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The human CAS protein which is homologous to the CSE1 yeast chromosome segregation gene product is associated with microtubules and mitotic spindle

(*Pseudomonas* exotoxin/tumor necrosis factor/microtubule-associated protein/apoptosis/cyclin B)

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ABSTRACT **Human *CAS* cDNA contains a 971-aa open reading frame that is homologous to the essential yeast gene *CSE1*. CSE1 is involved in chromosome segregation and is necessary for B-type cyclin degradation in mitosis. Using antibodies to CAS, it was shown that CAS levels are high in proliferating and low in nonproliferating cells. Here we describe the distribution of CAS in cells and tissues analyzed with antibodies against CAS. CAS is an ≈100-kDa protein present in the cytoplasm of proliferating cells at levels between 2×10^5 and 1×10^6 molecules per cell. The intracellular distribution of CAS resembles that of tubulin. In interphase cells, anti-CAS antibody shows microtubule-like patterns and in mitotic cells it labels the mitotic spindle. CAS is removed from microtubules by mild detergent treatment (cytoskeleton preparations) and in vincristine- or taxol-treated cells. CAS is diffusely distributed in the cytoplasm with only traces present in tubulin paracrystals or bundles. Thus, CAS appears to be associated with but not to be an integral part of microtubules. Immunohistochemical staining of frozen tissues shows elevated amounts of CAS in proliferating cells such as testicular spermatogonia and cells in the basal layer cells of the colon. CAS was also concentrated in the respiratory epithelium of the trachea and in axons and Purkinje cells in the cerebellum. These cells contain many microtubules. The cellular location of CAS is consistent with an important role in cell division as well as in ciliary movement and vesicular transport.**

CAS is the human homologue of the essential yeast chromosome segregation gene *CSE1* (1, 2). CAS appears to have a role in toxin or tumor necrosis factor (TNF)-induced apoptosis as well as in cell proliferation. Expression of CAS antisense mRNA was found to inhibit apoptosis induced by TNF, diphtheria toxin, and *Pseudomonas* exotoxin in MCF-7 breast cancer cells (1, 3). Furthermore, CAS expression correlates with the proliferation status of human cells. CAS mRNA is highly expressed in growing cells and in tumor cell lines, while it is expressed at very low levels in nonproliferating cells (1).

CAS cDNA encodes a 971-aa protein with a deduced molecular mass of ≈100 kDa. This is very similar to the predicted size of the homologue *CSE1* (960 aa). Neither CSE1 nor CAS shows significant homology to any other known protein. The only clues to the function of CSE1 come from studies of mutant CSE1, which leads to defects in chromosome segregation and B-type cyclin degradation in yeast (2, 4). To obtain more information about the function of the human CSE1 homologue, we raised antibodies against CAS and used these to determine the intracellular distribution of CAS.

Our results show that CAS is associated with microtubules and the mitotic spindle, although it is not an integral part of microtubules. A role of CAS in the formation or function of the mitotic spindle might explain why yeast with CSE1 mutations have a chromosome segregation-deficient phenotype.

MATERIALS AND METHODS

Construction of Plasmids. DNA fragments encoding N-terminal or internal sequences of CAS were obtained by PCR using *CAS* cDNA (1) as template. The primers 5′-GAGATCCTATACATATGGAACTCCAGCGATG-3′ with ATG translation initiation codon as part of a *Nde* I site (underlined), and 5′-TATCGCTGGAATTCTCATC-CTAC-3′ with an *Eco*RI site (underlined) for fusion to a hexahistidine tag were used to amplify the sequence for the first 284 aa. For the internal CAS fragment (aa 327–669) we used the primers 5′-CAGTTTGTCATATGCCTCATTATA-AAATC-3′ and 5′-GAAGCAAAGAATTCACTTGAAA-GACGTATG-3′. *Nde* I (start codon) and *Eco*RI (His_6) sites are underlined. PCR products were cloned into pCR II (Invitrogen). The inserts were isolated from these vectors as *Nde* I/*Eco*RI fragments and cloned into pET-23b(+) (Novagen). The resulting plasmids, pUS6 for expression of CAS_{1-284} and pUS7 for $CAS_{327-669}$ were confirmed to be correct by DNA sequencing.

