A 33 kDa protein with sequence homology to the 'laminin binding protein' is associated with the cytoskeleton in hydra and in mammalian cells

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Summary
In hydra and in mammalian cells the monoclonal antibody V recognises an epitope which colocalises with cytoskeletal structures. Using this antibody for expression screening, a cDNA clone (955 bp) was isolated from hydra, which covers an open reading frame for a protein of 294 amino acids with a calculated molecular mass of 32.8 kDa. Northern blot analysis of hydra RNA resulted in a single mRNA species of 1.2 kb, and primer extension experiments proved this to be the full length message. 218 residues at the amino terminus of the hydra protein show extensive homology (73.5%) to a human protein designated 'laminin binding protein'. The carboxyl-terminal 76 amino acids possess no significant similarity (20%). The monoclonal antibody V, which recognises an epitope in this carboxyl-terminal part, reacts in Western blots, both in hydra and in mammalian cells, with a protein of 33 kDa and not with the 45 kDa 'laminin binding protein'. The 33 kDa protein is not extracellular or transmembrane, but has a strictly intracellular location as indicated by its amino acid sequence and by immunocytochemical and cell fractionation studies.

In non-dividing mammalian cells the 33 kDa protein colocalises with filamentous structures; in dividing cells it dissociates from it and concentrates centrally. Presence of the SPLR-sequence, which is the consensus phosphorylation motif for the p34cdc2 kinase, links this 33 kDa protein to events occurring during the cell cycle.

Key words: hydra, cytoskeleton, expression screening, human 'laminin binding protein', p34cdc2 phosphorylation motif, cell division.

Introduction
In the freshwater coelenterate hydra, cellular growth is controlled by the nervous system. At appropriate times nerve cells release neuropeptides like head and foot activator which act on surrounding cells as growth factors (Schaller et al. 1989b). The effect of head activator on cell division is visible both in hydra and in mammalian cells 1 h after application as an increase of cells in mitosis (Schaller et al. 1989c). This indicates that within this short period of time signal transduction occurs via interaction of the peptide with a membrane receptor, followed by signal amplification over second messenger systems, leading to reorganisation of the cytoskeleton, condensation of chromosomes, nuclear envelope breakdown, formation of the mitotic apparatus, segregation of sister chromosomes and finally, cell division.

In an attempt to understand this pathway we investigated changes in cytoskeletal organisation preceding cell division. In this paper we describe a 33 kDa protein with its full length cDNA clone. This protein is associated with filamentous cytoskeletal elements in hydra and in mammalian cells. Like other cytoskeletal structures it undergoes reorganisation during mitosis. The possibility that phosphorylation of the 33 kDa protein might regulate this reorganisation is supported by the presence of the consensus phosphorylation motif for the cell cycle-specific p34cdc2 kinase.

Materials and methods

Animals
Hydra vulgaris (formerly named Hydra attenuata) was originally obtained from P. Tardent, Zürich. The multiheaded mutant of the species Chlorohydra viridissima was from H. M. Lenhoff, Irvine. The animals were cultured in a medium consisting of 1 mM CaCl2, 0.1 mM MgCl2, 0.1 mM KCl buffered with 0.5 mM sodium phosphate, pH 7.6, (hydra medium), at 19±2°C. Animals were fed daily between 9 and 10 a.m. and washed 5–7 h later. For experiments they were starved for at least one day.

Preparation of hydra cell fractions
Hydra were collected from the culture, sedimented and homogenised in a buffer containing 0.4 M sucrose in hydra medium to which 1 mM phenylmethylsulfonyl fluoride (PMSF) was added as protease inhibitor (buffer A). All steps were carried out at 4°C. On average, 1000 Hydra vulgaris yielded a volume of 1 ml containing 10 mg of protein. After the first gentle homogenisation the tissue was centrifuged at 1000 g for 10 min. The sediment was resuspended in buffer A, rehomogenised and centrifuged again at 1000 g for 10 min to yield the nuclear and cytoskeletal pellet.

Antibodies
The IgM monoclonal antibody V was originally produced and selected against head activator-containing conjugates (Schawal-
Immunocytochemistry

Actin detection

For colocalisation studies with actin, the rhodamine-labelled phalloidin (a kind gift from Michael Nassal) was used at a concentration of 0.5 μg ml⁻¹ (Wieland, 1981; Jahn, 1982).

