Selection of *Dictyostelium* mutants defective in cytoskeletal proteins: use of an antibody that binds to the ends of α -actinin rods

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Communicated by G.Gerisch

A monoclonal antibody, mAb 47-19-2, was used to study the subunit topology of the rod-shaped α -actinin molecules of *Dictyostelium discoideum* and to screen for mutants defective in the production of α -actinin. Electron microscopy of rotary-shadowed α -actinin-antibody complexes showed binding of mAb 47-19-2 to both ends of the α -actinin rods and cleavage of the rods into its subunits, indicating that the two subunits of α -actinin extend in an anti-parallel mode through the whole length of the rod. The antibody binding sites were located in close proximity to the sites responsible for actin cross-linking, which is consistent with the blocking activity of the antibody. In a mutant, HG1130, no antibody label was detected in colony blots, and by immunoblotting of mutant proteins separated by SDS-PAGE, only trace amounts of α -actinin were found. The mutant showed normal binding of antibodies directed against the actin-binding proteins severin and capping protein. The mutation responsible for the α -actinin defect was recessive and located on linkage group I of the genetic map of *D. discoideum*. HG1130 cells grew on bacteria at a normal rate and also axenically like cells of the parent strain AX2. After starvation the mutant cells expressed the contact site A glycoprotein, a marker of the aggregation-competent stage, and reacted chemotactically to cyclic AMP. The aggregation patterns and fruiting bodies of the mutant appeared to be normal. Patching and capping on the surface of HG1130 cells was induced by antibodies against the contact site A glycoprotein. It remains to be shown whether α -actinin has unique functions not observed under the conditions used or whether it can be functionally replaced by other actin cross-linking proteins.

Key words: cytoskeleton/ α -actinin-defective mutant/actin-binding proteins/function-blocking monoclonal antibody/*Dictyostelium*

Introduction

Among the methods used to elucidate the functions of cytoskeletal proteins, genetic analysis has played only a minor role because mutants defective in specific components of the cytoskeleton are scarce. *Dictyostelium discoideum* is a convenient organism for the investigation of cell motility by a combined biochemical and genetic approach. Parasexual genetics has been worked out (Williams and Newell, 1976; Newell *et al.*, 1977; Williams, 1978) and >10 proteins of the cytoskeleton have been characterized in this organism. *D. discoideum* possesses all components of a eukaryotic cell which are necessary for amoeboid movement and its regulation by chemo-, photo- and thermosensory mechanisms (Fisher *et al.*, 1984). Several accessory proteins of the cytoskeleton are comparable to those found in

vertebrate cells. An actin cross-linking protein from *D. discoideum* has been designated as α -actinin because it resembles α -actinin from vertebrate smooth and skeletal muscles (Fechheimer *et al.*, 1982; Condeelis and Vahey, 1982; Condeelis *et al.*, 1984). *D. discoideum* α -actinin has a molecular mass of ~210 kd and consists of two subunits of equal size. Its molecules are rod-shaped and subdivided into two side-by-side associated strands discernible in the electron microscope after low-angle shadowing (Condeelis *et al.*, 1984). Like α -actinin from porcine skeletal muscle (Jockusch and Isenberg, 1981) the 210-kd protein from *D. discoideum* binds with the ends of the rod to F-actin (Condeelis and Vahey, 1982).

In an attempt to select mutants defective in specific components of the motility system of *D. discoideum* we have screened mutagenized cells with monoclonal antibodies against various actin-binding proteins. Here we describe the colony blotting technique used for the screening of mutants, we report on the structure of complexes of α -actinin with an antibody used for the selection of a mutant defective in α -actinin production, and we characterize this mutant HG1130. In a subsequent paper we will report on a second mutant which is defective in severin, another actin-binding protein (Brown *et al.*, 1982).