Expression and Purification of CAS Protein Fragments. The expression plasmids pUS6 and pUS7 were transformed into *Escherichia coli* BL21 (λDE3) (5), expression induced at OD_{600} of 2 with 2 mM isopropyl β-D-thiogalactoside, and the cells were harvested 2 h later (6). Both recombinant proteins accumulated in cytoplasmic inclusion bodies (IBs), which were purified as described (7) but without preparation of spheroplasts prior to IB preparation. The IBs were solubilized in 6 M GuHCl/0.1 M sodium phosphate/0.01 M Tris·HCl, pH 8.0/5 mM 2-mercaptoethanol, and 10 mg was loaded onto a Ni-NTA column (Qiagen) (8). The column was washed with 50 ml of wash buffer (8 M urea/0.1 M sodium phosphate/0.01 M Tris·HCl/5 mM 2-mercaptoethanol) at pH 8.0, 20 ml of wash buffer at pH 6.3, and 20 ml of wash buffer at pH 5.9. The proteins were eluted with wash buffer at pH 4.5. Protein concentration was determined with Bradford assay reagent (Pierce) using bovine serum albumin (BSA) as a standard.

Immunizations. Each purified CAS protein fragment was diluted in phosphate-buffered saline (PBS), pH 7.4/1.5% Tween 20 (100 μg/ml or 180 μg/ml). Samples (250 μl) were diluted with 250 μl of Freund's adjuvant and 125 μl each was injected subcutaneously into four different sites of each rabbit [HM(NZW)FBR, Hare-Marland, Hewitt, NJ]. Two rabbits

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Abbreviations: TNF, tumor necrosis factor; DAB, 3,3′-diaminobenzidine; MAP, microtubule-associated protein.

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were injected with each protein. Injection on day 1 was with complete Freund's adjuvant, the booster injections on days 14 and 28 were with incomplete adjuvant. Test bleeds were obtained 7 days after each injection and in 2-week intervals after the last injection.

Antibody Purification. Antibodies from rabbit R2.6-I against the N-terminal portion CAS_{1-284} and from rabbit R46-I against the center portion $CAS_{327-669}$ of CAS were purified with immobilized protein A (Pierce). Anti-$CAS_{327-669}$ was further purified by affinity chromatography. For that we purified $CAS_{327-699}$ by Ni-NTA chromatography as described for preparation of immunogen but used 1 M GuHCl without 2-mercaptoethanol (pH 4.5) as elution buffer. Fractions containing pure CAS protein were concentrated by Centricon 30 (Amicon) to 1.4 mg/ml. Six milligrams of this protein was coupled to Affi-Gel 10 (Bio-Rad) activated with 0.1 M sodium acetate, pH 4.5/1 M GuHCl. Protein A purified anti-$CAS_{327-669}$ antibody from rabbit R46-I dialyzed against 0.1 M borate, pH 8.0/0.5 M NaCl was applied to the column equilibrated with dialysis buffer. After washing with dialysis buffer and 0.1 M sodium acetate, pH 4.8/0.5 M NaCl, the antibody was eluted in 1-ml steps with 0.2 M sodium-acetate (pH 2.5) and neutralized with 500 μl of 1 M Tris·HCl (pH 9.0). BSA was added to a final concentration of 1 mg/ml. Antibody fractions ($\approx$35 μg of affinity-purified antibody per ml of immune serum) were dialyzed against PBS (pH 7.4) and stored -20°C.

