Nucleus-specific and Temporally Restricted Localization of Proteins in *Tetrahymena* Macronuclei and Micronuclei

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Abstract. Labeled nuclear proteins were microinjected into the cytoplasm of *Tetrahymena thermophila*. Macronuclear H1, calf thymus H1, and the SV40 large T antigen nuclear localization signal linked to BSA accumulated specifically in macronuclei, even if cells were in micronuclear S phase or were nonreplicating. The way in which histone H4 localized to either the macronucleus or the micronucleus suggested that it accumulates in whichever nucleus is replicating. The inability of the micronucleus to accumulate *Tetrahymena* H1 or heterologous nuclear proteins, even at a period in the cell cycle when it is accumulating H4, suggests that it has a specialized transport system. These studies demonstrate that although the mechanism for localizing proteins to nuclei is highly conserved among eukaryotes, it can differ between two pore-containing nuclei lying in the same cytoplasm.

THE signal sequence–dependent import of proteins across the nuclear envelope is mediated by nuclear pore complexes, which are common to all eukaryotic nuclei (Gerace and Burke, 1988). The nuclear localization signal sequences from diverse organisms exhibit a common motif, suggesting that the mechanism for targeting proteins to nuclei is highly conserved (Dingwall and Laskey, 1986). In fact, the nuclear localization signal from the SV40 large T antigen, a primate viral protein, targets proteins to the nuclei of *Tetrahymena thermophila*, a ciliated protozoan (see below) and of yeast (Nelson and Silver, 1989).

In *Tetrahymena* and other ciliates nuclear targeting is complex because these organisms possess two structurally and functionally distinct nuclei that contain different proteins (Gorovsky, 1973). The macronucleus is an amitotically dividing, somatic nucleus that is responsible for the transcriptional activity of the cell. The micronucleus is a transcriptionally inert, mitotically dividing, germ-line nucleus. Both nuclei divide without breakdown of the nuclear envelope. Both nuclei contain the same major core histones. Each nucleus contains a specific subset of histone proteins. The micronucleus has four unusual linker histones that are proteolytically processed from a single polyprotein (Allis et al., 1984; Wu et al., 1988; unpublished observations), referred to as the micronuclear linker histone. The macronucleus has a more typical H1 as well as two additional core histone variants (see Gorovsky, 1986, for review). Thus, in these cells, certain proteins must be selectively accumulated in one of two nuclei.

During vegetative growth, macro- and micronuclei have non-overlapping periods of DNA synthesis (McDonald, 1962; Woodard et al., 1972; Wu et al., 1988). Previous studies indicated that expression of the gene for macronuclear H1 occurs only during macronuclear S, whereas that for micronuclear linker histone occurs only during micronuclear S, suggesting that replication-coupled expression might explain nucleus-specific localization (Wu et al., 1988). A prediction of the replication-expression hypothesis is that macronuclear H1 should accumulate in micronuclei if present during micronuclear S phase. We have used microinjection of fluorescein- or ³H-labeled proteins to test this hypothesis, with surprising results. We show here that macronuclear H1 accumulates specifically in macronuclei whenever it is injected, even if the cells are in micronuclear S phase or are nonreplicating.

We have investigated the conservation of the nuclear targeting mechanism by studying the SV40 large T antigen nuclear localization signal in *Tetrahymena*. Synthetic peptides containing SV40 sequence cross-linked to large nonnuclear proteins serve as nuclear localization signals when microinjected into *Xenopus* oocytes or mammalian cells (Goldfarb et al., 1986; Lanford et al., 1986; Yoneda et al., 1987). These peptide-BSA conjugates localize specifically to the *Tetrahymena* macronucleus. We discuss the implications of these findings in terms of the evolutionary conservation of the nuclear localization process, the specificity of transport across the nuclear membrane, and the functioning of the micronucleus.

Materials and Methods

Cells and Culture Conditions

Tetrahymena thermophila were grown axenically in enriched proteose pep-

tone at 28°C as described (Gorovsky et al., 1975). Starved cells were obtained by washing and resuspending a logarithmically growing culture in 10 mM Tris·HCl, pH 7.4 and incubating at 28°C for 24 h. Cells used for microinjection were of strain SB281, a mucoscyst-less strain (Maihle and Satir, 1985).

Protein Preparations

FITC-BSA, poly-L-lysine hydrobromide (5,200–9,200 D), and poly-L-lysine hydrobromide (18,300-24,000 D) were obtained from Sigma Chemical Co., St. Louis, MO. *Tetrahymena* macronuclei were isolated as described (White et al., 1988). Histones were prepared by sulfuric acid extraction of isolated nuclei as described (Allis et al., 1979). H1 and H4 were further purified by perchloric acid extraction (Glover et al., 1981) and size-exclusion chromatography on Bio-Gel P-60 (Bio-Rad Laboratories, Richmond, CA) as described (Johmann and Gorovsky, 1976). Calf thymus histones were prepared from frozen tissue as described (Johns, 1964). Calf thymus H1 was further purified by perchloric acid extraction of whole histone. SV40 large T antigen nuclear localization peptides used in this study have been described previously (Goldfarb et al., 1986). P_{LYS}^1 is a 12-amino acid peptide corresponding to residues 126–135 of the large T antigen with additional carboxy-terminal tyrosine and cysteine residues for radiolabeling and chemical cross-linking, respectively. P_{THR} is a mutant form of this peptide in which the critical lysine corresponding to position 128 has been changed to threonine. Peptides were prepared and linked to BSA (Sigma Chemical Co.) using m-maleimidobenzoyl-N-hydroxysuccinimide ester (Pierce Chemical Co., Rockford, IL) as described (Goldfarb et al., 1986), forming P_{LYS}-BSA and P_{THR}-BSA. Peptide/BSA ratios were ~10-15:1 as assayed by gel electrophoresis (data not shown).