Results

Monoclonal antibody that blocks α -actinin function

Blocking of the cross-linking activity of α -actinin by mAb 47-19-2 was tested using a low shear viscometry assay. The increase of viscosity of actin with rising concentrations of α -actinin was assayed in the presence and absence of the antibody. This increase was completely inhibited by 62 μ g of antibody IgG per ml (Figure 1). At the highest α -actinin concentration tested this cor-

Fig. 1. Blocking activity of mAb 47-19-2 as tested by low shear viscometry. The viscosity of an F-actin solution was raised by increasing concentrations of α -actinin (○). The antibody completely inhibited the rise of viscosity (●). Viscosity values are means of duplicates and are expressed in per cent relative to controls that contained actin only.

Fig. 2. Rotary-shadowed α -actinin molecules and their complexes with mAb 47-19-2. The angle of shadowing was 5° except for VIa where it was 8° .

Fig. 3. Proposed structure of some of the α -actinin/antibody complexes shown in Figure 2.

responded to a 4-fold molar excess of antibody. The antibody alone caused an increase of viscosity of the F-actin solution as was previously reported for various immunoglobulins by Fechner *et al.* (1979).

Structure of the complexes of α -actinin with mAb 47-19-2

α -Actinin molecules and their complexes with antibody were

visualized in the electron microscope after rotary shadowing. The purified α -actinin showed only the 95-kd subunit band in Coomassie blue-stained SDS-polyacrylamide gels; it was particularly free of detectable amounts of the 120-kd actin cross-linking protein which, like α -actinin, is a rod-shaped molecule (Condeelis *et al.*, 1984). Examples of α -actinin molecules and

Table I. Lengths of α -actinin molecules and of their complexes with mAb 47-19-2

Length measured		N	Mean $\pm$ standard error (nm)
Free α -actinin		45	55.7 $\pm$ 0.7
a	77	45.4 $\pm$ 0.6
b	75	31.9 $\pm$ 0.5
c	30	36.4 $\pm$ 0.7
d	61	50.5 $\pm$ 0.9
e	62	27.4 $\pm$ 0.7

The specimens were rotary shadowed at an angle of 8° and the measurements calibrated with a catalase crystal. N, number of measurements. Circles or triangles indicate antibody molecules. Length c was measured at selected complexes distinguished by clearly triangular antibody molecules that were located laterally at one end of the rods.

their complexes with mAb 47-19-2 are shown in Figure 2. Most of the free α -actinin molecules were extended rods which were often bent in the middle and not detectably thickened at their ends (Figure 2, Ia–e). Sometimes the molecules were shorter and had globular ends (Figure 2, If–g). α -Actinin-antibody complexes showed the extended configuration of the α -actinin (Figure 2, IIa–b) as well as a shortened one in which the rod was markedly condensed at the binding site of the antibody (Figure 2, IIc–g), suggesting that the antibody changes the configuration of the α -actinin at the end of the rod to which it binds. This was even more clear when the α -actinin rod was cleaved into its two subunits after antibody binding (Figure 2, IIIa–g). The subunits remained associated with each other only at the end opposite to the antibody binding site. Complexes formed by two antibody molecules and one α -actinin molecule showed that binding sites for mAb 47-19-2 exist at both ends of the rods (Figure 2, IVa–f).

The IgG molecule of the antibody was able to bind α -actinin molecules simultaneously to both of its antigen binding sites (Figure 2, IVg). Often complexes were seen in which two antibody molecules were connected by rods or their filamentous subunits, but we have never observed two antibody molecules bound to the same end of an α -actinin rod. Complexes in which two antibody molecules seemed to be connected by four α -actinin subunits are shown in Figure 2, Va–e. A complex in which three antibody molecules appeared to be associated with four α -actinin subunits is depicted in Figure 2, Vf, and a complex of two antibody molecules associated with probably three α -actinin molecules in Figure 2, Vg. Tetramers consisting of two antibody and two α -actinin molecules were clearly recognizable when the integrity of the rods was preserved (Figure 2, VIa–e) or when the subunits constituting the rods were only partially separated (Figure 2, VI f–g). Figure 3 helps to explain these more complicated configurations. The suggested models are based on the evidence provided that the two subunits of the α -actinin are aligned to each other in an anti-parallel fashion through the entire rod.

