mRNA Transport in Yeast: Time to Reinvestigate the Functions of the Nucleolus

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Nucleocytoplasmic transport of mRNA is vital to gene expression and may prove to be key to its regulation. Genetic approaches in *Saccharomyces cerevisiae* have led to the identification of conditional mutants defective in mRNA transport. Mutations in approximately two dozen genes result in accumulation of transcripts, trapped at various sites in the nucleus, as detected by in situ hybridization. Phenotypic and molecular analyses of many of these mRNA transport mutants suggest that, in yeast, the function of the nucleolus is not limited to the biogenesis of pre-ribosomes but may also be important for transport of poly(A)⁺ RNA. A similar function of the animal cell nucleolus is suggested by several observations.

INTRODUCTION

RNA transport from the nucleus to the cytoplasm comprises intranuclear steps that translocate the transport substrate to the nuclear envelope, followed by export through the nuclear pore complex to the cytoplasm. It is remarkable that for mRNAs, despite their enormous structural variety, transport appears to be efficient and orderly—in the sense that only mature mRNAs are customarily allowed to exit. Although several reviews of nucleocytoplasmic transport have been published recently (Garcia-Bustos *et al.*, 1991; Nigg *et al.*, 1991; Krug, 1993; Newmeyer, 1993; Elliott *et al.*, 1994; Izaurralde and Mattaj, 1995), none of these focused on mRNA transport in yeast or described the substantial number of gene products that have been shown only recently to participate in transport.

Studies of animal cells have defined some structural features of mRNA that are key for export and some properties of the “export apparatus.” For example, in vitro-transcribed RNAs microinjected into *Xenopus* oocyte nuclei are transported to the cytoplasm in a saturable and energy-dependent process (Zasloff, 1983; Dworetzky and Feldherr, 1988; Khanna-Gupta and Ware, 1989; Bataillé *et al.*, 1990). Competition experiments indicate that at least some of the steps of transport are specific for individual classes of RNA

(Dargemont and Kühn, 1992; Jarmolowski *et al.*, 1994). The presence of both the 5' m⁷G cap structure and the 3' poly(A) tail positively affect mRNA export in this system, but appear not to be essential (Hamm and Mattaj, 1990; Eckner *et al.*, 1991; Jarmolowski *et al.*, 1994). These microinjection experiments do not discriminate among the individual events of RNA transport: packaging into ribonucleoprotein particles, peripheralization, and interaction with and export through nuclear pores (Figure 1). It is possible that factors that are critical for transport actually associate with transcripts even before their completion. For example, there is evidence suggesting a connection between promoter function and RNA processing and/or transport of the corresponding transcript (de la Peña and Zasloff, 1987; Neuberger and Williams, 1988; Enssle *et al.*, 1993). Furthermore, as discussed below, coupling between transcription/processing and transport is suggested by the observation that two of the best characterized yeast RNA transport defective mutants, *mtr1* and *rna1*, synthesize oversized transcripts (Forrester *et al.*, 1992; Kadowaki *et al.*, 1993).

Some information concerning transport in animal cells also comes from steady-state localization of poly(A)⁺ RNA and individual transcripts to irregular domains (“speckles”) in the nucleoplasm (Carter *et al.*, 1991; for review see Lamond and Carmo-Fonseca, 1993; Rosbash and Singer, 1993; Spector, 1993; Xing and Lawrence, 1993). Although export of pre-mRNA from polytene nuclei of *Drosophila* occurs at rates con-

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Figure 1. Schematic model of steps of mRNA transport subsequent to transcription. Certain steps may be concurrent or involve several partial reactions (see text).

sistent with diffusion (Zachar *et al.*, 1993), nuclear pre-mRNA is not soluble but rather immobilized by attachment to the nuclear matrix in the form of tightly packed ribonucleoprotein complexes (for review see Verheijen *et al.*, 1988; Berezney, 1991; Dreyfuss *et al.*, 1993). Such immobilization may be related to the regulation of transport, which is observed in virally infected cells (Leppard and Shenk, 1989; Cullen and Malim, 1991; Alonso-Caplen *et al.*, 1992; Krug, 1993), in the activation of sea urchin embryonic RNA (Caldwell and Emerson, 1985), in amino acid-starved hepatoma cells (Laine *et al.*, 1994), and may also be related to the finding that certain polyadenylated RNA polymerase II transcripts apparently never leave the nucleus (Brown *et al.*, 1992; Hogan *et al.*, 1994). One further example of regulation is that assembly of splicing factors on newly synthesized transcripts can prevent transport of immature mRNA (Chang and Sharp, 1989; Legrain and Rosbash, 1989; He *et al.*, 1993).

mRNA transport thus depends on the presence of *cis*- and *trans*-acting factors. Although the *cis*-acting requirements of the substrate are best defined by taking advantage of the size of the *Xenopus* oocyte nucleus, no cell-free system is available to identify *trans*-acting factors. Such factors can, however, be identified using a genetic approach. In this essay, we describe such a genetic approach to identify components that are required for mRNA transport in yeast, briefly discuss what has been learned from the analysis of more than a dozen of the best characterized mutants and, in a more speculative part, present evidence that suggests that the nucleolus plays a role in mRNA transport.

