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The Capactins, a Class of Proteins that Cap the Ends of Actin Filaments

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The capactins, a class of proteins that cap the ends of actin filaments

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A number of proteins that bind specifically to the barbed ends of actin filaments in a cytochalasin-like manner have been purified to various degrees from a variety of muscle and non-muscle cells and tissues. Preliminary evidence also indicates that proteins that interact with the pointed ends of filaments are present in skeletal muscle. Because of their ability to cap one or the other end of an actin filament, we have designated this class of proteins as the 'capactins'. On the basis of their effect on actin filament assembly and interaction *in vitro*, we propose that the capactins play important roles in cellular regulation of actin-based cytoskeletal and contractile functions. Our finding that the disappearance of actin filament bundles in virally transformed fibroblasts can be correlated with an increase in capactin activity in the extracts of these cells is consistent with this hypothesis.

Introduction

An important question in the study of the molecular basis of cell locomotion is how various forms of cytoskeletal and contractile functions are regulated. Over the past several years, our laboratory has taken the following strategic steps to gather information relevant to this question. First, we wished to find out how the fungal metabolite cytochalasin B inhibits cell motility and affects cell morphology in a variety of animal cells. By identifying the target site (i.e. the high-affinity binding site) of this drug, we hoped to pinpoint a cellular component that functions as an on–off switch for the control of motile function. Next, we wished to see if animal cells contain endogenous substances that interact with cytochalasin binding sites in a specific way. It is possible that such substances play a physiological role in the control of cellular and intracellular movements. Finally, we wished to determine whether alterations in motile and morphological properties of a cell correlate with changes in the activity of the proposed regulatory substances. This type of study should help us to understand the nature of the molecules involved in cellular regulation of cytoskeletal and contractile activities. In this paper, we describe the work that led to the identification of the end of an actin filament as a target site of cytochalasin action, the discovery that a class of proteins (designated as the capactins) also recognizes this site, and the finding that the alteration of actin filament organization in virally transformed fibroblasts is correlated with increased capactin activity.

Interaction of cytochalasins with actin filament ends

Initial attempts to probe the cellular target sites of cytochalasin B were complicated by the fact that the drug affects sugar transport as well as cell motility (Tanenbaum 1978); the former effect has since been shown to be caused by the high-affinity binding of the drug to the sugar transport proteins in the plasma membrane (Lin & Spudich 1974; Jung and Rampal 1977).

To identify target sites related to the inhibitory effects of cytochalasin B on cell motility, we used a derivative of the drug, dihydrocytochalasin B, which affects cell movements but not sugar transport (Lin *et al.* 1978; Atlas & Lin 1978). In extracts of various types of cells and tissues (e.g. human erythrocytes, human platelets, bovine brain), we were able to identify protein complexes that contain high-affinity binding sites for [^{3}H]dihydrocytochalasin B (Lin & Lin 1979; Lin *et al.* 1981). These complexes, containing actin in stable, oligomeric form, accelerate the assembly of actin monomers (G-actin) into filamentous structures (F-actin) *in vitro* under ionic conditions that normally allow only a very slow rate of assembly. Furthermore, low concentrations of cytochalasins effectively slow down actin filament assembly under these conditions (figure 1) (see also Lin & Lin 1979). Subsequent experiments performed in our laboratory (Lin *et al.* 1980; Flanagan & Lin 1980; Lin 1981) and elsewhere (Brenner & Korn 1979; Brown & Spudich 1979; MacLean-Fletcher & Pollard 1980*a*) indicate that the above observations can be explained as a seeding phenomenon, with the oligomeric actin in the complexes serving as stable nuclei onto which G-actin can be rapidly added to form filaments, and with cytochalasins inhibiting monomer addition by capping the growing ends of the nuclei and filaments.

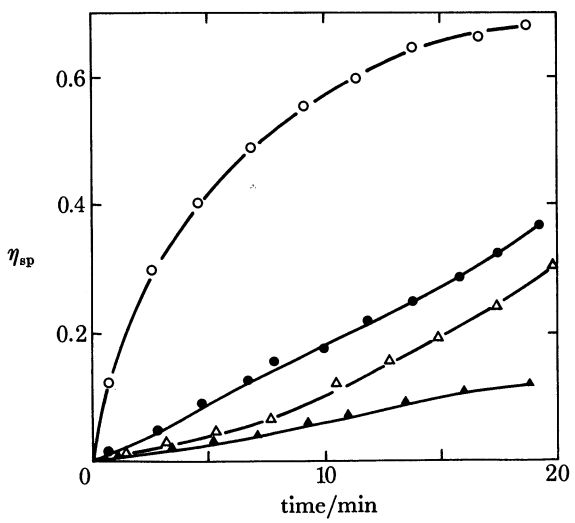

FIGURE 1. Inhibition of nucleated actin filament assembly by cytochalasin B. This experiment was performed essentially as previously described (Lin & Lin 1979). G-actin (1 mg ml^{-1}) in a buffer containing 0.4 mM $MgCl_2$, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM 2-mercaptoethanol, 5 mM Tris–HCl, pH 8.0, was induced to polymerize by addition of stable nuclei in the form of erythrocyte spectrin–4.1–actin complex (○, ●, ▲, 32 μg protein ml^{-1}). Filament assembly was monitored by measuring the increase in viscosity of the samples with an Ostwald viscometer at 25 °C. Under the conditions used, assembly proceeded at a negligible rate in the absence of the complex (see figure 2). The addition of cytochalasin B at 0.2 μM (●) or 2 μM (▲) to this system resulted in a slower rate of assembly, analogous to that observed when a smaller amount of erythrocyte complex (△, 8 μg protein ml^{-1}) was added to induce assembly.

