Acta virol. 33 : 193—205, 1989

MECHANISM OF ALTERED CYTOSKELETON ORGANIZATION IN INFLUENZA VIRUS INFECTION

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Received January 3, 1988

Summary. — We followed the autophosphorylation of cytoskeleton (CS) isolated from control chick embryo cell membranes (CS-C) and from these membranes after influenza virus adsorption (CS-V) under conditions allowing to determine the activity of a single type proteinkinase. The $Ca2^{+}$ dependent calmodulin (CaM) kinase used different substrates from CS-V than did the c'AMP dependent proteinkinase. The catalytic subunit (c-subunit) of the c'AMP dependent proteinkinase added from outside phosphorylated the same polypeptides than the endogenous c'AMP dependent proteinkinase, the further being more active than the latter. The purified influenza virus incorporated ^{32}P in the presence of the c-subunit only. Incubation of influenza virus with the c-subunit caused morphological changes visible by electron microscopy. The pleomorphy of the particles as well as their electron transmissibility were enhanced in result of structural alterations and rarefaction of surface spikes of the haemagglutinin and neuraminidase. The contractibility of CS isolated from normal CEC and of the CS from CEC by 15 min postinfection (p.i.) was determined according to the actomyosin ATPase activity. The ATPase activity of the cytoskeleton in the presence of the Ca^{2+}/CaM and that in the presence of c'AMP were used as controls. The virus as well as the Ca^{2+}/CaM increased the ATPase activity. EGTA had no effect but did not interfere with virus stimulation, while c'AMP blocked the virus-induced enhancement of the ATPase activity.

Key words: *influenza virus*; *cytoskeleton contraction*; *phosphorylation*; *actomyosin*; *ATPase*; *chick embryo cell membranes*

Introduction

The organization of actin changes due to different cell functions is regulated by different actin-binding proteins (ABP). Their activity, in turn, is also regulated at higher hierarchic level (Weber and Glenney, 1982; Rosenfeld *et al.*, 1985). Several authors assume that some ABP can bind the receptors

to cytoskeleton (CS) or that the extent of ABP phosphorylation regulates the extent of its interaction with the CS (Sato *et al.*, 1985; Zhuang *et al.*, 1984), and that phosphorylation of some ABP leads to reorganization of the CS (Kawamoto, 1984; Kometani *et al.*, 1986; Lichtfield and Ball, 1986). Phosphorylation is mediated by proteinkinase, which may be activated by binding of a ligand to appropriate receptors (Helmreich and Elson, 1984) or by proteinkinace C, transferred from cytosol to the membranes by the action of of 12-myristate 13-acetate (PMA) (Pontremoli *et al.*, 1987).

Modulation of the interaction between actin-binding proteins may influence the activation of myosin Mg^{2+} dependent ATPase and muscle contraction (Horiuchi *et al.*, 1986; Onii *et al.*, 1987). ABP, myosin and actin may be found in nonmuscle cells. They are all colocalized in moving or phagocytizing cells. The redistribution of F-actin as related to ABP or to myosin is important for rearrangement and orientation of forces involved in phagocytosis (Howard and Meyer, 1984). The distribution and function of CS is influenced also by the so called second messenger molecules. Contraction of CS prepared from human fibroblasts depends on Ca^{2+} and is followed by myosin light chain phosphorylation (20 kD band) by the Ca^{2+}/CaM activated myosin light chain kinase (MLC-kinase) (Masuda *et al.*, 1984). Actin polymerisation and its interaction with phosphorylated myosin and with ABP may be reversed with compounds enhancing the cytosol c'AMP concentration (Wheeler *et al.*, 1985).

We have shown that at early postinfection intervals influenza virus alters the organization and function at least of the submembraneous actin network (Križanová *et al.*, 1986). After adsorption influenza virus enhances the phosphorylation of certain CS proteins by means of an endogeneous proteinkinase, but its relationship to the reorganization of CS has not been elucidated. To approach this question, we tried to identify the proteinkinase involved in ^{32}P incorporation to the CS and the possible relationship of the phosphorylation of individual components to its contraction.

Materials and Methods

Chick embryo cells (*CEC*) were prepared by trypsinization of 11-day-old chick embryos. The cells were grown in Roux flasks in a modified Parker medium supplemented with 8 % inactivated bovine serum and 2 % native horse serum.

Influenza virus A/WSN (*H1N1*) purification, isolation of CEC plasma membranes, virus adsorption to the isolat d membranes, autophosphorylation of CS proteins, analysis of CS components by SDS-PAGE, their autoradiography and densitometric evaluation were described previously (Križanová *et al.*, 1985; Križanová *et al.*, 1986).

Cytoskeleton (*CS*) was prepared either from isolated CEC plasma membranes or from CEC monolayer as described (Meisheri and Rüegg, 1983).

Protein content was determined by the method of Lowry *et al.*, 1951.

ATPase activity of the cytoskeleton (*CS*). The CS prepared from CEC monolayers was incubated for 15 min at room temperature either with virus-infected allantoic fluid (10—20 virions per cell, CS-V) or with noninfected allantoic fluid (CS-NAF). Nonadsorbed virus was removed before isolation of CS. Closely before measuring the ATPase activity the isolated CS was incubated for 20 min at 25 °C in ATPase buffer (20 mmol/l imidazol-HCl pH 7.2, 60 mmol/l KCl, 1 mmol/l $MgCl_2$, 0.4 mmol/l DTT, 0.2 mmol/l EGTA) which enhanced the susceptibility to Ca^{2+} (Nag and Seidel, 1983).

