Vol. 118, No. 2, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1984 Pages 580-586

PRESENCE OF ACTIN IN ONCORNAVIRUSES

Lena STANISLAWSKY, Françoise MONGIAT and Vivaldo MOURA NETO*

Laboratoire de Médecine Expérimentale, Unité INSERM 112, et
**Laboratoire de Biochimie Cellulaire, Collège de France,*
11, Place Marcelin Berthelot, 75231 Paris Cedex 05, France

Received December 12, 1983

SUMMARY : A 43K protein present in avian myeloblastosis virus has been identified as actin by 2D gel electrophoresis and peptide mapping proteolysis. Electron microscopy of chicken embryo fibroblasts infected with different pseudotypes of oncornaviruses treated with anti-actin antibody showed positive staining at the level of the virions especially on buds.

Our results indicate that this actin is unlikely to have been artefactually absorbed at the virion surface during its preparation. It may therefore play a possible role in the budding of enveloped virions.

Various non-muscular eukaryotic cells have been found to contain cytoplasmic actin. The functions of actin in non-muscle cells are diverse : it has been involved in cell locomotion, transport, secretion and cytokinesis (for a review, see 1).

Recent data suggest that actin is also present in some enveloped viruses and that host-cell actin may be incorporated into the virion during the process of budding on the plasma membrane (2-4).

This report concerns oncornaviruses, that are enveloped viruses which form by budding from the cell surface. We provide biochemical and also morphological evidence that actin is indeed present in avian myeloblastosis virus (AMV) and other leucosarcomatosis viruses. This protein is most probably carried away from the host-cell during the process of virion formation.

MATERIAL AND METHODS

Viruses and cells. AMV purified by two successive centrifugations on sucrose gradient was kindly provided by Dr. D. Levy. RAV-1, RAV-2, Sr-RSV-B, RSV-RAV-2 were obtained from NIH (Bethesda 100214, USA). Chick embryo fibroblasts (CEF) were grown in culture and infected with the above viruses as described (5).

Immunoenzymatic staining. Actin was detected in infected CEF by indirect immunoperoxidase labelling. Anti-actin antibody was purified from a rabbit

0006-291X/84 $1.50

anti-actin serum by affinity chromatography (6). Purified rabbit muscle actin was a kind gift of Dr. C. Oriol-Audit. Peroxidase-conjugated anti-rabbit IgG antibody was from the Institut Pasteur (Paris). Infected CEF were washed rapidly with "cytoskeletal" buffer (7) and treated with 0.1 % Triton X-100 for 30 s, washed for 1 min and fixed with 1 % glutaraldehyde for 10 min. After a brief wash in PBS, the cells were treated with 0.1 M L-lysine for 1 h and then with trypsin 0.25 % for 15 min at 35°C. Treatment with both antibodies was for 1 h at 0.1 mg / ml for anti-actin and at 1/100 dilution for the peroxidase conjugate. Peroxidase activity was revealed with 3,3'-diamino-benzidine tetrahydrochloride (8). The cells were then fixed with 0.1 % OSO_4 for 20 min, dehydrated and embedded *in situ* as described (9). The sections were observed in a Siemens Elmiskop II. For conventional electron microscope observation the cells were fixed in 1 % glutaraldehyde and proceeded as above.

Gel analysis. Isoelectric focusing in 9.5 M urea (Schwarz Mann) was performed according to O'Farrell (10). The pH ranges of LKB ampholines used were 3.5-10 and 5-8 (1:4, v/v). Second dimension electrophoresis or slab gel electrophoresis (both at 12 % acrylamide) were carried out as described (11) with twice the concentration of Tris and glycine in the running buffer. Staining was done with Coomassie blue. For autoradiography the gels were processed for fluorographic detection of radioactivity (12).

Peptide mapping. Limited proteolysis with *S. aureus* V8 protease (1 ng per spot, Miles) and peptides revealed by silver coloration (13) were analysed according to Cleveland *et al.* (14).

