A *Dictyostelium* Mutant Deficient in Severin, an F-Actin Fragmenting Protein, Shows Normal Motility and Chemotaxis

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Abstract. A severin deficient mutant of *Dictyostelium discoideum* has been isolated by the use of colony immunoblotting after chemical mutagenesis. In homogenates of wild-type cells, severin is easily detected as a very active F-actin fragmenting protein. Tests for severin in the mutant, HG1132, included viscometry for the assay of F-actin fragmentation in fractions from DEAE-cellulose columns, labeling of blots with monoclonal and polyclonal antibodies, and immunofluorescent-labeling of cryosections. Severin could not be detected in the mutant using these methods. The mutation in HG1132 is recessive and has been mapped to linkage group VII.

The mutant failed to produce the normal severin mRNA, but small amounts of a transcript that was ~100 bases larger than the wild-type mRNA were detected in the mutant throughout all stages of development. On the DNA level a new Mbo II restriction site was found in the mutant within the coding region of the severin gene.

The severin deficient mutant cells grew at an approximately normal rate, aggregated and formed fruiting bodies with viable spores. By the use of an image processing system, speed of cell movement, turning rates, and precision of chemotactic orientation in a stable gradient of cyclic AMP were quantitated, and no significant differences between wild-type and mutant cells were found. Thus, under the culture conditions used, severin proved to be neither essential for growth of *D. discoideum* nor for any cell function that is important for aggregation or later development.

SEVERIN is a soluble F-actin fragmenting protein isolated from *Dictyostelium discoideum* (Brown et al., 1982; Yamamoto et al., 1982). It intercalates in the presence of 1–5 μM Ca^{2+} into actin filaments, and remains bound to the barbed end of the fragments even when free Ca^{2+} is removed by EGTA (Giffard et al., 1984). The 40-kD severin molecule (André et al., 1988) has sequence homologies not only with fragmin, a severing protein of *Physarum polycephalum* (Ampe and Vandekerckhove, 1987), but also with gelsolin and villin (Kwiatkowski et al., 1986; Bazari et al., 1988). Severin and fragmin may be closely related to an ancestral form of these vertebrate F-actin severing proteins (André et al., 1988).

The amount of F-actin in the cortex of *D. discoideum* cells decreases with increasing Ca^{2+} concentration, suggesting that the association of actin with the cortex is inhibited by severin or other Ca^{2+}-dependent proteins (Giffard et al., 1983). Mutual competition of severin with other actin binding proteins is suggested by the inhibitory effect of myosin S1-fragments on the interaction of severin with F-actin (Giffard et al., 1984). These in vitro assays as well as colocalization of severin and F-actin under certain conditions (Brock and Pardee, 1988) suggested that severin could be involved in cell motility and chemotactic orientation. To determine whether severin has such a function in cells, we screened mutagenized *D. discoideum* cells for the absence of reactivity with severin-specific monoclonal antibodies (Wallraff et al., 1986). One mutant, HG1132, was isolated in which no severin was detected. This mutant is analyzed in the present paper on the DNA, RNA, and protein level; we also report on genetic mapping of the mutation and on our attempts to discover alterations in cell growth, development, motility or chemotactic response in the mutant.

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Materials and Methods

Antibodies and Immunoblotting

Antibodies 42-65-23 (Schleicher et al., 1984), 101-460-2, 102-200-1, and 102-425-1 (André et al., 1988) are referred to in this paper as mAb 65, 460, 200, and 425. These antibodies were obtained by immunizing BALB/c mice with purified severin. Adjuvants were *Bordetella pertussis* antigen for mAb 65, Alugel S (Serva Fine Biochemicals, Heidelberg) for mAb 200 and 425, and Freund's adjuvant for mAb 460. Spleen cells were fused with myeloma NS-1 cells for mAb 65, with 63Ag8-653 cells for mAb 425 and 460, or with Sp2-01 cells for mAb 200. Monoclonal IgG was purified from hybridoma culture supernatants by ammonium sulfate precipitation and chromatography on Protein A-Sepharose. The IgG was labeled with ^{125}I by the chloramine T method. The iodinated antibodies were used for colony blotting according to Wallraff et al. (1986), for labeling of blots after SDS-PAGE,

for radioimmunocompetition assays, and for the screening of cDNA clones in expression vectors. Polyclonal antisera were raised in rabbits by subcutaneous injections of purified severin with Freund's adjuvant and used, together with ^{125}I goat anti-rabbit IgG, for indirect labeling of blots after SDS-PAGE.

Isolation and Genetic Analysis of the Mutant

Cells of *D. discoideum* AX2 clone 214, in this paper referred to as wild-type, were mutagenized with 1-methyl-3-nitro-1-nitrosoguanidine, cloned, and subjected to colony blotting essentially as described by Wallraff et al. (1986). By labeling the blots of 1.4×10^4 colonies with anti-severin mAb 65 one unlabeled colony was detected which gave rise to the mutant strain HG1132. The mutation responsible for the severin defect, *sevA1000*, was mapped using parasexual genetics. Heterozygous diploids were constructed from the mutant HG1132 and the tester strain HU1628 in the presence of $CaCl_2$ (Williams and Newell, 1976) and isolated by *tsg/cob* selection (Williams, 1978). In HU1628 all established linkage groups of *D. discoideum* are marked by mutations (Welker and Williams, 1985; Wallraff et al., 1986). Haploid segregants produced from one diploid according to Welker and Williams, (1980) were screened for linkage group markers (Wallraff et al., 1984) and for the severin defect by labeling colony blots with mAb 65 (Wallraff et al., 1986).

DNA and RNA Isolation from D. discoideum and Hybridization Analysis

DNA was isolated from purified nuclei as described (Noegel et al., 1985*b*). Restriction fragments of nuclear and plasmid DNA were separated on 1% agarose gels in Tris-phosphate buffer, pH 7.8 (Maniatis et al., 1982), transferred to nitrocellulose BA85 (Schleicher & Schuell, Inc., Keene, NH) and probed with nicktranslated cDNA inserts (André et al., 1988) in 50% formamide, 2× SSC, 1% Sarkosyl, 4 mM EDTA, 0.1% SDS, 4× Denhardt's solution, and 0.12 M sodium phosphate buffer, pH 6.8, at 37°C for 16 h. The filters were washed twice in 2× SSC and 0.01% SDS at room temperature and 1 h at 37°C under hybridization conditions. For isolation of RNA, cells were lysed with 1% SDS in the presence of 0.05% diethylpyrocarbonate and the RNA purified by several phenol-chloroform extractions (Noegel et al., 1985*a*).

For Northern blot analysis 10 μg of total RNA were loaded and separated on a 1.2% agarose gel in the presence of 2.2 M formaldehyde (Maniatis et al., 1982). The RNA was transferred to nitrocellulose filters and hybridized with nicktranslated cDNA probes as described above.

Expression of Severin cDNA Sequences in a Plasmid Expression Vector in E. coli

cDNA coding for fragments of or for the complete *Dictyostelium* severin were subcloned into pIMS1, 5 or 6 (Simon et al., 1988), depending on the reading frame, and transformed into *E. coli* JM83. Clones expressing immunoreactive material were identified as described (Simon et al., 1988) with a mixture of monoclonal antibodies directed against severin and used for further analysis.