ATPase activity was determined in 1 mg of isolated CS in the presence of 0.3 mmol/l $CaCl_2$, or 0.3 mmol/l $CaCl_2$ and 1.5 μmol/l CaM, or in the presence of 10^{-4} mol/l c'AMP or 0.5 mmol/l EGTA. The reaction started by adding of ATP in a final concentration of 2 mmol/l. After incubation for 0 or 15 min (25 °C) the proteins were precipitated with 2 vol of 15 % trichloroacetic acid (TCA) containing 1 % active carbon and centrifuged. The amount of released Pi was determined in the supernatant according to Fiske and Subbarow (1925). The ATPase activity was expressed as a difference in the amount of released Pi at 15 min and 0 min (Pi 15 min − Pi 0 min).

The effect of virus on the ATPase activity of the isolated CS was determined as follows: after incubation of CS in the imidazol buffer 1500 haemagglutination units per mg CS protein were added (CS-V). The control CS mixture (CS-C) was prepared by adding 10 mmol/l Tris-HCl and 150 mmol/l NaCl (pH 7.2). The ATPase activity was determined as described. Activity of the same amount of purified virus was substracted from that of CS-V.

Electron microscopy. Virus suspension preincubated for 10 min at 30 °C in phosphorylation buffer (40 mmol/l Hepes pH 6.9, 10 mmol/l $MgCl_2$, 0.1 mmol/l $CaCl_2$, 14 mmol/l DTT) under various conditions was layered on formwar coated or carbon coated grids. After attachment (2 min), the grids were stained with 2 % phosphowolfram acid (pH 7) for 1 min and investigated in the microscope Philips EM 300 at 80 kV.

Drugs. Cyclic AMP (c'AMP) and adenosin triphosphate (ATP) from Merck; imidazol, the catalytic subunit (c-subunit) of the c'AMP-dependent proteinkinase and c'AMP proteinkinase inhibitor (c'AMP Prki inhibitor) were from Sigma. Ethalenbisglycol tetraacetic acid (EGTA) were from Fluka. Calmodulin (CaM) was prepared from chick embryos as described by Križanová *et al.* (1979). Dithiothreitol (DTT) was from Calbiochem. All other compounds not listed here were analytic grade.

Results

^{32}P *incorporation into individual CS proteins after virus adsorption*

To determine the type of proteinkinase transferring ^{32}P from $(\gamma)^{32}P$-ATP to individual proteins, phosphorylation was accomplished: 1. in the presence of 1 μmol/l c'AMP and 2 mmol/l EGTA in phosphorylation buffer, 2. in the presence of 30 units of the c-subunit, 3. in the presence of 1 μmol/l CaM, and finally 4. in the presence of 1 μmol/l CaM and 30 μg of c'AMP dependent kinase inhibitor. The conditions under 1 and 2 allow to determine the c'AMP dependent proteinkinase while the conditions under 3 and 4 the Ca^{2+} dependent CaM kinase. Incorporation of ^{32}P into CS observed in the presence of 2 mmol/l EGTA or 2 mmol/l EGTA and 30 μg c'AMP kinase inhibitor corresponded to the basic activity provided by an unknown enzyme or by proteinkinase C-M, a new enzyme occurring after cleavage of proteinkinase C due to the Ca^{2+}-activated protease (Pontremoli *et al.*, 1987).

The results of CS analysis under above mentioned phosphorylation conditions are shown on Fig. 1. As visible in Fig. 1-a, in the presence of c'AMP ^{32}P has been incorporated into the following CS-V polypeptides: 170 kD, 100 kD, 85 kD, 51 kD, 47 kD, 41 kD, 37 kD, 34 kD, and 27—25 kD; in addition, one polypeptide incorporating ^{32}P moved at the front of the 10 % gel in SDS-PAGE revealing a M_r lower than 23 kD. The c-subunit of c'AMP dependent proteinkinase (Fig. 1-b) not only enhanced the phosphorylation of above mentioned polypeptides but also transferred ^{32}P, although to lower extent, from $(\gamma)^{32}P$-ATP to some of above mentioned polypeptides of CS-C (polypeptides 170 kD, 100 kD, 85 and 34 kD showed minimal labelling). When CS-C was incubated under similar conditions as CS-V in the absence of the c-subunit, ^{32}P had been incorporated to the 51 kD polypeptide only.

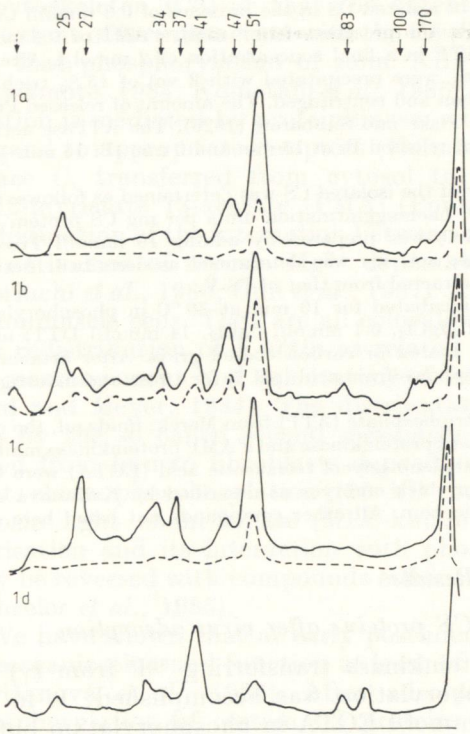


Fig. 1.