Western Blot Analysis, Immunofluorescence, and Immunohistochemical Staining. Western blot analysis was performed with the Vectastain ABC kit (Vector Laboratories) using biotinylated anti-rabbit IgG (H+L) as secondary antibody, avidin horseradish peroxidase, and 3,3′-diaminobenzidine (DAB) for color development. For immunofluorescence, affinity-purified anti-CAS preparations (10 μg/ml) or anti-tubulin monoclonal antibody (10 μg/ml) (clone YL 1/2-supernatant; Accurate Scientific, Westbury, NY) were incubated with MCF-7, A431, OVCAR3, or KB cells grown for 1 day in 35-mm dishes to subconfluency. Cells were fixed and permeabilized using 3.7% formaldehyde in PBS for 10 min followed by inclusion of 0.1% saponin in all subsequent incubations. Bound antibody was detected with goat anti-rabbit IgG-rhodamine for anti-CAS antibody and goat anti-rat IgG rhodamine for anti-tubulin antibody (9). For double-label immunofluorescence, MCF-7 cells were fixed and processed as described for single-label immunofluorescence. Cells were then sequentially incubated with rabbit anti-CAS and rat monoclonal anti-tubulin, followed by affinity-purified goat anti-rabbit rhodamine (minimal cross-reaction to rat) and goat anti-rat fluorescein (minimal cross-reaction to rabbit) both from Jackson ImmunoResearch. Controls demonstrated that the two antibody systems showed no cross-reactivity and no cross-detection between fluorescence channels (data not shown). Frozen tissue samples from the Hollings Cancer Center Tumor Bank Collection at the Medical University of South Carolina were cryostat sectioned and processed for immunohistochemical staining as described (10).

Cell Fractionation and Preparation of Cytoskeleton. MCF-7 or A431 cells (2×10^6) were chilled to 4°C, washed three times with ice-cold Dulbecco's phosphate buffered saline without Ca^{2+}/Mg^{2+}, scraped from the flasks, and suspended in 1 ml of hypotonic buffer (25 mM Tris·HCl, pH 7.4/2 mM $MgCl_2$/1 mM EDTA). After 15 min of incubation on ice, the suspension was homogenized with 20 strokes in a Dounce homogenizer and centrifuged at $1000 \times g$ at 4°C for 5 min. The pellet is the nuclear fraction. The supernatant was centrifuged again at $100{,}000 \times g$ at 4°C for 60 min. The supernatant from this centrifugation is the cytosolic fraction. The pellet was resuspended in hypotonic buffer and contains mainly membranes. Cytoskeletons of MCF-7 cells grown in 35-mm poly(L-lysine) dishes were prepared by washing them three times with 0.1 M Mes/1 mM EGTA/1 mM $MgCl_2$/4% PEG 8000, pH 6.5, incubation for 20 min with 0.2% Triton X-100 in the same buffer, and washing three times with PBS (11). The cytoskeletons still attached to the dishes were fixed with 3.7% formaldehyde for 10 min, washed three times with PBS, and subjected to immunofluorescence.

RESULTS

Production of Anti-CAS Antibodies. Antibodies were prepared by injecting rabbits with the N-terminal 284-aa fragment or an internal fragment containing aa 327–669. The proteins were produced in *E. coli* and purified to near homogeneity (>95% pure) as described. About 6 mg of purified recombinant protein was obtained from a 1-liter culture of *E. coli* expressing the N-terminal CAS_{1-284} fragment and 11 mg of the $CAS_{327-669}$ fragment per liter was obtained.

Test bleeds of rabbits immunized with each CAS fragment were evaluated by Western blot analysis using various concentrations of total cell extracts of *E. coli* expressing the appropriate antigen. CAS was detected at a 1:2000 dilution of all sera 3 weeks after the third injection. Sera from one animal immunized with CAS_{1-284} and from one immunized with $CAS_{327-669}$ were first purified on a protein A column. Antibodies from the $CAS_{327-669}$ immunized rabbit were additionally purified by antigen affinity chromatography. The yield of affinity-purified anti-$CAS_{327-669}$ was $\approx$1.5% of the total protein A-purified antibodies. Affinity purified anti-$CAS_{327-669}$ specifically detects $\leq$0.8 ng of CAS in Western blots developed with horseradish peroxidase and DAB as color substrate. A clear band was visible with 0.8 ng of purified recombinant CAS and 0.3 ng still showed a very weak band (data not shown).