The physiological implications of the experiments *in vitro* described above is that interference with assembly of actin filaments by affecting filament ends may be the basis for alteration of motile and morphological properties in certain cytochalasin-treated cells. Such a view is supported by the demonstration that the inhibition of platelet shape change by cytochalasin is correlated with a blockage of a shift of cellular G-actin to F-actin, which normally accompanies platelet activation (Casella *et al.* 1981).

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Proteins that interact with actin filament ends in a cytochalasin-like manner

The studies with cytochalasins indicated that interaction with actin filament ends is a highly effective way of controlling filament assembly. To search for cellular factors that affect actin filaments in this manner, we devised an assay based on the ability of a substance to inhibit the binding of [^{3}H]cytochalasin to stable actin nuclei, presumably by competitive displacement of the labelled ligand (Grumet & Lin 1980). Using this assay, we were able to purify from human platelets a 65 kDa protein that is cytochalasin-like in the following ways. First, the protein inhibits [^{3}H]cytochalasin binding to actin nuclei and filaments (Grumet & Lin 1980). Second, the protein affects actin filament assembly induced by the addition of stable nuclei in a manner closely resembling that of the cytochalasins (compare figure 2 with figure 1). Third, the protein nucleates actin filament assembly in the absence of actin nuclei (see below), as previously seen with cytochalasins (Howard & Lin 1979). Finally, the protein reduces the viscosity of F-actin solutions as measured with a low-shear falling-ball viscometer. This effect is similar to that produced by cytochalasins (see below). This type of reduction in F-actin viscosity is thought to be a result of interference of filament–filament interaction (MacLean-Fletcher & Pollard 1980*a*), the mechanism of which is not well defined at present.

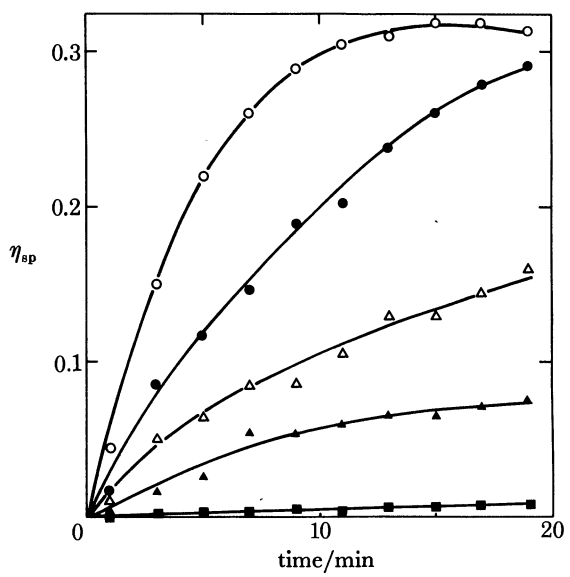

Figure 2. Inhibition of nucleated actin filament assembly by a 65 kDa protein isolated from human platelets. This experiment was performed essentially as described in figure 1, except that cytochalasin B was replaced by the platelet 65 kDa protein (see figure 3 for a brief description of this preparation). All samples contained G-actin (0.5 mg ml^{-1}) and 0 (○), 10 (●), 20 (△) or 40 (▲) μg ml^{-1} of the 65 kDa protein. Filament assembly was induced by addition of erythrocyte complex (50 μg ml^{-1}). In the control sample (■), G-actin but not the complex or the 65 kDa protein was present.

At about the same time as the 65 kDa protein from platelets was being studied in our laboratory, a number of proteins that have powerful effects on the length of actin filaments *in vitro* were being discovered by other investigators (see Weeds (1982) for review). One example is the protein villin, isolated from the microvilli of chicken intestinal brush borders (Bretscher & Weber 1980). In the presence of micromolar calcium ions, this 95 kDa protein has the ability to

