

Murine cDNAs coding for the centrosomal antigen centrosomin A*

GABY JOSWIG¹, CHRISTIAN PETZELT² and DIETER WERNER^{1,†}

¹*Institute of Cell and Tumor Biology, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG*

²*Laboratoire International de Biologie Cellulaire Marine, F-85 350 Ile d'Yeu, France*

*The nucleotide sequence of full-length centrosomin A cDNA published here has been deposited at the EMBL sequence data bank and is available under accession number X173737.

† Author for correspondence

Summary

Screening of an induced Ehrlich ascites cell-derived λ gt11 cDNA library with an antibody (GP1), immunoreacting specifically with centrosomal antigen(s) of interphase and mitotic cells of different species, released a partial cDNA clone (λ P10A) encoding the carboxy-terminal section of a centrosome-specific antigen. This specificity of the clone λ P10A could be verified by *lacZ*-directed antigen expression from *Escherichia coli* Y1089 lysogenized with the recombinant phage λ P10A and subsequent production of centrosome-specific antibodies by means of the recombinant antigen. Using the λ P10A insert as a probe, two types of cDNA clones were identified in a λ gt10 cDNA library by plaque-hybridization. The inserts of PN1 type clones were 1.2 kb (kilobases) and those of PN5 type clones were 2.2 kb in length. The DNA sequence of a PN1 type clone revealed its full-length cDNA nature. The open reading frame of PN1 encodes a rather hydrophilic and highly charged $34.5 \times 10^3 M_r$ polypeptide comprising short but appar-

ently significant strings of 100% sequence identity with the major nuclear lamina polypeptides lamins A/C and lamin B. Restriction enzyme mapping of PN1 and PN5 inserts, cross-hybridization experiments and comparison of overlapping DNA sequences indicate that the 1.2 kb and 2.2 kb cDNAs code for the same $34.5 \times 10^3 M_r$ polypeptide, termed centrosomin A. Western blots of Ehrlich ascites cell proteins show a second, larger GP1 antigen (centrosomin B) whose cDNA has not been cloned. It remains to be investigated whether centrosomin B is encoded by a second mRNA or whether it reflects an oligomeric or a postrationally modified form of centrosomin A.

Key words: centrosome, centrosomal antigens, lamins, monoclonal/polyclonal antibodies, recombinant DNA, cDNA libraries, λ gt10, λ gt11, cDNA clones, cDNA sequencing.

Introduction

One of the cellular organelles whose structure and function are still poorly understood is the centrosome. It has been a central enigma since its first description by Boveri (1900). On the morphological level it is known to be essential for mitosis where it forms the two poles for subsequent cell division. In interphase, it represents the central part of the 'cytocoenter' from which the microtubules emanate to guide the movement of particles and to form an ephemeral cytoskeleton (McIntosh, 1983; Vorobjev and Nadezhdina, 1987). Because of its obvious central functions in directing intracellular movements it has been termed 'traffic control center' of the cell (Mazia, 1987). Centrosomal elements have been found to undergo cyclic condensations and decondensations (Schatten *et al.* 1987), and evidence has been presented for a linear structure for the centrosome (Calarco-Gillam *et al.* 1983; Schatten *et al.* 1986). In contrast to the large bulk of morphological data, our knowledge of the molecular characteristics of intrinsic centrosomal components and their function at the molecular level are still scarce. It is evident that the introduction of recombinant DNA technology into this field could reveal new dimensions in centrosome research. As a step in this direction we cloned and sequenced cDNAs coding for a centrosomal antigen termed centrosomin A.

Materials and methods

Antibodies to centrosomal antigens

Lysates of PtK cells were fractionated on sucrose step gradients as described previously in detail (Mitchison and Kirschner, 1984; Bornens *et al.* 1987; Joswig and Petzelt, 1990). Fractions enriched in material immunoreactive with a monoclonal antibody (F5.1), which decorated centrosomal spheres exclusively (Joswig and Petzelt, 1990), were submitted to SDS-polyacrylamide gel electrophoresis. Gel slices corresponding to the position of the antigenic material ($32-34 \times 10^3 M_r$ region) were electroeluted and the antigen was used to immunize guinea pigs by conventional protocols. Preimmune serum was tested for the absence of antibodies to centrosomal antigens. Development of centrosome-specific antibodies was monitored by immunofluorescence techniques described previously in detail (Joswig and Petzelt, 1990) and the positive serum was termed GP1.

The recombinant antigen specified by λ P10A was electro-eluted from SDS-polyacrylamide gels, precipitated with 7 vol. acetone and used to immunize chickens whose eggs were pretested for the absence of centrosome-specific antibodies. Development of centrosome-specific antibodies after intramuscular injections of the recombinant antigen was detected by the immunofluorescence methods described for Fig. 1 (below). Antibodies were isolated from eggs according to the method of Hiepe *et al.* (1988).

cDNA and cDNA libraries

The λ gt11 cDNA library screened with the antibody was prepared

from RNA of Ehrlich ascites tumor cells grown in mice. A detailed description of the preparation and the quality of the library has been published (Lu and Werner, 1988; Lu *et al.* 1988). An Ehrlich ascites cell-derived cDNA library prepared in the λ gt10 vector (Lu and Werner, 1988; Lu *et al.* 1988) was used for screening experiments with nucleic acid probes.

