for gene therapy, new anti-bacterial drugs and to better understand the origin of the deoxyribonucleotide salvage-pathway enzymes.

The TK1 structure [9] has clearly illustrated that the catalysts of various biochemical reactions could be developed in nature using several alternative methods: employing different protein folds and engaging different amino acid residues in the active center. Although the dCK/dGK/TK2 family has relaxed substrate specificity, the TK1 family seems to be more conserved. However, in the future it will be important to determine whether TK1 could be mutated to change its nucleoside substrate preference, and if such mutations have also occurred in nature.

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References

1 Thelander, L. and Reichard, P. (1979) Reduction of ribonucleotides. *Annu. Rev. Biochem.* 48, 133–158
2 Arner, E.S.J. and Eriksson, S. (1995) Mammalian deoxyribonucleoside kinases. *Pharmacol. Ther.* 67, 155–186
3 Eriksson, S. *et al*. (2002) Structure and function of cellular deoxyribonucleoside kinases. *Cell. Mol. Life Sci.* 59, 1327–1346
4 Piskur, J. *et al*. (2004) Animal deoxyribonucleoside kinases: 'forward' and 'retrograde' evolution of their substrate specificity. *FEBS Lett.* 560, 3–6
5 Attardi, G. and Schatz, G. (1988) Biogenesis of mitochondria. *Annu. Rev. Cell Biol.* 4, 289–333
6 Mandel, H. *et al*. (2001) The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. *Nat. Genet.* 29, 337–341
7 Saada, A. *et al*. (2001) Mutant mitochondrial thymidine kinase in mitochondrial DNA depletion myopathy. *Nat. Genet.* 29, 342–344
8 Dobrovolsky, V.N. *et al*. (2003) Mice deficient for cytosolic thymidine kinase gene develop fatal kidney disease. *Mol. Genet. Metab.* 78, 1–10
9 Welin, M. *et al*. (2004) Structures of thymidine kinase 1 of human and mycoplasmic origin. *Proc. Natl. Acad. Sci. U. S. A.* 101, 17970–17975
10 Brown, D.G. *et al*. (1995) Crystal structures of the thymidine kinase from herpes simplex virus type-1 in complex with deoxythymidine and ganciclovir. *Nat. Struct. Biol.* 2, 876–881
11 Wild, K. *et al*. (1995) The three-dimensional structure of thymidine kinase from herpes simplex virus type 1. *FEBS Lett.* 368, 289–292
12 Munch-Petersen, B. *et al*. (1998) Four deoxynucleoside kinase activities from *Drosophila melanogaster* are contained within a single monomeric enzyme, a new multifunctional deoxynucleoside kinase. *J. Biol. Chem.* 273, 3926–3931
13 Johansson, K. *et al*. (2001) Structural basis for substrate specificities of cellular deoxyribonucleoside kinases. *Nat. Struct. Biol.* 8, 616–620
14 Lavie, A. *et al*. (1997) Structure of thymidylate kinase reveals the cause behind the limiting step in AZT activation. *Nat. Struct. Biol.* 4, 601–604
15 Sabini, E. *et al*. (2003) Structure of human dCK suggests strategies to improve anticancer and antiviral therapy. *Nat. Struct. Biol.* 10, 513–519
16 Sawaya, M.R. *et al*. (1999) Crystal structure of the helicase domain from the replicative helicase-primase of bacteriophage T7. *Cell* 99, 167–177
17 Knecht, W. *et al*. (2002) A few amino acid substitutions can convert deoxyribonucleoside kinase specificity from pyrimidines to purines. *EMBO J.* 21, 1873–1880
18 Rainov, N.G. (2000) A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. *Hum. Gene Ther.* 11, 2389–2401
19 Zheng, X. *et al*. (2000) Retroviral transduction of cancer cell lines with the gene encoding *Drosophila melanogaster* multisubstrate deoxyribonucleoside kinase. *J. Biol. Chem.* 275, 39125–39129
20 Greco, O. and Dachs, G.U. (2001) Gene directed enzyme/prodrug therapy of cancer: historical appraisal and future prospectives. *J. Cell. Physiol.* 187, 22–36
21 Black, M.E. *et al*. (1996) Creation of drug-specific herpes simplex virus type 1 thymidine kinase mutants for gene therapy. *Proc. Natl. Acad. Sci. U. S. A.* 93, 3525–3529
22 Ives, D.H. and Ikeda, S. (1998) Life on the salvage path: the deoxynucleoside kinase of Lactobacillus acidophilus R-26. *Prog. Nucleic Acid Res. Mol. Biol.* 59, 205–255
23 Andersen, R.B. and Neuhard, J. (2001) Deoxynucleoside kinases encoded by the yaaG and yaaF genes of *Bacillus subtilis*. Substrate specificity and kinetic analysis of deoxyguanosine kinase with UTP as the preferred phosphate donor. *J. Biol. Chem.* 276, 5518–5524
24 Wang, L. *et al*. (2001) Novel deoxynucleoside-phosphorylating enzymes in mycoplasmas: evidence for efficient utilization of deoxynucleosides. *Mol. Microbiol.* 42, 1065–1073

