ratio in the lower plume is high. Because of the higher pressure beneath the sea floor, we suspect that the ^{3}He in the dike is not released immediately but rather is partitioned into the liquid remaining in the dike interior. Upon solidification and continued hydrothermal cooling, ^{3}He from the dike may be transported to the lower plume. In the absence of additional magma, however, the ^{3}He/heat ratio there will gradually return to a lower level.

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Diverse Effects of the Guanine Nucleotide Exchange Factor RCC1 on RNA Transport

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Transport of RNAs within nuclei and through nuclear pore complexes (NPCs) are essential, but poorly understood, steps in gene expression. In experiments with mammalian cells, RCC1, the abundant nuclear guanine nucleotide exchange factor for the guanosine triphosphatase Ran/TC4, was shown to be required for nucleocytoplasmic transport of precursors of spliceosomal small nuclear RNAs (snRNAs), intranuclear transport of U3 snRNA, and processing of ribosomal RNAs, but not for export of transfer RNAs. It is proposed that guanosine triphosphate (GTP)–bound Ran/TC4 associates with ribonucleoprotein particles (RNPs) during intranuclear movement, and that GTP hydrolysis promotes deposition of RNPs at targeted sites such as NPCs or nucleoli.

Eukaryotic RNAs are generally processed in cell compartments different from those in which they function, making transport a crucial phase in their metabolism (*1*). Much remains to be learned about the mechanisms of transport of RNAs and RNPs (*2*) to and through NPCs (*3*).

An intriguing protein that appears to function in the export of mRNAs is the product of the *RCC1* gene in mammalian cells (*4*) and the homologous gene in yeast, *PRP20* (*5*), also known as *MTR1* (*6*); inactivation of RCC1 or Prp20 proteins results in accumulation of polyadenylated [poly(A)$^+$] RNAs within nuclei (*5*, *6*). The RCC1 protein, originally identified in tsBN2 mutant hamster cells as a regulator of chromosome condensation (*4*), is required for many processes of mammalian cells, including initiation of DNA synthesis and progression of the cell cycle (*7*). This nuclear protein functions as a guanine nucleotide exchange factor (GEF) for the Ras-like guanosine triphosphatase (GTPase) Ran/TC4 (*8*). Although a small but significant fraction of Ran/TC4 is present in the cytoplasm where it functions in protein import (*9*, *10*), the role of RCC1 in this process has not been established. Overproduction of the yeast homolog of Ran/TC4 can suppress certain *prp20/mtr1* mutations (*6*, *11*), and GTP hydrolysis by this protein is needed for export of poly(A)$^+$ RNAs from nuclei (*12*); by analogy, nuclear RCC1 and Ran/TC4 may collaborate in RNA transport in higher organisms.

To investigate the role of RCC1 protein in nuclear RNA transport, we analyzed the metabolism and intracellular distribution of several other classes of RNAs synthesized in tsBN2 cells depleted of this protein (*4*). We show here that RCC1 participates in the transport of some but not all RNAs within nuclei and propose that it does so by promoting the generation of GTP-bound Ran/TC4 [(GTP)-Ran/TC4], which complexes with RNPs and allows their movement through the nucleoplasm to specific sites. The accumulation of poly(A)$^+$ or other RNAs in nuclei depleted of RCC1 would result from inefficient delivery of the RNAs to NPCs in the absence of (GTP)-Ran/TC4.

Precursors of most spliceosomal small nuclear RNAs (pre-snRNAs) undergo maturation only after they have been exported to the cytoplasm and have bound a complex of proteins, the Sm antigens (*13*). The 5′ m^{7}G-caps of pre-snRNAs are then converted to hypermethylated m^{2,2,7}G-caps and several nucleotides are trimmed off the 3′ ends before the mature snRNAs are transported back into the nucleus as snRNPs. Because small RNAs leak out of nuclei during cell fractionation (*14*), we used these cytoplasmic modifications as indicators of whether the RNAs had been exported from the nuclei of intact cells.

A shift of tsBN2 cells to the nonper-

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missive temperature (40°C) resulted in a dramatic reduction in the accumulation of $m^{2,2,7}$G-capped mature spliceosomal snRNAs U1, U2, U4, and U5 in tsBN2 cells (*15*) (Fig. 1, compare lanes 1 and 2). This change was not observed in wild-type baby hamster kidney (BHK21) cells (Fig. 1, lanes 3 and 4), indicating that it was due to loss of RCC1. The levels of m^7G-capped, untrimmed pre-U1 and pre-U2 RNAs increased concomitantly in the mutant cells depleted of RCC1 but not in wild-type cells (lanes 5 and 7) (*16*).

