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A Polymorphic, Prespore-Specific Cell Surface Glycoprotein Is Present in the Extracellular Matrix of *Dictyostelium discoideum*

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Polymorphisms of a major developmentally regulated prespore-specific protein (PsA) in *Dictyostelium discoideum* slugs are described. These polymorphisms allowed discrimination between PsA (found on the cell surface and in the extracellular matrix) and a similar extracellular but nonpolymorphic protein, ShA. The two proteins were also distinguished by their differing reactivities with a range of monoclonal antibodies and by their sensitivity to release from the sheath with cellulase. The results are discussed in terms of the molecular and genetic relationships between the cell surface and the extracellular matrix during development.

Interactions between the cell surface and the extracellular matrix have been proposed to play an important role in developmental processes (e.g., sea urchin blastulation [6, 10]). In some cases (e.g., fibronectin), there are distinct cellular and extracellular forms of the molecules proposed to be involved in these interactions, raising questions concerning the structural and functional relationships between the cell surface and the extracellular matrix. Consequently, elucidation of the biochemical similarities and differences between cellular and extracellular molecules is a necessary prerequisite to resolve how such interactions occur.

The cellular slime mold *Dictyostelium discoideum* has been used extensively as a model system for the study of a wide range of developmental processes (see reference 9). During development, individual vegetative amoebae aggregate to form a multicellular mass which is then covered throughout the developmental process by a thin cellulose-protein sheath or extracellular matrix (16, 17). Although numerous roles for the sheath have been suggested, evaluation of these proposals has been hampered by the lack of information concerning the composition of the sheath. It contains cellulose fibrils (1) in a highly proteinaceous matrix (5, 18), and recent work with monoclonal antibodies (mAbs) has demonstrated shared antigenic determinants on a large number of prespore cell surface and extracellular matrix proteins (3; Grant et al., manuscript in preparation). Furthermore, there are a small number of specific cell surface proteins which are also apparently in the sheath, i.e., have cellular and extracellular forms.

The most prominent of these was a 32-kilodalton (kDa) antigen whose cellular and extracellular forms were immunochemically distinguishable. It was not determined whether this was due to (i) alternate processing pathways of a single precursor, (ii) similar processing of unrelated precursors, or (iii) the existence of related but genetically distinct proteins.

In this work we demonstrate, using genetic (13, 22), immunochemical, and biochemical criteria, that there are two 32-kDa proteins: one (termed PsA) which is common to the prespore cell surface and the sheath and is polymorphic and a second (termed ShA) which may be related to PsA but is encoded by a separate, nonpolymorphic locus.

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MATERIALS AND METHODS

Culture conditions and strains. Standard conditions were used for the growth of *D. discoideum* amoebae and the preparation of migrating slugs (18, 19). Sheath, sheath extracts, and slug cell extracts were prepared as previously described (3). The sheath has previously been rigorously shown to be free from cellular contamination by three criteria: (i) silver, (ii) concanavalin A-peroxidase, and (iii) immunochemical staining of sodium dodecyl sulfate (SDS)-polyacrylamide gels (reference 3, Fig. 1 and 3). This can also be seen in Fig. 3, where it is clear that cellular proteins of ca. 45 and 80 kDa recognized by mAb MUD50 (Fig. 3b) are not present in sheath extracts (Fig. 3e).

The strains NC4 (Raper), DD61, WS576, WS380B, and HU188 were all nonmutagenized wild isolates of *D. discoideum*. Strain HU1628 is a derivative of NC4 in which six of the seven linkage groups are genetically marked (23). HU1628 also carries the unstable, dominant *cob-354* cobalt resistance mutation essential for diploid construction with the wild isolate strains.

Genetic analysis. Diploids were constructed between HU1628 and each of the wild isolates (except NC4) by standard techniques (see reference 13). They were selected from mixed populations of the parental haploids on the basis of their dual ability to grow in the presence of cobalt and in association with *Bacillus subtilis* as a food source (both selector mutations, i.e., cobalt resistance and growth on *B. subtilis*, come from HU1628). Haploidization of the diploids was induced by growth on medium containing a low concentration of the microtubule inhibitor thiabendazole (21). The haploid segregants obtained were cloned several times, and their genotypes were established by standard techniques (13).

Mitotic recombinants were selected on nutrient agar containing cycloheximide (500 μg/ml) as described previously (22).

Biochemical techniques. One-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) and subsequent immunoblotting with mAbs were performed as previously described, with 12.5% (wt/vol) acrylamide in the resolving gels (3). Two-dimensional electrophoresis was carried out by the

TABLE 1. mAbs used in this study

mAb	Specificity as isolated[a]	Other specificity	Probable nature of determinant[b]	Reference
MUD1	Prespore cell surface	Sheath	Not determined	4,8
MUD50	Sheath	Prespore cell surface	Carbohydrate	3
MUD51	Sheath	None	Protein	3
MUD52	Sheath	Prespore cell surface	Carbohydrate	3

[a] MUD1 was initially isolated as a prespore-specific antibody by flow cytometry. MUD50, MUD51, and MUD52 were isolated as antisheath antibodies by an enzyme-linked immunosorbent assay.
[b] Based on pronase sensitivity (see reference 3).

method of O'Farrell (14) with a linear pH range of 4 to 8 in the first (isoelectric-focusing) dimension.

