essential hydrogen bond is associated with the N–H of Gly[9] of SP. What is not so clear is the location and catalytic function of this hydrogen bond. Two possibilities exist: (1) The hydrogen bond is intermolecular and bridges the substrate and enzyme; the role of such a hydrogen bond would be to stabilize catalytic transition states. (2) The hydrogen bond is intramolecular within SP (Chassaing et al., 1986); the role of this hydrogen bond would be to stabilize a population of substrate conformations that can be bound by SLN. The results of Table III suggest that the hydrogen bond associated with Gly[9] is not involved in stabilizing catalytically essential solution-phase conformations since binding constants for SP and SP(Sar-9) are identical. Rather, this hydrogen bond must be involved in specific interactions between the enzyme and substrate in the transition state.

The results of Table III and the foregoing discussion allow us to formulate a proposal for the mechanistic origin of SLN's substrate specificity. As we have pointed out, Table III indicates that SP, SP(Sar-9), SP^{4-11}, SP^{6-11}, and SP^{1-7} all form complexes of similar stability with SLN ($K_m = K_i \sim 0.4$ mM). But only SP is hydrolyzed. These results indicate that SLN manifests its substrate specificity in catalysis and not in substrate binding. SLN uses the energy that is available from favorable interactions with its substrate to stabilize catalytic transition states and does not use this energy to stabilize Michaelis complexes (Fersht, 1974; Jencks, 1975; Schowen, 1978).

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Tropomyosin Stabilizes the Pointed End of Actin Filaments by Slowing Depolymerization†

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ABSTRACT: Tropomyosin is postulated to confer stability to actin filaments in nonmuscle cells. We have found that a nonmuscle tropomyosin isolated from the intestinal epithelium can directly stabilize actin filaments by slowing depolymerization from the pointed, or slow-growing, filament end. Kinetics of elongation and depolymerization from the pointed end were measured in fluorescence assays using pyrenylactin filaments capped at the barbed end by villin. The initial pointed end depolymerization rate in the presence of tropomyosin averaged 56% of the control rate. Elongation from the pointed filament end in the presence of tropomyosin occurred at a lower free G-actin concentration, although the on rate constant, k_p^+, was not greatly affected. Furthermore, in the presence of tropomyosin, the free G-actin concentration was lower at steady state. Therefore, nonmuscle tropomyosin stabilizes the pointed filament end by lowering the off rate constant, k_p^-.

The polymerization of monomeric actin in vitro occurs rapidly after a slow nucleation step above the critical G-actin concentration [for reviews, see Frieden (1985), Korn (1982), and Pollard and Cooper (1986)]. The ends of the linear actin polymer are kinetically distinct. The barbed, or fast-growing, end elongates at a greater rate and at a lower monomer concentration than the pointed, or slow-growing, end of the filament. In vivo, actin filament assembly must be tightly regulated since the cellular pool of unpolymerized actin is much greater than the critical concentration for polymerization in vitro. The regulation of actin polymerization and organization

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is thought to be mediated by actin binding proteins (Pollard & Cooper, 1986; Stossel et al., 1985). Actin binding proteins affect actin assembly by at least two mechanisms: (1) binding to and blocking the barbed end of the actin filaments; (2) binding to G-actin, thereby reducing the pool of free actin monomers. The barbed filament end, which can be associated with the membrane in cells, is likely regulated by the abundant barbed end capping proteins which may regulate filament lengthening or shortening. However, regulation of the pointed end of actin filaments is not understood.

