Chapter 10

The Molecular Mechanisms of mRNA Export

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A general paradigm for nuclear transport was established primarily through studies of protein import and export. Until recently, this paradigm was generally presumed also to apply to the process of RNA export from the nucleus. In particular, it was assumed that general mRNA export was mediated by one or more transport receptors of the importin-β family and that the RanGTP/GDP gradient was required to impart directionality to the process. The highly abundant class of nuclear RNA-binding proteins—the hnRNP proteins—were regarded as primary candidates for mRNA export adapter proteins that could link mRNAs to importin-β family export factors. Within the past few years, however, an explosion of data has largely disproven prior assumptions about the mechanisms of mRNA export, permanently changing the face of the field. The dust is still settling, but what we now see, albeit incompletely, is the outline of a probable major route of mRNA export that is independent of the importin-β family and the Ran GTPase system.

Introduction

The distinguishing feature of eukaryotic cells is the segregation of RNA biogenesis and DNA replication in the nucleus, separate from the cytoplasmic machinery for protein synthesis. Communication between the nucleus and the cytoplasm occurs through aqueous channels in the nuclear envelope called nuclear pore complexes (NPCs).[22,92] Small molecules can pass through NPCs by diffusion, but there is a permeability barrier for larger molecules—those with a relative molecular mass of >40 kDa—which permits transport only of selected cargo with the help of transport receptors. The NPC is a gigantic proteinous complex ranging in size from approximately 50 MDa in the yeast *Saccharomyces cerevisiae* to 125 MDa in higher eukaryotes, and possessing an eight-fold symmetric structure. All nucleocytoplasmic transport occurs via the central aqueous channel found in NPCs. As the maximum diameter of this channel may be only ~25 nm, achieving nuclear transport of large complexes such as ribosomes and viral genomes presents a potentially formidable challenge and must involve a considerable change in the three-dimensional conformation of the transport cargo or of the pore itself.

Active transport through the NPC is a signal-mediated process involving recognition of cargo molecules by a large class of soluble transport factors.[30,66] Transport is bidirectional, energy dependent, and highly regulated. Research into the molecular mechanisms of nucleocytoplasmic transport has been initiated by studies of nuclear protein import. Protein export from the nucleus has been shown to utilize similar mechanisms as protein import, and now researchers are focusing on the transport of another class of macromolecules—RNAs. RNA export, especially export of mRNA, has been less understood because the process is more complex than that of protein export, involving the coordination of several post-transcriptional

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processing events with the formation of RNA-protein complexes (RNPs) that are the actual export cargoes. In the past few years, however, researchers have accumulated a vast amount of information that reveals the existence of a distinct transport system dedicated to mRNA export.[17,79,80] In this chapter, we review recent studies of the molecular mechanisms of nucleocytoplasmic transport, from 'classical' protein transport to the modern view of the mRNA export system.

Ran Dependent Nucleocytoplasmic Transport

Considerable progress has recently been made in understanding the mechanisms underlying the sequence-specific transport of proteins between the nucleus and the cytoplasm and the critical role played by the NPC in this process.[30,66] Nucleocytoplasmic protein transport is promoted by signal-receptor recognition process. Generally, cargo proteins contain peptide motifs that function as nuclear localization signals (NLSs) and/or nuclear export signals (NESs). These include the so called 'classical NLSs', such as SV40 large T antigen NLS, which consists of a short cluster of basic amino acids, the nonclassical M9 signal, which is a 38- amino acid domain of hnRNP A1 used both for nuclear import and export, and HIV Rev-type NESs consisting of an approximately 10-amino acid stretch rich in leucine residues that are found in many nuclear export cargos. Evolutionally conserved transport signal receptors specific for each transport signal have been identified. These members form a protein family known as the 'importin-β family' or as 'karyopherins' that includes more than 20 members in metazoans and 14 members in yeast belong (from now on, these will be referred to as β family receptors). The family members share a partial similarity in sequence and structure as well as a biochemical property of interaction with Ran, a Ras-related small GTP binding protein. Like other GTP-binding proteins, Ran has both GTP- and GDP-bound forms, and the switch between these two forms plays a crucial role in regulating transport by promoting the association and dissociation of transport receptors and their cargoes, as well regulating their interactions with the NPC. Ran requires two regulatory factors, the GTPase activating RanGAP and the guanine nucleotide exchange factor RanGEF, to switch between its two nucleotide bound states. At steady state, these regulators localize to the cytoplasm and the nucleus, respectively; this asymmetric distribution generates a RanGTP/GDP gradient across the nuclear envelope, which is essential for most nuclear transport pathways. Cooperation with the Ran GTPase system allows transport receptors to bind and subsequently release their substrates on opposite sides of the nuclear envelope, which in turn ensures directed nucleocytoplasmic transport.

