Mechanical Perturbation Elicits a Phenotypic Difference between *Dictyostelium* Wild-Type Cells and Cytoskeletal Mutants

Ludwig Eichinger,* Bernd Köppel,‡ Angelika A. Noegel,‡ Michael Schleicher,* Manfred Schliwa,* Kees Weijer,§ Walter Witke,¶ and Paul A. Janmey¶

*Institut für Zellbiologie, Ludwig-Maximilians-Universität München, 80336 München, Germany; ‡Max-Planck-Institut für Biochemie, 82152 Martinsried, Germany; §Zoologisches Institut, Ludwig-Maximilians-Universität München, 80333 München, Germany; and ¶Experimental Medicine Division, Brigham and Women's Hospital, Program in Biological and Biomedical Sciences, Harvard Medical School, Boston, Massachusetts 02115 USA

ABSTRACT To determine the specific contribution of cytoskeletal proteins to cellular viscoelasticity we performed rheological experiments with *Dictyostelium discoideum* wild-type cells (AX2) and mutant cells altered by homologous recombination to lack α-actinin (AHR), the ABP120 gelation factor (GHR), or both of these F-actin cross-linking proteins (AGHR). Oscillatory and steady flow measurements of *Dictyostelium* wild-type cells in a torsion pendulum showed that there is a large elastic component to the viscoelasticity of the cell pellet. Quantitative rheological measurements were performed with an electronic plate-and-cone rheometer, which allowed determination of G', the storage shear modulus, and G'', the viscous loss modulus, as a function of time, frequency, and strain, respectively. Whole cell viscoelasticity depends strongly on all three parameters, and comparison of wild-type and mutant strains under identical conditions generally produced significant differences. Especially stress relaxation experiments consistently revealed a clear difference between cells that lacked α-actinin as compared with wild-type cells or transformants without ABP120 gelation factor, indicating that α-actinin plays an important role in cell elasticity. Direct observation of cells undergoing shear deformation was done by incorporating a small number of AX2 cells expressing the green fluorescent protein of *Aequorea victoria* and visualizing the strained cell pellet by fluorescence and phase contrast microscopy. These observations confirmed that the shear strain imposed by the rheometer does not injure the cells and that the viscoelastic response of the cell pellet is due to deformation of individual cells.

INTRODUCTION

Cell locomotion requires a force-generating system, reversible formation of adhesion between cell and substratum, and structural components that mediate force transmission from the cell interior to the cell surface. The cellular structure responsible for force transmission is generally thought to be the cytoskeleton, a viscoelastic network of filaments lining the cell membrane and extending throughout the cytoplasmic space. A major component of the cytoskeleton are actin filaments, the length and attachment of which to each other or to the cell surface are governed by a number of actin-binding proteins. They respond, directly or indirectly, to signals generated at surface receptors when cells are stimulated to move or to change shape (reviewed by Schleicher and Noegel, 1992; Condeelis, 1993; Stossel, 1993). Several filament-binding proteins have large effects on the viscoelasticity of actin gels in vitro (Sato et al., 1987; Janmey et al., 1990; Wachsstock et al., 1993), but evidence that these proteins control the viscoelasticity of the cytoskeleton in vivo has been shown in only very few cases (Cunningham et al., 1992; Pasternak et al., 1995).

Dictyostelium is an excellent system to evaluate the role of actin-binding proteins in cell function, as a number of mutant strains exist in which expression of one or more actin-binding proteins has been suppressed by chemical mutagenesis or homologous recombination (Schleicher et al., 1995). Analysis of mutant *Dictyostelium* cells lacking α-actinin, ABP120 gelation factor, or severin led to the result that these cells exhibited only subtle changes with respect to growth, motility, chemotaxis, and development (Wallraff et al., 1986; Noegel and Witke, 1988; Schleicher et al., 1988; André et al., 1989; Brink et al., 1990; Cox et al., 1992). These findings were completely unexpected, as these proteins remained highly conserved during evolution and constitute major cellular actin-binding activities. The emerging hypothesis that these proteins at least under laboratory conditions form a functional network and are multiply guaranteed (Bray and Vasiliev, 1989) was strongly supported by experimental data from *Dictyostelium* transformants that lacked more than one actin-binding protein (Witke et al., 1992; Haugwitz et al., 1994). After deleting both proteins, α-actinin and the ABP120 gelation factor, the double-mutant cells showed clear differences in motility and development that could be rescued by re-introducing either one of the two F-actin-binding proteins (Witke et al., 1992; Noegel et al., 1995). This result, however, did not exclude the possibility that there were already subtle changes in the viscoelastic properties of the cytoskeleton in single mutants that might have escaped detection by the particular techniques applied.

Here we describe how macroscopic samples of whole cells can be assayed with rheological methods and show that significant differences between wild-type cells and cytoskeletal mutants can be detected by this approach. Our

Received for publication 3 July 1995 and in final form 4 November 1995.

Address reprint requests to Dr. Michael Schleicher, Institut für Zellbiologie, Ludwig-Maximilians-Universität München, 80336 München, Germany. Tel.: 49-89-5996-876; Fax: 49-89-5996-882; E-mail: schleicher@bio.med.uni-muenchen.de.