This study addresses the regulation of the pointed end of actin filaments by high-affinity low molecular weight tropomyosin isoforms present in the intestinal epithelial cell brush border (Broschat & Burgess, 1986). Brush border microvillar actin filaments are polarized with the pointed filament end in the cytoplasm and do not depolymerize significantly during brush border isolation in buffers which should promote rapid disassembly [see reviews by Burgess (1987) and Mooseker (1985)]. The pointed filament ends are apparently not capped since elongation occurs when exogenous actin monomer is added (Mooseker et al., 1982). Since the pointed ends are free to elongate, but not depolymerize, an alternative mechanism to a pointed end capping protein may function to stabilize brush border actin filaments. Since tropomyosin is bound to the rootlet actin filament at the pointed filament end (Bretscher & Weber, 1978; Drenckhahn & Groschel-Stewart, 1980), we have tested the effects of tropomyosin on pointed end elongation and depolymerization.

We have found that intestinal epithelial cell tropomyosin stabilizes the pointed filament end by lowering the k_p^-. Initial depolymerization rates from the pointed end were measured fluorometrically following dilution of villin-capped pyrenylactin filaments saturated with tropomyosin to far below the critical G-actin concentration. Tropomyosin did not block depolymerization completely and was not a pointed filament end capping protein. Elongation from the pointed filament end was promoted at a lower G-actin concentration in the presence of tropomyosin, and the steady-state concentration of G-actin was lower in the presence of tropomyosin. The net effect of tropomyosin was to stabilize the pointed filament end against disassembly.

Materials and Methods

Preparation of Proteins. Tropomyosin (Broschat & Burgess, 1986) and villin (Bretscher & Weber, 1980) were purified from isolated chicken intestinal epithelial cells (Matsudaira & Burgess, 1982). Tropomyosin was stored at −20 °C. Concentrated villin was stored on ice with weekly addition of 0.1 mM DTT. Villin concentration was determined by using an absorptivity coefficient of E_{280} = 123.5 mM^{-1} cm^{-1} (Northrop et al., 1986). Chicken breast muscle actin was purified as described (Pardee & Spudich, 1982). Nucleating activity was removed from twice-cycled G-actin by gel filtration (MacLean-Fletcher & Pollard, 1980). Actin was labeled at the penultimate cysteine by reaction with pyrenyliodoactetamide as described (Kouyama & Mihashi, 1981), incorporating the modifications of Bryan and Coluccio (1985).

The native G-actin concentration was determined by using an E_{290} = 24.9 mM^{-1} cm^{-1} (Wegner, 1976). The concentration of pyrenyl-G-actin was determined, compensating for the absorbance of the fluorescent probe as described (Seldon et al., 1983). The concentration of pyrene was calculated with a coefficient for pyrene of E_{344} = 22 mM^{-1} cm^{-1}. The ratio of pyrene/actin was calculated as described (Cooper et al., 1983); pyrenylactin contained 0.9–0.99 mol of pyrene/mol of actin. Column-purified G-actin was frozen in liquid N_2 and stored at −70 °C until use. Thawing was performed quickly in a 25 °C water bath with frequent mixing. The actin samples were allowed to equilibrate at 25 °C for 3 h prior to use in assays. G-Actin stocks treated in this manner had the same critical concentration and initial fluorescence as unfrozen G-actin.

Filament Formation. To measure elongation of actin filaments from the pointed end, short actin filaments (50–200 monomers long) were polymerized with villin to define filament length and cap the barbed filament end. Villin's high affinity for the barbed filament end stops polymerization and depolymerization events at the barbed end as measured in these assays (Northrop et al., 1986; Walsh et al., 1984a; Weber et al., 1987a,b). In all experiments, actin polymerization was initiated by adding 20× F-buffer (2.0 M KCl/40 mM $MgCl_2$) to achieve a final concentration of 0.1 M KCl/2 mM $MgCl_2$ in G-buffer (0.5 mM ATP, 0.2 mM $CaCl_2$, 10 mM HEPES, pH 7.5, 0.01% NaN_3, and 0.1 mM DTT). Filament concentration was subsequently determined by measuring the mean length of each filament preparation by negative-stain electron microscopy. Filaments (7 μM actin stock, varied villin concentrations) were rapidly diluted 10-fold, placed on formvar- and carbon-coated grids, and negatively stained with 1% uranyl acetate. Grids were viewed in a JEOL 100 CX electron microscope at 60 kV. The filament lengths were measured from micrographs, and mean filament length was calculated from $n > 75$. This value was used to calculate the filament concentration.