Length measurements of free α -actinin rods and of α -actinin-antibody complexes confirmed the notion that, on average, α -actinin molecules which were bound to antibody were shorter than the free ones (Table I). The average length of the shadowed α -actinin molecules was 56 nm under our conditions. The length of the 1 α -actinin + 1 antibody complexes was 45 nm when the size of the antibody was included (a in Table I). In cases where the triangular shape of the antibody was clearly seen the total rod length could be determined in the presence of an antibody

mutagenesis with MNNG and cloning of cells on agar plates

transfer of clones in a regular array of 56 inocula per plate

replication of plates with a toothpick multidispenser

Fig. 4. Diagram of the selection procedure for mutants defective in actin-binding proteins.

molecule, and was found to be 36 nm, about 20 nm shorter than rods of α -actinin without any bound antibody (c in Table I). Similar results have been obtained with tetramers consisting of 2 α -actinin + 2 antibody molecules whose total lengths were also less than that of the free α -actinin molecules (e in Table I).

We have chosen mAb 47-19-2 for mutant screening because this antibody binds close to the ends of the rods and blocks the α -actinin-induced cross-linking of F-actin. The prediction was that a mutant which does not bind this antibody either lacks the entire α -actinin or at least a functional site involved in the α -actinin-dependent actin gelation.

Screening for mutants defective in cytoskeletal proteins using colony blots

Clones from mutagenized cells were screened in colony blots similar to a procedure used for the selection of mutants defective in cell surface glycoproteins (Francis *et al.*, 1985). The colony blotting technique was adapted to the screening of mutants defective in intracellular proteins for which no selection procedure was available. Clones of mutagenized cells were plated in a regular array on agar plates and the colonies replicated as indicated in Figure 4 to allow the same clones to be screened by different antibodies. Cells of the blotted colonies were lysed on the nitrocellulose filters by freezing and thawing. Subsequently the colony blots were labeled with iodinated antibodies for autoradiography. The antibodies used were specific for either α -actinin, the actin depolymerizing protein severin, the 34-kD subunit of the actin-capping protein, or a membrane-associated

Fig. 5. Autoradiograms of colony blots labeled with mAb 47-19-2. **Top:** original blot of clones of mutagenized AX2 cells on which HG1130 (arrow) was discovered. **Bottom:** blot of seven subclones of HG1130 compared with control clones showing normal antibody binding.

17-kD actin-binding protein (Schleicher *et al.*, 1984). Among 5000 clones which were assayed for defects in the latter two proteins, no mutant was detected. Among 8000 or 9000 clones that were assayed for defects in the first two proteins, one clone was detected that did not bind the severin-specific mAb 42-65-23, and another clone that did not bind the α -actinin-specific mAb 47-19-2 (Figure 5). Both clones gave rise to stable mutant strains that grew on bacteria, aggregated and formed fruiting bodies. In the following we focus on the α -actinin-defective mutant HG1130 carrying the mutation *abpA1000*, where *abp* stands for a defect in actin-binding proteins.

Mutant HG1130 is specifically defective in α -actinin

AX2 wild-type and mutant HG1130 cells were harvested during growth or early development, and total cellular proteins were subjected to SDS-PAGE and immunoblotting. The blots were labeled with monoclonal antibodies against α -actinin, severin, the 34-kD subunit of actin capping protein, or the contact site A glycoprotein, a marker of development. With the anti- α -actinin antibody only a faint band was observed which had the same position as wild-type α -actinin (Figure 6A). The 40-kD band of severin (Figure 6B) and that of the capping protein (Figure 6C) were labeled in HG1130 with similar or only slightly reduced intensity compared with wild-type, and the 80-kD contact site A glycoprotein was normally expressed during development of the mutant (Figure 6D).