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A putative nuclear function for mammalian Staufen

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In addition to its role in rRNA processing and ribosome assembly, the nucleolus plays a part in the assembly of non-ribosomal ribonucleoprotein particles (RNPs) that are destined for cytoplasmic RNA delivery. Recent evidence indicates that mammalian Staufen2, a brain-specific RNA-binding protein involved in RNA localization, can – at least transiently – enter the nucleolus. Therefore, the assembly of Staufen2 into transport-competent RNPs might occur in the nucleus before their export into the cytoplasm. This could provide new insights into the mechanisms of subcellular RNA localization.

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Introduction

The primary function of the nucleolus is the synthesis and processing of rRNAs and their assembly into ribosomal subunits. Several observations suggest that the nucleolus might perform additional functions, including an involvement in export and/or degradation of mRNA, the processing of non-rRNAs transcribed by RNA polymerase III, the modification of small RNAs and the sequestration of regulatory molecules [1–3]. Moreover, a body of evidence suggests that non-ribosomal ribonucleoprotein particles (RNPs) destined for cytoplasmic RNA delivery might assemble in the nucleolus [3]. This is best documented for signal-recognition particles (SRPs) [2] and telomerase-containing particles [4]. Interestingly, a series of independent studies suggests that certain RNA-binding proteins, including nucleolin, La, the fragile-X mental retardation proteins FMRP, FXR1 and FXR2, and Modulo, might be components of non-ribosomal RNPs that transiently enter the nucleolus. These proteins have been previously described as nucleocytoplasmic-shuttling proteins and most of them have been implicated in cytoplasmic RNA localization [3,5–7].

The involvement of Staufen in cytoplasmic RNA localization

Staufen belongs to the family of the double-stranded RNA (dsRNA)-binding proteins [8], which contain three-to-five copies of the dsRNA-binding domain (dsRBD) consensus. In *Drosophila*, the product of the maternal effect gene *Stau* is required for the transport of *oskar* mRNA from the anterior to the posterior pole of the oocyte. During its movement, *Drosophila* Staufen co-localizes with the mRNA in an RNP called the *oskar* localization complex [9]. Mammals possess two different Staufen genes that encode two distinct Staufen (Stau) proteins, Stau1 and Stau2. Moreover, alternative splicing generates two isoforms for Stau1 and three isoforms for Stau2; the molecular and cellular localization of these proteins has been characterized [10,11]. In mammalian neurons, both Staufen proteins are predominantly located in the cytoplasm where the Staufen-containing RNPs move along microtubules from the cell body into dendrites, suggesting a functional role of Staufen proteins in the dendritic localization of mRNAs [12]. Two reports have suggested that the more ubiquitously expressed Stau1 protein might be able to enter the nucleus before the assembly into RNPs: (i) exogenously expressed Stau1 has been detected in the nucleus of mammalian cells; and (ii) interaction of Stau1 with RNAs in the nuclear compartment was reported ([4] and references therein).