Biological Assays

Rates of axenic growth of HG1132 and AX2 were determined under identical conditions in suspension cultures at 23°C in the nutrient medium of Watts and Ashworth (1970) with 1.8% maltose. Growth on a lawn of *E. coli* B/2 on low-nutrient (LN) agar (Wallraff et al., 1984) was determined at 21 and 27°C by inoculating Petri dishes in the center and measuring diameters of each colony in two directions at daily intervals.

Chemotactic stimulation with a micropipette was performed according to Gerisch et al. (1975*a*,*b*; Swanson and Taylor, 1982). Quantitative data on motility and chemotaxis were obtained using an image processing system (Segall et al., 1987) and a chamber with which the orientation of wild-type and mutant cells in stable linear gradients of cAMP can be determined (Fisher et al., 1989). Light scattering changes in response to cAMP were monitored in cell suspensions according to Gerisch and Hess (1974).

To test the capability of mutant cells to patch and cap membrane proteins, the cells were incubated for 15 min on ice in 17 mM Soerensen phosphate buffer, pH 6.0, with 250 μg IgG per milliliter of mAb 41-71-21 directed against the protein moiety of the contact site A glycoprotein (Bertholdt et al., 1985), washed and incubated under the same conditions with fluorescein/isothiocyanate-conjugated goat anti-mouse IgG (Jackson Immuno Research Laboratories Inc., Avondale, PA). After washing, the cells were transferred to 21°C and the distribution of the fluorescence label on their surface was observed.

Immunofluorescent Labeling of Cryosections

After 6 h of starvation cells were fixed in a mixture of 1% formaldehyde and 0.01% glutaraldehyde in 17 mM Soerensen phosphate buffer, pH 6.0, for 15 min at room temperature, followed by 1 h on ice. Fixed cells were pelleted in gelatin, postfixed in 1% formaldehyde for 1 h on ice and prepared for sectioning according to Tokuyasu (1973). Sections of ~0.5 μm were cut using a Reichert FC 4D cryoultramicrotome and treated for 5 min with 50 mM glycine in PBS, pH 7.4; 10 min with 2 mg/ml of sodium borotetrahydride in PBS; 2 × 10 min with 0.5% BSA and 0.2% gelatin in PBS (PBG); 1 h with a mixture of mAb 200, mAb 65, and mAb 460, containing 10 μg of each IgG in PBG. The fourth antibody, mAb 425, gave similar results with wild-type and mutant cells as the other antibodies, but was not added to the mixture because it bound strongly to material outside the cells, probably to the gelatin in which the cells were embedded. Subsequently the sections were treated for 7 × 2 min with PBG; 1 h with a mixture of fluorescein/isothiocyanate-labeled goat anti-mouse IgG (Jackson Immuno Research Laboratories Inc.; diluted 1:200), and 0.2 μg per ml of TRITC-labeled phalloidin (Sigma Chemical Co.); 7 × 2 min with PBG. Labeled sections were mounted in semisolid medium (Lennette, 1978) containing 25 μg/ml of 1,4-diazobicyclo-(2,2,2)-octane (DABCO) (Langanger et al., 1983) and photographed on Tri-X film (Eastman Kodak Co., Rochester, NY) using a Neofluar 100/1.3 Zeiss phase-contrast objective.

Immunocompetition Assays

Competition of antibodies for binding to severin was assayed either in microtiter wells or on nitrocellulose filters after blotting of total proteins from growth-phase AX2 cells separated by SDS-PAGE. Per microtiter well were added 50 μl of a solution of 130 μg severin per milliliter in buffer containing 10 mM potassium phosphate, pH 6.7, 0.2 mM DTT, 1 mM EDTA, and 0.02% NaN_3. After 14 h the wells were washed with 10 mM Tris-HCl, pH 7.8, 150 mM NaCl, 4% BSA, 0.05% Tween-20, 0.02% NaN_3, and incubated with 5×10^4 cpm per well of ^{125}I-mAb with or without 100 μg/ml of unlabeled mAb for competition. Bound radioactivity was determined in a γ-counter.

Alternatively, 20 μg of cellular proteins were separated per lane of polyacrylamide gels. Blots were incubated with 2.5×10^5 cpm/ml of iodinated mAb and 20 μg/ml of unlabeled mAb for competition. On the autoradiograms the areas of the severin band were scanned.

Protein Purification and Viscometry

Rabbit skeletal muscle actin was prepared according to Spudich and Watt (1971) and gel filtered on Sephacryl S300. Severin was isolated essentially as described by Schleicher et al. (1984). Protein was determined according to Lowry with BSA as standard. Low shear viscometry was carried out after 15 min of incubation at 25°C in a falling ball viscometer (MacLean-Fletcher and Pollard, 1980).

Results

Characterization of Mutant HG1132 and Genetic Mapping of its Severin Defect to Linkage Group VII

Mutant HG1132 was detected by its inability to bind mAb 65, an anti-severin antibody. Antibodies against three other actin binding proteins, α-actinin, the 120-kD gelation factor, and myosin, bound to blots of wild-type and HG1132 colonies (Fig. 1 *A*). Because HG1132 was selected with a single monoclonal antibody we have also tested the mutant with polyclonal antisera, and no severin was detected in immunoblots (Fig. 1 *B*). The mutant cells formed pseudopodia, aggregated and made fruiting bodies similar to wild type (Fig. 2).

The locus of the mutation responsible for the severin defect, termed *sevA1000*, was mapped to linkage group VII

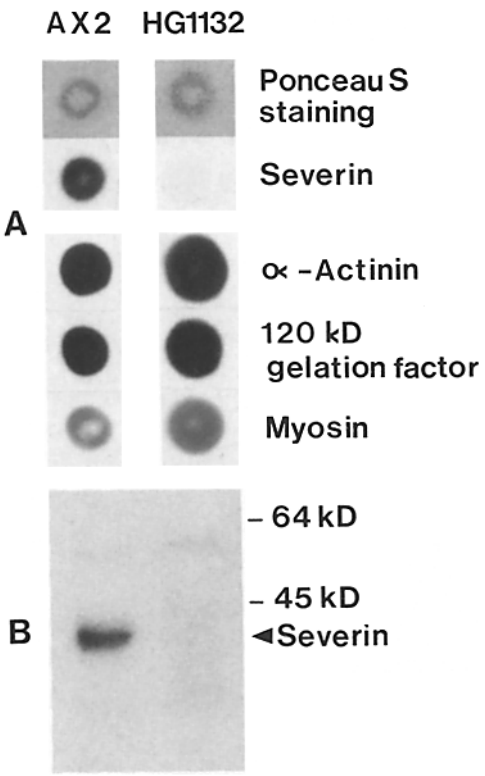

Figure 1. Immunoblots of wild-type AX2 (*left lane*) and mutant HG1132 (*right lane*). (*A*) Blots of colonies grown on SM agar in a lawn of *Klebsiella aerogenes*, labeled with ^{125}I-mAb 65 for severin followed by staining the proteins on the same blot with Ponceau S. The other colony blots were either labeled with iodinated mAb 47-19-2 for α-actinin (Schleicher et al., 1984), mAb 82-471-14 for the 120-kD gelation factor, or mAb 21-96-3 for the myosin heavy chain (Pagh and Gerisch, 1986). All proteins except severin were recognized in the mutant by the antibodies. (*B*) Blots of total cellular proteins separated by SDS-PAGE labeled with polyclonal rabbit antibodies against severin. The weakly cross reacting 55-kD protein provides an internal control for the absence of severin labeling in the mutant.