CAS Is an Abundant Cytosolic Protein. *CAS* cDNA contains an open reading frame of an $\approx$100-kDa protein (1). To analyze the specificity of the antibody preparation and to confirm the open reading frame deduced from *CAS* cDNA, we analyzed total cell extracts of MCF-7 and A431 cells by Western blots. If the deduced reading frame is correct, we should detect an $\approx$100-kDa band in the extracts with immune serum but not with preimmune serum. Fig. 1*A* shows immunoblot analyses of total cell protein of MCF-7 cells on 4–15% SDS gels with anti-CAS_{1-284} and anti-$CAS_{327-669}$ antibodies. It is evident that both anti-CAS antisera specifically detect an $\approx$100-kDa protein, while the corresponding preimmune sera do not. We conclude that CAS is a 100-kDa protein as predicted from the cDNA sequence.

To evaluate the cellular localization of CAS, we homogenized MCF-7 and A431 cells and prepared fractions containing membranes, cytosol, and nuclei. Western blot analysis with anti-CAS_{1-284} (not shown) and anti-$CAS_{327-669}$ antibodies (Fig. 1*B*) shows that CAS is present exclusively in the cytosolic fraction.

To obtain an estimate of the number of CAS molecules per cell, we compared the CAS signal intensity in the cytosolic fraction of MCF-7 cells with the signal intensity of known amounts of recombinant CAS. We found that the signal from protein present in 10^4 cells corresponded to $\approx$1 ng of CAS. Thus, one MCF-7 cell contains $\approx$0.1 pg of CAS; i.e., $\approx 10^{-18}$ mol or $\approx$600,000 molecules, indicating that CAS is an abundant protein. Taking into account the variations between different immunoblot analyses and extract preparations, we estimate the number of CAS molecules is between 2×10^5 and 1×10^6 per cell in actively growing cells.

CAS Is Associated with Microtubules. To obtain more information about the intracellular distribution of CAS, we analyzed permeabilized MCF-7, A431, OVCAR3, and KB cells by immunofluorescence with affinity-purified anti-$CAS_{327-669}$ antibody. Anti-CAS antibody shows a diffuse background staining as well as clear staining of structures that form an intracellular network in all cell lines (Fig. 2 *A* and *C* for MCF-7; data not shown for A431, OVCAR3, and KB). This

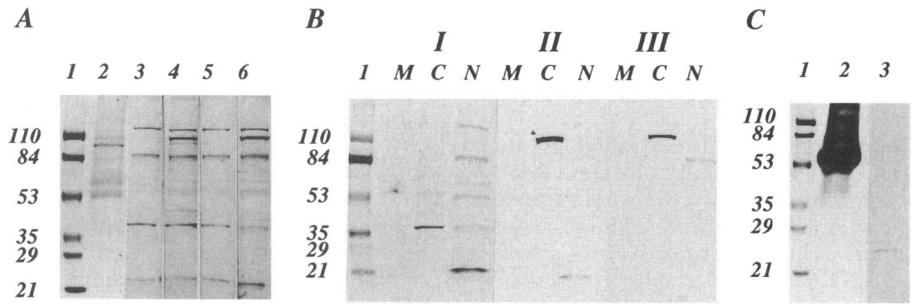

FIG. 1. Western blot analysis of MCF-7 cell fractions and of α-tubulin preparation. Reducing SDS/4–15% PAGE (*A* and *B*) or SDS/12% PAGE (*C*). (*A*) Evaluation of anti-CAS antisera. Lanes: 1, prestained protein marker; 2, 10 μg of total cell extracts of MCF-7 cells Coomassie blue R250 stained; 3–6, Western analysis with anti-CAS_{1-284} prebleed (lane 3), anti-CAS_{1-284} antiserum (lane 4), anti-$CAS_{327-669}$ prebleed (lane 5), and anti-$CAS_{327-669}$ antiserum (lane 6) at 1:2000 dilution each. (*B*) Determination of intracellular localization of CAS. Lane 1, prestained protein marker. Western blot analysis of MCF-7 membranes (lane M), cytosol (lane C), and nuclei (lane N) with anti-$CAS_{327-669}$ prebleed (1:2000) (I), anti-$CAS_{327-669}$ antiserum (1:2000) (II), and affinity-purified anti-$CAS_{327-669}$ antibody (20 ng/ml) (III). (*C*) Analysis of reactivity with tubulin. Lanes: 1, marker; 2, 60 μg of tubulin Coomassie blue R250 stained; 3, Western blot analysis of 60 μg of tubulin with 20 ng of affinity-purified anti-$CAS_{327-669}$ per ml. Numbers on left are kDa.

