Journal of Cell Science 112, 797-809 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
JCS3798

Conformational difference between nuclear and cytoplasmic actin as detected by a monoclonal antibody

Sabine M. Gonsior[1,*], Stefanie Platz[2], Sabine Buchmeier[3], Ulrich Scheer[2], Brigitte M. Jockusch[3] and Horst Hinssen[1,‡]

[1]Biochemical Cell Biology, University of Bielefeld, D-33501 Bielefeld, FRG
[2]Dept of Cell and Developmental Biology, Biocenter of the University of Würzburg, D-97074 Würzburg, FRG
[3]Cell Biology, Zoological Institute, Technical University of Braunschweig, D-38092 Braunschweig, FRG
*Present address: MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK
‡Author for correspondence

Accepted 8 January; published on WWW 25 February 1999

SUMMARY

Using a reconstituted complex of profilin and skeletal muscle actin as an antigen, we generated a monoclonal mouse antibody against actin, termed 2G2. As revealed by immunoblots of proteolytic actin fragments and by pepscan analysis, the antibody recognises a nonsequential epitope on actin which is located within three different regions of the sequence, consisting of aa131-139, aa155-169, and aa176-187. In the actin model derived from X-ray diffraction, these sequences lie spatially close together in the region of the nucleotide-binding cleft, but do not form a coherent patch. In immunoblots, 2G2 reacts with all SDS-denatured actin isoforms and with actins of many vertebrates. In contrast, its immunofluorescence reactivity is highly selective and fixation-dependent. In fibroblasts and myogenic cells, fixed and extracted by formaldehyde/detergent, stress fibres or myofibrils, respectively, remained unstained. Likewise, after microinjection into living cells, 2G2 did not bind to such microfilament bundles. Extraction of myosin and tropomyosin did not alter this pattern indicating that the lack in reactivity is probably not due to epitope-masking by actin-binding proteins. More likely, the reason for the lack of reactivity with filamentous actin is that its epitope is not accessible in F-actin.

However, the antibody revealed a distinct pattern of nuclear dots in differentiated myogenic cells but not in myoblasts, and of fibrillar structures in nuclei of *Xenopus* oocytes. In contrast, after methanol treatment, a 2G2-specific staining of stress fibres and myofibrils was observed, but no nuclear dot staining. We conclude that 2G2, in addition to binding to SDS- and methanol-denatured actin, recognises a specific conformation of native actin which is present in the nucleus and specified by compaction of the antibody-reactive region into a coherent patch. This conformation is apparently present in differentiated myogenic cells and oocytes, but not in cytoplasmic actin filament bundles.

Key words: Nuclear actin, Actin antibody, Epitope mapping, Myogenic cell, *Xenopus* oocyte

INTRODUCTION

Actin is one of the most abundant proteins in eucaryotic cells and is highly conserved during evolution (Hightower and Meagher, 1986). Since the actin isoforms of one organism (Herman, 1993) as well as the actins of different organisms differ only in a relatively small number of amino acids (for a review see Sheterline and Sparrow, 1994), the generation of actin antibodies has been notoriously difficult. Numerous attempts have been made to overcome the low immunogenicity of actin, and to obtain actin antibodies with high affinity, for example, by raising antibodies against SDS-denatured actin (Lazarides and Weber, 1974), against synthetic peptides derived from the actin sequence (Skalli et al., 1988; Gimona et al., 1994) and against complexes of actin with an actin-binding protein (Polzar et al., 1989). Moreover, even after mastering these difficulties, the anti-actins generated may be of limited applicability.

Since in vivo actin exists in different states (filamentous actin, non-filamentous actin or as a complex with actin binding proteins) the detection of actin with antibodies may be selective. In addition, actin is only one member of a large superfamily of proteins which also comprises actin-related proteins (arps) with highly homologous sequence regions (Fyrberg et al., 1994; Schroer et al., 1994; Mullins et al., 1996), and actin antibodies may therefore recognise actin-related proteins as well. With the exception of those antibodies which were generated against specific peptides, the locations of anti-actin epitopes are unknown. Therefore, the interest in epitope-mapped anti-actins of high affinity is scientifically justified. For example, they are more versatile in recognising different states and suprastructures of actin, as compared to the actin binding drug phalloidin which is restricted to F-actin binding. Thus, anti-actins have identified actin in the nucleus in addition to cytoplasmic actin.

In addition to its localisation in the cytoplasm, actin has frequently been reported to exist in interphase nuclei (e.g. Jockusch et al., 1974; Volkman et al., 1992; Milankow and deBoni, 1993; Sahlas et al., 1993; Amankwah and deBoni, 1994; Soyer-Gobillard et al., 1996; Rao et al., 1997; for further references see DeBoni, 1994) as well as nuclei of amphibian oocytes (e.g. Clark and Merriam, 1977; Clark and Rosenbaum, 1979; Gounon and Karsenti, 1981; Parfenov et al., 1995). However, the existence and possible function of actin in the nucleus in situ is still a matter of dispute since in many cases a contamination of the nuclei with cytoplasmic actin could not be excluded and filamentous actin was often detected only after special pretreatment of the cells, e.g. with DMSO (Fukui and Katsumaru, 1979; Sanger et al., 1980; Wehland et al., 1980) or heat (Welch and Suhan, 1985; Iida et al., 1986). Incubation with DMSO also induced the formation of actin-cofilin coaggregates (Abe et al., 1993). Regarding its function, it has been proposed that actin might be involved in cleaving nuclear membranes (Jockusch et al., 1974), in transcription (Scheer et al., 1984; Egly et al., 1984), in the activities of a motor system engaged in the dynamics of chromatin (Milankow and deBoni, 1993) and in the transport of RNA (Salhas et al., 1993). Today, none of these hypotheses can be considered proven or dismissed. Definitely, a more detailed knowledge on localisation, organisation, complex formation and structure of nuclear actin is required for the understanding of the function of nuclear actin.

In this paper we report on the characterisation of a monoclonal actin antibody, generated against a complex of profilin and skeletal muscle actin. We mapped its epitope, and we describe the fixation-dependent reactivity of the antibody with nuclear structures and with cytoplasmic actin, respectively. The results indicate that the antibody recognises a specific conformation of the actin molecule which apparently is not prevalent in the cytoplasm of living cells but is present in nuclei of certain cell types.

MATERIALS AND METHODS

Cells

Chicken myoblasts were prepared from legs and thighs of 11-day-old embryos (Konigsberg, 1979). To reduce fibroblasts, the cells were preplated twice, and the nonadherent myoblasts were then seeded on plastic tissue culture dishes containing collagenized coverslips and growth medium. The next day the medium was changed for differentiation medium. After 6 days, the majority of myotubes showed clear cross-striations in the phase contrast microscope. For details of cell density and medium components, see Dissmann and Hinssen (1994).

Cardiac myocytes were prepared from the hearts of 7-day-old chicken embryos according to the method of Dlugosz et al. (1984). Briefly, heart tissue from 20 embryos was minced in medium and incubated for 10 minutes at 37°C with 3 ml 0.05% trypsin (Gibco, Eggenstein, FRG) in phosphate-buffered saline (PBS) + 0.5 mM (EDTA). The medium was discarded and the tissue mince was placed in 1.0 mg/ml collagenase type 1A-S (Sigma) in minimal essential medium (MEM) with glutamine (Gibco), and 5% fetal calf serum (FCS) and incubated at 37°C for 30 minutes. The cells were collected by centrifugation (170 ***g***, 10 minutes) and resuspended in medium. After 2 preplating steps on collagen coated dishes (20 minutes, 37°C, 5% CO_2), cardiac cells were cultured on collagen-coated glass coverslips at an initial density of 4×10^5 cells per 60 mm tissue culture dish. Beating cardiac myocytes were observed after 24 hours, and 5-7 day old cultures were used for immunofluorescence studies. 2 days after plating, the medium was replaced by a glutamine-free medium to reduce fibroblast growth.

The myogenic cell line C2C12 (Blau et al., 1983) was propagated in Dulbecco's minimal essential medium (DMEM, Gibco) supplemented with 10% foetal calf serum. Fusion of cells was induced by reducing the serum content to 5%. For immunofluorescence, cells were replated on collagen-coated glass coverslips and maintained for 48 hours after plating. For isolation of nuclei and for SDS-samples, cells were replated on 100 mm and 140 mm tissue culture dishes at a density of $4\text{-}6\times10^5$ cells and kept in culture for 5 days. FH12 cells and 3T3 fibroblasts were cultivated by standard procedures.

Small pieces of *Xenopus laevis* ovary were obtained from anesthesised animals and immediately shock-frozen in isopentane cooled by liquid nitrogen.

Isolation of nuclei (modified from Compton et al., 1976)

C2C12 myoblasts were harvested from culture dishes by scraping, rinsed in phosphate-buffered saline (PBS) and pelleted by centrifugation at 160 ***g*** for 10 minutes. The pellet was resuspended in buffer C (10 mM Tris-HCl, pH 7.5, 1 mM $CaCl_2$) and incubated for 10 minutes on ice in this hypotonic solution. The cells were then lysed mechanically in a Dounce homogenizer (Wheaton, USA). The lysate was layered onto 3 ml of 1.7 M sucrose in buffer C and centrifuged for 1 hour at 650 ***g***. The supernatant was discarded and the pelleted nuclei were resuspended in 1 ml PBS, layered onto 3 ml of 2.0 M sucrose in buffer C and centrifuged until a sediment was obtained. This pellet was again resuspended in 1 ml PBS and layered onto 3 ml of 2.12 M sucrose in buffer C. The small nuclear pellet obtained after this procedure was used for immunofluorescence microscopy and biochemical analysis. One unit of benzonase (Benzon Pharma, Denmark) was added to the SDS-samples after heating.