Table I. Linkage Analysis of the sevA1000 Locus Using 94 Haploid Segregants of the Diploid Strain DG104

Linkage group	Genotype of segregants	No. of independent segregants	
		sevA1000	+
I	+	1	12
	cycA1	12	69
II	+	10	53
	acrA1823	3	28
III	+	1	18
	bsgA5	12	63
IV	+	3	37
	whiC351	9 +1*	41 +3*
VI	+	8	52
	manA2	5	29
VII	+	13	0
	tsgK21	0	81

DG104 is derived from the haploid mutant HG1132 carrying the *sevA1000* mutation responsible for the severin defect, and from the haploid tester strain HU1628 carrying the six mutations that mark linkage groups I–IV and VI–VII (Wallraff et al., 1984; Welker and Williams, 1985). For linkage group V no marker is available.

* In these segregants sorocarp color could not be determined because the cells did not aggregate.

using parasexual genetics. Only the marker for this linkage group (*tsgK2I*) and the defect in severin production excluded each other (Table I). Data shown later (see Figs. 9 and 10) indicate that the severin gene itself is altered in the mutant. Consequently, it is the structural gene rather than a regulatory gene that is located on linkage group VII. The mutation is recessive; heterozygous diploids showed severin labeling in colony blots with mAb 65. No revertant of the mutant was observed after ~200 generations of culture on SM agar plates when >200 colonies were scored for severin.

Severin Is Not Detected in Homogenates of Mutant HG1132 by Monoclonal Antibodies that Bind to Different Epitopes

In addition to mAb 65, three other anti-severin antibodies were applied to wild-type and mutant. Severin was not detected in HG1132 by SDS-PAGE and immunoblotting with any of these antibodies (Fig. 3). Binding of the antibodies to different epitopes of the severin molecule was tested by competition assays and by determining their binding to bacterially expressed severin polypeptides encoded by cDNA fragments of various lengths. In the competition assays, no or incomplete mutual inhibition of mAb 200 and mAb 460 for binding to severin was observed (Table II), and mAb 65 showed weak or no cross competition with any of the other antibodies (Fig. 4 *A*). Binding of the antibodies to the translation products of cDNA-fragments revealed that mAb 425 bound to an epitope distinct from those of the other antibodies (Fig. 4 *B*). These results show that four different epitopes of the severin molecule are recognized by the monoclonal antibodies used.

In Cryosections the Antibodies Brilliantly Label the Cytoplasm of Wild-Type and Not That of Mutant Cells

Monoclonal antibodies were employed for indirect fluorescence labeling of cryosections of fixed wild-type and mutant cells (Fig. 5). In specimens of the wild-type, the cytoplasm was strongly labeled. The severin label was almost uniformly distributed over the cytoplasm, with the nuclei and smaller organelles appearing as darker areas (Fig. 5 *B*). This distribution of the severin label was clearly distinct from that of F-actin. Fluorescent phalloidin labeled preferentially the

Table II. Competition Radioimmunoassay Showing that mAbs 200 and 460 Recognize Different Epitopes of Severin

Competing mAb	^{125}I-labeled mAb	
	200	460
200	4	52
460	93	2

^{125}I-labeled antibodies were incubated with or without competing, unlabeled antibody in severin-coated microtiter wells. Data represent radiolabel associated with severin as the percent of control without competing antibody.

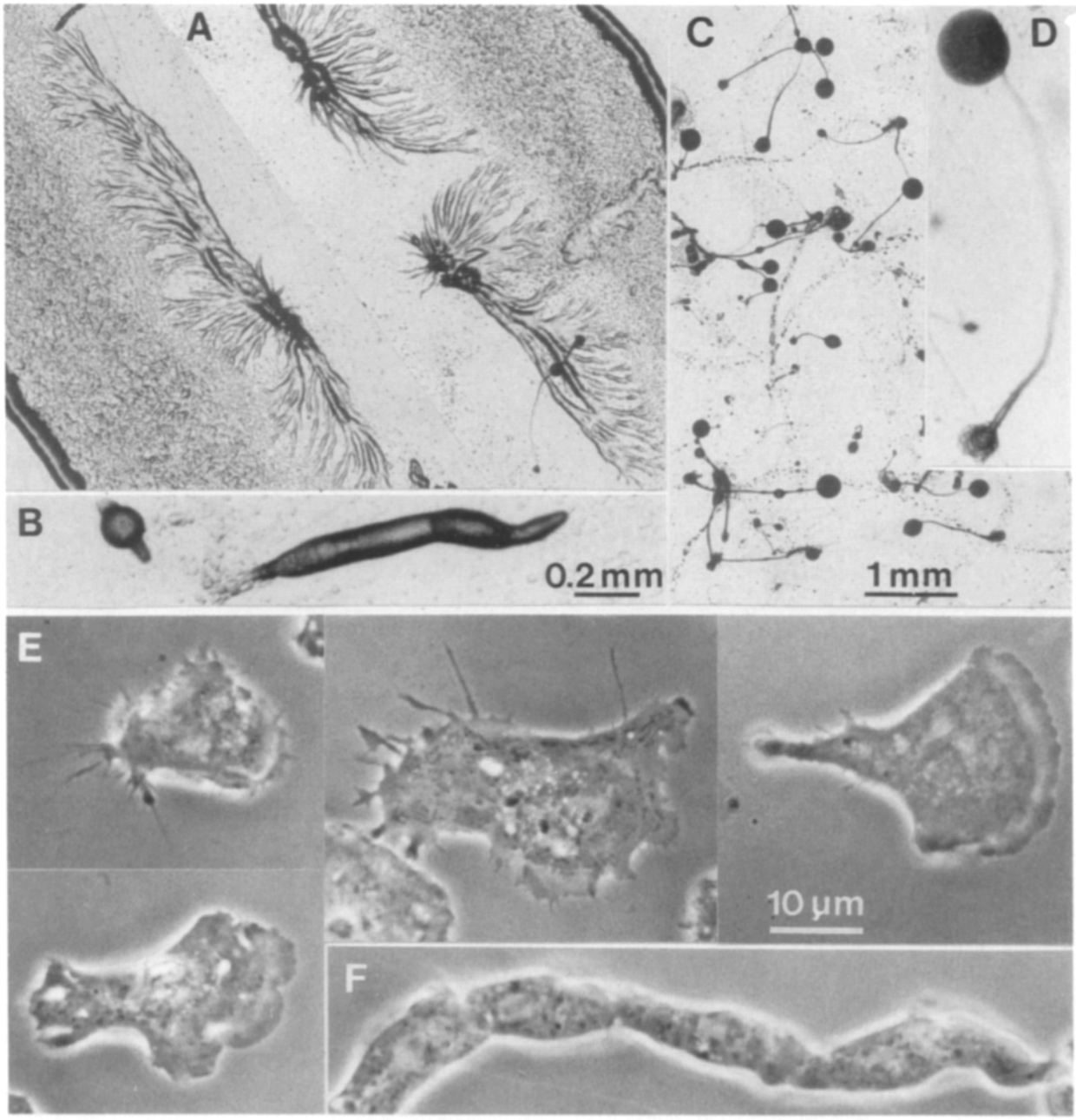

Figure 2. Developmental stages of mutant HG1132. (*A*) Colonies showing the wall of feeding amoebae, the preaggregation zone, and typical aggregates with centers and streams. (*B*) Migrating slug and early culmination stage. (*C*) Mature fruiting bodies. (*D*) Fruiting body showing typical foot disc, stalk, and spore head (attached to the agar surface). (*E*) Four preaggregative amoebae showing variability of cell shape similar to wild-type. (*F*) Chain of end-to-end connected, elongated cells at an early aggregation stage. For *A–D* photographs were taken from cultures on low-nutrient agar with *E. coli* B/2. For *E* and *F*, phase-contrast photographs were taken from axenically grown cells starved in phosphate buffer and transferred onto a glass surface. The bar for *A* is the same as in *C*, for *D* the same as in *B*, and for *F* the same as in *E*.