distribution resembles that of microtubules (9). We therefore compared cells stained with anti-CAS antibody to cells stained with anti-tubulin antibody and found that CAS is distributed in cells in a pattern similar to tubulin (Fig. 2 *B* and *D*). Thus, it is possible that CAS is associated with or part of microtubules. Anti-CAS staining of metaphase MCF-7 cells show that CAS is also associated with the chromosome segregation spindle in mitotic cells (Fig. 2 *E* and *F*). The spindle and the intercellular bridge between cells that have just divided stain strongly. The intracellular distribution of CAS, as defined with anti-$CAS_{327-669}$ antibody was confirmed with protein A-purified anti-CAS_{1-284} antibody, which shows the same CAS localization. Thus these tubular staining patterns reflect the intracellular CAS distribution. In addition to the similarity in patterns observed for microtubular-like structures using both anti-CAS and anti-tubulin antibodies, a separate double-label immunofluo-

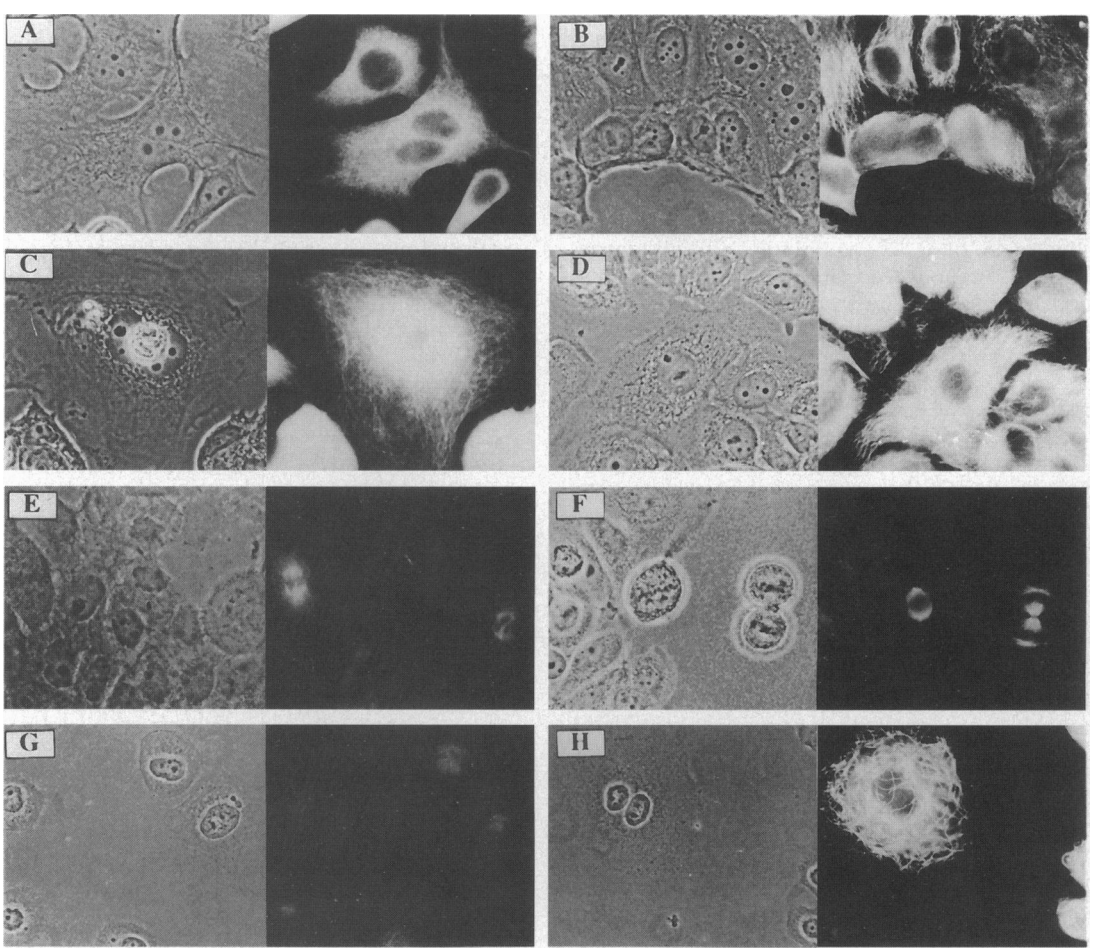

FIG. 2. Localization of CAS and α-tubulin in MCF-7 breast cancer cells. (*A–D*) Permeabilized whole cells stained with affinity-purified anti-$CAS_{327-669}$ (10 μg/ml) (*A* and *C*) or anti-tyrosinated α-tubulin (10 μg/ml) (*B* and *D*). Cells in *C* and *D* are very flat and therefore appear larger and their microtubule structure is more easily visible. (*E*) CAS and (*F*) tubulin distribution in mitotic cells. (*G*) Cytoskeleton preparation stained with anti-CAS. (*H*) Cytoskeletons stained with anti-α-tubulin. Prints in *E* and *F* are less exposed than the others to better visualize the spindle and intercellular bridges in the cytoskeleton background.

rescence experiment was performed as shown in Fig. 3. This result clearly demonstrates that the tubular structures demonstrated by anti-CAS and anti-tubulin antibodies are the same.

CAS Is Not an Integral Part of the Microtubules and Is Not Tightly Bound to Microtubules. Possible explanations for a tubulin-like intracellular distribution of CAS, as observed by immunofluorescence with anti-CAS antibody, are that (*i*) the polyclonal antibody preparations crossreact with tubulin, (*ii*) CAS is an integral part of microtubules, or (*iii*) CAS is associated with but not part of microtubules. To