Nucleocytoplasmic shuttling of Stau2

Recently, two independent studies have provided evidence that the brain-specific Stau2 might enter the nucleus in mammalian cells and behave as a nucleocytoplasmic-shuttling protein [13,14]. This is surprising because *Drosophila* Staufen has only been shown to have roles in RNA localization and translational control in the cytoplasm [9]. When a mutagenesis approach was performed in mice to render Stau2 incompetent for RNA-binding, mutant Stau2 proteins accumulated in the nucleus and, in particular, in nucleoli [13]. A closer inspection of the protein sequence revealed that Stau2 indeed contains a nuclear localization signal immediately following the third dsRBD, which binds RNA with high affinity.

Because Staufen proteins are predominantly located in the cytoplasm of mammalian cells, it is likely that Stau2 is efficiently exported upon nuclear import. Under normal circumstances, the export of Stau2 is not dependent on chromosomal region maintenance 1 (CRM1; or exportin-1) [13], an export factor that recognizes nuclear-export signals (NES). Instead, it depends on exportin-5 (exp-5), which is known to be responsible for the export of tRNAs, microRNAs and possibly dsRNA-binding proteins [15,16]. When exp-5 is downregulated by RNAi, wild-type Stau2 accumulates in nucleoli. Interestingly, only the largest isoform of Stau2 (Stau2^{62}, which contains five dsRBDs like the *Drosophila* homolog), but not the other two differentially spliced isoforms (Stau2^{59} and Stau2^{52}), changes its intracellular localization upon downregulation of exp-5 [13]. Recently, the Stau2^{59} isoform has been shown to leave the nuclear compartment in a CRM1-dependent manner [14]. This differential specificity originates by alternative splicing at the N terminus of Stau2, leading to the presence of an NES in the 59-kDa isoform, which is not present in the 62-kDa isoform [14].

Based on the previous observations, it is tempting to speculate that Stau2 enters the nucleus and possibly the nucleolus to bind its target RNAs (e.g. tRNAs, microRNAs and mRNAs). Because exp-5 has been proposed as the export factor for microRNAs [16], it is conceivable that Stau2^{62} might play a part in the assembly of translationally repressed RNPs that contain such microRNAs. Once assembled, the RNPs would be exported via the exp-5 (or the CRM1) pathway into the cytosol of mammalian cells where they are transported to their final destinations.

Nucleolar sequestration of ADAR proteins

ADAR (adenosine deaminases that act on RNA) proteins also belong to the family of dsRNA-binding proteins and have a role in RNA editing by changing adenosines to inosines in mRNAs. Whereas ADAR2 is mainly found in the nuclear compartment, ADAR1 is predominantly located in the cytoplasm of fibroblasts. Independent studies have shown that ADAR1 and ADAR2 both shuttle in and out of the nucleolus in living cells [17,18]. Further evidence for localization of ADAR proteins to the nucleolus came from an *in vitro* study showing binding to small nucleolar RNAs that form dsRNA structures with specific substrate rRNAs. This nucleolar sequestration of ADAR proteins could represent a regulatory mechanism by which a pool of editing activity is rapidly available by translocation of ADAR proteins from the nucleolus to the nucleoplasm in response to changes in the expression of potential RNA-editing substrates [18]. Another interesting parallel to Stau2 is that the RNA-binding activities of ADAR proteins are necessary for proper subcellular localization [18]. In contrast to Stau2, ADAR proteins additionally contain a nucleolar localization sequence.