CEF protein labelling and extraction. CEF were labelled with 15 µCi / ml of ^{35}S-methionine (Amersham, 1600 Ci / mmol) for 18 h. The cells were ethanol precipitated (15), the pellet was resuspended in actin depolymerization buffer (G buffer) : 2 mM Tris, 0.1 mM $CaCl_2$, 1 mM 2-mercaptoethanol and 1 mM ATP, pH 7.5 (16), and centrifuged (15 min, 27 000 *g*). The labelled CEF soluble extract (18 000 cpm / µl) was incubated with viral preparation as described in fig. 4.

RESULTS AND DISCUSSION

When AMV proteins were analysed by SDS-PAGE a 43K protein was observed (fig. 1B) which co-migrated with brain actin (fig. 1A). The p27, p29 and other viral proteins showed an usual pattern. In order to better identify this viral component, electrophoretic patterns of the AMV proteins and rabbit muscle actin were compared by 2D gel electrophoresis. Fig. 2a and 2c show that the viral 43K protein and actin share a similar molecular weight and isoelectric point. Moreover, when viral proteins and actin were mixed prior to 2D-gel analysis, the 43K viral protein and the actin were observed in same region, as expected for actin isoforms (fig. 2B). Further evidence was obtained from peptide mapping of these two proteins. After the second dimension electrophoresis of the viral proteins, the spot corresponding to 43K protein was cut out and subjected to digestion by protease V8 (fig. 3B). The pattern obtained was very similar to a digestion of rabbit muscle actin extracted from a 2D-gel electrophoresis also (fig. 3A). From this first

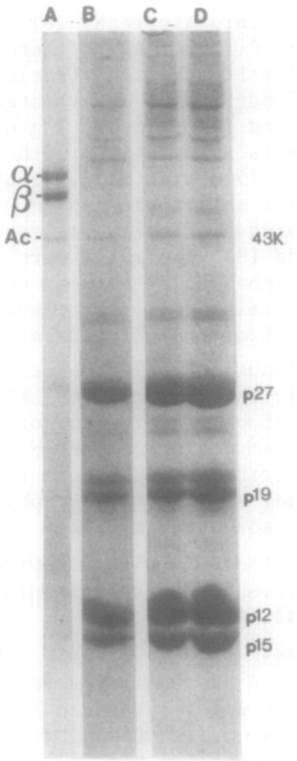

Fig. 1. SDS-PAGE of AMV proteins : A) Actin and tubulin enriched preparations from mice brain (a kind gift of Dr. A. Wolff) used here as molecular markers. B) AMV proteins after viral washing with PBS. C) AMV proteins from viral washed with G buffer. D) AMV proteins from viral washed with G buffer + 0.15 M NaCl. Ac corresponds to the actin, α and β to the tubulin subunits. Note the presence of 43K protein in the three conditions. Some viral proteins are indicated.

series of experiments it was concluded that viral 43K protein is identical to actin. Two experiments were performed in order to eliminate the possibility that actin could be absorbed onto the virions during the purification procedure.

First, the viral preparation was suspended in G buffer, with or without 0.15 M NaCl (actin polymerization conditions) (16), and centrifuged at high speed (90 minutes, 100 000 *g*). The viral pellet was washed twice in the same conditions before analysis in SDS-PAGE. Fig. 1 shows that repeated washes with an actin depolymerization buffer (fig. 1C) did not remove more actin than similar washes with PBS (fig. 1B). It therefore seems that actin is incorporated into the virion and is not likely to result from an adsorption on the viral envelope.