rule out the possibility that our antibody preparations cross-react with tubulin, we analyzed by Western blot analysis a tubulin preparation (T-4925; Sigma) derived from bovine brain, which contains ≈15% microtubule-associated proteins (MAPs). The gel was loaded with 60 μg of tubulin, and no reactivity with tubulin was detected. Also, no reactivity with tubulin-associated proteins was seen in immunoblots with anti-$CAS_{327-669}$ antibody (Fig. 1*C*). Although our antibodies were prepared against human CAS, we would expect to detect bovine CAS, since the sequence is highly conserved between humans and yeast, and since our antibody does detect mouse CAS. Thus, our antibody preparations do not cross-react with tubulin or with MAPs that are still present in this tubulin preparation. Furthermore, the fact that sera from animals that were independently immunized with different parts of CAS show the same distribution pattern and the same protein size (see above) makes it very unlikely that the signals observed are due to cross-reactivity. Thus, CAS is either an integral part of microtubules or is associated with microtubules.

To distinguish between these possibilities, we chose two approaches: First, we isolated cytoskeletal microtubules by an EGTA/Mg^{2+}/Triton X-100 method, which retains the microtubule network but removes loosely associated proteins. If CAS is an integral part of microtubules or very tightly bound to microtubules, the cytoskeletal preparation should stain with anti-CAS as well as with anti-tubulin antibodies. If CAS is associated loosely to microtubules, the anti-CAS staining pattern should be weaker than with anti-tubulin. Fig. 2 *G* and *H* show cytoskeletal preparations of MCF-7 cells stained with anti-$CAS_{327-669}$ and anti-tubulin antibodies. The distribution of CAS and tubulin is different. Anti-tubulin strongly stains the microtubule structures but anti-CAS staining results in a weak diffuse image. Only highly stable structures such as intercellular bridges still show some weak staining. These results indicate that, although CAS is associated with the microtubular cytoskeleton as seen by staining of whole cells (Fig. 2 *A* and *C*), it is not an integral part of it. Most of CAS is removed from the cytoskeleton during the EGTA/Triton X-100 preparation.