The mAbs (all immunoglobulin G) were purified, and immunoglobulin G was used at a concentration of 0.1 to 1.0 μg/ml. All of the mAbs have been described previously (see reference 4 for MUD1 and reference 3 for MUD50, MUD51, and MUD52). MUD50, MUD51, and MUD52 were all initially isolated as antisheath mAbs (3), and MUD50 and MUD52 were subsequently shown to also be prespore cell specific (3; Grant et al., in preparation). MUD1 was initially isolated as a prespore-cell-specific mAb (4) and subsequently shown to also recognize sheath (see below). The origins and specificities of the mAbs are summarized in Table 1. All of these mAbs recognize a 32-kDa antigen in either sheath or cellular extracts (or both).

Immunoaffinity chromatography was performed by a procedure based on that of McMaster and Williams (11). Briefly, 10 mg of IgG (obtained from ascites fluid) was coupled to 5 ml of CNBr-Sepharose (Pharmacia) following the manufacturer's instructions. Urea-extracted sheath proteins were dialyzed into column-running buffer (0.5 M NaCl, 10 mM Tris hydrochloride, 0.05% Tween 20, pH 8.0) and run onto the column(s). Unbound material was washed through in several volumes of running buffer, and the bound proteins were then eluted in 50 mM diethylamine (pH 11.5) and immediately neutralized with solid glycine. The recovery of protein and antigenic activity from the columns was generally >90%.

RESULTS

Polymorphisms of slug cell and sheath proteins. Slug cell and sheath extracts from 21 wild isolates of *D. discoideum* were separated by SDS-PAGE, immunoblotted, and reacted with the mAbs MUD1 (4) and MUD50 (3; see Materials and Methods). Six of these isolates showed some form of polymorphism of sheath or slug cell proteins or both (data not shown). In particular, four wild isolates showed either an increase or a decrease of ~2 kDa in the apparent molecular mass of the 32-kDa prespore-specific protein (termed PsA) recognized in cellular extracts by MUD1 and MUD50 (3, 4, 8). The same isolates also showed ~2-kDa shifts in the 32-kDa sheath protein recognized by the same mAbs. This protein had initially been reported as 30 kDa (8), but revised estimates of its molecular mass made as a part of this work suggest that 32 kDa is a more accurate estimate.

To facilitate the following description of the results, the proposed nomenclature for the various forms of the 32-kDa protein(s) and the loci coding for it (them) is set out in Table 2. The data supporting the table are described in the following sections.

PsA is encoded by a single locus *pspA*. The same gene is responsible for the polymorphism in both slug cells and the sheath described above, i.e., the same locus is responsible for the cellular and the extracellular forms of PsA. Evidence supporting this conclusion (from parasexual genetic studies [see reference 13]) is presented below.

A diploid, DU2885, was constructed between DD61 (a wild isolate which carries the 30-kDa *pspA351* allele) and the well-marked strain HU1628 (which carries the "wild-type" 32-kDA PsA allele *pspA350*). Haploid segregants were then obtained from the diploid, and their genotypes were established (Table 3).

Slug cells from a selection of segregant haploids from DU2885 covering a range of possible genotypes (Table 3) were immunoblotted and reacted with MUD1 (Fig. 1a). Only one form of the PsA protein was seen in each haploid, either the 30-kDa form (HU2245, HU2259) or the 32-kDa form (HU2250, HU2236, HU2237, and HU2267). This is particularly important for HU2245 (which contains only one DD61 linkage group) and for HU2250 (which contains only one HU1628 linkage group), since it shows that both forms of PsA are correlated with linkage group I and not with any other linkage group. Examination of the genotypes in Table 3 shows that all *pspA351* (30-kDa segregants are $cycA^+$ while all *pspA350* (wild-type, 32-kDa) segregants are $cycA^-$. The *cycA* locus is on linkage group I (13, 26), so the simplest explanation of the observed segregation of *pspA* is that it is also on linkage group I.

Sheath from the same segregants was also immunoblotted and reacted with MUD1 (Fig. 1b). The pattern of PsA polymorphism in the sheath of those segregants which could be examined (see the legend to Fig. 1) was exactly the same as that obtained with slug cells (Fig. 1a). Thus, the same locus is responsible for the PsA polymorphism in both sheath and slug cells, i.e., the cellular and extracellular PsA proteins are encoded by the same locus.

