^{-4} survival and were immediately plated into microtest plates at about 1 cell per well as described previously (6). Surviving clones were grown to a density of ca. 10^4 to 10^5 cells per ml, at which point 5-μl samples were spotted onto nitrocellulose filters (Schleicher & Schuell BA85, moistened with water). The filters were fixed for 5 min in 90% methanol-2% acetic acid, followed by three 5-min water washes. Binding of monoclonal antibody E28D8 was assayed by visualization of binding of ^{125}I-labeled staphylococcal protein A, followed by autoradiography as previously described (11, 18), except that (i) all solutions were at pH 8.0 to enhance the binding of protein A to E28D8 immunoglobulin G1 (IgG1) antibodies (2); (ii) incubation with E28D8 was for 2 to 4 h; and (iii) incubation with protein A was for 1 to 2 h. Autoradiograms generally were exposed overnight. Isolates which failed to react with E28D8 (i.e., did not darken the film) were recovered, grown, and retested. Those which were still negative were recloned by dilution onto plates in association with *Klebsiella aerogenes*, transferred on toothpicks from growing plaques to nitrocellulose filters, and retested as above. Two stable negative isolates were recovered from a total of 1,236 survivors of mutagenesis; they were designated strains HL205 and HL207, carrying mutations *modB501* and *modB502*, respectively.

Genetic analysis. General methods for parasexual analysis have been described previously (5, 8, 13, 16, 19, 20). Strains HL206 (*tsg506*) and HL208 (*tsg509*) are temperature-sensitive derivatives of strains HL205 and HL207, respectively. Strains HL206 and HL208 were crossed with tester strains XP99, HL204, and XP330, and diploids were selected by

TABLE 1. Genotypes of haploid strains

Strain	Linkage group					
	I	II	III	IV	VI	VII
Parents						
HL206[a]		*axeA1*	*axeB1*		*modB501*	
HL208[b]	*tsg509*	*axeA1*	*axeB1*	*tsgB3*	*modB502*	
HL204	*cycA*	*acrA1*	*bsgA1*	*ebr404*		
XP99[c]	*cycA*		*bsgA5*	*bwnA1*	*manA2*	*cobA1*
		acrA2				
XP30		*whiA1*	*bsgA5*		*manA2*	*cobA1*
		axeA1				
Segregants[d]						
HL211	*cycA*	*axeA1*	*axeB1*		*modB501*	
		acrA2				
HL216		*axeA1*	*bsgA5*		*modB501*	
HL220	*cycA*		*bsgA5*	*bwnA1*	*modB502*	
HL221	*tsg509*		*bsgA5*	*bwnA1*	*modB502*	

[a] The temperature-sensitive mutation in strain HL206 was not mapped.
[b] The temperature-sensitive mutation in strain HL208 (*tsg509*) was mapped to linkage group I (data not shown).
[c] Strain XP99 and its derivatives carry either *cycA1* or *cycA5*.
[d] The stock of strain AX3 which was mutagenized to obtain the *modB* mutations carried an undetected *agg* mutation (not mapped) which is also present in strains HL205, HL206, HL207, and HL208 but absent from strains HL216 and HL220.

growth of 27°C on *Bacillus subtilis* plates. The genotypes of the parental strains are given in Table 1 (8, 16; P. Newell, personal communication; this study). The tester strains carry *bsgA* mutations which render them unable to grow on *B. subtilis*, whereas strains HL206 and HL208 are unable to grow at 27°C. Diploidy was verified by spore size analysis. Diploidy was also indicated by wild-type phenotypes of parental mutant characters in the diploids and by the reappearance of these characters in haploid segregants. Haploid segregants were obtained by growth on cycloheximide plates (500 μg/ml), ben late plates, or 2% methanol plates (16, 19, 20). All segregants were examined for haploidy by genetic analysis (i.e., possession of recessive parental markers on more than one linkage group) or by spore size analysis or both. Segregants whose haploidy could not be verified were not included in the analysis. Markers were scored as previously described (8, 16, 20). α-Mannosidase activity (*manA*) was scored after heating the extract at 55°C for 10 min to inactivate residual activity of the mutant enzyme.

RESULTS

Isolation and mapping of *modB* mutations. We used a nitrocellulose filter binding assay to screen mutagenized vegetative cells of strain AX3 for mutant strains which no longer react with monoclonal antibody E28D8. Two independent isolates, designated HL205 and HL207, were recovered from 1,236 survivors of mutagenesis as described above. This screening technique should be applicable to the isolation of mutations affecting any determinant for which an antibody is available.