cortical region of the cells (Fig. 5 *C*). This difference in the distribution of severin and F-actin was observed after fixation with paraformaldehyde and also after fixation with a glutaraldehyde-formaldehyde mixture.

In sections of mutant HG1132 cells only very faint fluorescence, slightly above the background obtained with second antibody alone, was obtained with the same anti-severin antibodies as used for the wild-type (Fig. 5 *E*). The F-actin cortical layer in sections from mutant cells (Fig. 5 *F*) was indistinguishable from that of wild-type cells (Fig. 5 *C*). This result shows that the lack of severin does not result in an increase of F-actin concentration throughout the entire cytoplasmic space where severin is normally located.

No Severin-like Viscosity Decreasing Activity Is Found in HG1132 Fractions

The F-actin fragmenting activity of severin is the most prominent activity revealed by viscometry in the soluble fraction

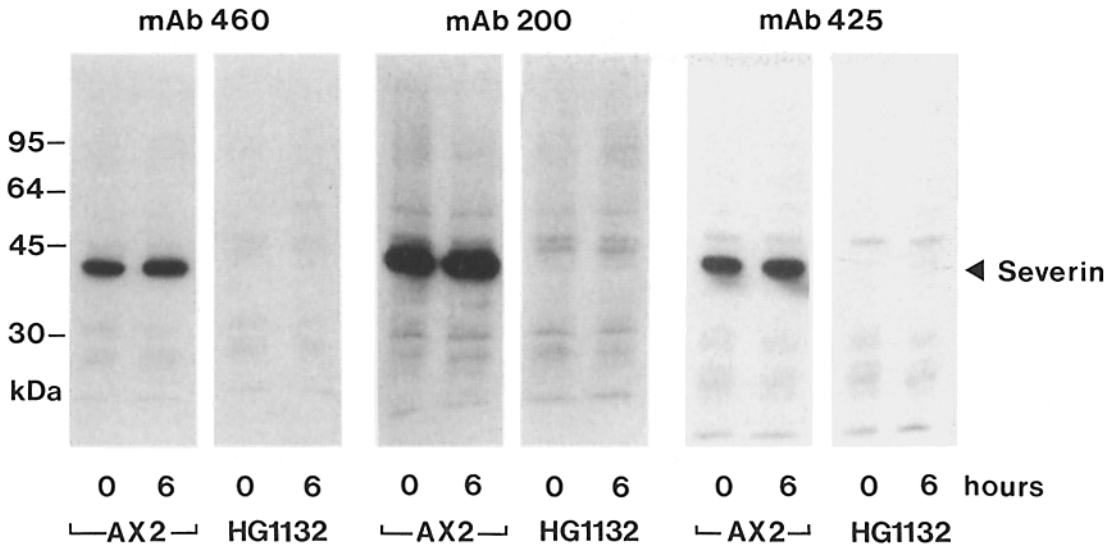

Figure 3. Labeling of total cellular proteins separated by SDS-PAGE with three anti-severin antibodies. The proteins of wild-type AX2 or mutant HG1132 cells harvested either at the end of growth (0 h) or at the aggregation competent stage (6 h of starvation) were blotted onto nitrocellulose and incubated with the ^{125}I-labeled antibodies. The autoradiograms were overexposed until unspecifically labeled bands became visible to demonstrate absence of any severin labeling in the mutant. All three antibodies used were raised in mice other than the one for mAb 65, which was used for selection of the mutant. Positions of molecular mass markers are shown on the left.

from wild-type cells. Severin can be separated from most of the other actin binding proteins by a single DEAE-cellulose fractionation step where severin appears in the flow-through (Brown et al., 1982). It precipitates between 50 and 80% saturated ammonium sulfate. In contrast to wild-type, there was no severin activity found in the ammonium sulfate precipitate of the flow through fraction of mutant HG1132 (Fig. 6). This result shows that the mutant does not produce normal severin but it does not exclude the possibility that the mutant produces severin with an altered chromatographic behavior. Therefore, the DEAE-cellulose column was eluted by a NaCl gradient and the fractions assayed by viscometry. In these fractions the Ca^{2+}-independent viscosity-decreasing activity of the F-actin capping protein (cap 32/34) was found at ~5 mS in the wild-type and mutant (Fig. 7). Increases of viscosity corresponded to the F-actin cross-linkers α-actinin and 120-kD gelation factor that are eluted in overlapping peaks between 11 and 14 mS. However, no Ca^{2+} requiring viscosity-decreasing activity was detected in the mutant fractions that would indicate the presence of a chromatographically altered but still active severin.

For the following reasons the other actin binding proteins would not mask severin if it were present in the eluted fractions. The activity of the capping protein is distinguished from the viscosity decreasing effect of severin by its Ca^{2+} independence. α-Actinin is inhibited by Ca^{2+} and thus does not interfere with severin which is only active in the presence of Ca^{2+}. To exclude the possibility that the 120-kD gelation factor would mask severin, a control experiment was performed in which severin was assayed in the presence of the gelation factor. Fig. 7 *C* shows that severin abolished the viscosity increasing effect of the factor, which means that the gelation factor would be masked in the presence of severin, but not vice versa.