A GENETIC APPROACH TO mRNA TRANSPORT USING *SACCHAROMYCES CEREVISIAE*

To identify genes that are essential for mRNA transport in yeast, our laboratory, and others, have turned to a genetic approach. A suicide enrichment procedure was used to establish a library of temperature-sensitive conditional mutants of *S. cerevisiae*, which, at the restrictive temperature, cease protein synthesis. This library was rescreened by fluorescent *in situ* hybridization using a biotinylated oligo-(dT) probe to identify mutants that accumulate poly(A)⁺ RNA in their nuclei (Amberg *et al.*, 1992; Kadowaki *et al.*, 1992). The use of this approach is based on the assumption that the mRNA that is not transported from the nucleus is nevertheless polyadenylated and sufficiently stable and abundant to be detected by *in situ* hybridization (Figure 2). These screens yielded 17 complementation groups of recessive mutants defective in mRNA transport (*mtr*; Kadowaki *et al.*, 1994a) and seven possibly different complementation groups affecting ribonucleic acid trafficking (*rat*; Amberg *et al.*, 1992). Although the extent of overlap between *mtr* and *rat* mutants is not known, the observation that most of the *mtr* complementation groups are represented by only one allele indicates that these screens are far from reaching saturation, making it unlikely that many of the *mtr* mutants are allelic to *rat* mutants. The early observation that a considerable number of *rat* mutants (Copeland *et al.*, 1991) affect the distribution of the nuclear pore complexes, on the other hand, anticipated an overlap between *rat* and pore complex mutants (Table 1; see below). Screens of temperature-sensitive mutant banks for defects in pre-mRNA processing (*prp*; for review see Rymond and Rosbash, 1992) or nuclear accumulation of poly(A)⁺ RNA show that the incidence of these two phenotypes is of comparable frequency (3.6% vs. 2%; Vijayraghavan *et al.*, 1989; Kadowaki *et al.*, 1994a). The number of genes required for mRNA transport may thus be of the same order of magnitude as those required for splicing. Only a few *prp* mutants have been shown to exhibit nuclear poly(A)⁺ RNA accumulation (Kadowaki *et al.*, 1992), suggesting that there is no mandatory coupling between the splicing of pre-mRNA and export of the average mRNA (which does not undergo splicing in yeast).

As for *PRP* genes, whose products may or may not be directly involved in pre-mRNA splicing, *MTR* and *RAT* products may affect transport only indirectly. Nevertheless, several are nuclear proteins and contain RNA-binding motifs (Table 1; see below). Direct interactions between RNA and *MTR* or *RAT* proteins have not yet been shown; however, because the transport substrate is likely to be a ribonucleoprotein complex, protein/protein interactions may be as important for

Figure 2. Nuclear accumulation of poly(A)⁺ RNA in a temperature-sensitive mutant for the largest subunit of RNA polymerase I, *rpa190-3* (Wittekind *et al.*, 1988). In situ hybridization using biotinylated oligo-d(T) followed by fluorescein-avidin detection, applied to Pol I mutant cells that were grown at the permissive (23°C; A), or the restrictive temperature (37°C, 5 h; B). Corresponding DAPI (C and D) and differential interference contrast pictures (DIC; E and F) of the same visual fields are shown below. Accumulated Poly(A)⁺ RNA is revealed by the brightly stained focal spots (arrowheads) within the nucleus of cells that were incubated at the restrictive temperature. Strikingly, regions containing the accumulated poly(A)⁺ RNA are visible even in the DIC picture (arrowheads in F). In situ hybridization applied to wild-type cells grown at either temperature does not result in detectable nuclear accumulation of poly(A)⁺ RNA (not shown). Parallel studies show that the sites of poly(A)⁺ RNA accumulation illustrated in panels B and F are enriched in the nucleolar antigen, NOP1.

transport as protein/RNA interactions. Even if some of the available mutants affect RNA transport only indirectly, they should prove useful for secondary screens that ultimately will lead us closer to the "transport apparatus." This should be true regardless of the extent to which transport includes significant diffusional or stochastic steps.

In analogy to the picture that is emerging from the characterization of *PRP* genes, we suspect that the *MTR* genes fall into several subfamilies that affect successive steps along the transport path. This possibility is consistent with the observation that different mRNA transport mutants accumulate poly(A)⁺ RNA in clearly distinguishable patterns that range from a granular distribution scattered throughout the nucleus (*mtr10* and *mtr16*) to a marginal crescent (*mtr3* and *mtr14*) or a continuous circle located at the nuclear perimeter (*mtr5* and *mtr12*) in cells depleted of the nuclear pore complex protein NUP145 (Fabre *et al.*, 1994) or in a *nup116* mutant (Wente and Blobel, 1993)).

YEAST GENES THAT AFFECT NUCLEOCYTOPLASMIC TRANSPORT OF mRNA

Fourteen conditional yeast mutants described below accumulate polyadenylated RNA in the nucleus at

the restrictive temperature. Although the corresponding wild-type genes have little in common with each other, the subcellular distribution of their products (Figure 3) suggests some properties of RNA transport: six of the mutants affect the function of the nuclear pore complex, confirming the central role of this structure in nucleocytoplasmic transport. Three of the products constitute a nucleocytoplasmic GTP/GDP-cycle. Interestingly, this GTPase cycle and the pore complex appear to interact. Five of the mutants display an unexpected phenotype: nuclear accumulation of RNA is accompanied by fragmentation of the crescent-shaped yeast nucleolus. It is this phenotype especially that led us to formulate the hypothesis presented in the last section of this essay and to consider a possible function of the nucleolus in RNA transport. As was mentioned above, several of these mutants were obtained by screening a bank of temperature-sensitive strains for conditional nuclear accumulation of poly(A)⁺ RNA and by use of a suicide enrichment procedure. Others were obtained by biochemical approaches, e.g. generation of mutant forms of purified nuclear pore proteins, followed by biochemical and genetic searches for interacting components.