Gel Electrophoresis

Protein preparations were run on one-dimensional acid-urea gels as described previously (Allis et al., 1980).

Fluorescein Labeling

Tetrahymena H1, calf thymus H1, P_{LYS}-BSA, P_{THR}-BSA, and both 18–24 and 5–9-kD poly-L-lysine samples were suspended at 1 mg/ml in H_2O. An equal volume of 100 µg/ml FITC (isomer I; Sigma Chemical Co.) in 0.5 M carbonate-bicarbonate buffer (3.7 g $NaHCO_3$, 0.6 g Na_2CO_3 to 100 ml, pH 8.8-9.0) was added, samples were wrapped in foil and shaken at room temperature for 2 h. *Tetrahymena* H1, calf thymus H1, and poly-L-lysine (18–24 kD) were concentrated and purified from free FITC by precipitation with 20% TCA on ice for 30 min. The precipitates were washed with 0.2% HCl in acetone followed by acetone, dried in a vacuum desiccator, and resuspended in H_2O at a concentration of ~10 mg/ml. P_{LYS}-BSA and P_{THR}-BSA were purified by centrifugation through a microconcentrator (Centricon 30; Amicon Corp., Danvers, MA) using 20 vol of H_2O to wash the samples. The samples were used at a final concentration of ~10 mg/ml. Poly-L-lysine (5–9 kD) was purified by fractionation over a prepacked, Sephadex G-25 column (PD-10; Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated with H_2O. Appropriate fractions were combined, dried in a vacuum desiccator and resuspended at a final concentration of ~10 mg/ml.

Rhodamine Labeling

Tetrahymena H1 and P_{LYS}-BSA were suspended at 1 mg/ml in H_2O. An equal volume of 1 mg/ml rhodamine isothiocyanate (RITC; mixed isomers) on Celite (Sigma Chemical Co.) in 0.5 M carbonate-bicarbonate buffer (pH 8.8-9.0) was added and the samples were shaken in the dark for 2 h. Celite was removed by centrifugation in a microfuge for 10 min followed by water washing and concentration through a Centricon-30 filter (P_{LYS}-BSA) or by TCA precipitation (H1). Rhodamine labeled proteins were microinjected at a concentration of 2–5 mg/ml in H_2O.

^{3}H-Labeling

Tetrahymena H1 and H4 were ^{3}H-labeled by reductive methylation as described (Wu et al., 1986b). Unbound radioactive material was removed by TCA precipitation as described above. Typical specific activities of labeled

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1. *Abbreviations used in this paper*: DAPI, 4'-6-diamidino-2-phenyl-dihydrochloride; P_{LYS}, 12-amino acid peptide corresponding to residues 126–135 of the large T antigen; P_{THR}, mutant form of this peptide.

proteins were 1–2 × 10^7 dpm/µg corresponding to approximately two to four methyl groups per histone molecule.

Microinjection of Labeled Proteins

Using a dissecting microscope, droplets of *Tetrahymena* cells in media were deposited under a large drop of paraffin oil on glass microscope slides treated with 3-aminopropyltriethoxysilane. Excess liquid was removed with a micropipette immobilizing the cells under the oil (Tondravi and Yao, 1986). Cells were microinjected utilizing an Olympus IMT-2 or Nikon Diaphot inverted microscope at magnifications of 15–40, and a micromanipulator (MO-120N; Narishige USA, Inc., Greenvale, NY) with oil hydraulics such that the flow of sample from the needles was constant. Injection needles were pulled on a needle puller (settings: heater = 5, magnet 1 = 0, and magnet 2 = 9, model PN-3; Narishige) from 1.0-mm thin-walled glass capillaries with filaments (World Precision Instruments, New Haven, CT).

Cell Fixation and Autoradiography

Injections of a series of cells in droplets generally took ~20 min. Cells injected with fluorescein- or rhodamine-labeled proteins were viewed immediately under fluorescent illumination and then fixed for photography. Some cells were viewed immediately after injection to determine how quickly the proteins localized. For most fluorescein-labeled cells, fixative (2:1 mixture of saturated $HgCl_2$/EtOH; Karrer, 1983) was added directly into the oil over the immobilized cells and left for 5 min at room temperature. Slides were then dipped in three changes of $CHCl_3$ to remove the oil, then allowed to dry for at least 30 min, during which time the fixative evaporated and the cells attached to the slide. Slides were then dipped in three changes of MeOH and allowed to dry. 15 µl of 2 µg/ml 4'-6-diamidino-2-phenyl-dihydrochloride (DAPI) (Polysciences, Inc., Warrington, PA) solution was placed on the slide to stain the nuclei. Some fluorescein-labeled cells and all rhodamine-labeled cells were fixed for 10 min in 4% paraformaldehyde in buffer A (Manuelides, 1985), treated in $CHCl_3$ as above to remove oil, baked for 2 h at 50°C, rinsed in 10 mM Tris (pH 7.4), and air dried. Formaldehyde fixed cells were stained and mounted in 15 ml of 0.4 mg/ml DAPI. Cells were photographed under DAPI, fluorescein, or rhodamine illumination with an Olympus C-35AD-2 camera using an Olympus BH-2 microscope at magnifications of 40 or 100 with Kodak TMAX-400 film that was push processed to 1,600.