Preparation of actin and its proteolytic fragments

Actin was purified from rabbit skeletal muscle acetone powder according to the method of Spudich and Watt (1971) with an additional gel filtration on Sephadex G-150. G-actin was stored in 2× G-buffer (2 mM Tris base, 0.4 mM ATP, 0.08 mM $CaCl_2$, 0.8 mM β-mercaptoethanol, 2 mM NaN_3, pH 8.2). The purity of the protein preparation was analysed on SDS-PAGE (Laemmli, 1970) and protein concentrations were determined using the micro-biuret method (Goa, 1953). Proteolytic digestions were carried out at a protein concentration of 24 μM G-actin at room temperature. At different time intervals, aliquots of the digests were taken and boiled for 2 minutes in SDS sample buffer (see below) to terminate proteolysis. Digestion products were analysed by SDS-PAGE on 15% or 20% slab gels.

V8-protease from *Staphylococcus aureus* (Sigma) was added to G-actin at a 1:5 weight ratio for 30 minutes (Drapeau et al., 1976). Thermolysin type X from *Bacillus thermolyticus* rokko (Sigma) was used at a 1:50 weight ratio to cleave G-actin for 0, 5, 10 and 15 minutes in the presence of 0.1% SDS. SDS increased the number of cleavage products. G-actin (Ca-actin) was cleaved with trypsin (type III, Sigma) (1:10, w/w) according to the method of Jacobson and Rosenbusch (1976), and also after preincubation with 0.1 mM $MgCl_2$ and 0.5 mM EGTA (Mg-actin) according to the method of Strzelecka-Golaszewska et al. (1993). In addition, actin was cleaved with the *E. coli* A2-protease (1:50, w/w, generous gift of Dr S. Khaitlina, St Petersburg) according to the method of Khaitalina et al. (1991). Subtilisin-cleaved actin (subtilisin type VIII, Carlsberg, 1:200, 1:1000, w/w) was prepared essentially as described by Schwyter et al. (1989).

Peptide sequencing

To determine the N-terminal amino acid sequence of the actin fragment after thermolysin/trypsin digestion, the samples were first subjected to SDS-PAGE, and then blotted to polyvinylidinedifluoride

(PVDF) membrane (Immobilon™, Millipore, USA; Hirano and Watanabe, 1990). Proteins were identified by staining with Ponceau S. The respective bands were excised from the blot and automatic amino acid sequencing was performed, using a gas-phase sequencer.

Antibodies

The monoclonal actin antibody 2G2 was generated by immunising mice with profilactin, a reconstituted complex of rabbit skeletal muscle α-actin and profilin. Profilin was isolated from calf thymus according to the method of Tanaka and Shibata (1985) with modifications. After hybridization and cloning, antibody producing hybridoma cells from clone 2G2 were grown either according to standard protocols or in serum-free medium in the presence of 500 mg/l albumin and 10 mg/l transferrin. The culture supernatant containing 2G2 was concentrated by membrane filtration about 50-fold and stored at −80°C either lyophilized or in the presence of 20% sucrose. Under these conditions, the antibody was stable. It was classified as an IgM-type.

The following primary antibodies were used for immunostaining: monoclonal anti-actin 2G2 (described above), and the monoclonal anti-actin A-4700 (Sigma, Deisenhofen, FRG) (IgG2a isotype). This antibody was raised against a synthetic actin C-terminal peptide, Ser-Gly-Pro-Ser-Ile-Val-His-Arg-Lys-Cys-Phe. For colocalisation studies, the following antibodies were also tested: another monoclonal anti-actin (Sigma A-2172) (IgM isotype), anti-fibrillarin (human autoimmune serum S4; Ochs et al., 1985), a monoclonal antibody against *Dictyostelium* actin (224-236-1, IgG isotype, generous gift from Dr G. Gerisch, Max-Planck Institute of Biochemistry, Martinsried), monoclonal anti-C8A2/-NDP52 (IgG isotype) specific against nuclear dots (generous gift of Dr J. Frey, Bielefeld) and the monoclonal anti-proliferation associated nuclear antigen (Clevenger and Epstein, 1984) which was obtained from ICN. FITC-conjugated goat anti-mouse-IgM (Sigma), rhodamine-conjugated goat anti-mouse (Dianova, Hamburg, FRG) and TRITC-conjugated goat anti-human (Sigma) were used as second antibodies.

Pepscan analysis

For the identification of 2G2-reactive amino acid residues, the sequence of the reactive proteolytic actin fragment was spot-synthesized as peptides of 15 aa in length and with an overlap of 12 aa, on cellulose membranes (Frank, 1992). 2G2-binding to this sequence on the membrane was monitored as described by Mayboroda et al. (1997).

Fluorescence microscopy

Myogenic and fibroblastic cells were extracted with 0.2% Triton X-100 in solution I (137 mM NaCl, 5 mM KCl, 1.1 mM Na_2HPO_4, 0.4 mM K_2HPO_4, 4 mM $NaHCO_3$, 5.5 mM glucose, 2 mM $MgCl_2$, 2 mM EGTA, 5 mM Pipes, pH 6.1) for 90 seconds at room temperature before or after fixation with 4% formaldehyde in solution I. In some cases, cells were extracted after Triton X-100 permeabilisation with a high ionic strength buffer (0.6 M KCl, 10 mM $Na_4P_2O_7$, 1 mM $MgCl_2$, 0.1 M KH_2PO_4, pH 6.4, 4°C) for 8-10 minutes (Hasselbach and Schneider, 1951). Some cells were fixed and permeabilised with −20°C cold methanol for 2 minutes. After permeabilisation, extraction and fixation, the cells were washed 3 times with solution I. For immunofluorescence, Tris-buffered saline (TBS: 10 mM Tris, 155 mM NaCl, pH 7.0) was used as washing buffer and dilution buffer for antibodies. Cryostat sections (5 μm thick) of shock-frozen *Xenopus* ovary were air-dried, fixed in 4% formaldehyde in PBS (10 minutes) and washed 3 times with PBS followed by antibody incubation. In this case PBS served as washing and dilution buffer. In addition to immunostaining, rhodamine-phalloidin (Sigma) was used for fluorescence staining of filamentous actin, Hoechst 33258 (Boehringer-Mannheim/FRG) was used as a nuclear stain. The cells were mounted in Mowiol 4.88 (Farbwerke Hoechst, Frankfurt, FRG) with 0.5% *n*-propylgallate to reduce photobleaching (Giloh and Sedat, 1982), and examined with a Zeiss Axiophot equipped with epifluorescence optics.

Microinjection

Anti-actin 2G2 was microinjected into the cytoplasm of FH12 rat fibroblasts at a concentration of 2 mg/ml. 20 minutes after microinjection the cells were fixed with 4% formaldehyde and subsequently incubated with a Cy3-conjugated affinity purified goat anti-mouse (IgG+IgM H+L) antibody (Dianova). Cytoplasmic F-actin was stained with FITC-phalloidin.

SDS-PAGE and immunoblotting

Samples were heated for 2 minutes in a boiling water bath in SDS-sample buffer (2% SDS, 10% β-mercaptoethanol, 0.0625 M Tris-HCl, pH 6.8, 5% glycerol, 4 mM EDTA, and 0.01% Bromophenol Blue). For electrophoresis, a discontinuous buffer system (Laemmli, 1970) and 15% or 20% acrylamide/0.1% bisacrylamide gels was used in a mini-gel system. The gels were either stained with Coomassie Brilliant Blue or electrophoretically transferred onto nitrocellulose (Kyhse-Anderson, 1984). The nitrocellulose was incubated with 3% fish gelatin (Serva, Heidelberg, FRG) in Tris-buffered saline (TBS) with 0.05% Tween-20 (TTBS) for 1 hour at room temperature and subsequently with 3% skimmed milk powder in TTBS overnight, 4°C, for blocking. The transfers were incubated with either anti-actin 2G2 (1:10,000 in TTBS containing 0.5% skimmed milk powder) or with anti-actin A-4700 (Sigma) (1:10,000) for 4 hours at room temperature, respectively, washed 3 times in TTBS, and subsequently incubated with horseradish peroxidase-conjugated goat anti-mouse and goat anti-mouse (IgG) antibodies (1:3,000). The enhanced chemoluminescence (ECL) system (Amersham, Braunschweig, FRG) was used with exposure times of 0.5-2 minutes.

Computer modelling

To identify the location of the 2G2-reactive sequences, modelling of the actin molecule was performed with the 'Ribbons'-programme (Carson, 1997).

RESULTS

The 2G2 anti-actin reacts with a wide variety of vertebrate actins

In immunoblots, 2G2 showed a broad spectrum of reactivity. Although raised against rabbit α-skeletal muscle actin, it cross-reacted equally well with other actin isoforms from rabbit, chicken and mouse (Table 1), both with isolated actins and with actin in whole tissue samples. Thus, in immunoblots with SDS-denatured samples, the antibody does not discriminate between the different actin isoforms of vertebrate actins. However, there was no cross-reactivity with actin from the slime mould *Physarum polycephalum*, and also no reaction with several invertebrate muscle tissues (not shown).