© 1996 by the Biophysical Society

0006-3495/96/02/1054/07 $2.00

studies on the rheological behavior of *Dictyostelium* transformants that lack α-actinin, the ABP120 gelation factor, or both cross-linking proteins suggest that α-actinin has a larger impact on the elastic properties of the cytoplasm.

MATERIALS AND METHODS

Growth of *Dictyostelium* cells

D. discoideum strains were cultivated axenically in liquid nutrient medium essentially as described (Claviez et al., 1982). For rheological measurements, cells were grown to a density of approximately 5×10^6 cells/ml, packed by low speed centrifugation ($180 \times g$ for 5 min), and washed twice in Soerensen phosphate buffer, pH 6.0.

Generation of mutant *Dictyostelium*

Three mutant *D. discoideum* strains deficient in α-actinin or ABP120 gelation factor or both proteins were generated by homologous recombination. The constructs used and the mutants generated will be described in detail elsewhere. Briefly, AX2–214 cells were transformed with the vector pDabgel 1.5 (Witke et al., 1992) to generate GHR (gelation factor homologous recombination), the ABP120 gelation factor minus mutant, or with pΔAA to replace the endogenous α-actinin gene. The vector pΔAA was constructed from pIC20H (Marsh et al., 1984) and contained the 0.8-kb 5′- and the 0.3-kb 3′-coding sequences of α-actinin interrupted with the gene for neomycin (G418) resistance. Before transformation, pΔAA was cut with *PstI*/*PvuII* to liberate the resistance gene flanked with the α-actinin coding sequences and briefly digested with T4 DNA polymerase in the presence of dGTP (Manstein et al., 1989) to avoid re-ligation of the vector. The resulting gene replacement mutant AHR (α-actinin homologous recombination) was transformed with pΔGF for disruption of the ABP120 gelation factor gene. pΔGF was constructed from pUC19 (Yanisch-Perron et al., 1985) and contained the 0.1-kb 5′-noncoding plus the 0.6-kb 5′-coding and 0.7-kb 3′-coding sequences from the ABP120 gelation factor gene, which were separated by the gene for phleomycin resistance (Leiting and Noegel, 1991). Before transformation, pΔGF was linearized with *BglII* and dephosphorylated with alkaline phosphatase. The resulting double mutant for α-actinin and the ABP120 gelation factor was named AGHR. The generation of the A3 mutant (α-actinin minus, T-abα 3) is described elsewhere (Witke et al., 1987).

Transformations were carried out essentially as described by Witke et al. (1987) and Haugwitz et al. (1994). In the case of pΔGF the selection medium was phleomycin-containing HL5 medium. The transformants were cloned on *Klebsiella aerogenes* by spreader dilutions, and the colonies were tested for expression of the ABP120 gelation factor or α-actinin with specific monoclonal antibodies (Schleicher et al., 1988; Brink et al., 1990).

D. discoideum AX2 transformants expressing the green fluorescent protein (AXGFP) of *Aequoria victoria* (Chalfie et al., 1994) were obtained by transforming AX2 cells with a vector (PB15GFP) driving the expression of the green fluorescent protein under the control of the *D. discoideum* actin 15 promotor. Cells were transformed by electroporation and selected for 4 days in the presence of 20 μg/ml G418. Clones were obtained by cloning the transformants on *Micrococcus luteus* bacteria on SB agar plates containing 15 μg/ml G418 (Wilczynska and Fisher, 1994). Brightly fluorescent clones were selected by visual inspection of the colonies under an epifluorescence microscope (using a Leitz H-Filterblock) with a low power (4×) objective.

Video microscopy with fluorescently labeled *D. discoideum* cells

To observe the behavior of individual cells in a torsion pendulum, a modified pendulum was designed and mounted onto a Zeiss IM-35 inverted microscope. As in the standard pendulum rheometer, the pendulum is attached to a thin filament suspended from an X, Y, Z positioner, which in turn is mounted onto the microscope stage. At the end of one of the torsion arms (length 5 cm) a 22-mm round coverslip was glued. This coverslip is positioned in front of the microscope objective and serves as the upper plate of the sample holder, the lower plate being a glass slide. The enclosed volume (packed cells) was approximately 400 μl. As in the standard torsion pendulum, the torsion arm is given a momentary displacement, and the resulting oscillations of the sample are observed in the light microscope and recorded. The actual dimensions of this microscope stage pendulum are not critical because it was used for simple qualitative observations only.

Rheology

The shear compliance $J(t)$ and the storage shear modulus $G'(\omega)$ were measured from creep experiments or free oscillations, respectively, using a torsion pendulum. Details of the instrument design and principle of operation are described elsewhere (Janmey, 1991). Briefly, in creep experiments, a constant shear stress is applied to the sample and its resulting deformation (strain) is measured as a function of time during application of stress and after its removal (creep recovery). The storage shear modulus G' is determined from the frequency of oscillations caused by a momentary impulse to the pendulum arm. This measurement is possible only if the material has a greater storage than loss modulus or, in other words, is possible only if the system is not overdamped. Stress relaxation measurements and forced oscillatory measurements of G' in which the strain and frequency can be independently varied were done with a Rheometrics RFS-II instrument using cone-and-plate geometry with titanium surfaces. The cone had a diameter of 50 mm and an angle of 0.02 radian (rad). Packed cells were gently placed onto the plate of the rheometer using a soft plastic pipette with a tip diameter of several millimeters. The plates of the rheometer (either glass disks in the case of the pendulum or titanium and aluminium in the RFS-II) were lowered slowly onto the cells. The volume between plate and cone was 652 μl and cell density was between 7×10^8 and 1×10^9 cells/ml. Similar sample volumes (400 μl) and cell numbers were used in the torsion pendulum.