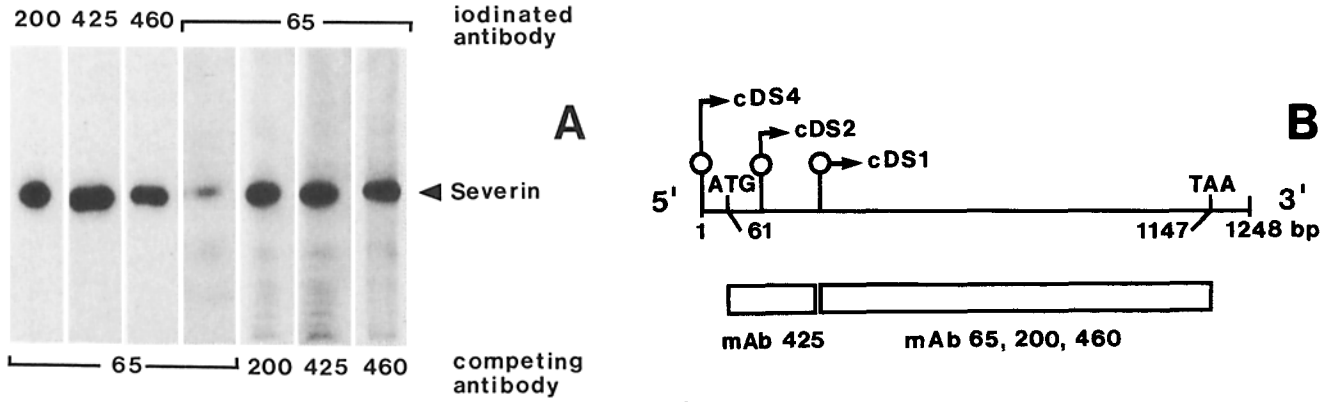

Figure 4. Mapping of antibody binding sites on the severin polypeptide. (*A*) Competition radioimmunoassay demonstrating that antibodies 200, 425, and 460 bind to other epitopes than mAb 65, the antibody with which mutant HG1132 was isolated. Substantial inhibition was only obtained in the combination of unlabeled mAb 65 with ^{125}I-labeled mAb 65. (*B*) Polypeptides encoded by the cDNA fragments, cDS1, cDS2, and cDS4 with different 5′ ends were subcloned in pIMS vectors, expressed in bacteria and probed with four monoclonal antibodies. mAb 425 bound only to the complete severin polypeptide chain encoded by the cDS4 cDNA. Length and coding region of this cDNA are indicated. The blocks represent the regions in which mAb binding sites are located.

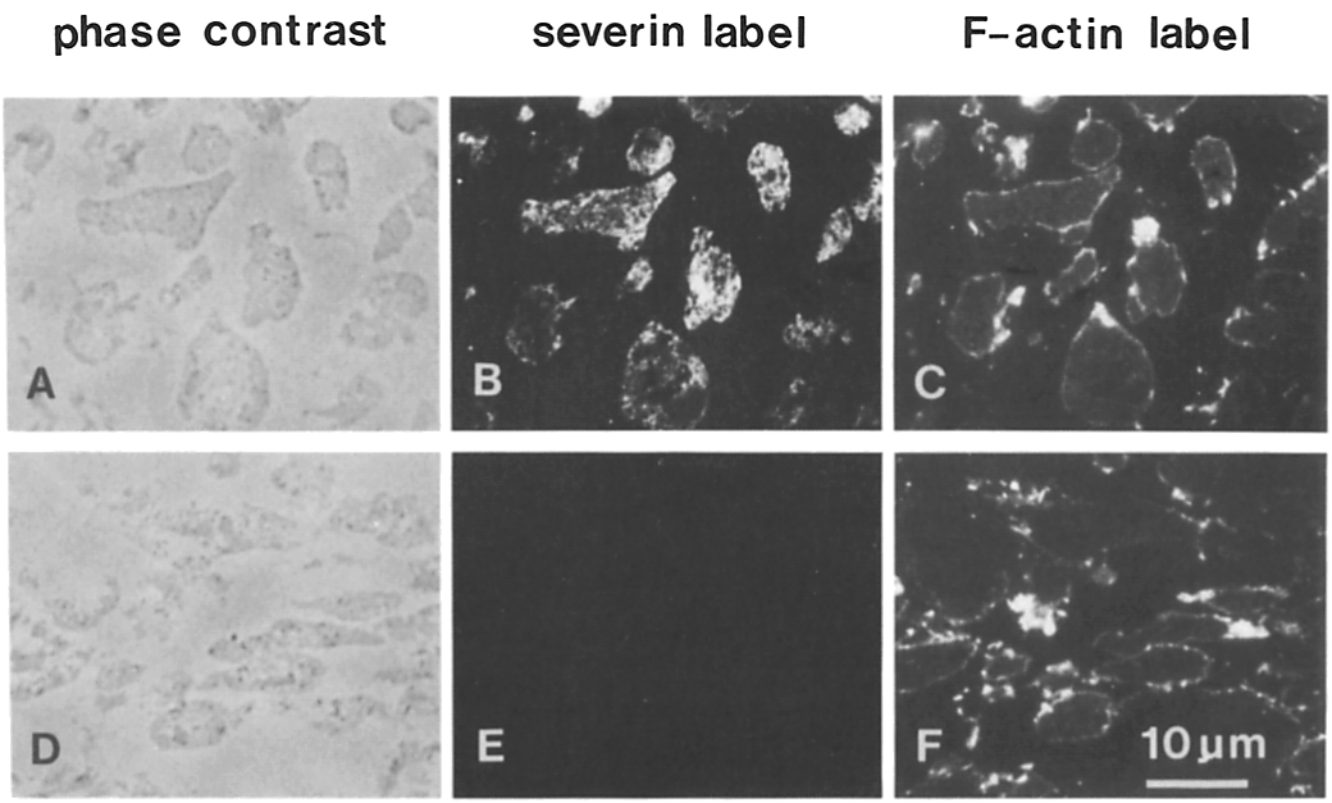

Figure 5. Cryosections of wild-type AX2 cells (*A–C*) and mutant HG1132 cells (*D–F*). From one field of either wild-type or mutant preparations phase-contrast images (*A* and *D*), labeling with a mixture of anti-severin antibodies mAb 65, 200, and 460 followed by FITC-conjugated goat anti-mouse IgG (*B* and *E*), and TRITC-conjugated phalloidin (*C* and *F*) are shown.

An Altered RNA Is Produced in the Mutant

RNA of HG1132 was compared in Northern blots with RNA of the parent AX2 strain by probing with a nick-translated severin cDNA fragment, cDS1 (Fig. 8). The severin transcript of HG1132 proved to be ~0.1 kb larger than the 1.4-kb severin mRNA of the AX2 wild-type strain. The mutant transcript was only weakly labeled by the probe, although the same amount of total wild-type and mutant RNA was loaded onto the gels. As an internal control, the mRNA of the 120-kD gelation factor was used. The cDNA probe for this transcript strongly labeled the mRNA in mutant cells, and the transcript had a size of ~3.2 kb as in the wild-type. The altered transcript of the mutant severin gene was, like the wild-type transcript, present throughout growth and development up to the slug and culmination stages. At no stage was a normal-sized severin transcript produced in the mutant (data not shown).

The Restriction Pattern Indicates an Altered Sequence in the Mutant Severin Gene

Genomic DNA of AX2 and HG1132 cells was cleaved with Eco RI, which had no cleavage site within the severin cDNA, with Hind III and Mbo II which had a single cleavage site, and with Rsa I which had more than one cleavage site. Southern blots were probed with the cDNA fragment cDS4 which spans the entire coding region of severin. Eco RI produced a labeled 3.8-kb fragment, Hind III two fragments, one of ≥9.4 and one of 2.3 kb, and Rsa I four fragments, the largest with a size of 1.6 kb (Fig. 9). With Eco RI, Hind III, and Rsa I no difference between wild-type and mutant DNA was observed. These results indicate that the mutant gene is not distinguished by a deletion or duplication of substantial size. With Mbo II a sequence change in the mutant gene was found. In the Mbo II digested DNA from AX2, a 1.4- and 0.5-kb fragment were labeled with the cDS4 probe (Fig. 9). The same result was obtained with DNA from the wild-type strain NC4 from which AX2 is derived (data not shown). In HG1132 DNA, however, 0.8, 0.6, and 0.5 kb fragments were

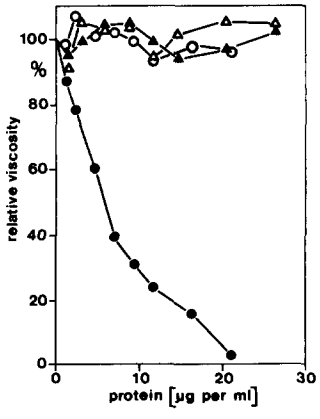

Figure 6. Assay for severing activity in mutant HG1132: lack of activity in the flow-through fraction of a DEAE-cellulose column. The decrease in viscosity of an F-actin solution caused by wild-type AX2 (● and ○) and mutant HG1132 (▲ and △) fractions was assayed in the presence of 0.2 mM Ca^{2+} (● and ▲) or 1.0 mM EGTA (○ and △).

