Identification of a spindle-associated protein in ciliate micronuclei

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Summary

Ciliated protozoa display a nuclear dualism, with germinal micronuclei and a somatic macronucleus. During mitosis, which proceeds without disruption of the nuclear envelope, a spindle is organized within the micronucleus from, presumably, intranuclear microtubule-organizing centres (MTOCs). In order to characterize these MTOCs, monoclonal antibodies generated against human centrosomes were screened on several ciliates and particularly on Paramecium tetraurelia. In this ciliate, the monoclonal antibody CTR 532, which decorates centrosomal and spindle-associated components in mammalian cells, specifically labelled the micronuclei during interphase. At the electron-microscope level, it stained a fibrous material surrounding microtubules localized on the inner face of the nuclear envelope. During mitosis this decoration extended all over the metaphase spindle. At all stages of the cell cycle, the decoration remained specific to the micronucleus and was absent not only from all of the various cytoplasmic and cortical microtubule arrays but also from the macronuclei, even at early stages of their development from the zygotic nucleus. CTR 532 recognizes a single 170×10^3 Mr polypeptide in the cytoskeletal fraction that contains micronuclei and this polypeptide is absent in the cytoskeletal fraction of amicronucleate cells.

Key words: spindle-associated protein, micronucleus, ciliates.

Introduction

Unlike metazoa, protozoa frequently display endomitosis, and the structures that are morphogenetically connected with mitotic spindle formation vary quite considerably. Several types of cytoplasmic or intranuclear microtubule-organizing centres (MTOCs) have been well described in ultrastructural terms (for reviews, see Kubai, 1975; Heath, 1980; Raikov, 1982). However, the biochemical nature of these MTOCs is unknown, as is their relationship to the nuclear envelope. Closed nuclear orthomitoses are characteristic of ciliates. These protozoa display a nuclear dualism with one or more diploid micronuclei and one 'polyploid' macronucleus. During interphase, besides the cytoplasmic and cortical microtubule networks, a layer of microtubules is found under the nuclear envelope in the micronucleus of a number of ciliates and is referred to as a 'residual spindle' (Raikov, 1982). During mitosis or meiosis several microtubule networks are organized into the micro- and the macronuclei (Tucker et al., 1980, 1985; Cohen & Beisson, 1988). Different classes of mitotic microtubules are assembled during successive mitotic stages and characterized by their differential stability to cold or anti-mitotic drugs (Eichenlaub-Ritter & Ruthman, 1982) and/or by their protofilament number (Eichenlaub-Ritter & Tucker, 1984). Besides the nuclear spindles, a cortical spindle develops during mitosis (Cohen et al., 1982). It has been suggested that microtubule-associated proteins (MAPs) could contribute to the stability of these different microtubule subclasses in protozoa (Eichenlaub-Ritter & Ruthman, 1982). However, nothing is known about these putative MAPs associated with internal microtubule networks in ciliates.

In order to analyse MTOCs and the spindle in ciliate micronuclei, a library of monoclonal antibodies, generated against purified human centrosomes (Bornens et al., 1987), was screened on Paramecium tetraurelia. One of these monoclonals, CTR 532, which decorates the centrosome and the mitotic spindle in mammalian cells, was observed to label specifically fibres in the micronucleus of interphase paramecia as well as the spindle during mitosis in both Paramecium and Tetrahymena. We describe here the ultrastructural localization and biochemical characterization of the antigen, a 170×10^3 Mr polypeptide that appears to be similar in both Paramecium and Tetrahymena and is restricted to the micronuclear compartment.

Materials and methods

Strains and culture conditions

Cells of stock d4-2 of Paramecium tetraurelia (Sonneborn,
Antibodies

The monoclonal antibody CTR 532 (IgM class) was obtained from a collection of monoclonal antibodies raised against centrosomes isolated from human lymphoblast (Bornens et al. 1987). It was used as a culture supernatant diluted 1/100 to 1/500 in phosphate-buffered saline (PBS) containing 3% bovine serum albumin (BSA). The anti-α-tubulin (Bloise et al. 1982) was obtained from Amersham (France), was used at 1/1000 dilution. The monoclonal antibody raised against Paramecium axonemal tubulin (Cohen et al. 1982) was used at 1/400 dilution.