For measurements in the RFS-II, the first measurement was started 4 min after the adjustment of the plates. The second measurement was started 14 min later. Measurements were done in two groups. Group 1 consisted of measurements of G' as a function of time or frequency. Time-dependent measurements were done for 300 s with one time point every 30 s at 1 rad/s and 1% strain. Frequency-dependent measurements were done at 1% maximal strain amplitude from 100 to 0.04 rad/s with five measurement points taken each decade. Group 2 consisted of a stress relaxation and a strain sweep experiment. Stress relaxation measurements were done at 2% strain, and 400 data points were collected at each of the following times: 5, 10, 30, and 150 s. Strain sweep, the second measurement, was done at 1 rad/s and from 0.2 to 10% strain with an increment of 0.2%. All measurements were done at room temperature. After the measurements, cell density was determined with a Thoma hemocytometer. For vital staining with trypan blue, an aliquot of the cells was incubated for 4 min in a 0.04% trypan blue solution (final concentration). The cells were then placed into a Thoma hemocytometer, and blue cells were counted under the microscope.

RESULTS

Dictyostelium cells are elastic

When a sedimented pellet of *Dictyostelium* cells was placed between the glass plates of a torsion pendulum and the pendulum arm received an angular displacement, a series of damped oscillations of the pendulum arm was observed, as shown in Fig. 1 *A*. The observation of oscillation rather than a single transient displacement implies that there was a large

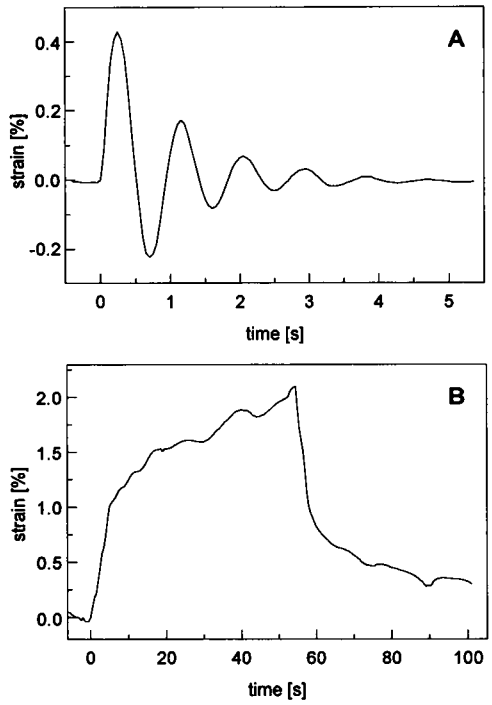

FIGURE 1 *Dictyostelium* AX2 cells are viscoelastic. (*A*) Free oscillations of sedimented AX2 cells. The deformation (strain) of a pellet of cells held in a torsion pendulum is shown after application of a momentary impulse to the pendulum arm at $t = 0$ s. The frequency and damping of the oscillating displacement are a measure of the shear moduli of the cell sample. (*B*) Creep of AX2 cells at constant stress and recovery after the deforming force is removed. The time course of deformation (shear strain) is shown after imposition of 0.075 Pa stress at $t = 0$ s followed by recovery after removal of stress at $t = 55$ s.

elastic component to the viscoelasticity of the cell pellet. The storage shear modulus, a measure of this elasticity, at 1 Hz calculated from these oscillations was 15 Pa. Control measurements of mechanically disrupted cells and of the growth medium alone produced only the simple, transient deformation characteristic of viscous liquids (data not shown). After the oscillations decayed, the sample rapidly returned to its undeformed state, suggesting that no irreversible change in the structure or arrangement of the closely packed cells occurred, and subsequent measurements of the storage shear modulus from free oscillations gave nearly identical results.

An independent measure of the viscoelasticity of the cells was derived from the slow deformation that occurred when a constant shear stress was applied to the cell pellet. Fig. 1 *B* shows the resulting shear strain (creep) after application of a shear stress and the recovery of strain after the stress was removed. A time-dependent shear compliance, $J(t)$, was determined from the ratio of strain to stress and gave a value of 0.2 Pa^{-1} at $t = 10$ s, roughly equal to the inverse of G' calculated from the data of Fig. 1 *A*. As expected from the elasticity of the sample, much of the strain recovered when the stress was released. The unrecovered strain is a measure of the amount of irreversible change in cell structure or of slippage of cells past each other that occurred at these stresses and times, both of which were much larger than those used for determining sample oscillations.

