Errata

Due to a printer error the following paper, which appeared in Volume 6, Issue 11, 1515–1534, was printed without the necessary color. It is being reprinted here in its entirety.

Nucleolar Accumulation of Poly (A)$^+$ RNA in Heat-shocked Yeast Cells: Implication of Nucleolar Involvement in mRNA Transport

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Transport of mRNA from the nucleus to the cytoplasm plays an important role in gene expression in eukaryotic cells. In wild-type *Schizosaccharomyces pombe* cells poly(A)$^+$ RNA is uniformly distributed throughout the nucleoplasm and cytoplasm. However, we found that a severe heat shock blocks mRNA transport in *S. pombe*, resulting in the accumulation of bulk poly(A)$^+$ RNA, as well as a specific intron-less transcript, in the nucleoli. Pretreatment of cells with a mild heat shock, which induces heat shock proteins, before a severe heat shock protects the mRNA transport machinery and allows mRNA transport to proceed unimpeded. In heat-shocked *S. pombe* cells, the nucleolar region condensed into a few compact structures. Interestingly, poly(A)$^+$ RNA accumulated predominantly in the condensed nucleolar regions of the heat-shocked cells. These data suggest that the yeast nucleolus may play a role in mRNA transport in addition to its roles in rRNA synthesis and preribosome assembly.

INTRODUCTION

In eukaryotic cells, domains for transcription and translation are spatially separated by the nuclear membrane. Transport of mRNA from the transcription sites (nucleus) to the translation sites (cytoplasm) is performed in a highly selective manner (Chang and Sharp, 1989, 1990; Legrain and Rosbash, 1989; He *et al.*, 1993). With few exceptions, only mature mRNAs are transported from the nucleus to the cytoplasm (for reviews see Maquat, 1991; Nigg *et al.*, 1991; Izaurralde and Mattaj, 1992). Therefore, post-transcriptional maturation of mRNA, which involves 5′ capping, 3′ polyadenylation, splicing, and internal methylation, is thought to be essential for mRNA transport (for review see Schröder *et al.*, 1987). In fact, the 7-methylguanosine (m^{7}G) cap structure of RNA polymerase II transcripts has been shown to constitute a part of the nuclear export signal (Hamm and Mattaj, 1990) and the 2,2,7-trimethylguanosine (m^{3}G) cap structure of small nuclear RNAs (snRNAs) has been shown to represent the nuclear import signal (Fischer and Lührmann, 1990; Marshallsay and Lührmann, 1994). In addition, mature 3′ end formation of histone mRNA stimulates RNA export from the nucleus (Eckner *et al.*, 1991). However, some RNA species such as the XIST RNA in human cells (Brockdoff *et al.*, 1992; Brown *et al.*, 1992), and the omega-n transcript in *Drosophila* (Hogan *et al.*, 1994) are retained in the nucleus. It is not clear if nuclear retention of these RNAs is receptor mediated, due to the association with other nuclear components, or the result of specific signals.

The process of nucleocytoplasmic transport of mRNA can be dissected into the following stages: release of mRNA from the transcription and processing sites, movement of the mRNA to the nuclear pore complex, docking of the mRNA at the nuclear pore complex, translocation of the mRNA through the nuclear pore complex, and potential binding of the translocated mRNA to cytoskeletal elements and movement to the proper location for translation. Therefore, transport of mRNA may involve sequential attach-

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ment and detachment processes at specific sites of the nuclear matrix, the nuclear envelope, and the cytoskeleton.

Evidence for the role of nuclear anchoring and release in pre-mRNA processing events comes from the study of yeast splicing mutants (Legrain and Rosbash, 1989). Mutations that disrupt or prevent the formation of presplicing complexes are found to lead to the aberrant export of precursor mRNAs, suggesting that once an RNA is released from its processing site (or transcription site), it enters a transport pathway and movement occurs irrespective of previous modifications to the RNA. The human immunodeficiency virus Rev protein is also thought to act to control cytoplasmic ratios of spliced and unspliced viral mRNAs by mediating the dissociation of intron-containing mRNAs from presplicing complexes (Chang and Sharp, 1989). Furthermore, Denome *et al.* (1989) have reported that nuclear mRNA exists in both salt-insoluble and salt-extractable fractions. Pre-mRNA is thought to be transcribed and processed in association with the salt-insoluble nuclear pool. Mature mRNA was found to move to the salt-extractable pool, which was proposed to be the direct precursor to transported mRNA.

Although it is not yet clear how mRNAs move from their sites of transcription to the nuclear envelope, two models have been presented. Several lines of evidence have suggested that some mRNAs may move along a directed "track" from their transcription site to the nuclear membrane (Lawrence *et al.*, 1989; Huang and Spector, 1991; Xing and Lawrence, 1991; Xing *et al.*, 1993). Although little is known about the substructural composition of the "tracks," it is possible that RNA movement occurs in association with components of the nucleoskeleton (for reviews see Verheijen *et al.*, 1988; Berezney, 1991). In fact, splicing components (Zeitlin *et al.*, 1987), functional spliceosomes (Zeitlin *et al.*, 1989), a putative splicing factor (Smith *et al.*, 1989), pre-mRNA (Ciejek *et al.*, 1982; Mariman *et al.*, 1982), and small nuclear ribonucleoprotein particles (snRNPs) (Vogelstein and Hunt, 1982; Spector *et al.*, 1983; Smith *et al.*, 1986) have all been shown to be associated with the nuclear matrix. On the other hand, Zachar *et al.* (1993) have examined the transport of mRNA in *Drosophila* polytene nuclei and have suggested that mRNAs move from their sites of transcription to the nuclear surface at rates that are consistent with diffusion. Based on this data they have proposed a channeled diffusion model for mRNA transport.

After movement to the nuclear membrane, mRNAs are translocated through the nuclear pore complexes. Several lines of evidence have implicated the nuclear pore complexes in RNA transport. Perhaps the most convincing report showed translocation of the Balbiani ring ribonucleoprotein particle through the nuclear pores (Mehlin *et al.*, 1992). RNA-coated colloidal gold particles injected into *Xenopus* oocyte nuclei were also shown to exit the nucleus through the nuclear pore complexes (Dworetzky and Feldherr, 1988) and all of the nuclear pores were shown to be capable of exporting RNA (Feldherr and Akin, 1990). Furthermore, Dworetzky and Feldherr (1988) showed that an individual nuclear pore is capable of both protein import and RNA export. More recently, electron microscopic in situ hybridization with an oligo dT probe has directly revealed endogenous poly $(A)^+$ RNA in the process of being transported through the nuclear pores (Huang *et al.*, 1994).

Upon translocation through the nuclear pore complexes, mRNAs are distributed to the appropriate sites for translation in the cytoplasm. In some cases, a specific distribution of mRNAs in the cytoplasm is thought to constitute an important means of localization of cytosolic proteins and a role for the 3′-untranslated regions of mRNA have been implicated in cytoplasmic sorting of mRNAs (for reviews see Singer, 1992; Ding and Lipshitz, 1993; Wilhelm and Vale, 1993). By injecting fluorescently labeled mRNA into oligodendrocytes, Ainger *et al.* (1993) showed that myelin basic protein mRNA, which initially appears dispersed in the perikaryon, is transported unidirectionally in the form of granules to the cell periphery, where the RNA distribution once again becomes dispersed.

Recently, temperature-sensitive mutants deficient in mRNA transport were obtained in *Saccharomyces cerevisiae* (Amberg *et al.*, 1992; Kadowaki *et al.*, 1992, 1994a,b; Schneiter *et al.*, 1995). Of these, *RAT1* encodes an essential 116-kDa protein with regions of homology to the protein encoded by *SEP1*, which is a nucleic acid binding protein, a 5′ → 3′ exonuclease, and catalyzes DNA strand transfer reactions in vitro (Amberg *et al.*, 1992). *RAT3/NUP133* encodes a 133-kDa nuclear pore protein and mutation of this gene results in constitutive clustering of nuclear pore complexes (Li *et al.*, 1995). Another mutant *mtr1* was found to be identical with the splicing mutant *prp20* and is an *S. cerevisiae* homologue of *RCC1* in mammalian cells and *pim1* in *S. pombe* (Kadowaki *et al.*, 1992, 1993). These mutants accumulate poly $(A)^+$ RNA in the nuclei at the nonpermissive temperature. The RCC1 protein is known to function as a guanine nucleotide exchange factor for the Ras-like guanosine triphosphatase (GTPase) Ran/TC4 in mammalian cells (Bischoff and Ponstingl, 1991). Furthermore, the product of the *RNA1* gene is required for RNA export (Shiokawa and Pogo, 1974) in addition to pre-tRNA processing (Hopper *et al.*, 1978) and polyadenylation of mRNA (Piper and Aamand, 1989). The RNA1 protein was recently shown to be a homologue of human Ran/TC4 GTPase-activating protein RanGAP1 (Bischoff *et al.*, 1995).

Several groups have examined the effects of heat shock on mRNA transport and have come up with differing results. Yost and Lindquist (1988) reported

that although heat shock resulted in a block in pre-mRNA splicing, RNAs were transported to the cytoplasm with similar efficiencies in *Drosophila* tissue culture cells at both control and heat shock temperatures. Several earlier studies in *Drosophila* (McKenzie and Meselson, 1977; Spradling *et al.*, 1977) have shown that stress reduces the accumulation of newly synthesized non-heat shock mRNA in the cytoplasm. However, Sadis *et al.* (1988) showed that newly synthesized cytoplasmic poly(A)$^+$ RNA was significantly reduced and the appearance of newly synthesized 18S and 28S rRNA was virtually blocked in HeLa cells after heat shock. Finally, Denome *et al.* (1989) found actin mRNA transport to be diminished threefold to more that 10-fold in heat-shocked HeLa cells.

In this study, we report that severe heat shock results in a block in the mRNA transport pathway in the fission yeast *S. pombe*. Both intron-containing and intron-less RNAs are not transported to the cytoplasm. Interestingly, poly(A)$^+$ RNA accumulated in the nucleolar region of the nucleus. Electron microscopic in situ hybridization confirmed that the poly(A)$^+$ RNA was localized to the nucleolar region and further demonstrated that these RNAs sublocalized within condensed nucleolar regions in the heat-shocked cells. Based on these results, we propose that the yeast nucleolus plays a role in mRNA transport in addition to its roles in RNA polymerase I–related activities.

MATERIALS AND METHODS

Immunoblot

Wild-type *S. pombe* cells (HM123) were grown to mid-log phase in YE medium (0.5% yeast extract and 3% glucose) at 30°C and were heat shocked at 42°C for 60 min or maintained at 30°C. Cells (5×10^6) were collected by centrifugation, spheroplasted, and suspended in 30 μl of sample buffer [62.5 mM Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, and 5% 2-β-mercaptoethanol]. The cell suspensions were boiled at 95°C for 4 min to make cell lysates. Ten microliters of cell lysate from the control cells or the heat-shocked cells was applied per lane of a 7.5% SDS polyacrylamide gel. After electrophoresis, proteins were electro-transferred to Immobilon (Millipore, Bedford, MA), and probed with antiserum 2–3 raised against a peptide corresponding to residues 209–224 of hsp104 (Parsell *et al.*, 1991).