The most well-characterized β family receptors are importin-β itself and CRM1/exportin-1.[30,66] Importin-β was the first nuclear transport receptor to be identified. It forms a heterodimeric complex with the adapter protein impotin-α, which mediates recognition of classical NLSs in nuclear import cargoes. The importin-α/-β-cargo complex is formed in the cytoplasm, then travels through the NPC to the nuclear interior, where the cargo is released from the receptor upon binding of RanGTP to importin-β. In contrast, RanGTP binding to the export receptor CRM1/exportin-1 is required for its association with proteins that contain leucine-rich NESs. After the ternary complex of CRM1-RanGTP-cargo is translocated to the cytoplasm, the cytoplasmic Ran activators RanGAP and Ran-binding protein 1/2 (RanBP1/2) stimulate GTP hydrolysis, resulting in the conversion of RanGTP to RanGDP. The switch to RanGDP results in the dissociation of the export complex and release of the export cargo. The export receptor then returns to the nucleus for another round of export. Thus, import and export are essentially reverse processes, with their directionality maintained by the presence of RanGTP in the nucleus and RanGDP in the cytoplasm. The interactions between soluble transport factors described in these examples suggested that it was not unlikely that a given receptor could function in the import of some substrates and in the export of others. In fact,

recent studies have demonstrated that some of the β family receptors do function both in import and in export.[67,101]

It is interactions between β family receptors and components of the NPC that mediate translocation through the pore, while adapters and substrates seem to behave as inert cargo. The β family receptors preferentially bind to FG (phenylalanine-glycine) dipeptide repeats contained in nucleoporins that line the aqueous channel of the NPC. Current models[9,81,83] propose that the consecutive array of FG nucleoporins provides transient docking sites for moving receptor-cargo complexes through the pore. It is also suggested that these FG repeats form a sieve-like structure that restricts the flow of large molecules unless they can compete for direct binding to the motifs. The interactions of these motifs with nuclear transport receptors would allow infiltration into the barrier and facilitate fast transport. This 'selective phase model' could explain how NPCs function as a permeability barrier for inert molecules and yet become selectively permeable for nuclear transport receptor-cargo complexes.[81] The two nucleotide-bound forms of Ran modulate the affinity of receptors for binding to FG motifs during the translocation process. For example, CRM1/exportin-1 efficiently interacts with the FG repeat-containing nucleoporins, Nup214/CAN and p62 in a RanGTP dependent manner.[3,50] In contrast, Pse1p/Kap121p, a yeast import receptor, shows increased affinity for binding to FG nucleoporins when the cellular RanGDP level is elevated.[88] In both cases, enhanced receptor-nucleporin interactions facilitate transport by these receptors. Thus, Ran acts as a molecular switch to regulate two key events of the transport process: formation of transport complexes and their interaction with the NPC.

RanGTPase Dependent RNA Exports

Nuclear export of some RNA species also requires the RanGTPase system. In addition to exporting NES substrates, CRM1/exportin-1 also exports U small nuclear RNA (U snRNA). In this case, CRM1/exportin-1 does not recognize U snRNA directly, but through an adapter protein called PHAX, which carries a leucine-rich NES. PHAX interacts with the cap-binding complex (CBC) bound to the cap structure of U snRNA, and phosphorylation of PHAX promotes the formation of the export complex.[75] In general, signals for nuclear export of RNAs are thought not to reside in the RNA molecules themselves, but rather in proteins that decorate the RNA. A notable exception is that of tRNAs. Exportin-t,[1,54] another member of the β family receptors, directly binds to the TΨC and acceptor arm structures of tRNAs,[2] which act as NES, to form an exportin-t-RanGTP-tRNA ternary export complex similar to that of CRM1-RanGTP-NES. Involvement of the RanGTPase system in export of 5S rRNA in higher eukaryotes,[20] and of both the 40S and 60S ribosomal subunits in yeast has been suggested,[27,37,40,69] however, further studies will be required to understand the precise mechanisms of these export processes.