DNA staining

Cells were incubated for 10 min at room temperature with 0.01 μg ml⁻¹ Hoechst 33258.

Immunocytochemistry

Hydra. Whole animals of the species Hydra vulgaris were fixed overnight in Lavdowsky's fixative, a solution containing 4% acetic acid, 3.7% formaldehyde and 50% ethanol (Dunne et al., 1985), or alternatively in 4% paraformaldehyde in phosphate-buffered saline (PBS: 140 mM sodium chloride, 10 mM sodium phosphate, pH 7.2). Lavdowsky's fixative was better for visualising filaments with either an α-actin antibody or with the monoclonal antibody V, whereas formaldehyde was better for staining with phalloidin. The animals were then washed three times for 30 min in PBS and then once with PBS, 0.1% Triton X-100. Nonspecific binding was blocked by incubation for 30 min at room temperature in PBS with 1% bovine serum albumin (BSA) and 0.1% Triton X-100. The monoclonal antibody V was used at a dilution of 1:100 in the same solution and incubated overnight at 4°C. After washing three times for 15 min in PBS, 0.1% Triton X-100 and blocking for 30 min, animals were incubated for 3–4 h at room temperature with fluorescein isothiocyanate (FITC)-labelled α-mouse IgM at a dilution of 1:100 in PBS, 0.1% Triton X-100, 1% BSA. The preparations were washed twice in PBS for 10 min and then counterstained with rhodamine-labelled phalloidin (0.5 μg ml⁻¹) for 1 h at room temperature.

NIH 3T3 mouse fibroblasts. The procedures described above for whole mounts of hydra were also used for the study of NIH 3T3 mouse fibroblasts grown on tissue culture slides.

Immunoblotting of proteins

Total hydra protein samples were prepared by boiling polyps in SDS sample buffer (62.5 mM Tris/HCl, pH 6.8, 2% SDS, 5% β-mercaptoethanol, 5% glycerol, 0.01% bromphenol blue). Cell fractions were either dissolved directly in sample buffer or after concentration by ethanol precipitation. Proteins were fractionated by electrophoresis on an SDS-polyacrylamide gel (Laemmli, 1970) and transferred onto nitrocellulose filters (Towbin et al., 1979). Blots were saturated for 30 min in blocking buffer containing PBS, 10% fetal calf serum (FCS), 1% BSA, 0.2% casein, and then incubated overnight at 4°C with the first antibody, V dilution 1:5000 in the same buffer. After three washes with PBS containing 0.05% Triton X-100, the second antibody (Sigma, rabbit cr-mouse IgM, alkaline phosphatase-coupled) was applied at a dilution of 1:1000 in blocking buffer and the incubation was continued for 3 h at room temperature. The nitrocellulose was then washed again three times with PBS, 0.05% Triton X-100, then once in PBS without Triton X-100, and finally with a buffer containing 100 mM Tris/HCl, 100 mM sodium chloride, 5 mM MgCl₂, pH 9.5 (AP buffer). The alkaline phosphatase-linked second antibody was then detected via a color reaction by incubation of the nitrocellulose in AP buffer containing 0.33 g l⁻¹ p-nitro blue tetrazolium chloride, 0.17 g l⁻¹ 5-bromo-4-chloro-3-indolyl phosphate. The enzyme reaction was stopped with 20 mM Tris/HCl, pH 8, 5 mM EDTA.

Molecular biology

Two cDNA libraries of the multihedated mutant of the species Chlorohydra viridissima were constructed following the method of Huynt et al. (1985), using the expression vector λgt11. The λgt11 library was screened with the V antibody at a dilution of 1:5000. Antibody binding was detected using an alkaline phosphatase-labeled rabbit α-mouse IgM (Sigma, 1:1000).

To obtain the full length clone a cDNA insert, isolated via the λgt11 screening, was radiolabelled with 3²P and used for further screening of an oligo-dT-primed λgt10 library of the multihedated mutant Chlorohydra viridissima, constructed as described in Benton and Davis (1977). For sequence determination, cDNA inserts were cloned into the plasmid vector PBS. DNA sequencing was done using the chain termination method (Sanger et al. 1977). DNA and amino acid data were analysed using the HUSAR software package (Heidelberg Unix Sequence Analysis Resources).