Integrity of samples during oscillatory measurements

Relating the viscoelasticity of whole cell pellets to the rheological properties of single cells requires that the resistance to motion of the macroscopic sample is derived mainly from deformation of individual cells and not from sliding of cells past each other. To determine the behavior of individual cells in the cell pellet during oscillations, the position of a small number of AX2 cells expressing the green fluorescent protein of *Aequorea victoria* incorporated in trace amounts within the sample of wild-type *Dictyostelium* cells was observed by low light level video microscopy. The distribution and orientation of the fluorescent cells were nearly identical before and after applying a small oscillatory deformation corresponding to a strain of approximately 0.01 (Fig. 2). Consistent with the oscillatory deformation of the macroscopic sample, each of the labeled cells shown in Fig. 2 *A* underwent identical oscillatory deformations with an initial amplitude of more than 300 μm and returned to their original positions after the oscillations decayed. Representative traces of the movements of four cells are shown in Fig. 2 *B* in which individual video frames are purposely displaced to show the damped oscillatory motion of the individual cell as a function of time. From the distance of the focal plane from the fixed glass surface and the lateral dimension of the displacement, these deformations corresponded to a strain of several percent. When larger stresses were applied for longer times, the cells did not return to their original positions (data not shown), consistent with the creep experiments of Fig. 1 *B* showing a

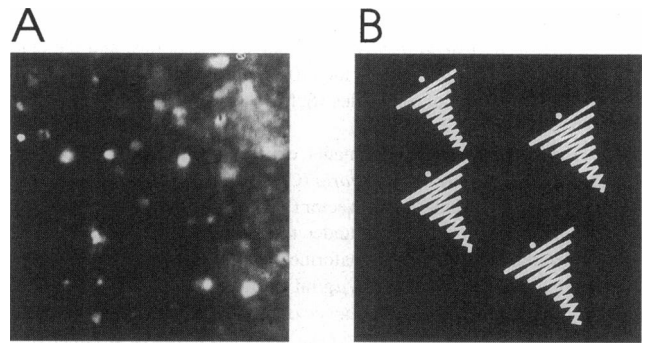

FIGURE 2 Visualization of single fluorescent cells within a sample of AX2 cells undergoing shear deformation. (*A*) Representative picture of fluorescent *Dictyostelium* cells (bright points) among AX2 cells in a torsion pendulum. (*B*) Schematic depiction of the positions of fluorescent *Dictyostelium* cells during oscillation. Individual video frames are displaced and superimposed to give an impression of their movement as the cell pellet freely oscillates in a measurement similar to that shown in Fig. 1 *A*. The location of four cells before and after the oscillations shows that the cells did not significantly change their position.

significant amount of unrecovered strain. To keep cell-cell adhesion at a minimum, only *Dictyostelium* cells from growing cultures were used.

Viscoelasticity of cell pellets depends on the frequency, strain, and time of the measurement

Preliminary experiments using the torsion pendulum and mutant cells lacking either α-actinin, ABP120 gelation factor, or both suggested that these cell lines, especially those lacking α-actinin, had lower storage shear moduli and larger compliances than wild-type cells. However, reproducibility of such measurements is hampered by the strong dependence of cell viscoelasticity on the frequency and strain of the measurements (Fig. 3). Neither frequency nor strain can be accurately controlled or varied with the torsion pendulum. Therefore, comparisons of cell types were done with a Rheometrics RFS-II instrument that imposes on the sample forced oscillations of determined strain and frequency, or rapid step deformations of known strain, from which stress relaxation can be determined. Accurate measurements of the rheology of cell pellets also depended on the length of time that the cells were kept in the rheometer. Experiments such as those of Fig. 3 were qualitatively but not quantitatively similar when they were repeated after the approximately 15 min required to do the measurement.

Changes in cell rheology over time are shown in Fig. 4. Immediately after placing the cells in the rheometer, the storage shear modulus of wild-type *Dictyostelium* cells was approximately 200 Pa but began to decrease rapidly. These first measurements are especially interesting because the time-dependent decrease of G' during the experiment could be a consequence of the mechanical stress or of oxygen deprivation of the cells. For initial time points, the storage shear modulus of wild-type cells was significantly higher

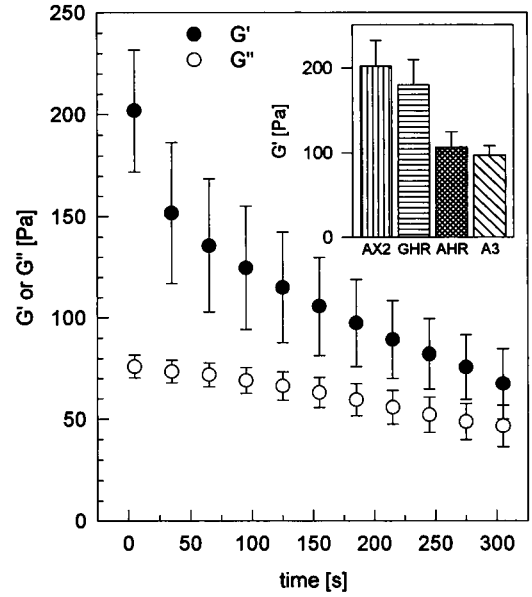

FIGURE 4 Time dependence of the storage shear modulus G' and the viscous loss modulus G'' of AX2 wild-type cells. Packed cells were taken from the centrifuge tube and introduced into the rheometer. The measurements with a frequency of 1 rad/s and 1% strain started 4 min later and continued at 30-s intervals for 5 min. The mean values and standard errors of seven experiments are shown. The inset shows G' of AX2, GHR, AHR, and A3 measured at the first time point. Mean values and standard errors of seven (AX2 and GHR), five (AHR), and four (A3) experiments are shown.