Export of mRNA

The transport field had initially assumed that one or more members of the well-studied importin-β receptor family would be responsible for the bulk of mRNA export. In fact, one such family member, CRM1/exportin-1, was the first mRNA export receptor to be identified. Human immunodeficiency virus (HIV) Rev protein binds to a structured RNA element called the Rev-responsive element (RRE) within an HIV intron, and CRM1/exportin-1 forms an export complex via a leucine-rich NES contained in Rev, thereby facilitating export of HIV pre-mRNA.[4,25] It was proposed that this mechanism might also apply to endogenous cellular mRNA export, but experiments with leptomycin B, a specific antibiotic inhibitor of CRM1/exportin-1, suggested that this was unlikely, since leptomycin B did not affect the export of bulk mRNA.[73] There are individual mRNAs, however, whose export requires the RanGTPase

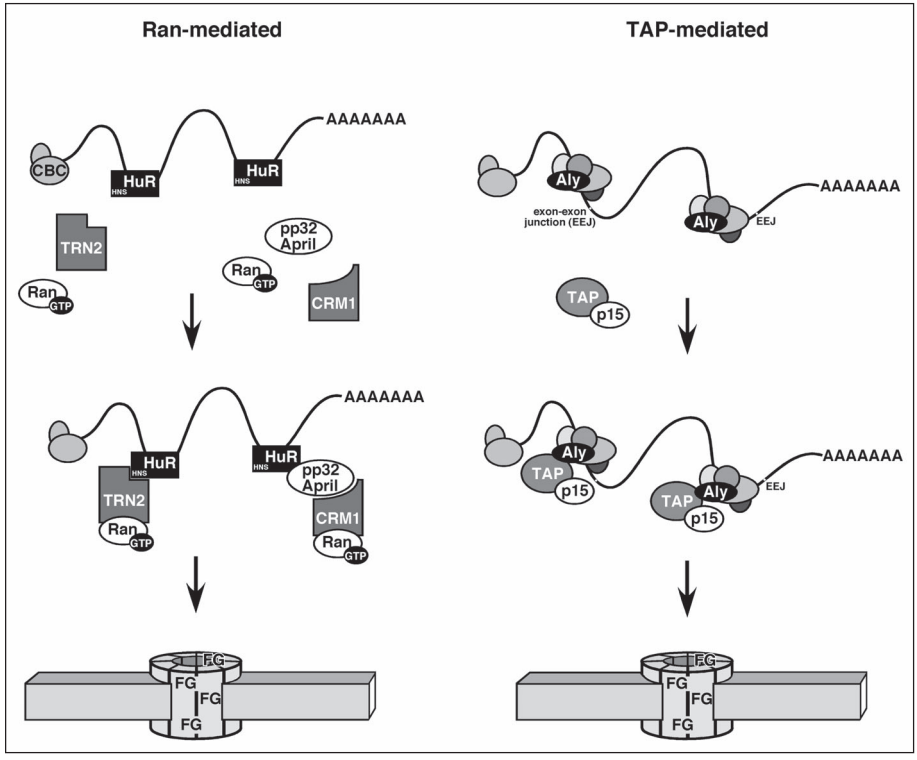

Figure 1. Diverse mechanisms of cellular mRNA export. Cellular mRNAs are exported by at least two independent mechanisms after maturation in nucleus. In the case of the Ran-mediated pathway of c-fos mRNA, export receptors CRM1/exportin-1 or TRN2/transportin-2 and GTP-bound form of Ran may organize a ternary export complex with mRNPs being exported. An adaptor molecule HuR has a critical function to connect the mRNA to the Ran-receptor complex by associating with specific sequence in mRNA and with receptors either directly or indirectly through ligand proteins pp32 and APRIL (Ran-mediated). 'Spliced' mRNAs are exported by TAP-mediated mechanism (TAP-mediated). During splicing, RNA-protein complexes are formed at 20-22 nucleotides upstream of exon-exon junction (EEJ). A RNA binding protein Aly is recruited into the the exon-exon junction complex (EJC) by function of U2AF associating factor UAP56, and Aly deposits export receptor TAP onto the EJC. Both in Ran- and TAP-mediated pathways, mRNA-export machinery complexes recognize the phenylalanine-glycine (FG) dipeptide repeat in nucleoporins. TAP forms a heterodimer with p15 in order to recognize the FG repeats, whereas CRM1/exportin-1 and TRN2/transportin-2 can directly bind to these repeats.

system and importin-β family members. For example, the protein HuR, which is involved in stabilization of RNAs containing AU-rich elements (AREs) containing RNA, acts as a transport adaptor for the mRNA transcript of the early response gene *c-fos* (Fig. 1). The *c-fos* mRNA/HuR complex is either directly recognized by TRN2/transportin-2,[91] another β family receptor, via an export signal in HuR called HNS,[23] or indirectly by CRM1/exportin-1 via leucine-rich NESs in the HuR ligands pp32 and APRIL.[12,28]

Until recently, the primary candidates for cellular mRNA export factors were the highly abundant heterogeneous nuclear ribonucleoproteins (hnRNP) proteins. Nascent pre-mRNAs are bound by a subset of hnRNP proteins. The approximately 20 classes of hnRNP proteins (hnRNP A to U) are divided into two types: those that shuttle between the nucleus and the