Fluorescence Measurements. Fluorescence measurements utilized a Perkin-Elmer 650-10s fluorescence spectrophotometer calibrated with a standard solution of 0.1 μM pyrenyliodoacetamide in DMSO (excitation, 365 nm with a 4-nm slit; emission, 407 nm and 6-nm slit). Elongation experiments were conducted by using a maximum of 1 μM G-actin containing 5–15% pyrenylactin; depolymerization experiments were conducted with 40 nM pyrenylactin. For elongation experiments, G-actin was converted from Ca^{2+}- to Mg^{2+}-actin by incubation with 0.2 mM EGTA and 2 mM $MgCl_2$ for 10 min in G-buffer; G-actin fluorescence was measured before and after exchange to ensure that polymerization did not occur. At the highest G-actin concentration (1 μM G-actin), less than 5% of the fluorescence increase during the course of the assay was due to spontaneous polymerization. A fluorescence increase of 23–30-fold was observed upon polymerization of actin. The sample was illuminated only intermittenly during the fluorescence measurements to minimize photobleaching.

The concentration of F-actin in samples containing a mixture of F- and G-actin was calculated as described (Northrop et al., 1986). The fluorescence coefficients and critical concentrations of the barbed and pointed ends were determined for each preparation of native and pyrenylactin from steady-state concentration curves. The critical concentration at the barbed end was 0.15–0.17 μM G-actin, and the pointed end critical concentration ranged between 0.6 and 0.8 μM G-actin, in agreement with previous reports (Bonder et al., 1983; Coluccio & Tilney, 1983; Pollard & Mooseker, 1981).

Results

The effect of tropomyosin on depolymerization from the pointed end of actin filaments was tested using villin-capped pyrenylactin filaments. As shown in Figure 1, actin filaments preincubated with tropomyosin depolymerized more slowly than control filaments. Initial depolymerization rates were calculated for each assay from the rate of fluorescence decrease. The off rate constant for the pointed filament end, k_p^-, was calculated by dividing the measured initial off rate

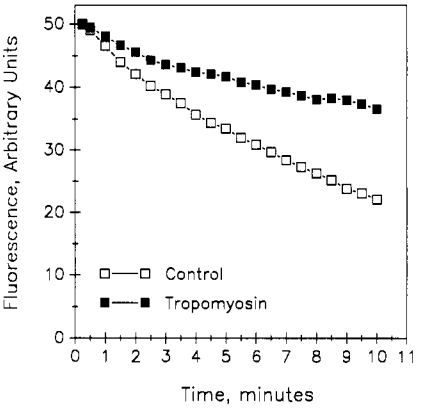

FIGURE 1: Depolymerization of capped filaments in the presence and absence of tropomyosin. Villin-capped filaments were prepared by initiating polymerization of pyrenyl-G-actin (7.5 μM) in the presence of 37 nM villin. The filament lengths were measured by negative-stain EM; the mean length was 231 ± 17 monomers (standard error of the mean). The filaments were incubated (7 μM actin) in the presence and absence of 1.25 μM tropomyosin overnight. The lengths of capped filaments in the presence or absence of tropomyosin did not differ by a statistically significant margin (data not shown). Depolymerization was monitored by the decreasing fluorescence of filaments diluted rapidly into F-buffer (0.1 M KCl, 2 mM $MgCl_2$, 0.5 mM ATP, 0.2 mM $CaCl_2$, 10 mM HEPES, pH 7.5, 0.1 mM DTT, 0.01% NaN_3, and 40 nM total actin, plus or minus 0.5 μM tropomyosin). Filaments depolymerized from the pointed end at a slower rate in the presence of tropomyosin.