The block in maturation was not general to all pre-snRNAs synthesized by RNA polymerase II, as shown by analysis of the newly made U3 RNA. Unlike the spliceosomal snRNAs, pre-U3 RNA is not transported to the cytoplasm but is matured in the nucleus (*17*). Both 5′ cap hypermethylation and 3′ end trimming of U3 RNA proceeded normally in the absence of RCC1 (Fig. 1, lane 1), although the level of accumulated RNA was slightly reduced, perhaps because of changes in the location of U3 RNA within the nucleus (see below). Thus, RCC1 function is required for maturation of only those pre-snRNAs that must first exit the nucleus.

Hypermethylation of the 5′ caps of spliceosomal pre-snRNAs, but not of pre-U3 RNA, requires association of the RNAs with Sm proteins (*13*). The spliceosomal pre-snRNAs that accumulated after RCC1 depletion were not complexed with Sm proteins (Fig. 2A) (*18*), explaining why the RNAs were not modified. These complexes may not have formed either because the RNAs were sequestered in the nucleus or because the Sm proteins were not available in the cytoplasm. The latter possibility is unlikely, as Sm proteins continued to be synthesized in mutant cells for several hours after the processing of precursor snRNAs had ceased (Fig. 2B). Furthermore, the Sm proteins present in cytoplasmic extracts of tsBN2 cells depleted of RCC1 were functional, as demonstrated by their ability to assemble with in vitro–made U1 RNA into snRNPs precipitable with antibodies to Sm protein (Fig. 2C). Thus, the absence of 5′ cap hypermethylation is best explained by blockage of transport of pre-snRNAs to the cytoplasm, and we conclude that RCC1 is required for this process.

The absence of maturation of spliceosomal pre-snRNAs in tsBN2 cells is one of the earliest changes observed after inactivation of RCC1. Within 60 min of the shift to the nonpermissive temperature, the pre-U2 RNA accumulated as a faster migrating form (*19*) (Fig. 3, bottom). A higher steady-state level of accumulated pre-U2 RNA was reached within 2 hours of temperature shift and persisted for several hours (Fig. 3, top). The time course of these transport-related changes closely paralleled that of RCC1 disappearance (*4*), indicating that the loss of RCC1 has an immediate effect on the transport of this RNA.

U3 RNA, which is matured by enzymes in the nucleoplasm (*17*), participates in ribosomal RNA (rRNA) processing in the nucleolus (*20*). Upon cell fractionation, U3 RNA normally is found in the nuclear rather than the cytoplasmic fraction (Fig. 4A, lanes 3 to 8), presumably because it is sequestered in nucleoli (*21*). However, when extracts were prepared from tsBN2 cells incubated at 40°C, over half of the newly made, $m^{2,2,7}$G-capped U3 RNA was released into the cytoplasmic fraction (Fig. 4A, lanes 1 and 2), indicating that this U3 RNA was not in nucleoli. This aberrant fractionation did not result from a general disintegration of nucleoli, because previously synthesized U3 RNA remained in the nuclear fraction (Fig. 4B)

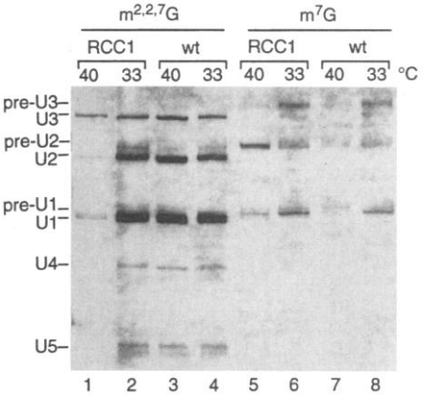

Fig. 1. Synthesis and processing of snRNAs in the absence of RCC1 protein. Temperature-sensitive tsBN2 (RCC1) and wild-type BHK21 (wt) cells were pulse-labeled for 2 hours with [^{3}H]uridine (150 μCi/ml) (Amersham) after 5 hours of incubation at the permissive (33°C) or nonpermissive (40°C) temperature to deplete RCC1 from tsBN2 cells (*4*). The mature, $m^{2,2,7}$G-capped and precursor, m^7G-capped forms of snRNAs were analyzed in an 8% polyacylamide gel and visualized by fluorography (*15*). The differential accumulation of precursors reflects the stabilities of individual types of RNAs (*16*).