Preparation of Probes

Oligonucleotides containing 50 residues of deoxythymidine [oligo $(dT)_{50}$] were labeled at their 3′ ends with digoxigenin-11-dUTP, biotin-16-dUTP, or rhodamine-6-dUTP using an oligonucleotide tailing kit (Boehringer Mannheim, Indianapolis, IN) according to the manufacturer's instructions. The labeling reaction was performed without dATP to generate a short tail that consisted of labeled dUTP only.

To prepare a probe for the alcohol dehydrogenase (ADH) mRNA, we amplified a region of the ADH gene by the polymerase chain reaction using oligonucleotide primers corresponding to nucleotides 91–110 (ADH1) and 878–897 (ADH2) of the ADH gene. The amplified 806-bp fragment containing most of the protein coding region of the ADH gene was subcloned into the pCR vector (Invitrogen, San Diego, CA). The plasmid was then nick translated in the presence of 50 mM digoxigenin-11-dUTP, dATP, dCTP, and dGTP. The labeled probe was then purified through a G-50 Sephadex spin column (Boehringer Mannheim) and stored at −20°C.

The nucleotide sequences of the oligonucleotides ADH1 and 2 are as follows.

ADH1: 5′-GGTCAAG ACGAGGTCTTGGT-3′
ADH2: 5′-GTTACCGACGTGAGAACCGC-3′

In Situ Hybridization Using the Oligo $(dT)_{50}$ Probe

S. pombe haploid strain HM123 (*h*$^-$, *leu1*) was grown to mid-log phase at 30°C in YE medium and either maintained at 30°C or exposed to heat shock at 42°C for the indicated times. Cells were then fixed for 1 h in freshly made 4% formaldehyde in 0.1 M Sörensen's phosphate buffer (pH 6.0). After washing three times in 0.1 M Sörensen's phosphate buffer containing 0.3 M glycine, the cells were resuspended in PEMS buffer [100 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), pH 6.9, 0.1 mM $MgCl_2$, 1 mM EGTA, 1.2 M sorbitol] containing 1 mg/ml of Novozym 234 (Novo Nordisk) and Zymolyase 100T (Seikagaku Corporation), and incubated at 37°C for 5–20 min. Resultant spheroplasts were washed three times in ice-cold PEMS and adhered to the wells of Teflon-faced slides (Polysciences, Warrington, PA) that had been precoated with poly-[L]-lysine (m.w. = 150,000–300,000; Sigma, St. Louis, MO). The slides were then plunged into 70, 90, and 100% ethanol for 5 min successively. Subsequently, cells were incubated in prehybridization buffer containing 4× SSC, 5× Denhardt's solution, and 1 mg/ml of tRNA for 30 min at room temperature. Hybridization was done in the above prehybridization buffer containing 1 ng/μl of the digoxigenin-labeled oligo$(dT)_{50}$ for 16–20 h at 42°C in a humidified chamber. After hybridization, cells were washed three times in 4× SSC (20 min per wash), once in 2× SSC for 20 min, and once in 1× SSC for 10 min. All washing steps were done at room temperature. After washing, the cells were equilibrated in phosphate-buffered saline (PBS)-BAG (PBS containing 1% bovine serum albumin, 0.1% sodium azide, and 0.5% cold water fish skin gelatin; Sigma) for 30 min, and were incubated with mouse monoclonal anti-digoxin antibody (Sigma) in PBS-BAG for 60 min at room temperature. After washing with PBS, the cells were incubated with goat anti-mouse IgG antibody conjugated with fluorescein for 60 min at room temperature. The nuclei were counterstained with 1 μg/ml of 4′,6-diamido-2-phenylindole (DAPI). The slides were mounted in medium composed of 90% glycerol, 10% PBS, and 0.1% *p*-phenylenediamine, and were examined with a Nikon FXA epifluorescence microscope.

For triple staining, fixed and permeabilized cells were hybridized with the biotin-labeled or rhodamine-labeled oligo $(dT)_{50}$ probe under the same conditions as described above. For the biotin-labeled probe, the cells were washed three times in 4× SSC at 37°C for 20 min, and once in 2× SSC at room temperature for 20 min. After washing, the cells were incubated with Texas red-avidin (Leinco Technologies) for 30 min at room temperature and washed with 4× SSC, 4× SSC/0.1% Triton X-100, and 4× SSC as described (Kadowaki *et al.*, 1992). The cells were then incubated with D77 primary antibody at a dilution of 1:20 (Aris and Blobel, 1988) in PBS-BAG for 60 min at room temperature in a humidified chamber. After antibody incubation, cells were washed three times in PBS and incubated with an affinity-purified fluorescein-conjugated goat anti-mouse IgG secondary antibody (Organon Teknika, Durham, NC) at a dilution of 1:50 in PBS-BAG for 60 min at room temperature in a humidified chamber. The cells were washed four times in PBS, counterstained with DAPI. For the rhodamine-labeled probe, the cells were washed as described above and treated with anti-m^3G antibody at a dilution of 1:20 (Krainer, 1988) followed by the treatment with an affinity-purified fluorescein-conjugated goat anti-mouse IgG secondary antibody (Organon-Teknika) at a dilution of 1:50 in PBS-BAG. The triply stained specimens were observed on a computer-controlled microscope system based on an Olympus inverted microscope IMT-2 (Hiraoka *et al.*, 1991; Chikashige *et al.*, 1994) using an Olympus oil immersion objective lens (DPlanApo

100/NA = 1.3). High-selectivity excitation and barrier filter combinations (Omega Optical, Brattleboro, VT; Chroma Technology, Brattleboro, VT) for DAPI, fluorescein, and Texas Red mounted on revolving wheels were used with a single dichroic mirror with triple-band pass properties designed for DAPI, fluorescein, and Texas Red (Chroma Technology). Microscopic images were obtained on a Peltier-cooled charge-coupled device (CCD; Photometrics, Tucson, AZ) camera with a 1340 × 1037 pixel CCD chip (KAF1400). Microscope shutter, focus movement, CCD data collection, and filter combinations are controlled by a Silicon Graphics workstation Personal Iris 4D35/TG. Triple-color images were superimposed on a Silicon Graphics workstation Crimson using an image processing and display software package Priism (Hiraoka *et al.*, 1991).

In Situ Hybridization to ADH mRNA

Fixation and preparation of spheroplasts were done as described above. After washing three times in ice-cold PEMS, fixed spheroplasts were resuspended in PEMS containing 1% Triton X-100 and kept at room temperature for 30 s. Permeabilized spheroplasts were then collected by centrifugation and washed three times in PEMS. Hybridization was done in a solution containing 4× SSC, 1% bovine serum albumin, 1 mg/ml tRNA, 10% dextran sulfate, 50% deionized formamide, and 6 ng/μl of heat-denatured digoxigenin-labeled ADH probe at 42°C for 16–20 h. The spheroplasts were washed three times in a solution containing 4× SSC and 50% formamide at 42°C (20 min per wash), twice in 2× SSC, and once in 1× SSC for 20 min each at room temperature. The hybridized digoxigenin-labeled ADH probe was detected as described above, except that the reactions with antibodies and subsequent washing were carried out in suspension. After final washing, the cells were adhered to the wells of Teflon-faced slides that had been precoated with poly-L-lysine. The nuclei were counterstained with DAPI.

Electron Microscopic In Situ Hybridization

S. pombe HM123 cells were grown to mid-log phase at 30°C in YE medium. The cells were collected by centrifugation and washed once in 0.1 M Sörensen's phosphate buffer (pH 6.0). Fixation of the cells was carried out in 4% formaldehyde and 0.01% glutaraldehyde in 0.1 M Sörensen's phosphate buffer at pH 6.0 for 60 min. Formaldehyde solution was made fresh from paraformaldehyde before use. After washing three times with 0.1 M Sörensen's phosphate buffer containing 0.3 M glycine, the cells were suspended in PEMS containing 0.5 mg/ml of Novozym 234 and Zymolyase 100T, and were incubated at 37°C for 5–10 min to prepare spheroplasts. The spheroplasts were washed three times in ice-cold PEMS and were dehydrated in 50% (45 min at 4°C), 70%, 90%, and 100% ethanol (60 min per wash at −20°C). The dehydrated spheroplasts were then embedded in Lowicryl K4 M according to the manufacturer's instructions (Chemische Werke Lowi GmbH). Polymerization was done under 360 nm long-wavelength UV light (Ted Pella) for 2 days at −20°C and for 3 days at room temperature. Thin sections (80 nm) were cut using a Diatome diamond knife on a Reichert-Jung Ultracut-E ultramicrotome and were picked up on 300 mesh gold grids.

The gold grids bearing Lowicryl sections were floated face down in 2× SSC and incubated at 70°C for 30 min. The grids were then transferred onto small drops (30 μl) of hybridization solution containing 2× SSC, 1.4% bovine serum albumin (Boehringer Mannheim), 10 mM vanadyl-ribonucleoside complex, 1% dextran sulfate, and 15 ng/μl of digoxigenin-labeled oligo $(dT)_{50}$ probe, and were incubated at 37°C for about 20 h in a humid chamber. The grids were then washed twice in 2× SSC and 1× SSC for 10 min each. After rinsing with dH_2O for 10 min, the grids were incubated with mouse anti-digoxin monoclonal antibody (Sigma) in PBS containing 1% normal goat serum at 4°C overnight. Subsequently, the grids were washed with Tris-buffered saline (TBS; 20 mM Tris [pH 7.6], 150 mM NaCl, 20 mM sodium azide) containing 1% Tween 20 and were incubated with anti-mouse IgG antibody conjugated with 15 nm colloidal gold particles (Amersham) in TBS containing 1% Tween 20 for 60 min at room temperature. The grids were then washed with the same buffer for 10 min, rinsed with dH_2O for 10 min, and air dried. The sections were counterstained with uranyl acetate for 10 min and Reynolds lead citrate (Reynolds, 1963) for 2 min. Sections were examined at 75 kV in a Hitachi H-7000 transmission electron microscope.

RESULTS

Severe Heat Shock Results in the Accumulation of Bulk Poly(A)$^+$ RNA in S. pombe Nuclei

To examine the effects of heat shock on mRNA transport in *S. pombe*, cells standardly grown at 30°C were incubated at various increased temperatures for 1 h. After fixation and permeabilization, the cells were subjected to in situ hybridization using a digoxigenin-labeled oligo $(dT)_{50}$ probe, which hybridizes to the poly(A) tail of pre-mRNA and mRNA. Poly(A)$^+$ RNA was uniformly distributed in the cytoplasm of cells maintained at 30°C (Figure 1a) and in cells shifted to 37°C (Figure 1c). A low level of labeling was observed uniformly throughout the nuclei (Figure 1a) suggesting that upon polyadenylation, mRNA is rapidly transported to the cytoplasm. In contrast, a severe heat shock at 42°C results in the accumulation of a significant fraction of poly(A)$^+$ RNA in the nuclei with little to no cytoplasmic poly(A)$^+$ RNA observed (Figure 1e). To be sure that we elicited the heat shock response extracts from *S. pombe*, cells grown at 30°C and at 42°C for 1 h were immunoblotted with an antiserum raised against the C-terminal 15 residues of hsp104 (Parsell *et al.*, 1991). This antibody recognizes a heat-inducible protein of 105K in *S. pombe* cells (Parsell *et al.*, 1991). As shown in Figure 2, induction of the 105K protein was observed in *S. pombe* cells heat shocked at 42°C for 1 h.

