

## SHORT NOTE

# Viscoelasticity in Wild-Type and Vinculin-Deficient (5.51) Mouse F9 Embryonic Carcinoma Cells Examined by Atomic Force Microscopy and Rheology

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We have been studying mouse F9 embryonic carcinoma cells which contain no detectable vinculin protein (5.51 cells), and compared them with F9 wild-type cells. Employing atomic force microscopy, we probed the elastic properties of individual F9 wild-type and 5.51 cells by measuring the dynamic response of controlled loads of the cantilever tip. An elastic modulus (Young) of  $\sim 3.8$  and  $\sim 2.5$  kPa was calculated for wild-type and 5.51 cells, respectively. Using disc rheometry, we detected a marked change in shear of a 1000g pellet of  $\sim 55 \times 10^6$  cells between wild-type and 5.51 mutants. These differences are attributed to the loss of vinculin and altered cytoskeletal organization in these cells. © 1996 Academic Press, Inc.

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### INTRODUCTION

The linkage between microfilaments to areas of the cell membrane consists of a complex of proteins that assemble at sites of attachment of the cell to the extracellular matrix. The focal adhesion complex is a critical point for the regulation of cytoskeletal filament assembly and mechanical signal transfer. A number of proteins are found in focal adhesions at the intracellular face of the plasma membrane, including vinculin, talin, paxillin, and  $\alpha$ -actinin [1].

We have been studying wild-type mouse F9 embryonic cells, which can attach and spread well on extracellular matrix, and vinculin-deficient (5.51) cells, which contain no detectable vinculin protein and spread poorly on substrates [2]. In wild-type cells, F-actin is organized into bundles (stress fibers) which terminate at focal contacts, while 5.51 cells exhibit

no actin stress fibers; instead F-actin is concentrated under the plasma membrane. Though 5.51 cells contain normal amounts of talin and  $\alpha$ -actinin, two focal adhesion proteins capable of binding actin, their presence alone is not sufficient to induce stress fiber formation, suggesting that vinculin is required for stress fiber formation and cell attachment, spreading, and shape [cf. 3].

Intensive investigation into understanding how the cytoskeleton responds to chemical stimuli has been carried out; however, the mechanism by which external forces are transmitted across the cell surface and transduced into a cytoskeletal response is poorly understood. Mechanical properties of cell surfaces have been measured with micropipette aspiration [4], cell poker techniques [5], and optical tweezers [6]. Intracellular viscoelasticity and motility have also been quantitated using magnetometry [7], and Wang *et al.* [8] reported on a magnetic twisting device in which controlled mechanical stresses can be applied directly to cell surface receptors. Recently, Evans *et al.* [9] developed a micropipette suction method combined with reflection interference microscopy to measure local mechanical compliance at cell surfaces, and Radmacher *et al.* [10] imaged soft samples by atomic force microscopy (AFM).

In this investigation we have exposed F9 wild-type and 5.51 cells to controlled cantilever loads of the AFM and defined forces in the rotation disc rheometer to determine differences in their viscoelastic properties.

### MATERIALS AND METHODS

*Cell culture.* Wild-type F9 cells were maintained on 1% gelatin-coated charged plastic culture dishes in high-glucose (4.5 g/liter) Dulbecco's modified Eagle's medium supplemented with 10% calf serum and 1% L-glutamine, 1 mg/ml penicillin, and streptomycin. The 5.51 cells were cultured in suspension in the same medium. Prior to AFM studies both cell lines were plated on 24-mm<sup>2</sup> No.

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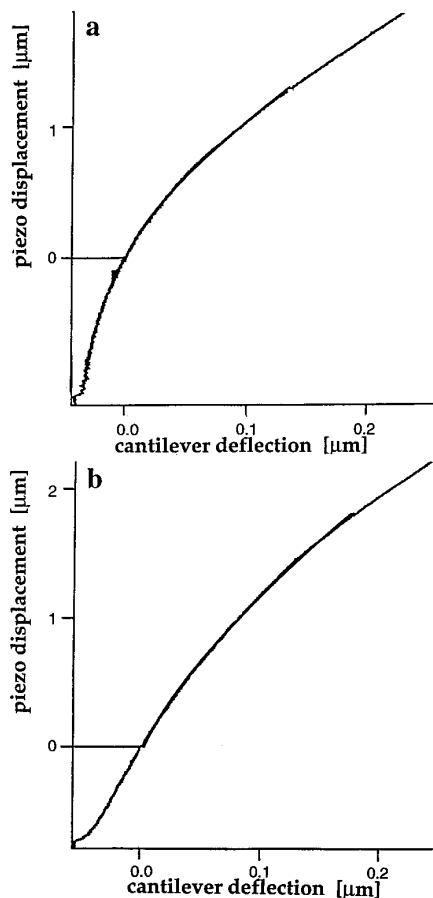


FIG. 1. Individual AFM force curves taken of a F9 wild-type cell (a) and vinculin-deficient (5.51) cell (b) adhering to glass coverslips coated with fibronectin/poly-D-lysine and with the best fit superimposed.