The assignment of *pspA* to linkage group I was confirmed by mitotic recombination (see reference 22). These experiments utilized heterozygous diploids (e.g., DU2885; Table 4) which were constructed between the polymorphic wild isolates and the well-marked cycloheximide-resistant strain HU1628. Diploid mitotic recombinants on linkage group I were derived from the heterozygous diploids by selection on growth plates containing cycloheximide.

When slug cell (Fig. 2a) and sheath (Fig. 2b) extracts of the heterozygous diploids were subjected to PAGE, transferred to nitrocellulose, and reacted with MUD1, all clearly expressed both forms of the PsA protein in the sheath and on

TABLE 2. Nomenclature, molecular mass, and mAb reactivity of polymorphic and nonpolymorphic proteins

Allele	Mol wt of protein[a]	Wild isolate	Protein	mAb[b]
pspA350	32[c]	NC4	PsA	MUD1, MUD50
pspA351	30	DD61	PsA	MUD1, MUD50
pspA352	30	WS380B	PsA	MUD1, MUD50
pspA353	34	WS576	PsA	MUD1, MUD50
pspA354	34	HU188	PsA	MUD1, MUD50
shpA350	32[d]	NC4	ShA	MUD50, MUD51, MUD52

[a] Molecular mass of protein in SDS-PAGE (kilodaltons).
[b] mAbs which recognize the protein.
[c] Fifteen other wild isolates, of 21 examined, also have a 32-kDa PsA protein, so this is the wild-type size of this protein.
[d] All isolates examined have a 32-kDa ShA protein.

TABLE 3. Partial genotypes of parental haploids (DD61, HU1628), diploid DU2885, and selected haploid segregants of DU2885[a]

Isolate	Linkage group						
	? (*pspA351*)	I (*cycA1*)	II (*acrA1823*)	III (*bsgA5*)	IV (*whiC351*)	VI (*manA2*)	VII (*couA351*)
Haploid parents							
HU1628	+	*cycA1*	acrA1823	bsgA5	whiC351	manA2	couA351
DD61	*pspA351*	+	+	+	+	+	+
Diploid							
DU2885	+/*pspA351*	+/*cycA1*	+/*acrA1823*	+/*bsgA5*	+/*whiC351*	+/*manA2*	+/*couA351*
Haploid segregants from DU2885							
HU2245	*pspA351*	+	*acrA1823*	bsgA5	whiC351	manA2	couA351
HU2250	+	*cycA1*	+	+	+	+	+
HU2236	+	*cycA1*	+	+	*whiC351*	+	+
HU2237	+	*cycA1*	+	*bsgA5*	+	*manA2*	*couA351*
HU2259	*pspA351*	+	+	*bsgA5*	+	*manA2*	couA351
HU2267	+	*cycA1*	+	+	*whiC351*	manA2	couA351

[a] The diploid DU2885 is heterozygous at all loci shown including *pspA*. HU1628 carries the unstable, dominant cobalt resistance mutation *cob-354* (12, 25). For the complete genotype of HU1628; see reference 23. Markers scored are: *cycA1*, cycloheximide resistance (500 μg/ml); *acrA1823*, methanol resistance (2%, vol/vol); *bsgA5*, inability to grow on *B. subtilis*; *whiC351*, white spores;*manA2*, absence of α-mannosidase-1; *couA351*, inability to grow on coumarin (1.3 mM), also associated with growth temperature sensitivity. A + denotes wild-type alleles.

the cell surface (Fig. 2a and b, lanes 1, 3, 5, and 7). In contrast, recombinant diploids selected on the basis of cycloheximide resistance (i.e., diploids which have become homozygous for *cycA1* on linkage group I via mitotic recombination) expressed only the 32-kDa form of PsA (Fig. 2a and b, lanes, 2, 4, 6, and 8). Thus, mitotic recombination on linkage group I leading to homozygosity at the *cycA* locus concurrently leads to homozygosity at *pspA*, supporting the conclusion that the *pspA* polymorphism is due to multiple alleles at a single locus on linkage group I. In addition, the pattern of PsA in the recombinants is the same in sheath as in slug cells, thereby strengthening the conclusion that the sheath and the cellular forms of PsA are encoded by the same locus (compare Fig. 2a and b).

A nonpolymorphic, sheath-specific, 32-kDa protein: ShA. Previous work had shown that a third antibody, MUD51 (Table 1), recognizes a 32-kDa protein in the sheath but does not recognize any cellular protein at all (3), i.e., there is a sheath-specific 32-kDa protein which is immunologically distinct from the cellular 32-kDa protein. To determine whether this protein is encoded by the same locus as the cellular-extracellular PsA protein, slug cell and sheath extracts from DD61, HU1628, and two segregants from the DD61 × HU1628 diploid (HU2245 and HU2250, see above and Table 3) were immunoblotted with the antisheath mAbs MUD50 and MUD51.