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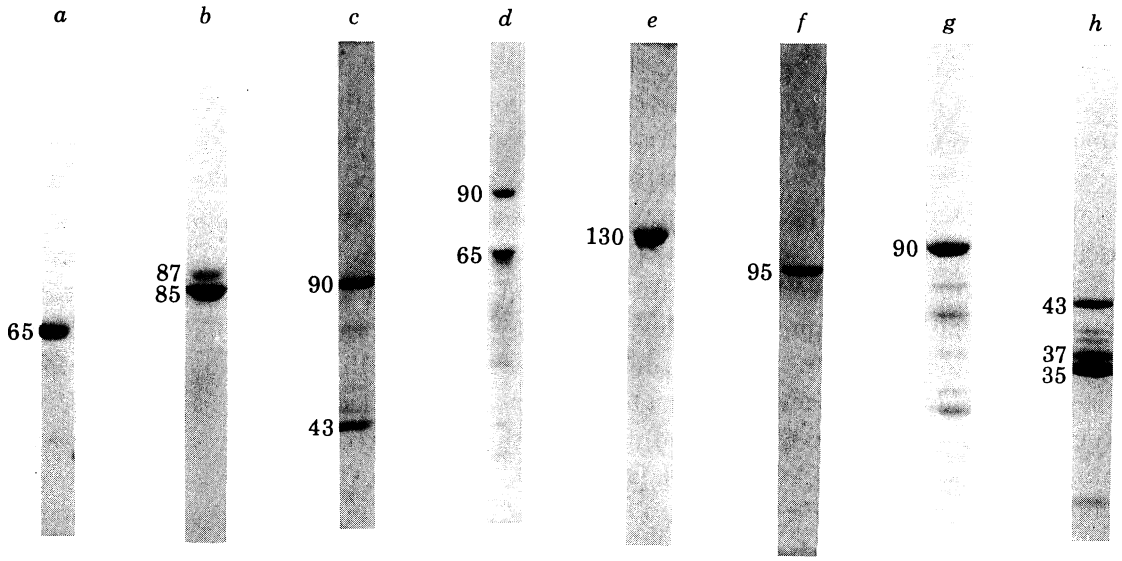

FIGURE 3. Electrophoretic analysis of the capactin preparations described in this paper. Samples were separated by electrophoresis in polyacrylamide slab gels containing sodium dodecyl sulphate, as described by Laemmli (1970). All of the gels contained 7% acrylamide except gel (*h*), which contained 9% acrylamide. Because the gels are from different experiments, the relative position of bands from gel to gel is not significant. The numbers beside each gel designate approximate molecular mass in kilodaltons as determined by comparison with mobility of standard proteins in that particular experiment. Gel (*a*), platelet 65 kDa protein preparation. The DEAE-cellulose column fractions containing capactin activity as described previously (Grumet & Lin 1980) were pooled, concentrated and chromatographed on a Sephacryl S-200 column equilibrated in 50 mM KCl, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM 2-mercaptoethanol, 5 mM Tris-HCl, pH 8.0. Fractions rich in the 65 kDa protein were pooled, concentrated and re-chromatographed on the same column. Gel (*b*), platelet preparation containing 87 and 85 kDa polypeptides. This preparation was obtained as described for the 65 kDa protein, except that fractions from the Sephacryl S-200 column rich in these two polypeptides were pooled, concentrated and re-chromatographed. It is uncertain whether these two polypeptides are related to the 90 kDa protein in gel (*c*). However, we have shown that the two preparations are similar in their ability to inhibit filament assembly nucleated by the spectrin–4.1–actin complex and to decrease F-actin viscosity measured with a low-shear viscometer. Gel (*c*), a platelet preparation enriched in 90 kDa protein obtained by affinity chromatography on an actin–DNase column, as previously described (Lin *et al.* 1981; Wang & Bryan 1981). Note that this preparation contained a mixture of 90 and 43 kDa polypeptides in the molar ratio of about 1:2. These two components are apparently part of a complex and cannot be separated by gel-filtration chromatography. The 43 kDa polypeptide has the same electrophoretic mobility as actin. Gel (*d*), a partly purified capactin preparation from bovine adrenal medulla (Grumet & Lin 1981). Gel (*e*), vinculin isolated from chicken gizzard, as previously described (Feramisco & Burridge 1980; Wilkins & Lin 1982). Gel (*f*), villin, from chicken intestinal brush border microvilli, provided by Dr Klaus Weber and Dr John R. Glenney, prepared as described (Bretscher & Weber 1980; Cribbs *et al.* 1982). Gel (*g*), a partly purified preparation of brevin from human serum. The purification procedure involves fractionation of serum proteins by ammonium sulphate precipitation, followed by chromatography on a DEAE-cellulose column and on a blue agarose column (Lees & Lin 1982). This brevin preparation is similar to that described by Harris & Schwartz (1981) in its ability to inhibit actin filament assembly nucleated by the spectrin–4.1–actin complex and to decrease F-actin viscosity as measured with a low-shear viscometer. Gel (*h*), muscle protein preparation highly enriched in proteins that appear to interact with the pointed ends of actin filaments (Lin *et al.* 1982). This preparation was derived from an acetone-powder extract of rabbit skeletal muscle containing β-actinin (Maruyama *et al.* 1977).