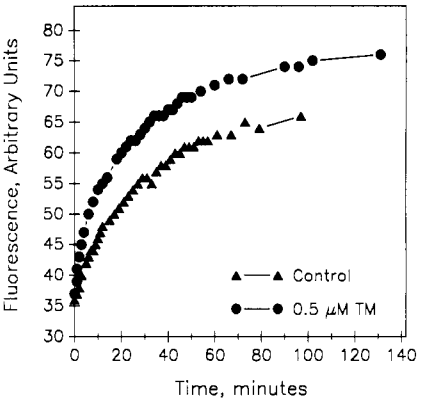

FIGURE 2: Effect of tropomyosin upon the elongation of capped filaments in 1 μM G-actin. Filaments (5% prenylactin) were incubated for 3 h in the absence or presence of tropomyosin. Elongation was initiated by adding filaments (0.5 μM F-actin) and 20× F-buffer to G-actin in G-buffer as described under Materials and Methods. The villin:actin ratio was 1:100. In the presence of tropomyosin, the fluorescence increases at a greater rate and plateaus at a higher level.

Table I: Initial Pointed End Depolymerization Rates[a]

expt	k_p^-, control (s^{-1})	k_p^-, +tropomyosin (s^{-1})
1	0.153	0.102
2	0.214	0.106
3	0.158	0.082
	av 0.175	0.097

[a] The off rate constants were calculated from the fluorescence decrease measured upon rapid dilution of actin filaments into F-buffer. The average depolymerization rate (dF/dt, μM/s, calculated from 30-s time points over a 5-min time course) was divided by the filament concentration to obtain the initial off rate constants shown.

(from 0 to 5 min) by the filament concentration. The results of three separate experiments are shown in Table I. In the presence of 0.5 μM free tropomyosin, the measured k_p^- averaged 56% of the control k_p^-.

Tropomyosin increased the initial rate of elongation from the pointed end of preformed filaments. A time course of filament elongation in 1 μM G-actin is shown in Figure 2. In

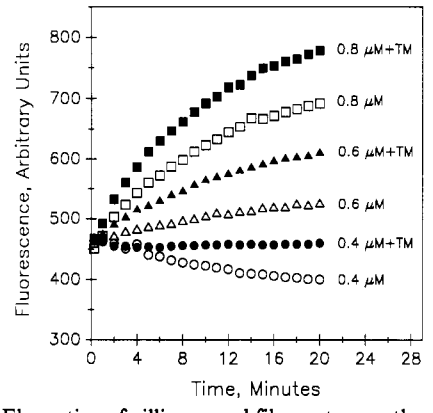

FIGURE 3: Elongation of villin-capped filaments near the pointed end critical G-actin concentration. Filaments (mean length 69 ± 4 monomers, standard error of the mean) were incubated for 3 h with or without tropomyosin) and subsequently diluted (0.5 μM total actin, 7.2 nM filaments) into 0.4 (●), 0.6 (▲), and 0.8 (■) μM G-actin. Control elongations have open symbols; samples containing tropomyosin have solid symbols. At each G-actin concentration, the rate of elongation is greater in the presence of tropomyosin.

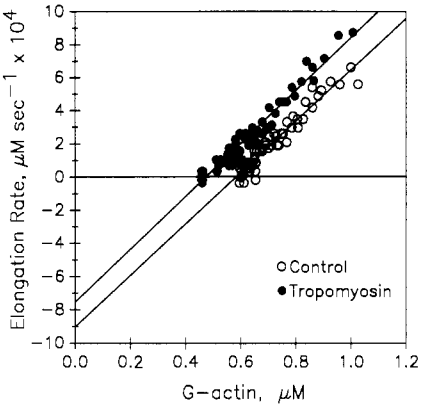

FIGURE 4: Net elongation rates from the pointed end plotted as a function of G-actin concentration. The net elongation rates of filaments plus (solid symbols) or minus (open symbols) tropomyosin diluted into initial concentrations of 0.6, 0.8, and 1 μM G-actin (shown in Figure 3) are plotted vs the free G-actin concentration. A regression line is drawn through each set of points (correlation coefficients: control = 0.962; tropomyosin = 0.978). The slopes of the rate vs concentration plots from the linear regression are not significantly different in the presence or absence of tropomyosin (see Table II, set 3). The critical concentration (x intercept) in the presence of tropomyosin was 0.48 μM vs 0.59 μM G-actin in the control.