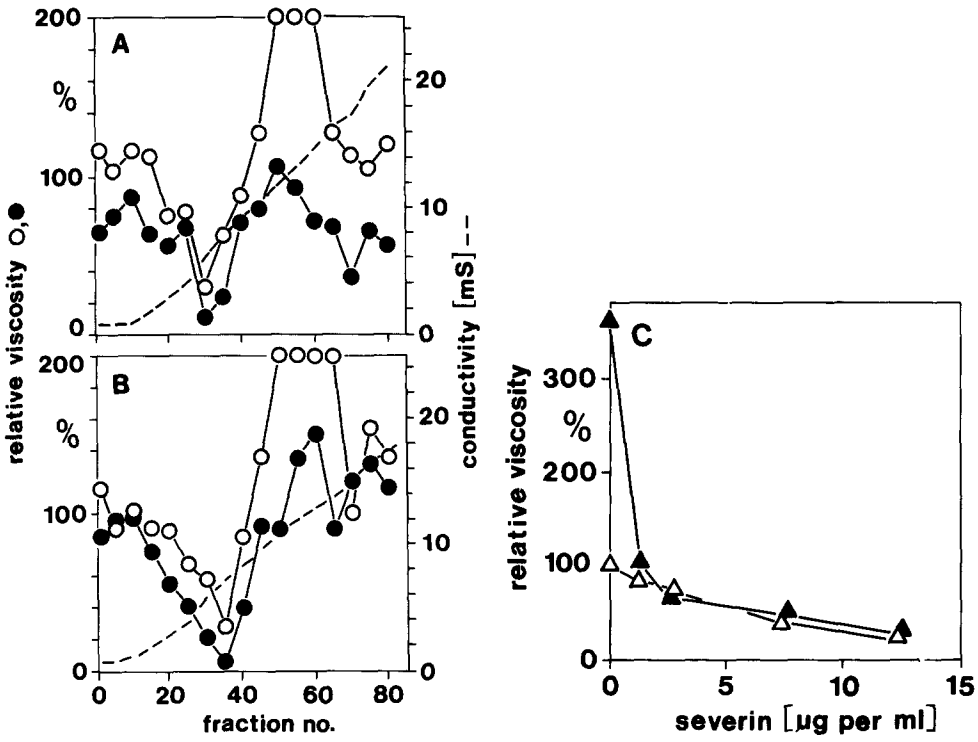

Figure 7. Assay for severing activity in mutant HG1132: lack of the activity in fractions eluted from DEAE-cellulose. Wild-type (*A*) or mutant (*B*) fractions were assayed in the presence of 0.2 mM Ca^{2+} (●) or 1.0 mM EGTA (○). The Ca^{2+}-independent viscosity decreasing activity found in fractions 20–40 is due to the 32/34-kD capping protein, the viscosity increase in fractions 43–63 primarily to the Ca^{2+}-inhibited α-actinin, and to a minor extent to the 120-kD cross-linking protein. To prove that severin activity is not masked by the 120-kD cross-linker (*C*), the viscosity decreasing effect of purified severin was assayed with F-actin in the presence (▲) or absence (△) of 12 µg per ml of this cross-linker.

detected. This result indicates that the *sevA1000* mutation of HG1132 has created a new Mbo II restriction site in the severin gene (Fig. 10). Using cDNA probes specific for the 5′ or 3′ portions of the severin gene the new Mbo II site of the mutant was localized to the 5′ region of the severin coding sequence (Figs. 9 and 10).

Growth Rates, Cell Motility, Chemoresponses and Cell Surface Capping of Mutant Cells Resemble those of the Wild-Type

Growth was determined on agar plates where the cells phagocytose bacteria, and in shaken cell suspensions where nutrients from liquid medium are pinocytosed (Table III). The cells were cultivated at optimal temperatures of 21 or 23°C and, on the agar plates, also at 27°C, the upper limit of growth for wild-type strains. No significant difference between the growth rates of wild-type and mutant cells was observed under these conditions.

The motility of HG1132 cells was analyzed using a computer-controlled image processing system. Speed and turning rate were determined in mutant and wild-type cells and no difference was found (Table IV). Chemotactic orientation was measured in a chamber that generates a stable linear gradient of 2.5×10^{-8} M cAMP/mm. As a measure of orientation the mean fraction of cell movement in the direction of the gradient was calculated. The orientation of HG1132 cells, and also their speed and turning rate in the presence of a cAMP gradient, were similar to those of AX2 cells. When stimulated through a micropipette filled with cAMP, HG1132 cells contracted and extended new pseudopods towards the pipette (Fig. 11) as do wild-type cells (Claviez et al., 1986).

Another assay for chemoresponses of *D. discoideum* cells makes use of changes in the light scattering of cell populations (Gerisch and Hess, 1974). Again, in HG1132 cell suspensions responses to cAMP were similar in amplitude and duration to those seen with AX2 cells (Fig. 12).

The redistribution of a cell surface protein, the contact site A glycoprotein of aggregating cells, was investigated by incubating cells with monoclonal IgG directed against its protein moiety, followed by fluorescent anti-mouse IgG. The

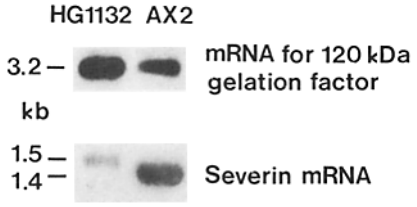

Figure 8. Northern blot showing the 1.4-kb severin mRNA of wild-type AX2 and the 1.5-kb transcript of mutant HG1132. The RNA was labeled with the severin specific probe cDS1. For a control, this blot was also probed with a cDNA probe recognizing the 120-kD gelation factor.

Table III. Growth Rates of Wild-Type AX2 and Mutant HG1132 Cells on Agar with Bacteria and under Axenic Conditions

	On low-nutrient agar		In axenic suspension culture
	21°C	27°C	23°C
AX2	10.4	9.0	7.7
HG1132	10.5	9.3	8.3

Data on low-nutrient agar with *E. coli* B/2 as a food represent increases of colony diameters in millimeters per day (averages of three measurements), data in axenic suspension culture are generation times in hours.