Cells injected with ^{3}H-labeled proteins were allowed to incubate at room temperature for 30 min to 3 h after the completion of microinjection and
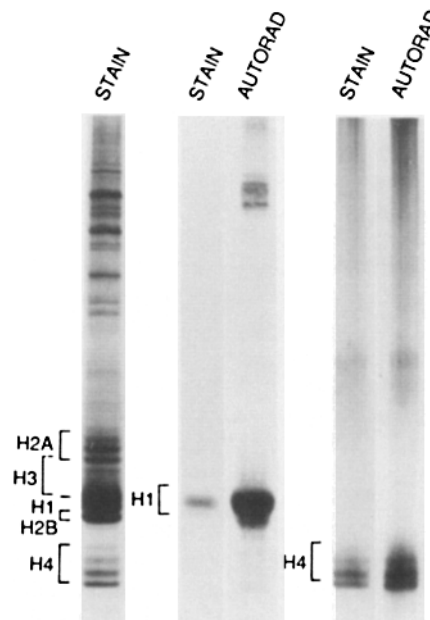

Figure 1. Purified histones used for microinjection. Lane *1* shows a histone preparation from *Tetrahymena* macronuclei stained with Coomassie brilliant blue. The other lanes show stained profiles of purified preparations of *Tetrahymena* H1 and H4, and autoradiographic exposures of these proteins labeled with ^{3}H.

TETRAHYMENA H1

GROWING

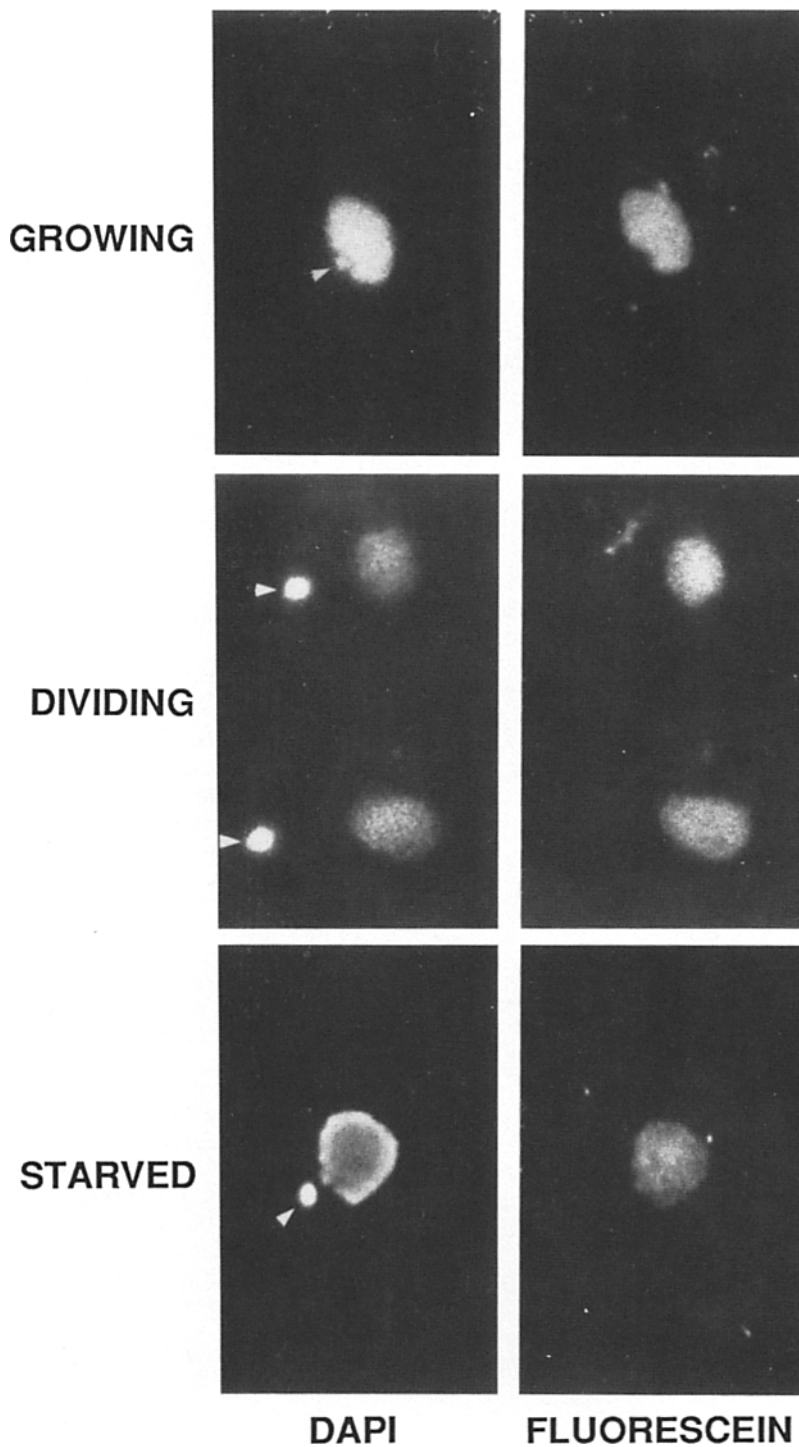DIVIDING

STARVED

DAPI   FLUORESCEIN

Figure 2. *Tetrahymena* H1 accumulates specifically in macronuclei. Fluorescein-labeled H1 was microinjected into the cytoplasm of growing (nondividing), dividing, and starved cells. DAPI stain shows the location of the macro- and micronuclei in the injected cells. Micronuclei are indicated by arrows.

were then fixed essentially as described above except that the dried cells were washed with 70% EtOH and then fixed in a 3:1 mixture of EtOH/acetic acid instead of MeOH. Cells were then prepared for autoradiography as described previously (Vavra et al., 1982; Allis and Wiggins, 1984). Slides were developed after an average of 30 d, stained with DAPI, and photographed.