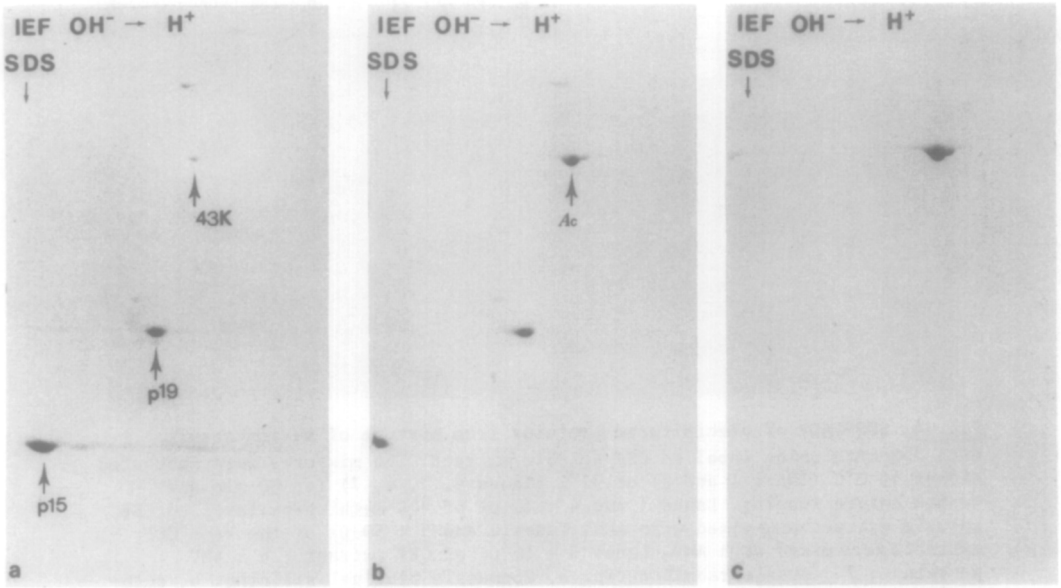

Fig. 2. 2-Dimensional gel separation : (a) AMV proteins. (b) Mixture AMV proteins and muscle rabbit actin prior to running. (c) Muscle rabbit actin. The p15 and p19 were identified by peptide mapping. Ac, actin.

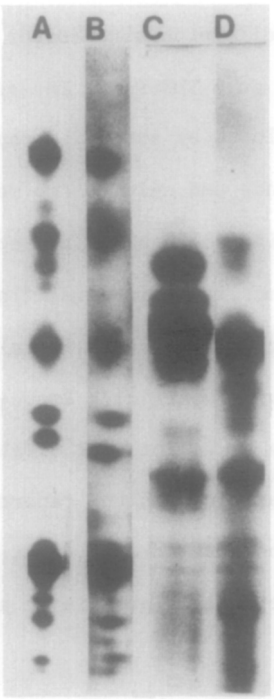

Fig. 3. Protease V8 digest peptide analysis of purified actin (A), 43K AMV protein (B), p15 AMV protein (c), p19 AMV protein (D).

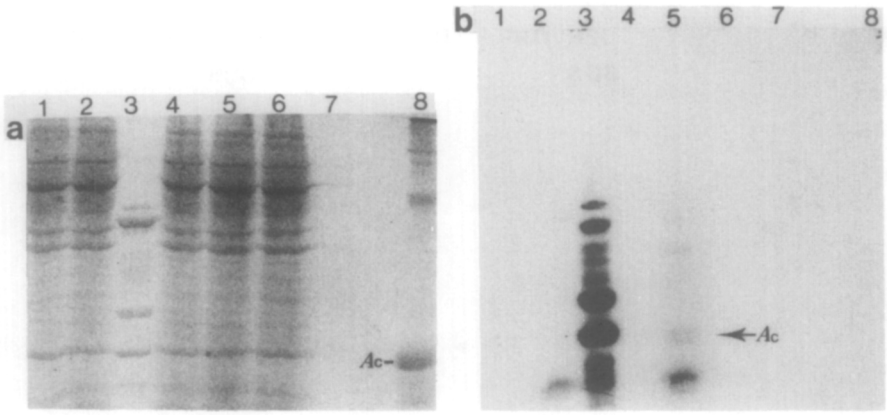

Fig. 4. SDS-PAGE of precipitated proteins from mixture of virion samples with ^{35}S-methionine labelled CEF soluble extract. The mixtures were incubated either at 0°C (lanes 1 and 2) or 37°C (lanes 4, 5, 6, 7) for 60 min and washed before running. Lanes 1 and 4 : 10 µl of ^{35}S-methionine labelled CEF soluble extract were mixed with AMV. Lanes 2 and 5 : 50 µl of the same CEF extracts were mixed with AMV. Lanes 3 : 10 µl of CEF extract ; 6 : AMV proteins ; 7 : muscle rabbit actin. a, Coomassie blue gel staining. b, autoradiogram of the gel. The picture (a, b) shows the upper part of the gel.