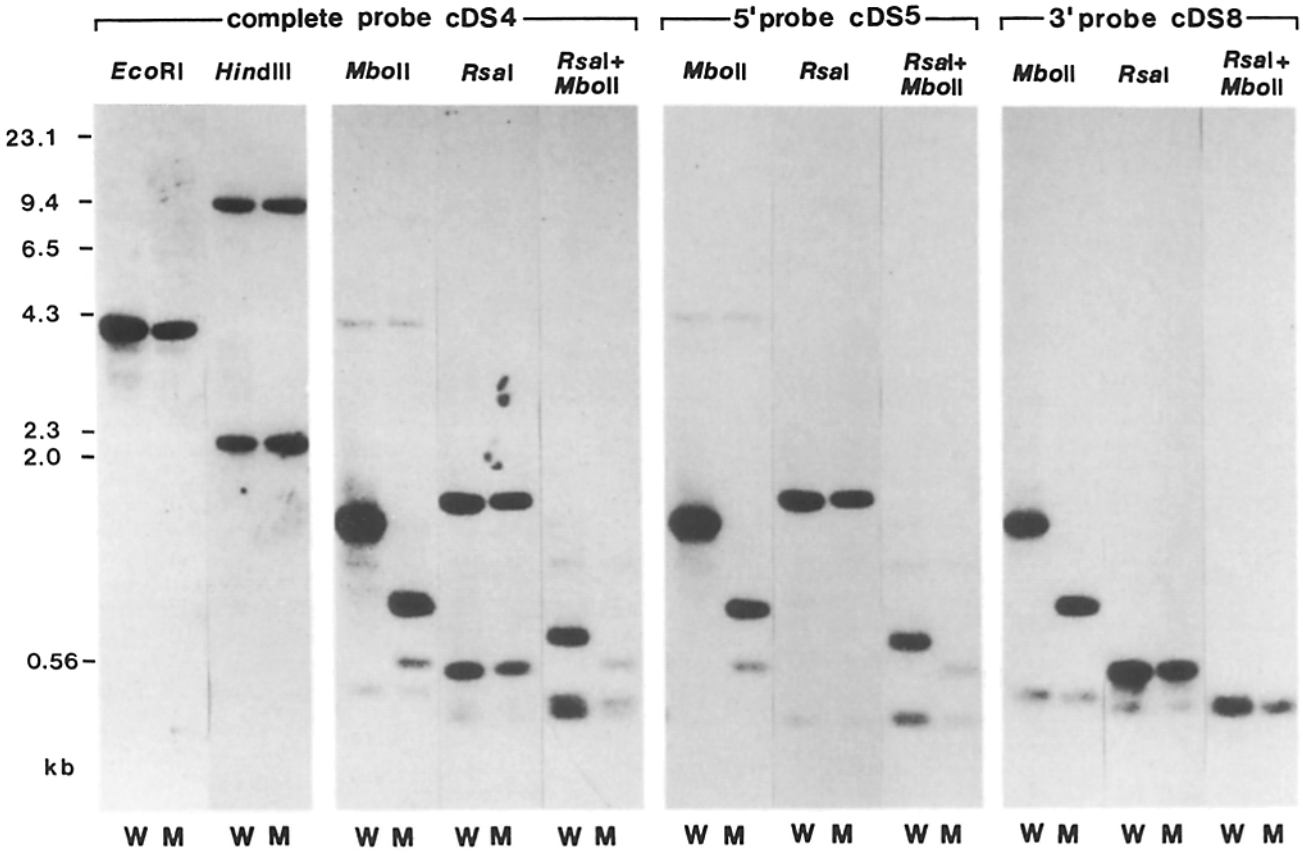

Figure 9. Southern blots of nuclear DNA from wild-type AX2 cells (*W*) or mutant HG1132 cells (*M*). The restriction enzymes and cDNA probes used are indicated on top. Alignment of the cDNA probes within the gene is shown in Fig. 10. The band at 4.3 kb that hybridizes weakly to cDS4 and cDS5 in the Mbo II digests seems to represent related sequences in the genome.

glycoprotein formed patches on the surface of HG1132 cells, and accumulated in a cap within 15 min at 21°C, similar to AX2.

Discussion

Severin is by far the most prominent protein that decreases the viscosity of F-actin in cell homogenates of *D. discoideum*. Its enrichment in tips of advancing pseudopods of actively moving growth-phase cells has suggested a function for severin in the control of cell motility (Brock and Pardee, 1988). Moreover, the sequence homologies between severin (André et al., 1988), gelsolin (Kwiatkowski et al., 1986), and villin (Bazari et al., 1988) suggest that essential regions of F-actin fragmenting proteins have been conserved during evolution, implying that these proteins serve functions important not only for *Dictyostelium* but also for vertebrate cells.

In the light of these results the finding of a severin-less mutant that grows and develops normally was unexpected. Our results indicate that the mutant HG1132 does not produce detectable amounts of normal severin nor does it synthesize an altered one that has preserved the F-actin fragmenting activity. The lack of severin is accompanied by reduced levels of severin-specific RNA in the mutant. This transcript is ~100 bases larger than the wild-type mRNA, suggesting that the mutant RNA is improperly spliced. Comparison of Southern blots of cDNA and genomic DNA revealed the presence of an intron in a severin-specific Rsa I fragment. Sizes of the corresponding fragments were 250 bp in the cDNA and

Table IV. Motility and Chemotactic Orientation of Wild-Type AX2 and Mutant HG1132 Cells

	Speed	Orientation in gradient direction	Turning rate
	$\mu m \times min^{-1}$		$rad^2 \times min^{-1}$
A. In buffer			
AX2	7.8	0.10	0.97
HG1132	8.3	0.03	1.10
B. In a cAMP gradient			
AX2	12.0	0.32	0.86
HG1132	12.1	0.36	0.83

Data are averages of two independent experiments in which cells starved for 6 h were deposited on a glass surface for recording of their motility behavior. (*A*) Speed of cell movement, turning rate, and orientation before the gradient was applied was recorded for 30 min. (*B*) 30 min after formation of the cAMP gradient recording was continued for another 30 min.

Steepness of the cAMP gradient was 2.5×10^{-8} M $\times$ mm^{-1}, the mean concentration in the area recorded was 2.5×10^{-8} M. The turning rate describes the rate at which the direction of movement of a cell changes as a rotational diffusion coefficient (Segall et al., 1987). Orientation is the fraction of the distance travelled per time-lapse interval that is in the direction of the gradient. The approximate number of cells from which data were collected was 40–100 per experiment.

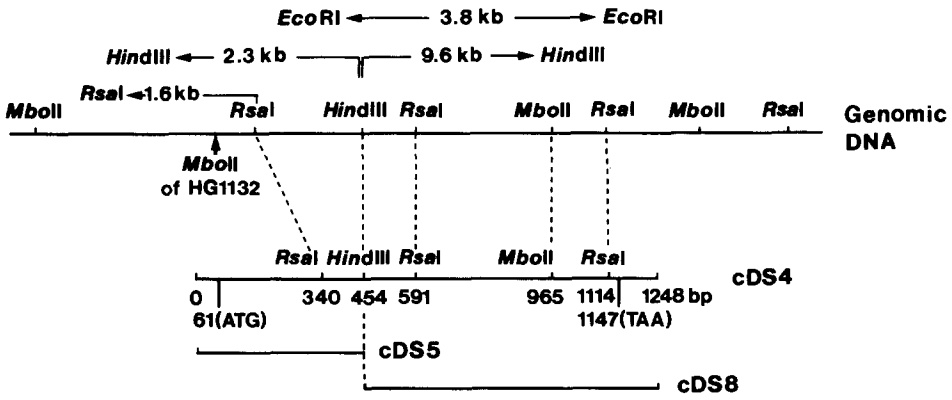

Figure 10. Restriction sites and location of an intron in the severin gene of wild-type AX2 and mutant HG1132 (*top line*), and regions covered by various cDNA clones (*below*). Sizes of the genomic fragments were estimated using Hind III-generated fragments of DNA as size markers and adjusted to the lengths of the cDNA-fragments as calculated from the sequence (André et al., 1988).