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shorten actin filaments *in vitro* at sub-stoichiometric levels. In collaboration with Weber and co-workers, we found that although villin does not inhibit cytochalasin binding to F-actin, it does inhibit nucleated actin filament assembly in a cytochalasin-like manner (Cribbs *et al.* 1982) (table 1). Other examples of proteins found to have this type of activity are (see table 1): vinculin, a 130 kDa protein purified from chicken smooth muscle (Wilkins & Lin, 1982); brevin (also called actin depolymerizing factor), a 90 kDa protein purified from human plasma (Harris & Schwartz 1981; Lees & Lin 1982); and another 90 kDa protein purified from platelets by affinity chromatography on an actin-DNase column (Lin *et al.* 1981; Wang & Bryan 1981). In addition, proteins with cytochalasin-like activity on actin filament assembly have also been partly purified from bovine adrenal medulla (Grumet & Lin 1981), and detected in extracts of rabbit skeletal muscle (Lin *et al.* 1982) and chicken embryo fibroblasts (Magargal & Lin 1982). Thus it appears that the ability to affect actin filament assembly by capping the end of the filament is shared by many different proteins from a wide spectrum of muscle and non-muscle sources. To unify the nomenclature for this class of proteins, we propose the name 'capactin' for describing any protein that caps the ends of actin filaments (see figure 3 for

TABLE 1. INHIBITION OF NUCLEATED ACTIN FILAMENT ASSEMBLY BY CAPACTINS

capactin preparation†	effective concentration range‡/(μg ml^{-1})
villin	0.5–8
platelet 90 kDa protein	0.5–8
brevin	≤1.5
adrenal medulla preparation	≤24§
platelet 65 kDa protein	4–60
vinculin	6–50

† See legend to figure 3 for description of these preparations.

‡ The approximate level of capactin preparations found to cause a significant inhibition of actin filament assembly nucleated with erythrocyte spectrin–actin–band-4.1 complex. In general, the assay used is similar to that described in figures 1 and 2, with the exception that villin and platelet 90 kDa protein were assayed in 3 mM EGTA, 3 mM $CaCl_2$, 0.2 mM ATP, 0.5 mM 2-mercaptoethanol, 5 mM Tris–HCl, pH 8.0 (Cribbs *et al.* 1982).

§ Actin filament assembly was nucleated by small amounts of F-actin (Grumet & Lin 1981).

electrophoretic analysis of various capactin preparations being studied in our laboratory). This is to distinguish this particular class of proteins from actin-binding proteins that cross-link actin filaments (e.g. filamin, α-actinin, spectrin, macrophage ABP) (see Weeds (1982) for references).

Besides inhibiting actin filament assembly at sub-stoichiometric levels, the capactins also have other effects on actin filaments. Villin has previously been found to shorten preformed actin filaments by what is thought of as a direct severing action (Bretscher & Weber 1980). This type of effect can be assayed quantitatively by measuring the release of radioactively labelled F-actin attached to inside-out erythrocyte membranes (Cribbs *et al.* 1982) (figure 4). Using this assay, we found that villin and brevin (data not shown) are both highly effective in releasing large amounts of actin from the membranes. By comparison, vinculin is far less effective than villin. It is interesting to note that relatively high concentrations of cytochalasins also decrease membrane-associated actin in this assay, although it is not clear whether this type of effect is mechanistically related to that produced by villin.

As mentioned above, cytochalasins and the 65 kDa capactin from platelets are highly effective in reducing the viscosity of F-actin as measured with a low-shear viscometer. This property is

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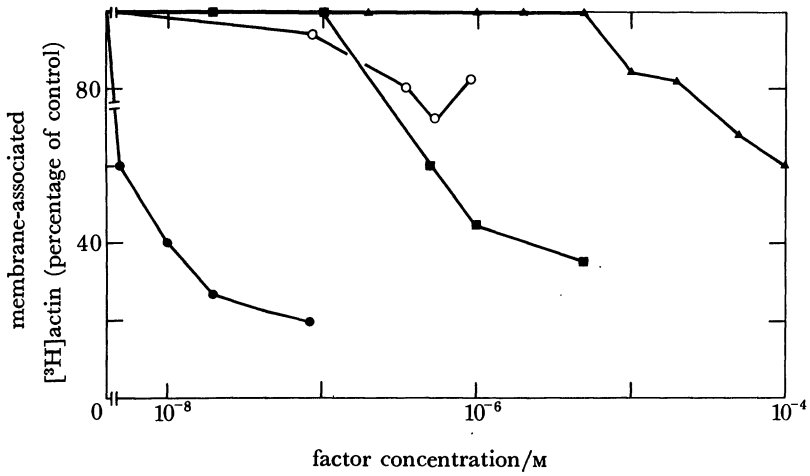

FIGURE 4. Effect of capactins and cytochalasins on actin filaments attached to membranes. The purpose of this study is to measure the effect of different factors on preformed filaments. Radioactively labelled G-actin was allowed to assemble onto stable nuclei bound to the outside surface of inside-out erythrocyte membranes, which results in formation of membrane-attached filaments. Various concentrations of villin (●), vinculin (○), cytochalasin D (■) or cytochalasin B (▲) were then added to the samples. After sedimentation of the membranes by centrifugation through a 20 % sucrose solution, the amount of membrane-associated actin in each sample was determined by scintillation counting. The procedure used is essentially as previously described (Cribbs *et al.* 1982), with the following exceptions: (*a*) the samples with cytochalasins contained 50 μg ml^{-1} of actin in 1 mM ATP, 0.75 mM 2-mercaptoethanol, 0.4 mM $MgCl_2$ and 5 mM sodium phosphate, pH 6.5, and (*b*) the samples with vinculin contained 100 μg ml^{-1} of actin in 1 mM ATP, 0.75 mM 2-mercaptoethanol, 20 mM KCl and 5 mM sodium phosphate, pH 6.5.