Immunofluorescence microscopy

Immunofluorescence on mammalian cells was performed as follows: the cells were rinsed briefly in PHEM (60 mM-Pipes, 25 mM-Hepes, 10 mM-EGTA, 5 mM-MgCl₂, pH 6.9) buffer as described by Schliwa & Van Blerkom (1981) and permeabilized with 0.5% Triton X-100 in PHEM buffer for 1 min; they were fixed with cold methanol for 5 min at -20°C. Immunostaining with CTR 532 was done in PBS for 30-45 min, followed by three washes in PBS containing 0.1% Tween 20. Then the cells were incubated for 30 min with a rhodamine-conjugated goat anti-mouse immunoglobulin (from Cappell), diluted 1/150 in PBS plus 1% BSA. The cells were washed and mounted as described below for ciliates.

Immunofluorescence was performed on paramecia permeabilized in a 1% Triton X-100-containing PHEM buffer. Two procedures were used for immunostaining. In the first one (Cohen et al. 1982), the permeabilized cells were washed once in the same buffer for antibody incubation and washings. This method provided quite a satisfactory conservation of the cytoskeleton organization and an apparently good labelling specificity as controlled by electron microscopy. However, for stringent antigen–antibody affinity requirement we have used a second procedure: the permeabilized cells were fixed with 2% paraformaldehyde in PHEM buffer for 1 h at room temperature, rinsed in PBS containing 2 mM-MgCl₂ and 10 mM-EGTA (modified PBS, PBSm) then transferred into the same buffer plus 3% BSA. In both procedures the cells were incubated for 45 min with the primary antibody. In order to eliminate the strong fluorescence of cilia and visualize the internal microtubules with the monoclonal anti-α-tubulin antibody raised against *Paramecium* axonemal tubulin (Cohen et al. 1982), then the monoclonal antibody was added and the incubation continued for 45 min as described by Cohen & Beisson (1988). The cells were washed three times for 10 min each in PBSm or PHEM containing 0.1% Tween 20 and 3% BSA. They were incubated for 45 min with a fluorescein-conjugated goat anti-mouse immunoglobulin (Pasteur Institute, France), diluted to 1/100 with PBSm plus 3% BSA. After the second antibody incubation the samples were washed twice and mounted in Citifluor mounting medium (City University, London, England) and photographed under a Zeiss epifluorescence photomicroscope using an ×63 phase, 1:25 plan neofluorescence photomicroscope. The same protocols were applied to *Tetrahymena* and *Euplotes*.

Cold treatment of cells

Culture dishes were cooled at 4°C and maintained at this temperature for 45-60 min. The survival of the treated cells was ascertained by observation of these cells after return to room temperature. Cells were permeabilized in the cold with 1% Triton X-100 in PHEM buffer, transferred to room temperature and then fixed.

Immunoelectron microscopy

Immunoelectron microscopy was performed on permeabilized cells in PHEM buffer plus 3% BSA. Either the cells were fixed with 2% paraformaldehyde as described for immunofluorescence studies or they were directly incubated with the first antibody (at a dilution of 1/500) in PHEM buffer without fixation. The same immunogold staining was observed for both protocols although poor microtubule preservation was obtained after paraformaldehyde fixation. Then the cells were washed with PHEM buffer containing 0.1% Tween 20 and 3% BSA. The secondary antibody (goat anti-mouse IgM) coupled to 5 nm gold (GAM IgM from Janssen Life Sciences Products) was used at a dilution of 1/5 in PHEM buffer plus 3% BSA. The cells were fixed in 2% glutaraldehyde in 0.05 M-cacodylate buffer (pH 7.2) for 45 min, washed and post-fixed with 1% osmium tetroxide in cacodylate buffer. After washing, the cells were dehydrated in ethanol and embedded in Epon. Thin-sectioned cells were examined with a Philips EM301 electron microscope. The specificity of the decoration was ascertained by comparison with results of parallel experiments carried out in the laboratory using either the second antibody alone or other monoclonal antibodies from the same collection.