Screening of λ gt11 cDNA recombinants with the GP1 antibody probe

The λ gt11 recombinants were screened with the antibody probe as plaques on a lawn of *Escherichia coli* Y1090. The procedure was essentially that recommended by Huynh *et al.* (1986). In the first round, about 20 000 plaque-forming units (p.f.u.) of λ gt11 recombinants were plated per 130 cm² dish and incubated at 42°C until small plaques became visible. Production of fusion proteins was induced by application of nitrocellulose filters (Schleicher & Schuell, BA85) soaked with 10 mM β -D-thiogalactopyranoside (IPTG) in water. After 5 h at 37°C the first set of filters was replaced by a second set and incubation was continued for another 5 h at 37°C. All phosphate-buffered saline solutions (NaCl/P_i) used in the following steps were supplemented with 0.02% sodium azide. After two washes with NaCl/P_i containing 0.05% Tween 20, the filters were dried for 30 s on filter paper and soaked in NaCl/P_i/1% bovine serum albumin (BSA) for 30 min. Guinea pig serum containing antibodies against the GP1 antigen(s) was precipitated with ammonium sulfate (40%, w/v, final concentration) and the pellet was redissolved in 20 original volumes of NaCl/P_i/1% BSA. Each of the filters was incubated for 10 h at 4°C with 10 ml of this antibody solution. Following removal of the first antibody, the filters were washed twice in NaCl/P_i/0.05% Tween 20 (3×50 ml), placed for 30 s on filter paper to remove excess NaCl/P_i and then soaked in 10 ml NaCl/P_i/1% BSA for 30 min. The alkaline phosphatase-conjugated second antibody was applied according to the instructions of the producer (PicoBlue, Stratagene). The positively stained regions on parallel filters were localized on the agar plate. Agar was taken from this site and extracted with phage diluent. Appropriate dilutions of this phage stock were screened by the same procedure until λ P10A was plaque-purified.

Screening of the λ gt10 cDNA library with nucleic acid probes

Approximately 20 000 p.f.u. of λ gt10 recombinants were plated per 130 cm² plate using *E. coli* C600 hfl⁻ as host strain. Following plaque growth the plaques were lifted (Hybond-N, Amersham) and the phage DNA retained on plaque-lifts was denatured according to standard protocols (Sambrook *et al.* 1989). The plaque-lifts were vacuum-dried and crosslinked under an ultraviolet lamp. The insert of pP10A specifying GP1 antigen was excised from a 0.6% low-melting-point agarose gel and radio-labelled to give a specific radioactivity of 10⁶–10⁷ Bq μ g⁻¹ using the multiprimer-labelling procedure of Feinberg and Vogelstein (1983, 1984). Prehybridizations (3 h) and hybridizations (18 h) were performed at 68°C in rotating tubes using 7% sodium dodecyl sulfate, 1 mM EDTA, 0.5 M sodium phosphate, pH 7.2, as prehybridization and hybridization solution (Church and Gilbert, 1984). Following hybridization the replica filters were washed at 68°C under stringent salt conditions (0.1×citrate buffered saline). After drying, they were exposed to Kodak X-Omat films and intensifier screens. Agar from regions of interest was extracted by λ diluent and rescreened by the same procedure until the clones were plaque-purified.

cDNA recloning and sequencing

Plaque-purified recombinants were amplified on agar plates using *E. coli* Y1088 for λ gt11-recombinants and *E. coli* C600 hfl⁻ for λ gt10-recombinants. Phage DNA was isolated by standard techniques (Sambrook *et al.* 1989). Following digestion with *Eco*RI, the inserts were recovered from 0.6% low-melting agarose gels by phenol extraction, recloned in the *Eco*RI site of the pBluescript vector (Stratagene) and sequenced according to Sanger *et al.* (1977), by means of a DNA sequencing kit from Pharmacia. cDNA fragments were sequenced in both strands

using commercially available M13 as well as custom primers and reverse primers.

Production of fusion proteins by recombinant λ gt11 lysogens

E. coli Y1089 cells were lysogenized with the λ P10A recombinant and, for control, with non-recombinant λ gt11. Approximately 10⁶ cells of exponentially growing *E. coli* Y1089 cultures supplemented with 0.2% maltose were incubated at 32°C for 30 min with 100 μ l phage stocks containing about 10⁹ p.f.u. ml⁻¹. The cells were plated on LB plates and incubated at 32°C. Single colonies were inspected for growth at 32°C and for temperature sensitivity at 42°C. For production of fusion proteins, the lysogens were grown to high cell density at 32°C followed by a rapid shift to 42°C. *lacZ*-directed expression was induced by addition of IPTG to a final concentration of 10 mM and incubation was continued at 37°C for 18 h. Proteins of induced cultures and of non-induced cultures were analyzed by SDS-polyacrylamide gel electrophoresis followed by protein blotting.

Other methods

The cytosol fraction of Ehrlich ascites cells was prepared according to the method of Favalaro *et al.* (1980) and the proteins contained in this fraction were used for protein blots. The computer program package HUSAR (Heidelberg Unix Sequence Analysis Resources) provided by S. Suhai, German Cancer Research Center, Heidelberg, was used for data-base searches, sequence conversions and sequence comparisons.

Results

Antibodies to centrosomal antigens

The monoclonal antibody (F5.1) prepared against a crude centrosome fraction from PtK cells has been shown to react exclusively with centrosomes (Joswig and Petzelt, 1990). Using protein blotting techniques, the major antigen in PtK cells was identified as a polypeptide of about 32×10³ M_r (Joswig and Petzelt, 1990). In combination with a method for the isolation of active centrosomes (Mitchison and Kirschner, 1984; Bornens *et al.* 1987) the F5.1 antibody was used to identify cellular fractions enriched in centrosomes (Joswig and Petzelt, 1990). Fractions of sucrose step-gradients loaded with extracts of PtK cells were screened with the antibody and it was found that a 32–34×10³ M_r polypeptide band of enriched fractions contained the immunoreactive material. This material was recovered from SDS-polyacrylamide gels and used to immunize guinea pigs pre-tested for the absence of centrosomal antibodies. A positive serum (GP1 antibody) was obtained that immunoreacted specifically with centrosomes of interphase and mitotic cells of different species including PtK cells and fertilized sea urchin eggs (Fig. 1).