In slug cells, PsA was recognized by MUD1 and MUD50 (Fig. 3a and b, solid arrows), and, as previously reported (3),

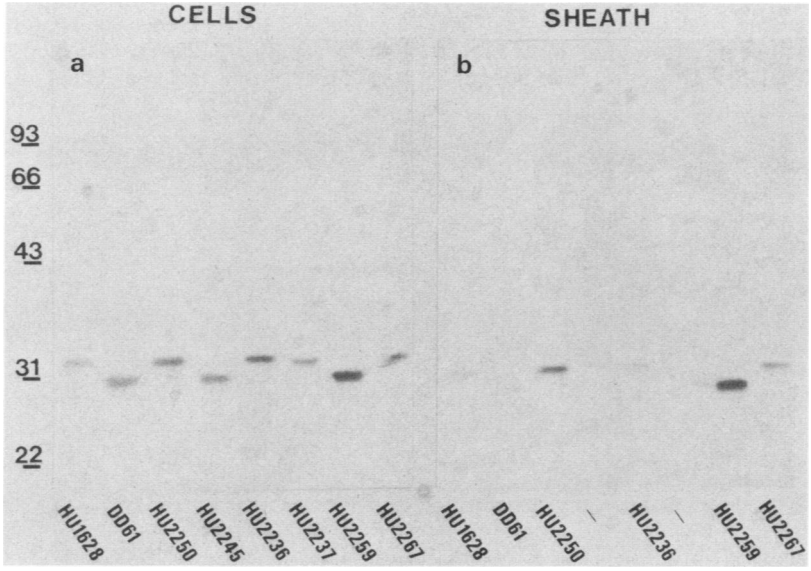

FIG. 1. mAb analysis of PsA in haploid segregants obtained from the diploid DU2885 (HU1628 × DD61). Slug cell (a) and sheath extracts (b) immunoblotted and reacted with MUD1. The parental haploids (HU1628, DD61) are included for reference; all other strains are haploid segregants from DU2885 (Table 3). Sheaths from HU2245 and HU2237 could not be collected, since these strains do not form migrating slugs. Numbers on the left refer to molecular mass ($\times 10^3$ daltons) of standard proteins (Bio-Rad Laboratories).

TABLE 4. Haploid parents, heterozygous diploids, and homozygous recombinant diploids on linkage group I

Haploid parents	Diploid	Genotype[a]	Recombinant	Genotype[a]
HU1628/DD61	DU2885	+/*pspA351*	DU2914	+/+
HU1628/HU188	DU2873	+/*pspA354*	DU2913	+/+
HU1628/WS380B	DU2889	+/*pspA352*	DU2915	+/+
HU1628/WS576	DU2900	+/*pspA353*	DU2916	+/+

[a] Genotype of the *pspA* locus only, where + is the *pspA350* allele.

MUD51 did not react with slug cell extracts. A series of lower-molecular-mass proteins which underwent a 2-kDa shift in molecular mass similar to that of PsA were also stained by MUD50 in slug cells (Fig. 3b, open arrows). These proteins may be either precursors or degradation products of PsA.

In the sheath the situation is more complex. The PsA protein was clearly present in MUD1- and MUD50-stained immunoblots (Fig. 3d and e, solid arrows). However, in addition to PsA there was clearly a 32-kDa protein in the sheath of DD61 and HU2245 which was recognized by MUD50 and MUD51 but not by MUD1 (Fig. 3e and f, asterisks). No 32-kDa protein was present in the slug cell immunoblots of slug cell extracts of these strains (Fig. 3b and c). Consequently, there must be a sheath-specific, nonpolymorphic, 32-kDa protein which is recognized by MUD50 and MUD51 but not by MUD1. This protein has been termed ShA (Table 2). It is also recognized by a third antisheath mAb, MUD52, which does not react with PsA (data not shown).

PsA and ShA can be resolved in nonpolymorphic isolates. In wild isolates nonpolymorphic for PsA (i.e., in which PsA is 32 kDa), PsA and ShA would comigrate in SDS-PAGE and not be resolved on immunoblots. To demonstrate that ShA can also be resolved in nonpolymorphic isolates and is therefore independent of the PsA polymorphism, two alternative approaches were used.

First, sheath proteins were passed over either MUD50-Sepharose or MUD51-Sepharose immunoaffinity columns, and then the bound and unbound material from each column was immunoblotted and reacted with antisheath mAbs. The MUD50-Sepharose column bound all material able to react with either MUD1 or MUD51. However, the unbound fraction from the MUD51-Sepharose column contained material which reacted with MUD1 and MUD50, whereas the bound fraction from the same column did not react with MUD1. It was, therefore, possible by using a MUD51-Sepharose column to separate the 32-kDa protein from a nonpolymorphic strain into a PsA fraction (reacting with MUD1 and MUD50) and a ShA fraction (reacting with MUD51 and MUD50) (data not shown).