Southern and Northern blot analysis

Genomic DNA was digested with the restriction endonucleases EcoR I and HindIII, fractionated by agarose gel electrophoresis and transferred to nitrocellulose filters. Hybridisation to filter-bound DNA (Southern, 1975) was carried out in a solution containing 43% formamide, 5×SSC (150 mM sodium chloride, 15 mM sodium citrate), 50 mM sodium phosphate, pH 7.0, 0.1% sodium dodecyl sulfate (SDS), 200 μg ml⁻¹ tRNA, 10×Denhardt's (0.02% BSA, 0.02% Ficol, 0.02% polyvinylpyrrolidone) at 42°C overnight. After hybridisation, filters were washed with 2×SSC, 0.1% SDS at room temperature and twice with 1×SSC, 0.1% SDS at 42°C. For Northern blot analysis, 5 μg poly(A⁺) RNA was electrophoresed through a 1% agarose gel in 3.7% formaldehyde, 20 mM morpholine propane sulfonic acid, 50 mM sodium acetate and 10 mM EDTA, pH 7.0. The RNA was transferred to a Hyb N membrane (Amerham) and hybridised with the radiolabelled cDNA clone (Thomas, 1980) in a solution containing 50% formamide, 5 mM sodium phosphate, pH 6.7, 5×SSC, 0.1% SDS, 1×EDTA, 1×Denhardt's, 200 μg ml⁻¹ salmon sperm DNA. After an overnight incubation at 50°C the membrane was washed with 1×SSC, 1% SDS. The DNA fragments used as hybridisation probes were labelled with 3²P by using the multirandom primer method (Feinberg and Vogelstein, 1983).

Results

Immunocytochemical colocalisation of the V antigen with cytoskeletal structures in hydra

The freshwater coelenterate hydra consists of two cell layers, ectoderm and endoderm, which contain epithelio-muscular cells as main constituents. Such cells have an apico-basal polarity with muscular processes located at their base (Kanaev, 1952). In these processes, actin-containing fibers are oriented parallel to the body axis in the ectoderm and perpendicular to it in the endoderm (Lentz, 1966). Fig. 1 shows these fibres in whole polyps stained with rhodamine-labelled phalloidin, a phytotoxin which binds filamentous actin (Wieland, 1981). The monoclonal antibody V also recognises these actin-containing fibers, shown as a double stain in Fig. 2. In addition to this filamentous appearance of the V-antigen, we also detected a diffuse cytoplasmic staining of dividing interstitial cells in hydra.

Identification of a 33 kDa protein as a target for the monoclonal antibody V in hydra

To establish the identity of the protein containing the epitope for the monoclonal antibody V, Western blot analyses of extracts from whole hydra were performed. One major area of immunoreactivity was detected as a doublet band with a molecular mass around 33 kDa (Fig. 3). The extract derived from one hydra sufficed to detect this band, indicating that the 33 kDa protein is relatively abundant (0.1–1% of all proteins).

Cloning and characterisation of the cDNA coding for the 33 kDa protein from hydra

The monoclonal antibody V was used for immunoscreen-
Fig. 1. Visualisation of actin filaments in whole mounts of *Hydra vulgaris*. Animals were fixed with 4% paraformaldehyde and stained with rhodamine-coupled phalloidin. (A) Young bud still attached to the mother animal. (B) Tentacles surrounding a hypostome with longitudinal fibres in the ectoderm and horizontal fibres in the endoderm around the mouth opening. (C) Head region with two tentacle bases. (D) Part of a tentacle in which both longitudinal and horizontal fibres are visible. Bars, A, 100 μm; B, 35 μm; C, 30 μm; D, 25 μm.