Table 1. Mutations affecting mRNA transport in yeast

Gene	Protein structure/location	Phenotype at restrictive temperature/function/comments	References
<i>CNR1/GSP1</i>	25 kDa; nuclear GTPase of Ras superfamily; essential	Also required for protein import in mammalian cells	Belhumeur <i>et al.</i> , 1993; Kadowaki <i>et al.</i> , 1993; our unpublished observation
<i>HKE1/RAT1/TAP1</i>	116 kDa; RNase activity in vitro; nuclear; essential	5' trimming of 5.8S rRNA	Amberg <i>et al.</i> , 1992; Aldrich <i>et al.</i> , 1993; Kenna <i>et al.</i> , 1993
<i>MAS3</i>	93-kDa heat-shock transcription factor; essential	Mitochondrial protein import; cell division cycle; nucleolar fragmentation	Sorger and Pelham, 1988; Wiederrecht <i>et al.</i> , 1988; Smith and Yaffe, 1991; Kadowaki <i>et al.</i> , 1994a
<i>MTR1/PRP20/SRM1</i>	52-kDa homologue of <i>RCC1</i> ; guanine nucleotide release protein; binds to ds-DNA in vitro through a multicomponent complex; nuclear; essential	G1 arrest; pre-mRNA splicing; pre-rRNA and pre-tRNA processing; oversized transcripts; nucleolar fragmentation; suppressed by overexpression of <i>CNR1/GSP1</i> , <i>CNR2/GSP2</i>	Clark and Sprague, 1989; Aebi <i>et al.</i> , 1990; Fleischmann <i>et al.</i> , 1991; Forrester <i>et al.</i> , 1992; Amberg <i>et al.</i> , 1993; Lee <i>et al.</i> , 1993
<i>MTR2</i>	21 kDa; homology to <i>mbeA</i> ; nuclear; essential	Slow pre-rRNA processing; nucleolar fragmentation	Kadowaki <i>et al.</i> , 1993
<i>MTR13/MTS1</i> <i>NAB1/NOP3/NPL3</i>	45 kDa; RRM and GAR domains; four proline rich N-terminal repeats; nucleoplasm and nucleolus; binds poly(A) RNA in vivo shuttles between nucleus and cytoplasm; essential	Block in protein import; pre-rRNA processing; electron dense granulae in nucleoplasm	Kadowaki <i>et al.</i> , 1994b; Bossie <i>et al.</i> , 1992; Russell and Tollervey, 1992, 1995; Ellis and Reid, 1993; Wilson <i>et al.</i> , 1994; Flach <i>et al.</i> , 1994; Singleton <i>et al.</i> , 1995
<i>NUP1</i>	113-kDa nuclear pore complex protein; XFXFG repeats; essential	Synthetic lethal interaction with <i>NUP2</i> , <i>NUP133</i> , <i>NSP1</i> , <i>RNA1</i> and <i>SRP1</i> ; block in protein import	Davis and Fink, 1990; Belanger <i>et al.</i> , 1994; Boegerd <i>et al.</i> , 1994
<i>NUP49</i>	49-kDa nuclear pore complex protein; GLFG repeats; essential	Temperature-sensitive allele displays block in protein import and RNA export; synthetic lethal interaction with <i>NSP1</i> and <i>NUP133</i>	Wente <i>et al.</i> , 1992; Wimmer <i>et al.</i> , 1992; our unpublished observation
<i>NUP116</i>	116-kDa nuclear pore complex protein; GLFG repeats; RNP-1-like octapeptide sequence; nonessential	Null-allele shows double membrane seal over pore complex at the restrictive temperature; synthetic lethal interaction with <i>NSP1</i> , <i>NUP100</i> , and <i>NUP145</i>	Wimmer <i>et al.</i> , 1992; Wente and Blobel, 1993, 1994
<i>NUP133/RAT3</i>	133-kDa nuclear pore complex protein; nonessential	Synthetic lethal interaction with <i>NSP1</i> , <i>NUP1</i> , <i>NUP49</i> , and <i>NUP158</i> ; block in protein import; clustering of pore complexes	Doye <i>et al.</i> , 1994; Li <i>et al.</i> , 1995
<i>NUP145/RAT10</i>	145-kDa nuclear pore complex protein; GLFG repeats; RNP-1-like octapeptide sequence; essential	Synthetic lethal interaction with <i>NSP1</i> , <i>NUP100</i> , and <i>NUP116</i> ; binds homopolymeric RNA in vitro; block in protein import; N-terminal deletion results in herniation of the nuclear envelope	Doye <i>et al.</i> , 1994; Fabre <i>et al.</i> , 1994; Wente and Blobel, 1994
<i>NUP158/RAT7</i>	158-kDa nuclear pore complex protein	Synthetic lethal interaction with <i>NUP133</i> ; clustering of pore complexes	Gorsch <i>et al.</i> , 1995
<i>RNA1</i>	40-kDa hydrophilic protein with acidic C-terminal domain; leucine rich repeat; cytoplasmic; essential	Oversized transcripts; accumulation of 35S pre-rRNA and pre-tRNA; nucleolar fragmentation; functional interaction with <i>NUP1</i>	Hopper <i>et al.</i> , 1978; Traglia <i>et al.</i> , 1989; Hopper <i>et al.</i> , 1990; Kadowaki <i>et al.</i> , 1994a; Boegerd <i>et al.</i> , 1994
<i>RPA190</i>	186 kDa; largest subunit of RNA Pol I; zinc-binding finger; essential	Nucleolar fragmentation; suppressed by <i>SRP1</i>	Wittekind <i>et al.</i> , 1988; Oakes <i>et al.</i> , 1993; Figure 2

Fourteen genes required to ensure nucleocytoplasmic transport of poly(A)⁺ RNA in *Saccharomyces cerevisiae* are listed.