than that of GHR and especially AHR as well as A3 (Witke et al., 1987), a different α-actinin minus mutant that was obtained by gene disruption (Fig. 4, inset). Mutant cells appeared to be less affected by incubation within the rheometer; therefore, the difference between wild-type and mu-

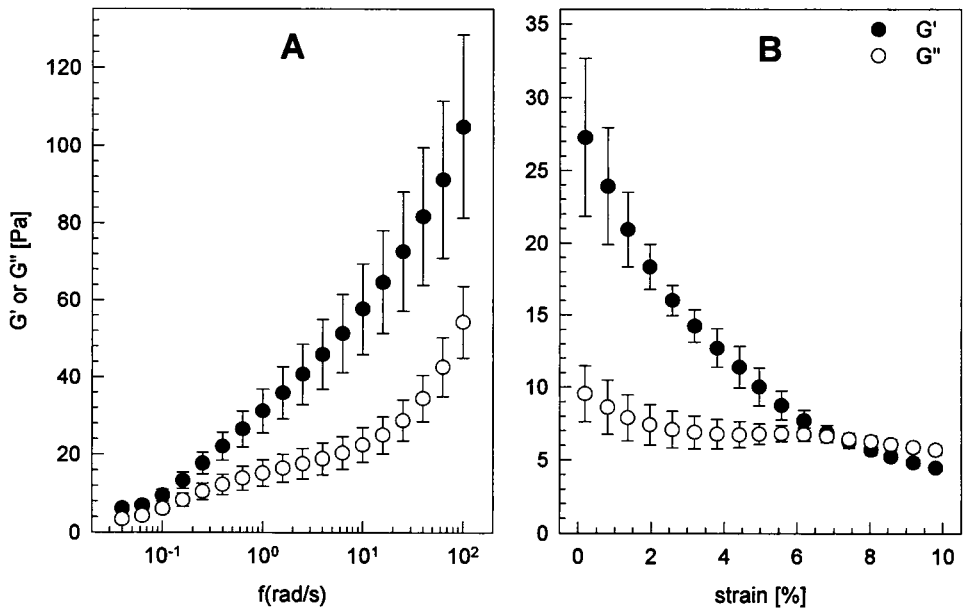

FIGURE 3 Viscoelasticity of AX2 wild-type cells as functions of frequency (*A*) and strain (*B*). (*A*) The storage shear modulus G' and the viscous loss modulus G''were measured at 1% strain over a range of oscillation frequencies. (*B*) G' and G'' were measured at a frequency of 1 rad/s over a range of strain amplitudes. For clarity, only every third value is shown. In *A*, mean values and standard errors were derived from seven separate cell samples, and in *B*, they represent three separate experiments.

tant cells seen at the beginning of the measurement was obscured at later time points.

To assess whether cells were mechanically ruptured during the measurements, an aliquot of the cell pellet was taken before and after the measurements and stained with trypan blue. Only a very small fraction (less than 1%) of the cells was stained and there was no difference in the number of stained cells before and after the measurements (data not shown). The morphology of the cells, however, had changed during the experiment. After the measurements, the cells had rounded up, which could have been a consequence of mechanical stress, oxygen deprivation, or both.

Mutant *Dictyostelium cells* showed less resistance in stress relaxation experiments

A third independent measurement of the rheology of wild-type *Dictyostelium* cells is shown in Fig. 5 where the time course of stress after a rapid deformation of the sample is shown for wild-type and mutant cells. Cells lacking either the ABP120 gelation factor, α-actinin, or both proteins developed less resistance to the imposed strain. The largest difference was between wild-type cells and those lacking α-actinin, which had relaxation moduli nearly 50% lower than wild-type cells, indicating that α-actinin plays an important role in cell elasticity. These data also show the reproducibility of measurements, which renders the Rheometrics instrument as superior over the torsion pendulum largely because the strain can be accurately controlled. In all cases, the stress decayed nearly to zero within several minutes, suggesting that the cells remained fluid for long times due either to passive viscous flow or active remodeling of their cytoskeletons.

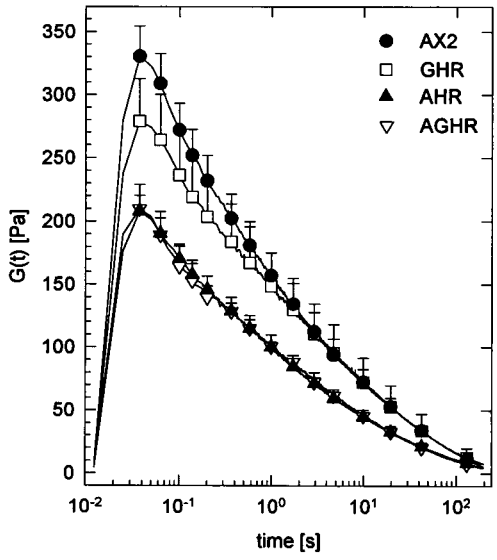

FIGURE 5 Stress relaxation of AX2, GHR, AHR, and AGHR cells. The relaxation modulus ($G(t)$, the ratio of stress (t) to strain) of wild-type cells and cytoskeletal mutants is shown following the rapid imposition of 2% strain. Mean values and standard errors are derived from six (AX2), five (GHR), eight (AHR), and three (AGHR) experiments. In each experiment the values of $G(t)$ were stored at 400 evenly spaced intervals during each of the four following sequential time zones: 5, 10, 30, and 150 s. For the calculation of standard errors (symbols with error bars in the figure) a regular subset of these values was selected.