To partially separate the new mutations from their mutagenized backgrounds and to map them with respect to the established parasexual linkage groups of *D. discoideum* (5, 13), we crossed temperature-sensitive derivatives of each strain with the well-marked tester strains XP99, HL204, and XP330 (8, 16; P. Newell, personal communication). The resulting diploids all reacted with E28D8, indicating that both *modB* mutations are recessive (data not shown). Haploid segregants of these diploids were screened for reactivity with E28D8 and for other genetic markers (Table 2, Fig. 1). Both mutations were found to segregate with linkage group VI in opposition to the *manA* marker and independently of markers on the other linkage groups tested.

Strain HL211 (derived from HL205) was crossed with strain HL220 (derived from HL207). The resultant diploids did not react with E28D8 (Fig. 1). We conclude that both mutations lie within the same complementation group. For the reasons given below, we have designated the two mutations *modB501* (from HL205) and *modB502* (from HL207).

Characterization of *modB* mutants. The original AX3 strain from which the *modB* mutations were isolated contained an inadvertant mutation(s) which blocks development at the aggregate stage. We tried to separate the *modB* mutations from this background by segregation from the diploids formed with the tester strains XP99 and HL204. The deduced genotypes of several of the segregants are given in Table 1. For both *modB501* and *modB502*, several segregants were recovered which failed to react with E28D8 but which formed fruits containing stalks and detergent-resistant spores. Cells grown from such detergent-treated spores failed to react with E28D8. Thus the presence of the determinant recognized by this monoclonal antibody is not essential for the growth or development of *D. discoideum* under the conditions employed in our laboratory. Of course, more subtle effects on development cannot be ruled out.

The fruiting segregants HL216 (carrying *modB501*), HL220 (carrying *modB502*), and HL221 (carrying *modB502*) all developed the ability to form EDTA-resistant adhesions at approximately the same stage of development as did bacterially grown *modB*$^+$ strains such as AX3 or XP99, and the formation of these adhesions was blocked by rabbit antibodies raised against gp80 (Table 3). Thus these cells could still form contact sites A-mediated adhesions (defined as EDTA-resistant adhesions sensitive to anti-gp80 antibodies), although the percent single cells was higher in strains HL220 and HL221. Furthermore, the blocking ability of the antibodies against either mutant or wild-type cells could be removed by absorption with either mutant or wild-type cells (Table 2), showing that mutant and wild-type cells share the target determinants for our adhesion-blocking antibodies.

TABLE 2. Linkage analysis of *modB*[a]

Diploid	Parental strains	Haploid segregants	I		II		III		IV		VI		VII	
			Cyc$^+$	Cyc	Acr$^+$	Acr	Bsg$^+$	Bsg	Bwn$^+$	Bwn	Man$^+$	Man	Cob$^+$	Cob
DL 102	HL206	*modB*$^+$	—[b]	3			0	3	3	0	0	3	0	3
	XP99	*modB501*	—	19			17	2	1	0	19	0	17	1
DL 103	HL208	*modB*$^+$	23	6			8	21	25	1	0	29	27	2
	XP99	*modB502*	32	22			23	31	45	3	54	0	48	6
DL 114	HL206	*modB*$^+$			—	27	18	9			0	24		
	XP330	*modB501*			—	53	52	1			39	0		
DL 115	HL208	*modB*$^+$			—	41	15	26						
	XP330	*modB502*			—	17	14	3						

[a] All of the markers used are recessive, including both *modB* mutations, and were scored as described in the text. Crosses of HL206 with HL204 also indicated that *modB501* is not on linkage groups I, II, or III (data not shown).
[b] —, Marker used in haploid selection technique.

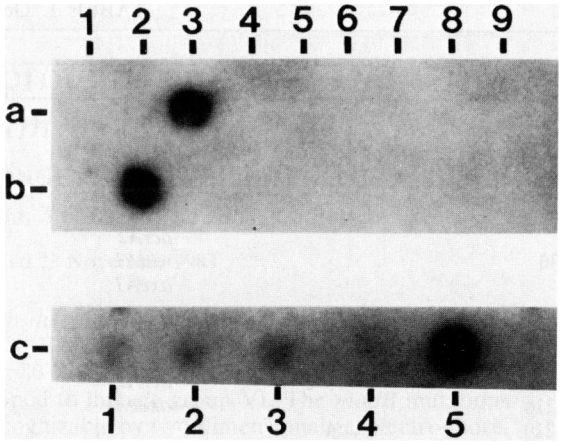

FIG. 1. Segregation and complementation analysis of *modB*-carrying strains. Strains were assayed for the ability to react with monoclonal antibody E28D8 by a nitrocellulose filter binding assay. A photograph of the autoradiogram is shown. Row a: strains HL211 through HL219 in lanes 1 through 9, respectively. These strains are segregants from diploids formed by crossing strain HL205 (*modB501*) with tester strain XP99 (strains HL211 to HL215) or HL204 (strains HL216 to HL219). Strain HL213 carries the wild-type allele, and the remaining strains carry *modB501*. Row b: strain HL208 (*modB502*) in lane 1 and strain HL80 (*modB*$^+$) (wild-type control) in lane 2. Row c: strains HL211 (*modB501*) (lane 1), HL220 (*modB502*) (lane 2), two diploids derived by crossing strains HL211 and HL220 (lanes 3 and 4), and HL80 (lane 5). The diploids were shown to segregate haploids carrying *bwnA5* (from HL220) but were not themselves brown, verifying that they arose from fusions between the indicated parental strains. Other mating experiments, including those which generated strains HL211 through HL219, demonstrated that both *modB501* and *modB502* are recessive to the wild-type allele (data not shown).