One-dimensional SDS–polyacrylamide gels and identification of antibody-reactive polypeptides

Triton X-100-extracted cells were prepared as follows. After two washes in 3 mM-EDTA, 20 mM-Tris–maltate buffer (pH 7.8), cells were permeabilized for 10 min in PHEM buffer with 1% Triton X-100 at room temperature in the presence of a cocktail of protease inhibitors: 20 μg leupeptin, 1 mM PMSF, 1 mM-O-phenanthrolin, 1 mM-1-L-chloro-3-tosylamido-7-aminohexan-2-heptanone, 1 mM-1-chloro-3-tosylamido-4-phenyl-2-butanone, 20 μg/ml pepstatin and chymotrypsin and 1 μg/ml aprotinin. The extracted cells were pelleted at low speed in a clinical centrifuge and the pellet (insoluble fraction) was solubilized as indicated below. Proteins in the supernatant (soluble fraction) were precipitated with 70% cold methanol and solubilized as described. Other *Paramecium* subfractions (cortex and gullets) were isolated by centrifugation of homogenized cells on a sucrose gradient as described by Keryer et al. (unpublished data). Triton-extracted cells, purified cortices or gullets were solubilized in the presence of protease inhibitors in sample buffer according to Laemmli (1970). The polypeptides were separated by electrophoresis and electrophoretically transferred to nitrocellulose paper for 1 h (transblot apparatus from Biotron, France) at 800 mA in Towbin's buffer (Towbin et al. 1979). The polypeptides were visualized on nitrocellulose filters after electroblotting with 0.2% Ponceau Red in 3% trichloroacetic acid. After three washes with distilled water the nitrocellulose filters were soaked for 1 h at room temperature in 0.15 M-NaCl, 101.2 mM-Tris–HCl (pH 7.4) (TBS) with either 5% BSA or 5% non-fat dry milk according to Johnson et al. (1984), then incubated for 1 h in the presence of CTR 532 diluted 1/5 to 1/1000 in the same buffer containing 0.1% Tween 20 (TBST). After three washes in the same buffer the immunoblots were
incubated again with anti-mouse immunoglobulin conjugated to alkaline phosphatase diluted 1/7500 (Promega, Biotech, Madison, USA) in TBST for 1 h. They were washed three times in TBST and once in the alkaline phosphatase buffer and the colour reaction was started by addition of nitroblue tetrazolium (NBT, Sigma, 50 mg ml⁻¹ in 70 % dimethylformamide) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP, Boehringer-Mannheim, 50 mg ml⁻¹ in dimethylformamide) in alkaline phosphatase buffer (100 mM-Tris-HCl, pH 9.5, 100 mM- NaCl, 5 mM-MgCl₂).

**Results**

The monoclonal antibody CTR 532 decorates the metaphase spindle in mammalian cells

Among a library of monoclonal antibodies raised against human lymphoblast centrosomes (Bailly et al. unpublished data), CTR 532 was selected, as it decorated the mitotic spindle in several types of mammalian cells, the centrosome in interphasic Vero and CHO cells and the centrosome-associated ciliary rootlets in Vero or PtK₂ cells (Fig. 1). It is noteworthy that depolymerization of spindle microtubules by cold or by high calcium concentrations did not modify the decoration pattern observed with CTR 532 (not shown), suggesting that CTR 532 antigen could be associated with a non-microtubule component in the spindle.

CTR 532 recognizes microtubule-embedding material in the interphase and mitotic micronucleus of Paramecium

In Paramecium internal microtubule arrays can be visu-

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**Fig. 1.** CTR 532 decoration in HeLa and Vero cells. A–B. In HeLa cells, the mitotic poles and the spindle are labelled. C,D. In Vero cells, CTR 532 decorates the centrosome and ciliary rootlets (arrows in D) during interphase while during mitosis (insets) the staining is observed on poles of the metaphase spindle (arrowhead) but not during anaphase (small arrow). Bar, 10 μm.
Fig. 2. Monoclonal anti-α-tubulin antibody decoration of microtubules inside the nuclei of Paramecium. A. In interphase cells the dense cytoplasmic microtubule network does not permit good observation of microtubules in the micronuclei (small arrows) near the macronucleus. B. During mitosis this antibody brightly decorates the barrel-shaped metaphase spindles in micronuclei (arrows). ng, new gullet; og, old gullet. C. At anaphase B a long separation spindle separates each daughter micronucleus (long arrows) and longitudinal microtubules are observed in the macronucleus as it elongates (arrowhead). Bar, 10 μm.

Fig. 3. Immunofluorescence decoration of micronuclei in Paramecium with CTR 532 during interphase and mitosis. A,B. Interphase; C–G, mitosis. A. When the antibody staining is performed on permeabilized and unfixed cells in PHEM buffer, CTR 532 recognizes the micronuclei and slightly the basal bodies. B. Higher magnification shows CTR 532 decoration of some filamentous material. During metaphase the mitotic spindle is brightly labelled. C. Phase-contrast image of an early stage of division. D. Immunofluorescence on the same cell with CTR 532. E. Higher magnification of micronuclear spindles at the same stage of division. The CTR 532 staining is mainly localized at the poles. During anaphase (F and G), only the polar parts of the micronuclei are stained. The long separation spindle is not or faintly decorated. Bars: A,C,D,F,G. 10 μm; B, 5 μm; E, 3–5 μm.