DISCUSSION

This report describes for the first time rheological studies on the viscoelastic properties of whole cells, the actin cytoskeletons of which had been altered by directed gene disruption of the F-actin cross-linking proteins α-actinin and ABP120 gelation factor. By using green fluorescent marker cells it could be shown that the applied forces in the rheometer did not slide the cells against each other in the cell mass and that the values obtained reflect the deformation of individual cells. These comparisons of wild-type cells with cytoskeletal mutants led to the following conclusions:

1) Living cells can be used in a torsion pendulum and in a plate-and-cone rheometer for measuring the strength of the cytoskeleton and the impact of distinct cytoskeletal proteins on the viscoelastic properties of the cytoplasm.

2) Considering the difficulties to find clearly aberrant phenotypes in *Dictyostelium* mutants without certain actin-binding proteins, the rheometric assays used in this study offer an excellent additional tool for measuring differences in the cytoskeleton.

3) α-Actinin turned out to be more important for the viscoelastic properties of a cell than the second major F-actin cross-linking protein ABP120 gelation factor.

The general approach of performing rheological measurements of macroscopic cell samples to ascertain the viscoelasticity of individual cells may be applicable to other systems and has some potential advantages over single-cell measurements. One advantage of macroscopic measurements is the relative simplicity of the method and the fact that it averages over the entire cell volume and over many cells. Therefore, cell-to-cell variability and differences between different regions of the same cell are avoided. This method therefore provides a representative measurement of a whole cell population but is insensitive to heterogeneity within or between cells and therefore can complement but not duplicate rheological measurements made by other methods. There are very elegant ways to measure viscoelastic properties on single cells by poking (Petersen et al., 1982; Elson, 1988), by aspiration into micropipettes (Evans and Yeung, 1989), by passage through pores (Hochmuth and Needham, 1990), by osmotic measurements (Menkel and Jockusch, 1991), by optical trapping in the form of a cell optical displacement assay (Grabski et al., 1994), or by using magnetic beads (Wang et al., 1993). Very often, however, studies on single cells are impossible because the forces to be measured are too small, and rigid attachment that itself perturbs the cytoskeleton is required. Furthermore, with the macroscopic method one obtains absolute values of viscoelastic parameters that cannot be determined for single cells because of their complicated geometry (Za-

halak et al., 1990, 1993). A quantitative interpretation of macroscopic cell samples is not without its own complication and is likely to produce an underestimate of the cell elasticity. To relate the macroscopic viscoelasticity to that of an individual cytoskeleton, it must be assumed that cells are not freely sliding past each other during the measurement and that the elastic response is due to deformation of individual cell interiors rather than to stretching of the planar interface between cells. The direct observation of individual cells within a dense sample by fluorescence microscopy and the fact that deletion of a protein localized to the three-dimensional cytoskeleton strongly affects the rheology of the cell pellet suggest that these assumptions are appropriate.

Genetic ablation of several cytoskeletal proteins has often led to surprisingly small changes in phenotype. *Dictyostelium* cells devoid of actin-binding proteins such as α-actinin (Wallraff et al., 1986, Witke et al., 1987, Schleicher et al., 1988), ABP120 gelation factor (Brink et al., 1990, Cox et al., 1992), severin (André et al., 1989), or single profilin isoforms (Haugwitz et al., 1994) are able to grow, move, and divide in a manner often indistinguishable from that of wild-type cells. One possible explanation for the negligible effects was functional redundancy by a number of proteins, at least under the near-optimal conditions at which cultured cells are usually studied. This principle is most clearly seen with double mutations that resulted in the concurrent lack of two F-actin cross-linking proteins (Witke et al., 1992) or both profilin isoforms (Haugwitz et al., 1994). A network of cytoskeletal proteins with overlapping or antagonistic functions apparently guarantees normal cellular behavior as long as a tolerable limit of viscous and elastic conditions in the cytoplasm is retained; the input of a distinct protein usually remains unknown.

The rheometric measurements, however, indicated that in this concert of functions in a living cell the influence on viscoelasticity by α-actinin is more pronounced than that of ABP120 gelation factor. The natural habitat of *Dictyostelium* amoebae is forest soil and leaf litter where the cells feed on bacteria, grow, and divide. To survive in the wild it is necessary for the cells to be capable of squeezing through tiny openings to prey on bacteria and to be sufficiently elastic to withstand sudden and strong impacts from outside. A deficiency in α-actinin causes significantly reduced elasticity in the cells due to which these mutant cells would be at a vital disadvantage in the wild.