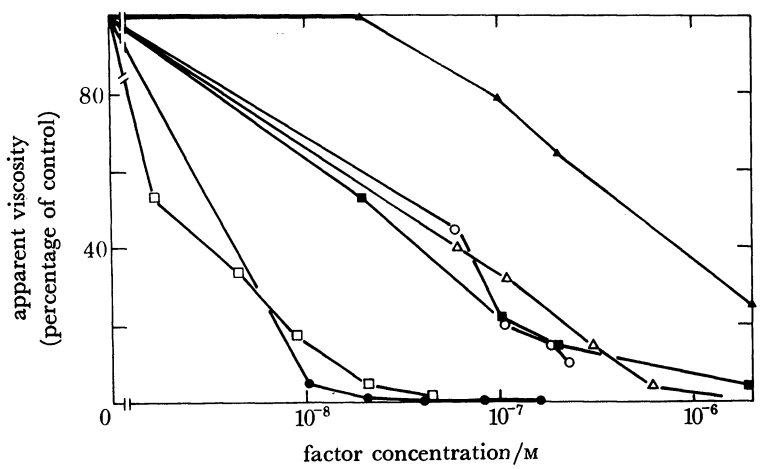

FIGURE 5. Effect of capactins and cytochalasins on the viscosity of F-actin as measured with a low-shear viscometer. In these experiments, the actin prepared by the Spudich & Watt (1971) procedure was further purified by chromatography on a Sephacryl S-200 column (Wilkins & Lin 1982). The apparent viscosity of F-actin was measured with the falling-ball viscometer (MacLean-Fletcher & Pollard 1980*b*; Wilkins & Lin 1982) in the presence of various concentrations of platelet 90 kDa protein (□), villin (●), vinculin (○), platelet 65 kDa protein (△), cytochalasin D (■) or cytochalasin B (▲). All of the samples were in 2 mM $MgCl_2$, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM 2-mercaptoethanol, 5 mM Tris–HCl, pH 8.0; in addition, the samples with villin and platelet 90 kDa protein contained 3 mM EGTA and 3 mM $CaCl_2$ to give a free Ca^{2+} concentration of 10 μM. In some samples (■, ▲, △), the factors were added before the actin was induced to polymerize by $MgCl_2$; the viscosity was measured after incubation at 30 °C for 30 min. In other samples, the factors were added directly to F-actin and the viscosity measured after incubation at 25 °C for 2 h (○), or at 30 °C for 4 h (●, □). The results presented have been normalized by comparing the apparent viscosity of the samples containing the factors with samples containing only actin measured under identical conditions. We have found that the percentage decrease in viscosity caused by a given factor is similar whether it was added to the actin before or after polymerization.

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shared by all of the capactin preparations described above (figure 5). In addition, brevin, the adrenal medulla preparation, and certain fractions derived from extracts of skeletal muscle and chicken embryo fibroblasts are all active as measured with this type of assay.

Proteins that appear to interact with the pointed end of actin filaments

Actin filaments have a polarity easily demonstrable by decoration of the filaments with proteolytic fragments of myosin, which results in the formation of arrowhead-like structures. When filaments are being assembled, monomer addition at the barbed end is up to ten times faster than addition at the pointed end (Pollard & Mooseker 1981). Detailed studies on the effect of cytochalasins on filament assembly indicated that the drug selectively caps the barbed end of filaments and nuclei (MacLean-Fletcher & Pollard 1980*a*; Wilkins & Lin 1981; Pollard & Mooseker 1981). By analogy, one can conclude that all of the capactins described in the preceding section also act on this end of the actin filament.

Although the barbed end is the preferred end for monomer addition, a filament can still grow extremely rapidly under physiological salt conditions (on the order of 1 μm per 20 s) if the barbed, but not the pointed, end is capped by an inhibitor. Monomer addition at both ends of the filament must therefore be controlled if the length of a filament is to be regulated effectively. To study proteins that might cap the pointed end of a filament, we examined a muscle extract shown to be rich in β-actinin, a protein previously thought to act at this end of the filament (Maruyama *et al.* 1977). The assay we developed to measure capping of the pointed end is based on the inhibition of filament elongation in the presence of an inhibitor that caps the barbed end (i.e. cytochalasin or one of the capactins described in the preceding section). Using this assay, we were able to obtain a partly purified preparation enriched in what can be regarded as pointed-end inhibitory activity (Lin *et al.* 1982) (see figure 3 for an electrophoretic analysis of this preparation). As shown in figure 6, the addition of a combination of platelet 65 kDa capactin and the muscle preparation led to a more complete inhibition of salt-induced filament assembly than that produced by the platelet protein alone. Although other types of action cannot be ruled out at this point, our working hypothesis is that the muscle preparation contained molecules that cap the ends of actin filaments not affected by the 65 kDa capactin (i.e. the pointed end). Whether the active molecules in the muscle preparation are those previously designated as the α and β subunits of the protein designated as β-actinin (molecular masses 37 and 35 kDa) (Maruyama *et al.* 1977) remains to be determined. However, it is interesting that in a related study we found that a different fraction derived from the β-actinin-rich extract contained capactins that affect the barbed end of filaments (Lin *et al.* 1982). It is likely that the earlier studies performed on the β-actinin preparation described by Maruyama *et al.* (1977) may in fact contain a combination of two different types of capactin that act on opposite ends of the filament.