the presence of 0.5 μM tropomyosin, filaments elongated at a higher net rate than in the control. Upon approaching steady state, the sample containing tropomyosin contained a higher concentration of F-actin. At G-actin concentrations near the pointed end critical concentration (Figure 3), elongation was promoted in the presence of tropomyosin. A lower concentration of exogenous G-actin was required for elongation in the presence of tropomyosin, indicating that tropomyosin lowered the critical concentration for pointed end elongation.

Tropomyosin increased the net elongation rate, a result which could reflect changes in either rate constant, k_p^- or k_p^+. Rate concentration (J/c) plots of the elongation data in Figure 3 were used to examine the rate constants for elongation (Oosawa & Asakura, 1975; Pollard, 1986; Weber et al., 1987b). As shown in Figure 4, the net elongation rate was linear with respect to the free G-actin concentration in the presence or absence of tropomyosin. The primary effect of tropomyosin is on the apparent k_p^-. The slopes of either plot did not differ significantly in the presence or absence of tropomyosin. However, the intercepts differ; in the presence of tropomyosin, the apparent off rate constant, k_p^-, is lower than

Table II: Summary of Pointed End Rate Constants Measured by Elongation Assays

expt	k_p^+ (μM^{-1} s^{-1})	k_p^- (s^{-1})	[critical] (μM)
(1) control	0.254 ± 0.012	0.154 ± 0.004	0.606
(1) tropomyosin	0.239 ± 0.011	0.115 ± 0.007	0.481
(2) control	0.328 ± 0.010	0.238 ± 0.004	0.726
(2) tropomyosin	0.290 ± 0.017	0.165 ± 0.006	0.569
(3) control	0.219 ± 0.010	0.130 ± 0.007	0.594
(3) tropomyosin	0.226 ± 0.007	0.109 ± 0.006	0.482

Table III: Measured Free G-Actin Concentrations at Steady State

	micromolar G-actin concn	
expt	control (n)	+tropomyosin (n)
1	0.783 (6)	0.538 (6)
2	0.572 (6)	0.459 (6)
3	0.813 (4)	0.577 (4)
4	0.810 (5)	0.544 (5)
	av 0.744	0.530

the control. The result is that the critical concentration is lowered by the presence of tropomyosin. The values obtained for the k_p^+, k_p^-, and the critical concentration from elongation assays using three separate actin, villin, and tropomyosin preparations are summarized in Table II. In each of these experiments, the slope (k_p^+) was not significantly different in the presence of tropomyosin. The k_p^-, determined by extrapolating the curve to the ordinate, was an average of 24% lower in the presence of tropomyosin. Tropomyosin did not inhibit the on rate constant for the pointed end in contrast to the effect of skeletal tropomyosin on barbed end elongation (Lal & Korn, 1986; Wegner & Ruhnau, 1988).

The critical concentration for polymerization at the pointed end was estimated from steady-state fluorescence measurements of each sample from the elongation experiments. As shown in Table III, the measured free G-actin concentration at steady state was lower in the presence of tropomyosin by an average of 28%, from 0.74 to 0.53 μM G-actin. This result verifies the findings from the elongation assays that the pointed end critical concentration was lowered by tropomyosin by lowering the k_p^-.

Discussion

Our results show that a nonmuscle tropomyosin stabilizes the point end of actin filaments by slowing depolymerization. Tropomyosin does not have a significant effect on the k_p^+, but lowers the k_p^-, and thus lowers the critical concentration for elongation at the pointed filament end. Our findings confirm a recent report in which tropomyosin was shown to have no effect on the k_p^+ as measured by electron microscopic assays (Hitchcock-DeGregori et al., 1988). The present study is the first to examine the effect of tropomyosin on the depolymerization of actin filaments from the pointed end.