2G2 recognises a central sequence region in actin

For characterisation of the binding epitope of 2G2 on actin, rabbit skeletal muscle actin was subjected to limited proteolytic cleavage and subsequent immunoblot analysis. Most proteases cleave native actin at the N- or C-terminal region yielding a protease-resistant 'core' fragment of about 33-35 kDa (reviewed by Sheterline and Sparrow, 1994). Fig. 1A summarises the results of immunoblots from actin fragments reacting with 2G2. Cleavage of (Ca-) actin with trypsin (Jacobson and Rosenbush, 1976), *E. coli* A2 protease (Khaitlina et al., 1991) or subtilisin (Schwyter et al., 1989)

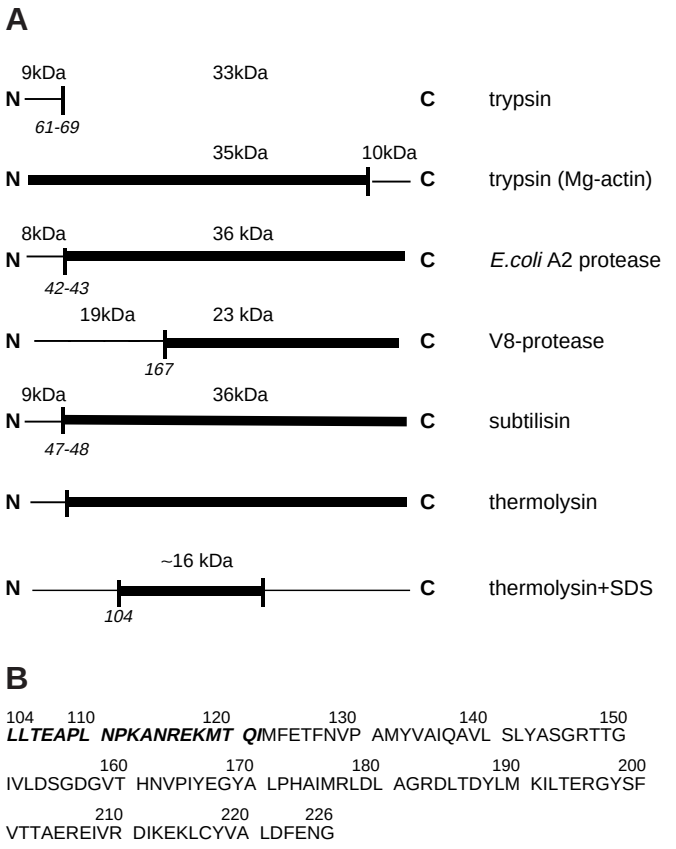

Fig. 1. (A) Schematic representation of 2G2-reactivity with proteolytic cleavage products of G-actin generated by various proteases. The bars represent the actin sequence with the cleavage sites of the proteases. The bold parts indicate the polypeptide recognised by 2G2 while the thin line represents the region which did not react. Numbers above the bars indicate the size of the cleavage products in kDa, numbers below the cleavage position (for references see Materials and Methods). (B) Sequence of the 16 kDa proteolytic actin fragment obtained by thermolysin cleavage in the presence of SDS. The first 20 N-terminal amino acid residues (bold letters) of this fragment were determined by sequencing.

resulted in a large C-terminal fragment while cleavage of (Mg-) actin with trypsin yielded a large N-terminal fragment (Strelecka-Golaszewska et al., 1993). Both large fragments were recognised by 2G2 indicating that the epitope was neither located in the N- nor C-terminal 60-70 amino acids. V8 protease generated smaller 2G2-postive actin fragments. Since the main cleavage site of V8 is at Glu226 (Mornet and Ue, 1984), the initial products were a 26 kDa N-terminal fragment and a 16 kDa C-terminal fragment. After V8 protease cleavage, 2G2 reacted with a fragment of approximately 25 kDa but not with any smaller fragment. Taken together these results imply that the epitope is located in the sequence region between ~aa70 and aa226.

For further analysis, we used thermolysin cleavage under denaturing conditions (Fig. 2). The number of cleavage products increased proportionally with the concentration of SDS. At a thermolysin/actin ratio of 1:50 (w/w) and in 0.1% SDS, a 17-18 kDa 2G2-reactive peptide was generated (Fig. 2) which did not react with an actin-antibody specific for the C terminus (A4700, see Materials and Methods). This fragment was subjected to peptide sequencing and its first N-terminal 20 amino acid residues corresponded to aa104-aa123 of actin (Fig. 1B). Its location within the sequence is indicated in Fig. 1A. We concluded that the 2G2 binding epitope is located somewhere between aa104 and ~aa260. Together with the results from V8-cleavage (see above) we were able to restrict the reactive peptide to the region between aa104 and aa226.

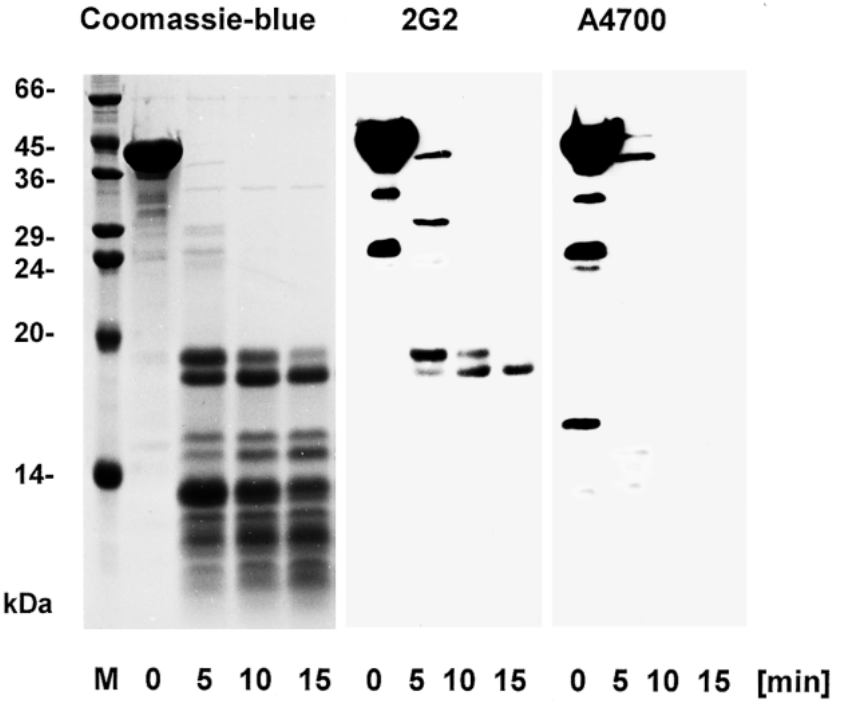

Fig. 2. Time course of the limited cleavage of G-actin with thermolysin (1:50, w/w) at 0, 5, 10 and 15 minutes in the presence of 0.1% SDS. Coomassie Blue staining (left) and detection of the reactive peptide after immunoblotting with the monoclonal 2G2 (middle) and A4700 antibodies (right). A fragment of about 17 kDa was recognised by the 2G2 antibody (at 15 minutes) but not by A4700 indicating that this fragment did not contain the C-terminal part of G-actin.

B

aa104
peptide 1.-6:

7: IMFETFNVPAMYVAI
8: ETFNVP**AMYVAIQAV**
9: NVP**AMYVAIQAV**LSL ← **aa 131-139**
10: **AMYVAIQAV**LSLYAS
11: VAIQAVLSLYASGRT

peptide 12. - 16.

17: VLDSGDGVTHNVPIY
18: **SGDGVTHNVPIYEGY** ← **aa 155-169**
19: GVTHNVPIYEGYALP
20: HNVPIYEGYALPHAI
21: PIYEGYALPHAIMRL
22: EGYALPHAIMRLDLA
23: ALPHAIMRLDLAGRD
24: HAI**MRLDLAGRDLTD** ← **aa 176-187**
25: **MRLDLAGRDLTD**YLM
26: DLAGRDLTDYLMKIL

peptide 27. - 37.

Fig. 3. Pepscan analysis of 2G2 reactivity. (A) 37 peptides (15 aa each) covering the range from aa104 to 226 on the actin sequence with 12 aa overlap between two consecutive peptides were generated on a nitrocellulose membrane. After incubation with 2G2 and development, the peptides, 8, 9 10,18, 24, 25 showed a clear positive reaction. (B) The sequences of some of the peptides in the region of positive reactions. Taking into consideration the overlap with the non-reactive peptides, those sequence regions calculated to contain part of the antibody binding epitope are written in bold letters.

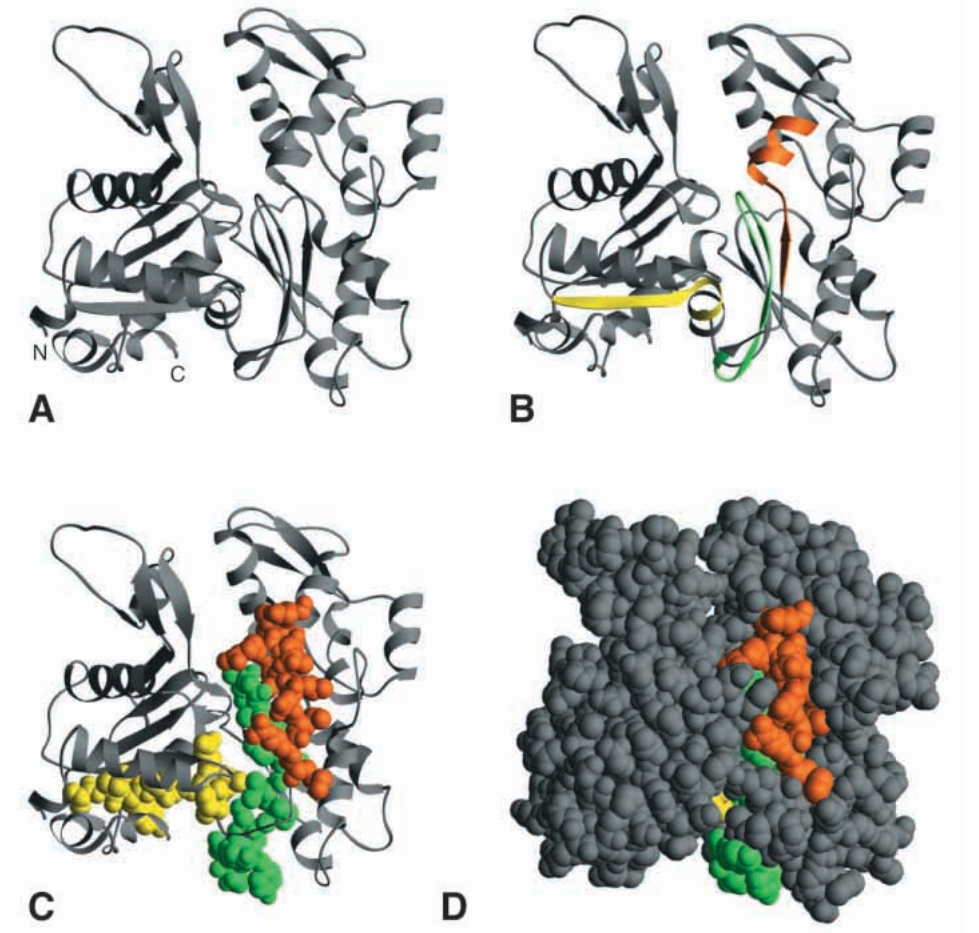

Fig. 4. Modelling of the 2G2 binding epitope. (A) Ribbon model of the actin molecule as generated from the data of Kabsch et al. (1990). Subdomain 1 is on the left. (B) Ribbon model as in A but with the three reactive sequences coloured (orange: aa131-139, yellow: aa155-169, green: aa176-187). (C) Model as in B, but coloured sequences in space filling mode, D. Space filling model of the actin molecule with the reactive sequences coloured as in B.