Figure 3. Steady-state locations of proteins implicated in mRNA transport. The largest subunit of RNA polymerase I (RPA190) is a nucleolar component; NUP1, NUP49, NUP116, NUP133, NUP145, and NUP158 form part of the nuclear pore complex; MTR13 shuttles between nucleus and cytoplasm; RNA1 is cytoplasmic; CNR1/2, MTR1, MTR2, and RAT1 are nuclear proteins. Localization of MAS3 to the nucleus is based on the presence of a putative nuclear localization signal in its sequence and the fact that it directly binds DNA in vitro. For references see Table 1.

Cytoplasmic Components

The first and best characterized heat-sensitive conditional mutant reported to be required for mRNA export, *rna1*, also affects processing of rRNA, tRNA, and pre-mRNA; pre-mRNA splicing, however, appears to be unaffected (Hutchison *et al.*, 1969; Shiokawa and Pogo, 1974; Hopper *et al.*, 1978; Piper and Aamand, 1989; Forrester *et al.*, 1992). RNA1 and its fission yeast (Melchior *et al.*, 1993b) and mouse (DeGregori *et al.*, 1994) homologues are essential for mitotic growth. Unexpectedly, the RNA1 gene product, which lacks apparent homology to characterized proteins, is found in the cytoplasm near to the nuclear envelope (Hopper *et al.*, 1990). The striking phenotypic similarity between *rna1* and *mtr1* with regard to their pleiotropic involvement in the processing of all three classes of RNAs (Forrester *et al.*, 1992) and in chromosome segregation (Atkinson *et al.*, 1985; Matsumoto and Beach, 1991) suggested that MTR1 might interact with RNA1 (Forrester *et al.*, 1992; DeGregori *et al.*, 1994). A link between RNA1 and MTR1 via a small nuclear GTPase has now been established (see below).

Components of the Nuclear Pore Complex

The external dimensions of nuclear pores in yeast seem significantly smaller than in vertebrates (~100 nm diameter compared with ~120 nm); however, they do show octagonal symmetry and the diameters of their central structures are similar. In addition to the approximately one dozen known nuclear pore complex proteins (NSP or NUP nucleoporins), many re-

main to be identified (Rout and Blobel, 1993; for review see Rout and Wentz, 1994). Although the recent rate of discovery of new pore components is astounding, only one (nonessential) integral membrane protein of the pore domain has been characterized in yeast (Wozniak *et al.*, 1994). Because RNA export occurs at nuclear pores, some nucleoporin mutants should exhibit nuclear accumulation of poly(A)⁺ RNA. Indeed, this has been documented for four conditional nucleoporin mutants, at least one of which, *nsp49*, is also defective in import of nuclear localization signal (NLS)-containing substrates. More remarkable, however, is the observation that some nucleoporin mutants preferentially or initially affect only protein import (Schlenstedt *et al.*, 1993; Doye *et al.*, 1994; Kadowaki *et al.*, 1994a). NUP116 and NUP145 both harbor a solitary RNP-1-like octamer domain and bind homopolymeric RNA in vitro, suggesting that they directly interact with RNA in vivo (Wentz and Blobel, 1993; Fabre *et al.*, 1994). Cells bearing an amino-terminal deletion/disruption of NUP145 display successive herniations of the nuclear envelope, leading to the formation of "grape-like" structures (Wentz and Blobel, 1994). Related ultrastructural alterations of the nuclear envelope are also observed in cells lacking the nonessential nucleoporin NUP116 (Wentz and Blobel, 1993). In *nup133* and *nup158* mutants, a striking clustering of the pore complexes is observed (Doye *et al.*, 1994; Gorsch *et al.*, 1995; Li *et al.*, 1995). Interestingly, NUP1, the only XFXFG repeat-containing nucleoporin known to be required for RNA transport, shows a genetic interaction (synthetic lethality) with RNA1 and thus with the nucleocytoplasmic GTPase cycle (see below; Boegerd *et al.*, 1994).

Nucleoplasmic Components

A mutant bearing a temperature-sensitive allele of the heat shock transcription factor, *mas3*, accumulates nuclear poly(A)⁺ RNA at the restrictive temperature (Kadowaki *et al.*, 1994a), suggesting that transcriptional activation of heat shock genes is required to continue export of RNA at elevated temperature. Examination of a number of strains that have deficiencies in individual heat shock proteins has not, however, led to the identification of a single heat shock mutant that displays conspicuous nuclear poly(A)⁺ RNA accumulation. The importance of heat shock proteins for RNA export might parallel observations on protein import into the nucleus, where the heat shock protein hsp70, or its cognate hsc70, are implicated (Imamoto *et al.*, 1992; Shi and Thomas, 1992). Moreover, *Xenopus* Hsc70 shuttles between the nucleus and the cytoplasm (Mandell and Feldherr, 1990). A direct interaction of heat shock proteins with nuclear and cytoplasmic poly(A)⁺ RNA-containing complexes has indeed been observed in *Drosophila* Kc cells (Kloetzel and

Bautz, 1983). By analogy with other members of the hsp70 family (for review see Craig, 1992), these proteins may act as molecular chaperones that (by virtue of their interaction with protein components of mRNA-protein complexes) cause these complexes to fold or refold into a conformation that is compatible with export.