When these strains were analyzed at several different times of development, no proteins were detected which reacted with monoclonal antibody E28D8 or with polyclonal anti-gp80 rabbit antibodies which had been absorbed with vegetative AX3 cells (Fig. 2). We have shown previously that both of these antibody preparations react with periodate-sensitive (presumably carbohydrate) determinants on gp80 and on other proteins in vegetative and developed cells (11). Neither antibody preparation can block EDTA-resistant adhesion of wild-type or *modB* cells (11; data not shown). The unabsorbed rabbit antibodies raised against gp80, which do block adhesion, retain the ability to bind to many proteins even in *modB* cells (Fig. 2).

When total cell proteins or membrane proteins were examined by two-dimensional gel electrophoresis at several times during development, spots corresponding to gp80 (11, 12) were not detected, either by silver staining (to detect accumulated proteins) (Fig. 2) or by autoradiography of material from [^{35}S]methionine-labeled cells (to detect newly made proteins; data not shown). We estimate that we would have been able to detect spots with intensities 10% of those from wild-type (strain AX3 or HL80) cells. We were unable to identify any new spots present for the mutants but absent for the wild-type cells. Strains HL220 and HL216 normally synthesized and accumulated spore coat proteins (data not shown), which in the wild-type strain appear later in development than does gp80 (1). This is consistent with the ability of the mutant strains to complete development and to produce viable spores.

TABLE 3. Adhesiveness[a]

Strain and method	*modB* allele	Blocking antibody	Antibody absorption	% Single cells	
				Range	Avg
A					
AX3	+			48–64	54
XP99	+			50–57	53
HL216	501			36–43	41
HL220	502			72–85	78
B					
AX3	+	None		32–33	33
AX3	+	Anti-gp80		59–71	66
HL216	501	None		29–31	29
HL216	501	Anti-gp80		63–71	67
C					
AX3	+	Preimmune	None	31–33	32
AX3	+	Anti-gp80	None	66–74	69
AX3	+	Anti-gp80	0 h AX3	29–34	32
AX3	+	Anti-gp80	10 h AX3	29–34	31
AX3	+	Anti-gp80	0 h HL221	31–39	35
AX3	+	Anti-gp80	10 h HL221	36–39	37
HL221	502	Preimmune	None	68–82	76
HL221	502	Anti-gp80	None	88–92	90
HL221	502	Anti-gp80	0 h AX3	71–78	74
HL221	502	Anti-gp80	10 h AX3	72–73	73
HL221	502	Anti-gp80	0 h HL221	72–81	76
HL221	502	Anti-gp80	10 h HL221	78–94	85

[a] Cells were collected, washed, and gyrated at 200 rpm for 20 min at 22°C as described previously (12). Adhesion was assayed by following the disappearance of single cells, using an electronic particle counter (12). The mean and range of triplicate determinations are presented. Methods: A, cells which had been grown on bacteria were collected at 12 h of development; B, cells were collected at 12 h of development, washed, and incubated on ice for 30 min at 10^7 cells per ml in the presence of 0 or 100 μg of rabbit anti-gp80 antibodies per ml before being assayed for adhesion (12, 17); C, cells (collected at 10 h of development) were incubated before assay as in B with 111 μg of preimmune rabbit IgG per ml, 183 μg of unabsorbed rabbit anti-gp80 IgG per ml, or 183 μg of unabsorbed rabbit anti-gp80 IgG per ml which had been absorbed twice with 5×10^7 of the indicated cells per 75 μg of IgG for each absorption.

DISCUSSION

Because the antigenic determinant recognized by monoclonal antibody E28D8 is present on different proteins with different developmental expressions, we favor the hypothesis that it is associated with a post-translational modification (probably involving carbohydrate) common to a number of proteins (11). Since a single mutation simultaneously abolishes the expression of this determinant on all of these proteins, the mutation is likely to affect a modification system required for the establishment or maintenance of the determinant. Thus, we have designated the locus involved as *modB* by analogy with the previously described locus *modA* (3). None of the proteins carrying the antigenic determinant recognized by E28D8 could be detected in the mutant strains, either immunologically or by two-dimensional gel electrophoresis. Nevertheless, the mutant strains are capable of contact sites A-mediated intercellular adhesion (defined as EDTA-resistant intercellular adhesion sensitive to anti-gp80 antibodies) although it is weaker than that of wild-type strains and are capable of development into spores and stalk cells. We cannot rule out the possibility that these mutants may show developmental defects under conditions of development other than those used in our laboratory.