CTR 532 decorates the meiotic spindle in situ but not after isolation
In Paramecium, meiosis is induced by conjugation synchronously in both conjugants, the following sequence of events taking place (Jones, 1956; Jurand, 1976): at meiotic prophase, the nuclei become crescent-shaped, then undergo first and second meiotic divisions, yielding eight haploid products. A single one of them
Spindle-associated protein in micronuclei
As populations of synchronous conjugants can be obtained, isolation of meiotic spindle was carried out from Triton X-100-permeabilized conjugants using the technique described for mammalian spindles (Kuriyama et al. 1984). Although the presence of microtubules could be confirmed with an anti-tubulin antibody, no staining with CTR 532 was observed in isolated spindles. This result could be due either to denaturation or to solubilization of the CTR 532 immunoreactive antigen in the low ionic strength medium used for spindle isolation.

**CTR 532 decoration disappears during macronuclear differentiation**

After the haploid nucleus that survives in the paroral cone has divided, a second phase of the nuclear story takes place. The two conjugants exchange one haploid nucleus and retain the sister one. In each conjugant, a zygotic diploid nucleus is then formed. Two post-zygotic divisions take place and, in each conjugant, two of the four resulting nuclei retain their micronuclear condition,
Fig. 6. CTR 532 micronuclei decoration during nuclear differentiation. The decoration by CTR 532 persists in the zygotic nuclei throughout the two post-zygotic divisions but is lost in the macronuclear anlagen as soon as they begin to differentiate. A. Zygotic nuclei (arrows), still in the paroral cones of the paired conjugants, are seen in mitosis; and then, in B, as a pair of nuclei in each conjugant. At this stage the conjugants separate and the second post-zygotic division takes place (C). Of the four resulting nuclei (D), two will differentiate into macronuclei and two will remain as micronuclei. Less than half an hour later, already differentiated macronuclear anlagen can be identified. E. Phase-contrast image; mic, micronucleus; maa, macronuclear anlagen; F, CTR 532 staining of the same cell revealing only the micronuclei. The chromatin mass occupies only the centre of the nucleus, CTR 532 decorates the periphery of the micronucleus. G. Hoechst staining showing the fragments of the old macronucleus, the two macronuclear anlagen (maa) and one micronucleus (mic). Bars, 10 μm.

Fig. 7. Immunofluorescence decoration of the micronuclei with CTR 532 and anti-α-tubulin antibodies after cold treatment of the cells. A,B. Phase-contrast and immunofluorescent images of the same cold-treated cell at an early stage of division, stained with anti-α-tubulin antibody. The micronuclei are labelled (arrows). C. Immunostaining with CTR 532 of a different cell from the same cold-treated population: metaphase spindle-like structures are indicated by arrows. D. Immunofluorescent image of conjugants at an early stage of meiosis, stained with anti-α-tubulin antibody. The crescent-shaped micronuclei are labelled. mic, micronucleus; g, gullet; cv, contractile vacuole. E,F. CTR 532 and Hoechst staining of a different pair of conjugants from the same cold-treated population: metaphase spindle-like structures are observed. mac, macronucleus. Arrows indicate the chromatin at the equatorial plate. Bar, 10 μm.

Spindle-associated protein in micronuclei 295
while the other two differentiate into macronuclei. In the macronuclei, transcription is activated, genomic organization is remodelled and, over the subsequent vegetative divisions of the exconjugant clones, no true mitotic spindles but only bundles of longitudinal microtubules (Tucker et al. 1980) will support the distribution of chromatin between daughter macronuclei. Fig. 6 shows that CTR 532 decoration persists in micronuclei throughout post-zygotic divisions (Fig. 6A–D) but is absent even from very young macronuclear anlagen while still being present in the micronuclei (Fig. 6F–G).