HKE1/RAT1/TAP1, an essential gene required for poly(A)⁺ RNA export, encodes a nuclear protein with 5'→3' exoribonuclease (RNase) activity in vitro (Amberg *et al.*, 1992; Aldrich *et al.*, 1993; Kenna *et al.*, 1993). It displays homology to DST2/KEM1/RAR5/SEP1/XRN1 and also has DNase and DNA strand exchange activity, a possible role in recombination, DNA replication and cleavage of tetrastranded G4-DNA, RNA processing and turnover, microtubule function and karyogamy (for review see Kearsey and Kipling, 1991; Liu and Gilbert, 1994). Mutants in *HKE1/RAT1/TAP1* were independently isolated as suppressors of a tRNA promoter mutation as well as a suppressor of overproduction of a mutant form of transcription factor IID (Aldrich *et al.*, 1993; Di Segni *et al.*, 1993), suggesting that the activity exerted by *HKE1/RAT1/TAP1* is not limited to polymerase II transcribed genes. The poly(A)⁺ accumulation phenotype of *rat1* may result from its inability to degrade a class of (uncapped?) nuclear polymerase II transcripts that normally is unstable.

MTR1/PRP20/SRM1 encodes a homologue of the mammalian and *Schizosaccharomyces pombe* proteins, *RCC1* and *pim1/dcd* (Clark and Sprague, 1989; Aebi *et al.*, 1990; Matsumoto and Beach, 1991; Sazer and Nurse, 1994). Temperature-sensitive point mutations in these homologues also lead to nuclear accumulation of poly(A)⁺ RNA at the restrictive temperature (Amberg *et al.*, 1993; Kadowaki *et al.*, 1993). The *S. pombe* and hamster homologues are also involved in controlling progression through mitosis and chromosome condensation (Matsumoto and Beach, 1991; for review see Dasso, 1993; Sazer and Nurse, 1994), whereas the *S. cerevisiae* *MTR1* gene does not appear to be closely related to cell cycle progression. Nevertheless, human *RCC1* partially complements yeast strains deficient in *MTR1* (Fleischmann *et al.*, 1991). Reminiscent of the phenotype of *rna1* mutants, *mtr1* pleiotropically affects accumulation and processing of all classes of RNA (see above). The mutant *MTR1* protein is rapidly lost from the nucleus at the restrictive temperature (Amberg *et al.*, 1993). Judging from our experiments on epitope-tagged *MTR1* expressed in one nucleus of *kar1* × *wt* dikaryons, it is unlikely that *MTR1* itself shuttles in and out of the nucleus. Wild-type *MTR1* binds at least three GTP-binding proteins (Lee *et al.*, 1993). Most important, human *RCC1* acts as a guanine nucleotide exchange protein for Ran/TC4, a small, predominantly nuclear, GTPase of the Ras superfamily (Bischoff and Ponstingl, 1991). Much of the analysis of

RCC1 has been facilitated by the availability of the temperature-sensitive BHK cell mutant, tsBN2, which has a point mutation in *RCC1* (Nishimoto *et al.*, 1978).

Ran/TC4 is one of the most abundant cellular proteins. It is bidirectionally implicated in transport in the sense that poly(A)⁺ RNA accumulates in the nucleus upon depletion of its *S. cerevisiae* homologues, *CNR1/2* (Kadowaki *et al.*, 1993) and Ran/TC4 is required for import of NLS-bearing proteins into the nucleus of animal cells in vitro (Melchior *et al.*, 1993a; Moore and Blobel, 1993; for review see Moore and Blobel, 1994; Tartakoff and Schneider, 1995). The observations that the *MTR1* homologue *RCC1* acts as a guanine nucleotide exchange factor for Ran/TC4 (Bischoff and Ponstingl, 1991) and that a homologue of RNA1 acts as a GTPase-activating protein for Ran/TC4 (Melchior *et al.*, 1994; Bischoff *et al.*, 1995) are compatible with a simple model for Ran/TC4 function in export (Figure 4). This model postulates that Ran/TC4 shuttles in and out of the nucleus in association with cargo (Moore and Blobel, 1994). Thus, Ran/TC4 would assist cargo loading onto a putative carrier when nuclear *RCC1* promotes GTP binding to Ran/TC4, whereas the cytoplasmic RNA1 homologue would generate the GDP-bound form and therefore facilitate dissociation of the cargo-carrier complex. An equivalent model might explain Ran/TC4 function in import (Moore and Blobel, 1994). Consistent with such models, overexpression of the yeast homologues of Ran/TC4, *CNR1/GSP1*, and *CNR2/GSP2*, suppresses mutations in *MTR1/PRP20/SRM1* in allele-specific fashion (Belhumeur *et al.*, 1993; Kadowaki *et al.*, 1993). Interestingly, a mammalian protein that specifically interacts with GTP-charged Ran, termed Ran binding protein, contains a potential RNA-binding site (Coutavas *et al.*, 1993) and thus may constitute a missing link between carrier and cargo. A functional yeast homologue of this protein interacts with the GTP-bound form of *CNR1/GSP1* in vivo (Ouspenski *et al.*, 1994).