Isolation and characterization of a partial GP1 antigen cDNA clone (P10A) and its fusion protein

An induced λ gt11 cDNA library prepared from RNA of Ehrlich ascites tumor cells was screened with the GP1 antibody probe. Inspection of 200 000 recombinants released a positive clone, which was plaque-purified in further rounds and saved as purified phage stock (λ P10A). Immunoblot analysis of cell lysates from lysogens prepared by infection of *E. coli* Y1089 with λ P10A, and for control with λ gt11, verified that clone P10A specified a polypeptide with antigenicity to the GP1 antibody probe (Fig. 2). Gels stained with Coomassie Brilliant Blue revealed the inducible expression of β -galactosidase by λ gt11 lysogens, which proved to be non-reactive with the

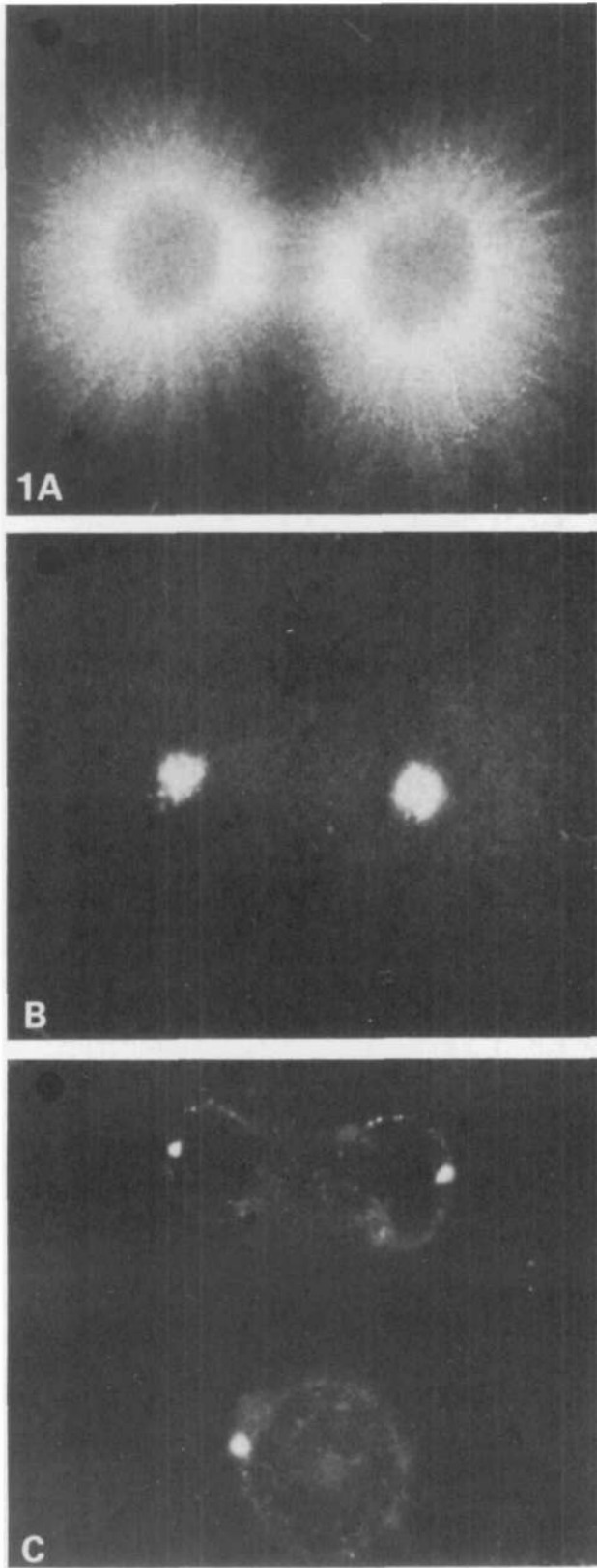


Fig. 1. Immunoreaction of the GP1 antibody with centrosomes of different cells in different cell cycle phases. (A) Fertilized sea urchin egg in mitosis. Spindle asters immunostained by means of a tubulin antibody. (B) The same object as shown in A, immunostained with the GP1 antibody. (C) An interphase and a dividing PtK cell immunostained with the GP1 antibody.

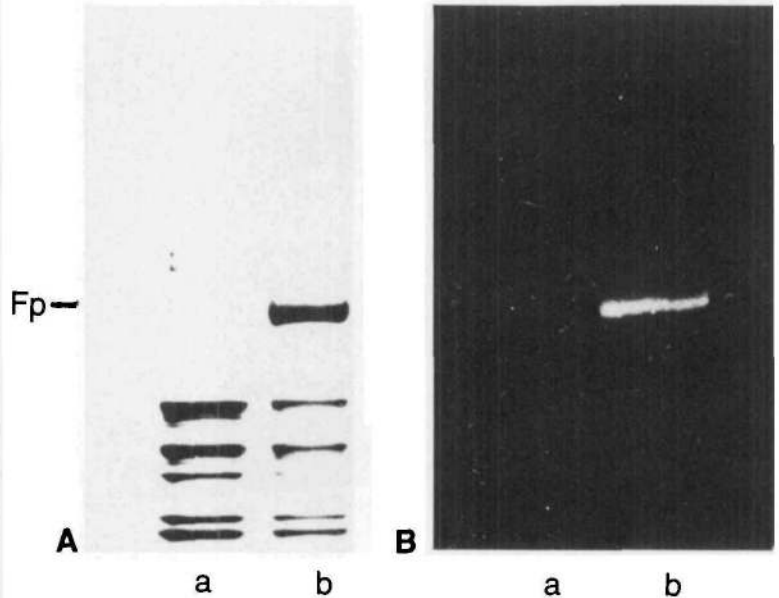


Fig. 2. Analysis of recombinant GP1 antigen expressed by induced λ P10A lysogens. (A) Lysates of *E. coli* Y1089 lysogenized with λ P10A were analysed by SDS-polyacrylamide gels stained with Coomassie Brilliant Blue. A lysate of the non-induced lysogen is shown in lane a and a lysate of the IPTG-induced lysogen is shown in lane b. (B) Autoradiography of a protein blot of an identical gel to that shown in A, immunostained with the GP1 antibody and ^{125}I -labelled protein A. Fp, the position of the fusion protein specified by the λ P10A recombinant.