cytoplasm and those that are restricted to the nucleus.[53,72] hnRNP A1 was thought to be the major player in mRNA export because this protein belongs to the shuttling class of hnRNP proteins and contains an export signal M9. In addition, hnRNP A1-like proteins have been shown to bind to the giant Balbiani ring mRNA in *Chironomus tentans* and to accompany this mRNA to the cytoplasm.[98] Furthermore, excess hnRNP A1 or M9 peptides inhibit export of DHFR mRNA.[41] To date, however, no export receptor has been identified for hnRNP A1, although its M9-dependent import by a β family receptor TRN1/transportin-1, has been well-characterized.[77] Thus, no direct connection between hnRNP A1 and mRNA export still has yet been established.

From Gene to Nuclear Pore to Cytoplasm

Studies over the past several years indicate that there is extensive coupling between the different steps in gene expression.[21,35] Before mRNA can leave the nucleus, proper processing events, such as capping, polyadenylation, and splicing must occur. These processing events are thought to occur cotranscriptionally as the mRNA is synthesized. As will be discussed in the following sections, a splicing-dependent mRNP complex specifically targets mature mRNA for export.[43] There is also evidence linking proper 3'-end formation to mRNA export. Thus, it is likely that very early mRNA maturation events determine export competence of the transcript.[59,65] In addition, various proteins become associated with transcripts concomitant with transcription and behave differently during RNA transport according to the particular function of each protein. These functions are not limited to early nuclear events, but may even affect the cytoplasmic fate of exported mRNA molecules, impinging on such aspects as transport of mRNA within the cytoplasm, translational efficiency, and mRNA turnover. Thus, proteins loaded cotranscriptionally on pre-mRNA determine to a large extent the fate of mRNA in both the nucleus and the cytoplasm.

TAP-Mediated mRNA Export: Ran Independent Nucleocytoplasmic Transport

While it is clear that the RanGTPase system is required for some types of mRNA export, new studies have shifted the focus of attention to several highly conserved proteins that function in general mRNA export independent of Ran. The major export receptor is TAP (also called as NXF1), which connects messenger ribonuleoprotein particles (mRNPs) to nuclear pores. TAP was originally identified as a cellular factor that stimulated the nuclear export of RNAs containing constitutive transport elements (CTE) from type D retroviruses.[11,31,45] These studies revealed that TAP binds directly to the CTE, and that titration of TAP by excess CTE causes nuclear retention of bulk cellular mRNA, suggesting that TAP is likely to be the primary export receptor for cellular mRNA. TAP is a nucleocytoplasmic shuttling protein that associates with cellular poly(A)+ RNA and interacts with the NPC.[5,8,45,47,87] Genetic experiments involving the *S. cerevisiae* TAP homolog Mex67p and TAP homologs in *Caenorhabditis elegans* and *Drosophila melanogaster* (called NXF-1) have provided evidence of a crucial role for TAP in bulk mRNA export.[34,89,97,100] TAP is now recognized as a major cellular mRNA export receptor, and these TAP homologs comprise a family called the nuclear export factor (NXF) family.