Both α-actinin and ABP120 gelation factor share the same actin-binding motif; they are elongated molecules and exhibit in vitro F-actin cross-linking activity. The molecular organization, however, suggests a slight functional difference; the rod portion in α-actinin is rich in α-helix, which renders the molecule as rather rigid, whereas the corresponding regions in the ABP120 gelation factor consist mostly of cross-β conformation causing fairly high flexibility (Noegel et al., 1987, 1989). This difference is reflected in the geometry of filamentous networks formed by these proteins. α-Actinin has a tendency to connect two actin filaments in a ladder-like structure, and sometimes they even form filament bundles (Meyer and Aebi, 1990). On the other hand, the more flexible ABP120 gelation factor seems to be responsible for the formation of three-dimensional networks (Cox et al., 1995). Our data suggest that in *Dictyostelium* amoebae α-actinin is responsible for organizing the cytoskeleton against fast and strong impacts from outside, whereas the ABP120 gelation factor primarily helps to build a three-dimensional meshwork of filaments. This view is corroborated by independent experimental approaches from other groups. In in vitro studies on *Acanthamoeba* α-actinin, it could be shown that the on/off rate of α-actinin interaction with F-actin is very high and that movement of filaments at low frequency allows α-actinin continuously to readjust. Impacts at higher frequency, however, showed that momentarily bound α-actinin tremendously increased the elastic properties of the sample (Sato et al., 1987). On the other hand, the time scale of the redistribution of ABP120 gelation factor to newly formed pseudopods indicates a structural function in these rather slow moving compartments (Cox et al., 1995). It is consistent with these observations that in relatively fast relaxation experiments ABP120 gelation factor seems less important than α-actinin.

We thank Dr. Otto Müller, Dr. Josef Käs, Dr. Phil Allen, Harald Felgner, and Klaus-Peter Janssen for stimulating discussions and advice during the rheological experiments and Daniela Rieger for excellent assistance with cell culture.

The work was supported by grants from the Deutsche Forschungsgemeinschaft, National Institutes of Health (AR38910 to P. Janmey), and NATO (940277).

REFERENCES

André, E., M. Brink, G. Gerisch, G. Isenberg, A. Noegel, M. Schleicher, J. E. Segall, and E. Wallraff. 1989. A *Dictyostelium* mutant deficient in severin, an F-actin fragmenting protein, shows normal motility and chemotaxis. *J. Cell Biol.* 108:985–995.

Bray, D., and J. Vasiliev. 1989. Networks from mutants. *Nature.* 338: 203–204.

Brink, M., G. Gerisch, G. Isenberg, A. A. Noegel, J. E. Segall, E. Wallraff, and M. Schleicher. 1990. A *Dictyostelium* mutant lacking an F-actin cross-linking protein, the 120-kD gelation factor. *J. Cell Biol.* 111: 1477–1489.

Chalfie, M., Y. Tu, G. Euskirchen, W. W. Ward, and D. C. Prasher. 1994. Green fluorescent protein as a marker for gene expression. *Science.* 263:802–805.

Claviez, M., K. Pagh, H. Maruta, W. Baltes, P. Fisher, and G. Gerisch. 1982. Electron microscopic mapping of monoclonal antibodies on the tail region of *Dictyostelium* myosin. *EMBO J.* 1:1017–1022.

Condeelis, J. 1993. Life at the leading edge: the formation of cell protrusions. *Annu. Rev. Cell Biol.* 9:411–444.

Cox, D., J. Condeelis, D. Wessels, D. Soll, H. Kern, and D. A. Knecht. 1992. Targeted disruption of the ABP120 gene leads to cells with altered motility. *J. Cell Biol.* 116:943–955.

Cox, D., J. A. Ridsdale, J. Condeelis, and J. Hartwig. 1995. Genetic deletion of ABP-120 alters the three-dimensional organization of actin filaments in *Dictyostelium* pseudopods. *J. Cell Biol.* 128:819–835.

Cunningham, C. C., J. B. Gorlin, D. J. Kwiatkowski, J. H. Hartwig, P. A. Janmey, H. R. Byers, and T. P. Stossel. 1992. Actin-binding protein requirement for cortical stability and efficient locomotion. *Science.* 255:325–327.

Elson, E. 1988. Cellular mechanics as an indicator of cytoskeletal structure and function. *Annu. Rev. Biophys. Chem.* 17:397–430.

Evans, E., and A. Yeung. 1989. Apparent viscosity and cortical tension of blood granulocytes determined by micropipet aspiration. *Biophys. J.* 56:151–160.

Grabski, S., X. G. Xie, J. F. Holland, and M. Schindler. 1994. Lipids trigger changes in the elasticity of the cytoskeleton in plant cells: a cell optical displacement assay for live cell measurements. *J. Cell Biol.* 126:713–726.

Haugwitz, M., A. A. Noegel, J. Karakesisoglou, and M. Schleicher. 1994. *Dictyostelium* amoebae that lack G-actin-sequestering profilins show defects in F-actin content, cytokinesis, and development. *Cell.* 79: 303–314.

Hochmuth, R. M., and D. Needham. 1990. The viscosity of neutrophils and their transit times through small pores. *Biorheology.* 27:817–828.

Janmey, P. A. 1991. A torsion pendulum for measurement of the viscoelasticity of biopolymers and its application to actin networks. *J. Biochem. Biophys. Methods.* 22:41–53.

Janmey, P. A., S. Hvidt, J. Lamb, and T. P. Stossel. 1990. Resemblance of actin-binding protein/actin gels to covalently crosslinked networks. *Nature.* 345:89–92.

Leiting, B., and A. A. Noegel. 1991. The *ble* gene of *Streptoalloteichus hindustanus* as a new selectable marker for *Dictyostelium discoideum* confers resistance to phleomycin. *Biochem. Biophys. Res. Commun.* 180:1403–1407.