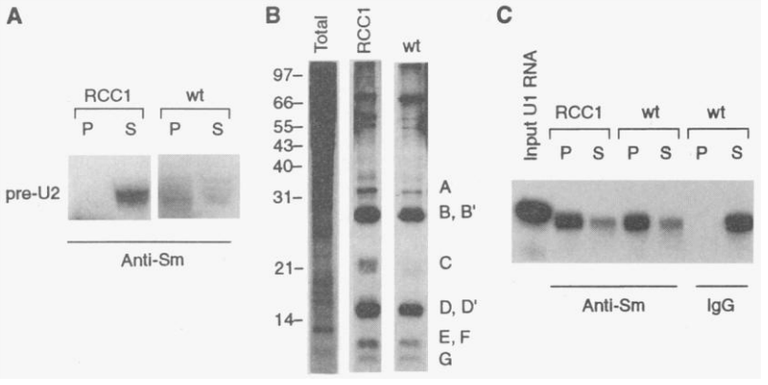

Fig. 2. Restricted access of pre-snRNAs to cytoplasmic Sm proteins in RCC1-deficient cells. (**A**) Absence of complex formation between pre-U2 RNA and Sm proteins. The snRNAs complexed with Sm proteins were precipitated with Y12 anti-Sm monoclonal antibodies (*28*). Pre-U2 RNA in the precipitate (P) and supernatant (S) fractions was separated from mature U2 RNA and detected by RNA blot analysis with a ^{32}P-labeled antisense U2 RNA probe. (**B**) Synthesis of Sm proteins after 4 hours of preincubation at 40°C as monitored by labeling with [^{35}S]methionine (*28*). Molecular size markers (in kilodaltons) are noted on the left. (**C**) In vitro assembly of U1 snRNPs. ^{32}P-labeled, m^7G-capped U1 RNA made by in vitro transcription with SP6 RNA polymerase (*17*) was reconstituted into U1 snRNPs by incubation with cytoplasmic extracts of tsBN2 or wild-type cells (*29*). The efficiency of snRNP assembly was determined by immunoprecipitation with Y12 antibodies. Nonspecific immunoglobulin G (IgG) was used as a control.

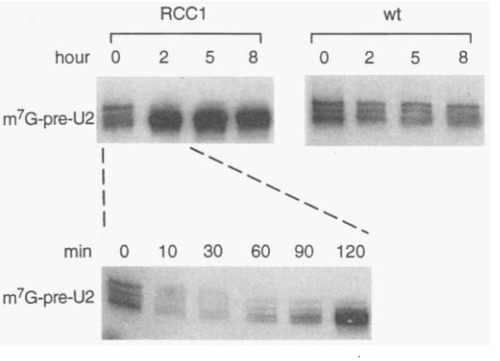

Fig. 3. Kinetics of accumulation of pre-U2 RNA upon loss of the RCC1 protein. Total RNA was isolated after tsBN2 and wild-type BHK21 cells were incubated at 40°C for the indicated periods. The immunoprecipitated m^7G-capped pre-U2 RNA (*15*) from 2.5 × 10^5 cell equivalents of total RNAs was separated by electrophoresis in a high-resolution gel and analyzed by RNA blot hybridization as in Fig. 2A.

and the nucleolar structure appeared to be essentially unaltered, as visualized by staining with antibodies to fibrillarin (Fig. 4C, α-Fib) and by electron microscopy (*22*). Because it is unlikely that RCC1 is required for nucleolar retention of the newly made but not the preexisting U3 RNA, our data are best explained by a model in which intranuclear transport of the RNA to the nucleolus requires RCC1 function.

Also apparent was a redistribution of $m^{2,2,7}$G-capped spliceosomal snRNAs to larger speckles (Fig. 4C, α-TMG), consistent with the results of others who have shown changes in the location of splicing components such as Sm and SC-35 proteins (*22*) and of nuclear poly(A)$^+$ RNA (*6*). Thus, the intranuclear trafficking of several nuclear RNPs appears to be disrupted upon depletion of RCC1.