GP1 antibody (not shown). In contrast, the larger fusion protein expressed by recombinant λ P10A lysogens was significantly immuno-stained by the GP1 antibody. Injection of this fusion protein into chickens induced the production of centrosome-specific antibodies with the GP1 characteristics shown in Fig. 1. Consequently, clone λ P10A could be considered as a *bona fide* probe specifying a centrosome-specific antigen.

The 349 bp insert of clone λ P10A was recloned in the *Eco*RI site of the pBluescript vector and designated pP10A. The nucleotide sequence of the pP10A insert (shown in Fig. 4, from nucleotide position 904 to the end) revealed that this clone could code for the carboxy-terminal portion of a GP1 antigen. It was found to comprise an open reading frame of 86 amino acid residues followed by a terminal in-frame stop codon, 51 nucleotides of 3' non-translated region including a polyadenylation signal and the adjacent poly(A) tail. These parameters characterized this clone as a 3'-terminal section of a cDNA.

Isolation of full-length GP1 antigen cDNA clones

In order to obtain complete GP1 antigen cDNA clones the 349 bp insert of pP10A was used as a probe to rescreen an Ehrlich ascites cell-derived λ gt10 cDNA library by plaque hybridization. Screening of 120 000 plaques released five positive ones indicating that transcripts encoding polypeptides with antigenicity to the GP1 antibody are not extremely rare in Ehrlich ascites cells.

With respect to their insert lengths, two types of clones could be distinguished. The inserts of PN1 type clones were about 1.2 kb in size while PN5 type clones showed inserts of the order of 2.2 kb (Fig. 3). Southern blots of the

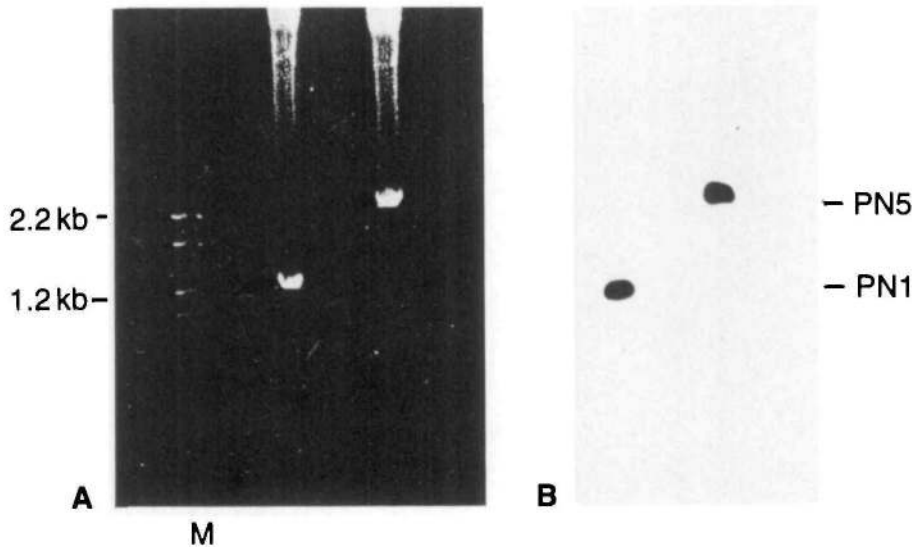


Fig. 3. Homology between the cDNA clones λ P10A, λ PN1 and λ PN5. (A) Phage DNA of clones λ PN1 and λ PN5 was digested with *Eco*RI, submitted to agarose gel electrophoresis and stained with ethidium bromide. (B) Southern blot of the gel shown in A hybridized with the radiolabelled insert of clone λ P10A.

inserts with different lengths hybridized significantly with the radiolabelled P10A insert, indicating that the 3'-terminal sections of the PN1 and PN5 cDNA clones could be considered to be at least highly homologous (Fig. 3).

Sequencing of the GPI antigen cDNA clone PN1

The complete 1.2 kb insert of the clone λ PN1 was recloned in the *Eco*RI site of the pBluescript vector and designated pPN1. The nucleotide sequence of pPN1 revealed that its insert reflects a complete cDNA to a mRNA (Fig. 4). The sequence shows an open reading frame of 289 amino acid residues. The start codon is preceded by several in-frame stop codons in the 294 base pair 5' non-translated region. The 3'-terminal sequence of pPN1 was found to be 100% identical with that of the partial cDNA clone P10A. Consequently, the inserts of the clones PN1 and pP10A could be oriented as indicated in Fig. 5.

Characteristics of the polypeptide encoded by pPN1

The nucleotide sequence of pPN1 codes for a rather hydrophilic $34.5 \times 10^3 M_r$ polypeptide (Fig. 4). Charged amino acid residues amount to 45.33 mol%. The most frequent amino acid residue is glutamic acid (50 residues, 17.3 mol%) followed by arginine (31 residues, 10.7 mol%). The calculated isoelectric point is 6.61. The amino acid sequence comprises potential consensus sequences for phosphorylation sites (positions 61, 71, 211, 270), one site for N-glycosylation (position 134), and two potential myristinylation sites (positions 26, 121); however, there is no known sequence motif for enzymatic activity.