Results

H1 Localizes Specifically to the Macronucleus

Tetrahymena macronuclear H1 was purified by perchloric

acid extraction from whole histone preparations followed by size exclusion chromatography on Bio-Gel P-60. Fig. 1 shows the purity of the preparation used for ^{3}H labeling. A sample similar to that shown was labeled with fluorescein and microinjected into the cytoplasm of growing and starved (nongrowing) *Tetrahymena* cells. The results of these studies are shown in Fig. 2. In all such figures, the micrograph on the left is the injected cell, stained with DAPI, under UV illumination to show the location of the macronucleus and the micronucleus (*arrows*). The micrograph on the right is fluorescein or rhodamine fluorescence or is an autoradiograph
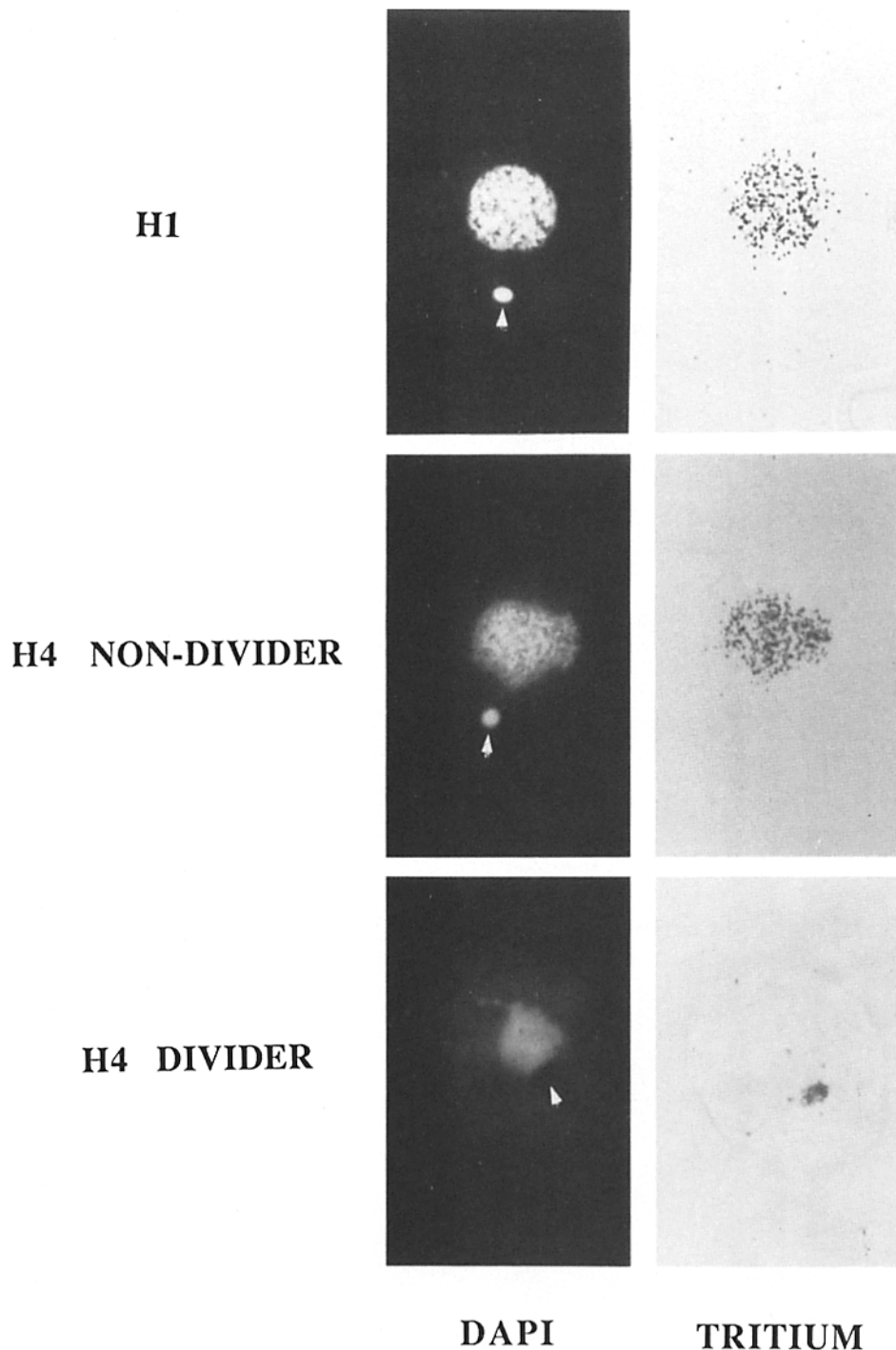
H1

H4 NON-DIVIDER

H4 DIVIDER

DAPI   **TRITIUM**

Figure 3. Radiolabeled H1 localizes to macronuclei, whereas radiolabeled H4 localizes to macronuclei or micronuclei. ^{3}H-labeled H1 or H4 was microinjected into the cytoplasm of growing cells. (*Top*) A cell injected with H1 as a divider and allowed to recover for ∼3 h; only the macronucleus is labeled. (*Middle*) Cell injected with H4 as a nondivider, whereas that in the lower panel was injected as a divider but completed division in the 30–60 min that elapsed between injection and fixation. Micronuclear S occurs in dividing and recently divided cells (see Wu et al., 1988). DAPI stain shows the location of the macro- and micronuclei in the injected cells. Micronuclei are indicated by arrows. The micronuclear DAPI stain in the bottom panel is masked by silver grains.

to show the location of the injected protein. The top panels of Fig. 2 show a nondividing cell (either in macronuclear S or nonreplicating), the second a dividing cell (in micronuclear S), and the third a starved cell (undergoing no DNA synthesis). In all cases, the H1 protein was localized specifically to the macronucleus. Localization was extremely rapid; accumulation in the macronucleus of growing cells occurred within 1 min after injection. ^{3}H-labeled (Fig. 3) and rhodamine-labeled *Tetrahymena* H1 (data not shown) were also found to localize specifically to the macronucleus of dividing and nondividing cells. In experiments where

records were kept, 101 of 103 cells showed macronuclear accumulation of injected H1. These included eight dividing cells. Two cells showed similar staining intensities in the macronucleus and the cytoplasm. In no case was micronuclear accumulation of H1 observed.