To demonstrate that the in situ hybridization assay specifically detected poly(A)$^+$ RNA, we performed several control experiments using heat-shocked cells. Addition of a 500-fold molar excess of an unlabeled oligo$(dT)_{50}$ oligonucleotide to the hybridization buffer abolished the nuclear signal completely in the heat-shocked cells (Figure 3A), whereas addition of the same amount of an unlabeled oligonucleotide (ADH2) complementary to a region of ADH mRNA did not affect the intensity of the poly(A)$^+$ signal (Figure 3B). The competence of the homologous but not the heterologous oligonucleotide to block the hybridization signal demonstrated the specificity of the hybridization. In addition, pretreatment of cells with RNase T2 (adenosine-specific ribonuclease) before hybridization abolished the nuclear signal, probably by digestion of the poly(A) sequences capable of hybridizing to the oligo $(dT)_{50}$ probe (Figure 3C). In contrast, pretreatment of the cells with DNase I did not diminish the signal intensity (Figure 3D). Furthermore, when cells

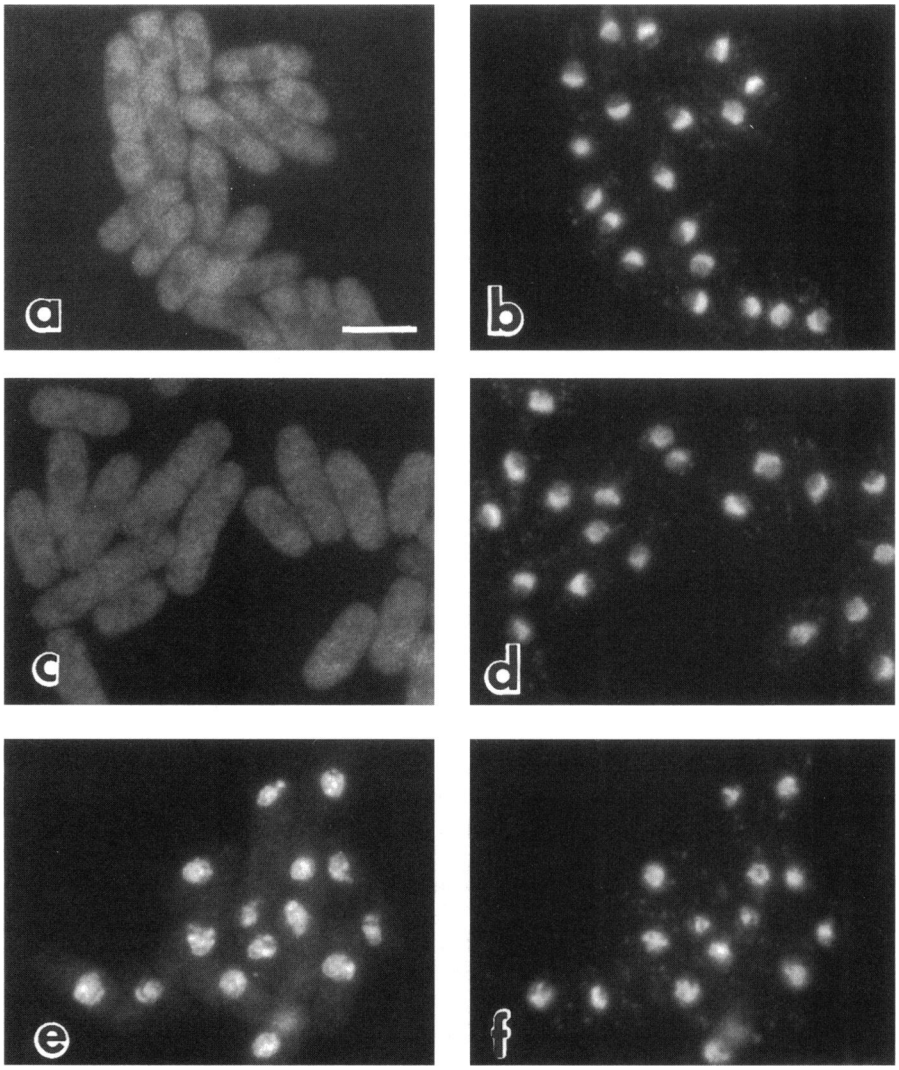

Figure 1. Severe heat shock results in accumulation of poly(A)$^+$ RNA in *S. pombe* nuclei. Cells were cultured at 30°C and either maintained at 30°C (a and b), or heat shocked at 37°C (c and d) or at 42°C (e and f) for 1 h, and then subjected to in situ hybridization with the digoxigenin-labeled oligo $(dT)_{50}$ probe. Hybridized probe was detected by treatment with the mouse anti-digoxin antibody followed by the fluorescein-conjugated goat anti-mouse IgG antibody. Left column, in situ hybridization; right column, DAPI staining. Bar, 10 μm.

were heat shocked in the presence of 200 μg/ml of 1,10-phenanthroline, which is a broad RNA polymerase inhibitor in yeast (Santiago *et al.*, 1986), the nuclear signal in heat-shocked cells was not observed (Figure 3E). In addition, the *rpb3* mutant, which has a thermolabile RNA polymerase II (Azuma *et al.*, 1995), showed very weak nuclear signals after heat shock at 42°C for 1 h (Figure 3F), demonstrating that the nuclear signal is dependent on transcription by RNA polymerase II. Taken together, these results demonstrate that in situ hybridization with the oligo $(dT)_{50}$ probe detected poly(A)$^+$ mRNA in the *S. pombe* cells.

We next examined the kinetics of poly(A)$^+$ RNA accumulation in the nuclei of heat-shocked cells. Nuclear accumulation was first observed at 5 min after heat shock (Figure 4B) and the intensity of the nuclear signal increased with increased incubation time at 42°C, whereas the intensity of the cytoplasmic signal decreased (Figure 4, C–F). These results suggest that transcription continued at a substantial level in the heat-shocked cells and newly transcribed poly(A)$^+$ RNAs accumulated in the nuclei over time. Based upon these results, we conclude that the steady state distribution of poly(A)$^+$ RNA in the heat-shocked cells (bright nucleus and dark cytoplasm) is not only a consequence of degradation of cytoplasmic poly(A)$^+$ RNA, but is due to the accumulation of poly(A)$^+$ RNA in the nuclei by the inhibition of mRNA transport.

An Inhibition of Protein Synthesis Does Not Block Transport of mRNA

The rate of protein synthesis is sharply reduced by heat shock (Lindquist, 1980). To test the possibility that the block in mRNA transport might simply be a consequence of a block in protein synthesis, cyclohex-

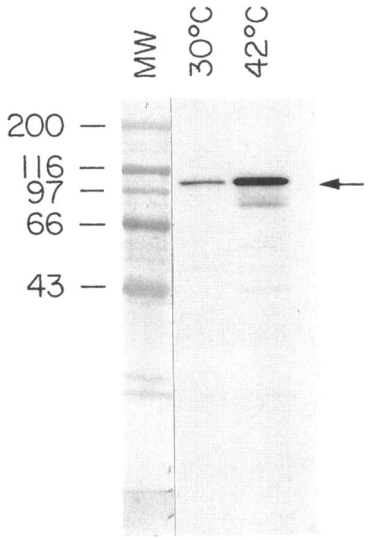

Figure 2. Heat-shocked *S. pombe* cells produce a 105K protein. Total cellular proteins from control (30°C) and heat-shocked (42°C, 1 h) cells were probed with antiserum 2–3, which recognizes a member of the hsp104 family of heat-shock proteins (Parsell *et al.*, 1991). The arrow indicates the position of the 105K protein.

imide (which is an inhibitor of protein synthesis) was added to *S. pombe* cells to prevent protein synthesis at the normal growth temperature (30°C). The inhibition of protein synthesis by cycloheximide did not result in the accumulation of poly(A)$^+$ RNA in the nuclei (Figure 5D), suggesting that the block in mRNA transport at high temperature is not a consequence of a block in normal protein synthesis. This result also suggests that no labile proteins are involved in the pathway of mRNA transport. The block in mRNA transport seems to be a primary effect of severe heat shock.

Mild Heat Pretreatment Protects mRNA Transport

In *Drosophila* cells and *S. cerevisiae*, mild heat pretreatment of cells protects pre-mRNA splicing from disruption by a subsequent severe heat shock (Yost and Lindquist, 1986, 1991). To test whether a mild heat pretreatment has a similar effect on the observed block of mRNA transport by severe heat shock in *S. pombe*, we incubated cells at 37°C for 45 min before exposing them to a subsequent severe heat shock at 42°C. After incubation, cells were examined for the localization of poly(A)$^+$ RNA by in situ hybridization with the digoxigenin-labeled oligo $(dT)_{50}$ probe. The cells pretreated with a mild heat shock showed a significant reduction in the nuclear hybridization signal (Figure 5A), suggesting that mild heat pretreatment protects the mRNA transport machinery from disruption by the severe heat shock.

To examine whether protein synthesis during the pretreatment is required for the protection of mRNA transport, we treated the cells with 10 μg/ml of cycloheximide and subjected them to in situ hybridization with the oligo $(dT)_{50}$ probe. Addition of cycloheximide to the culture before mild heat pretreatment blocks the protective effects of the pretreatment and results in accumulation of poly(A)$^+$ RNA in the nuclei (Figure 5B). In contrast, treatment with cycloheximide after the mild heat pretreatment did not affect the protective effects of the pretreatment (Figure 5C). These results demonstrate that synthesis of protein factors, probably the heat shock proteins, during the pretreatment is necessary for the protection of the mRNA transport machinery.

Poly(A)$^+$ RNA Accumulates Predominantly in the Condensed Nucleolar Region

Electron microscopic studies have revealed that there are two readily distinguishable regions of the fission yeast nucleus, an electron-dense region and a region that is less electron-dense (McCully and Robinow, 1971; Tanaka and Kanbe, 1986; Robinow and Hyams, 1989; Potashkin *et al.*, 1990). The electron-dense region that occupies about one-third to one-half of the nucleus has been referred to as the nucleolar region and the less electron-dense region as the chromatin-enriched region. Both the 37S and 28S rRNA precursors (Sillevis Smitt *et al.*, 1972, 1973) as well as the nucleolus-specific protein fibrillarin (Potashkin *et al.*, 1990), localize to the electron-dense region of the nucleus.