A detailed inspection of the pPN1-encoded polypeptide revealed repeated sequence motifs that appear to be non-random because amino acid sequences four residues in length should occur, on average, only once among 8000 random peptide bonds (Table 1). Screening of protein data-

bases revealed that these sequences are indeed rare. Their repeated occurrence in one protein may reflect functional sites that remain to be elucidated.

Identical blocks up to five amino acid residues in length were found to occur in the pPN1-encoded polypeptide as well in A-type and B-type lamins (Fig. 6). It should be noted that the strings LQEKE and EEERL are of special significance because they belong to the short regions of high homology between A-type and B-type lamins (Riedel and Werner, 1989). In A-type lamins these strings are flanking the α -helical regions that appear to be essential for the interaction of the lamins (McKeon *et al.* 1986). As in A/C-type lamins these strings are also flanking the α -helical region predicted by the method of Fasman (1989) for the secondary structure of centrosomin A (plots not shown). These two strings were not found together in any of the other 16 524 protein sequences (4.6×10^6 residues) comprising protein sequence data-bases. However, in contrast to the lamins and as expected for a cytoplasmic polypeptide, centrosomin A contains no sequence motif specifying karyophilic proteins (Kalderon *et al.* 1984; Kleinschmidt and Seiter, 1988; Løwinger and McKeon, 1988; Riedel and Werner, 1989).

Characterization of the GPI antigen cDNA clone PN5

Cross-hybridization between the cDNA inserts of the clones PN1 and PN5 with the radiolabelled insert of clone P10A (Fig. 3) and their restriction-site mapping (Fig. 5) indicate that the nucleotide sequence of clone PN1 is apparently included in that of clone PN5. From this it could be suggested that the cDNA clones PN1 and PN5 encode two polypeptides of different size that share a long carboxy-terminal section. This suggestion was favoured by the protein blot shown in Fig. 7. The GPI antibody detects a major antigen in the cytoplasmic protein fraction of Ehrlich ascites cells that is larger than the $34.5 \times 10^3 M_r$ polypeptide encoded by PN1 and whose size fits well with the 2.2 kb PN5 cDNA. Alternatively, on the assumption that there is a long (>1 kb) 5' non-translated region in PN5, the two cDNAs could code for the same $34.5 \times 10^3 M_r$ antigen. In order to test these alternative possibilities a custom primer complementary to the PN1 sequence downstream from the PN1 translation initiation region was used for PN5 DNA sequencing. It was found that the

Table 1. Significant repeats in the amino acid sequence deduced from the nucleotide sequence of clone PN1

Sequence	Occurrence	Positions
MQLE	2×	1 235
QLEK	2×	2 175
EKEE	2×	45 90
EEER	2×	108 228
EERL	2×	109 279

	10	20	30	40	50
	ACT AAA ACT CTG TGA GAG AGT TAC AAA GGT ATT AAA TTG GGT TAG GGA ACA				
	ACC TGA AAA GAA CCA GAA TTG CAA CAA TAT GTA CCA CAA CTC CAG AAC AAT				
	ACC ATA CTC CGC CTT CTG CAA CAG GTG GCA CAG ATT TAT CAG AGC ATT GAG				
	TTT TCT CGT TTG ACT TCT CTG GTT CCT TTT GTT GAT GCT TTC CAA CTG GAC				
	GGG CCA TAG TAG ATG CAG CCA GGC ACT GTG ACC TGC AGG TAC GTA TTG ACC				
	ATA CTT CCC GGA CTC TGA GTT TTG GAT CAG ATT TGA ATT ATG CAA CTC GAG				
1		***		***	M Q L E
	AAG ATG CCC CAG TTG GCC CTC ATC TGC AGA GAT GCC TTC GGA GCA GAT AAG				
5	K M P Q L A L I C R D A F G A D K				
	AAA CCA GCT CAC GGC CAT GTC CTC AGT GCT TGC AAC TGT GAA GTC ATC AGA				
22	K P A H G H V L S A C N C E V I R				
	CCT GCT CAC ATT CTG CAA GAG AAA GAA GAA CAG CAT CAG TTG GCA GTT AAT				
39	P A H I <u>L Q E K E</u> E Q H Q L A V N				
	CGA TAT CTT AAA AAT TCA AGA AAA GAG CAC CAG AGG ATC CTG GCT CGG AGA				
56	R Y L K N S R K E H Q R I L A R R				
	CAG ACA ATT GAG GAA AGA AAA GAG CGT CTT GAG AGT CTG AAT ATT CAA CGT				
73	Q T I E E R K E R L E S L N I Q R				
	GAG AAG GAA GAA CTC GAG CAG AGG GAA GCT GAA CTC CAG AAA GTA CGA AAG				
90	E <u>K E E L</u> E Q R E A E L Q K V R K				
	GCT GAA GAA GAA AGG CTG CCA AGA GGC AAA GGA GCG AGA GAA GGA ACG AAT				
107	A <u>E E E R L</u> P R G K G A R E G T N				
	CCT TCA AGA ACA CGA GCA AAT CAA GAA GAA AAC TGT TCG GGA GCG GTT AGA				
124	P S R T R A N Q E E N C S G A V R				
	GCA GAT CAA GAA GAC AGA CTG GGC GCC AAA GCA TTT AAA GAT ATT GAC ATT				
141	A D Q E D R L G A K A F K D I D I				
	GAA GAC CTT GAA GAA CTG GAT CCA GAT TTT ATT ATG GCC AAA CAG GTT GAA				
158	E D L E E L D P D F I M A K <u>Q V E</u>				
	CAA CTG GAG AAA GAA AAG AAG GAT TAC CAG GAA CGC CTG AAG AAT CAA GAA				
175	<u>Q</u> L E K E K K D Y Q E R L K N Q E				
	AAG AAG ATT GAC TAT TTT GAG AGA GCT AAG CGT TTG GAA GAA ATT CCT TTA				
192	K K I D Y F E R A K R L E E I P L				
	ATA AAG AGT GCT TAT GAA GAA CAC AGG GTT AAA GAC ATG GAC CTG TGG GAA				
209	I K S A Y E E H R V K D M D L W E				
	CAG CAA GAA GAA GAA AGA ATC ACT ACA ATG CAG CTA GAA CGA GAA AAA GCT				
226	Q Q E E E R I T T M Q L E R E K A				
	CTG GAG CAT AAG AAT AGG ATG TCA CGA ATG TTG GAA GAC AGA GAT CTA TTT				
243	L E H K N R M S R M L E D R D L F				
	GTG ATG CGG CTC AAA GTC GCC CGG CAG TCT GTC TAC GAG GAA AAA CTG AAA				
260	V M R L K V <u>A R Q S</u> V Y E E K L K				
	CAG TTT GAA GAG CGC TTA GCA GAA GAA AGC ATA GTC GCC TAG AAG ATC GGA				
277	Q F E E R L A E E S I V A ***				
	AAA GGC AGC GGA AAG AAG AAC GCA AAA TAA CTT ATT ACA AAA AAA AAA A				