This nucleocytoplasmic GTPase cycle may also function in intranuclear steps of RNA transport: for example poly(A)⁺ RNA accumulates throughout the nucleoplasm (not at nuclear pores) in *mtr1* and tsBN2 cells (Kadowaki *et al.*, 1992, 1993; Amberg *et al.*, 1993) and newly-synthesized U3 RNA does not arrive in the nucleolus in tsBN2 cells at the restrictive temperature (Cheng *et al.*, 1995).

MTR2 displays weak homology to *mbeA*, one of four proteins encoded by the overlapping gene cluster of the *E. coli* plasmid ColE1, which is required for transfer of single-stranded plasmid DNA during bacterial conjugation (Kadowaki *et al.*, 1994b). Proteins essential for plasmid mobilization are conserved between related classes of plasmids (ColE1/ColK/ColA) and are thought to compose the machinery that specifically

Figure 4. Model for how the nucleocytoplasmic GTP/GDP-cycle might function. The small GTPase, CNR1/2, shuttles between the nucleus and the cytoplasm in association with a putative carrier of transport "cargo." In this model, the chromatin-bound guanine nucleotide exchange factor for CNR1/2, MTR1, promotes loading of the carrier with export substrate. The poly(A)⁺ RNA-loaded carrier is transported to the nuclear envelope by a diffusional or motor-driven mechanism and is exported through the nuclear pore complex. In the cytoplasm, GTPase activity stimulated by RNA1 is required for unloading cargo from the carrier which, in its GDP-bound state, shuttles back into the nucleus to begin an additional transport cycle. This model does not include the participation of the GTP/GDP-cycle in protein import or intranucleoplasmic events.

nicks plasmids at *oriT*, "pilots" the 5' end of the nicked strand through the pilus, recircularizes the DNA, and primes synthesis of the complementary strand in the recipient cell (Boyd *et al.*, 1989). It is too soon to judge whether there is a meaningful relation between transfer of single-stranded bacterial DNA and transport of mRNA from the eukaryotic nucleus. Two observations on *mtr2-1* are especially striking: 1) the nucleolus fragments to produce two to five foci that contain poly(A)⁺ RNA, and 2) this fragmentation is observed only when RNA polymerase II is active. The latter observation may reflect perturbation of an ongoing interaction between polymerase II transcripts and nucleolar proteins (see below).

MTR13/MTS1/NAB1/NOP3/NPL3 (Bossie *et al.*, 1992; Russell and Tollervey, 1992, 1995; Ellis and Reid, 1993; Wilson *et al.*, 1994; Singleton *et al.*, 1995), a protein implicated in both import and export from the nucleus (as well as mitochondrial protein targeting) contains two consensus RNA recognition motifs (RRM) as well as a glycine/arginine rich C-terminal domain (GAR domain) similar to that which is often found in nucleolar proteins (Girard *et al.*, 1992). In *mtr13-1* cells grown at the non-permissive temperature, nuclear accumulation of poly(A)⁺ RNA can be detected before the block in protein import, consistent with the possibility that the latter is a consequence of the former (Singleton *et al.*, 1995). Observations coming from *in vitro* protein import assays using semi-intact cells prepared from *npl3* mutants indicate that NPL3 is important for an early step in protein import: the association of NLS-bearing proteins with the nu-

clear envelope (Schlenstedt *et al.*, 1993). Considering that MTR13 is required for import and export and shuttles between cytoplasm and nucleus (Flach *et al.*, 1994), it may form part of the putative bidirectional transporter, regulated by the nucleocytoplasmic GTPase cycle, which we have discussed above (see Figure 4). The recent report that a functional NLS is required both for protein import into the nucleus and for export of proteins from the nucleus (Guiochon-Mantel *et al.*, 1994) also suggests mechanistic similarities between export and import.

A FUNCTION FOR THE YEAST NUCLEOLUS IN mRNA TRANSPORT?

The yeast nucleolus normally appears as a "gray" or "dense" crescent-shaped structure occupying more than one-third of the nuclear volume (Sillevis Smitt *et al.*, 1972, 1973). It lacks the obvious subcompartmentalization typically observed in nucleoli of higher eukaryotic cells and is much more extensively in contact with the nuclear envelope. Nevertheless, it appears functionally comparable to the mammalian cell nucleolus in that it is the compartment for ribosomal subunit assembly (for review see Busch and Smetana, 1970; Goessens, 1984; Bourgeois and Hubert, 1988; Warner, 1989; Scheer and Benavente, 1990; Woolford, 1991; Hurt *et al.*, 1992; Scheer *et al.*, 1993).

Ribosome biogenesis involves the assembly of rRNAs synthesized by RNA polymerase I (18S, 25S, and 5.8S) and by RNA polymerase III (5S) together with approximately 77 proteins translated from mRNAs

synthesized by RNA polymerase II. Not only must the synthesis and processing of these components be coordinated, but those that are synthesized in the cytoplasm or elsewhere in the nucleoplasm must be concentrated in the nucleolus (for review see Woolford and Warner, 1991). After assembly in the nucleolus, the large ribosomal subunits are released to (or possibly guided to) the nuclear pores at an estimated frequency of ~40 ribosomal subunits per second per pore in exponentially growing yeast (Tollervey *et al.*, 1991).