To examine the sublocalization of the poly(A)$^+$ RNA that accumulated in the nuclei of the heat-shocked cells, we first performed triple labeling with the oligo $(dT)_{50}$ probe, the D77 antibody that specifically labels nucleoli (Potashkin *et al.*, 1990), and DAPI, which labels DNA. The D77 antibody recognizes a nucleolar protein of 38 kDa in *S. cerevisiae* (Aris and Blobel, 1988). The p38 protein is a yeast homologue of fibrillarin, which is a protein found in the fibrillar region of the metazoan nucleolus (Ochs *et al.*, 1985) and is associated with several nucleolar specific snoRNPs including U3 snoRNP (Aris and Blobel, 1988; Schimmang *et al.*, 1989). The D77 antibody has been shown to specifically label nucleoli in *S. pombe* by immunofluorescence and immunoelectron microscopy (Potashkin *et al.*, 1990). This antibody recognizes a single band of about 37 kDa on immunoblot using *S. pombe* extracts (Tani and Spector, unpublished results). In normal *S. pombe* cells grown at 30°C, the biotin-labeled oligo (dT) probe uniformly stains the cytoplasm and the nuclei (Figure 6A). The D77 antibody labels about one-half of the nucleus (the nucleolar region) (Figure 6B) and DAPI stains the other half, the chroma-

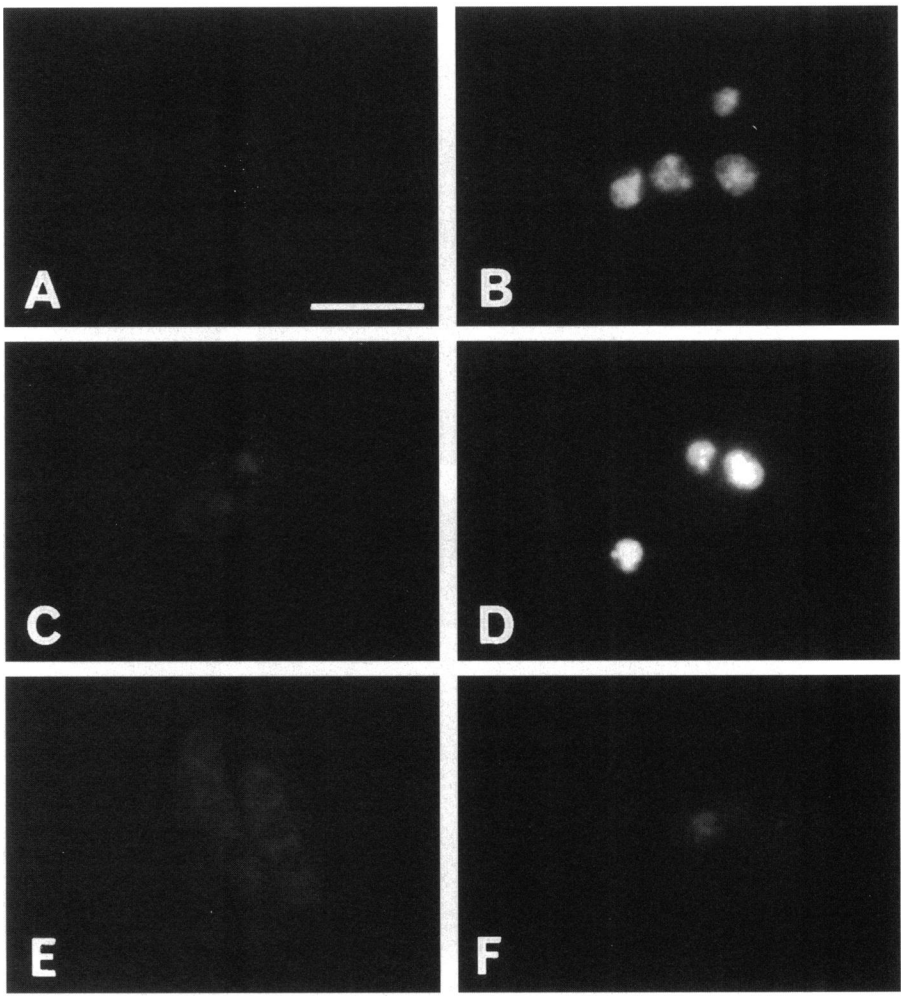

Figure 3. The oligo $(dT)_{50}$ probe specifically detects poly(A)$^+$ RNA. Cells were heat shocked at 42°C for 1 h and were then subjected to in situ hybridization. (A) Cells prehybridized with a 500-fold molar excess of unlabeled oligo $(dT)_{50}$ showed no hybridization signal. (B) Cells prehybridized with a 500-fold molar excess of unlabeled ADH2 probe, however, showed no reduction in the hybridization signal. (C) Cells treated with 600 u/ml of RNase T2 (Life Technologies, Gaithersburg, MD) at 37°C for 2 h before hybridization showed almost no hybridization signal. (D) Pretreatment with 1 mg/ml of DNase I (Life Technologies) at 37°C for 2 h, however, did not effect the hybridization signal. (E) Addition of 200 μg/ml of the transcription inhibitor 1,10-phenanthroline to the culture before shifting to 42°C resulted in no hybridization signal. (F) The *rpb3* mutant cells heat shocked at 42°C for 1 h gave very weak hybridization signals. Bar, 10 μm.

tin-enriched crescent shaped region of the same nucleus (Figure 6C). In the cells heat shocked for 1 h, the region where the D77 antibody stains was redistributed into a few compact structures (Figure 6F). In addition, the configuration of the chromatin region changed significantly from the crescent shape (Figure 6C) to an irregular shape in the heat-shocked cells (Figure 6G). We do not know whether the change in the morphology of the chromatin region is a reflection of disruption of the nucleoskeleton or is due to chromatin condensation. Interestingly, overlapping the staining pattern of the D77 antibody and that of the oligo $(dT)_{50}$ probe in the heat-shocked cells clearly showed that the accumulation site of poly(A)$^+$ RNA coincides with the region stained by the D77 antibody (Figure 6H, yellow color). However, the accumulated poly(A)$^+$ RNA occupies a region that is a little larger than that labeled by the D77 antibody.

In a previous study, Potashkin *et al.* (1990) reported that most of the abundant snRNAs are localized to the nucleolar region in yeast by using antibodies specific for the 2,2,7-trimethylguanosine cap (m^3G) structure of snRNAs and for a protein component of snRNPs. To demonstrate further that poly(A)$^+$ RNA is accumulated in the nucleolar region of the heat-shocked cells, we carried out triple staining of the heat-shocked cells with the rhodamine-labeled oligo $(dT)_{50}$ probe, the anti-m^3G antibody, and DAPI. After heat shock, the anti-m^3G antibody labeled several condensed structures (Figure 7B) that were also enriched in poly(A)$^+$ RNA (Figure 7D).

pim1, Which Has a Defect in mRNA Transport, also Accumulates Poly(A)$^+$ RNA in the Nucleolar Region

pim1$^+$ is an *S. pombe* homologue of *RCC1* in mammalian cells and *PRP20/MTR1/SRM1* in *S. cerevisiae* (Aebi *et al.*, 1990; Matsumoto and Beach, 1991). A temperature-sensitive mutation in the *pim1* gene

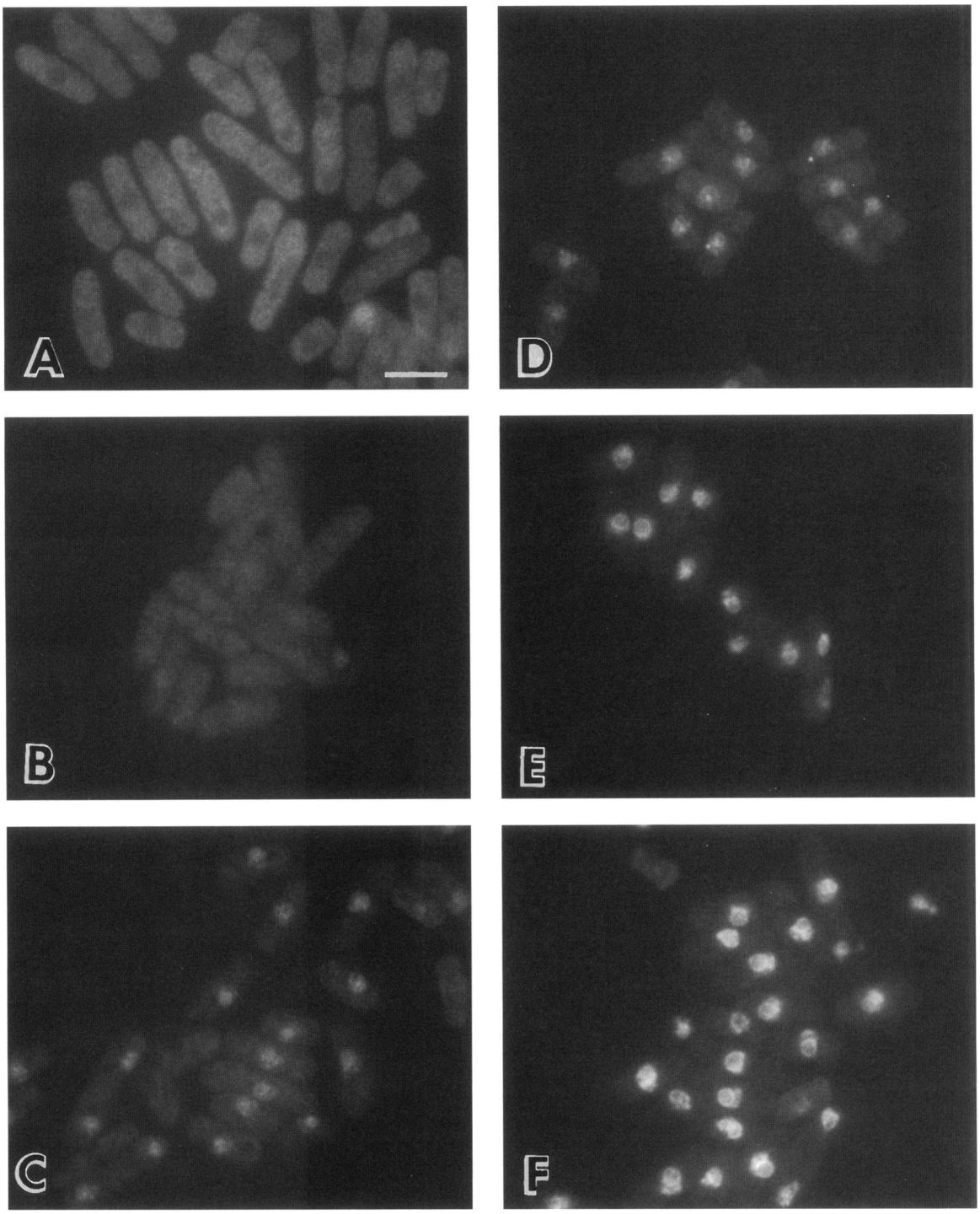

Figure 4. Poly(A)$^+$ RNA rapidly accumulates in the nuclei upon inhibition of mRNA transport. Cells were heat shocked at 42°C and an aliquot of cells was removed for in situ hybridization with an oligo(dT)$_{50}$ probe at 0 min (A), 5 min (B), 10 min (C), 20 min (D), 40 min (E), and 60 min (F). Photographs were taken with the same exposure time. Bar, 10 μm.

