Talin decreases the bending elasticity of actin filaments.

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The interplay between the proteins of the cytoskeleton is a central feature and of vital importance for cell architecture. Previous encouraging work on the dynamics of filamentous actin in solution by quasi-elastic light scattering (QELS) [1], on low shear rate viscometry in the dilute to semidilute concentration regime [2] and by Flicker spectroscopy [3] triggered off this work of wave-vector-dependent bending stiffness measurements of freely flickering actin filaments in the presence of talin.

Actin was prepared from rabbit back muscle as described in [4] and further purified by gel filtration on Sephacryl S-300. Talin was isolated from outdated human thrombocytes by the Collier and Wang method [5]. Fluorescent labelling of actin was performed as follows: polymerization of 5µM globular actin for at least 2 hours in F-buffer (2mM Tris/HCl, pH 7.4; 1mM ATP, 2mM $MgCl_2$, 100mM KCl, 0.5mM DTT) in the presence of rhodamine labelled phalloidin. Prior use, the samples were diluted with F-buffer to a ratio of 1:2000 and then placed between two glass cover slips coated with DMPC lipid bilayers (Fig. 1).

For observation an inverted Zeiss microscope Axiovert 10 equipped with a filter set for rhodamine fluorescence, a Zeiss Plan Neofluar 63 x Ph3 objective (nA=1.4) and a Zeiss HBO100 as light source was used.

For documentation the microscope was connected to a SIT camera (Hamamatsu C2400). The images were recorded on videotape by a SVHS recorder (Panasonic) and analysed on a Macintosh IIfx, supplemented with a fast frame grabber (PixelPipeline, Perceptics, Knoxville, TN, USA). Image analysis was performed on a modified version of the image processing software Image1.44 (Wayne Rasband, National Institute of Health, USA).

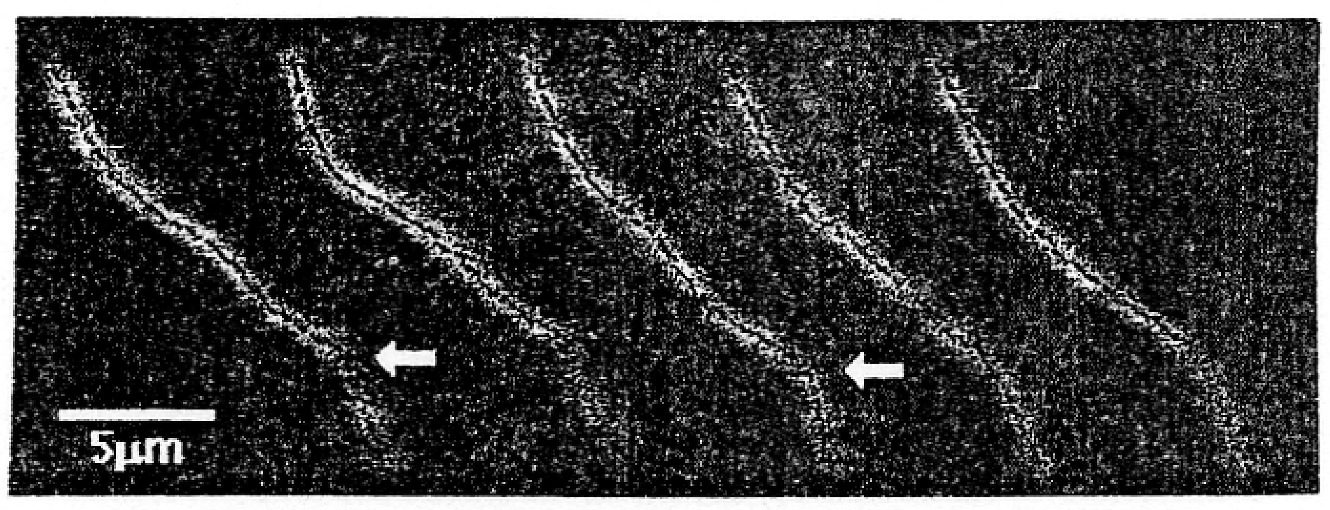

Fig. 1 Rhodamine-phalloidin labelled F-actin filaments taken at Δt=0.2s intervals; bends marked by arrow.