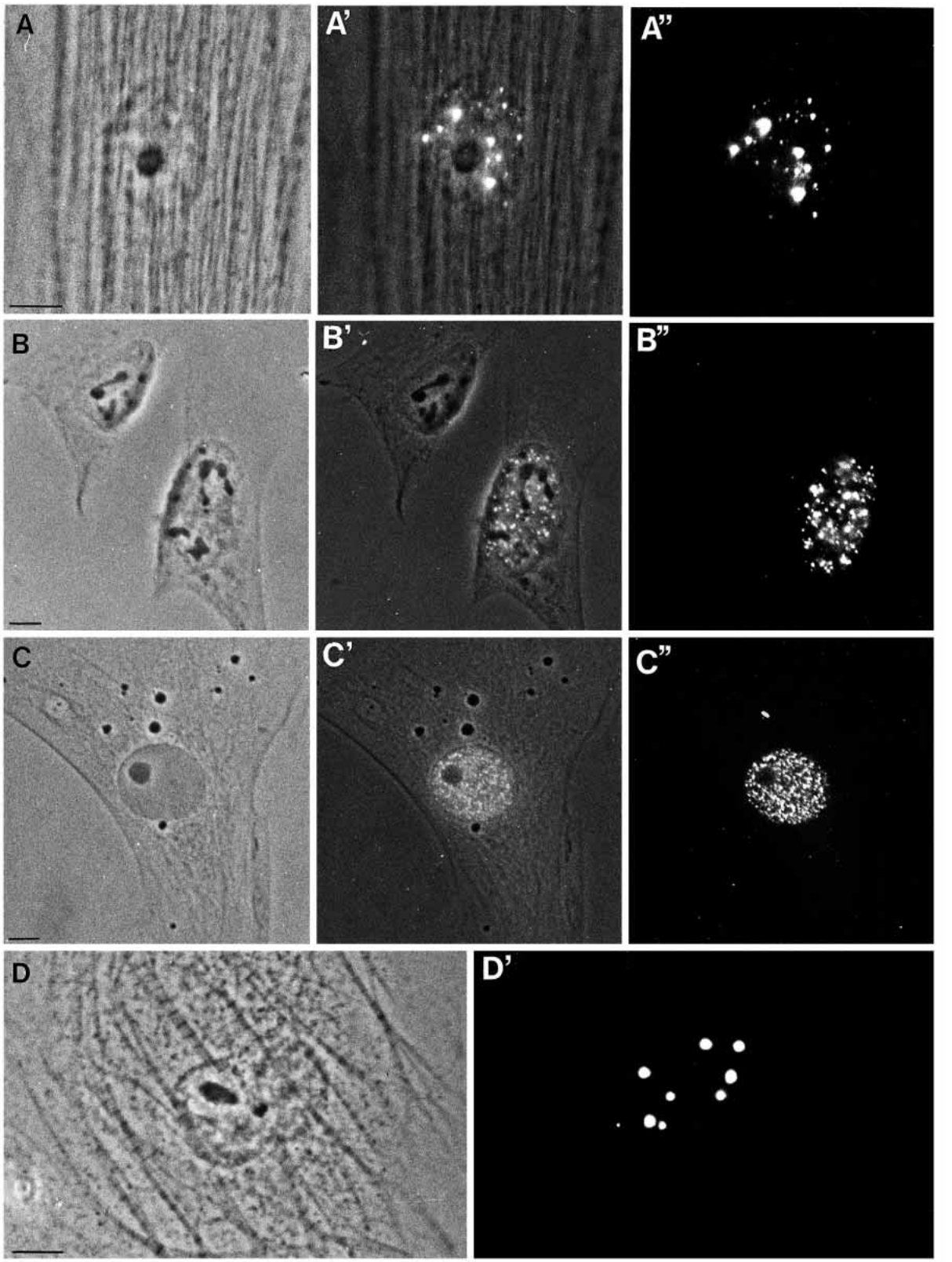

Fig. 5. Immunofluorescence microscopy of different myogenic cells using 2G2 after formaldehyde fixation. A differentiated chicken myotube (A,A′,A″), C2C12-myogenic cells (B,B′,B″) and chicken cardiac myocytes (C,C′,C″, D,D′) were permeabilised by Triton X-100 before (A,A′,A″, B,B′,B″, D,D′) or after (C,C′,C″) fixation and stained with anti-actin 2G2. (A,B,C,D) Phase contrast images, showing cross-striated myofibrils in A and D. (A′,B′,C′) Combined phase contrast and epifluorescence illumination. (A″,B″,C″,D′) Fluorescence illumination. After formaldehyde fixation the antibody stained only distinct regions in the nucleus of the cells excluding the nucleolus. Not all nuclei are stained by 2G2 (B′,B″), and myofibrillar actin was not recognised by the antibody under these conditions (A′,C′,D′). Bar, 5 μm.

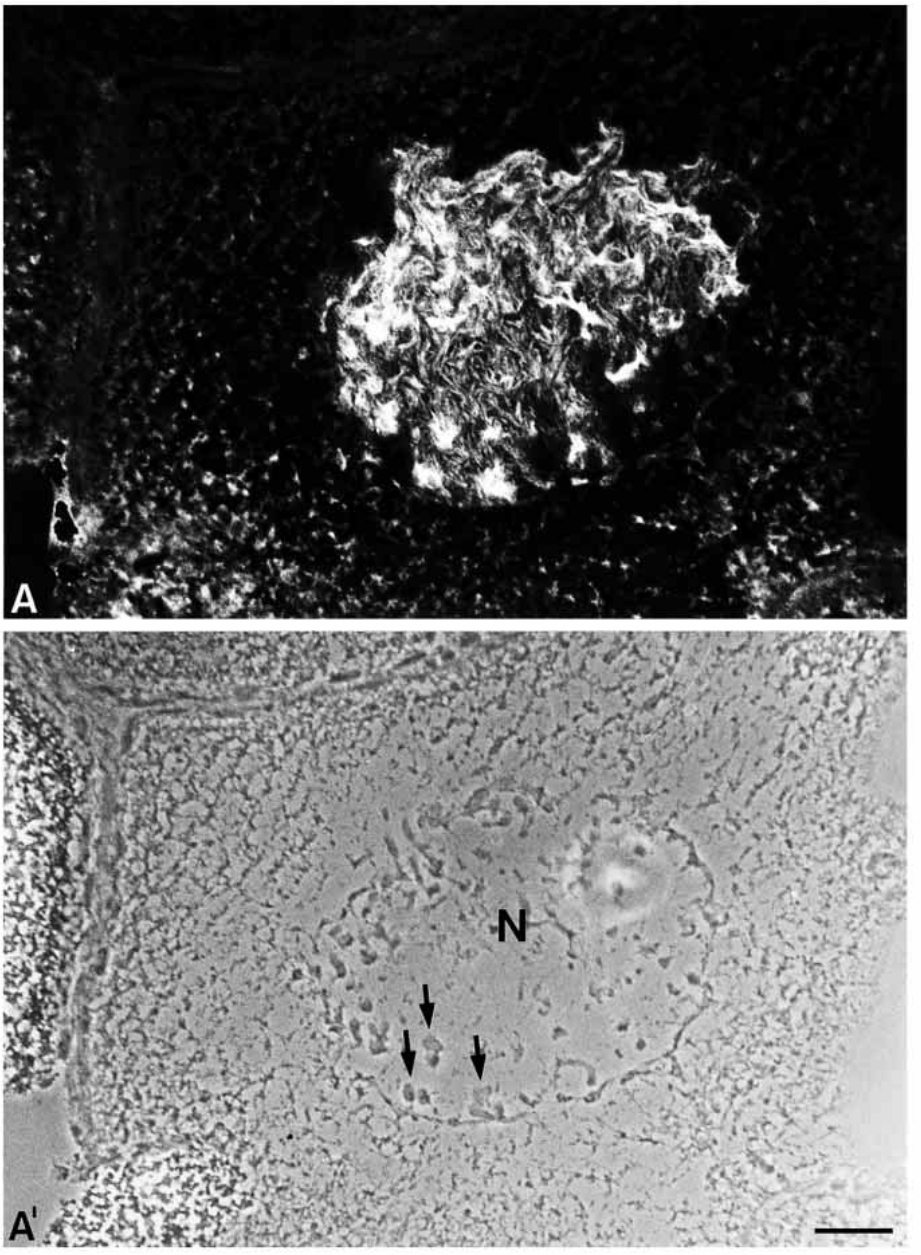

Fig. 6. Immunofluorescence microscopy of a cryosection through a previtellogenic *Xenopus laevis* oocyte stained with 2G2 anti-actin. The antibodies decorate a filamentous meshwork extending throughout the nucleus (A). The fluorescent foci seen in A correspond to amplified nucleoli which are recognised in the corresponding phase contrast image (A′, arrows). N, oocyte nucleus. Bar, 25 μm.

2G2 recognizes a conformational epitope

For a precise localisation of the 2G2 epitope, a series of 37 synthetic peptides of the actin sequence (aa104-aa227) was synthesised using the Pepscan method (Frank, 1992). Each peptide was 15 aa in length, with an overlap of 12 aa between adjacent peptides. After incubation of the membrane with 2G2, clear positive signals were obtained with six peptides in 3 different regions of the sequence (Fig. 3A). Taking into consideration the 12aa overlap, we concluded that only those residues shared between adjacent 2G2-reactive peptides contained the binding motifs. These regions consisted of residues aa131-139, 155-169 and 176-187 (Fig. 3B). Hence, rather than binding to a continuous sequence epitope, 2G2 reacts with 3 regions well spaced in the sequence.