(*pim1–46*) was shown to result in accumulation of poly(A)$^+$ RNA in the nuclei in addition to chromosome condensation at the nonpermissive temperature (Kadowaki, 1993). The sublocalization of the poly(A)$^+$ RNA that accumulated in the nuclei of *pim1–46* at the nonpermissive temperature was analyzed by using

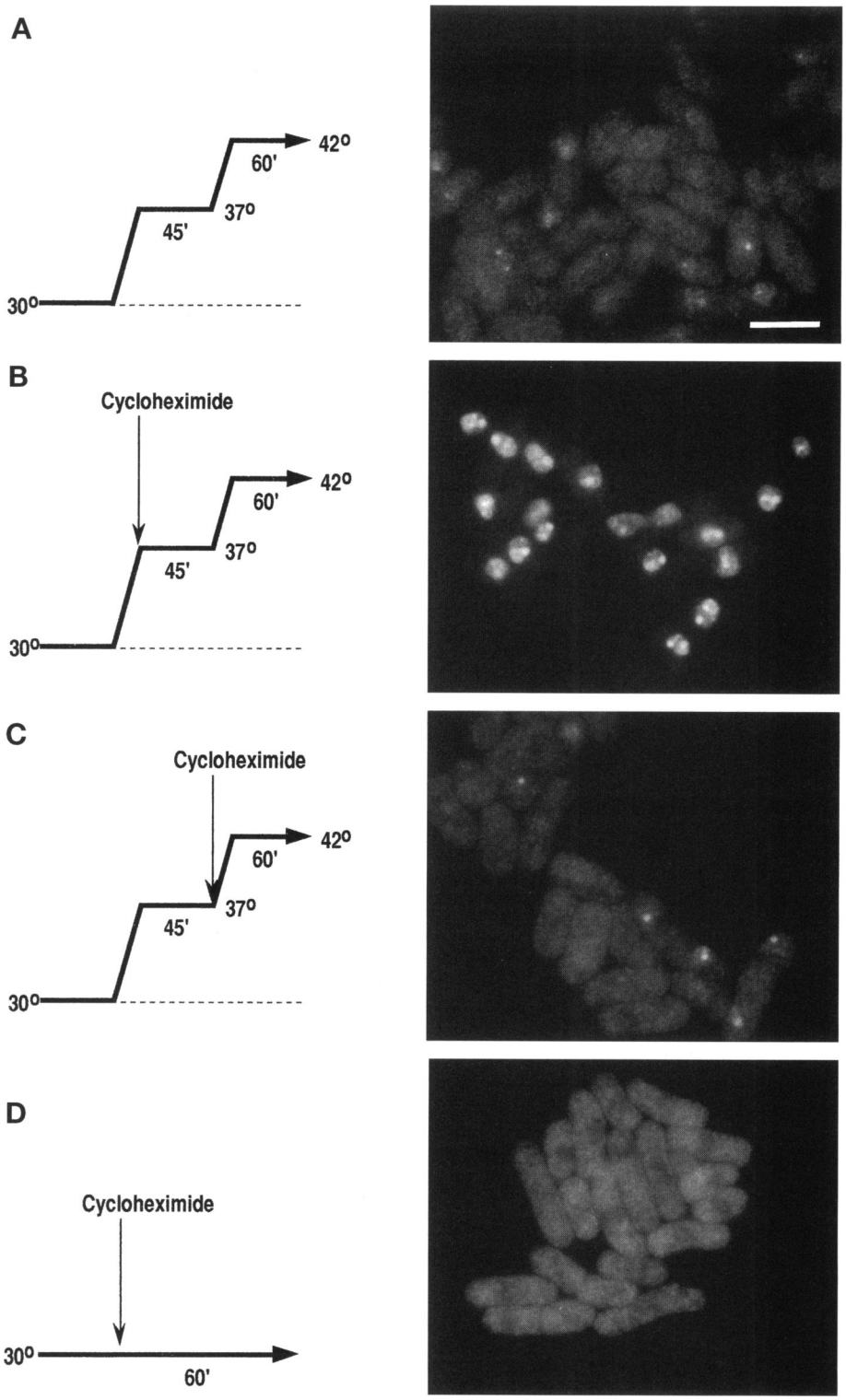

Figure 5. The mRNA transport machinery is protected by the previous synthesis of heat shock proteins. (A) When cells grown at 30°C were given a pretreatment at 37°C for 45 min and then shifted to 42°C for 1 h, mRNA transport was not altered. (B) Addition of cycloheximide blocks the protection of mRNA transport by the mild heat pretreatment. (C) Addition of cycloheximide after the mild heat pretreatment has no effect on protection of mRNA transport. (D) An inhibition of protein synthesis by cycloheximide does not block mRNA transport. In situ hybridization was performed using the digoxigenin-labeled oligo $(dT)_{50}$ probe. Bar, 10 μm.

triple labeling with the oligo $(dT)_{50}$ probe (Figure 8E), the D77 antibody (Figure 8F), and DAPI (Figure 8G). Overlapping these images showed that poly(A)$^+$ RNA accumulated preferentially in the nucleolar region of *pim1–46* after shifting to 37°C to block mRNA transport (Figure 8H, yellow region).

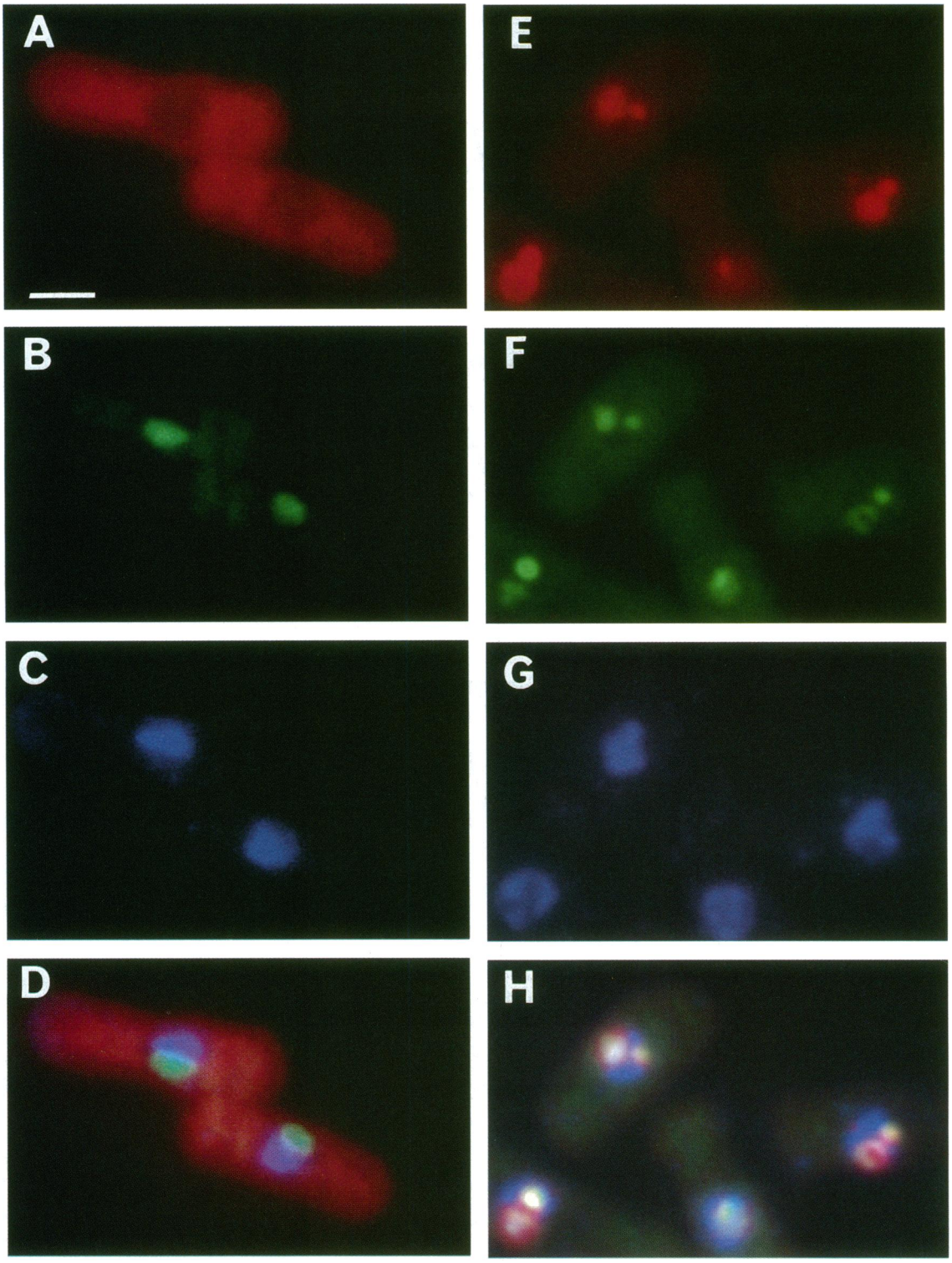

Figure 6. Poly(A)$^+$ RNA accumulates in the nucleolar region of heat-shocked *S. pombe* cells. Cells were grown at 30°C and either maintained at 30°C (left panels) or shifted to 42°C for 60 min (right panels), and then subjected to triple staining with the biotin-labeled oligo $(dT)_{50}$ probe (A and E), the D77 antibody (B and F), and DAPI (C and G). Images were taken using a Peltier-cooled CCD camera. In merged images (D and H), red denotes poly(A)$^+$ RNA, green denotes D77 antibody, blue denotes DAPI, and yellow denotes colocalization of poly(A)$^+$ RNA and D77 antibody. Bar, 5 μm.