The intensity of antibody labeling of wild-type α -actinin was retained when homogenates of AX2 and HG1130 were mixed. In order to quantify α -actinin in HG1130 relative to wild-type, proteins of both strains and mixtures of them were applied to SDS-polyacrylamide gels at various dilutions and the antibody-labeled bands were cut out from the blots for γ -counting. The

Fig. 6. Autoradiograms of blots of total cellular proteins of the AX2 strain and of mutant HG1130. The proteins were separated by SDS-PAGE, blotted and labeled with mAb 47-19-2 for α -actinin (A), with mAb 42-65-23 for severin (B), with mAb 43-442-1 for the 34-kD subunit of the actin-capping protein (C), or with mAb 33-294-17 for the contact site A glycoprotein (D). The band below the major one in D belongs to a 68-kD precursor of the contact site A glycoprotein (Hohmann *et al.*, 1985). Numbers on bottom indicate hours of starvation without (C) or with stimulation of development by pulses of cyclic AMP (P). Proteins equivalent to 1×10^6 cells were applied to each slot.

α -actinin content of the mutant was estimated to be $< 1\%$ of that in the wild-type. To exclude contamination of the mutant by a revertant that might produce the α -actinin, cells of the HG1130 cultures were plated and 400 clones were screened by colony blotting and labeling with [125 I]mAb 47-19-2. No revertant was detected.

Undisturbed cell functions in HG1130

The mutant grew at a normal rate on agar plates with *Escherichia coli* at various temperatures (Figure 7). This result indicates that phagocytosis of bacteria was not affected by the mutation at a temperature optimal for growth (21°C), nor was cold sensitivity

or high temperature sensitivity of phagocytosis associated with the mutation. HG1130 grew also axenically at 23°C with a generation time of 8 h which is similar to that of the AX2 strain.

Fig. 7. Growth of HG1130 (open symbols) and of its parent strain AX2 (closed symbols) on low nutrient agar plates with *E. coli* B/2 at different temperatures. $\triangle$, $\blacktriangle$, 27°C; $\circ$, $\bullet$, 21°C; $\square$, $\blacksquare$, 12°C. Data are means of duplicates.

Aggregating wild-type cells of *D. discoideum* become elongated and they assemble into streams by cell-to-cell adhesion during their chemotactic movement to aggregation centers. These activities were clearly exhibited by HG1130 cells as shown in Figure 8. Following aggregation, mutant HG1130 formed slugs and fruiting bodies containing elliptical spores, indicating that the defect in α -actinin production had no substantial effect on cell motility, changes of cell shape or cell differentiation as they occur during late development.

α -Actinin is supposed to be involved in the surface capping of plasma membrane antigens (Carboni and Condeelis, 1985). Therefore, we incubated aggregation-competent cells of HG1130 with mAb 41-71-21, an antibody specific for the contact site A glycoprotein, and subsequently with fluorescent labeled anti-mouse IgG. After transferring the labeled HG1130 cells to room temperature strongly fluorescent caps were observed within 15 min, indicating that capping of the antigen was as efficient in the mutant as it was in the wild-type.

Genetic analysis of mutant HG1130

Of the seven chromosomes of *D. discoideum*, six are genetically labeled by linkage group markers. In order to map the mutation *abpA1000* responsible for the defect in α -actinin production, mutant HG1130 carrying this mutation was crossed with the haploid strain HU1628 in which all the six established linkage groups are marked by recessive mutations (Tables II and III). Two independently derived diploids, DG98 and DG100, were selected from these crosses. In both diploids α -actinin was recognized by mAb 47-19-2 after SDS-PAGE as a strong band in the correct 95-kd position, indicating that the *abpA1000* muta-

Fig. 8. Chemotactic response of HG1130 cells. Cells were washed at 6 h of starvation in non-nutrient buffer and transferred onto a Teflon surface. A, B, cells stimulated by a micropipette filled with 1 mM cyclic AMP and photographed shortly after insertion of the pipette (A) or 6 min later (B). C, aggregates formed without stimulation.

tion was recessive.