Fig. 4. Nucleotide sequence of the cDNA clone pPN1 and its deduced amino acid sequence. The nucleotide sequence of clone P10A is 100% identical with that of pPN1 from nucleotide position 901. Underlined letters indicate strings of 100% sequence identity in the PN1-encoded polypeptide and in lamins (see Fig. 6).

PN5 sequence corresponding to the translation initiation region of PN1 is 100% identical with the PN1 sequence and includes the in-frame stop codons preceding the ATG start codon (Fig. 4). This indicates that the cDNA PN5 does not encode a larger polypeptide than PN1. Most likely, PN1 and PN5-type cDNA clones reflect two mRNAs of different size coding for the same $34.5 \times 10^3 M_r$ GP1 antigen.

Discussion

There has been little sequence information available about intrinsic centrosomal components. Indeed, only one partial cDNA has been detected in a sea urchin-derived cDNA library specifying a $50 \times 10^3 M_r$ polypeptide that seems to be located or enriched in centrosomal spheres (Kuriyama *et al.* 1990). The antigen sequence predicted

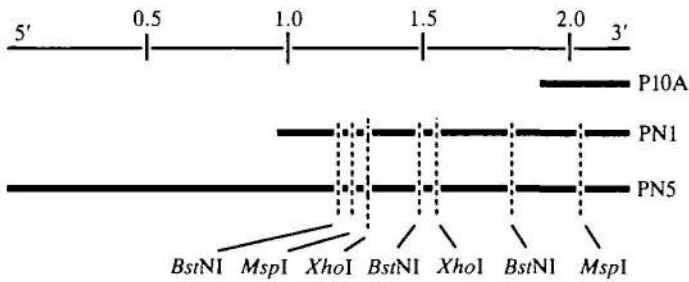


Fig. 5. Overlapping cDNA clones P10A, PN1, PN5. The top line specifies the approximate lengths of the cDNA inserts (kb) and the orientation of their coding strands (5'→3'). Bars reflect inserts of different cDNA clones. P10A is the partial cDNA clone identified by antibody recognition. PN1 and PN5 reflect cDNA clones detected by the P10A probe in the λ gt10 cDNA library by plaque-hybridization. The relative positions of P10A and PN1 are proved by their nucleotide sequences. The relative positions of clones PN1 and PN5 were deduced from the results of the cross-hybridization experiment shown in Fig. 3 and from restriction enzyme mapping. Restriction enzyme analysis of the inserts of clones PN1 and PN5 indicated that the PN1 insert is essentially identical with the 3' section of the insert of clone PN5. Fragmentation of the two inserts by different enzymes released the DNA fragments from the two inserts that had to be expected on the basis of the nucleotide sequence of the insert of clone PN1 (gels not shown).

	43	91	108	172	266
Centrosomin A	LQEKE..KEEL..EERL..QVEQ..ARQS				
	29	201	383	255	274
Mouse lamin C	LQEKE..KEEL..EERL..QVEQ..ARQS				
	31	262	385		
Mouse lamin B	LQEKE..KEEL..EERL				

Fig. 6. Blocks of 100% sequence identity in the amino acid sequences of centrosomin A (PN1-encoded polypeptide), murine lamin C (Riedel and Werner, 1989) and murine lamin B (Hoeger *et al.* 1988).

from this cDNA shows a high percentage of positional identities with elongation factors EF-1 α from different species. Consequently, it is not known whether this previously cloned cDNA codes for the EF-1 α of the sea urchin, which may be enriched in the centrosomal sphere, or whether that cDNA specifies an intrinsic centrosomal polypeptide closely related to the EF-1 α (Kuriyama *et al.* 1990). In contrast, the $34.5 \times 10^3 M_r$ centrosomal antigen characterized in this manuscript by its cDNA has not yet been sequenced at the DNA or the protein level. Screening of the most recent releases of the data-bases indicates that neither identical nor closely related sequences are known. Significant but minor identities with the sequences of nuclear lamina proteins will be discussed below. In contrast to many other centrosome-specific antibodies, the GPI antibody used in this work detects the centrosome-specific antigen(s) in cells of various species exclusively at the centrosomal location and throughout the cell cycle. It is highly unlikely that cellular components that are not intrinsically involved in the centrosome structure remain associated with it under different physiological conditions,