Fig. 2. Colocalisation of the V antigen with actin filaments. Extended tentacles from *Hydra vulgaris* were stained with rhodamine-coupled phalloidin to visualise actin (A) and with the V antibody which was made visible by a FITC-coupled second antibody (B). For fixation, 4% paraformaldehyde was used. Bar, 20 μm.
This putative initiation codon was followed by an open untranslated region. From this it is concluded that the 35 Chlorohydra ing of an oligo-dT-primed Agt11 library from Chlorohydra viridissima of 282 bp was used as a probe to screen an oligo-dT-primed consensus translation initiation sequence (Kozak, 1984). The motif CCA at position 27-29, which conformed to the position 32-34. This start triplet was preceded by the region (Fig. 4). The first in frame ATG was found at inserts. One clone with 955 bp contained the whole coding region for longer AgtlO cDNA library of Chlorohydra viridissima p34cdc2 protein kinase (S/T-P-X-R) (Peter et al. 1989). This putative initiation codon was followed by an open reading frame was 294 residues long with a possible polyadenylation signals, AATAAA, (Proudfoot et al. 1989). Both have been shown to be very A+T-rich, actin containing 75% and src-related protein 82% A+T. The coding region was followed by 14-18 bases of a 3' untranslated sequence containing two possible polyadenylation signals. Thick underlining indicates the potential phosphorylation site of the ORF. Thin lines indicate the position of start and stop codons. The ORF. Thin lines indicate the position of start and stop codons of the two possible polyadenylation signals. Thick underlining indicates the potential phosphorylation site of the p34cdc2 kinase. Stars indicate possible serine/threonine phosphorylation sites. Arrowheads label the tryptophan residues. The open circle indicates a putative myristoylation site.

Further computer analysis revealed the following features. The protein sequence contained no glycosylation sites, but several putative serine/threonine phosphorylation sites. Four of these, located at positions 78, 190, 221 and 286, could be signals for protein kinase C; one at residue 252 could be a signal for casein kinase II, and one located at position 162 contained the motif SPLR, which corresponds to the consensus phosphorylation site for the p34cdc2 protein kinase (S/T-P-X-R) (Peter et al. 1990). Very striking was the relatively high abundance and the pattern of the usually rare amino acid tryptophane: 10 tryptophane residues were present in the 294 amino acid sequence, 6 of them, preceded by aspartic acid in 5 out of 6 cases, were located with a relatively even spacing of 6-10 residues in the last 50 amino acids at the carboxyl terminus of the molecule. The results of hydropathy analysis, performed according to the method of Kyte and Doolittle (1982) confirmed the hypothesis that this is a hydrophilic protein containing some weak hydrophobic regions (Fig. 5A). Hydropathy analysis performed according to the method of Engelman et al. (1986) (Fig. 5B) revealed that there was no stretch of amino acids long and hydrophobic enough to serve as a transmembrane region. A putative myristoylation site at amino acid position 4, a glycine residue, followed by a serine at position 5, may indicate membrane anchoring and/or protein–protein interaction (McIlhinney, 1990).

So far two genes have been cloned from hydra, actin (Fisher and Bode, 1989) and src-related protein (Bosch et al. 1989). Both have been shown to be very A+T-rich, actin containing 75% and src-related protein 82% A+T. The 882 coding bases for the 33 kDa protein consist of 250 A, 198 G, 164 C and 270 T, corresponding to a total A+T content of 59%. This is not distributed randomly, but

Fig. 3. Western blot analysis of extracts from whole hydra of the species Hydra vulgaris with the monoclonal antibody V. Lane 1 was a negative control with the second antibody alone. Lanes 2–4 were incubated with the monoclonal antibody V at a dilution of 1:5000. Lanes 1, 2, 3 and 4 contained 50 μg, 10 μg, 30 μg and 100 μg of protein, respectively. Molecular mass standards are depicted by bars.

Fig. 4. Nucleotide sequence and predicted amino acid sequence of the cDNA clone encoding the 59 kDa protein. The nucleotide sequence was determined by the dideoxy method (Sanger et al. 1977). Numbering of bases starts at the first nucleotide, that of amino acids at the first methionine as potential start site of the ORF. Thin lines indicate the position of start and stop codons and of the two possible polyadenylation signals. Thick underlining indicates the potential phosphorylation site of the p34cdc2 kinase. Stars indicate possible serine/threonine phosphorylation sites. Arrowheads label the tryptophan residues. The open circle indicates a putative myristoylation site.
preferentially (75%) located at the third position of a codon, indicating a preference for A or T at the wobble position for the hydra codon usage.

Northern blot analysis of poly(A)⁺ RNA from hydra, in which the radiolabelled cDNA of the 33 kDa protein was used as a probe, revealed the presence of a single RNA species of about 1.2 kb (Fig. 6). Primer extension experiments showed this to be the full length mRNA. No larger mRNA species were detected.