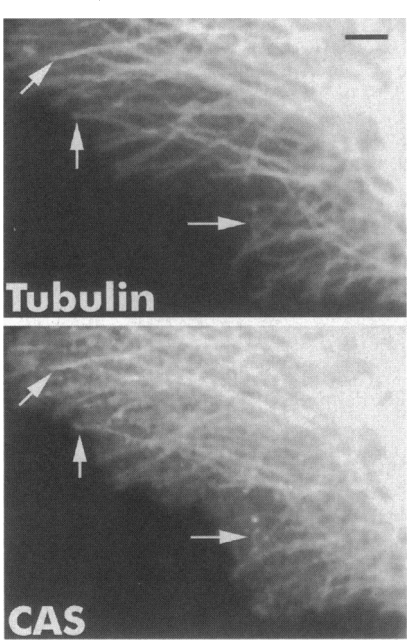

FIG. 3. Coincidence of CAS and tubulin in microtubular structures by double-label immunofluorescence. MCF-7 cells were processed for double-label immunofluorescence using antibodies to CAS and tubulin. Microtubular structures labeled using anti-CAS can be clearly seen to be the same as those detected using anti-tubulin (arrows) (×1000; bar = 8 μm.)

We also analyzed CAS distribution in cells that were treated with vincristine or taxol. Vincristine treatment results in formation of tubulin paracrystals and taxol produces tubulin bundles. If CAS were an integral part of microtubules, we would expect similar images of vincristine- and taxol-treated cells stained with anti-tubulin or anti-CAS. However, we found that in contrast to strong anti-tubulin staining of paracrystals and microtubule bundles in vincristine- and taxol-treated MCF-7 cells, staining with anti-CAS yields a strong diffuse cytoplasmic staining with only a weak immunofluorescence signal associated with paracrystals and bundles (data not shown). These results indicate that CAS is associated with but not an integral part of microtubules. CAS that is bound to microtubules is released by the EGTA/Triton X-100 incubation during preparation of the cytoskeleton and also when cells are treated with vincristine or taxol.

Distribution of CAS in Human Tissues. We previously showed by Northern blot analyses that CAS is preferentially expressed in human tissues that contain proliferating cells, particularly in testis, and is weakly expressed in nonproliferating tissues (1). To gain more information of the distribution of CAS, frozen tissue sections were stained with anti-$CAS_{327-669}$. Fig. 4*A* shows testis, which contains the largest amount of CAS RNA (1). CAS is present in many cells of the testis but the spermatogonia, the actively dividing cells in the testis, and the Sertoli cells adhering to the basal lamina are preferentially marked. Another example of a correlation of CAS expression with cell proliferation was found in colon. Fig. 4*B* shows a section of epithelial cells of large intestine. The actively dividing cells close to the basal layer in colon are highly labeled with anti-CAS antibody. The anti-CAS staining decreases toward the upper part of the intestinal crypt, which contains cells that are not dividing.

We also found elevated amounts of CAS in some cells that are not proliferating. One is the respiratory epithelium of the trachea (Fig. 4*C*), where CAS is present in the cilia and basal bodies of the columnar epithelium cells. Cilia contain many microtubules. CAS is also present in the cerebellum (Fig. 4*D*); Purkinje cells, Koerner cells, and axons are rich in CAS protein. They contain large numbers of microtubules, which are components of the axonal transport system.

DISCUSSION

We have raised antibodies that specifically recognize CAS protein and used these antibodies to analyze the distribution of CAS in human cancer cells and human tissues. We found that CAS is an ≈100-kDa protein, consistent with the deduced reading frame of *CAS* cDNA. CAS is a very abundant protein (2×10^5–1×10^6 molecules per cell) located in the cytoplasm where it appears as part of the intracellular microtubule network. CAS is also associated with the mitotic chromosome segregation spindle.

Our observation that CAS is associated with microtubules and the chromosome segregation spindle suggests a function for CAS and CSE1 in cell proliferation and cell division. It is therefore reasonable to assume that mutations in the *CSE1* gene in yeast, which is the *CAS* homologue, cause a chromosome segregation-deficient phenotype, because CSE1 is necessary for proper assembly or function of the mitotic spindle.