The defect in intranuclear transport of U3 RNA observed in the absence of RCC1 (Fig. 4A) prompted us to ask if nucleolar function was also affected. Strikingly, the accumulation of 18*S*, 28*S*, and 5.8*S* rRNAs, all of which are processed in the nucleolus from a single 45*S* rRNA precursor, was severely curtailed (Fig. 5, A and B), as has also been observed in yeast (*6*). Very little, if any, mature 18*S* and 28*S* rRNA was exported to the cytoplasm (Fig. 5A, lane 3) and processing of large RNAs appeared to be incomplete (Fig. 5, lane 2). This disruption of ribosome maturation and export could occur if transport of labile factors or ribosomal proteins to the nucleolus were impaired, although a direct role of RCC1 in the formation of rRNA processing structures cannot be ruled out.

Surprisingly, depletion of the RCC1 protein appeared to have little effect on the accumulation and the nucleocytoplasmic distribution of tRNAs (Fig. 5B, lanes 2 to 5). To distinguish between bona fide nuclear export of tRNAs in intact cells and leakage of tRNAs from nuclei during cell fractionation, we determined if newly made tRNAs in the cytoplasmic fractions cosedimented with polyribosomes. Transfer RNAs that had been exported would have the opportunity to be recruited onto polyribosomes, whereas those that had leaked from nuclei during cell fractionation would not. Radioactively labeled tRNAs made in the absence of RCC1 were present in the polyribosome fraction (*23*) (Fig. 5C, lane 1), demonstrating that export of tRNA to the cytoplasm was independent of RCC1 function. Moreover, the ratios of labeled tRNA to either 5*S* or 5.8*S* rRNAs was much higher in tsBN2 cells than in wild-type cells (compare lanes 1 and 2), as would be expected if loss of RCC1 disrupted the processing and export