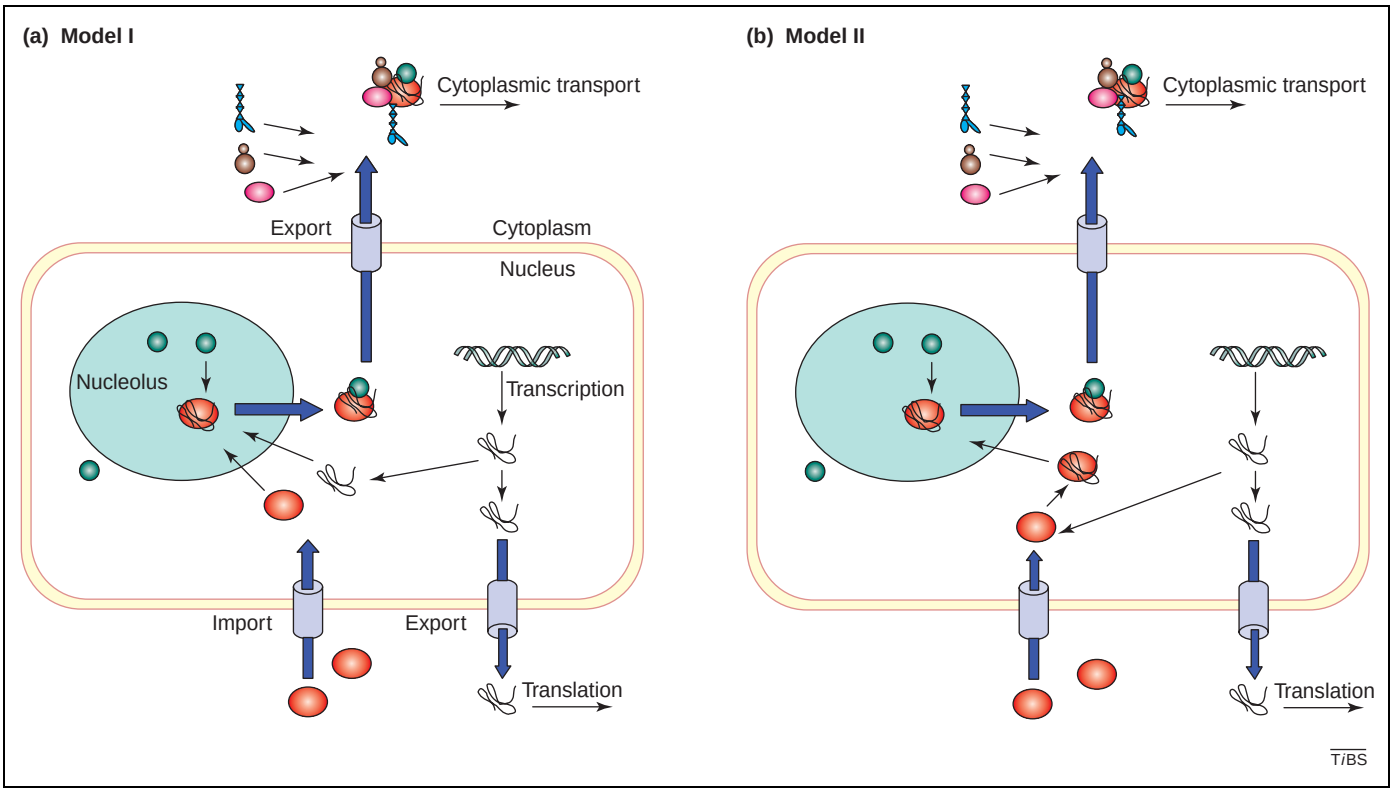

Figure 1. Alternative models for RNP biogenesis in mammalian cells. RNA precursors are generated in the nucleus by transcription and are subsequently exported for translation or recruited to the nucleolus. RNA-binding proteins (red), for example, Stau2 and ADAR, are imported into the nucleus. **(a)** In model I, RNA-binding proteins and RNA precursors independently localize to the nucleolus where they assemble into RNPs. **(b)** In model II, the RNA-binding proteins assemble with their cargo RNA precursors into RNPs in the nucleoplasm and then localize to the nucleolus. Upon addition of nucleolar proteins (green), the RNPs mature, leave the nucleolus and subsequently become exported from the nucleus into the cytoplasm. In a final step, further cytosolic factors such as additional RNA-binding proteins, accessory proteins and molecular motor proteins are recruited to these RNPs and transport to the final destinations inside the cell occurs.