Influence of phosphorylation conditions of ^{32}P incorporation into CS proteins

CS proteins (45—50 μg) were isolated from plasma membranes of CEC, incubated for 10 min at 30 °C in the phosphorylation buffer (40 mmol/l Hepes, 10 mmol/l $MgCl_2$, 0.1 mmol/l $CaCl_2$, 0.1 mmol/l DTT, pH 6.9) to which some of substances indicated below had been added in combination with $2 \times 10^6 - 2 \times 20^7$ cpn of $(\gamma)^{32}P$-ATP. The reaction was stopped by adding the solubilizing solution. After heating for 3 min (100 °C) the CS proteins were separated in SDS-PAGE. Phosphorylated proteins were detected by autoradiography and by densitometry. Sold arrows indicate the apparent M_r in kD, interrupted arrow points at polypeptides moving with the front. Phosphorylation conditions:

1—a: in the presence of μmol/l c'AMP and 0.5 mmol/l EGTA (c'AMP dependent proteinkinase)

1—b: 30 units of the c- subunit of c'AMP proteinkinase

1—c: 1 mmol/l CaM and 30 μg c'AMP proteinkinase inhibitor (Ca^{2+}/CaM dependent proteinkinase activity)

1—d: purified influenza virus in the presence c'AMP-subunit

CS-C — cytoskeleton from control membranes (— — — —)

CS-V — cytoskeleton from virus treated membranes (————)

These results indicate that adsorption of the virus to the CEC membranes may enhance the activity of c'AMP dependent proteinkinase in the isolated CS.

During phosphorylation of CS-V by the Ca^{2+}/CaM dependent proteinkinase no incorporation of ^{32}P into polypeptides of M_r 170 kD, 100 kD, 85 kD, 41 kD and 25 kD was observed (Fig. 1-c). The phosphorylation of the 47 kD protein was decreased, while the phosphorylation of proteins M_r 37 kD, 34 kD and 27 kD, and proteins moving at the front in SDS-PAGE was increased indicating that the Ca^{2+}/CaM dependent proteinkinase acts on other substrates in CS-V than the c'AMP dependent proteinkinase. When in the phosphorylation mixture, Ca^{2+} was chelated with EGTA or CaM by chlorpromazine, or the activity of c'AMP dependent proteinkinase was blocked by an inhibitor of the c'AMP dependent proteinkinase, the ^{32}P accumulated in similar CS-V polypeptides as under action of c'AMP dependent proteinkinase (results not shown).

The purified virus alone showed no proteinkinase activity. Under various phosphorylation conditions in the absence of exogenous proteinkinase, no

Table 1. Influence of influenza virus on the cytoskeleton ATPase activity

ATPase activity in the presence of	Experiment	nmol Pi released per mg CS/15 min		
		from CS-NAF	from CS-V	enhancement by the virus
0.3 mmol/l Ca^{2+}	A	79.3*	117.7	148 %
0.3 mmol/l Ca^{2+} and 1.5 μmol/l CaM		96.3	127.2	131 %
0.5 mmol/l EGTA		50.7	87.5	172 %
0.1 mmol/l c′ AMP		54	52.4	97 %
		nmol Pi released per mg CS/15 min		
		from CS-C	from CS-V	enhancement
0.3 mmol/l Ca^{2+} and 1.5 μmol/l CaM	B	53.2**	60.4	113.5 %
0.5 mmol/l EGTA		37	56.2	150 %
0.1 mmol/l c′ AMP		32	37	115.3 %

A — CS isolated from 48-hr-old CEC monolayers to which influenza virus had been adsorbed (CS-V); NAF = normal allantoic fluid;
* average of 4 measurements
B — Purified influenza virus (1500 HAU/mg CS) or physiological saline were added to CS (CS-V or CS-C). Viral ATPase activity was substracted from CS-V values.
** average of 3 measurements
For further details see *Mat. and Methods.*

labelled protein bands were seen in SDS-PAGE. In the presence of c-subunit of the c′AMP dependent proteinkinase we observed ^{32}P incorporation into several viral proteins. The 97 kD polypeptide was not labelled in CS-V and the amount of the labels in the 41 kD CS-V polypeptide was decreased; in the presence of Ca^{2+}/CaM no such CS-V band became labelled. The 34 kD band was especially prominent when CS-V was phosphorylated in the presence of Ca^{2+}/CaM and the c′AMP dependent proteinkinase inhibitor.

To determine whether phosphorylation of viral components may influence the virion morphology, we investigated by electron microscopy two previously purified virion samples incubated under similar conditions with the c-subunit and with ATP. Virus samples incubated with the c-subunit only were used as controls; another controls were the virus incubated with ATP or with the buffer above. In parallel, the ^{32}P incorporation from (γ) ^{32}P-ATP was checked in the presence of c-subunit.