One of the diploids, DG98, was haploidized and 63 progeny clones were checked for the presence or absence of the linkage group markers as well as for binding of mAb 47-19-2. The *abpA1000* mutation segregated independently from all markers with the exception of cycloheximide resistance which never co-segregated with the defect in antibody binding (Table II). Therefore *abpA1000* is located on the linkage group marked by *cycA1*, which is linkage group I.

Discussion

A monoclonal antibody which blocks the α -actinin-induced cross-linking of F-actin has been used in this study to gain more insight into the topological relationship of the two subunits of α -actinin by electron microscopy and for the selection of a mutant defective in α -actinin production. The fact that the antibody bound to the two ends of the rod-shaped molecules indicates the presence of identical epitopes at both ends of the α -actinin rod and confirms that the rod is composed of two anti-parallel subunit strands. The finding that binding of an antibody to one end may split the rod into two individual subunits which remain attached to each other only at the opposite end, indicates that each subunit extends through the entire length of the rod.

Our search for mutants was based on the assumption that defects in actin-binding proteins may not always be fatal to the cells. We have used monoclonal rather than polyclonal antibodies because they provided the opportunity of obtaining mutants which lack only a certain epitope in addition to mutants which are defec-

tive in the synthesis of the entire protein. Two mutants were obtained; one was defective in the binding of an antibody specific for α -actinin, the other in the binding of an antibody specific for severin, an actin-depolymerizing protein. The viability of mutants carrying these defects does not necessarily disprove the possibility that the affected proteins are essential since the mutant proteins may lack only an epitope which is not required for their function. In the case of the α -actinin defect in mutant HG1130 this is unlikely, however, because we have used an antibody for mutant screening which binds to the ends of the α -actinin rods and blocks their cross-linking function. Furthermore, the detection of a very faint 95-kd band in immunoblots indicates that the mutation caused drastic quantitative differences rather than structural changes of α -actinin.

Diglycerides and fatty acids are associated with α -actinin from platelets and are required for its function (Burn *et al.*, 1985). These lipids remain bound to the polypeptide even after SDS-PAGE. One has to consider that binding of an antibody to the α -actinin may be influenced by the lipids tightly associated with the polypeptide, and that the primary defect in the mutant resides in an altered lipid composition of the cells. This is unlikely, however, for HG1130 since two other monoclonal antibodies that did not recognize the same epitope as mAb 47-19-2, and also polyclonal antisera from rabbits, showed little or no binding to the 95-kd band of α -actinin in this mutant (M. Schleicher and E. André, unpublished results).

Under our laboratory conditions no significant impairment of growth or development was detected in HG1130, and no inhibition of antibody-induced capping on the cell surface was observed. Two possibilities cannot be ruled out. One is that the trace amounts of α -actinin are sufficient to fulfil its basic functions in the mutant cells; the other is that slight quantitative changes in cellular functions are detectable with more precise methods than we have used. An effect found which might be related to the defect in α -actinin production is the drastically reduced number of haploid segregants carrying the *abpA1000* mutation (Table II). It seems that the normal allele supports the viability of cells during the process of haploidization when diploids are treated with mitotic blockers.

Assuming that α -actinin is functionally replaced in the cells by another actin cross-linker, e.g., the 120-kd gelation factor (Condeelis *et al.*, 1982, 1984), a behavioral defect would be seen only when both cross-linkers are defective. The fact that a strong reduction of the cellular α -actinin concentration does not severely disturb the balance between proteins that regulate the assembly state of actin suggests that crucial cell functions are multiply guaranteed by similarly acting proteins. If this is the case it would not be surprising if the microinjection of antibodies against α -actinin or other cytoskeletal proteins had no effect on cell morph-

Table II. Linkage analysis of the *abpA1000* mutation using haploid segregants of DG98

Linkage group	Haploid parents of DG98		Independently derived segregants of DG98		
	No.	Genotype	<i>abpA1000</i>	+	Total
I	HG1130	+	12	0	12
	HU1628	<i>cycA1</i>	0	51	51
II	HG1130	+	10	16	26
	HU1628	<i>acrA1823</i>	2	35	37
III	HG1130	+	11	27	38
	HU1628	<i>bsgA5</i>	1	24	25
IV	HG1130	+	5	37	42
	HU1628	<i>whiC351</i>	7	14	21
VI	HG1130	+	10	21	31
	HU1628	<i>manA2</i>	2	30	32
VII	HG1130	+	3	29	32
	HU1628	<i>tsgK21</i>	9	22	31