To characterise the genomic organisation of the gene encoding the 33 kDa protein, Southern blot analysis was performed using genomic DNA of hydra, digested either with the restriction enzyme EcoRI or with HindIII, and hybridised with the radiolabelled 955bp-long cDNA encoding the 33 kDa protein. The EcoRI-digested DNA (Fig. 7, lane 1) showed one single band in the range of 6000 bp. The HindIII digest resulted in two bands with sizes of 4000 and 1400 bp, respectively (Fig. 7 lane 2). This is in agreement with an internal HindIII site within the sequence encoding the 33 kDa protein. From this we conclude that the gene encoding the 33 kDa protein occurs only once in the hydra genome.

Sequence homology of the hydra 33 kDa protein with a 'laminin binding protein'

A computer-directed homology search revealed a striking homology between the first 218 amino acids of the 33 kDa hydra protein and a human 'laminin binding protein' (Wewer et al. 1986; Yow et al. 1988), amounting to 73.5% homology at the amino acid level and to 65% at the nucleotide level (Fig. 8). The carboxyl-terminal part (76 amino acids), which in the human protein is supposed to be responsible for laminin binding (Wewer et al. 1986), and which in hydra contains the V epitope, did not show any sequence homology.
Fig. 7. Southern blot analysis of genomic DNA from the multiheaded mutant Chlorohydra viridissima. Genomic DNA digested with the restriction enzyme EcoRI (lane 1) or HindIII (lane 2) was separated on an agarose gel, blotted and hybridised with the radiolabelled cDNA clone encoding the 33 kDa protein.

Is the 33 kDa protein a laminin binding protein?

To establish a possible relationship between the human and hydra proteins the V antibody was assayed in Western blots with extracts from human cell lines. In contrast to the putative 'laminin binding protein' which was shown to migrate to a position of 40–45 kDa (Van den Ouweland et al. 1989; Rabacchi et al. 1990) the V antibody reacted in human as well as in hydra with a 33–35 kDa protein (data not shown).

Immunocytochemistry revealed no binding of the V antibody to the cell surface on living cells. This indicated that the carboxyl part of the 33 kDa protein which was recognised by the V antibody and which, in the case of the human 'laminin binding protein' was predicted to bind laminin, was not outside the cell. To prove the intracellular localisation of the V antigen, cell fractionation studies were performed. Analyses of the different hydra cell fractions showed that under mild iso-osmolar homogenisation conditions about 90% of the 33 kDa protein cosedimented with nuclear and cytoskeletal components at 1000 g, 5–10% cosedimented with the membrane fraction at 50000 g, and 0–5% were present in the 50000 g supernatant in a soluble form (Fig. 9). By extracting the 1000 g sediment with salt (80 mM NaCl), or 0.6 M KI, which is supposed to depolymerise filamentous actin (Jahn, 1982) and other cytoskeletal components, or by homogenising cells at acidic pH (10 mM ammonium acetate, pH 6) the 33 kDa protein was converted quantitatively into the soluble form. This suggests that the 33 kDa protein is an intracellular component, most of which is

Fig. 8. Comparison of the deduced amino acid sequence of the hydra 33 kDa protein (upper line) with the human 'laminin binding protein' (lower line).

Fig. 9. Western blot analysis of cell fractions from Hydra vulgaris with the monoclonal antibody V. Cell fractionation was performed as described in Materials and methods. Lanes 1–3 contained the 1000 g sediment with nuclear and cytoskeletal components, lanes 4–6 contained the 1000 g supernatant with the soluble proteins, lanes 7–9 contained the 50000 g sediment with the membrane components. Lane 10 contained the 50000 g supernatant. Different amounts of hydra equivalents were separated on a 12.5% SDS–polyacrylamide gel, as follows: 3 (lane 1), 5 (lane 2), 10 (lanes 3, 4, 7), 30 (lanes 5 and 8), 50 (lanes 6, 9 and 10).