Incubation of influenza A/WSN in phosphorylation buffer in the presence of ATP (Fig. 2-II) did not cause morphologic changes of virions. They retained a spherical shape with a small pleomorphic variability, showed neither changes in electron density nor in the distribution of haemagglutinin and

neuraminidase spikes. Only a few particles revealed a clear-cut envelope structure. Viral particles incubated with the c-subunit (Fig. 2-III) and those incubated with the c-subunit in the presence of ATP (Fig. 2-IV) showed more evident morphologic alterations as compared with control specimens (Figs. 2-I; 2-II). Virions became more pleomorphic, more electron translucent due to rarefaction of the haemagglutinin and neuraminidase spikes. All particles incubated with the c-subunit in the presence of ATP showed more prominent envelope structure and an enlarged volume.

Fig. 3 shows (in accord with previous experiments) that viruses lacking the c-subunit did not incorporate ^{32}P from (γ) ^{32}P-ATP. In contrast, several virion components were phosphorylated in the presence of the c-subunit. Whether these changes participate in the uncoating of virions remains unclear.

Cytoskeleton contraction

The biochemical corrélate of CS contraction are superprecipitation and activation of actinomyosin ATPase (Hidaka *et al.*, 1979; Kanamori *et al.*, 1981). The Ca^{2+}/CaM stimulates the contraction of isolated CS (Rüegg and Paul, 1982; Rüegg *et al.*, 1984; Masuda *et al.*, 1984), while c'AMP and CaM antagonists cause CS relaxation (Nagai *et al.*, 1985; Hidaka *et al.*, 1979; Rüegg and Paul, 1982; Meisheri and Rüegg, 1983; Sobue *et al.*, 1985). As shown by preliminary experiments, CS prepared from 48-hr-old CEC monolayers is sensitive to Ca^{2+}/CaM. Tab. 1 shows that such CS (CS-NAF) responded to Ca^{2+}/CaM by increasing its ATPase activity. If influenza virus had been adsorbed to CEC before CS isolation, ATPase activity of CS increased also in the absence of CaM or even in the presence of EGTA. The activity detected in the presence of 10^{-4} mol/l c'AMP was practically identical in CS-NAF and/or CS-V (Tab. 1). Therefore, we assume that the virus stimulated CS contraction.

Similar results were obtained when purified virus was added to already isolated CS (Tab. 1B). CS preparations which did not respond to Ca^{2+}/CaM revealed no enhancement of their ATPase activity not even after adding of the virus (results not shown).

Discussion

The results of this work have shown that Ca^{2+}/CaM increased the ATPase activity of isolated CS. In the CS isolated from chick embryo fibroblasts with adsorbed virus (CS-V) the specific activity of ATPase has been increased in the absence of CaM and even in the presence of EGTA. We believe that after adsorption to corresponding cell receptors influenza virus causes reorganization of CS leading to its contraction.

The prerequisite of Ca^{2+} dependent contraction in the CS model prepared from human fibroblasts is the phosphorylation of myosin light chain (MLC) by the Ca^{2+}/CaM dependent MLC-kinase (Masuda *et al.*, 1984). The necessity of MLC phosphorylation has been described by several authors.

Electrophoresis under our conditions did not allow to determine the M_r of proteins when lower than 23 kD. In the CS isolated from CEC plasma membranes with adsorbed virus (CS-V) these polypeptides became phosphorylated also in the absence of CaM, but in the presence of Ca^{2+}/CaM their phosphorylation has more increased. The surface delineated by the phosphorylation curve in the presence of c'AMP proteinkinase was 0.11, while that of outlined by the phosphorylation curve in the presence of Ca^{2+}/CaM was 0.4. In the CS isolated from noninfected plasma membranes (CS-C) the surface outlined by c'AMP kinase phosphorylation was 0.05 and that by Ca^{2+}/CaM phosphorylation 0.09. We assume that this fraction contains the phosphorylated MLC important for the Ca^{2+}-dependent contraction.

In addition to MLC phosphorylation, Ca^{2+} related contraction depends on the organization of actin filaments (Coleman and Mooseker, 1985). Several Ca^{2+}-sensitive actin binding proteins have been described which confer $Ca2^{+}$ sensitivity for the regulation of filamentous or network CS structures. Just recently, the CaM-binding and/or CS-binding activity of two polypeptides, 34 kD and 35—36 kD has been characterized (Takahashi *et al.*, 1986; Sobue *et al.*, 1987). It should be cleared, however, whether the increased incorporation of ^{32}P into polypeptides 27 kD, 35 kD and 37 kD in the presence of Ca^{2+}/CaM is associated with a similar activity. Ca^{2+} induced CS contraction may be reversed (relaxed) by c'AMP, their analogues and by substances enhancing cellular c'AMP levels (Rüegg and Paul, 1982).

Interaction actin-myosin (contraction — relaxation) may be regulated also by phosphorylation — dephosphorylation of certain actin-binding proteins. Relaxation occurs when the 150 kD protein becomes phosphorylated to bind actin and myosin (Hartzell and Glass, 1984). In turn, phosphorylation of caldesmon (M_r 70 kD or 150 kD) hinders its interaction with myosin and actin (Horiuchi *et al.*, 1986). A good substrate for the c'AMP dependent proteinkinase as well as for proteinkinase C is the membrane skeleton-associated calmodulin-binding protein (CaM-BP 103/97) (Ling *et al.*, 1986). It binds to the spectrin-actin complex and enhances the capacity of actin filaments to interact with spectrin. The significance of its phosphorylation is unknown, however. In the presence of c'AMP and EGTA or in the presence of the c-subunit of the c'AMP dependent proteinkinase we found that proteins of the infected cytoskeleton (CS-V) of apparent M_r 170 kD, 100 kD, 85 kD and 41 kD were phosphorylated; their role in the reorganization of CS is still unknown. Selective phosphorylation of proteins by proteinase C is considered for a mechanism transferring environmental signals to cell interior (Pontremoli *et al.*, 1987).