Second, sheath proteins from polymorphic and nonpolymorphic wild isolates were separated on two-dimensional gels (14), immunoblotted, and reacted with MUD50 and MUD51 (Fig. 4). The center panels (c and d) show the nonpolymorphic form, where the single 32-kDa band from the one-dimensional gel is resolved into at least three spots of pI ~4 to 5 when reacted with MUD50 (Fig. 4c). Comparison of Fig. 4c with Fig. 4a and e shows that the PsA protein(s) corresponds to the most acidic two spots, since it is these which are of altered molecular mass in the polymorphic wild isolates. The most basic spot(s) reacts with MUD51 and is nonpolymorphic and therefore corresponds to ShA (see Fig. 4b, d, and f). The antigenic separation achieved above with immunoaffinity techniques can, therefore, be duplicated by physicochemical methods.

It is striking that (i) the shift of 2kDa in the molecular mass of the polymorphic forms of PsA is not accompanied by any detectable change in pI or change in the number of PsA spots, (ii) both PsA and ShA are very similar in pI as well as molecular mass and are both resolved into similar numbers of evenly spaced spots, suggesting that they may be processed in similar ways, and (iii) the changes in PsA are not observed in other proteins, i.e., the effect of *pspA* is specific for the PsA protein. Examination of concanavalin A-binding glycoproteins in polymorphic wild isolates also showed no detectable widespread changes in glycosylation (data not shown). Point iii suggests that *pspA* is the structural gene for the PsA protein.

ShA and PsA are retained differently in the sheath. A subset of sheath proteins is released by mild cellulase digestion of the sheath, implying that they are held in place by cellulose-protein interactions (3). The 32-kDa sheath protein(s) was originally included in this group.

Figure 5 shows immunoblots of SDS and cellulase extracts of the sheath of NP84 (32 kDa) and PsA polymorphic isolates DD61 (30 kDa) and WS576 (34 kDa) reacted with MUD1,

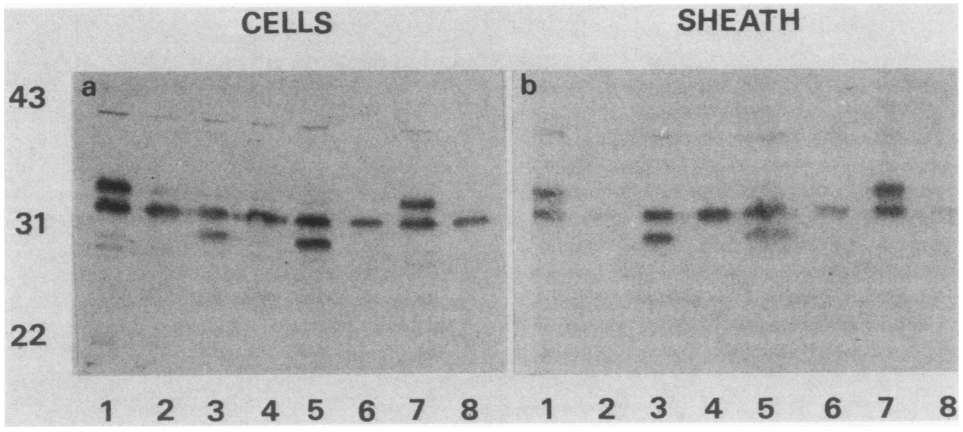

FIG. 2. Heterozygous diploids and mitotic recombinants for linkage group I. Odd-numbered lanes are heterozygous diploids and even-numbered lanes are recombinant homozygous diploids obtained from them (Table 4). Immunoblots of slug cell (a) and sheath extracts (b) reacted with MUD1. Lanes: 1, DU2900; 2, DU2916; 3, DU2889; 4, DU2915; 5, DU2885; 6, DU2914; 7, DU2873; 8, DU2913. Numbers on left refer to molecular masses ($\times 10^3$ daltons) of standard proteins.

MUD50, and MUD51. It is clear that PsA and ShA differ in their release by cellulase. No PsA could be detected in cellulase extracts (odd-numbered lanes), although it could be detected in SDS extracts. In contrast, ShA could be detected in both cellulase and SDS extracts. This is particularly clear in Fig. 5a, since MUD1 specifically recognizes PsA, and in Fig. 5c, since MUD51 recognizes ShA and not PsA. Thus, ShA but not PsA is released by cellulase treatment.

DISCUSSION

PsA and ShA. The results presented here are the first unequivocal demonstration of a direct molecular relationship between the surface of a specific, differentiating cell type and the extracellular matrix in *D. discoideum*. Previous work had demonstrated that sheath free from cellular contaminants contains its own complement of proteins (3) and that it is possible to define these sheath proteins and cell-type-specific cell surface molecules with monoclonal antibodies (3, 4, 8). Here we have extended this previous work and provide convincing genetic, immunological, and biochemical evidence for a close link between specific cell surface differentiation antigens and the extracellular matrix and have shown that this link can be resolved into two distinct components.