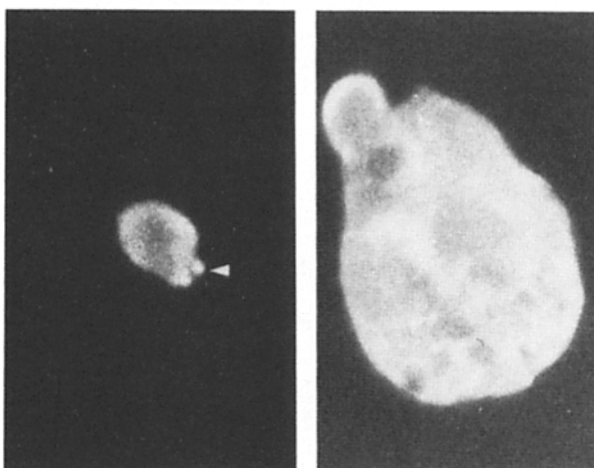## H4 Localizes to the Macronucleus or the Micronucleus

To show that a microinjected protein could accumulate in micronuclei, histone H4, which is common to both nuclei, was labeled with tritium (see Fig. 1) and microinjected into
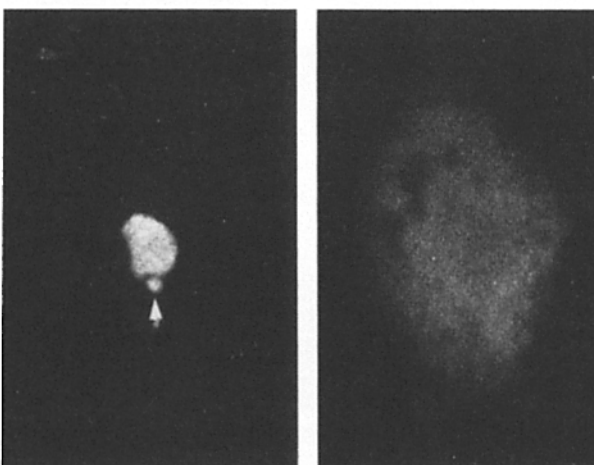
CONTROLS

18-24KD
POLYLYSINE
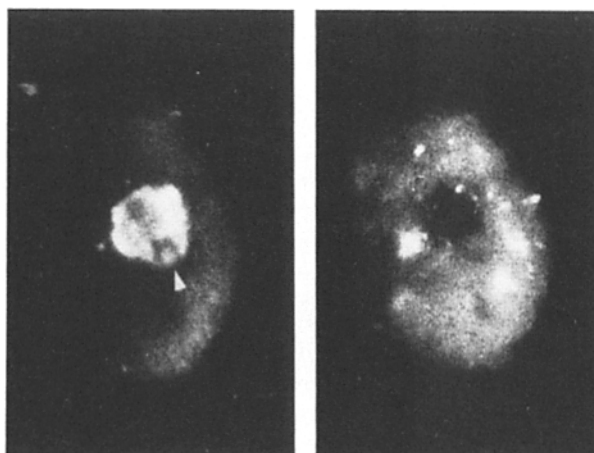
5-9KD
POLYLYSINE

FREE FITC

DAPI   FLUORESCEIN

Figure 4. Controls show specificity of local-
ization. Fluorescein-labeled polylysines of
18–24 or 5–9 kD and free FITC were in-
jected into the cytoplasm of growing cells.
DAPI stain shows the location of the macro-
and micronuclei in the injected cells. Mi-
cronuclei are indicated by arrows.

growing cells. Fig. 3 shows that in nondividing cells, the pro-
tein is targeted specifically to the macronucleus. In cells un-
dergoing micronuclear DNA replication, such as small cells
that have just completed division (Fig. 3), and dividing cells
(data not shown), H4 accumulated in the micronucleus. Note
that silver grains above strongly labeled nuclei in Fig. 3 mask
the DAPI fluorescence.

Polylysine, Free FITC, and Free Fluorescein Do Not Accumulate in the Macronucleus

To control for the possibility that the H1 localization was a
nonspecific charge- or size-dependent phenomenon, or re-
sulted from redistribution of fluorochrome after injection,
free fluorescein, FITC, and fluorescein-labeled polylysine of

18–24 or 5–9 kD were injected into the cytoplasm of growing cells. Large (18–24 kD) polylysine remained in the cytoplasm (Fig. 4), even if the microinjected cells were left for 1 h at room temperature. Note that large polylysine showed a tendency to aggregate to varying degrees inside injected cells. However, nuclear labeling was never observed, even in cells containing significant amounts of uniformly dispersed fluoresceinated polylysine. Smaller (5–9 kD) polylysine and free FITC (Fig. 4) and free fluorescein (data not shown) distributed more evenly throughout the cell, without accumulating in the macronucleus. Note that in these (and other) cases where the cytoplasm is labeled, it is not possible to tell whether micronuclei are labeled or are simply embedded in fluorescent cytoplasm.