Several authors found that proteinkinase C is translocated from the cytosol to the plasma membranes during cell activation. The membranes release a neutral protease (Ca^{2+}-activated protease called calpain) which cleaves proteinkinase C and transforms it to a new form, the C-M proteinkinase. This is fully active in the absence of phospholipids and Ca^{2+} and does not bind to the plasma membranes (Pontremoli *et al.*, 1987). CS extracted with Triton X-100 is an unsuitable substrate for proteinkinase C but a good substrate for proteinkinase C-M, which phosphorylates more CS proteins. We found an en-

hanced phosphorylation of CS proteins in the virus-infected CS even in the presence of EGTA or of the c'AMP dependent proteinkinase inhibitor.

Based on our previous observations (Križanová *et al.*, 1976; Križanová *et al.*, 1982) we believe that influenza virus at early intervals post-adsorption activates the adenylatcyclase system increasing the level of cellular c'AMP, which, in turn, activates the c'AMP dependent proteinkinase. Phosphorylation by means of the latter enzyme opens the calcium channel (Hescheler *et al.*, 1987) and Ca^{2+} ions enter the cytoplasm. In addition, phosphorylation of other membrane proteins and of actin binding proteins may translocate the CaM from the membrane to cytosol (phosphorylated CaM binding proteins show a lower affinity to CaM) (Malenčik, 1982) and interrupt the association of CS with actin binding proteins in the vicinity of plasma membrane allowing rearrangement of CS subunits (or of actin microfilaments). Intracellular Ca^{2+}/CaM activates the MLC kinase which phosphorylates MLC as well as other actin binding proteins (C-M proteinkinase may be phosphorylated as well). Stress (contractory) filaments are being formed allowing ATP-associated contraction inportant for signal transfer.

The proposed scheme may reflect early postinfection events; its validity should be investigated using specific antiglobulins.

Acknowledgement. The authors thank Mrs. A. Demová for the excellent technical assistance.

References

Coleman, T. R., and Mooseker, M. S. (1985): Effects of actin filament cross-linking and filament length on actin-myosin interaction. *J. Cell Biol.* **101**, 1850—1857.

Fiske, C. H., and Subbarow, Y. (1925): The colometric determination o phosphorus. *J. biol. Chem.* **66**, 375—378.

Hartzell, H. C., and Glass, D. B. (1984): Phosphorylation of purified cardiac muscle C-protein by purified cAMP-dependent and endogenous Ca^{2+}-calmodulin-dependent protein kinases. *J. biol. Chem.* **259**, 15587—15596.

Helmreich, E. J. M., and Elson, E. L. (1984): Mobility of proteins and lipids in membranes. *Adv. cyclic Nucleot. Protein phosphoryl. Res.* **18**, 1—61.

Hescheler, J., Kameyama, M., Trautwein, W., Mieskes, G., and Soling, H. D. (1987): Regulation of the cardiac calcium chanel by protein phosphatases. *Eur. J. Biochem.* **165**, 261—266.

Hidaka, H., Yamaki, T., Totsuka, T., and Asano, M. (1979): Selective inhibitors of Ca^{2+}-binding modulator of phosphodiesterase produce vascular relaxation and inhibit actin-myosin interaction. *Mol. Pharmacol.* **15**, 49—59.

Horiuchi, K. Y., Miyata, H., and Chacko, S. (1986): Modulation of smooth muscle actomyosin ATPase by thin filament associated proteins. *Biochem. Biophys. Res. Commun.* **136**, 962—968.

Howard, T. H., and Meyer, W. H. (1984): Chemotactic peptide modulation of actin assembly and locomotion in neutrophils. *J. Cell Biol.* **98**, 1265—1271.

Kanamori, M., Naka, M., Asano, M., and Hidaka, H. (1981): Effects of N-(6-aminohexyl)-5-chloro-1-naphtalenesulfonamide and other calmodulin antagonists (calmodulin interacting agents) on calcium-induced contraction in rabbit aortic strips. *J. Pharmacol. exp. Ther.* **217**, 494—499.

Kawamoto, S., and Hidaka, H. (1984): Ca^{2+}-activated, phospholipid-dependent protein kinase catalyzes the phosphorylation of actin-binding proteins. *Biochem. Biophys. Res. Commun.* **118**, 736—742.

Kometani, M., Sato, T., and Fujii, T. (1986): Effect of membraneinteracting amphiphiles on association of membrane glycoproteins with assembled cytoskeletal proteins in concanavalin A-activated rabbit platelets. *Thromb. Res.* **42**, 567—577.

Križanová, O., Kočišková, D., and Ondrušková, J. (1976): Interaction of plasma membranes with influenza virus. VI. The possible role of the adenylate cyclase system. *Acta virol.* **20**, 283—290.