Table III. Genotypes of the haploid strains used for parasexual genetics

Strain	Linkage group						Locus of interest	Source ^a
	I	II	III	IV	VI	VII		
HG1130	+	+	+	+	+	+	<i>abpA1000</i>	1
HU1628	<i>cycA1</i>	<i>acrA1823</i>	<i>bsgA5</i>	<i>whiC351</i>	<i>manA2</i>	<i>tsgK21</i>	+	2
	(<i>enhA351</i>)	(<i>axeA1</i> ?)				(<i>couA351</i>)		
		(<i>axeC1</i> ?)				(<i>ftrB353</i>)		
		(<i>oaaA1</i> ?)						

The strain contains *cob-354* responsible for dominant cobalt resistance

^aSource 1: This work; source 2: Welker and Williams (1985).

The linkage group markers used were *cycA1*, cycloheximide resistance; *acrA1823*, methanol resistance; *bsgA5*, inability to grow on *Bacillus subtilis*; *whiC351*, white spore colour; *manA2*, absence of α -mannosidase-1; *tsgK21*, temperature sensitivity of growth. Linkage group markers not scored in this work were put into brackets; they are explained by Wallraff *et al.* (1984). No markers are known for linkage group V.

ology or function.

The defect in HG1130 was localized to linkage group I. It remains to be established that the structural gene for α -actinin maps to this linkage group. The faint band at the position of α -actinin, as it is observed in immunoblots, suggests that the defect of HG1130 may reside in a regulatory region rather than in the coding sequence of the α -actinin gene. The question as to whether the structural gene for α -actinin is mutated in HG1130 will be answered by cloning the gene.

Materials and methods

Cultures of *D. discoideum* strains AX2 (clone 214) and HG1130

Cells were cultivated axenically at 23°C in nutrient medium with 1.8% maltose according to Watts and Ashworth (1970) up to a density of not more than 5×10^6 cells/ml. For induction of development, cells were washed and resuspended at a density of 1×10^7 /ml in 17 mM Soerensen phosphate buffer, pH 6.0 ('non-nutrient buffer'). For stimulation of development, pulses of 20 nM cyclic AMP were applied at intervals of 6 min. For agar plate cultures either SM agar and *Klebsiella aerogenes* (Williams and Newell, 1976) or low nutrient (LN) agar and *E. coli* B/2 were used (Wallraff *et al.*, 1984).

Monoclonal antibody preparation and immunolabeling procedures

Antibodies against actin-binding proteins were described by Schleicher *et al.* (1984), mAb 33-294-17 and mAb 41-71-21 against the contact site A glycoprotein by Bertholdt *et al.* (1985) and Hohmann *et al.* (1985). All antibodies used were IgGs and were prepared from hybridoma culture supernatants by ammonium sulfate precipitation and purification on protein A-Sepharose. The anti- α -actinin mAb 47-19-2 was an IgG₁.

The purified antibodies were directly iodinated with ¹²⁵I using chloramine T as described by Stadler *et al.* (1984) and diluted to 1×10^6 c.p.m./ml for labeling of blotted proteins after SDS-PAGE or to $\sim 1 \times 10^5$ c.p.m./ml for labeling of colony blots.

For the induction of capping on the cell surface, HG1130 cells were starved for 6 h, washed and incubated for 15 min on ice with 100 μ g/ml of mAb 41-71-21 in non-nutrient buffer. After washing, the cells were labeled similarly with 50-fold diluted FITC-conjugated sheep anti-mouse IgG (Institute Pasteur Production). Patching and capping was observed after warming up the cells in a wet chamber.