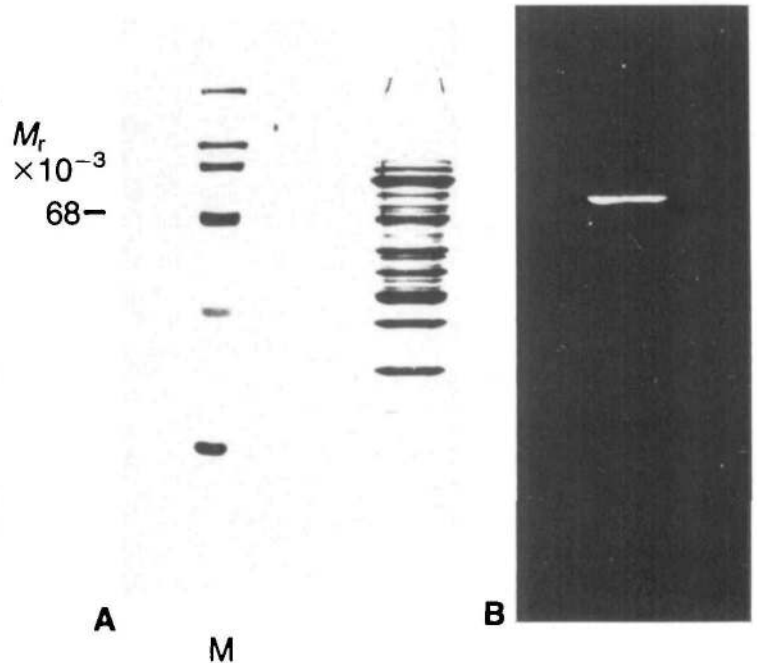


Fig. 7. Western blot of cytoplasmic polypeptides of Ehrlich ascites cells indicating the prevalence of a GPI antigen larger than the $34.5 \times 10^3 M_r$ antigen encoded by PN1 and PN5 cDNAs. The $34.5 \times 10^3 M_r$ region is only weakly immunostained and escapes photographic presentation. M, Size markers. (A) SDS-polyacrylamide gel of cytoplasmic polypeptides stained with Coomassie Brilliant Blue. (B) Autoradiography of the Western blot of A immunostained with the GPI antibody and ^{125}I -labelled protein A.

e.g. in different cell cycle phases. From this we conclude that the centrosome-specific antigens detected by the GPI antibody do not reflect non-related molecules that are only transiently or accidentally associated with centrosomes.

Our results point to the existence of two centrosomal antigens of different size immunoreacting with the GPI antibody. The $34.5 \times 10^3 M_r$ antigen (centrosomin A), now characterized by its cDNA sequence, is the major antigen in PtK cells (Joswig and Petzelt, 1990) while the larger antigen (centrosomin B) appears to be prevalent in Ehrlich ascites cells. If centrosomin B is encoded by an intrinsic mRNA it is somewhat surprising that we first detected the cDNA clones for the less-abundant antigen in the Ehrlich ascites cell-derived cDNA libraries. However, this may be explained, e.g. by a high turnover rate for the $34.5 \times 10^3 M_r$ antigen and the longevity of the larger antigen. Alternatively, the possibility cannot be ruled out that centrosomin B reflects an unusually stable dimeric or an otherwise post-translationally modified form of centrosomin A. Rescreening of various cDNA libraries with centrosomin A probes, which is in progress, should help to clarify this point.

The value of the cDNA clones for centrosomin A is mainly seen in their usefulness in elucidating aspects of centrosomal functions by recombinant DNA technology. However, the amino acid sequence deduced from the nucleotide sequences of the cDNA clones reveals interesting characteristics of the sequence itself. For instance, the blocks of 100% sequence identity found in centrosomin A and in the major nuclear lamina proteins suggest that similar structural principles may be involved in centrosome and nuclear lamina assembly.

These structural similarities draw attention to other relationships between the nuclear lamina and centrosomes that have not yet been discussed explicitly. Morphological data indicate that centrosomes are frequently found close to the cytoplasmic side of the cell nucleus (see Fig. 1C, and Paweletz *et al.* 1987; Paweletz and Mazia, 1989). This conspicuous closeness becomes especially evident in sea urchin embryos where the centrosome in early prophase is visible as a complex filamentous network around the cell nucleus (Mazia, 1987). These morphological data do not even rule out the possibility that centrosomes may be transiently interconnected across the nuclear membrane to the nuclear lamina. Finally, bearing in mind that the two organelles undergo dramatic and similar morphological changes that are strictly cell cycle-regulated, it is possible to conclude that these structures may be more closely related than they were thought to be. The disassembly and assembly of the nuclear lamina have recently been shown to be due to phosphorylations and dephosphorylations (Heald and McKeon, 1990). It remains to be investigated whether similarities also exist at this level and whether these mechanisms could also control the morphology and activity of the centrosome.