Križanová, O., Soláriková, L., and Hána, L. (1979): Role of calcium-dependent regulator protein (CDR) in inhibition of 3′,5′-c′AMP-phosphodiesterase by influenza virus. I. Isolation and purification of CDR and CDR-dependent 3′,5′-c′ AMP-phosphodiesterase from chick embryos. *Acta virol.* **23**, 295—302.

Križanová, O., Čiampor, F., and Veber, P. (1982): Influence of chloropromazine on the replication of influenza virus in chick embryo cells. *Acta virol.* **26**, 209—216.

Križanová, O., Matis, J., Závodská, E., and Stanček, D. (1985): Effect of influenza virus on protein phosphorylation in isolated membranes of chick embryo cells. *Acta virol.* **29**, 1—10.

Križanová, O., Čiampor, F., Závodská, E., Matis, J., and Stanček, D. (1986): Effect of influenza virus on the interaction of cytoskeleton with cell membranes. *Acta virol.* **30**, 273—280.

Ling, E., Gardner, K., and Bennett, V. (1986): Protein kinase C phosphorylates a recently identified membrane skeleton-associated calmodulin-binding protein in human erythrocytes. *J. biol. Chem.* **261**, 13875—13878.

Litchfield, D. W., and Ball, E. H. (1986): Phosphorylation of the cytoskeletal protein talin by protein kinase C. *Biochem. Biophys. Res. Commun.* **134**, 1276—1283.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 165—175.

Masuda, H., Owaribe, K., Hayashi, H., and Hatano, S. (1984): Ca^{2+}-dependent contraction of human lung fibroblasts treated with Triton X-100: A role of Ca^{2+} -calmodulin-dependent phosphorylation of myosin 20,000-Dalton light chain. *Cell Motility* **4**, 315—331.

Malencik, D. A., Anderson, S. R., Bohnert, J. L., and Shalitin, Y. (1982): Functional interactions between smooth muscle myosin light chain kinase and calmodulin. *Biochemistry* **21**, 4031—4039.

Meisheri, K. D., and Rüegg, J. C. (1983): Dependence of cyclic-AMP induced relaxation on Ca^{2+} and calmodulin in skinned smooth muscle of guinea pig *Taenia coli*. *Pflügers Arch.* **399**, 315—320.

Nag, S., and Seidel, J. C. (1983): Dependence on Ca^{2+} and tropomyosin of the actin-activated ATPase activity of phosphorylated gizzard myosin in the presence of low concentrations of Mg^{2+}. *J. biol. Chem.* **258**, 6444—6449.

Nagai, H., Yamada, H., Goto, S., Inagaki, N., and Koda, A. (1985): Reduction of antigen-induced contraction of sensitized guinea-pig tracheal smooth muscle in vitro by calmodulin inhibitors. *Int. Archs Allergy appl. Immun.* **76**, 251—255.

Onji, T., Tagaki, M., and Shibata, N. (1987): Calpain abolishes the effect of filamin on the actomyosin system in platelets. *Biochim. Biophys. Acta* **912**, 283—286.

Pontremoli, S., Melloni, E., Michetti, M., Sparatore, B., Salamino, F., Sacco, O., and Horecker, B. L. (1987): Phosphorylation and proteolytic modification of specific cytoskeletal proteins in human neutrophils stimulated by phorbol 12-myristate 13-acetate. *Proc. natn. Acad. Sci. U.S.A.* **84**, 3604—3608.

Rosenfeld, G. C., Hou, D. C., Dingus, J., Meza, I., and Bryan, J. (1985): Isolation and partial characterization of human platelet vinculin. *J. Cell Biol.* **100**, 669—676.

Rüegg, J. C., and Paul, R. J. (1982): Vascular smooth muscle. Calmodulin and cyclic AMP-dependent protein kinase alter calcium sensitivity in porcine carotid skinned fibers. *Circ. Res.* **50**, 394—399.

Rüegg, J. C., Pfitzer, G., Zimmer, M., and Hofmann, F. (1984): The calmodulin fraction responsible for contraction in a intestinal smooth muscle. *FEBS Letts.* **170**, 383—386.

Sato, Ch., Nishizawa, K., Nakayama, T., and Kobayashi, T. (1985): Effect upon mitogenic stimulation of calcium-dependent phosphorylation of cytoskeleton-associated 350,000- and 80,000-mol-wt polypeptides in quiescent 3Y1 cells. *J. Cell Biol.* **100**, 748—753.

Sobue, K., Tanaka, T., Kanda, K., Takahashi, K., Ito, K., and Kakiuchi, S. (1985): A dual regulation of the actin-myosin interaction in adrenal medullary actomyosin by actin-linked and myosin-linked systems. *Biomed. Res.* **6**, 93—102.

Sobue, K., Okabe, T., Kadowaki, K., Itoh, K., Tanaka, T., and Fujio, Y. (1987): Cytosynalin: A M_r 35,000 cytoskeleton-interacting and calmodulin-binding protein. *Proc. natn. Acad. Sci. U.S.A.* **84**, 1916—1920.

Takahashi, K., Hiwada, K., and Kokubu, T. (1986): Isolation and characterization of a 34 000-Dalton calmodulin- and F-actin-binding protein from chicken gizzard smoth muscle. *Bicchem. Biophys. Res. Commun.* **141**, 20—26.