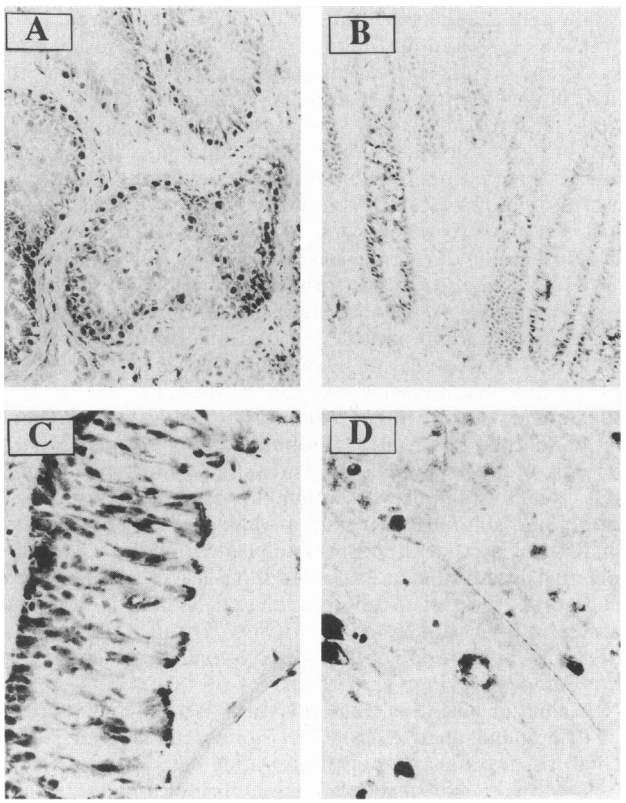

FIG. 4. Distribution of CAS in human tissues. Frozen tissue sections were stained with 10 μg of anti-$CAS_{327-669}$, goat-α-rabbit horseradish peroxidase as secondary antibody and DAB. (*A*) Testis. (*B*) Colon. (*C*) Trachea. (*D*) Cerebellum.

Our data suggest that CAS has the same function in human cells.

So far the molecular function of CAS is still unknown. CAS may be related to other MAPs or kinesins, even though its sequence is not homologous to any known MAP or kinesin. One indication for this is that, in addition to its association with microtubules, CAS contains a sequence at its N terminus that is similar to a MAP kinase (ERK) phosphorylation site (1). Another similarity of CAS with MAPs is that CAS has a repetitive sequence motif at its C terminus. MAPs are known to contain at their C terminus repetitive sequences that are involved in tubulin binding (but these are different from those of CAS). Finally, circumstantial evidence for a relation between CAS and MAPs emerges from recent observations (4, 12) that yeast CSE1 and MAP4 are necessary for cyclin B degradation. Neither CSE1 nor CAS is homologous to MAP4, but taken together these data might suggest that CAS is another component of a microtubule-associated complex (13, 14) involved in cyclin B degradation. Since CAS is associated with microtubules, varies in concentration as a function of cell proliferation, and possesses a putative ERK phosphorylation sequence, it is possible that CAS is linked like other MAPs, into the MAPK (ERK)-dependent signaling cascade (15) that regulates proliferation, differentiation, and development. Such an association with a MAP kinase pathway would also be consistent with the observation that CAS appears to play a role in TNF- and toxin-mediated apoptosis. CAS antisense renders cells partially resistant to apoptosis induced by TNF and some bacterial toxins (1, 3). It has recently been shown that the MAP kinase pathway is involved in TNF signaling as well as neuronal apoptotic cell death (16–18).

A further clue to the molecular function of CAS may also come from its tissue distribution. CAS was found not only in proliferating cells (testis, basal cells of colon) but also in some nonproliferating cells that show high microtubule-associated transport and dynamic function (axons, neurons, and ciliary cells). This distribution is very similar to the distribution of motor proteins (kinesins) that are, like CAS, associated with microtubules. Kinesins have size similar to CAS but they are not homologous (19). A link between CAS and kinesins may be suggested by the observation that kinesin antisense mRNA renders cells resistant to apoptosis induced by chemotherapy agents (20). This phenotype resembles the resistance of CAS antisense containing MCF-7 cells to toxin- and TNF-induced apoptosis (3).

In summary, our data suggest that CAS is a MAP with a function in cell proliferation (and apoptosis), which might be linked to the MAP kinase regulation system of cell proliferation.

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