1 glass coverslips coated with 10  $\mu\text{g/ml}$  bovine fibronectin and 2  $\mu\text{g/ml}$  poly-D-lysine and examined approximately 3 h later. For rheologic studies,  $\sim 55 \times 10^6$  F9 wild-type and 5.51 cells were washed three times in phosphate-buffered saline at pH 7.4 and centrifuged at 1000g for 10 min, at 37°C. The homogeneous mass of cells was then immediately transferred to the measuring chamber of the rheometer.

**Atomic force microscopy.** The atomic force microscope (AFM) is a purpose-built instrument described in detail by Radmacher *et al.* [11]. In brief, this device is combined with an epi-light microscope which allows the exact positioning of the cantilever tip. The deflection of the cantilever is measured by a position-sensitive two-segment photo diode, and the extent to which the applied force indents the cells depends on their elastic properties. The Hertz model was used here to describe the elastic response of F9 cell lines indented by the AFM cantilever which predicts the following relation between indentation and loading force:  $F = \delta^2 \cdot \pi/2 \cdot E/(1 - \nu^2) \cdot \tan(\alpha)$ , where  $F$  is the loading force,  $E$  is the elastic or Young's modulus,  $\nu$  is the Poisson ratio,  $\alpha$  is the opening angle of the cylindrical cone of the AFM tip, and  $\delta$  is the indentation depth.

**Rotation disc rheometer.** The apparatus and details of the data analysis have been described in detail by Müller *et al.* [12]. Basi-

cally, the apparatus consists of a cylindrical glass cuvette surrounded by two perpendicularly oriented magnetic coils. One of these serves to fix the orientation of the disc and the other (the deflection coil) to apply an oscillatory shear force to the cell pellet. The "in-phase" and "out-phase" components of the rotational amplitude,  $\alpha(t)$ , of the disc are analyzed as follows: The beam of a He-Ne-laser incident light in a vertical direction along the rotational axis of the disc is horizontally deflected by the mirror mounted on the disc and its horizontal orientation is recorded by a gradient photo diode. The complex shear modulus  $G^*(\omega) = G'(\omega) + G''(\omega)$ , where  $G'(\omega)$  is the frequency-dependent storage modulus and  $G''(\omega)$  is the loss modulus, was determined for a pellet of  $\sim 55 \times 10^6$  F9 wild-type and 5.51 cells.

## RESULTS AND DISCUSSION

Figures 1a and 1b represent single scans at constant force of F9 wild-type and 5.51 cells with the best fit to the data superimposed. Since the deflection of the cantilever was proportional to the loading force, the deviation of the deflection from the slope obtained on glass equaled the cell indentation. The elastic indentation versus loading force curve was then calculated from the measured force curves. The average calculated elastic values of controlled cantilever loads for wild-type and 5.51 cells are  $\sim 3.83$  and  $\sim 2.47$  kPa, respectively (Fig. 2).

The relation between the shear moduli of a pellet of  $\sim 55 \times 10^6$  wild-type and 5.51 cells was determined by rotation disc rheometry. A total of three measurements were performed over five frequency decades (log scale) recording the storage (elastic)  $G'(\omega)$  modulus (Fig. 3a) and the loss (viscous) modulus  $G''(\omega)$  (Fig. 3b). For better comparison, traces of both cell lines were plotted in the same graphs showing the following relative changes: (i) 5.51 cells have a

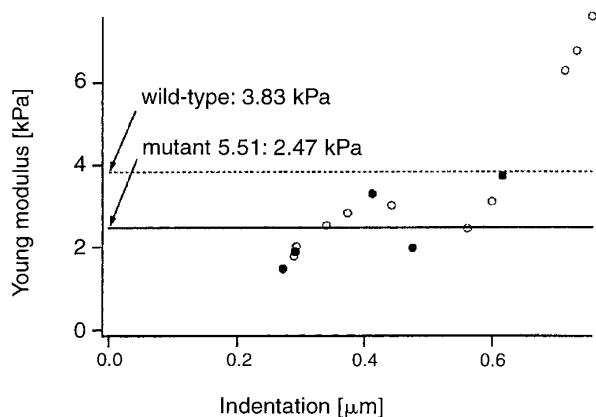


FIG. 2. The straight lines show the best fit to the average elastic values (Young modulus) for wild-type (open circles) and for vinculin-deficient 5.51 cells (filled circles) of  $3.83 (\pm 1; 10)$  and  $2.47 (\pm 1; 5)$  kPa, respectively. The standard deviation and number of measurements are given in parentheses. Experimental conditions as in Fig. 1.