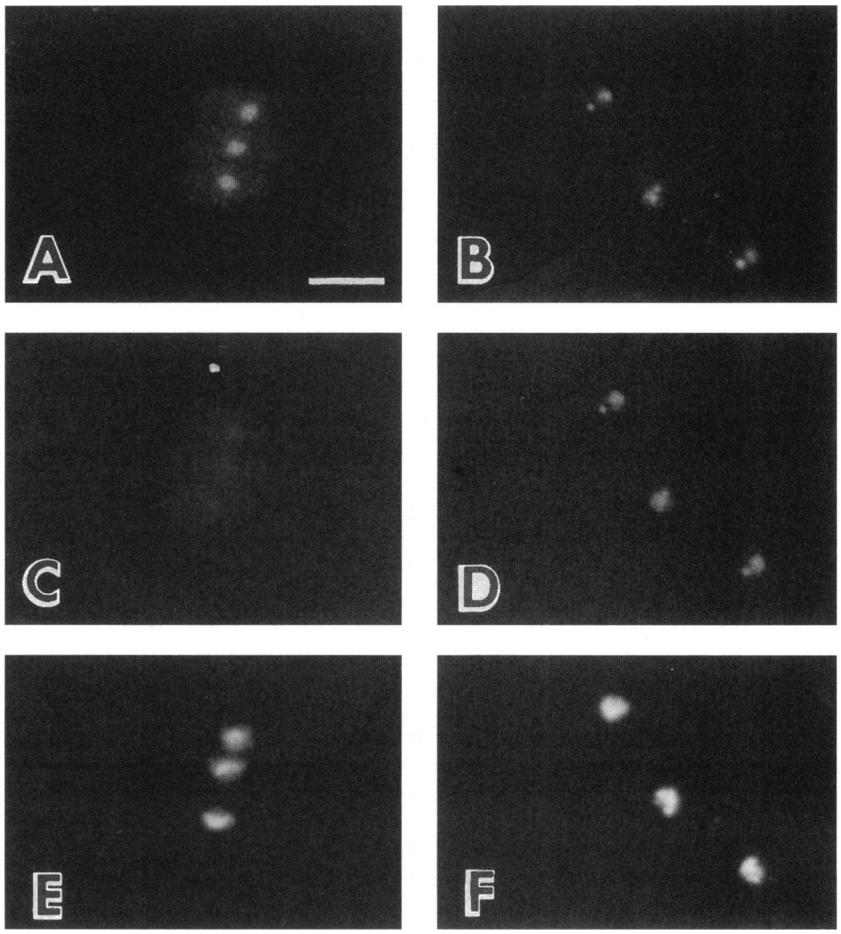

Figure 7. Poly(A)$^+$ RNA accumulates predominantly in the nuclear region where the abundant snRNAs are localized. Cells were grown at 30°C and either maintained at 30°C (left panels) or shifted to 42°C for 60 min (right panels), and then subjected to triple staining with the anti-m^{3}G antibody (A and B), the rhodamine-labeled oligo $(dT)_{50}$ probe (C and D), and DAPI (E and F). Bar, 10 μm.

Transport of mRNA from an Intron-less Gene Is Blocked in Heat-shocked Cells

The pre-mRNA splicing reaction was previously shown to be interrupted by severe heat shock in *Drosophila* cells (Yost and Lindquist, 1986), HeLa cells (Bond, 1988), *S. cerevisiae* (Yost and Lindquist, 1991), *S. pombe* (Tani and Ohshima, 1989; Frendewey *et al.*, 1990), and in two other yeast species (Tani and Ohshima, 1991). To exclude the possibility that nuclear accumulation of poly(A)$^+$ RNA in the heat-shocked cells is simply due to retention of unspliced precursor mRNA in the nuclei, we performed in situ hybridization using a probe for an RNA that does not contain introns. We choose the ADH mRNA because this mRNA is highly expressed in *S. pombe* (Russell and Hall, 1983). In normal cells grown at 30°C, we observed a single hybridization signal (1 dot) per nucleus in these haploid cells with the ADH probe (Figure 9A). The dot is frequently found at the periphery of the nucleus and is localized at the boundary between the chromatin-enriched region and the nucleolar region (Figure 9C). The dot may represent the transcription site of the ADH gene. To our knowledge, this is the first report of the detection of a specific endogenous mRNA in yeast cells by in situ hybridization. In the heat-shocked cells an accumulation of the ADH mRNA in the nuclei was clearly observed (Figure 9D), although all the cells did not necessarily show bright nuclear signals. In situ hybridization using a vector plasmid as a control probe gave no signal at the control (Figure 9F) or heat shock (Figure 9H) temperatures, demonstrating the specificity of the hybridization. These results show that the accumulation of poly(A)$^+$ RNA in the nuclei of the heat-shocked cells is not specifically due to the interruption of pre-mRNA splicing, but is a consequence of a block of the mRNA transport pathway.

Electron Microscopic In Situ Hybridization

To learn more about the precise localization of poly(A)$^+$ RNA in normal and heat-shocked cells, we performed electron microscopic in situ hybridization with the oligo $(dT)_{50}$ probe. Detection of the hybridized probe was achieved by incubation with mouse monoclonal anti-digoxin antibody followed by anti-

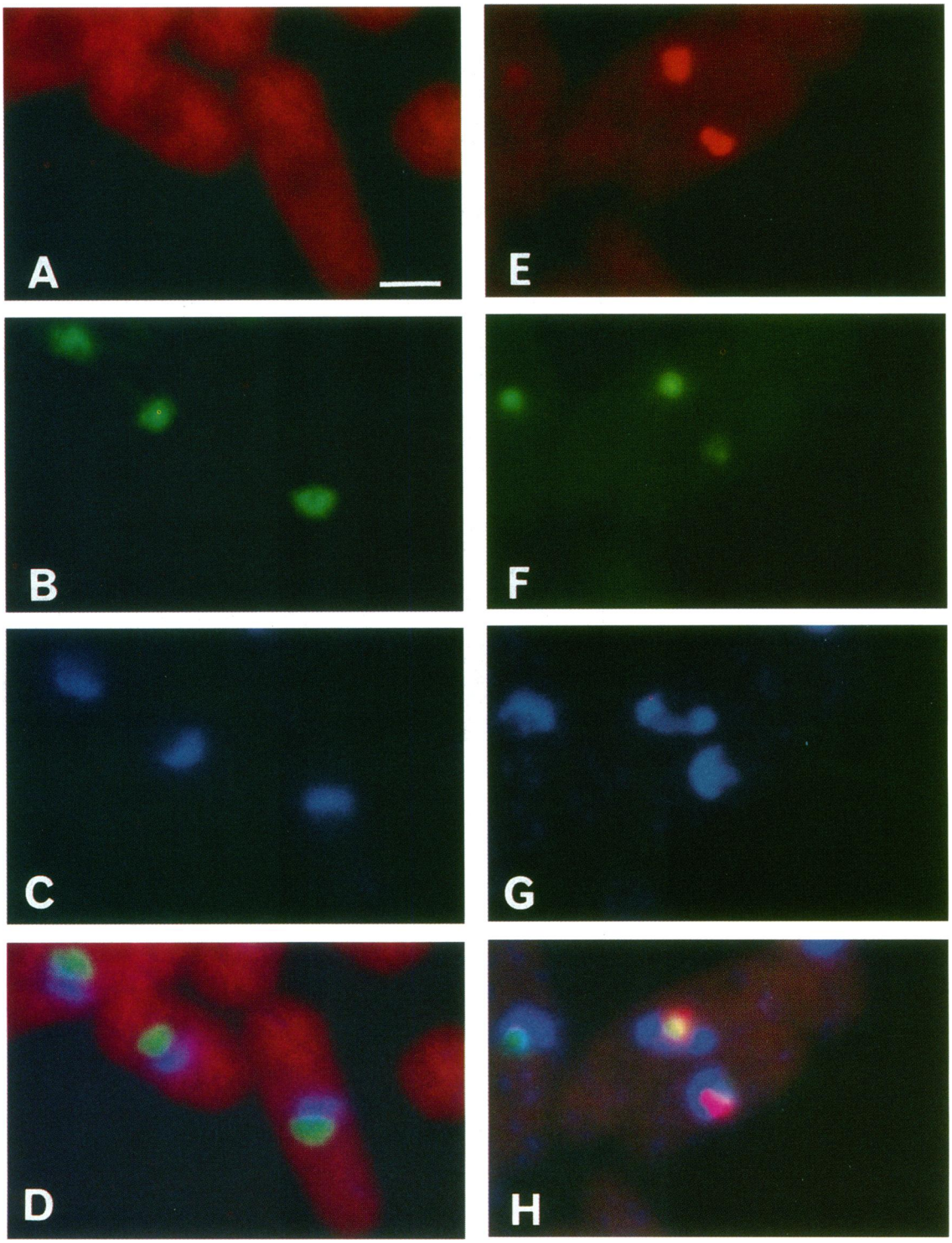

Figure 8. Poly(A)$^+$ RNA accumulates in the nucleolar region of *pim1–46* cells at the nonpermissive temperature. Cells were grown at 26°C and either maintained at 26°C (left panels) or shifted to 37°C for 2 h (right panels), and then subjected to triple staining with the biotin-labeled oligo $(dT)_{50}$ probe (A and E), the D77 antibody (B and F), and DAPI (C and G). In merged images (D and H), red denotes poly(A)$^+$ RNA, green denotes D77 antibody, blue denotes DAPI, and yellow/pink denotes colocalization of poly(A)$^+$ RNA and D77 antibody. Bar, 5 μm.

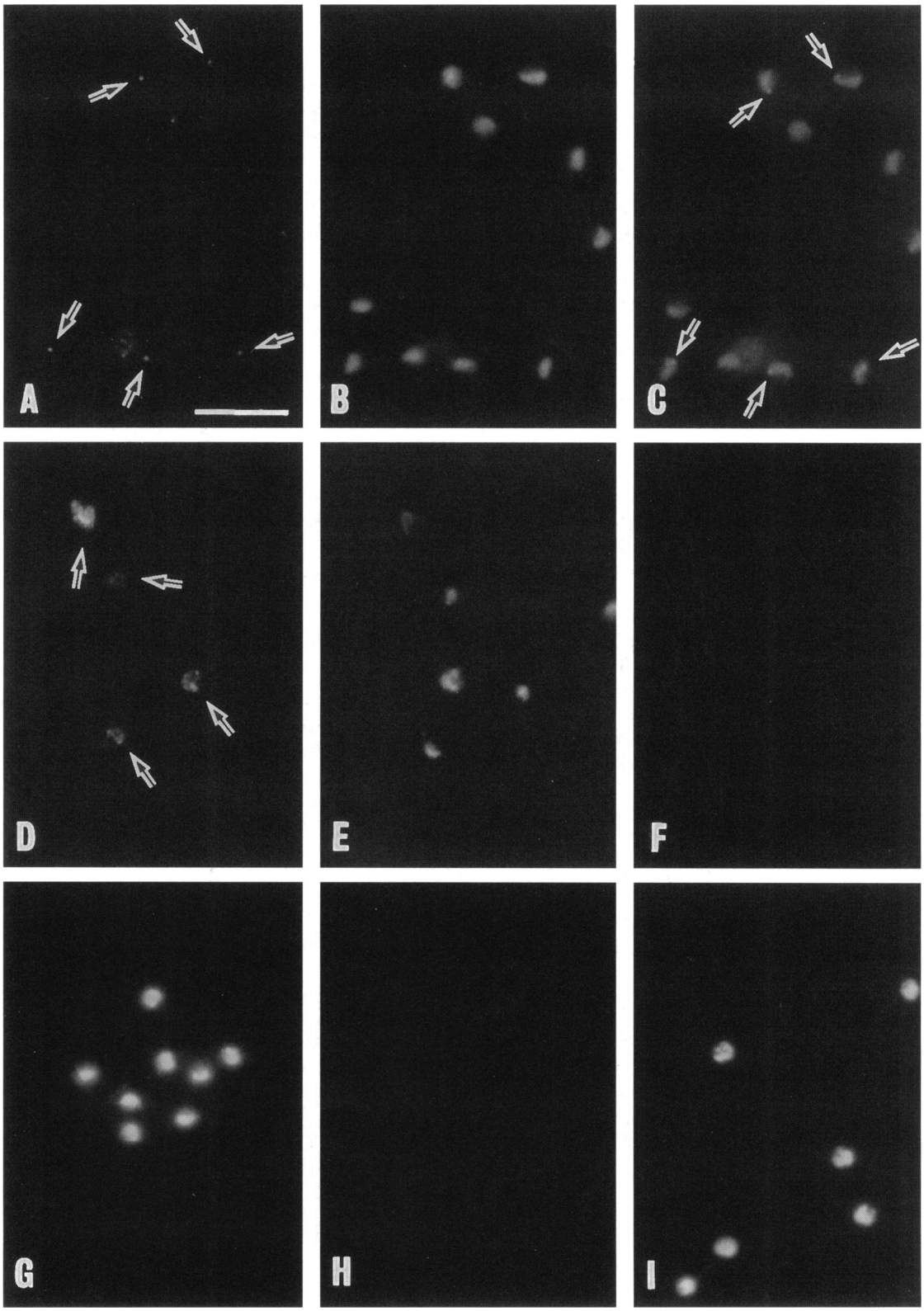

Figure 9. Transcripts from the intron-less ADH gene accumulate in the nuclei of heat-shocked cells. Cells were either maintained at 30°C (A–C, F, and G) or shifted to 42°C for 60 min (D, E, H, and I), and then subjected to in situ hybridization with the ADH probe (A, C, and D)