References

- BORNENS, M., PAINTRAND, M., BERGES, J., MARTY, M.-CH. AND KARSENTI, E. (1987). Structural and chemical characterization of isolated centrosomes. *Cell Motil. Cytoskel.* **8**, 238–249.
- BOVERI, T. (1900). Über die Natur der Centrosomen. In *Zellstudien*, Heft 4, G. Fischer Verlag, Jena.
- CALARCO-GILLAM, P. D., SIEBERT, M. C., HUBBLE, R., MITCHISON, T. AND KIRSCHNER, M. (1983). Centrosome development in early mouse embryos as defined by an autoantibody against pericentriolar material. *Cell* **35**, 621–629.
- CHURCH, G. M. AND GILBERT, W. (1984). Genomic sequencing. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1991–1995.
- FASMAN, G. D. (1989). Protein conformation prediction. *Trends biochem. Sci.* **14**, 295–299.
- FAVALORO, J., TREISMAN, R. AND KAMEN, R. (1980). Transcription maps of polyoma virus specific RNA: analysis by two dimensional nuclease S1 gel mapping. *Meth. Enzym.* **65**, 718–749.
- FEINBERG, A. P. AND VOGELSTEIN, B. (1983). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Analyt. Biochem.* **132**, 6–13.
- FEINBERG, A. P. AND VOGELSTEIN, B. (1984). Addendum. *Analyt. Biochem.* **137**, 266–267.
- HEALD, R. AND MCKEON, F. (1990). Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. *Cell* **61**, 579–589.
- HIEPE, F., SCHWARZ, G. AND HIEPE, TH. (1988). Untersuchungen zur Antikörpergewinnung aus dem Eigelb immunisierter Hühner. *Mh. vet. Med.* **43**, 680–682.
- HÖGER, T. H., KROHNE, G. AND FRANKE, W. W. (1988). Amino acid sequence and molecular characterization of murine lamin B as deduced from cDNA clones. *Eur. J. Cell Biol.* **47**, 282–290.
- HUYNH, T. V. M., YOUNG, R. A. AND DAVIS, R. W. (1986). Construction and screening of cDNA libraries in λ gt10 and λ gt11. In *DNA Cloning*, vol. I (Glover, D. M., ed.), IRL Press, Oxford.
- JOSWIG, G. AND PETZELT, CH. (1990). The centrosomal cycle. Its visualization in PtK cells by a monoclonal antibody to a 32 kD protein. *Cell Motil. Cytoskel.* **15**, 181–192.
- KALDERON, D., ROBERTS, B. L., RICHARDSON, W. D. AND SMITH, A. E. (1984). A short amino acid sequence able to specify nuclear location. *Cell* **39**, 499–509.
- KLEINSCHMIDT, J. A. AND SEITER, A. (1988). Identification of domains involved in nuclear uptake and histone binding of protein N1 of *Xenopus laevis*. *EMBO J.* **7**, 1605–1614.
- KURIYAMA, R., SAVEREIDE, P., LEFEBRE, P. AND DASGUPTA, S. (1990). The predicted amino acid sequence of a centrosphere protein in dividing sea urchin eggs is similar to elongation factor (EF-1a). *J. Cell Sci.* **95**, 231–236.
- LOEWINGER, L. AND MCKEON, F. D. (1988). Mutations in the nuclear lamin proteins resulting in aberrant assembly in the cytoplasm. *EMBO J.* **7**, 2301–2309.
- LU, X., KOPUN, M. AND WERNER, D. (1988). Cell cycle phase-specific cDNA libraries reflecting phase-specific gene expression of Ehrlich ascites cells growing *in vivo*. *Expl Cell Res.* **174**, 199–214.
- LU, X. AND WERNER, D. (1988). Construction and quality of cDNA libraries from cytoplasmic RNA not enriched in poly(A)⁺ RNA. *Gene* **71**, 157–164.
- MAZIA, D. (1987). The chromosome cycle and the centrosome cycle in the mitotic cycle. *Int. Rev. Cytol.* **100**, 49–92.
- MCINTOSH, J. R. (1983). The centrosome as an organizer of the cytoskeleton. In *Modern Cell Biology*, vol. 2, pp. 115–142. Alan Liss, New York.
- MCKEON, F. D., KIRSCHNER, M. W. AND CAPUT, D. (1986). Primary and secondary structural homologies between major nuclear envelope and cytoplasmic intermediate filament proteins. *Nature* **319**, 463–468.
- MITCHISON, T. AND KIRSCHNER, M. (1984). Microtubule assembly nucleated by isolated centrosomes. *Nature* **312**, 232–242.
- PAWELETZ, N. AND MAZIA, D. (1989). The fine structure of the formation of mitotic poles in fertilized eggs. In *The Cell Biology of Fertilization*, pp. 165–187, Academic Press, Inc., New York, London.
- PAWELETZ, N., MAZIA, D. AND FINZE, E.-M. (1987). Fine structural studies of the bipolarization of the mitotic apparatus in the fertilized sea urchin egg. II. Bipolarization before the first mitosis. *Eur. J. Cell Biol.* **44**, 205–213.
- RIEDEL, W. AND WERNER, D. (1989). Nucleotide sequence of the full-length mouse lamin C cDNA and its deduced amino-acid sequence. *Biochim. biophys. Acta* **1008**, 119–122.
- SAMBROOK, J., FRITSCH, E. F. AND MANIATIS, T. (1989). In *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY.
- SANGER, F., NICKLEN, S. AND COULSON, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467.
- SCHATTEN, H., MAZIA, D., BALCZON, R. AND SIMERLY, C. (1986). Behavior of centrosomes during fertilization and cell division in mouse oocytes and in sea urchin eggs. *Proc. natn. Acad. Sci. U.S.A.* **83**, 105–109.
- SCHATTEN, H., WALTER, M., MAZIA, D., BISSMANN, H., PAWELETZ, N., COFFKE, G. AND SCHATTEN, G. (1987). Centrosome detection in sea urchin eggs with monoclonal antibody against *Drosophila* intermediate filament proteins: characterization of stages of the cell cycle of centrosomes. *Proc. natn. Acad. Sci. U.S.A.* **84**, 8488–8492.
- VOROBYEV, I. A. AND NADEZHINA, E. S. (1987). The centrosome and its role in the organization of microtubules. *Int. Rev. Cytol.* **106**, 227–293.

(Received 10 September 1990 – Accepted 10 October 1990)