Weber, K., and Glenney, J. R. (1982): Calcium-modulated multifunctional proteins regulating F-actin organization. *Cold Spring Harbor Symp.* **46**, 541–552.

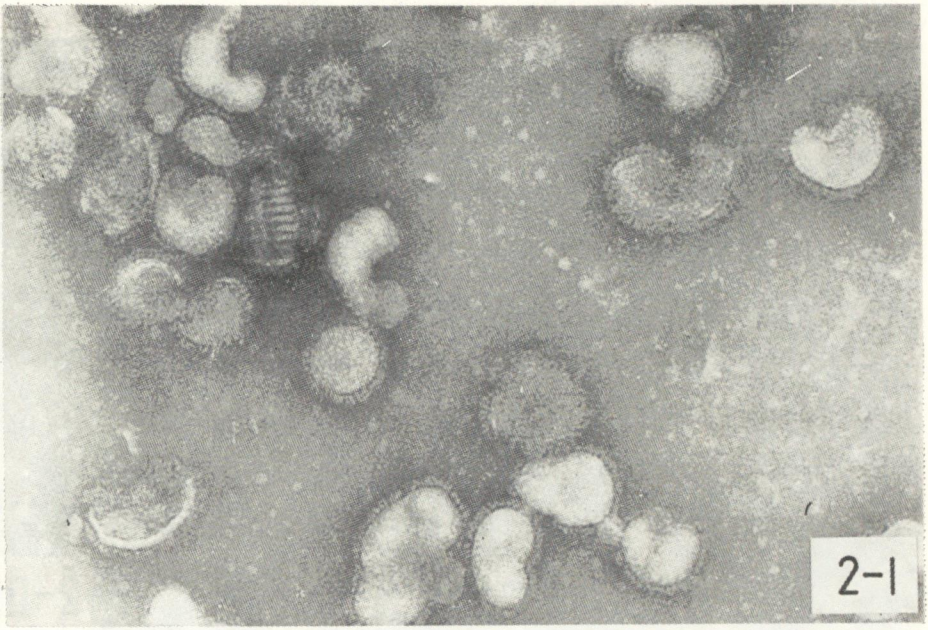
Wheeler, M. E., Gerrard, J. M., and Carroll, R. C. (1985): Reciprocal transmembranous receptor-cytoskeleton interactions in concanavalin A-activated platelets. *J. Cell Biol.* **101**, 993–1000.

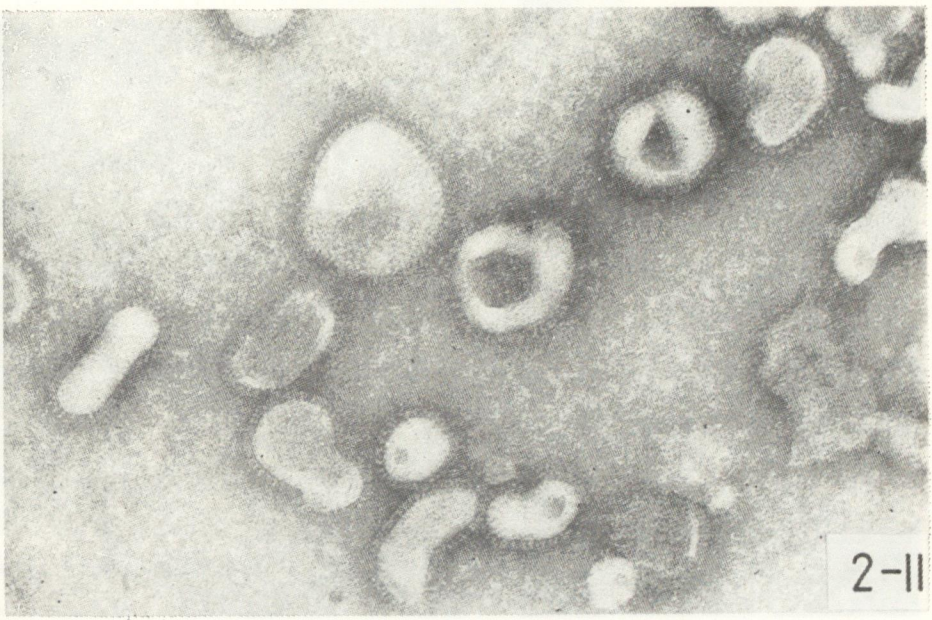
Zhuang, Q. Q., Rosenberg, S., Lawrence, J., and Stracher, A. (1984): Role of actin binding protein phosphorylation in platelet cytoskeleton assembly. *Biochem. Biophys, Res. Commun.* **118**, 508–513.