SV40 Peptide-BSA Conjugates Localize Specifically to the Macronucleus

Like *Tetrahymena* H1, fluorescein-labeled calf thymus H1 also accumulated only in macronuclei (data not shown). The macronuclear-specific localization of calf thymus H1 suggested that *Tetrahymena* macronuclei might contain an evolutionarily conserved nuclear transport system. Perhaps the best characterized nuclear localization signal is that of the SV40 large T antigen. To see if the transport system recognizing this signal is present in *Tetrahymena*, peptides containing the wild-type SV40 large T antigen nuclear localization sequence (P_{LYS}) were conjugated to BSA, labeled with fluorescein, and injected into the cytoplasm of *Tetrahymena* cells. Fig. 5 shows that the wild-type peptide-BSA conjugates accumulated specifically in the macronucleus (note the black spot under fluorescein illumination in the position of the micronucleus). Cells injected with peptide-BSA conjugates made with mutant peptides in which the critical lysine was changed to a threonine (P_{THR}) are also shown in Fig. 5. These conjugates are much less effective in localizing to *Xenopus* oocyte nuclei (Goldfarb et al., 1986). Mutant peptide-BSA conjugates remained in the cytoplasm of *Tetrahymena*. As a control, injected fluorescein-labeled BSA was shown to remain in the cytoplasm (Fig. 5). Wild-type peptide-BSA conjugates also accumulated in macronuclei when injected into (dividing) cells in micronuclear S (Fig. 6), whereas mutant constructs were excluded from macronuclei (data not shown). In experiments where cells were actually tallied 114 of 131 cells injected with P_{LYS}-BSA, including 7 dividing cells, showed accumulation in macronuclei. No cells showed labeled micronuclei. These studies indicate that the *Tetrahymena* macronucleus, but not the micronucleus, is capable of recognizing a mammalian nuclear localization signal.

Discussion

Surprisingly little is known about histone transport into nuclei. Early work by Gurdon (1970) and Bonner (1975a, b) demonstrated that nuclear uptake of proteins is extremely selective. A model was developed that suggested that small proteins could freely diffuse into the nucleus, but only nuclear proteins would be retained by specifically binding to nondiffusible nuclear components. Until recently, it had generally been assumed that histones conform to this model. Because the majority of histone synthesis is tightly coupled

to DNA replication, it also seemed likely that newly replicated DNA was the nuclear component to which histones bound. However, there is little direct evidence to support this model. Yeast histone H2B contains a small domain that acts as a nuclear localization signal capable of facilitating the transport of larger proteins into nuclei (Moreland et al., 1987), and core histones and histone H1 microinjected into *Xenopus* oocytes accumulated in germinal vesicles that were not replicating DNA (Bonner, 1975a; Dingwall and Allan, 1984). Also, some quantitatively minor histone subtypes (so-called basal or replacement variants) are synthesized and deposited in nuclei throughout the cell cycle (see Wu and Bonner, 1981). Thus, although individual histones are theoretically small enough to diffuse through nuclear pores and can bind to DNA, they may still utilize a facilitated transport system like that described for larger nuclear proteins (see Dingwall and Laskey, 1986; Gerace and Burke, 1988 for review).

Tetrahymena thermophila (like most ciliated protozoa) has two structurally and functionally distinct nuclei that reside in the same cytoplasm and that must acquire distinct complements of nuclear proteins. Specifically, histone complements of the two nuclei are distinctive (see Gorovsky, 1986). Both nuclei contain all the core histones, but their linker-associated histones differ significantly. Previous work (Wu et al., 1988) suggested a model for nucleus-specific localization of these proteins based on their expression patterns. Expression of the gene for macromolecular H1 occurred only during macronuclear S phase, whereas that of the micronuclear linker histone occurred only during micronuclear S phase, suggesting that the specific nuclear localization of each may be dependent on replication-coupled expression. One prediction of this model is that macronuclear H1 should accumulate in micronuclei when introduced into cells in micronuclear S. Our results, however, argue that the mechanism of specific nuclear localization in *Tetrahymena* is more complicated and more interesting than this. Macronuclear H1, when microinjected into the cytoplasm under a variety of physiological conditions, localized to the macronucleus, and was excluded from the micronucleus. This was true for nondividing growing cells (in macronuclear S or nonreplicating), dividing cells (in micronuclear S), and starved cells (nonreplicating). H1 thus is able to localize to nuclei without any histone-free DNA available for binding, and is excluded even from replicating micronuclei, which must have newly synthesized (free) DNA available to bind histones that enter the micronucleus. Therefore, there exists a specific mechanism for accumulating H1 in the macronucleus that is independent of DNA replication.

The possibility that macronuclear localization was a property associated with the diffusion and binding of any small, highly positively charged molecule was ruled out by injecting cells with small polylysines. *Tetrahymena* H1 has a molecular weight of ~18 kD (Wu et al., 1986a). Under the experimental conditions used here, 18–24-kD polylysine remained in the cytoplasm of injected cells. 5–9-kD polylysine distributed evenly throughout the cell without accumulating detectably in either nucleus, as might have been expected if small, highly basic molecules simply diffused into nuclei and bound to newly replicated DNA or to other nondiffusible nuclear anions. For such experiments where the cytoplasm is labeled, it was impossible to determine the labeling condi-
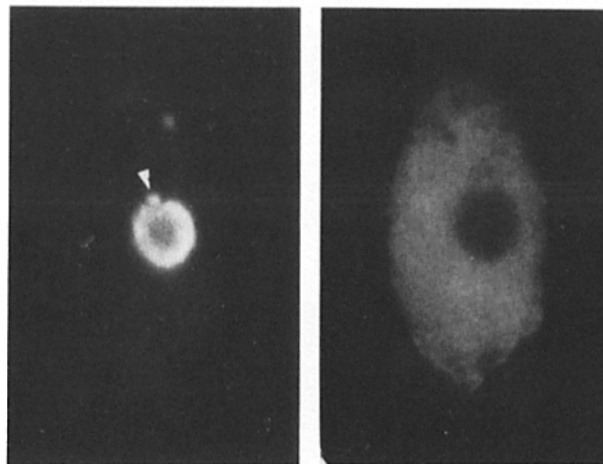
SV40 LARGE T ANTIGEN - GROWING CELLS