strong influence on  $G'(\omega)$  over the entire frequency range. (ii) A plateau regime of  $G'(\omega)$  is detected for wild-type cells at lower frequencies ( $\omega = 5 \times 10^{-3} - 10^{-2}$  rad/s), and for higher frequencies ( $\omega = 10^1 - 5 \times 10^1$  rad/s) a power-law behavior of  $G' \sim \omega^{1/5}$ . The 5.51 cells show no plateau at  $G'$ , and the power-law behavior is reduced to  $G' \sim \omega^{1/8}$ . This is probably due to a change in cytoskeletal network organization. (iii) The moduli  $G'$  and  $G''$  are clearly lower for 5.51 than for wild-type cells, indicating differences in shear.

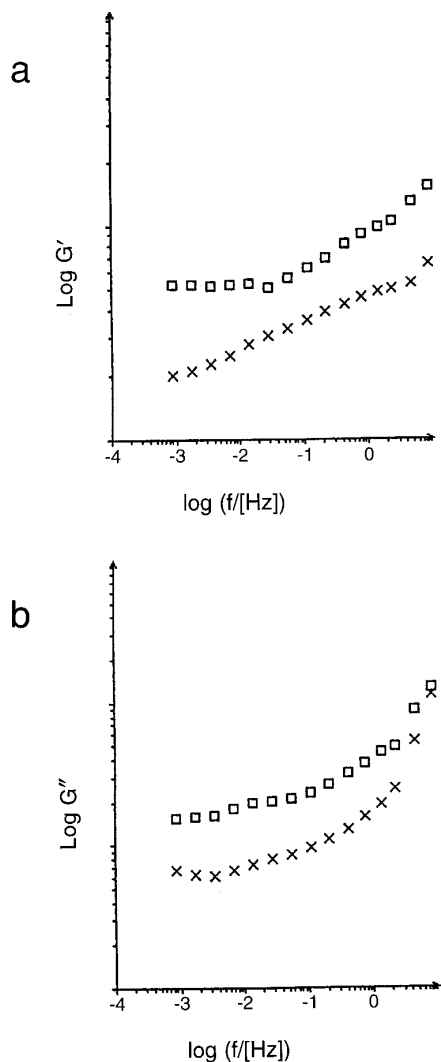


FIG. 3. The log-log plots  $G'(\omega)$  and  $G''(\omega)$  against frequency represent the average of three reproducible measurements of F9 wild-type cells (open squares) and vinculin-deficient (5.51) cells (crosses), demonstrating only the *relative* differences between the storage (= elastic;  $G'$ ) modulus (a) and the loss (= viscous;  $G''$ ) modulus (b) of a pellet of  $\sim 55 \times 10^6$  F9 wild-type and vinculin-deficient (5.51) cells. (Note: to measure *absolute* values for  $G'$  and  $G''$  much higher shear forces need to be applied.)

TABLE 1

The Influence of Vinculin in F9 Cell Lines Demonstrated by Three Independent Methods Measuring Different Physical Parameters

Cells	AFM	Magnetometry [13]	Rheology
Wild-type	(++)	(++)	(++)
5.51 cell	(+)	(+)	(+)
Parameter	Elasticity	Stress/strain	Shear

This study shows that the (visco)elasticity of F9 cell lines is markedly influenced by the presence/absence of vinculin, which raises the question whether vinculin provides a mechanical link between the cell surface and the cytoskeleton. Since vinculin seems to promote stress fiber formation, cell spreading, and focal adhesion complexes that couple integrins to the cytoskeleton in F9 wild-type cells [cf. 3], the loss of vinculin in 5.51 cells could account for the decreased resistance to external force. Support for this notion comes from recent observations of integrin-linked cytoskeletal stiffness (ratio of stress to strain) in F9 wild-type and 5.51 cells. Using the magnetic twisting device (described in [8]), in which controlled mechanical loads are applied directly to  $\beta$ 1-integrins via bound RGD-coated magnetic microbeads, 5.51 cells are less resistant to mechanical force, and thus deform more under the same stress compared to the wild-type cells [13].

In summary, the findings by the three different physical methods (Table 1) suggest that the linkage between integrins and the cytoskeleton could be affected by the presence/absence of functional vinculin and demonstrate its importance in cells. In addition to vinculin, subtle and complex regulatory interactions with other components in the cytoskeletal network are probably necessary for cell shear/elasticity.

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