The topography of these peptides was analysed in a computer-generated model of the actin molecule derived from the data of Kabsch et al. (1990). Fig. 4 shows that these sequence regions are located in the central part of the actin molecule, in the position of the cleft between the large and small domain, and that they lie adjacent to each other. This becomes especially obvious in the partly space filling model (Fig. 4C) rather than in the ribbon model (Fig. 4B). However, it is evident from the space filling model (Fig. 4D) that the three sequences do not form a coherent patch in this model.

2G2 recognises nuclear actin

To investigate the reactivity of the 2G2 antibody with actin in situ, cultured myogenic cells and fibroblasts were fixed either with formaldehyde or with methanol before incubating with 2G2 and processing for immunofluorescence. Distinct staining patterns were observed for each fixation method. After formaldehyde fixation, dot-like structures in the nuclei of

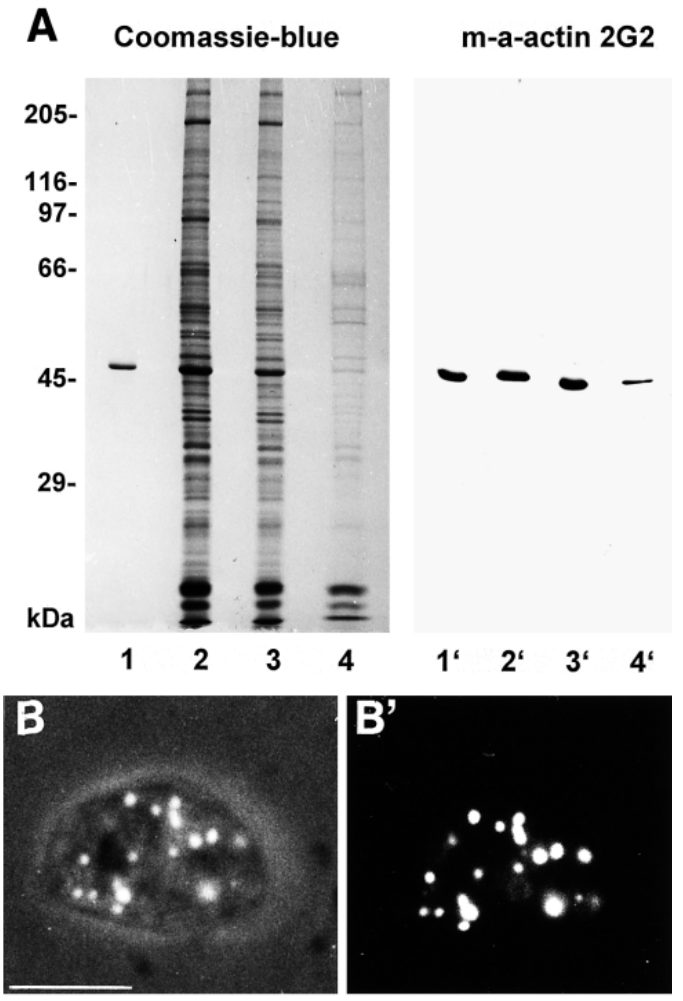

Fig. 7. (A) SDS-PAGE and immunoblot analysis of anti-actin 2G2 reactivity with purified rabbit α-skeletal muscle actin (lane 1), cultured C2C12-myoblasts (lane 2), cultured C2C12-myotubes (lane 3) and isolated nuclei of C2C12-myoblasts after centrifugation over 2.12 M sucrose in buffer C. Lanes 1-4, Coomassie-blue stain; lanes 1′-4′, immunoblot of the same samples as in lanes 1-4 probed with anti-actin 2G2. The antibody reacts exclusively with actin, even in the nuclear fraction (lane 4′), no cross reactivity with other proteins was observed. (B,B′) Immunofluorescence microscopy of isolated nuclei from C2C12 myoblasts with anti actin 2G2. Isolated nuclei were adhered on collagen coated coverslips, fixed with 4% formaldehyde, permeabilised with Triton X-100 and stained with 2G2. (B) Fluorescence and (B′) a mixture of phase contrast and fluorescence of the same nucleus indicating the location of the fluorescent dots. Bar, 5 μm.

myogenic cells were found 2G2-positive whereas cytoplasmic microfilament bundles (e.g. myofibrils and stress-fibers) remained unstained. As shown in Fig. 5, 2G2 reacted with the nuclei of skeletal myotubes and cardiac myocytes permeabilised either before or after formaldehyde fixation and with C2C12 cells. However, as shown in Table 2, only skeletal muscle cells of at least 5 days in culture clearly displayed the nuclear dots which persisted to the state of fully differentiated myotubes. The nuclei of myoblasts were consistently negative.

Formaldehyde-fixed fibroblastic cells, present in the same cultures, showed neither distinct nuclear dots nor a staining of cytoplasmic actin structures, but we observed nuclear dots in a number of cell lines of fibroblastic origin like Cos-cells, NIH-3T3 cells, and NRK Bristol cells. In these cells the dots appeared as numerous fine punctations in contrast to the large and spectacular dots of the myogenic cells. We also observed nuclear staining with the 2G2 antibody in formaldehyde-fixed sections of *Xenopus* oocytes. In this case, large fibrillar aggregates were seen rather than dots (Fig. 6). Neither in myogenic cells nor in oocytes were the 2G2-positive nuclear structures labelled by fluorescent phalloidin (not shown).

From the experiments described so far the nuclear structures recognised by 2G2 might either represent nuclear actin or actin-related proteins (arps), some of which have been localised in the nucleus (Weber et al., 1995). Since the 2G2-reactive epitope is localised within a sequence region which is highly conserved between actin and arps (Schroer et al., 1994), we tested the possibility that the observed nuclear staining might be caused by an arp. To this end, nuclei from C2C12 myoblasts were purified, and used for both immunoblot analysis and immunofluorescence. In

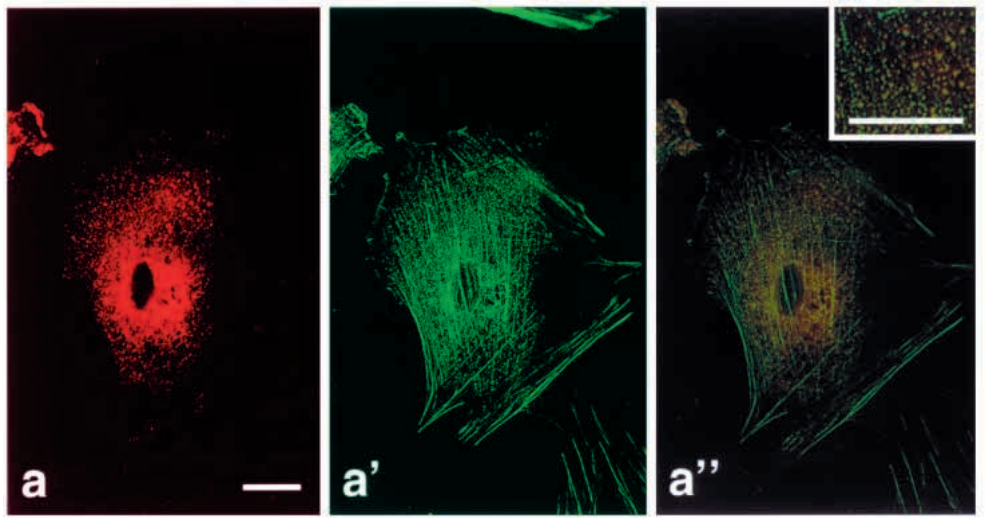

Fig. 8. 2G2 antibody was microinjected into FH12 rat-fibroblasts. After 20 minutes cells were fixed with formaldehyde and permeabilised. Cells were incubated with a Cy3-labelled goat anti-mouse antibody (a, red) and counterstained with FITC-phalloidin (a′, green) to detect F-actin. (a″) Double labelling with FITC-phalloidin and antibody. Pictures obtained with a confocal laser scanning microscope. Bar, 10 μm.