Viscometric assay

To test the blocking activity of mAb 47-19-2, 62 μ g/ml of the IgG were added together with increasing concentrations of α -actinin to a solution of rabbit skeletal muscle G-actin and incubated for 30 min at 4°C. The cross-linking activity of α -actinin and of the α -actinin/IgG-complexes was compared by low shear viscometry as described by MacLean-Fletcher and Pollard (1980). The reaction mixture contained 0.4 mg/ml of actin, and polymerization was started by the addition of 1 mM MgCl₂ in the presence of 1 mM EGTA.

Electron microscopy

α -Actinin was purified as described by Schleicher *et al.* (1984), and α -actinin/mAb 47-19-2 complexes rotary shadowed and subjected to length measurements essentially as described for myosin/antibody complexes by Claviez *et al.* (1982). Purified α -actinin and antibody were diluted into a solution containing 85 volumes of 50 mM NH₄HCO₃ and 15 volumes of glycerol yielding final concentrations of 15 μ g/ml of each protein. After incubation for 18–22 h on ice, 50 volumes of glycerol were added, the mixture was sprayed at room temperature onto mica and allowed to dry in a vacuum. The angle of rotary shadowing was either 5° or 8° as specified in Results.

Mutant selection and genetic analysis

Growth phase cells of AX2-214 were mutagenized by incubation with 1 mg/ml of 1-methyl-3-nitro-1-nitrosoguanidine for 20 min in non-nutrient buffer. The survival rate was ~2%. The mutagenized cells were plated on SM agar. Cells from single colonies were deposited in a regular array of 56 inocula per SM agar plate of 9 cm diameter and cultivated at 21°C (Figure 4). Colonies were replicated with a toothpick multidispenser onto SM agar followed by cultivation up to a colony size of 5–7 mm. Colonies were blotted onto BA85 nitrocellulose filters (Schleicher and Schüll, D-3354 Dassel, FRG). The filters were immediately frozen on a dry metal plate cooled on dry ice and thawed to lyse cells and to allow internal proteins to get access to the nitrocellulose. The filters were washed in a solution containing 10 mM Tris buffer, pH 7.8, 150 mM NaCl, 0.05% Tween 20, and 0.02% NaN₃ (Tris-Tween buffer). The blots were labeled using [¹²⁵I]mAb solutions in Tris-Tween buffer and autoradiographed on X-Omat AR film (Kodak).

The mutation *abpA1000* of HG1130 was mapped using HU1628 as tester strain (Table III). Diploids were selected using the dominant *cob-354* resistance mutation and the recessive *tsgK21* mutation (Newell *et al.*, 1977; Williams and Newell, 1976; Williams, 1978). Diploids were verified by spore size and by checking

wild-type phenotype for all recessive markers. Diploids were haploidized with thiabendazole according to Welker and Williams (1980) as described by Wallraff *et al.* (1984).

Miscellaneous techniques

For the assay of chemotaxis, axenically grown cells of HG1130 were starved in non-nutrient buffer for 6 h, washed in the buffer and transferred to the Teflon surface of Petriperm dishes (Heraeus, D6450 Hanau, FRG). The cells were chemotactically stimulated according to Gerisch and Keller (1981). Growth of AX2 and HG1130 cells was compared by measuring at intervals the colony diameters on LN agar plates.

Proteins were separated by SDS-polyacrylamide electrophoresis in 10% gels according to Laemmli (1970) and transferred to nitrocellulose according to Towbin *et al.* (1979) as specified by Stadler *et al.* (1984). Protein was determined according to Lowry with bovine serum albumin as standard except for antibody which was determined spectrophotometrically assuming an OD_{280 nm} (1 cm, 1% IgG) of 15.

Acknowledgements

We thank Dr. J. Stadler for the assay of chemotaxis, Dr. S. Bozzaro for mutagenesis, Mrs. I. Sailer and U. Weinhart for technical help, and Mrs. B. Book for organizing the manuscript. The work was supported by grant Is 25/4 provided by the Deutsche Forschungsgemeinschaft to G. Isenberg and G. Gerisch.

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Received on 28 October 1985; revised on 13 November 1985