First, a single protein (PsA) is found both on the cell surface and in the sheath, thereby at least partially accounting for the cell surface-sheath sharing of a 32-kDa protein bearing the MUD50 antigenic determinant. Both the cellular and the extracellular forms of PsA are polymorphic and are therefore almost certainly products of the same gene (*pspA*). This does not, however, mean that the cellular and extracellular forms of PsA are identical. For example, surface and secreted immunoglobulin molecules are structurally different despite being products of the same gene (15).

The second component is ShA, a 32-kDa protein which is also recognized by MUD50 but which is nonpolymorphic and sheath specific. On an antigenic level, ShA is different from PsA, lacking the MUD1 determinant but carrying the MUD51 (and MUD52) determinants. The work presented here unequivocally demonstrates that this antigenic difference has a genetic basis and is not due to differential processing of a single polypeptide (see reference 3). In addition to the genetic and antigenic differences there is also a difference in the release of PsA and ShA by cellulase treatment, and this may be of functional significance.

Aside from these differences, PsA and ShA share many common characteristics. Both are developmentally regulated (3, 4, 7); they are very similar in pI and molecular mass; and they are probably processed similarly, since both react with MUD50. In addition, preliminary data from peptide mapping studies suggest that they are structurally related (Grant, unpublished data). The simplest explanation is that PsA and ShA are related and may have arisen via duplication of a common ancestral gene. As such, PsA and ShA may be members of a small multigene family.

Function of PsA and ShA. While the functional relationships between PsA and ShA and between the cellular and extracellular forms of PsA are not known, there is some information concerning function. Cellular PsA is restricted to the prespore cells of slugs (4), implying that extracellular PsA may be restricted to the sheath adjacent to the prespore region of the slug (i.e., it may define a spatial specialization of the sheath). PsA is also a component of the mature spore coat (Grant et al., in preparation) where it probably corresponds to the disulphide-cross-linked 33-kDa spore coat protein reported by Wilkinson and Hames (24). While PsA in the sheath and on the surface of prespore cells is not disulphide cross-linked or bound covalently, it may play a role in maintaining the structural integrity of the slug. A wild isolate, WS584, which lacks PsA (and several other sheath and prespore proteins) forms tiny slugs which migrate for a short time and construct small fruiting bodies (Grant, unpublished data). Slugs migrating on agar containing cellulase (which releases ShA from the sheath) cease migrating and prematurely form fruiting bodies. Both of the above studies, while far from conclusive, imply that sheath and sheath proteins play a role in the slug-fruit switch, which governs