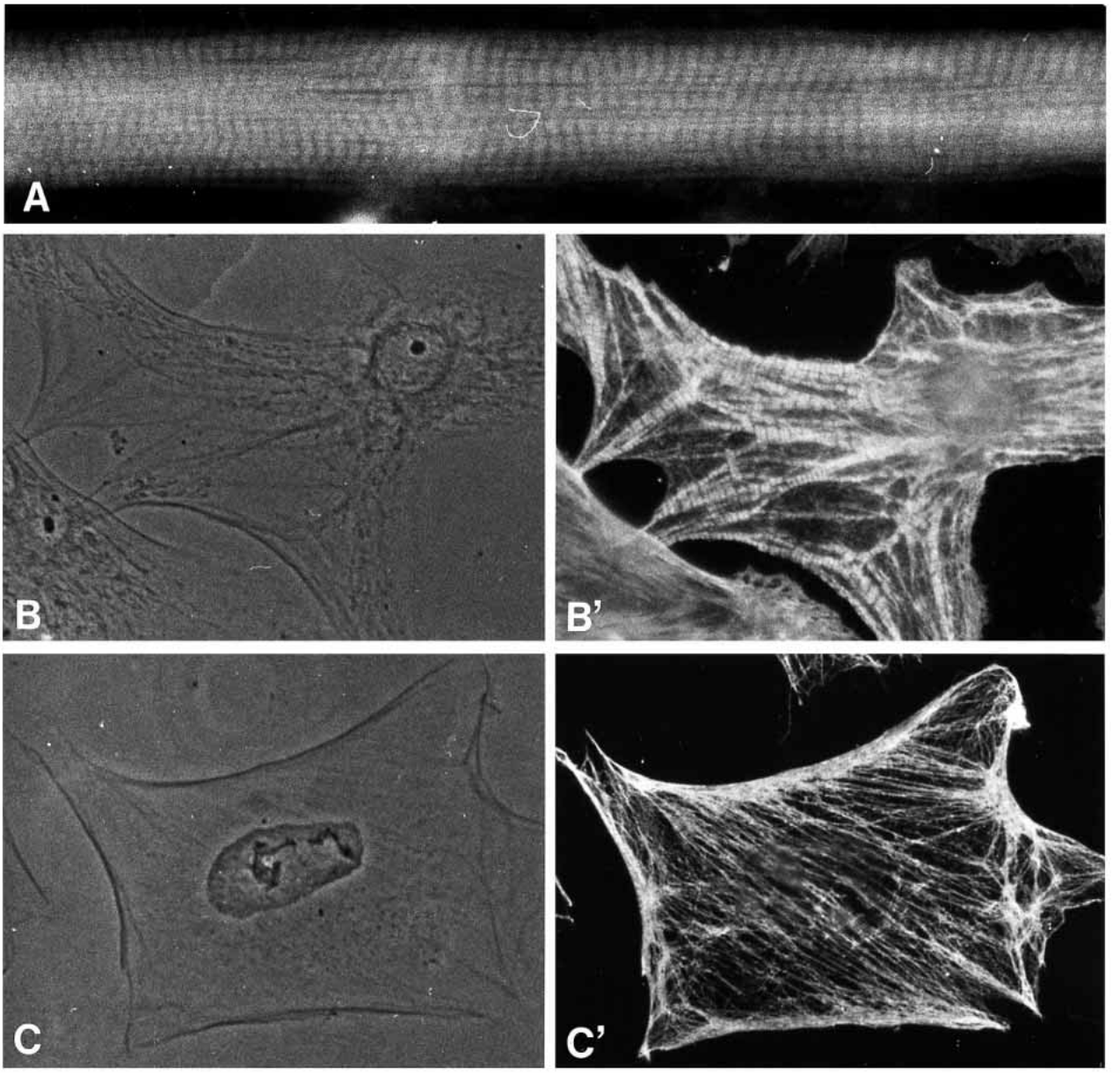

Fig. 9. Immunofluorescence localisation of myofibrillar and stress-fibre actin by 2G2 in methanol-fixed cells (A,B′,C′). (A) differentiated skeletal muscle myotubes (B,B′) cardiac myocytes and (C,C′), primary chicken fibroblasts after fixation with methanol. (B,C) phase contrast images of the samples shown in B′,C′. Myofibrils and stress-fibres are stained by 2G2, myofibrils revealing the typical cross-striation pattern, whereas the nuclei are completely negative under these conditions. Bar, 10 μm.

immunoblots, 2G2 reacted exclusively with one single polypeptide of about 42 kDa, supposedly actin (Fig. 7A). In total cell homogenates derived from C2C12 myoblasts and myotubes as well as in the nuclear fractions, only the 42 kDa polypeptide was recognised. In immunoblots obtained from 2-D-electrophoresis gels of isolated nuclei of differentiated myotubes, the antibody detected one spot at 42 kDa at a position corresponding to pH 5.5, the isoelectric point of actin (data not shown). No immunoreactive spots were found in more basic regions, especially around pH 6.4 where arps would be expected. As shown in Fig. 7B,B′, isolated nuclei revealed a staining pattern quite similar to that seen in situ, i.e. in the nuclei of formaldehyde-fixed cells, indicating that the isolation procedure did not affect this pattern. Hence, we conclude that the dots labelled by 2G2 contain nuclear actin. To compare these actin-positive dots with known nuclear structures, we carried out colocalisation studies with antibodies against the nucleolar protein fibrillarin, the nuclear dot-specific protein (Korioth et al., 1995) and the proliferation-associated nuclear antigen which gives a dotted stain as well (Clevenger and Epstein, 1984). In no case, a colocalisation with the 2G2 antibody was observed (data not shown). Furthermore, double-labelling with 2G2 and the DNA-specific Hoechst stain indicated that the actin dots did not correspond to nuclear domains of particularly high or low DNA concentrations.

Table 1. Reactivity of the 2G2 antibody with actin isoforms in immunoblots

Sample	Reactivity with 2G2
Purified rabbit skeletal muscle α-actin	+
Chicken smooth muscle γ- and α-actin	+
Cytoplasmic β-actin (chicken)	+
Whole chicken gizzard	+
Whole chicken erythrocytes	+
Chicken erythrocyte membrane skeleton	+
C2-C12C2C12 myoblasts (mouse)	+
C2-C12C2C12 myotubes (mouse)	+
Skeletal muscle in primary culture (chicken)	+
Heart muscle in primary culture (chicken)	+
Purified *Physarum* actin	-

Proteins and tissues were subjected to SDS-PAGE as described in Materials and Methods, transferred electrophoretically onto nitrocellulose membranes and incubated with 2G2 anti-actin. A HRP-conjugated anti mouse IgM was used to detect reactivity employing the ECL-luminiscence system.

Table 2. Reactivity of 2G2 antibody with different myogenic cells in culture after formaldehyde or methanol fixation

	Formaldehyde fixation		Methanol fixation	
Cell type	Nucleus	MF/SF	Nucleus	MF/SF
Primary skeletal muscle myoblasts	-	-	n.d.	+
Primary skeletal muscle myotubes (5-6 days in culture)	+	-/-	n.d.	n.d.
Fully differentiated skeletal muscle myotubes (7 days in culture)	+	-/-	-	+
Primary cardiocytes	+	-	-	+
C2C12 myogenic cells	+	-	n.d.	n.d.

MF, myofibrils; SF, stress fibres.
+, punctated staining of nuclei or staining of myofibrillar actin, respectively.
-, no staining; n.d., not determined.

The 2G2-epitope is not exposed on cytoplasmic actin filament bundles in living cells

To test whether the failure of 2G2 to recognise cytoplasmic actin in formaldehyde-fixed cells might be caused by the formaldehyde treatment, we injected the antibody into the cytoplasm of fibroblasts and allowed for antigen-antibody interaction by an additional incubation prior to formaldehyde fixation. After injection into rat fibroblasts, fixation and staining, 2G2 was localised in small cytoplasmic dots (Fig. 8a) but not associated with the typical prominent stress fibers as revealed by FITC-phalloidin staining (Fig. 8a′). Some of these dots were also phalloidin-positive as indicated by the yellow colour in superimposed images (Fig. 8a″). Hence, microinjected 2G2 does not recognise cytoplasmic actin filament bundles in living cells. The small dots in the cytoplasm may represent antigen-antibody complexes with either G-actin or with actin associated to monomer-stabilising proteins.

We also tested the possibility that the lack of recognition of actin in stress fibres of fibroblasts and myofibrils is caused by actin-binding proteins like tropomyosin, myosin, or, in skeletal muscle, possibly nebulin. Differentiated myotubes were extracted with a buffer of high ionic strength before formaldehyde fixation, thereby removing most of the actin-associated proteins. However, after labelling with 2G2 there was again no reaction with myofibrillar actin, although prominent myofibrillar staining was seen with rhodamine-phalloidin (not shown)

Denaturation by methanol exposes the 2G2-epitope on cytoplasmic actin

As described above, nuclear staining but no distinct cytoplasmic staining was observed after formaldehyde fixation. When myogenic cells and fibroblasts were fixed and permeabilised with methanol, nuclei were not labelled by 2G2 (Fig. 9). Instead, and again in contrast to formaldehyde-fixed cells, in methanol-treated skeletal muscle cells and cardiocytes, myofibrillar actin was labelled in the form of a regular cross-striated pattern (Fig. 9A,B′). In addition, in fibroblasts a typical stress-fibre actin staining was observed (Fig. 9C′). The pattern was coincident with the rhodamine-phalloidin staining and also with the localisation obtained with the other actin antibodies used for reference (data not shown).

DISCUSSION

In this study we describe a monoclonal actin antibody which is highly selective for a specific conformational state of actin that occurs in nuclei. Nuclear actin has been reported by others and has been identified by a variety of methods (see Introduction). In addition, several actin-binding proteins have been detected in the nucleus, like cofilin (Nishida et al., 1987; Abe et al., 1993), CapZ (Ankenbauer et al., 1989), CapG (Onoda et al., 1993) and a nuclear actin binding protein related to myosin I (Rimm and Pollard, 1989), indicating that nuclear actin is in a functional, though not necessarily filamentous, state. Our finding that neither the nuclear dots of myogenic cells nor the intranuclear filaments of *Xenopus* oocytes were stained by fluorescent phalloidin argues for a specific organization of these nuclear actin-containing structures. While the nuclear dots may contain actin in an unpolymerised or oligomeric form which is not recognised by phalloidin, the nuclear filaments of the oocytes apparently represent a type of actin organization which does not exist outside the nucleus and is probably caused by nuclear proteins that create the filamentous matrix.

With the nuclear actin dots detected by 2G2 we show for the first time by light microscopy that actin occurs in untreated nuclei in the form of discrete suprastructures, and that the presence of actin in this form is differentiation-dependent. The massive occurrence of large nuclear actin dots is characteristic for myogenic cells, but we also observed analogous dots in various fibroblastic cell lines (NIH 3T3, NRK Bristol, COS cells), albeit less distinct and less spectacular since the dots are smaller and fluorescence is weaker (results not shown). The number of dots varies from 5 to over hundred, and they were observed in more than 90% of the myotubes whereas myoblasts were completely negative. Hence, it may be assumed that the dots reflect a specific conformational state of actin which is induced by binding to nuclear structures or to nuclear actin binding proteins, depending on the physiological state of the cell. Actin which is not in this state may escape detection by 2G2, as does cytoplasmic actin under these fixation conditions.

Actin containing nuclear fibrils have also escaped detection by immunocytochemistry of untreated amphibian oocytes so far. Our results demonstrate the presence of a surprisingly

densely packed meshwork of such fibrils extending throughout the nuclear interior of *Xenopus* oocytes. Since the 2G2-antibody does not recognise F-actin it appears most likely that the fibrillar structures are not based on actin filaments but that actin is associated with the fibrils. Since we have used cryosections of freshly prepared and untreated oocytes, we can also rule out that these filaments described here are artefacts resulting from polymerisation during nuclear isolation or oocyte extraction (see Clark and Rosenbaum, 1979; Parfenov et al., 1995). Our data support the view that actin-containing nuclear fibrils represent a general component of amphibian oocyte nuclei (Clark and Rosenbaum, 1979; Gounon and Karsenti, 1981; Parfenov et al., 1995) and may impart structure to the nuclear interior.