Transformation-dependent increase in capactin activity in fibroblasts

When cultured fibroblasts are transformed by oncogenic viruses, one of the most dramatic changes in their phenotype is the disappearance of prominent actin filament bundles that are characteristic of normal cells spread on a flat substrate (Pollack *et al.* 1975). Although the

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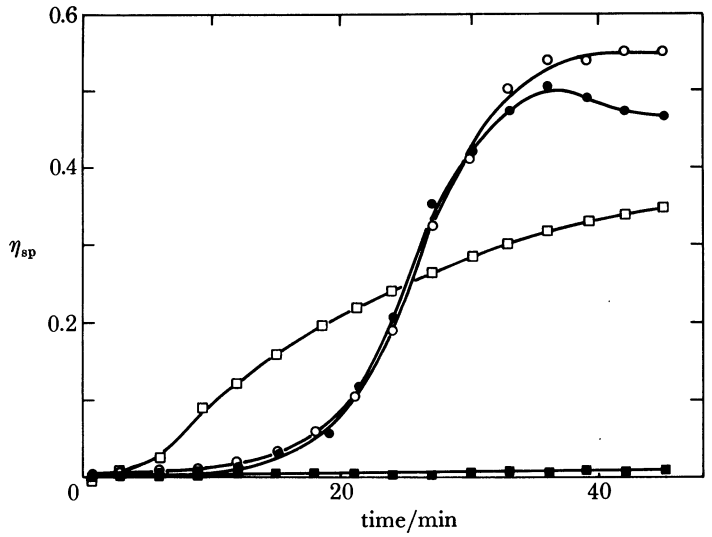

FIGURE 6. Effects of the muscle protein preparation on salt-induced actin filament assembly. The preparation used in this experiment was prepared as described in figure 3, with the exception that urea was omitted from the elution buffer for the DEAE-cellulose column and the active column fractions were then further chromatographed on a Sepharose 4B column to remove residual barbed-end inhibitor activity. In this experiment, G-actin (chromatographed on a Sephacryl S-200 column) in a buffer containing 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM 2-mercaptoethanol, 5 mM Tris–HCl, pH 8.0, was induced to polymerize by the addition of 50 mM KCl at time zero. The samples contained: (●), actin (0.5 mg ml^{-1}) alone; (□), actin and the platelet 65 kDa protein (165 μg ml^{-1}); (○), actin and the muscle protein preparation (154 μg protein ml^{-1}); (■), actin and both the muscle protein preparation and the 65 kDa protein. Filament assembly was monitored by measuring the increase in viscosity of the samples with an Ostwald viscometer at 25 °C.

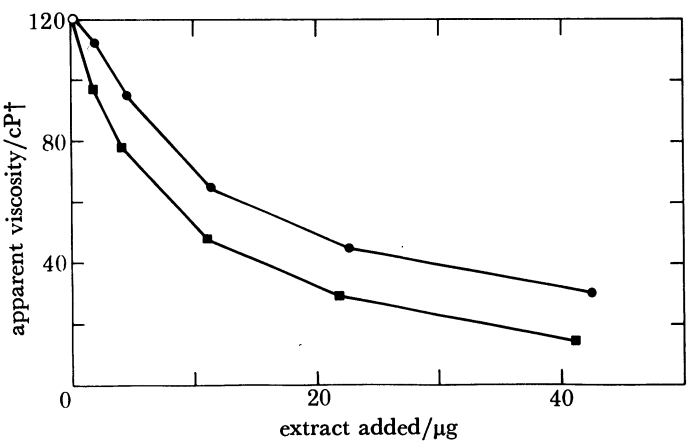

FIGURE 7. Effect of chicken-embryo fibroblast extracts on F-actin viscosity measured with a low-shear viscometer. Tertiary cultures of normal chicken-embryo fibroblasts or fibroblasts infected with the Schmidt Ruppin A strain of Rous sarcoma virus were gently scraped off culture dishes into phosphate-buffered saline, pelleted by centrifugation, and then resuspended in 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM 2-mercaptoethanol, 5 mM Tris–HCl, pH 8.0. The cells were disrupted by brief sonication, and the suspension was centrifuged at 250000 *g* for 30 min at 4 °C. The resultant supernatant (extract) was dialysed against the above buffer at 4 °C overnight. For assaying for capactin activity in the extracts, various amounts of samples were added to G-actin (0.5 mg ml^{-1}) in a total volume of 0.25 ml of the same buffer, and $MgCl_2$ (to 2 mM) was added to induce polymerization of the actin. The apparent viscosity of the samples (●, extract of normal cells; ■, extract of infected cells) was measured after incubation at 30 °C for 30 min (procedure similar to that described in figure 5). Each point represents the mean value obtained from duplicate determinations. †, 1cP = 1 mPa s.