**CTR 532 recognizes a cold-stable component associated with the spindle**

When cells were held at 4°C for 45 min no metaphase spindles were formed in the micronuclei of cells entering mitosis and those already present before cold treatment were depolymerized. In the cold-treated cells, microtubules were present and were detected by the antitubulin antibody only in micronuclei at prophase (Fig. 7A,B) and at later stages of division (Cohen & Beisson, 1988). In contrast, by CTR 532 staining all stages of mitosis were observed, including spindle-shaped figures (Fig. 7C) where no microtubule spindles were detected with the polyclonal anti-axonemal tubulin (double-labelling experiments, not shown). This was also observed during meiosis where the microtubules in the crescent-shaped micronuclei during prophase were not sensitive to cold (Fig. 7D), but no metaphase spindles were detected. However, metaphase figures were observed with CTR 532 (Fig. 7E,F). This suggests, as has been observed in mammalian cells, that CTR 532 antigen could remain localized in the remnant of the mitotic spindle after cold depolymerization of microtubules.

**CTR 532 recognizes a 170 000 M_r polypeptide in the Triton X-100-insoluble fraction**

Polypeptides from Triton X-100-insoluble and -soluble fractions of whole cells and from purified cortices and gullets were separated on SDS–5% to 15% polyacrylamide gradient gels and electrophoretically transferred onto nitrocellulose. When the filters were incubated under stringent conditions (Fisher et al. 1982) in the presence of 0.25% SDS and 0.1% Triton X-100, 5% BSA in TBS, three major polypeptides were recognized by CTR 532 in the Triton X-100-insoluble fraction of whole cells (Fig. 8B, lane 2a). However, when BSA was replaced by non-fat dry milk in TBS, only the 170K

![Fig. 8. CTR 532 immunostaining of proteins of *Paramecium* subfractions after SDS–PAGE and electro-transfer. Proteins of the Triton X-100-insoluble and -soluble fractions of whole cells, as well as purified cortex or gullets of *Paramecium* were electrophoresed on 5% to 15% polyacrylamide gradient gels and electrophoretically transferred onto nitrocellulose. The immunostaining was performed in TBS, either with 5% non-fat dry milk (B, lanes 2b, 3, 4, 5) or with 0.25% SDS, 0.1% Triton X-100 and 5% BSA (B, lane 2a). A. Ponceau Red coloration of *Paramecium* polypeptides (Tub, tubulin) given in M_r 10^6; Lane 1, molecular weight standards: myosin, β-galactosidase, phosphorylase B, BSA, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme; lane 2, Triton X-100-insoluble fraction of whole *Paramecium* (150 μg protein); lane 3, purified *Paramecium* cortex (50 μg protein); lane 4, purified *Paramecium* gullets (12 μg protein); B, CTR 532 immunostaining using anti-mouse alkaline phosphatase conjugate antibody. Lanes 2 (a and b), 3 and 4 are the same as in A, except lane 5; lane 5, Triton X-100-soluble fraction of whole *Paramecium* (150 μg).
Fig. 9. CTR 532 immunostaining of Triton X-100-insoluble fraction of micronucleate and amicronucleate Paramecium cell lines. A 20 μg protein sample (in each slot) of the Triton X-100-insoluble fraction of whole cells were electrophoresed on a 10% polyacrylamide minigel and electrophoretically transferred onto nitrocellulose. The immunostaining was performed as described for Fig. 8. Lane 1, micronucleate cells from stock d4-2; lane 2, micronucleate cells from stock d4-94 (pawn); lane 3, amicronucleate cells from stock d4-94 (C113) (obtained by removal of the two micronuclei at young clonal age). The 170K polypeptide is only present in micronucleate cell lines.

(K = 10^{13} M_2) polypeptide was reactive in the insoluble fraction (Fig. 8B, lane 2b). Nothing was observed in the purified cortex fraction and a faint band around 68K was present in the gullet fraction (Fig. 8B, lanes 3 and 4). We demonstrated indirectly that this antigen belonged to the micronucleus. Indeed the 170K polypeptide was not detected in the Triton X-100-insoluble fraction when the cells were amicronucleate (by removal of the two micronuclei at young clonal ages of about 20 fissions; Ng, 1981) (Fig. 9). An immunoreactive polypeptide with similar molecular weight was also present in the micronucleate T. thermophila strain (not shown) and absent from the amicronucleate T. pyriformis strain GL100.