Possible interpretations of our observations include: (i) gp80 (and other proteins bearing the monoclonal antibody-reactive determinant) are not essential for contact sites A-mediated adhesion; (ii) normal cellular levels of gp80 are considerably (at least 10-fold) in excess of those sufficient for adhesion, so that enough undetected gp80 may remain in the mutant strains to allow adhesion to occur; or (iii) gp80 is present and functioning in adhesion in the mutants, but it is so altered as to be unrecognized by the immunological and biochemical methods we used. Whether or not gp80 itself is required for adhesion, it carries the determinants recognized by our adhesion-blocking rabbit antibodies, since affinity-purified (using E28D8) gp80 can neutralize the adhesion-blocking ability of these antibodies (11). The target determinants for adhesion blocking are not identical with the determinant recognized by E28D8 since absorption with mutant cells (which lack the E28D8 determinant) removes the adhesion-blocking antibodies. Furthermore, the target determinants are not found only on gp80 as absorption with vegetative cells (which do not express gp80) also removes the adhesion-blocking antibodies. We conclude that gp80 and other molecules share antigenic determinants which are targets for adhesion-blocking antibodies. We hope to examine the role of gp80 itself in the adhesion process by isolating mutations in the structural gene for the glycoprotein. Alternatively, it should be possible to purify molecules from *modB* cells which can neutralize the adhesion-blocking activity of our antibodies. Mutants can then be sought in these molecules to determine whether they are physiological targets for the blocking of adhesion.

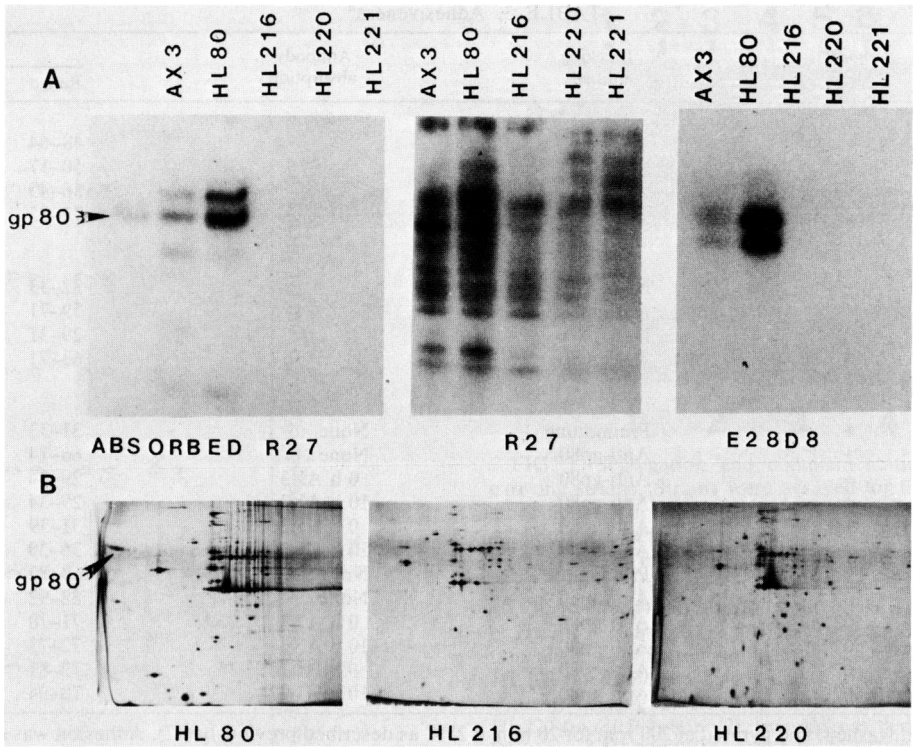

FIG. 2. Lack of gp80 in mutant cells. Cells of wild-type and mutant strains were allowed to develop on filters for 12 h before collection. For panel A, total cellular proteins were analyzed by one-dimensional electrophoresis, followed by transfer to nitrocellulose (12, 18) and staining with absorbed (left) or unabsorbed (middle) rabbit polyclonal antibody (R27) directed against gp80 or with monoclonal antibody E28D8 (right). For panel B, membranes were isolated from the cells (12) and were analyzed by two-dimensional electrophoresis, followed by silver staining as previously described (1, 7). The position of gp80 at the acidic end is indicated.

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