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relation between this effect and other virally induced changes (e.g. changes in cell morphology, growth control, membrane-related properties) has not yet been defined, a high level of correlation between abnormal actin distribution and tumorigenicity of transformed cells has been demonstrated (Pollack *et al.* 1975).

Because cytochalasin-treated cells and virally transformed cells both have an altered distribution of actin filaments, we reasoned that the latter may contain a higher level of capactins with cytochalasin-like activity. To test this hypothesis, we compared the ability of extracts of normal and transformed fibroblasts to decrease the viscosity of F-actin measured with a low-shear viscometer. As shown in figure 7, an extract of chicken embryo fibroblasts infected with Rous sarcoma virus had a significantly higher specific activity than a comparable extract of uninfected cells measured with this assay. Moreover, when cells were infected with a temperature-sensitive mutant Rous sarcoma virus (NY68), a higher level of specific activity was found in extracts of infected cells grown at the permissive temperature (37 °C), but not in extracts of cells grown at a restrictive temperature (41 °C) (see Magargal & Lin 1982). The results of this series of studies suggest that the product of the transforming gene of the virus may have a direct or an indirect effect on the total level of capactin activity in the cell. This increased activity may contribute to the abnormal distribution of actin filaments observed in the transformed phenotype.

Conclusion

Studies on the mechanism of action of cytochalasins on cell motility have led to the discovery that a number of muscle and non-muscle proteins have the capability of binding specifically to the barbed end of an actin filament *in vitro*. In addition, preliminary evidence suggests that proteins that bind to the pointed end of a filament are present in extracts of skeletal muscle. On the basis of their ability to cap one or the other end of an actin filament, we have designated this class of proteins as the 'capactins'.

Capactins that interact with the barbed end affect actin filament assembly and interactions in a number of ways. First, these proteins inhibit addition of actin monomers to the barbed end of an actin filament during filament elongation. Second, these proteins decrease the viscosity of F-actin as measured with a low-shear viscometer. Third, some of these proteins (e.g. villin) are very effective in severing preformed filaments, whereas others (e.g. vinculin) are not. For a given protein preparation there seems to be a correlation in its potency in different assays relative to that of other capactin preparations.

Besides the experiments showing a correlation between a higher level of capactin activity with a decrease in actin filament bundles in virally transformed cells, there is currently little information on the physiological function of the capactins. However, their potent effects on actin filament assembly and interactions *in vitro* strongly suggest that these proteins play important roles in regulating actin-dependent functions in the cell. For instance, by limiting the length of actin filaments, the capactins could effectively control cell morphology and locomotion. In addition, since the capactins also nucleate actin polymerization they might function as starting points of filament assembly. Finally, a capactin such as vinculin could conceivably anchor filaments to the plasma membrane in some systems as proposed by Geiger *et al.* (1980) by serving as a link between the barbed ends of the filaments and certain membrane components. Whether the capactins do in fact function in one or more of the above ways will have to be determined in future studies.