Manstein, D. J., M. A. Titus, A. de Lozanne, and J. A. Spudich. 1989. Gene replacement in *Dictyostelium*: generation of myosin null mutants. *EMBO J.* 8:923–932.

Marsh, J. L., M. Erfle, and E. J. Wykes. 1984. The pIC plasmid and phage vectors with versatile cloning sites for recombinant selection by insertional inactivation. *Gene.* 32:481–485.

Menkel, A., and H. Jockusch. 1991. Decreased osmotic stability of dystrophin-less muscle cells from the mdx mouse. *Nature.* 349:69–71.

Meyer, R. K., and U. Aebi. 1990. Bundling of actin filaments by α-actinin depends on its molecular length. *J. Cell Biol.* 110:2013–2024.

Noegel, A. A., B. Köppel, U. Gottwald, W. Witke, R. Albrecht, and M. Schleicher. 1995. Actin and actin-binding proteins in the motility of *Dictyostelium*. *In* The Cytoskeleton, 45: Colloquium-Mosbach 1994. Springer-Verlag, Berlin, 117–126.

Noegel, A. A., S. Rapp, F. Lottspeich, M. Schleicher, and M. Stewart. 1989. The *Dictyostelium* gelation factor shares a putative actin-binding site with α-actinins and dystrophin and also has a rod domain containing six 100-residue motifs that appear to have a cross-β conformation. *J. Cell Biol.* 109:607–618.

Noegel A. A., and W. Witke. 1988. Inactivation of the α-actinin gene in *Dictyostelium*. *Dev. Genet.* 9:531–538.

Noegel A., W. Witke, and M. Schleicher. 1987. Calcium-sensitive nonmuscle α-actinin contains EF-hand structures and highly conserved regions. *FEBS Lett.* 221:391–396.

Pasternak, C., S. Wong, and E. L. Elson. 1995. Mechanical function of dystrophin in muscle cells. *J. Cell Biol.* 128:355–361.

Petersen, N. O., W. B. McConnaughey, and E. L. Elson. 1982. Dependence of locally measured cellular deformability on position on the cell, temperature, and cytochalasin B. *Proc. Natl. Acad. Sci. USA.* 79: 5327–5331.

Sato, M., W. H. Schwarz, and T. D. Pollard. 1987. Dependence of the mechanical properties of actin/α-actinin gels on deformation rate. *Nature.* 325:828–830.

Schleicher, M., B. André, C. Andréoli, L. Eichinger, M. Haugwitz, A. Hofmann, J. Karakesisoglou, M. Stöckelhuber, and A. A. Noegel. 1995. Structure/function studies on cytoskeletal proteins in *Dictyostelium* amoebae as a paradigm. *FEBS Lett.* 369:38–42.

Schleicher, M., and A. A. Noegel. 1992. Dynamics of the *Dictyostelium* cytoskeleton during chemotaxis. *New Biol.* 4:461–472.

Schleicher, M., A. Noegel, T. Schwarz, E. Wallraff, M. Brink, J. Faix, G. Gerisch, and G. Isenberg. 1988. A *Dictyostelium* mutant with severe defects in α-actinin: its characterization by cDNA probes and monoclonal antibodies. *J. Cell Sci.* 90:59–71.

Stossel, T. P. 1993. On the crawling of animal cells. *Science.* 260: 1086–1094.

Wachsstock, D. H., W. H. Schwartz, and T. D. Pollard. 1993. Affinity of α-actinin for actin determines the structure and mechanical properties of actin filament gels. *Biophys. J.* 65:205–214.

Wallraff, E., M. Schleicher, M. Modersitzki, D. Rieger, G. Isenberg, and G. Gerisch. 1986. Selection of *Dictyostelium* mutants defective in cytoskeletal proteins: use of an antibody that binds to the ends of α-actinin rods. *EMBO J.* 5:61–67.

Wang, N., J. P. Butler, and D. E. Ingber. 1993. Mechanotransduction across the cell surface and through the cytoskeleton. *Science.* 260: 1124–1127.

Wilczynska, Z., and P. R. Fisher. 1994. Analysis of a complex plasmid insertion in a phototaxis-deficient transformant of *Dictyostelium discoideum* selected on a *Micrococcus luteus* lawn. *Plasmid.* 32:182–194.

Witke, W., W. Nellen, and A. Noegel. 1987. Homologous recombination in the *Dictyostelium* α-actinin gene leads to an altered mRNA and lack of the protein. *EMBO J.* 6:4143–4148.

Witke, W., M. Schleicher, and A. A. Noegel. 1992. Redundancy in the microfilament system: abnormal development of *Dictyostelium* cells lacking two F-actin crosslinking proteins. *Cell.* 68:53–62.

Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13, mp18, and pUC19 vectors. *Gene.* 33:103–119.

Zahalak, G. I. 1993. On the ambiguity of viscoelastic-fluid representations of cytoplasm subjected to large deformations. *Biorheology.* 30:471–472.

Zahalak, G. I., W. B. McConnaughey, and E. L. Elson. 1990. Determination of cellular mechanical properties by cell poking, with an application to leukocytes. *J. Biomech. Eng.* 112:283–294.