Despite the high conservation of the actin molecule, many actin antibodies show a selectivity for certain actin isoforms or for actins from certain species (Gimona et al., 1994; Skalli et al., 1988). In addition, actin antibodies with a selectivity for a specific structural state of actin have been reported previously, e.g. for actin in brush borders (Schrader et al., 1994; Hartmann et al., 1989). It is well documented now that in vivo actin can assume different conformational states, and the epitope for 2G2 may be present in only one of these conformations. The conformation of the actin molecule changes, i.e. upon binding of nucleotides (Lepault et al., 1994), toxins (Drewes and Faulstich, 1991), ionic conditions (Muhlrad et al., 1994) and binding of an actin-associated protein like fimbrin (Hanein et al., 1997) or profilin (Schutt et al., 1993; Chik et al., 1996). Cofilin changes the twist of F-actin (McGough et al., 1997), and the binding of gelsolin (Orlova et al., 1995; Khaitlina and Hinssen, 1997) apparently even induces conformational changes in actin which are propagated along the filament.

The putative binding partners of nuclear actin are as yet unknown. In the context of this study, profilin may be of particular interest since the 2G2 antibody was generated against a profilactin complex. Profilin acts as a nucleotide exchange factor for actin (Sohn and Goldschmidt-Clermont, 1994), and it is suspected to open the actin molecular structure at the nucleotide binding cleft where the 2G2 epitope resides. Although profilin is primarily described as a cytoplasmic protein and colocalises with actin at the cell periphery, it has been detected in the nuclei of formaldahyde-fixed cells (Mayboroda et al., 1997). Such a dual location, suggesting a shuttle between the cytoplasmic and the nuclear compartment, has been previously described for other cytoskeletal proteins such as plakophilin and pinin (Mertens et al., 1996; Schmidt et al.,1997; Brandner et al., 1997).

Since all three 2G2-reactive sequences are located in the vicinity of the nucleotide binding cleft where the actin molecule can easily undergo conformational changes (Adams and Reisler, 1994), it is conceivable that the nuclear actin conformation is distorted in such a way that the reactive sequences will join and form a patch exposed to the environment. Such conformational changes must be quite substantial as the 2G2 antibody is of the IgM type which normally requires epitopes on the surface of the antigen.

Regardless of the conformational nature of the 2G2 epitope, the antibody also recognises SDS-denatured actin in immunoblots, indicating that parts of the epitope are sufficient for binding. Indeed, the pepscan analysis showed that recognition by 2G2 does not require the complete epitope in its native conformation but that 2G2 also reacts with epitope parts in a presumably unfolded state. Apparently, less than the nominal 7-15 amino acids generally assumed to form an epitope are sufficient for complex formation between 2G2 and actin.

The assumption that only a specific state of actin is detected by 2G2, is corroborated by the fact that it does not bind to cytoplasmic F-actin neither in formaldehyde-fixed cells nor after microinjection into living cells. Visualization of the 2G2 epitope in a structural model of F-actin (derived from the model of Lorenz et al., 1993) revealed that it is located at the inner surface of the actin filament where the two strands oppose each other and is therefore most likely not accessible for the antibody (data not shown). 2G2 recognises cytoplasmic actin in methanol-treated cells possibly because of the denaturing effect of methanol which leads to a deterioration of the filaments and hence an exposure of the epitope, analogous to the situation after SDS-denaturation. The disappearence of nuclear dots after methanol treatment may be due to rigid nuclear extraction by methanol. Similar conclusions were drawn from the lack of nuclear reactivity with antibodies against plakophilin (Mertens et al., 1996).

Because of its specific properties described above, 2G2 may be a useful tool for the study of the structural state of actin in the nucleus. The nature and function of the nuclear dots remains unclear yet but their massive occurrence in myogenic cells and their differentiation-dependence point to a physiological function possibly connected with myogenesis. Similarly, the structural and functional role of the actin-containing fibrillar system in the amphibian oocyte nuclei remains to be established.

We are grateful to Drs J. Wehland and R. Frank, GBF, Braunschweig, for the spot synthesis of an actin fragment, Dr K. Schlüter (TU Braunschweig) for the computer modelling of actin, and to Dr K. Weber (Göttingen) for actin sequencing. Fermentation of 2G2 in serum-free medium was carried out by Dr J. Lehmann (Bielefeld). The work was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

Abe, H., Nagaoka, R. and Obinata, T. (1993). Cytoplasmic localisation and nuclear transport of cofilin in cultured myotubes. *Exp. Cell Res*. **206**, 1-10.

Adams, S. B. and Reisler, E. (1994). Sequence 18-29 on actin. antibody and spectroscopic probing of conformational changes. *Biochemistry* **33**, 14426-14433.

Amankawah, K. S. and De Boni, U. (1994). Ultrastructural localisation of filamentous actin within neuronal interphase nuclei in situ. *Exp. Cell Res.* **210**, 315-325.

Ankenbauer, T., Kleinschmidt, J. A., Walsh, M. J., Weiner, O. H., and Franke, W. W. (1989). Identification of a widespread nuclear actin binding protein. *Nature* **342**, 822-825.

Blau, H. M., Chin, C.-P. and Webster, C. (1983). Cytoplasmic activation of human nuclear genes in stable heterocaryons. *Cell* **32**, 1171-1180.

Brandner, J. M., Reichenbach, S. and Franke, W. W. (1997). Evidence that 'pinin', reportedly a differentiation-specific desmosomal protein, is actually a widespread nuclear protein. *Differentiation* **62**, 119-127.

Carson, M. (1997). Ribbons. *Meth. Enzymol.* **227**, 493-505.

Chik, J. K., Lindberg, U. and Schutt, C. E. (1996). The structure of an open state of beta-actin at 2.65 A resolution. *J. Mol. Biol.* **263**, 607-623.

Clark, T. G. and Merriam, R. W. (1977). Diffusible and bound actin in nuclei of Xenopus laevis oocytes. *Cell* **12**, 883-891.

Clark, T. G. and Rosenbaum, J. L. (1979). An actin filament matrix in hand-isolated nuclei of Xenopus laevis oocytes. *Cell* **18**, 1101-1108.

Clevenger, C. and Epstein, A. (1984). Identification of a nuclear protein

component of interchromatin granules using a monoclonal antibody and immunogold electron microscopy. *Exp. Cell Res.* **151**, 194-207.

Compton, J. L., Bellard, M. and Chambon, P. (1976). Biochemical evidence of variability in the DNA repeat length in the chromatin of higher eukaryotes. *Proc. Nat. Acad. Sci. USA* **73**, 4382-4386.

DeBoni, U. (1994). The interphase nucleus as a dynamic structure. *Int. Rev. Cytol.* **150**, 149-171.

Dissmann, E. and Hinssen, H. (1994) Immunocytochemical localisation of gelsolin in fibroblasts, myogenic cells, and isolated myofibrils. *Eur. J. Cell Biol.* **63**, 336-344.

Dlugosz, A. A., Antin, P. B., Nachmias, V. T. and Holtzer, H. (1984). The relationship between stress fiber-like structures and nascent myofibrils in cultured cardiac myocytes. *J. Cell Biol.* **99**, 2268-2278.

Drapeau, G. R. (1976). Protease from Staphylococcus aureus. *Meth. Enzymol.* **45**, 469-475.

Drewes, G. and Faulstich, H. (1991). A reversible conformational transition in muscle actin is caused by nucleotide exchange and uncovers cysteine in position 10. *J. Biol. Chem.* **266**, 5508-5513.

Egly, J. M., Miyamoto, N. G., Mancollin, V. and Chanton, P. (1984). Is actin a transcription factor for RNA polymerase B? *EMBO J.* **3**, 2363-2371.

Frank, R. (1992). Spot-synthesis. An easy technique for the positionally addressable, parallel chemical synthesis on a membrane support. *Tetrahydron* **48**, 9217-9232.

Fukui, Y. and Katsumaru, H. (1979). Nuclear actin bundles in Amoeba, Dictyostelium and human HeLa cells induced by dimethyl sulfoxide. *Exp. Cell Res.* **120**, 451-455.

Fyrberg, C., Ryan, L., McNally, L., Kenton, M., and Fyrberg, E. (1994). The actin protein superfamily. In *Molecular Evolution of Physiological Processes* (ed. D. M. Fambrough), pp. 173-178. New York. Rockefeller University Press.

Giloh, H. and Sedat, J. W. (1982). Fluorescence microscopy. Reduced photobleaching of rhodamine and fluorescein conjugated protein by n-propyl gallate. *Science* **217**, 1252-1255.

Gimona, M., Vandekerckhove, J., Goethals, M., Herzog, M., Lando, Z. and Small, J. V. (1994). Beta-actin specific monoclonal antibody. *Cell Motil. Cytoskel.* **27**, 108-116.

Goa, I. (1953). A micro-biuret method for protein determination. *Scand. I. Clin. Lab. Invest.* **5**, 218-222.

Gounon, P. and Karsenti, E. (1981). Involvement of contractile proteins in the changes in consistency of oocyte nucleoplasm of the newt Pleurodeles waltlii. *J. Cell Biol.* **88**, 410-421.

Hanein, D., Matsudaira, P. and DeRosier, D. J. (1997). Evidence for a conformational change in actin induced by fimbrin (N375) binding. *J. Cell Biol.* **139**, 387-396.

Hartmann, A. L., Sawtell, N. M. and Lessard, J. L. (1989). Expression of actin isoforms in developing rat intestinal epithelium. *J. Histochem. Cytochem.* **37**, 1225-1233.

Hasselbach, W. and Schneider, G. (1951). Der L-Myosin und Aktingehalt des Kaninchenmuskels. *Biochem. Zeitg.* **321**, 462-475.

Herman, I. M. (1993). Actin isoforms. *Curr. Opin. Cell Biol.* **5**, 48-55.

Hirano, H. and Watanabe, T. (1990). Microsequencing of proteins electrotransferred onto immobilizing matrices from polyacrylamide gel electrophoresis. application to an insoluble protein. *Electrophoresis* **11**, 573-580.