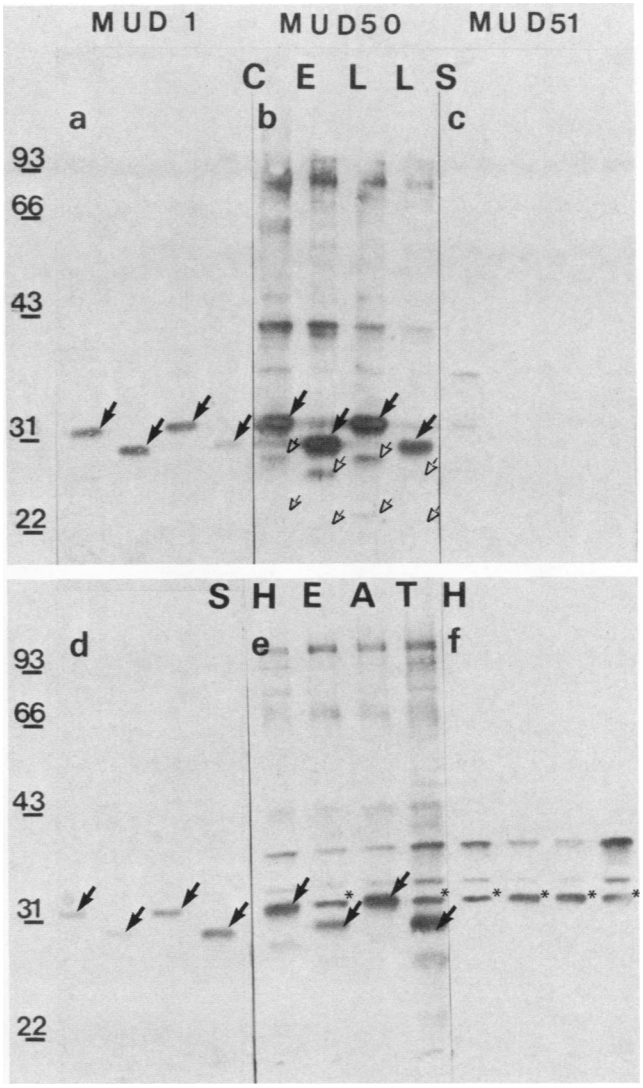

FIG. 3. Immunoblots of slug cell and sheath extracts of parental and segregant haploids reacted with MUD1 (a and d), MUD50 (b and e), and MUD51 (c and f). Sample order, left to right, in panels a through c is HU1628, DD61, HU2250, and HU2245. In panels d through f (sheath extracts), HU2245 is replaced by HU2259 (Fig. 1). The slight reactivity of MUD51 with slug cell extracts of HU1628 (panel c, left lane) is due to the presence of early fruiting bodies, which contain ShA (Grant et al., in preparation). In all panels, solid arrows indicate PsA and open arrows indicate possible precursors or degradation products of PsA. Asterisks indicate ShA (note: PsA and ShA are not resolved in sheath extracts of HU1628 and HU2259, since these strains have a 32-kDa PsA; panel e, lanes 1 and 3). Numbers on the left refer to molecular masses ($\times 10^3$ daltons) of standard proteins.

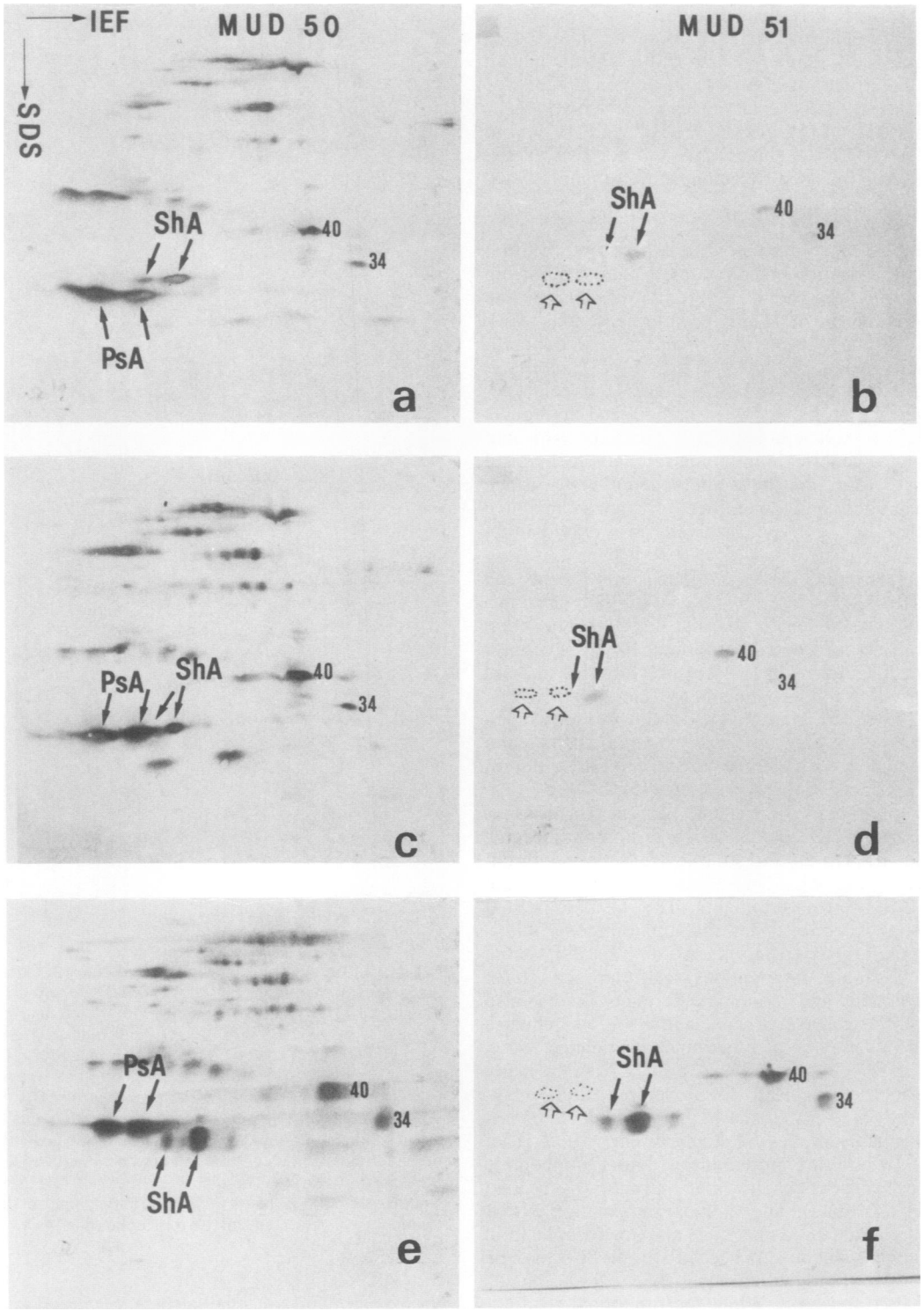

FIG. 4. Two-dimensional immunoblots of sheath extracts of polymorphic wild-type isolates. (a and b) DD61; 30-kDa PsA; (c and d) NP84; 32-kDa PsA; (e and f) WS576; 34-kDa PsA. Materials shown in panels a, c, and e were reacted with MUD50, and materials shown in panels b, d, and f were reacted with MUD51. The pH range in the first dimension was pH 4 to 8 with the acidic end to the left. The broken circles and open arrows in panels b, d, and f indicate the expected position of PsA, which does not react with MUD51. IEF, Isoelectric focusing.

the transition between migratory and fruiting developmental pathways.