Results of our wave-vector-dependent k_c-measurements for pure F-actin and for F-actin polymerized in the presence of talin at a molar ratio of 3:1 are shown in Fig. 2a+b, respectively, exhibiting two regimes: for $q > 4 \times 10^6$ m^{-1} and for $q < 4 \times 10^6$ m^{-1}. The binding of talin to actin filaments leads to a significant increase of the

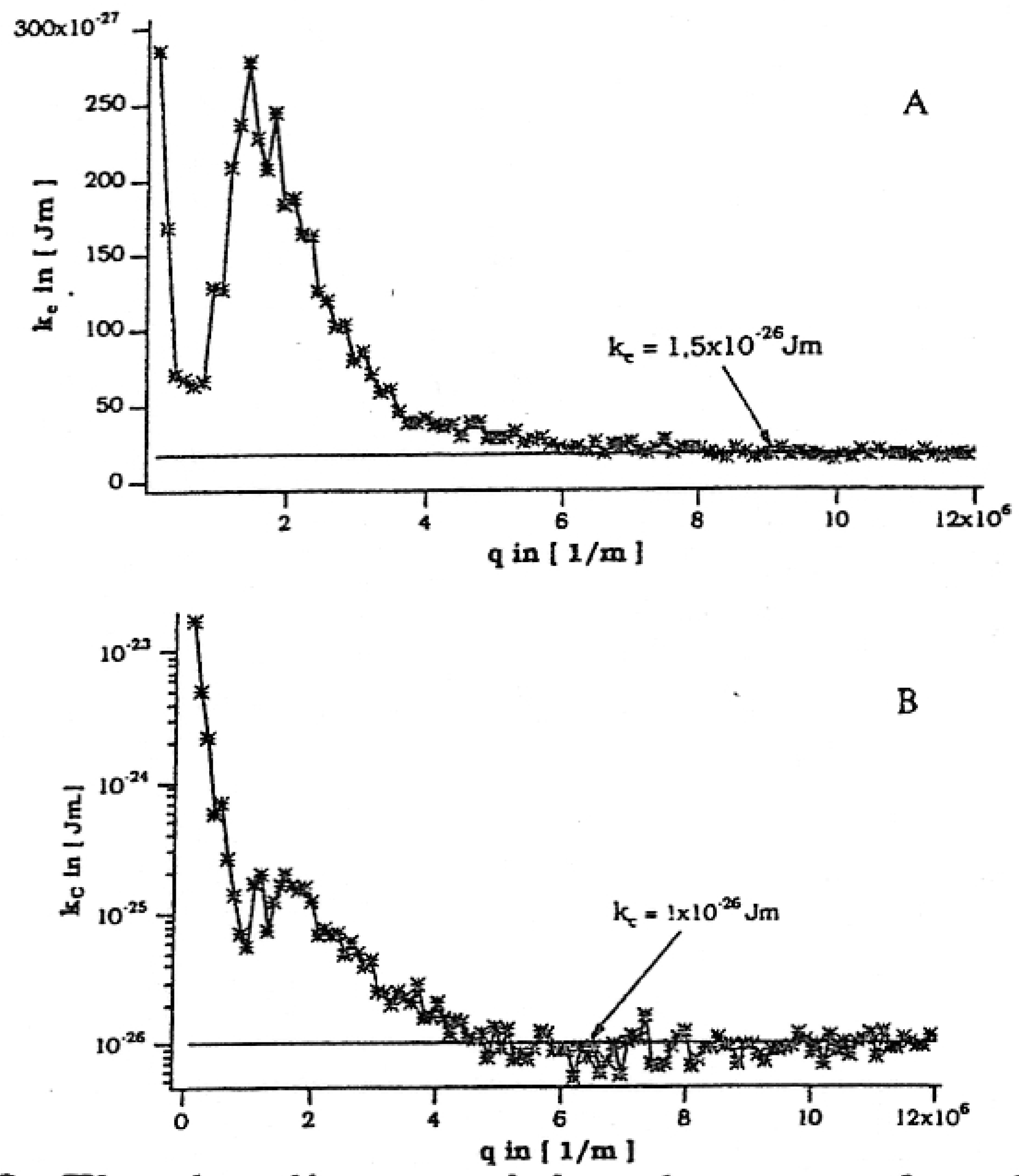

Fig. 2 The bending modulus k_c as a function of the wavevector $q=\pi n/L$ [3]. a) pure F-actin. b) F-actin in the presence of talin at molar ratio of 3:1 shows a sharp increase in filament stiffness for $q < 4 \times 10^6$ m^{-1}. Note: For large q the bending modulus (k_c) of 1-1.5 x 10^{-26} Jm is similar for pure actin and actin complexed with talin.

bending modulus for $q < 4 \times 10^6$ m^{-1} with a persistence length L_p of several milimeters. For short wave length the bending modulus equals $k_c = 1 \times 10^{-26}$ Jm which is similar to pure F-actin. Video sequences even show clearly bends after 12 h of polymerization. These bends distort the results insofar as bends are a prerequisite for analysis. Though sharp bends should only decrease the value of k_c, the fact that the presence of talin increases the bending stiffness of actin filament on long wavelength remains valid. It was noted that filaments between bends appeared like stiff rods. The binding of talin to actin seems to change the structure of actin filaments in such a way that filaments possibly stretch due to a long-range interaction between monomers.

These findings compare well with our recent results of the viscoelasticity of actin filaments in the presence of talin in terms of Rouse-like behaviour [6]. We observed a remarkable increase in stiffness of actin filament chains (expressed in terms of the effective segment length). This stiffening is paralled by an increase in bending stiffness as suggested by QELS [7].

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