mouse IgG antibody conjugated with 15-nm colloidal gold particles. We detected poly(A)$^+$ RNA in the nucleolar region (dark-staining region) as well as in the chromatin-enriched region (light-staining region) in normal cells grown at 30°C (Figure 10A). This data demonstrates that in normal cells, a portion of poly(A)$^+$ mRNA is distributed in the nucleolar region in addition to being present in the chromatin-enriched region of the yeast nucleus. Control sections incubated without the probe showed no labeling (Figure 10B), demonstrating the specificity of the hybridization signal. These results raise the possibility that some (or all) of the yeast mRNAs are transported through the nucleolar region of the nucleus under normal conditions. In the heat-shocked cells, the dark-staining nucleolar region is fragmented into one or two concentrated regions consisting of electron-dense components. In this case, most of the poly(A)$^+$ RNA was found within these nucleolar regions (Figure 11). Predominant labeling of the condensed nucleolar region with the oligo $(dT)_{50}$ probe is consistent with the fluorescent in situ hybridization data. Nucleolar fragmentation has been previously observed in the analysis of yeast mutants deficient in mRNA transport (*mtr* mutants) (Kadowaki *et al.*, 1994a,b; Schneiter *et al.*, 1995). Poly(A)$^+$ RNA has been observed to accumulate in *mtr* mutants in whole nuclear crescent-like foci, dots, or circular patterns, suggesting that RNA is being trapped at different steps along the transport pathway (Kadowaki *et al.*, 1994a). Mammalian nucleoli have also been reported to appear swollen and unraveled after heat shock (Welch and Suhan, 1985).

DISCUSSION

Heat Shock Blocks mRNA Transport in Yeast

In this study, we have demonstrated that under normal growth conditions, poly(A)$^+$ RNA is localized within both the chromatin-enriched and the nucleolar regions of the yeast nucleus. Furthermore, we have shown that the nucleocytoplasmic transport of poly(A)$^+$ mRNA in *S. pombe* is blocked by a severe heat shock. During heat shock, poly(A)$^+$ RNA accumulates in the nucleolar region of the yeast nucleus. To our knowledge, this is the first example that heat shock affects mRNA transport in yeast cells. These data suggest that the nucleolar region of the yeast nucleus may be involved in other functions such as mRNA transport, in addition to those associated with RNA polymerase I activity.

In yeast, the nuclear accumulation of poly(A)$^+$ mRNA by the inhibition of mRNA transport was easily visualized in the in situ hybridization assay, as most of the yeast mRNAs in the cytoplasm have relatively short half lives as compared with those of mammalian mRNAs and the accumulated nuclear mRNAs are considerably more stable. Half-lives ranging from 16 to 23 min have been measured for the average turnover rate of the poly(A)$^+$ RNA population in the yeast *S. cerevisiae* (Hynes and Phillips, 1976; Santiago *et al.*, 1986; Herrick *et al.*, 1990). Moreover, yeast mRNAs have been shown to exit the nucleus within 2 min after synthesis under normal growth conditions (Groner and Phillips, 1975). Therefore, blockage of the mRNA transport pathway in yeast cells leads to a dramatic change in the steady state distribution of mRNA, which can be easily detected by in situ hybridization with an oligo $(dT)_{50}$ probe. Such an in situ hybridization assay was previously applied to identifying mutants defective in mRNA transport in *S. cerevisiae* (Amberg *et al.*, 1992; Kadowaki *et al.*, 1992, 1994a,b; Schneiter *et al.*, 1995).

Heat Shock Proteins Protect the mRNA Transport Pathway

We have also demonstrated that pretreatment of cells at a temperature that induces heat shock proteins before the severe heat shock makes the cells tolerant or protects the mRNA transport machinery and allows mRNA transport to proceed unimpeded. A similar observation was previously observed in relation to pre-mRNA splicing (Yost and Lindquist, 1986). The protective effects of pretreatment were sensitive to cycloheximide. Because cycloheximide itself does not affect mRNA transport at low temperatures, nor at high temperatures when heat shock proteins have already been produced, it is most likely that synthesis of heat shock proteins is required to protect mRNA transport. Although we do not know that the hsp protein(s) involved in protection of mRNA transport is induced at 42°C as is hsp 104, it is likely that such an induction, simultaneously with the exposure to severe heat shock, is not sufficient to protect the mRNA transport pathway. Instead, it seems that protection of the mRNA transport pathway requires the presence of a considerable amount of the hsp protein(s) at the time that the cells are exposed to severe heat shock.

Novel Role of Yeast Nucleoli in mRNA Transport

Interestingly, the triple labeling experiments clearly showed that in heat-shocked cells, poly(A)$^+$ RNA accumulated predominantly in the nuclear regions, which have previously been referred to as the nucle-

(Figure 9 cont.) or a control probe lacking insert (F and H) uniformly labeled with digoxigenin. The hybridization signals were detected by treatment with the mouse monoclonal anti-digoxin antibody followed by fluorescein-conjugated goat anti mouse IgG antibody. The nuclei were counterstained by DAPI (B, C, E, G, and I). (C) A photograph taken using a triple band-pass filter so that FITC and DAPI labeling can be observed simultaneously. The arrowheads denote the hybridization signals. Bar, 10 μm.

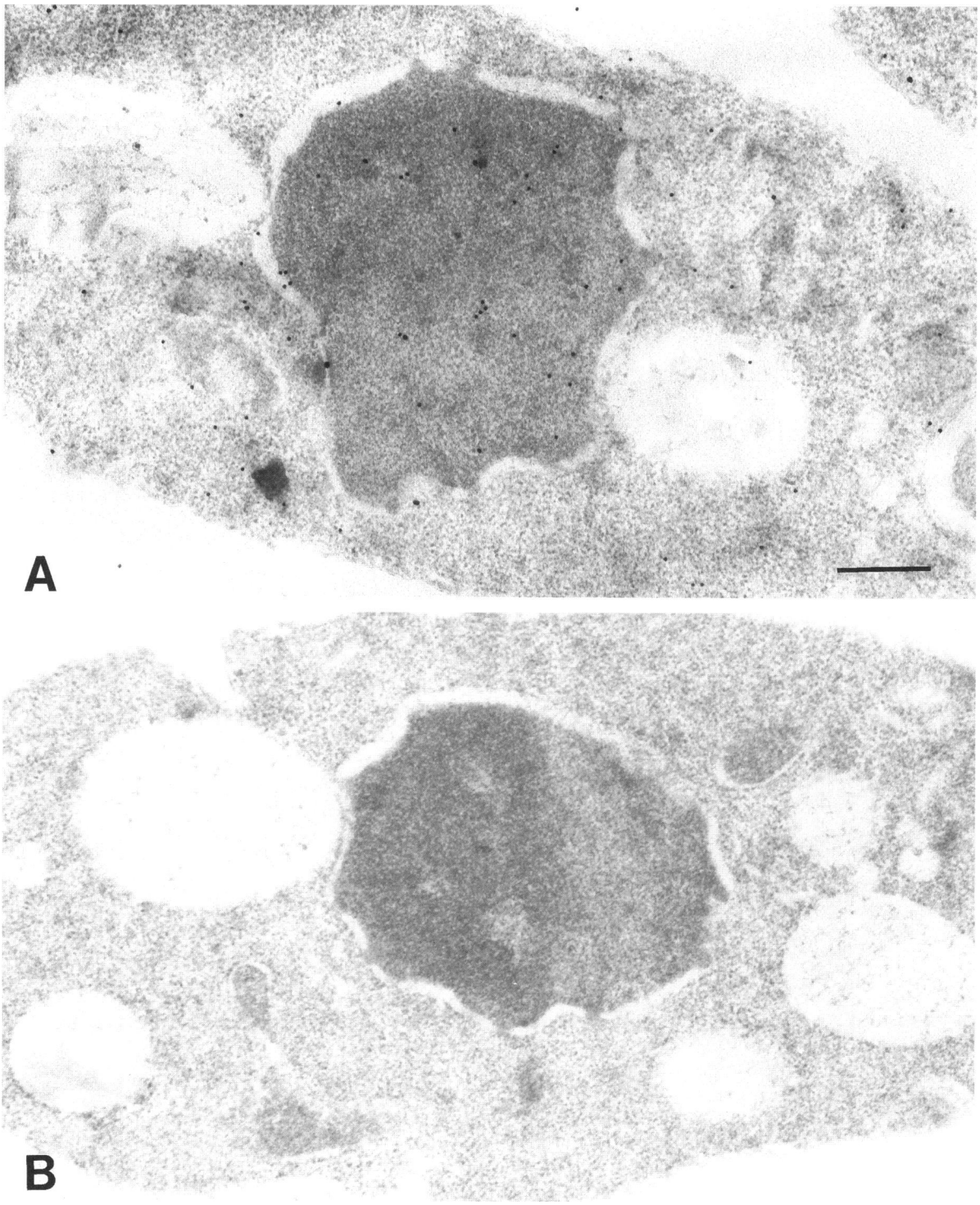

Figure 10. Ultrastructural localization of poly(A)$^+$ RNA in the nucleus of *S. pombe*. (A) Poly(A)$^+$ RNA is localized throughout the *S. pombe* nucleus and cytoplasm when cells are grown at 30°C. (B) Control sections hybridized without probe show no colloidal gold labeling. Bar, 500 nm.

olus on the basis of ultrastructural studies (Molenaar *et al.*, 1970; Sillevis Smitt *et al.*, 1973; Tanaka and Kanbe, 1986; Potashkin *et al.*, 1990) and localization of rRNA precursors (Sillevis Smitt *et al.*, 1972, 1973;