Hightower, R. C. and Meagher, R. B. (1986). The molecular evolution of actin. *Genetics* **144**, 315-332.

Iida, K., Iida, H. and Yahara, I. (1986). Heat shock induction of intranuclear actin rods in cultured mammalian cells. *Exp. Cell Res.* **165**, 207-215.

Jacobson, G. R. and Rosenbusch, J. P. (1976). ATP binding to a protease-resistant core of actin. *Proc. Nat. Acad. Sci. USA* **73**, 2742-2746.

Jockusch, B. M., Becker, M., Hindennach, I. and Jockusch, H. (1974). Slime mold actin. homology to vertebrate actin and presence in the nucleus. *Exp. Cell Res.* **89**, 241-246.

Kabsch, W., Mannherz, H. G., Suck, D. and Pai, E. F. (1990). Atomic structure of the actin-DNase I complex. *Nature* **347**, 37-44.

Khaitalina, S. Y., Collins, J. H., Kuznetseva, J. M., Pershina, V. P., Synakevich, I. G., Turoverov, K. K. and Usmanova, A. M. (1991). Physico-chemical properties of actin cleaved with bacterial protease from E. coli A2. *FEBS Lett.* **279**, 49-51.

Khaitlina, S. and Hinssen, H. (1997). Conformal changes in actin induced by its interaction with gelsolin. *Biophys. J.* **73**, 929-937.

Konigsberg, I. R. (1979). Skeletal myoblasts in culture. *Meth. Enzymol.* **58**, 511-527.

Korioth, F., Gieffers, C., Maul, G. G., and Frey, J. (1995). Molecular characterization of NDP 52, a novel protein of the nuclear domain 10 which is redistributed upon virus infection and interferon treatment. *J. Cell Biol.* **130**, 1-13.

Kyhse-Anderson, J. (1984). Electroblotting of multiple gels. A simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. *J. Biochem. Biophys. Meth.* **10**, 203-209.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Lazarides, E. and Weber, K. (1974). Actin antibody. The specific visualization of actin filaments in non-muscle cells. *Proc. Nat. Acad. Sci. USA* **71**, 2268-2272.

Lepault, J., Ranck, J. L,. Erk, I, and Carlier, M. F. (1994). Small angle X-ray scattering and electron cryomicroscopy study of actin filaments. role of the bound nucleotide in the structure of F-actin. *J. Struct. Biol.* **112**, 79-91.

Lorenz, M., Popp, D. and Holmes, K. C. (1993). Refinement of the F-actin model against X-ray fiber diffraction data by the use of a directed mutation algorithm. *J. Mol. Biol.* **234**, 826-836.

Mayboroda, O., Schlüter, K. and Jockusch, B. M. (1997). Different colocalisation of profilins with microfilaments in PtK_2 cells. *Cell Motil. Cytoskel.* **37**, 166-177.

McGough, A., Pope, B., Chiu, W. and Weeds, A. (1997). Cofilin changes the twist of F-actin: implications for actin filament dynamics and cellular function. *J. Cell Biol.* **138**, 771-781.

Mertens, C., Kubu, C. and Franke, W. W. (1996). Plakophilins 2a and 2b. constitutive proteins of dual location in the karyoplasm and the desmosomal plaque. *J. Cell Biol.* **135**, 1009-1025.

Milankov, K. and De Boni, U. (1993). Cytochemical localisation of actin and myosin aggregates in interphase nuclei in situ. *Exp. Cell Res.* **209**, 189-199.

Mornet, D. and Ue, K. (1984). Proteolysis and structure of skeletal muscle actin. *Proc. Nat. Acad. Sci. USA* **81**, 3680-3684.

Mullins, R. D., Kelleher, J. F. and Pollard, T. D. (1996). Actin' like actin? *Trends Cell Biol.* **6**, 208-212.

Muhlrad, A., P. Cheung, B. C. Phan, C. Miller, and Reisler, E. (1994). Dynamic properties of actin. Structural changes induced by beryllium fluoride. *J. Biol. Chem.* **269**, 11852-11858.

Nishida, E., Iida, K., Yonezawa, N., Koyasu, S., Yahara, I. Sakai, H. (1987). Cofilin is a component of intranuclear and cytoplasmic actin rods induced in cultured cells. *Proc. Nat. Acad. Sci. USA* **84**, 5262-5266.

Ochs, R. L., Lischwe, M. A., Spohn, W. H. and Busch, H. (1985). Fibrillarin. a new protein of the nucleolus identified by autoimmune sera. *Biol. Cell.* **54**, 123-134.

Onoda, K., F. X. Yu, and. Yin, H. L. (1993). gCap39 is a nuclear and cytoplasmic protein. *Cell Motil. Cytoskel.* **26**, 227-238.

Orlova, A., Prochniewicz, E. and Egelman, E. A. (1995). Structural dynamics of F-actin. II. Cooperativity in structural transitions. *J. Mol. Biol.* **245**, 598-607.

Parfenov, V. N., Davis, D. S., Pochukalina, G. N., Sample, C. E., Bugaeva, E. A. and Murti, K. G. (1995). Nuclear actin filaments and their topological changes in frog oocytes. *Exp. Cell Res.* **217**, 385-394.

Polzar, B., A. Rosch and Mannherz H. G. (1989). A simple procedure to produce monospecific polyclonal antibodies of high affinity against actin from muscular sources. *Eur. J. Cell Biol.* **50**, 220-229.

Rao, J. Y., Bonner, R. B., Hurst, R. E. and Liang, Y. Y. (1997). Quantitative changes in cytoskeletal and nuclear actins during cellular transformation. *Int. J. Cancer* **70**, 423-429.

Rimm, D. L. and Pollard, T. D. (1989). Purification and characterization of an Acanthamoeba nuclear actin-binding protein. *J. Cell Biol.* **109**, 585-591.

Sahlas, D. J., Milankov, K., Park, P. C., Be Boni, U. (1993). Distribution of snRNPs, splicing factor SC-35 and actin in interphase nuclei. immuncytochemical evidence for differential distribution during changes in functional states. *J. Cell Sci.* **105**, 347-357.

Sanger, J. W., Sanger, J. M., Kreis, T. E. and Jockusch, B. M. (1980). Reversible translocation of cytoplasmic actin into the nucleus caused by dimethyl sulfoxide. *Proc. Nat. Acad. Sci. USA* **77**, 5268-5272

Scheer, U., Hinssen, H., Franke, W. W. and Jockusch, B. M. (1984). Microinjection of actin-binding proteins and actin antibodies demonstrates involvement of nuclear actin in transcription of lampbrush chromosomes. *Cell* **39**, 111-122.

Schmidt, H., Langbein, L., Rode, M., Pretzel, S., Zwiebelmann, R. and Franke, W. W. (1997). Plakophilins 1a and 1b. widespread nuclear proteins recruited in specific epithelial cells as desmosomal plaque components. *Cell Tiss. Res.* **290**, 481-496.

Schrader, M., C. J. Temm Grove, J. L. Lessard, and Jockusch, B. M.

(1994). Chicken antibodies to rabbit muscle actin with a restricted repertoire of F-actin recognition. *Eur. J. Cell Biol.* **63**, 326-335.

Schroer, T. A., Fyrberg, E., Cooper, J. A., Waterson, R. H., Helfman, D. M., Pollard, T. D. and Meyer, D. I. (1994). Actin-related protein. nomenclature and classification. *J. Cell Biol.* **127**, 1777-1778.

Schutt, C. E., Myslik, J. C., Rozycki, M. D., Goonesekere, N. C. W. and Lindberg, U. (1993). The structure of cristalline profilin-—actin. *Nature* **365**, 810-816.

Schwyter, D. H., Phillips, M. and Reisler, E. (1989). Subtilisin-cleaved actin. polymerization and interaction with myosin subfragment 1. *Biochemistry* **28**, 5889-5895.

Sheterline, P. and Sparrow, J. C. (1994). Actin. *Protein Profile* **1**, 1-121.

Skalli, O., Gabbiani, G., Babai, F., Seemayer, T. A., Pizzolato, G. and Schurch, W. (1988). Intermediate filament proteins and actin isoforms as markers for soft tissue tumor differentiation and origin. II. Rhabdomyosarcomas. *Am. J. Pathol.* **130**, 515-531.

Sohn, R. J. and Goldschmidt-Clermont, P. J. (1994). Profilin. at the crossroads of signal transduction and the actin cytoskeleton. *BioEssays* **16**, 465-472.

Soyer-Gobillard, M.-O., Ausseil, J. and Géraud, M.-L. (1996). Nuclear and cytoplasmic actin in dinoflagellates. *Biol. Cell* **87**, 17-35.

Spudich, J. A. and Watt, S. (1971). The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin-complex with actin and the proteolytic fragments of myosin. *J. Biol. Chem.* **246**, 4866-4871.

Strzelecka-Golaszewska, H., Moraczewska, J., Khaitalina, S. Y. and Mossakowska, M. (1993). Localisation of the tightly bound divalent-cation-dependent and nucleotide-dependent conformation changes in G-actin using limited proteolytic digestion. *Eur. J. Biochem.* **211**, 731-742.

Tanaka, M. and Shibata, H. (1985). Poly(L-proline)-binding proteins from chick embryos are a profilin and a profilactin. *Eur. J. Biochem.* **151**, 291-297.

Volkmann, L. E., Talhouk, S. N., Oppenheimer, D. I. and Charlton, C. A. (1992). Nuclear F-actin. a functional component of baculovirus-infected lepidopteran cells? *J. Cell Sci.* **103**, 15-22.

Weber, V., Harata, M., Hauser, H., Wintersberger, U. (1995). The actin-related protein Act3p of Saccharomyces cerevisiae is located in the nucleus. *Mol. Biol. Cell* **6**, 1263-1270.

Wehland, J., Weber, K. and Osborn, M. (1980). Translocation of actin from the cytoplasm into the nucleus in mammalian cells exposed to dimethylsulfoxide. *Biol. Cell.* **39**, 109-111.

Welch, W. J. and Suhan, J. P. (1985). Morphological study of the mammalian stress response. characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. *J. Cell Biol.* **101**, 1198-1211.