WILD TYPE PEPTIDE/BSA
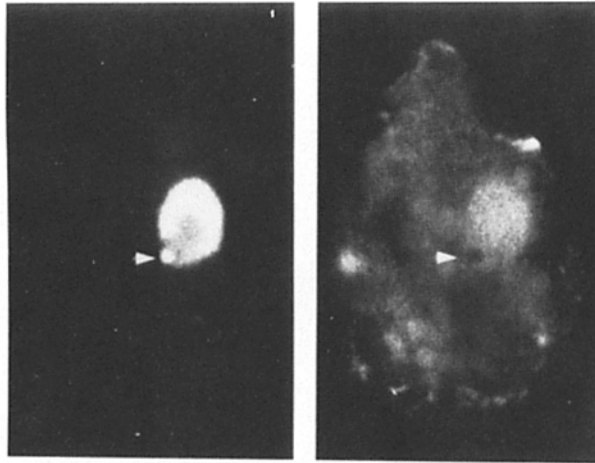
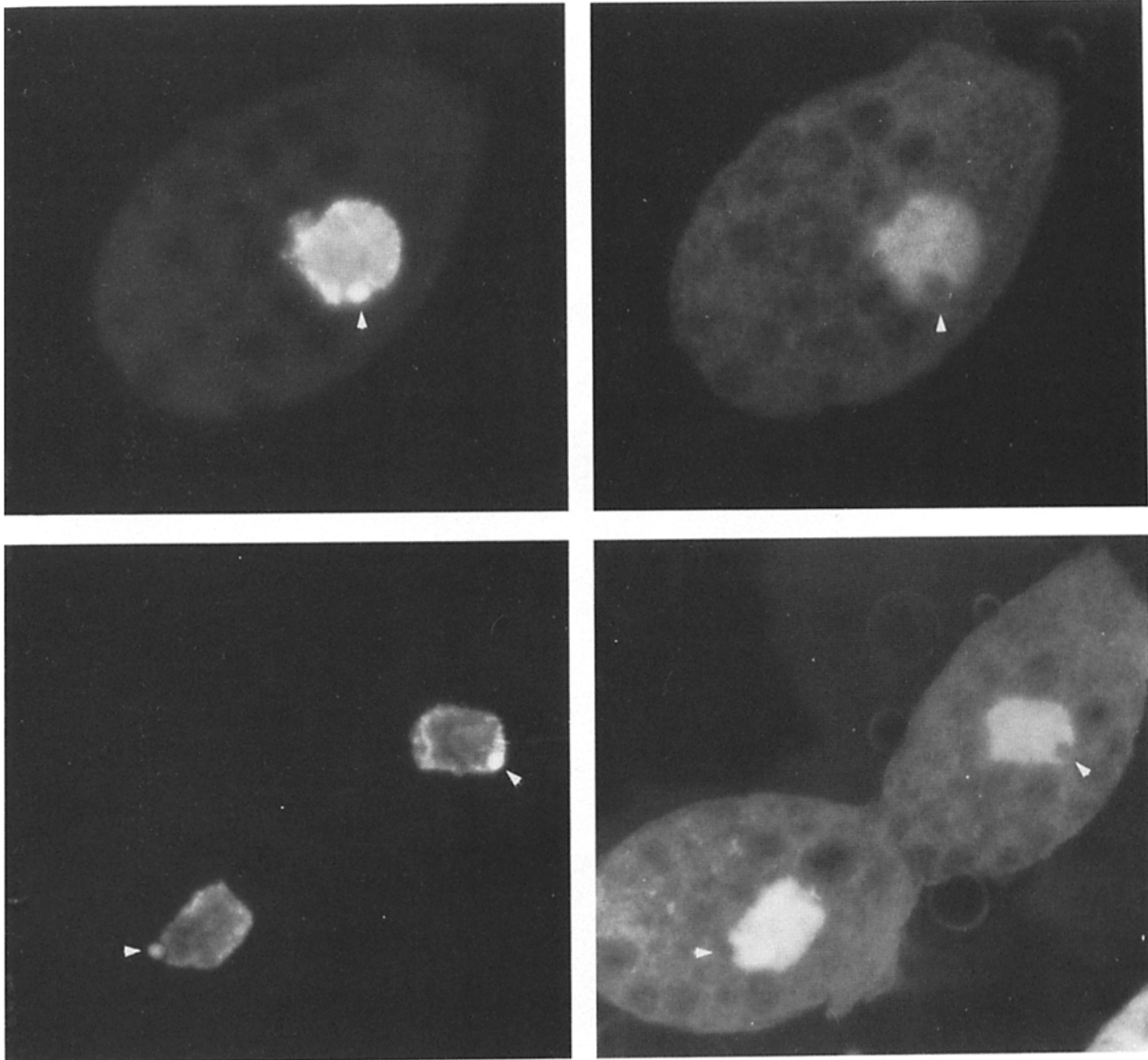**MUTANT PEPTIDE/BSA**

BSA
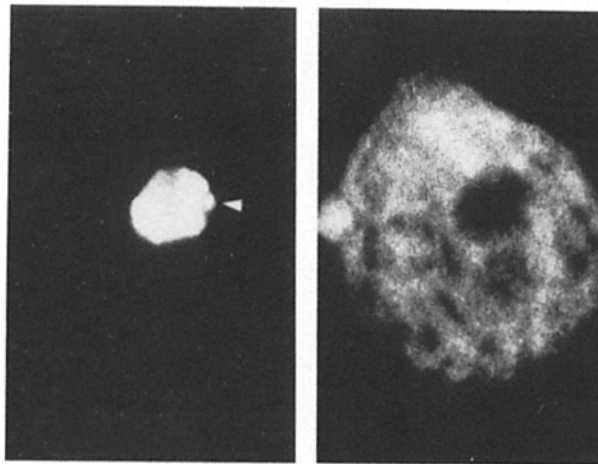
DAPI   **FLUORESCEIN**

Figure 5. SV40 large T antigen peptide/BSA conjugates accumulate specifically in macronuclei of nondividing, growing cells. Fluorescein-labeled wild-type or mutant peptides conjugated to BSA, or BSA alone, were microinjected into the cytoplasm of growing cells. DAPI stain shows the location of the macro- and micronuclei in the injected cells. Micronuclei are indicated by arrows. Note the clear absence of micronuclear stain for the fluorescein-labeled wild-type peptide/BSA.

tion of the small micronucleus. We also showed that free FITC and free fluorescein distributed evenly throughout the cell, with no nuclear accumulation, eliminating the possibility that excess bound FITC or fluorescein released from degraded microinjected proteins had simply labeled nuclear proteins after microinjection. These results suggest either that the conformation or binding properties of polylysine

differ from those of H1 or macronuclear-specific localization of H1 requires information specified in the H1 sequence itself.