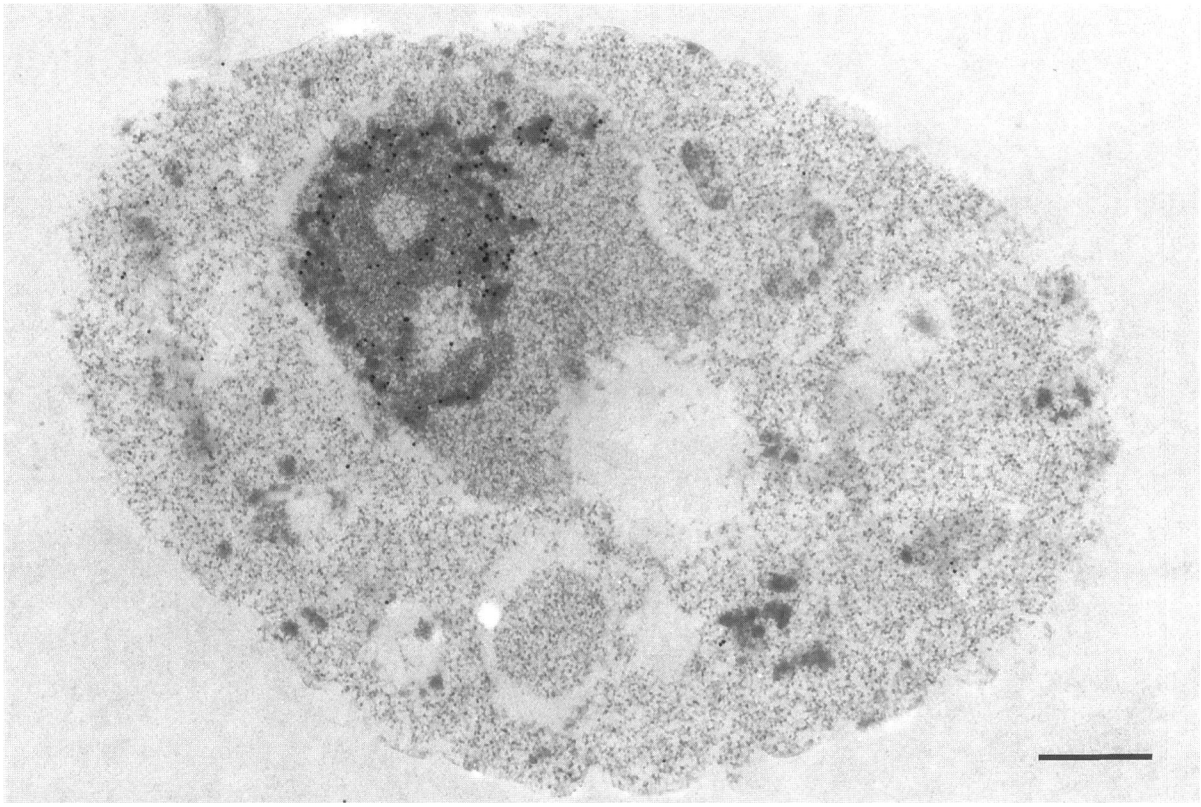

Figure 11. Ultrastructural localization of poly(A)$^+$ RNA in the nucleus of *S. pombe*. When cells are heat-shocked at 42°C for 1 h, poly(A)$^+$ RNA accumulates in the fragmented nucleolar region of the *S. pombe* nucleus. Bar, 500 nm.

Dvorkin *et al.*, 1991). The nucleolar region labeled by the D77 antibody and anti-m^{3}G antibody reorganizes into a few condensed structures after cells are heat shocked. The accumulation sites of poly(A)$^+$ RNA coincide with these condensed structures after a 1-h heat shock.

However, nucleolar labeling of poly(A)$^+$ RNA is not limited to heat-shocked cells. *pim1*, one of two *S. pombe* mutants defective in mRNA transport (Matsumoto and Beach, 1991; Brown *et al.*, 1995), was also shown to accumulate poly(A)$^+$ RNA in the nucleolar region at the nonpermissive temperature. It is a homologue of the mammalian *RCC1*, the nuclear guanine nucleotide exchange factor for the guanosine triphosphatase Ran/TC4. In mammalian cells, the RCC1 protein is required for many processes including nucleocytoplasmic transport of mRNA and precursors of spliceosomal snRNAs as well as progression of the cell cycle (Cheng *et al.*, 1995).

In addition to the observations made in mutant and heat-shocked cells, the nuclei of cells growing at their normal temperatures were labeled uniformly by the oligo $(dT)_{50}$ probe by fluorescence in situ hybridization. We could not detect any differential staining between the chromatin-enriched and nucleolar regions, demonstrating that poly(A)$^+$ RNA is localized in both nuclear regions under normal physiological conditions. Furthermore, we detected the specific hybridization signal in the nucleolar region as well as the chromatin-enriched region in normal cells by electron microscopic in situ hybridization. Therefore, the localization of poly(A)$^+$ RNA in the nucleolar region is not simply due to a nonspecific recompartmentalization of nuclear components after heat shock. These results raise an interesting possibility that some (or all) species of RNA polymerase II transcripts in *S. pombe* are exported to the cytoplasm through the nucleolus.

However, we cannot exclude an alternate, but less likely possibility, that poly(A)$^+$ RNAs found in the nucleolus are destined for turnover in the nucleolus and are not exported to the cytoplasm. After shifting cells back to 30°C for 2 h, however, the nuclear poly(A) signal in the heat-shocked cells almost disappeared and the cytoplasmic signal increased (Tani and Spector, unpublished results). A pulse-chase experiment will be necessary to further clarify the destiny of the accumulated poly(A)$^+$ RNA. However, other examples suggesting that nucleoli may have a potential role in mRNA transport come from observations made in *S. cerevisiae* and higher eukaryotic cells. Several

mRNA transport mutants in *S. cerevisiae* including *mtr1–1*, *mtr2–1*, and *rpa190–3* were found to accumulate poly(A)$^+$ RNA in the fragmented nucleoli at the nonpermissive temperature, suggesting a possible role of the nucleolus in mRNA export (Kadowaki *et al.*, 1994b; Schneiter *et al.*, 1995). Earlier studies in higher eukaryotic cells also showed that inactivation of the HeLa and BSC-1 nucleoli by UV irradiation inhibits the export of nonribosomal RNAs from the nucleus to the cytoplasm (Sidebottom and Harris, 1969; Deák, 1973). In a recent study, Bond and Wold (1993) reported that c-*myc*, N-myc, and MyoD transcripts localize in the nucleoli of mammalian cells. However, c-*myc* transcripts containing intron 1 were not found in the nucleoli. Based on these data, a new role for the nucleolus in transport and/or turnover of mRNAs was proposed. Nucleolar localization of RNA polymerase II transcripts has also been shown for viroid RNAs (Harders *et al.*, 1989) and HTLV-I env transcripts (Kalland *et al.*, 1991). Furthermore, the HIV and HTLV-I proteins Rev and Rex were shown to be localized in nucleoli (Cullen *et al.*, 1988; Siomi *et al.*, 1988). This localization was reported to be essential for the cytoplasmic accumulation of unspliced or partially spliced viral RNA (Siomi *et al.*, 1988; Felber *et al.*, 1989; Nosaka *et al.*, 1989). Expression of the Rex protein in COS cells increased the nucleolar accumulation of the HTLV-I env RNA (Kalland *et al.*, 1991). However, bulk poly(A)$^+$ RNA and several other specific endogenous mRNAs have not been localized in mammalian nucleoli (Lawrence *et al.*, 1989; Carter *et al.*, 1991; Huang and Spector, 1991; Huang *et al.*, 1994; Visa *et al.*, 1993; Xing *et al.*, 1993). Therefore, it is likely that only some species of mRNA are associated with the nucleoli in mammalian cells. In addition, accumulation of mRNA in the nucleoli by heat shock has not been observed in mammalian cells (Visa *et al.*, 1993). Thus far, nucleolar accumulation of mRNA by heat shock seems to be specific to yeast.

Two Functional Domains in the Yeast Nucleolar Region

The nucleolus is a subnuclear organelle that differs from other organelles in lacking a membrane boundary (Hadjiolov, 1985). In yeast, the nucleolus is an electron dense region that is directly in contact with the nuclear membrane. In this respect, the localization of the yeast nucleolus differs from that of some mammalian nucleoli that are located more centrally within the nucleoplasm. rDNA, rRNA precursors, RNA polymerase I, and the nucleolar snoRNP protein fibrillarin have all been localized to the nucleolar half of the yeast nucleus (Hirano *et al.*, 1989; Clark *et al.*, 1990; Potashkin *et al.*, 1990; Dvorkin *et al.*, 1991). Another unique feature of the yeast nucleolus is the localization of abundant snRNPs, which are involved in pre-mRNA splicing (Potashkin *et al.*, 1990). This finding led to the hypothesis that the yeast nucleolus contains a functional domain for pre-mRNA splicing in addition to those associated with the activities of RNA polymerase I. The present findings would suggest that a rigid functional boundary does not separate a distinct nucleolus from the rest of the nucleoplasm in yeast. The electron dense region of the yeast nucleus may function in nucleolar as well as other nuclear processes.

In mammalian cells, the snRNPs are localized in a specific distribution within the nucleus, which has been termed the speckled pattern. This pattern corresponds to structures called interchromatin granules and perichromatin fibrils at the electron microscopic level (for a review see Spector, 1993). In a temperature-sensitive hamster cell line tsBN2, which has a point mutation in the RCC1 gene (Uchida *et al.*, 1990), poly(A)$^+$ RNA is accumulated in the nuclei at the nonpermissive temperature, suggesting that the integrity of the RCC1 gene product is required for mRNA transport (Amberg *et al.*, 1993; Kadowaki *et al.*, 1993). Interestingly, poly(A)$^+$ RNA is accumulated in the interchromatin granule clusters, which decreased in number and increased in size, in tsBN2 cells at the nonpermissive temperature (Huang and Spector, unpublished data). However, poly(A)$^+$ RNA localization was not observed in nucleoli.

The electron-dense region of the *S. pombe* nucleus that is classically referred to as the nucleolus has similarities to the interchromatin granules of mammalian cells in two respects: localization of abundant snRNPs and accumulation of poly(A)$^+$ RNA after a block in mRNA transport. The electron-dense region of the yeast nucleus may have a functional domain similar to mammalian interchromatin granules. Thus far, the macromolecular structures resembling interchromatin granules, perichromatin fibrils, or coiled bodies have not been identified within the yeast nucleus. Potashkin *et al.* (1990) have suggested that the electron-dense region that has been called the nucleolar region should be referred to as the nonchromatin-enriched region of the nucleus rather than the nucleolus, because other functional domains may be localized in this nuclear region. We propose that two macromolecular functional domains are present in the same electron-dense region of the yeast nucleus: one is the domain associated with RNA polymerase I activity and the other is the domain involved in mRNA splicing and mRNA transport. Together, these domains comprise the electron-dense region of the yeast nucleus. Future studies will continue to elucidate the functional organization of the yeast cell nucleus and how communication occurs between functional domains.

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