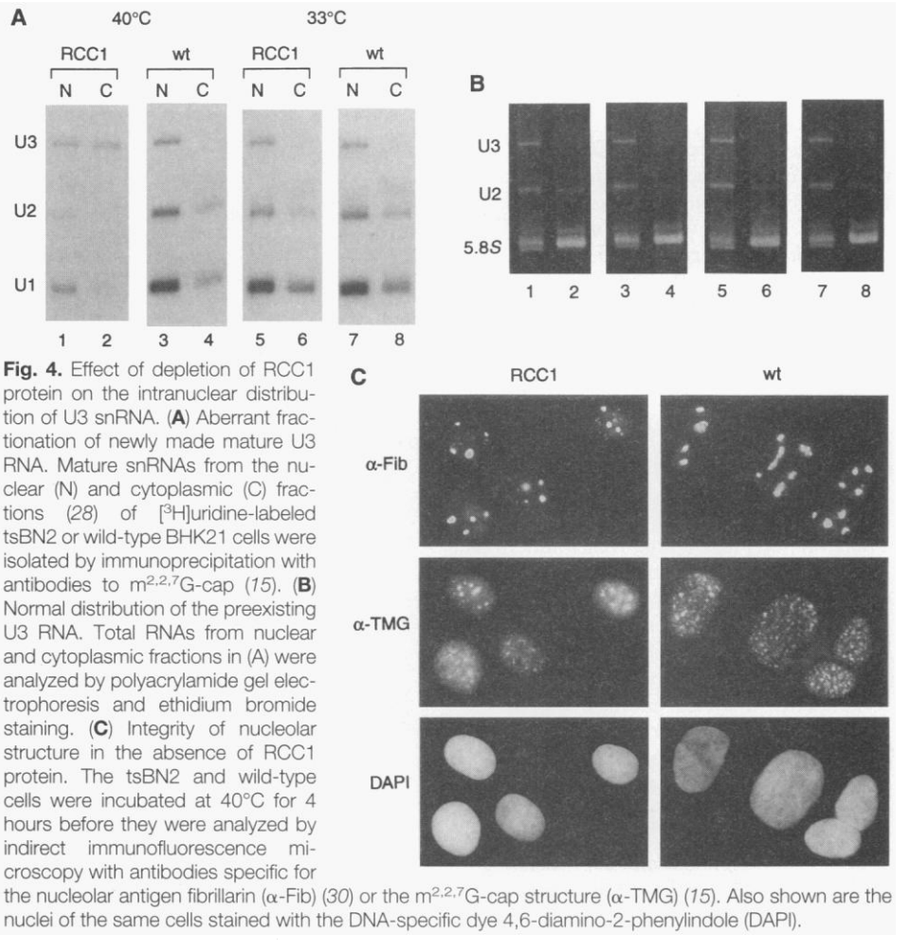

Fig. 4. Effect of depletion of RCC1 protein on the intranuclear distribution of U3 snRNA. (**A**) Aberrant fractionation of newly made mature U3 RNA. Mature snRNAs from the nuclear (N) and cytoplasmic (C) fractions (*28*) of [^{3}H]uridine-labeled tsBN2 or wild-type BHK21 cells were isolated by immunoprecipitation with antibodies to $m^{2,2,7}$G-cap (*15*). (**B**) Normal distribution of the preexisting U3 RNA. Total RNAs from nuclear and cytoplasmic fractions in (A) were analyzed by polyacrylamide gel electrophoresis and ethidium bromide staining. (**C**) Integrity of nucleolar structure in the absence of RCC1 protein. The tsBN2 and wild-type cells were incubated at 40°C for 4 hours before they were analyzed by indirect immunofluorescence microscopy with antibodies specific for the nucleolar antigen fibrillarin (α-Fib) (*30*) or the $m^{2,2,7}$G-cap structure (α-TMG) (*15*). Also shown are the nuclei of the same cells stained with the DNA-specific dye 4,6-diamino-2-phenylindole (DAPI).

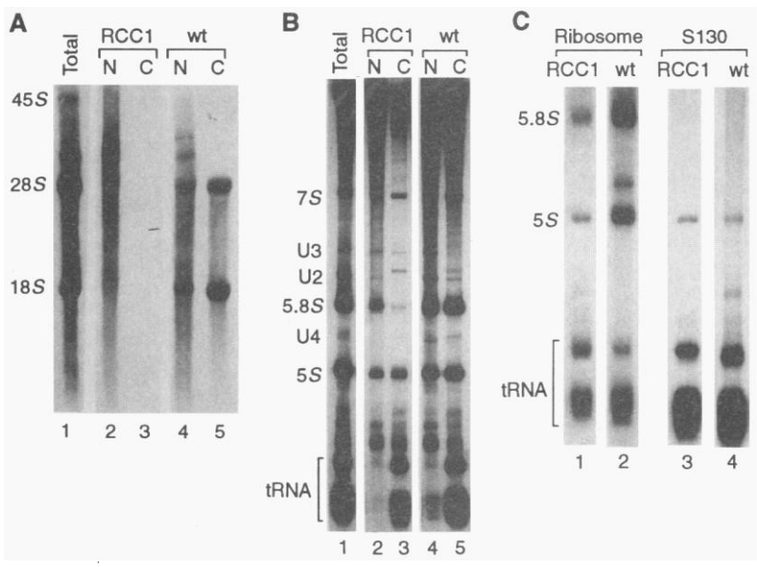

Fig. 5. Synthesis and distribution of ribosomal and transfer RNAs made in the absence of RCC1 protein. The newly made RNAs from nuclear (N) and cytoplasmic (C) fractions (*28*) were separated on a 1% agarose-formaldehyde denaturing gel (**A**) or an 8% polyacrylamide gel (**B**) and visualized by fluorography. In (A), twice as much total RNA was loaded in lanes 2 and 3 to compensate for the reduction in synthesis of rRNA in tsBN2 cells at the nonpermissive temperature. (**C**) Association of newly made tRNAs with polyribosomes. The tsBN2 or wild-type BHK21 cells were preincubated for 4 hours at 40°C and labeled with [H^3]uridine (100 μCi/ml) for 6 hours. The newly made transfer and ribosomal (5*S* and 5.8*S*) RNAs in the ribosome pellet and cytosolic supernatant (S130) fractions were analyzed by electrophoresis in an 8% polyacrylamide gel (*23*).

of rRNAs and ribosomal subunits (Fig. 5, A and B) but not of tRNA. Thus, in contrast to pre-snRNAs, tRNAs do not require RCC1 function for transport through the NPCs.