Perturbations of RNA polymerase I (Pol I) result in alterations of the nucleolus of both yeast and mammalian cells (Benavente *et al.*, 1987; Hirano *et al.*, 1989; Oakes *et al.*, 1993). For example, in yeast strains bearing temperature-sensitive alleles of the largest subunit of Pol I, RPA190 (Wittekind *et al.*, 1988), the intact nucleolar structure is replaced by "mininucleolar bodies" (Oakes *et al.*, 1993; Figure 2). In animal cells, pharmacological inhibition of Pol I causes "segregation" of the otherwise intermixed domains of the nucleolus (Busch and Smetana, 1970).

Several observations suggest that association of poly(A)⁺ RNA with yeast nucleolar Ags is functionally significant; they are as follows: 1) In five of the RNA transport mutants listed in Table 1, nuclear accumulation of RNA coincides with fragmentation of the crescent-shaped yeast nucleolus. Because inhibition of RNA polymerase II itself does not cause such fragmentation (Oakes *et al.*, 1993; Kadowaki *et al.*, 1994b), this phenotype is not simply the result of a lack of availability of ribosomal proteins. Thus, the yeast nucleolus may have some more direct function in RNA transport (see Table 1; Figure 2). Moreover, in *mtr1-1*, *mtr2-1*, and *rpa190-3* (ts for the nucleolar protein, Pol I) nucleolar Ags colocalize with foci containing accumulated poly(A)⁺ RNA (Kadowaki *et al.*, 1994b; see Table 1; Figure 2). Mutations in several other proteins which affect rRNA processing, however, do not cause nuclear accumulation of poly(A)⁺ RNA (Kadowaki *et al.*, 1994b).

2) Nucleolar fragmentation is observed in *mtr1-1* and *mtr2-1* only if RNA polymerase II is active. This observation suggests that nucleolar fragmentation is a consequence of the nuclear accumulation of poly(A)⁺ RNA (Kadowaki *et al.*, 1994b).

3) The poly(A)⁺ RNA accumulation in *mtr3*, *mtr14*, and *mtr17* is actually coincident with a nonfragmented nucleolus (Kadowaki *et al.*, 1994a).

4) In yeast, at least some snRNAs, possibly including those which are essential for pre-mRNA processing, are contained within the nucleolus (Potashkin *et al.*, 1990). The splicing protein PRP6, on the other hand, is concentrated within 8–12 discrete subregions of the yeast nucleoplasm, a pattern similar to the speckled distribution typically observed for mammalian splicing components (Elliott *et al.*, 1992; for review see Lamond and Carmo-Fonseca, 1993; Spector, 1993).

5) The incidence of introns among ribosomal protein transcripts is disproportionately high in yeast (Woolford and Warner, 1991; Rymond and Rosbash, 1992) and specific ribosomal proteins, known to be concentrated in the nucleolus, do regulate their own splicing (Li and Woolford, 1994; Vilardell and Warner, 1994). Although nucleolar components may possess binding sites for all classes of RNA and the nucleolus might be a default destination for RNAs that are not properly transported, these observations are also consistent with the possibility that nucleolar proteins are critical for mRNA export.

DOES THE METAZOAN CELL NUCLEOLUS FUNCTION IN mRNA MATURATION AND/OR TRANSPORT?

Although it is possible that the yeast nucleolus and animal cell nucleolus are not functionally equivalent, a possible role of the nucleolus of mammalian cells in maturation and/or transport of Pol II transcripts is suggested by several observations: 1) A possible function of the nucleolus in mRNA export was proposed 25 years ago based on observations of interspecies heterokaryons obtained from fusing chick erythrocytes with mouse cells. In these experiments the dormant chick nucleus was observed to initiate gene expression at precisely the time when a nucleolus became detectable (Sidebottom and Harris, 1969; Deák *et al.*, 1972; Harris, 1972). Furthermore, UV irradiation of the chick nucleolus in these heterokaryons greatly suppressed chick-specific gene expression (Perry *et al.*, 1961; Deák *et al.*, 1972).

2) Processed *myc* and *myoD* transcripts, unlike actin or lactate dehydrogenase transcripts, have been localized by in situ hybridization to the nucleolus of several cell types (Bond and Wold, 1993). In these experiments, *myc* intron 1-containing pre-mRNA was absent from nucleoli and instead was detected in the nucleoplasm. It was suggested that nucleolar localization of Pol II transcripts is a general phenomenon, detectable only for transcripts that have a rapid cytoplasmic turnover (Bond and Wold, 1993). Moreover, in cells from sea urchins to humans, nuclear poly(A)⁺ RNA is found primarily in discrete "transcript domains", which often concentrate around nucleoli (Carter *et al.*, 1991) and at least one hnRNP protein (hnRNP I, the polypyrimidine tract-binding protein) is also found adjacent to the nucleolus (Ghetti *et al.*, 1992). Thus, the region around the nucleolus, as well as the nucleolus itself, might play a role in mRNA transport and/or processing (Carter *et al.*, 1991).