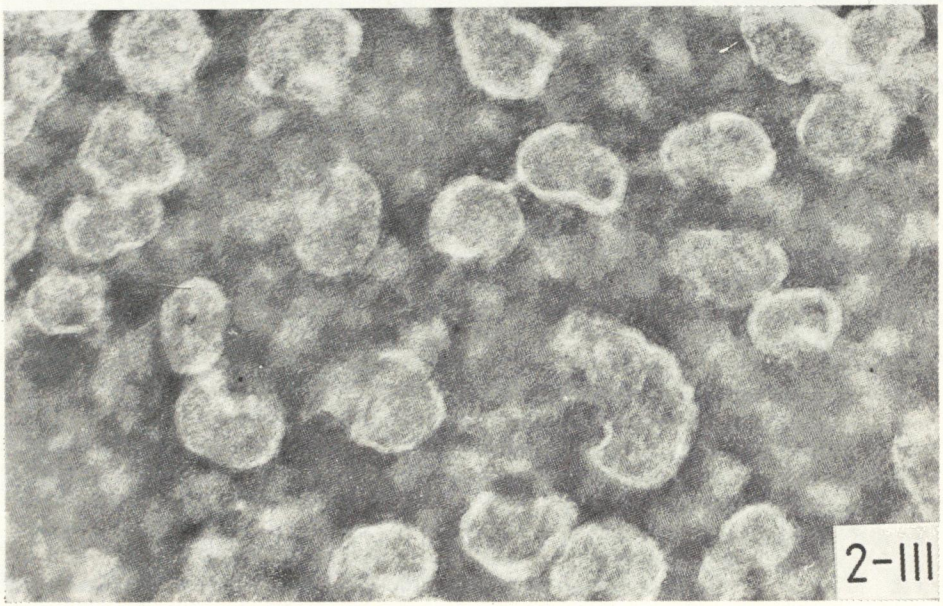
Legends to the Figures (pages 203–205):

Fig. 2. Electron microscopy of phosphorylated virions.
I — Influenza virus A/WSN in the phosphorylation buffer, contrasted with 2 % PTA, magn. × 99 000.
II — Influenza virus A/WSN incubated in the presence of 0.57 mmol/l ATP; magn. × 99 000.
III — Influenza virus A/WSN incubated with 30 units of the c-subunit; magn. × 99 000.
IV — Influenza virus A/WSN incubated in the presence of 30 units of the c-subunit and of 0.57 mmol/l ATP; magn. × 99 000.

Fig. 3. Phosphorylation of influenza virus in the presence or absence of the c-subunit of c′AMP-dependent proteinkinase.
Two purified influenza A/WSN (H1N1) samples were tested
a, b — purified virions stored at 4 °C for 7 months
c, d — purified virions stored for 7 days (at 4 °C)
For phosphorylation conditions see Legend to Fig. 1.
As the c-subunit had been dissolved in 100 mmol/l DDT, the same amount of DDT was added to the virus, phosphorylated in the absence of the c-subunit.
a, c — virus autophosporylation in the absence of the c-subunit
b, d — virus phosphorylation in the presence of 30 units of the c-subunit


2-I


2-II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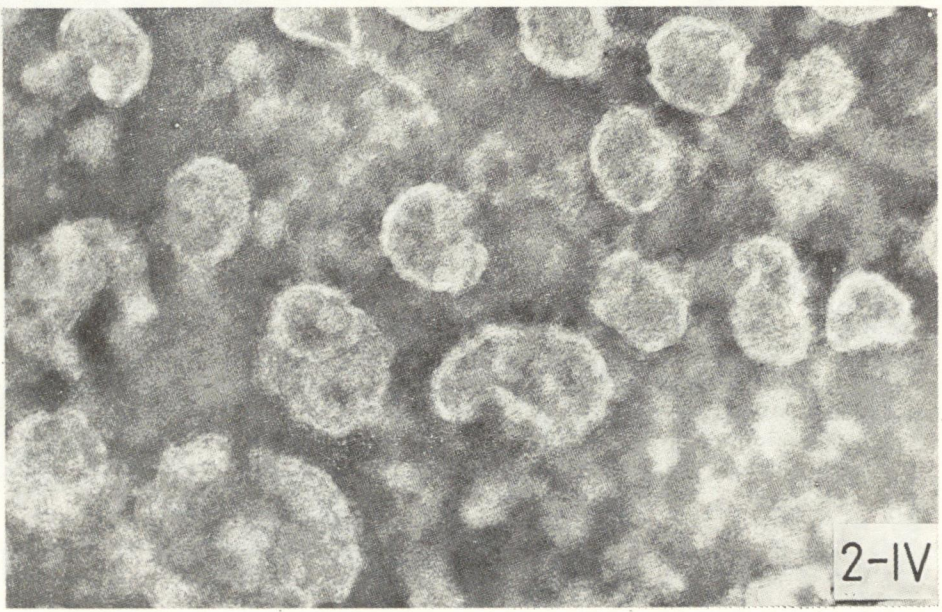
2-IV

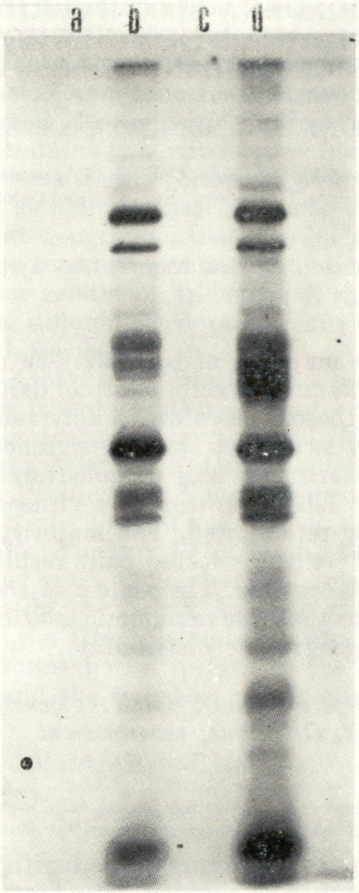

Fig. 3

For legend see page 202.