Second, to determine if virus could absorb extraneous actin, viral samples were mixed with various concentrations of ^{35}S-methionine labelled CEF soluble extract, as a source of extraneous actin, and incubated either at 0°C or 37°C for 60 minutes. The pellets obtained after two centrifugations (90 minutes, 100 000 *g*) were run on a SDS-PAGE. The gels were stained with Coomassie blue (fig. 4a) followed by autoradiography (fig. 4b). The autoradiogram showed no actin bands and only traces were seen in the 37°C incubated samples (lane 5, fig. 4b) besides the actin bands displayed similar intensity in the Coomassie-stained gel (lanes 1 to 6, fig. 4a).

These experiments indicate that actin is unlikely to be artefactually absorbed onto the enveloped viral particles during its purification and suggest rather that actin may be an integral viral constituent.

These results are compatible with the reported strong association of an actin-like protein with the Rous sarcome virus *gs* antigen (17) as well as with the description of an actin-like protein in anti *gs* immunoprecipitates of AMV-infected CEF (18).

We obtained additional data on the presence of actin in virus particles using an ultrastructural approach. CEF were infected with AMV,

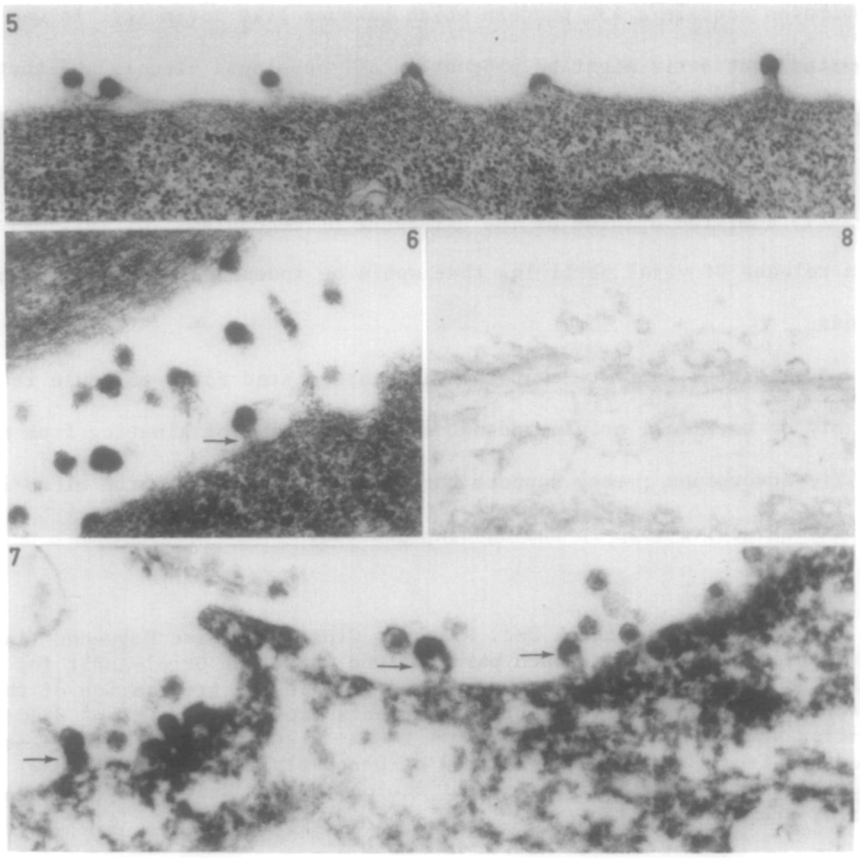

Fig. 5, 6. CEF infected with RSV-RAV-2 treated as for conventional electron microscopy. In fig. 6, microfilaments (→) may be discerned in the bud stems. X 30 000, X 37 500.

Fig. 7, 8. CEF infected with RSV-RAV-2 and stained with anti-actin antibody (7) or normal rabbit globulins (8). Note strong positive reaction on buds (→).