Discussion

Raikov (1982) has reviewed work on the ultrastructure of the spindle and the localization of microtubule-organizing centres associated with the spindle in protozoa. In these lower eukaryotes, the MTOCs that organize the mitotic spindle are localized either inside the nucleus or in the cytoplasm. These mitotic MTOCs are not always associated with centrioles or equivalent structures. The biochemical nature of these MTOCs is unknown. As an approach to the analysis of the MTOCs and spindle in protozoa, a collection of monoclonal antibodies generated against purified human centrosomes (Bornens et al. 1987) was screened on ciliates and in particular on P. tetraurelia. Like other ciliates, this protozoon displays two interesting characteristics. First, at least two types of MTOCs are present. The cortical ones correspond to the numerous basal bodies all over the cortex and in the oral apparatus, while submembrane, intranuclear MTOCs present in the micro- and the macronuclei are not associated with any structure equivalent to the centriole. Second, three spindle-like structures are organized during mitosis: the micro- and macronuclear spindles and a cortical spindle, the cytospindle (Cohen et al. 1982).

In this paper we report the presence of a mitotic spindle-associated protein in the micronucleus of several ciliates: Paramecium, Tetrahymena and Euplotes. This protein was identified with the monoclonal antibody CTR 532 that decorates the metaphase spindle of mammalian cells. Immunofluorescence observations on Paramecium showed that CTR 532 staining displayed four main characteristics: (1) it was observed during mitosis and meiosis; (2) it persisted in interphase micronuclei; (3) the staining resisted cold treatment but not spindle isolation at low ionic strength; (4) the staining disappeared during macronuclear differentiation.

As observed by electron microscopy in several Paramecium species, interphase micronuclei contain microtubules (Schwartz, 1965; Lewis, 1977; Stevenson & Lloyd, 1971). In close association with both the nuclear envelope and some microtubules, a fibrillar material was labelled with CTR 532. During mitotic or meiotic prophase, the CTR 532-reactive material was associated only with spindle poles, although microtubules are known in Paramecium (Jurand & Selman, 1970; Jurand, 1976) and in Tetrahymena (Wolfe et al. 1976) to run parallel to each other as a single layer under the nuclear envelope. During metaphase the whole spindle contained CTR 532-reactive material, suggesting that the antigen had now migrated down to the equatorial plane. During anaphase B, the spindle undergoes a 12-fold elongation (Tucker et al. 1985) and no spreading of CTR 532 antigen was observed on the separation spindle. Microtubules could be instrumental in the distribution of CTR 532 antigen all over the spindle. However, once the antigen is so distributed, microtubules are not necessary to hold it permanently in place, as cold or high-calcium microtubule extraction has no effect on its localization. A similar result was observed in mammalian cells. This result recalls the behaviour of sea urchin kinesin that has also been shown to be associated with a non-microtubule component of the spindle (Leslie et al. 1987).

In P. tetraurelia a polyplid macronucleus develops at each sexual process. While decoration of micronuclei with CTR 532 was consistently observed during and after the post-zygotoc divisions, the staining had completely disappeared in the macronuclear anlagen shortly after they had been triggered by their brief positioning at the posterior cell pole (Grandchamp & Beisson, 1981) to differentiate into macronuclei. Whether this antigen is directly related to the process of nuclear differentiation deserves further study.

A polypeptide of M_r 170 000 was recognized by CTR 532 in the Triton X-100-insoluble fraction of whole cells. It was not associated with the cortical or the gullet.
fractions. Direct proof that the 170K polypeptide was a micronuclear antigen could not be obtained by isolation of micronuclei. We demonstrated indirectly that this antigen belonged to the micronucleus by comparing the same Paramecium strain with and without micronuclei. An immunoreactive polypeptide with a similar molecular weight was also present in the micronucleate Tetrahymena strain but not in the amicronucleate one.

In conclusion, although the antigen recognized in situ in mammalian cells could not be identified in its de-natured state on immunoblots, the fact remains that CTR 532 decorates the mitotic spindle in both mammals and ciliates. This cross-reactivity may be fortuitous, since monoclonal antibodies and often IgM can react with similar epitopes on otherwise unrelated proteins, particularly in the case of cytoskeletal constituents (Nigg et al. 1982). However, CTR 532 has been generated against isolated microtubule-organizing centres from mammalian cells and specifically decorates the centrosome and the spindle. The very restricted and precise localization of the antigen in ciliates supports the hypothesis that this protein could be a specific spindle-associated component involved in the association of the MTOC with the nuclear envelope. As the antigen is present inside the micronucleus during the whole of the cell cycle, this suggests a putative role also for this antigen in nuclear envelope, microtubule and chromatin interactions. Functional studies will be necessary to decide between these possibilities. In summary, we have identified a micronuclear marker in Paramecium and we are currently exploring the possibility that it is involved in nuclear differentiation.

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