These observations cannot be explained by the loss of the villin cap from the barbed filament end. Although tropomyosin inhibits villin's severing ability in the presence of high calcium levels (Bonder & Mooseker, 1983; Walsh et al., 1984b) and villin's binding and bundling activities at low calcium levels (Burgess et al., 1987), our results indicate that the barbed ends remained capped in the presence of tropomyosin. Since the k_b^+ is 10-fold greater than the k_p^+ (Northrop et al., 1986), release of villin from the barbed end would increase the apparent k^+, which we did not observe. Furthermore, the k_b^- is greater than the k_p^- under these assay conditions (Walsh et al., 1984a; Pollard, 1986). Thus, as reported previously, tropomyosin does not cause the release of the villin cap from the barbed filament end (Walsh et al., 1984b). Therefore, the measured effects of tropomyosin on filament elongation and depolymerization reflect events solely at the pointed filament end.

The kinetics of elongation and depolymerization of tropomyosin–actin filaments are not simple since the apparent k_p^- changes in the presence of tropomyosin. The ability of tropomyosin to lower the k_p^- requires that tropomyosin be bound to the very end of the actin filament. The magnitude of tropomyosin's effect on the k_p^- differed between the two methods used to measure the off rate constant. That difference may reflect differences in the levels of tropomyosin saturation at the filament ends. Since an elongating filament must also be adding tropomyosin, filament elongation cannot exceed the rate of tropomyosin addition in order to observe a difference between control and experimental samples. Alternatively, depolymerizing filaments presaturated with tropomyosin would be limited by the rate of tropomyosin dissociation. Therefore, during depolymerization of tropomyosin-saturated filaments, the measured depolymerization rate reflects dissociation of both the tropomyosin and actin. Wegner demonstrated that muscle tropomyosin binding to an actin filament and one adjacent tropomyosin molecule is weaker than binding to the filament and two adjacent tropomyosins by a factor of 10^3 (Wegner, 1979). Since intestinal epithelial cell tropomyosin binds filaments with high cooperativity (Broschat & Burgess, 1986) similar to skeletal tropomyosin, its affinity for the filament end must be weaker than internal sites. Although tropomyosin was present in excess of the amount required to fully saturate the filaments according to sedimentation assays (Broschat & Burgess, 1986), the degree to which filament ends are saturated with tropomyosin is difficult to determine. At a free concentration of 0.5 μM tropomyosin, it seems unlikely that all sites at the filament ends were saturated with tropomyosin in our assays. Therefore, it is possible that the effect of tropomyosin could be markedly greater should all filaments have a bound tropomyosin at the very end. Due to the large differences in tropomyosin's affinity for contiguous versus end sites, it is unlikely that saturation of the end site could be achieved in vitro by increasing the tropomyosin concentration.

The effects of this nonmuscle tropomyosin on the pointed end differ from the reported effects of skeletal muscle tropomyosin on the barbed filament end. Skeletal tropomyosin has no effect on the critical concentration at the barbed filament end and inhibits elongation of the filament from the barbed end (Hitchcock-DeGregori et al., 1988; Lal & Korn, 1986; Wegner, 1982; Wegner & Ruhnau, 1988). Intestinal epithelial cell tropomyosin clearly does not inhibit elongation of filaments from the pointed end. The proposal that tropomyosin inhibits filament shearing (Hitchcock-DeGregori et al., 1988), thus inhibiting elongation, should not be a factor in our assays since short actin filaments were used as nuclei, tropomyosin was bound subsequent to filament formation, and the presence of newly formed barbed ends would be detected (Northrop et al., 1986). The lack of inhibition on pointed end elongation may reflect a difference between tropomyosin isoforms, since this nonmuscle tropomyosin is nonpolymerizable at low ionic strength as measured by low shear viscometry (Broschat & Burgess, 1986). Nonmuscle tropomyosin may not have the ability to extend past the end of the elongating filament according to the model proposed by Wegner and Ruhnau (1988). We did not test the effects of this nonmuscle tropomyosin on the barbed end. Another possibility remains: since the binding of tropomyosin to the actin filament is polarized, addition of tropomyosin to the ends of an elongating filament may be kinetically distinct. Due to the highly cooperative binding of