One possibility is that the micronucleus may, at times, simply be deficient in nuclear-cytoplasmic trafficking. *Tetrahymena* micronuclei, as well as macronuclei, have nuclear pores as shown by thin-section electron microscopy (Gorov-

PLYS-BSA

DAPI   **RHODAMINE**

Figure 6. SV40 large T antigen peptide/BSA conjugates accumulate in macronuclei of dividing cells. Rhodamine-labeled P_{LYS}-BSA conjugates were microinjected into the cytoplasm of nondividing (*top figures*) or dividing (*bottom figures*) cells. DAPI stain shows the location of the macro- and micronuclei in the injected cells. Micronuclei are pointed out by arrows.

sky, 1970; Wolfe et al., 1976). Although a rigorous quantitative analysis has not been done, perusal of a small number of published and unpublished (Gorovsky, M., unpublished observations) micrographs does not reveal any striking differences in pore density between the envelopes of the two nuclei. In a related ciliate, *Paramecium aurelia*, Stephenson and Lloyd (1971a,b) have shown macro- and micronuclear membranes have similar pore densities. Also it should be noted that the surface to volume ratio of the micronucleus is approximately five times greater than that of the macronu-

cleus, so that it would be even easier to detect proteins in micronuclei if micronuclear pores had similar spacing and similar transport properties as macronuclear pores.

To show that under our experimental conditions a protein could be transported into micronuclei, we injected histone H4, a protein found in both macro- and micronuclei. In nondividing growing cells this protein accumulated only in the macronucleus, whereas in dividing and recently divided cells it was localized specifically to the micronucleus. Thus, *Tetrahymena thermophila* exhibits temporally restricted nu-

clear specific localization of histone H4. The simplest interpretation of these observations is that the nuclear accumulation of H4 is coupled to DNA replication, i.e., H4 localization is that expected based on the replication expression hypothesis of protein targeting into nuclei. Consistent with this hypothesis is the observation that H4 mRNA accumulates twice in the *Tetrahymena* cell cycle, once during macronuclear S and once during micronuclear S (Yu et al., 1987). Perhaps more importantly, that H4 can enter micronuclei of dividing and recently divided cells while H1 cannot argues that micronuclei can somehow accumulate one histone and exclude another at the same time in the cell cycle.

Conservation of a general nuclear transport mechanism between mammals and protozoa was shown by the macronuclear accumulation of P_{LYS}-BSA. As in mammalian cells, peptide-mediated macronuclear import in *Tetrahymena* was sequence specific: mutant P_{THR}-BSA remains in the cytoplasm. Because they show a similar macronuclear specificity and do not depend on DNA replication for their accumulation, it seems likely that macronuclear H1, calf thymus H1, and P_{LYS}-BSA use the same nuclear transport system. Curiously, both heterologous proteins were excluded from the micronucleus. Although signal-mediated nuclear transport appears to be highly conserved, a mechanism thus exists that allows selective import into either of two pore-containing nuclei lying in the same cytoplasm and it is the micronucleus that appears to have an unusual, specialized mechanism for protein import.

Previous studies have suggested the possibility of physiological or developmental variability in the permeability properties of nuclear envelopes. However, these have either described small quantitative changes in the uptake of nonphysiological molecules (Feldherr, 1968; Jiang and Schindler, 1988) or differences in the uptake of nuclear proteins in cells of different species (Richter et al., 1985) or in different stages of development (Dreyer et al., 1981, 1982, 1983; Dequin et al., 1984) that could be explained by changes affecting the transported proteins (cell- or stage-specific secondary modifications or associations with proteins containing a nuclear localization signal) and not the nuclear transport system. The striking nucleus-specific accumulation of *Tetrahymena* H1, calf thymus H1, and the SV40 large T antigen nuclear localization signal represents the strongest suggestion to date of functional differentiation of the nuclear envelope transport apparatus because these differences cannot easily be explained except by postulating intrinsic differences in the protein transport systems of the nuclei themselves. Although the differences obtained here are spatial (i.e., distinguish two different nuclei lying in the same cytoplasm), temporal differentiation of a kind that could occur in a variety of cell types occurs during the sexual phase of the life cycle (conjugation), when the germinal micronucleus gives rise to both new macro- and micronuclei. Differentiation of nuclear pore complex–mediated transport processes thus may be an important mechanism in developmental regulation of nucleocytoplasmic exchange.

The mechanism(s) underlying the remarkable selectivity of transport into *Tetrahymena* macro- and micronuclei is unknown. It is worth noting that every protein that enters the micronucleus cannot simply have a micronuclear-specific targeting sequence because the H4-II gene, which is the only H4 gene expressed during micronuclear S (Yu et al., 1987),

encodes the same protein sequence as that which enters the macronucleus during macronuclear S (Horowitz et al., 1987). Another possibility is selective binding of micronuclear-destined proteins to one or more cytoplasmic factors that can themselves interact with a micronucleus-specific transport system. Experiments are now underway to test this hypothesis.

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