The diverse effects of RCC1 on RNA transport could be accounted for by its ability to regenerate the GTP-associated form of Ran/TC4 (*8*). Both RCC1 and Ran/TC4 are predominantly nuclear proteins (*4*, *8*) and the yeast homolog of Ran/TC4 has been directly implicated in the export of poly(A)$^+$ RNAs (*12*). The speed with which nuclear export of pre-snRNAs is inhibited (Fig. 3) indicates a close coupling of RNA transport and RCC1 function. However, transit of RNAs through the NPCs can occur in the absence of RCC1, as tRNAs made in RCC1-depleted cells accumulated in the cytoplasm (Fig. 5, B and C). Therefore, we propose that RCC1 functions in a step that is a prerequisite for the export of pre-snRNAs and poly(A)$^+$ RNAs, that is, transport to—rather than through—the NPCs. Similarly, RCC1 may be required for intranuclear transport to the nucleolus of components that are essential for rRNA processing or for ribosome assembly and transport to the cytoplasm. Export of tRNAs in the absence of RCC1 (Fig. 5C) may be facilitated by their small size and the localization of some tRNA processing enzymes near the nuclear envelope (*24*).

The proposal that RCC1 is required for intranuclear trafficking introduces a mechanism for specificity of RNA movement that is compatible with a channeled diffusion model (*25*). A model for the role of Ran/TC4 in translocation of proteins and RNAs through NPCs was proposed (*10*); by analogy, we suggest that (GTP)-Ran/TC4, generated by the chromatin-associated RCC1, forms complexes with newly made RNPs, promoting their diffusion through the nucleoplasm. Interaction of such complexes with an RNP-specific GTPase-activating protein (GAP) at a target site would result in GTP hydrolysis by Ran/TC4, leading to deposition of the RNP. Thus, the locations of GAP proteins would impose a net direction of movement of RNAs, resulting in high local concentrations of RNAs at certain sites such as the NPC or nucleolus. Several GAPs specific for Ran/TC4 have been identified in nuclei (*26*), but their precise locations and substrate specificities remain to be established.

Finally, we note that loss of RCC1 affects the maturation of pre-snRNAs in a manner closely resembling changes in host snRNA metabolism that occur in cells infected by vesicular stomatitis virus (*27*). We hypothesize that this cytoplasmic virus could inactivate the RCC1-Ran/TC4 system through production of a specific gene product(s).

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23. [H^3]-uridine labeled cells (4×10^7) were homogenized in 2 ml of 0.1% Triton-X100, 10 mM tris-acetate (pH 7.6), 1 mM potassium acetate, 2 mM magnesium acetate, 2 mM dithiothreitol (DTT). Polyribosomes were pelleted from the homogenates by sedimentation through a 30% sucrose cushion at 130,000*g* (*31*), and RNAs were prepared from the ribosome pellet and S130 supernatant fractions. The overall rate of protein synthesis in tsBN2 cells at 40°C declined by a factor of 5 within 10 hours after temperature shift (*4*, *18*), probably because of lower levels of cytoplasmic mRNAs. To compensate for the reduction in protein synthesis and active polyribosomes, we exposed the autoradiograph of lane 1 in Fig. 5C five times longer than that of other lanes. This normalization shows that comparable levels of newly made tRNAs were present in the pools of functional cytoplasmic tRNAs in both cell types (compare lanes 1 and 2). In other experiments (*18*) it was shown that the labeled tRNAs cosedimented with polyribosomes in a sucrose gradient and were released by treatment with EDTA. Furthermore, labeled tRNAs added to unlabeled cytoplasmic extracts did not associate with polyribosomes during cell fractionation.
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28. Nuclear and cytoplasmic fractions were prepared from tsBN2 or wild-type BHK21 cells that had been incubated at 40°C for 5 hours. Cells were lysed on ice by treatment with 0.5% NP-40 in TD buffer [25 mM tris-HCl (pH 7.6), 136 mM NaCl, 5 mM KCl, and 0.37 mM Na_2HPO_4] containing 0.5 unit/μl RNasin, 2 mM dithiothreitol, and 2 μg/ml of aprotinin, leupepsin, and pepstatin. Nuclei were isolated by pelleting at 1000*g*, at 4°C. The supernatant, which contained cytoplasmic RNAs plus those snRNAs that had leaked from the nuclei during preparation (*14*), was taken as the cytoplasmic extract. SnRNPs in cytoplasmic extracts were immunoprecipitated with the Y12 anti-Sm antibody [E. A. Lerner, C. A. Janeway Jr., J. A. Steitz, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2737 (1981)]. For analysis of Sm proteins, cells were labeled with [^{35}S]methionine (83 μCi/ml) for 3 hours at 40°C and cytoplasmic extracts were prepared. The immunoprecipitated Sm proteins were separated by electrophoresis in a 12% SDS-polyacrylamide gel and visualized by fluorography.
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