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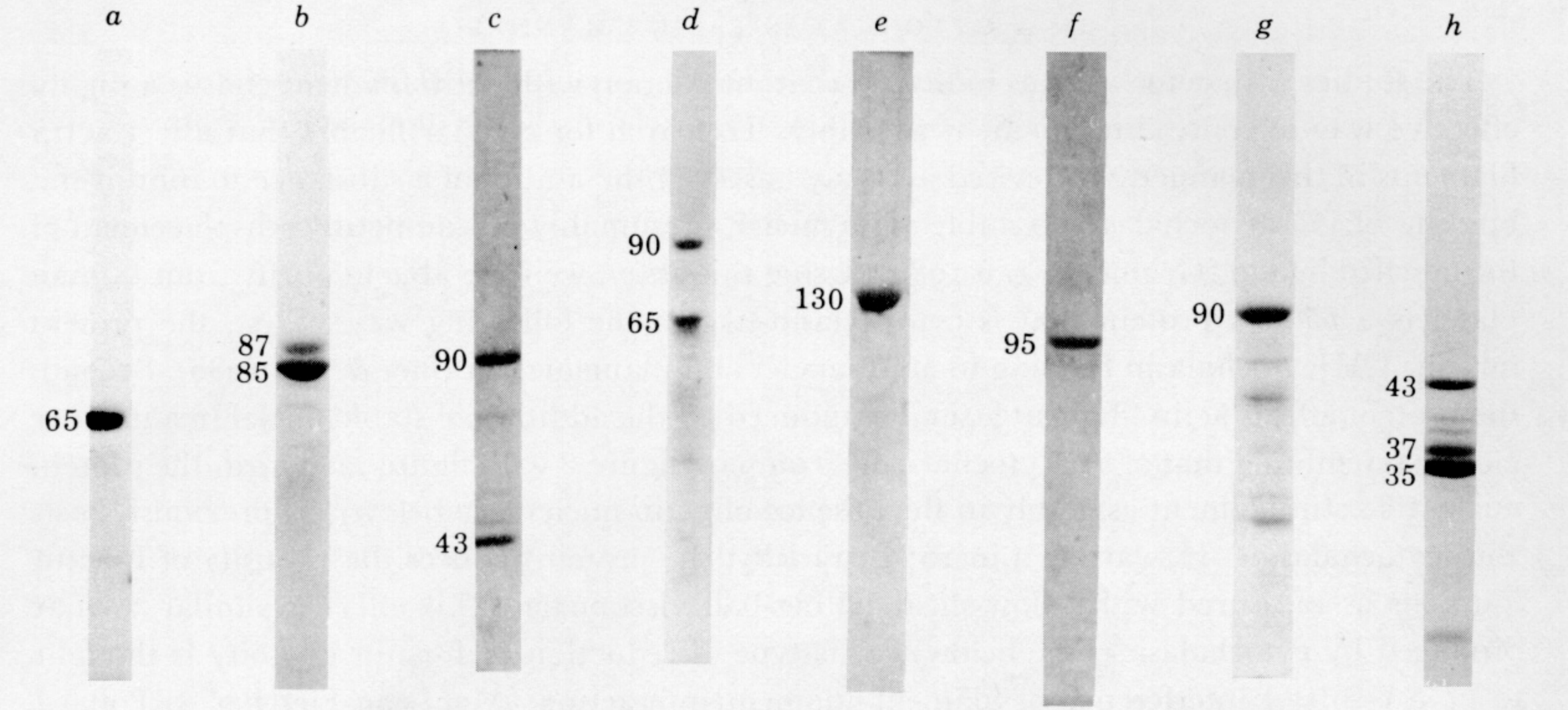

FIGURE 3. Electrophoretic analysis of the capactin preparations described in this paper. Samples were separated by electrophoresis in polyacrylamide slab gels containing sodium dodecyl sulphate, as described by Laemmli (1970). All of the gels contained 7% acrylamide except gel (*h*), which contained 9% acrylamide. Because the gels are from different experiments, the relative position of bands from gel to gel is not significant. The numbers beside each gel designate approximate molecular mass in kilodaltons as determined by comparison with mobility of standard proteins in that particular experiment. Gel (*a*), platelet 65 kDa protein preparation. The DEAE-cellulose column fractions containing capactin activity as described previously (Grumet & Lin 1980) were pooled, concentrated and chromatographed on a Sephacryl S-200 column equilibrated in 50 mM KCl, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM 2-mercaptoethanol, 5 mM Tris-HCl, pH 8.0. Fractions rich in the 65 kDa protein were pooled, concentrated and re-chromatographed on the same column. Gel (*b*), platelet preparation containing 87 and 85 kDa polypeptides. This preparation was obtained as described for the 65 kDa protein, except that fractions from the Sephacryl S-200 column rich in these two polypeptides were pooled, concentrated and re-chromatographed. It is uncertain whether these two polypeptides are related to the 90 kDa protein in gel (*c*). However, we have shown that the two preparations are similar in their ability to inhibit filament assembly nucleated by the spectrin–4.1–actin complex and to decrease F-actin viscosity measured with a low-shear viscometer. Gel (*c*), a platelet preparation enriched in 90 kDa protein obtained by affinity chromatography on an actin–DNase column, as previously described (Lin *et al.* 1981; Wang & Bryan 1981). Note that this preparation contained a mixture of 90 and 43 kDa polypeptides in the molar ratio of about 1:2. These two components are apparently part of a complex and cannot be separated by gel-filtration chromatography. The 43 kDa polypeptide has the same electrophoretic mobility as actin. Gel (*d*), a partly purified capactin preparation from bovine adrenal medulla (Grumet & Lin 1981). Gel (*e*), vinculin isolated from chicken gizzard, as previously described (Feramisco & Burridge 1980; Wilkins & Lin 1982). Gel (*f*), villin, from chicken intestinal brush border microvilli, provided by Dr Klaus Weber and Dr John R. Glenney, prepared as described (Bretscher & Weber 1980; Cribbs *et al.* 1982). Gel (*g*), a partly purified preparation of brevin from human serum. The purification procedure involves fractionation of serum proteins by ammonium sulphate precipitation, followed by chromatography on a DEAE-cellulose column and on a blue agarose column (Lees & Lin 1982). This brevin preparation is similar to that described by Harris & Schwartz (1981) in its ability to inhibit actin filament assembly nucleated by the spectrin–4.1–actin complex and to decrease F-actin viscosity as measured with a low-shear viscometer. Gel (*h*), muscle protein preparation highly enriched in proteins that appear to interact with the pointed ends of actin filaments (Lin *et al.* 1982). This preparation was derived from an acetone-powder extract of rabbit skeletal muscle containing β-actinin (Maruyama *et al.* 1977).