3) The posttranscriptional regulators of human immunodeficiency virus HIV and of human T-cell leukemia virus HTLV-I, Rev and Rex proteins, promote the export of certain unspliced pre-mRNAs. *Cis*-acting elements within these viral RNAs, called Rev-respon-

sive elements (RRE; Rosen *et al.*, 1988) or Rex-responsive regions (RxRE; Seiki *et al.*, 1988) are required for Rev and Rex binding. It has been suggested that these proteins either dissociate spliceosomes before the splicing reaction is complete (Chang and Sharp, 1989), or establish a rapid, splicing-independent pathway for pre-mRNA transport (Felber *et al.*, 1989; Malim and Cullen, 1993; Fischer *et al.*, 1994). What is striking is that Rev (Cullen *et al.*, 1988; Cochrane *et al.*, 1990) and Rex (Siomi *et al.*, 1988) localize to the nucleolus, thus raising the possibility that the path of transport of unspliced viral RNA actually involves the nucleolus. Moreover, Rev has a high affinity for the nucleolar protein B23 (Frankhauser *et al.*, 1991) and in the presence of Rex, significant nucleolar localization of unspliced (*env*-encoding) transcripts is observed (Kalland *et al.*, 1991). Nevertheless, more recent investigations of the relevance of their nucleolar localization indicates that these viral regulators can function independent of having a predominant steady-state nucleolar localization (McDonald *et al.*, 1992). Because Rev-dependent regulation of RRE-containing transcripts can be partly recapitulated in yeast, it may be possible to investigate this export path with the aid of yeast genetics (Stutz and Rosbash, 1994).

There are a number of additional observations which, without being conclusive, hint at a possible function of the mammalian nucleolus in processing and/or transport of RNA polymerase II transcripts. Resolving some of them may prove relevant to our future understanding of the nucleolus. 1) hnRNP proteins (Piñol-Roma and Dreyfuss, 1993), the U1A snRNP protein (Kambach and Mattaj, 1992), several nucleolar proteins (Borer *et al.*, 1989; Meier and Blobel, 1992), and Rev (Kalland *et al.*, 1994; Meyer and Malim, 1994) shuttle between nucleus and cytoplasm. In the case of hnRNP protein A1 and the nucleolar protein B23, nuclear relocation is dependent on ongoing transcription by RNA polymerase II. Considering that at least the hnRNP proteins associate with poly(A)⁺ RNA in both the nucleus and the cytoplasm, it will be interesting to learn whether any of the "nucleolar" proteins also bind poly(A)⁺ RNA and may function in mRNA transport, either in or outside the nucleolus (for review see Piñol-Roma and Dreyfuss, 1993).

2) Heat shock disrupts the mammalian cell nucleolus (Simard and Bernhard, 1967), causes uridine-labeled RNA of unknown identity to accumulate in the nucleolus (Simard *et al.*, 1968), and promotes re-localization of hsp70 to the nucleus and the nucleolus (Welch and Feramisco, 1984; Lewis and Pelham, 1985). Judging from these observations, and our finding that heat shock transcription factor is required for RNA transport in yeast (see above; Table 1) it will be important to learn how heat shock affects the subcellular distribution of poly(A)⁺ RNA.

3) The influenza virus NS1 protein, which induces a generalized block of mRNA export from the nucleus by binding to the poly(A) tail (Alonso-Caplen *et al.*, 1992; Lu *et al.*, 1994; Qiu and Krug, 1994), is concentrated at defined sites within the nucleus (Fortes *et al.*, 1994). It is not known whether these sites include the nucleolus.

Another, more biochemical, literature also bears on the question of whether the nucleolus might have a function in mRNA transport. Nonsense mutations resulting in premature termination of translation of certain mRNAs, e.g. dihydrofolate reductase and triosephosphate isomerase, give rise to reduced levels of nuclear and cytoplasmic message *without* reducing the rate of their transcription or enhancing their cytoplasmic decay. This yet unexplained observation might reflect "nuclear scanning" of transcripts by (pre)ribosomal particles, or some sort of coordination between translocation and translation of newly exported mRNA (Urlaub *et al.*, 1989; Cheng and Maquat, 1993). In the case of triosephosphate isomerase, tRNA and ribosomes coordinately mediate these effects (Belgrader *et al.*, 1993). It is equally intriguing that nonsense mutations induce exon skipping in an allele of the fibrillin gene of a patient with Marfan syndrome (Dietz *et al.*, 1993) and affect splicing in *cis* of the R2 transcript of minute virus of mice (Naeger *et al.*, 1992). Both of these surprising observations show that exon recognition is influenced by the coding capacity of the (processed) transcript. Although these phenomena may reflect translation within the chromatin-rich nucleoplasm (Goidl and Allen, 1978), we hypothesize that scanning actually occurs at the site where ribosomal subunits are already concentrated and the minute virus replicates (Walton *et al.*, 1989), the nucleolus.

The isolation and characterization of a dozen yeast mutants defective in mRNA transport has considerably broadened our understanding of this process. The fact that four of the mutants are defective in components of the nuclear pore complex confirms the central role of this macromolecular structure in nuclear import and export. The finding that several of the components required for export are equally important for import led to the identification of a nucleocytoplasmic GTPase cycle, thus paralleling the central role that GTPase-based molecular switches play in other intracellular transport events, e.g. protein translocation and vesicular transport. Finally, the observation that many transport mutants affect the structure of the nucleolus might point to a novel function of this subnuclear organelle. Whether this transport function is restricted to yeast or is a general property of the nucleolus, as suggested by the several lines of circumstantial evidence outlined above, remains to be established.

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