tropomyosin to actin (Broschat & Burgess, 1986; Wegner, 1979), tropomyosin binding to an elongating actin filament may be similar to polymerization.

How are actin filaments in the intestinal epithelial cell brush border stabilized? We suggest that the barbed filament ends are likely capped by villin. At submicromolar calcium concentrations, villin has a very high affinity for the barbed filament end, as compared to the relatively weak affinity for binding to the sides of actin filaments (Burgess et al., 1987; Northrop et al., 1986; Walsh et al., 1984a). Villin is present in a 1:10 molar ratio to actin in the microvillar cores, in vast excess of the concentration of barbed filament ends. Therefore, unless a mechanism exists to prevent villin from binding to the barbed end of microvillar actin filaments, the barbed ends should all be capped, leaving the pointed end active to elongate or depolymerize. Mooseker et al. (1982) have shown that EGTA pretreatment of isolated brush border microvilli is necessary for polymerization of exogenous actin onto the barbed ends. Since villin dissociates from the barbed filament end at free calcium levels below 10 nM (Northrop et al., 1986), the elongation of microvilli after treatment with EGTA is consistent with a villin cap at the barbed end. Conversely, since the pointed ends are free to elongate upon addition of exogenous actin under polymerizing conditions, they probably are not capped (Mooseker et al., 1982). Tropomyosin is present on the rootlet portion of the microvillar actin filament (Bretscher & Weber, 1978; Drenckhahn & Groschel-Stewart, 1980) and therefore likely slows actin depolymerization and stabilizes the rootlets.

The implications of our results reach beyond the intestinal epithelial cell. The ability of tropomyosin to slow disassembly from the pointed filament end may be an important function of tropomyosin in other cell types. Only two actin binding proteins have been described previously which may affect the pointed end, acumentin and β-actinin (Southwick & Hartwig, 1982; Yokota & Maruyama, 1983). Our results indicate that tropomyosin by itself does not act as a capping protein for the pointed filament end. However, regulation of tropomyosin's affinity for the pointed filament end could be a mechanism to regulate filament stability. If another protein were to increase the affinity of tropomyosin for the filament end, then tropomyosin might further stabilize the pointed end against depolymerization. Caldesmon, present in smooth muscle and nonmuscle cells, has been shown to increase the affinity of nonmuscle tropomyosin for filamentous actin; its binding is also regulated by Ca^{2+}-calmodulin (Yamashiro-Matsumura & Matsumura, 1988). In skeletal muscle, troponin increases the actin binding affinity of tropomyosin (Wegner & Walsh, 1981). We are currently testing the effects of caldesmon and skeletal troponin upon tropomyosin's ability to stabilize the actin filament pointed end. The ability of tropomyosin to regulate actin filament stability at the pointed end could have a major impact on actin filament stability in many cells.

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Effects of CapZ, an Actin Capping Protein of Muscle, on the Polymerization of Actin†