~430 bp in the genomic wild-type or mutant DNA (Fig. 10). The size of the intron, ~180 bp, agrees well with the sizes of introns in other *Dictyostelium* genes (Pears et al., 1985). Analysis of restriction enzyme digests of AX2 and HG1132 DNA with severin-specific cDNA sequences indicated the presence of an additional Mbo II site in the coding sequence of HG1132. This additional Mbo II site is located ~200 bp downstream of the start codon and close to the 5′ end of the Rsa I fragment that carries the intron. However, the Mbo II site is not located within this fragment and thus not immediately situated at this particular splice site. The evidence suggesting improper splicing and the creation of a new Mbo II site both indicate a change in the 5′ coding region of the severin gene. This change probably leads to an untranslatable transcript. Splicing defects that are associated with the lack of a protein have been described for albumin (Ruffner and Dugaiczyk, 1988) and the cytochrome P450dbl (Gonzalez et al., 1988).

Even at 27°C, the uppermost temperature at which *D. discoideum* can develop, no difference in growth rate of the mutant as compared to wild-type was observed (Table III). These data and the results suggesting that severin is unneces-

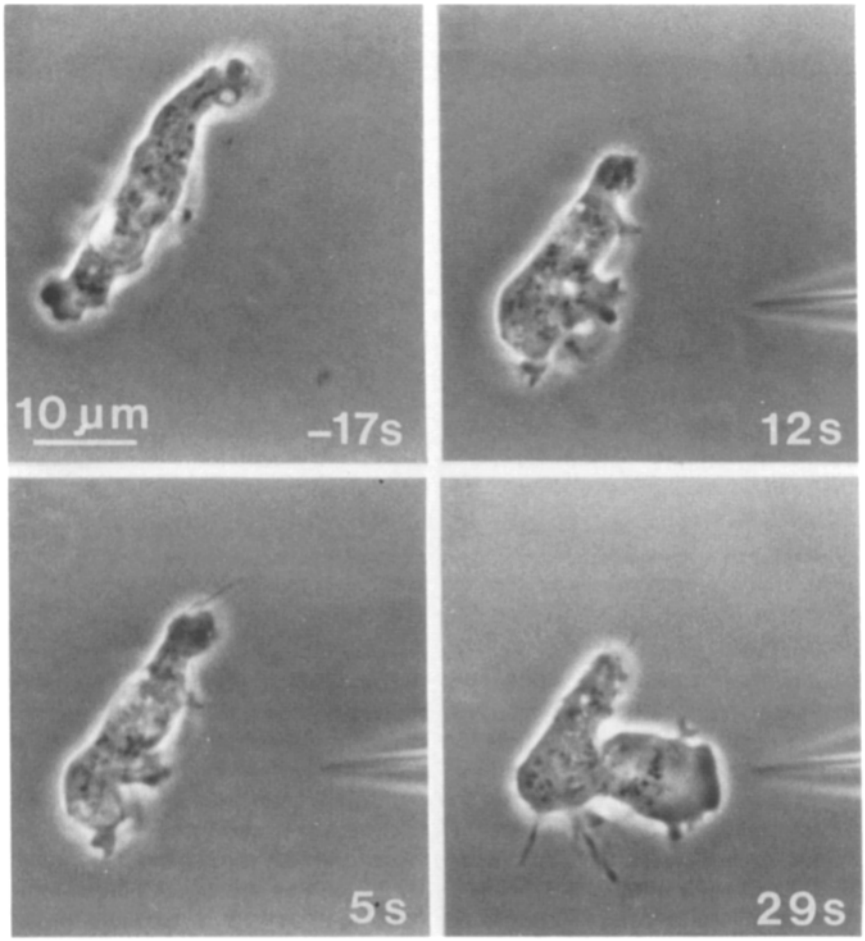

Figure 11. Chemotaxis of a single HG1132 cell. Before stimulation (*−17s*) the front of the elongated, aggregation competent cell pointed to the lower left. At zero time the cell was stimulated by a micropipette filled with 1×10^{-4} M cAMP. Within 5 s the cell contracted and extended multiple pseudopods into the direction of the diffusion gradient. After 12 s the cell was further shortened. One of the competing pseudopods gave rise to a new front which after 29 s was fully established.

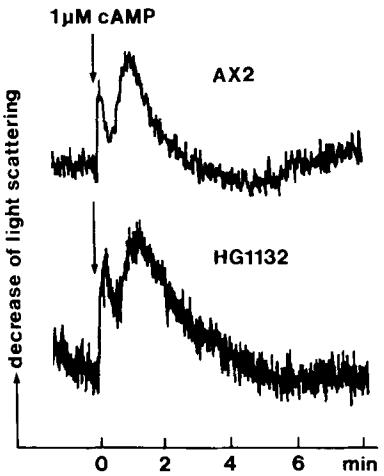

Figure 12. Light scattering, changes of wild-type (*top*) and mutant (*bottom*) cells in response to cAMP. Cells were starved for 6 h and stimulated in suspensions of 5×10^7 cells/ml.

sary for cell motility and chemotactic orientation should be taken together with biochemical results that are hard to reconcile with a reversible effect of severin on actin polymerization. Severin forms a stable complex with G-actin, thus preventing its polymerization (Giffard et al., 1984) and effectively counteracts the cross-linking activity of other actin binding proteins (Fig. 7 *C*). Thus the network of microfilaments shown to exist in *D. discoideum* cells (Claviez et al., 1986) would be destroyed if severin activity were unrestricted in the intact cells. It must be concluded therefore that the severin has, under normal circumstances, little if any F-actin severing activity within the cells.

When cryosections of fixed, aggregation competent cells were labeled for immunofluorescence, the severin appeared to be quite uniformly distributed throughout the cytoplasm of wild-type cells (Fig. 5). Rhodamine-phalloidin labeled F-actin was concentrated at the periphery of the cells (Fig. 5 *C*). These results are similar to those of Brock and Pardee (1988), who found uniform distribution of severin when they labeled whole cells in the aggregation stage that had been fixed and permeabilized with methanol. The different distributions of severin and F-actin in sections from wild-type cells suggest that a major portion of the severin is not bound to filamentous actin. The F-actin of HG1132 cells was concentrated in a cortical layer as in wild-type cells (Fig. 5 *F*). This means it is not severin that prevents actin from polymerizing uniformly throughout the cell.

Taken together, the data suggest that severin serves a function that is not required for normal growth, cell shape and movement, and for development of *D. discoideum* under the laboratory conditions used. We can only guess what this function in a natural environment might be. One possibility is that severin is only activated under adverse conditions, e.g., upon exposure of the cells to heat or to an extreme pH regime, and destroys conglomerates of actin filaments that under these circumstances may accumulate within the cells. During normal movement the polymerization of actin may be regulated by F-actin capping proteins rather than by severin. Mutant HG1132 will help to specify the role that these capping proteins play within the cells.

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