***pspA* polymorphism.** The regular ±2-kDa changes in molecular mass associated with all the *pspA* alleles are best explained by the addition or subtraction of identical 2-kDa units from the wild-type 32-kDa form of the protein. Indirect arguments which suggest that this unit is a peptide are: (i) diploids express both forms of the protein, implying that the polymorphism is not due to a processing defect; (ii) there is no change in pI or the number of PsA spots on two-

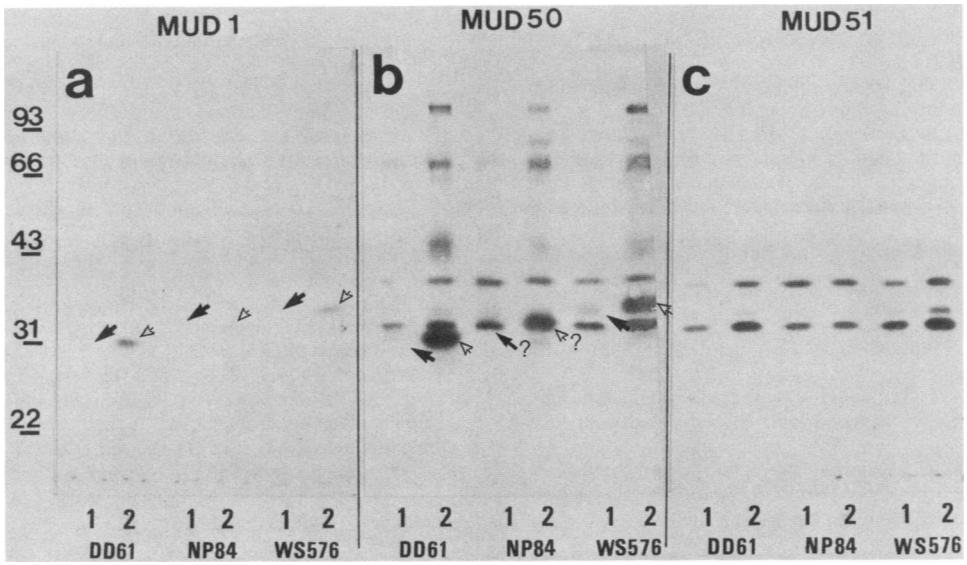

FIG. 5. SDS and cellulase extraction of PsA and ShA from the sheath. For each isolate, lane 1 is the cellulase extract and lane 2 is the SDS extract, with closed arrows indicating the expected position of PsA in cellulase extracts and open arrows indicating its observed position in SDS extracts. Question marks refer to cases in which PsA and ShA have the same molecular mass and therefore cannot be distinguished by using MUD50. Loading in all cases is the same. However, MUD1 is less sensitive on immunoblots than MUD50. Numbers on the left refer to molecular masses ($\times 10^3$ daltons) of standard proteins.

dimensional gels; (iii) all *pspA* alleles map to a single region of linkage group I and probably to a single gene.

At the DNA sequence level these polymorphisms may, therefore, have arisen by the addition or deletion of a specific sequence coding for a peptide 12 to 15 amino acids in length. The wild-type gene must contain at least one such sequence, the WS576 and HU188 genes at least two, and the DD61 and WS380B genes none. It is plausible that there may be several such peptides in PsA. For example, the circumsporozoite coat protein from the sporozoite stage of the malarial parasite *Plasmodium knowlesi* contains a tandemly repeated 12-amino acid peptide (2), and a sea urchin sperm acrosomal protein (bindin) contains at least three repeats of a 10-amino acid peptide (20). If PsA does contain such a repeated peptide it should show some form of periodic structure, which may have implications for its function (see reference 2) and should be readily detectable.

There are two basic approaches to studying the role of developmentally regulated cell surface and matrix proteins in morphogenesis. The first is to start with a hypothesis about function and to try to identify the relevant components. The second is to characterize the components of the system at a molecular level and then seek to assign functional roles to these molecules, using probes whose specificity is well defined. We have chosen the latter approach in our studies on *D. discoideum* morphogenesis. This report brings us to the end of the first stage in which two molecules, a polymorphic protein, PsA, and a nonpolymorphic protein, ShA, have been identified and located.

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