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ABSTRACT: We have studied the interaction of CapZ, a barbed-end actin capping protein from the Z line of skeletal muscle, with actin. CapZ blocks actin polymerization and depolymerization (i.e., it "caps") at the barbed end with a K_d of approximately 0.5–1 nM or less, measured by three different assays. CapZ inhibits the polymerization of ATP–actin onto filament ends with ATP subunits slightly less than onto ends with ADP subunits, and onto ends with ADP–BeF_3^- subunits about as much as ends with ADP subunits. No effect of CapZ is seen at the pointed end by measurements either of polymerization from acrosomal processes or of the critical concentration for polymerization at steady state. CapZ has no measureable ability to sever actin filaments in a filament dilution assay. CapZ nucleates actin polymerization at a rate proportional to the first power of the CapZ concentration and the 2.5 power of the actin concentration. No significant binding is observed between CapZ and rhodamine-labeled actin monomers by fluorescence photobleaching recovery. These new experiments are consistent with but do not distinguish between three models for nucleation proposed previously (Cooper & Pollard, 1985). As a prelude to the functional studies, the purification protocol for CapZ was refined to yield 2 mg/kg of chicken breast muscle in 1 week. The activity is stable in solution and can be lyophilized. The native molecular weight is 59 600 ± 2000 by equilibrium ultracentrifugation, and the extinction coefficient is 1.25 mL mg^{-1} cm^{-1} by interference optics. Polymorphism of the α and β subunits has been detected by isoelectric focusing and reverse-phase chromatography. CapZ contains no phosphate (<0.1 mol/mol).

CapZ is a heterodimeric protein with subunits of M_r 36 000 (α subunit) and 32 000 (β subunit) (Casella et al., 1986). CapZ binds to the barbed end of actin filaments in vitro and is located at the Z line in skeletal muscle (Casella et al., 1987). Since the barbed ends of actin filaments are also located at the Z line, CapZ may attach the actin filaments to the Z line. CapZ is part of a family of capping proteins (Stossel et al., 1985; Pollard & Cooper, 1986) that are heterodimers, bind the barbed ends of actin filaments, and do not require Ca^{2+} for activity. These proteins are widely distributed, having been purified from *Acanthamoeba* (Isenberg et al., 1980; Cooper et al., 1984), *Dictyostelium* (Schleicher et al., 1984), bovine brain (Kiliman & Isenberg, 1982), and chicken skeletal muscle (Casella et al., 1986). The α and β subunits are not similar to each other by antibody cross-reactivity, peptide maps (Casella et al., 1986; Cooper et al., 1984, 1986), or primary structure (Casella et al., 1989; Caldwell et al., 1989). The primary structures of CapZ α and β show no similarity to those of other actin binding proteins or actin itself (Casella et al., 1989; Caldwell et al., 1989).

Understanding the mechanism of action of CapZ on actin in vitro is important because it will allow us to make and test predictions about the role of CapZ in cells. In muscle, actin filaments in sarcomeres have a defined location, polarity, and length. In nonmuscle cells, actin filaments often have a well-defined spatial arrangement that can change over time. Actin assembly itself may provide the motive force for cell movement. CapZ and its nonmuscle analogues may regulate actin assembly in cells.

Actin contains a bound nucleotide that can be ATP, ADP–P_i, or ADP. If capping proteins bind differently to actin with different nucleotides, this might be a mechanism for regulating actin polymerization in cells. Actin monomers contain ATP, which is converted to ADP–P_i and then ADP after the monomer adds to a filament end (Korn et al., 1987). Previous work on the mechanism of action of capping proteins with actin has shown that they bind tightly to the barbed end of actin filaments (Kiliman & Isenberg, 1982; Cooper et al., 1984; Casella et al., 1986), with a K_d of 0.5 nM for brain capping protein (Wanger & Wegner, 1985). In this report, we extend these studies by determining the K_d with different assays using filaments that are either polymerizing, at steady state, or depolymerizing. One of the assays is used to directly compare barbed ends with subunits that contain ATP or ADP or ADP–BeF_3^-. BeF_3^- binds to actin filaments as a phosphate analogue and induces a state resembling ADP–P_i* (Combeau & Carlier, 1988). We find little or no difference in the ability of CapZ to bind to barbed ends with different nucleotides.

Capping proteins accelerate the polymerization of actin from monomers. The time course of actin polymerization induced by *Acanthamoeba* capping protein, analyzed by kinetic modeling, showed that capping protein accelerates but does not

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