A putative role of the nucleolus in the assembly of non-ribosomal RNPs

In addition to its role in rRNA synthesis, processing, ribosome assembly and maturation, it is now widely accepted that the nucleolus is involved in the biogenesis of SRP and telomerase RNPs, the sequestration of regulatory molecules and possibly even the control of aging. There are several independent observations that a series of non-nucleolar RNA-binding proteins (e.g. La, FMRP, FXR1, FXR 2, Modulo, ADAR1, ADAR2, Stau1 and Stau2) are transiently present in the nucleolus. It is, therefore, tempting to speculate that the nucleolus has a more general role in the assembly of non-ribosomal RNP particles destined for cytoplasmic RNA delivery (Figure 1).

There are several possibilities to explain a nucleolar transit of Stau2 and possibly additional RNA-binding proteins. The observed translocation of RNA-binding proteins, such as Stau2 and ADAR (Figure 1), from the cytoplasm to the nucleolus might be mediated by binding to abundant nuclear dsRNAs. However, because several of the aforementioned proteins still accumulate in nucleoli in their mutant form (i.e. when deficient in their RNA-binding activity), other mechanisms seem more likely. These RNA-binding proteins might bind their cargo RNAs either in the nucleoplasm (Figure 1a) or even in the nucleolus (Figure 1b) to assemble with additional RNAs and proteins, including ribosomes. The nucleolus might, thus, function as a checkpoint to verify the potential functional integrity of RNP complexes. Once the RNPs have matured and passed this quality control, they leave the nucleolus. In the cytosol, additional cytosolic factors, such as additional RNA-binding proteins, accessory proteins and molecular motor proteins, are recruited to these RNPs and transport to the final destinations inside the cell occurs (Figure 1).

Another plausible hypothesis is that the nucleolus functions as a regulatory or quality-control compartment that certain proteins or protein complexes only transiently enter. The recruitment of nuclear or cytoplasmic proteins to the nucleolus might, therefore, represent a mechanism to tightly regulate their activity. Visintin and Amon [19] have convincingly demonstrated, for cell-division cycle 14 (CDC14), a protein phosphatase that is kept inactive by sequestration in the nucleolus, thereby preventing the premature onset of mitosis. Based on these observations, the authors put forward the interesting hypothesis that the nucleolus might function as a 'prison' [19]. It will be interesting to see whether this concept also holds for RNA-binding proteins, such as Stau2 and others, that are involved in cytoplasmic RNA delivery.

Future perspectives

The biological role for the nucleocytoplasmic shuttling of dsRNA-binding proteins, including Stau2, remains to be elucidated. One important goal will, therefore, be to visualize the transit of wild-type Stau2 into the nucleolus of living cells under normal conditions. Furthermore, it will be interesting to unravel the precise location at which

Stau2 recognizes its cargo RNAs and the assembly into transport-competent RNPs occurs. One possible approach could be to interfere with the assembly of Stau2 into RNP particles and to investigate whether this results in the trapping of other components of the localization machinery in the nucleolus. Promising candidates will be cargo RNAs in addition to other protein components of RNPs. These represent, however, only a few of the many possible avenues of future research. We anticipate that the underlying mechanism(s) of RNP assembly in the nucleolus will soon emerge.