Sr-RSV-B, RSV-RAV-2, RAV-1 or RAV-2, stained with specifically-purified anti-actin antibodies and examined by electron microscopy. Positive staining was observed not only on the microfilaments, but also on the virus particles, particularly intense on buds (fig. 7 and 8). Furthermore, conventional electron micrograph showed the microfilaments to join the buds (fig. 5 and 6). This intense staining of the virus buds additionally argues against a non-specific absorption of actin.

A 43K protein had been previously observed in SDS-PAGE from AMV proteins. However it was considered as a cellular contaminant (19). More recently it has been shown that some Rous associated viruses and also para-

myxoviruses contain a 43K protein which behaves like actin (2). It was suggested that actin might be present in all enveloped viruses and that microfilament might act as an actomyosin system to provide the motile force for the extrusion of virions (2, 3). This would be in contradiction with the currently admitted opinion of the existence of mechanisms for self-assembly and a release of viral particles that would be independent of a contractile process.

Our results indicate that the virus-associated actin molecule is most probably of host-cell origin and not a contamination originating from non-specific adsorption ; they support the notion (2, 3) that actin microfilaments may play an active role in the budding process.

ACKNOWLEDGEMENTS

We would like to thank Drs. François Gros, Françoise Haguenau (in whose laboratories this work has been pursued) and Christine Oriol-Audit for their continuous support, critical remarks, and help in the preparation of the manuscript. This work was supported by grants from INSERM, CRL n° 826011, and Fondation pour la Recherche Médicale. VMN is a recipient of a long-term fellowship from the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico and from the Institute of Biology, State University, Rio de Janeiro, Brazil.

REFERENCES

1. Goldman, R., Pollard, T. and Rosenbaum, Y. (eds.) (1976) Cell Mobility, Cold Spring Harbor Conferences on Cell Proliferation, Cold Spring Harbor Laboratory, New York.
2. Wang, E., Wolf, B.A., Lamb, R.A., Choppin, P.W. and Goldberg, A.R. (1976) *In* Cell Mobility (Goldman, R., Pollard, T. and Rosenbaum, Y., eds.), Cold Spring Harbor Press, Cold Spring Harbor, New York, pp. 589-600.
3. Damsky, C.H., Sheffield, J.B., Tuszynski, G.P. and Warren, L. (1977) J. Cell Biol. 75, 593-605.
4. Naito, S. and Matsumoto, S. (1978) Virology 91, 151-163.
5. Stanislawski, L. (1983) J. Ultrastr. Res. 82, 134-142.
6. Jockusch, B.M., Kelley, K.H., Meyer, R.K. and Burger, M.M. (1978) Histochemistry 5, 177-184.
7. Schliwa, M., Blerkom, J. and Pryzwasisky, K.B. (1981) Cold Spring Harbor Symp. Quant. Biol. 46, 51-67.
8. Graham, R.C. and Karnovsky, M.J. (1966) J. Histochem. Cytochem. 14,291-302.
9. Brinkley, B.R., Murphy, P. and Richardson, C. (1967) J. Cell Biol. 35,279-283.
10. O'Farrel, P.H. (1975) J. Biol. Chem. 250, 4007-4021.
11. Sheir-Neiss, G., Lai, M.H. and Morris, N.R. (1978) Cell 15, 639-647.
12. Chamberlain, J.P. (1978) Anal. Biochem. 98, 132-135.
13. Oakley, B.R., Kirsch, D.R. and Morris, N.R. (1980) Anal. Biochem. 105,361-363.
14. Cleveland, D.W., Fischer, S.G., Kirschner, M.W. and Laemmli, U.K. (1977) J. Biol. Chem. 252, 1102-1106.
15. Lazarides, E. and Weber, K. (1974) Proc. Natl. Acad. Sci. USA 71, 2268-2272.
16. Spudich, J. and Watt, S.J. (1971) Biochem. Chem. 246, 4866-4871.
17. Fleissner, E. and Tress, E. (1973) J. Virol. 11, 250-262.
18. Vogt, V.M., Eisenman, R. and Diggelman, H. (1975) J. Mol. Biol. 96, 471-493.
19. Bolognesi, D.P. and Bauer, H. (1970) Virology 42, 1097-1112.