Fig. 10. Association of the V antigen with filamentous structures in NIH 3T3 mouse fibroblasts. Cells were fixed with Lavdowsky's fixative. Bar, 15 μm.
Fig. 11. Change in the V epitope localisation during cell division in mouse fibroblasts. NIH 3T3 cells were fixed with Lawdowsky's fixative. Shown are phase contrast (A, D, G, J) and fluorescence micrographs of cells stained either with the V antibody in conjunction with a FITC-labeled second antibody (B, E, H, K) or with Hoechst to visualise the DNA (C, F, I, L). Different mitotic stages are indicated by arrowheads. Bar, 35 μm.
attached in vivo to cytoskeletal structures. This is supported by immunocytochemistry on fixed NIH 3T3 mouse fibroblasts, where the V epitope is associated with cytoskeletal structures (Fig. 10). In interphase cells the V antibody reacted with thin filaments extending over the whole cell (Fig. 10 and Fig. 11). Staining of the filamentous structures disappeared when the cells started to enter mitosis, as shown in Fig. 11 where different mitotic stages were visualized by staining the cells with Hoechst (arrowheads). There was an accumulating immunoreactivity around the nucleus when chromosome condensation occurred. As mitosis proceeded through metaphase and anaphase, the V antigen appeared as a bright, diffuse cytoplastic staining (Fig. 11 D-I). When cytokinesis was almost complete filamentous structure became visible again (Fig. 11 J-L). These observations indicate that the change in localisation of the 33 kDa protein is correlated with changes occurring during the cell cycle.

Discussion

Eucaryotic cells have a high degree of internal organisation brought about by a complex network of protein filaments which are responsible for distinct cell shapes. Different parts of the cytoskeleton must be linked together and their functions must be coordinated in order to mediate intracellular events. During mitosis the cytoskeletal network undergoes a series of dramatic structural changes, which finally leads to cell division.

Several lines of evidence presented in this study have allowed us to conclude that a 33 kDa protein, which is recognised by the monoclonal antibody V, is involved in cytoskeletal rearrangements during mitosis. In whole mounts of hydra, this antibody reacted on the one hand with actin-containing filaments in muscular processes of epithelial cells, and on the other hand appeared as a diffuse cytoplasmic pattern in dividing interstitial cells. Similar results were obtained with mammalian cells, where in differentiated NIH 3T3 fibroblasts, for example, the V epitope was associated with cytoskeletal structures containing actin filaments, but in dividing cells it dissociated from the filaments and concentrated centrally. The immunocytochemical colocalisation with intracellular filamentous structures was confirmed by cell fractionation studies, which showed a copurification of the V immunoreactivity with cytoskeletal elements.

To characterise the immunoreactive 33 kDa antigen at the nucleotide level, we used the V antibody for expression screening of a Agt11 cDNA library from hydra. The full length cDNA clone obtained encoded 294 amino acids. Primer extension experiments confirmed the fact that the clone contained the whole coding region for a protein with a calculated molecular mass of 32.8 kDa. This corresponded to the V immunoreactive protein which in Western blot analysis migrated with 33 kDa.

Comparison of the putative amino acid sequence with other known proteins revealed a striking homology between this hydra protein and a human protein designated ‘laminin binding protein’ (Yow et al. 1988). At the amino acid level, this homology amounted to 73.5 %, at the nucleotide level to 65 %. The homology between these two proteins was restricted to the 218 amino-terminal amino acids. The remaining 76 amino acids showed no significant homology. This part of the human protein was originally described as the laminin binding domain of a 67 kDa ‘laminin receptor’ (Wewer et al. 1986). For the human and the hydra protein only a single mRNA species of 1.2 kb was detected and no larger transcripts. 67 kDa would exceed this coding capacity. Since the encoded proteins have similar general features, including lack of a signal peptide and an internal hydrophobic stretch long enough for transmembrane spanning, we consider it unlikely that they are components of the outer cell membrane. We confirmed this by demonstrating that living cells do not expose the protein at their outer cell surface and that the 33 kDa protein copurified with cytoskeletal and not with membrane fractions.

The hydra and the human protein contained several conserved serines and threonines as putative phosphorylation sites for kinases, which was in accordance with the appearance of the 33 kDa as a doublet band in Western blots. Increased levels of phosphorylation, for example of vimentin or laminin, brought about by interaction with the p34cdc2 kinase during mitosis, are accompanied by filament disassembly and nuclear envelope breakdown (Ottaviano and Gerace, 1985; Miike-Lye and Kirschner, 1985; Dessev and Goldman, 1988; Chou et al. 1990). Especially interesting for the hydra protein in this context was the presence of the sequence SPLR, which corresponds to the consensus phosphorylation motif for this cell cycle-specific kinase. We suggest that phosphorylation of this site may trigger dissociation of the 33 kDa protein from actin-containing filaments, thus initiating its reorganisation during mitosis.

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