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References

1 Lamond, A.I. and Earnshaw, W.C. (1998) Structure and function in the nucleus. *Science* 280, 547–553
2 Pederson, T. (1998) The plurifunctional nucleolus. *Nucleic Acids Res.* 26, 3871–3876
3 Olson, M.O. *et al*. (2000) The nucleolus: an old factory with unexpected capabilities. *Trends Cell Biol.* 10, 189–196
4 Le, S. *et al*. (2000) Identification of two RNA-binding proteins associated with human telomerase RNA. *Mol. Biol. Cell* 11, 999–1010
5 Intine, R.V. *et al*. (2003) Differential phosphorylation and subcellular localization of La RNPs associated with precursor tRNAs and translation-related mRNAs. *Mol. Cell* 12, 1301–1307
6 Tamanini, F. *et al*. (2000) The fragile X-related proteins FXR1P and FXR2P contain a functional nucleolar-targeting signal equivalent to the HIV-1 regulatory proteins. *Hum. Mol. Genet.* 9, 1487–1493
7 Perrin, L. *et al*. (1999) The *Drosophila* modifier of variegation modulo gene product binds specific RNA sequences at the nucleolus and interacts with DNA and chromatin in a phosphorylation-dependent manner. *J. Biol. Chem.* 274, 6315–6323
8 Saunders, L.R. and Barber, G.N. (2003) The dsRNA-binding protein family: critical roles, diverse cellular functions. *FASEB J.* 17, 961–983
9 Palacios, I.M. and St Johnston, D. (2001) Getting the message across: the intracellular localization of mRNAs in higher eukaryotes. *Annu. Rev. Cell Dev. Biol.* 17, 569–614
10 Kiebler, M.A. *et al*. (1999) The mammalian Staufen protein localizes to the somatodendritic domain of cultured hippocampal neurons: implications for its involvement in mRNA transport. *J. Neurosci.* 19, 288–297
11 Tang, S.J. *et al*. (2001) A role for a rat homolog of Staufen in the transport of RNA to neuronal dendrites. *Neuron* 32, 463–475
12 Köhrmann, M. *et al*. (1999) Microtubule-dependent recruitment of Staufen-green fluorescent protein into large RNA-containing granules and subsequent dendritic transport in living hippocampal neurons. *Mol. Biol. Cell* 10, 2945–2953
13 Macchi, P. *et al*. (2004) The brain specific double-stranded RNA-binding protein Staufen2: nucleolar accumulation and isoform specific exportin-5 dependent export. *J. Biol. Chem.* 279, 31440–31444
14 Miki, T. and Yoneda, Y. (2004) Alternative splicing of Staufen2 creates the nuclear export signal for CRM1 (exportin 1). *J. Biol. Chem.* 279, 47473–47479
15 Brownawell, A.M. and Macara, I.G. (2002) Exportin-5, a novel karyopherin, mediates nuclear export of double-stranded RNA-binding proteins. *J. Cell Biol.* 156, 53–64
16 Kim, V.N. (2004) MicroRNA precursors in motion: exportin-5 mediates their nuclear export. *Trends Cell Biol.* 14, 156–159
17 Desterro, J.M. *et al*. (2003) Dynamic association of RNA-editing enzymes with the nucleolus. *J. Cell Sci.* 116, 1805–1818
18 Sansam, C.L. *et al*. (2003) Modulation of RNA editing by functional nucleolar sequestration of ADAR2. *Proc. Natl. Acad. Sci. U. S. A.* 100, 14018–14023
19 Visintin, R. and Amon, A. (2000) The nucleolus: the magician's hat for cell cycle tricks. *Curr. Opin. Cell Biol.* 12, 752

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DNA aptamers as potential anti-HIV agents

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Guanine (G)-rich DNA sequences can adopt stable G-quadruplex structures by G-tetrad hydrogen-bonding and hydrophobic stacking. Recently, it has been shown that a DNA sequence forms an aptamer (termed *93del*) and adopts a novel dimeric quadruplex folding topology in K^+ solution. This aptamer exhibits anti-HIV1 integrase activity in the nanomolar range *in vitro*. A docking-based model of the *93del*-integrase complex positions the DNA aptamer within a channel of the tetrameric integrase. This mutual fitting blocks several catalytic amino acid residues that are essential for integrase function, and accounts for the anti-HIV1 activity of the *93del* aptamer.

Versatile DNA structures and functions

DNA sequences can form a variety of unique structures [1,2], of these, quadruplexes are particularly interesting because guanine (G)-rich sequences in the human genome, such as telomere [3,4], promoter [5,6] and regions associated with human disease [7], have been suggested to adopt such structures *in vivo*. Indeed, several proteins that bind to G-quadruplexes have been identified [8]. Stabilization of these quadruplex structures with small chemical agents might inhibit the function of telomerase that is active in cancer cells [4], or decrease the transcription of the *c-myc*

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