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 Ca²⁺ into actin filaments, and remains bound to the barbed end of the fragments even when free Ca²⁺ 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 Ca²⁺ concentration, suggesting that the association of actin with the cortex is inhibited by severin or other Ca²⁺-dependent proteins (Giffard et al., 1983). Mutual competition of severin with other actin binding proteins is suggested by the inhibitory effect of myosin SI-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.

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, Aluigel 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 460, and 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 125I 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 radioimmununcompetition 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 125I 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 × 106 colonies with anti-severin mAb 65 one unlabelled colony was detected which gave rise to the mutant strain HG1132. The mutation responsible for the severin defect, *sevA1000*, was mapped using parasexual genetics. Haploid diploids were constructed from the mutant HG1132 and the tester strain HU1628 in the presence of CaCl2 (Williams and Newell, 1976) and isolated by ttg/ccob 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., 1985b). 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 0.1% 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., 1985a).

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 pMMS5, 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 identi
cal 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 at two directions at daily intervals. Chemotactic stimulation with a micropipette was performed according to Gerisch et al. (1975a,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, these were isolated for 15 min on ice in 17 mM Sorenson's phosphate buffer, pH 6.0, with 250 µg IgG per milliliter of mAb 417-21 directed against the protein moiety of the contact site A glycoprotein (Bertholdt et al., 1985), washed and incubated under the same conditions with fluorescence-
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 |
|---------------|------------------------|------------------------------|
| I             | +                      | 12                           |
| II            | +                      | 12                           |
| III           | +                      | 12                           |
| IV            | +                      | 12                           |
| V              | +                      | 12                           |
| VI            | +                      | 12                           |
| VII           | +                      | 12                           |

DGI04 is derived from the haploid mutant HG1132 carrying the sevAl000 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.

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**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 125I-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.

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**Figure 2.** 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 125I-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.

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**Figure 3.** 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 125I-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.

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**Figure 4.** 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 125I-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.

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**Table I.** Linkage Analysis of the sevAl000 Locus Using 94 Haploid Segregants of the Diploid Strain DG104

| Linkage group | Genotype of segregants | No. of independent segregants |
|---------------|------------------------|------------------------------|
| I             | +                      | 12                           |
| II            | +                      | 12                           |
| III           | +                      | 12                           |
| IV            | +                      | 12                           |
| V              | +                      | 12                           |
| VI            | +                      | 12                           |
| VII           | +                      | 12                           |

DGI04 is derived from the haploid mutant HG1132 carrying the sevAl000 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.

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**Figure 5.** 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 125I-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.

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

| Competing mAb | 125I-labeled mAb |
|---------------|------------------|
| 200           | 4                |
| 460           | 2                |

125I-labeled antibodies were incubated with or without competing, unlabeled antibody in severin-coated microtiter wells. Data represent radioactivity 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 $\alpha$-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. $\alpha$-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 Ca²⁺ (● and ▲) or 1.0 mM EGTA (○ and △).
detected. This result indicates that the severin sequence 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, Chemoreponses 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 chemoreponses 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

| 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.
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 260 bp in the genomic DNA.

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

|                | Speed (μm × min⁻¹) | Orientation in gradient direction | Turning rate (rad × 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 × 10⁻⁴ M × mm⁻¹, the mean concentration in the area recorded was 2.5 × 10⁻⁵ 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.
\( \sim 430 \text{ bp} \) in the genomic wild-type or mutant DNA (Fig. 10). The size of the intron, \( \sim 180 \text{ bp} \), 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 \( \sim 200 \text{ bp} \) 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-

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**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).

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**Figure II.** Chemotaxis of a single HG1132 cell. Before stimulation \((-17\text{s})\) 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 \( 1 \times 10^{-4} \text{ M cAMP} \). 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.

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