The importance of TAP for bulk mRNA export was unexpected because the protein is structurally distinct from the Ran-dependent β family receptors, and the TAP export pathway is in fact independent of the RanGTPase system.[16] TAP forms a heterodimer with a small protein designated p15 (also called as NXT1).[47] Recent work has shown that the TAP-p15 heterodimer directly stimulates the export of cellular mRNAs.[10,32,47] p15 has sequence similarity to NTF2, a protein that mediates nuclear import of Ran and interacts with nucleoporin FG repeats[15,76]

TAP alone can bind weakly to nucleoporins carrying FG repeats, but Tap-p15 heterodimer formations significantly stimulates this association.[60,99] Structural studies have revealed that p15 and a NTF2-like domain found in TAP form a single structural domain that binds to an FG motif.[26] Thus, p15 can modulate the affinity of the TAP-mRNA export complex for binding to the NPC. The Ran GTPase system is not required for nuclear shuttling of TAP, but the TAP-p15 interaction is essential for the process.[16,46] These findings suggest that transport mediated by TAP-p15 may also involve sequential nucleoporin docking and release steps as proposed for translocation of β family receptors. However, given that Ran is not required for general mRNA export, directionality of mRNA transport must be determined by another mechanism. In principle, the TAP-p15 heterodimer can translocate across the nuclear pores in both directions, and it remains to be determined how directionality of mRNA export is established.

As in higher eukaryotes, the yeast TAP ortholog Mex67p also interacts with FG repeat-containing nucleoporins as a heterodimer, but interestingly, its partner protein, Mtr2p,[44,85] does not show any sequence similarity to p15. However, the *C. elegans* TAP-p15 heterodimer can replace the function of Mex67p-Mtr2p in yeast cells that lack these endogenous export factors, indicating that the function of these heterodimers is evolutionally maintained.[47]

An Adaptor Protein and Other Conserved mRNA Export Factors

TAP itself does not bind strongly to RNA, but interacts predominantly with other components of the mRNP, which will be referred to as adapters. The most conspicuous adapter is a shuttling protein called Aly/REF (Yra1p in yeast). Aly was originally discovered as a hnRNP-type coactivator of the transcription factors LEF-1 and AML-1 in human cells and is evolutionally conserved.[14,96] In *Xenopus* oocytes, microinjected recombinant Aly enhances cellular mRNA export and, conversely, inhibition of Aly function by microinjected Aly antibody specifically blocks mRNA export. These data strongly suggest that Aly is directly involved in the export of cellular mRNA. Aly simultaneously interact with both TAP and mRNA through distinct domains, further supporting the conclusion that Aly serves as an adapter molecule to link mRNA to its export receptor, TAP.

Another important aspect of Aly function is to link splicing events to the downstream process of mRNA export. Aly was originally found to colocalize with structures called nuclear speckles, which are sites of accumulation of factors required for pre-mRNA maturation.[82,103] Aly and several other proteins are recruited into mRNP particles coincident with pre-mRNA splicing, forming a complex that is situated about 20-24 nucleotides upstream of the exon-exon junction (EEJ) and is termed the exon-exon junction complex (EJC).[57] The EJC is thought to serve as a marker that designates mRNP particles as export-competent. Subsequent to EJC deposition, Aly is presumed to recruit the export receptor TAP, thus directly coupling the processes of pre-mRNA splicing and export of mature mRNAs.[94,103]

A component of the spliceosome, the evolutionally conserved DEAD-box RNA helicase UAP56, binds directly to Aly and plays a critical role in its recruitment to the spliced mRNA. UAP56 associates with the splicing factor U2AF65, a large subunit of U2AF, and is required for the U2 snRNP interaction with pre-mRNA.[24] Although Aly has some intrinsic RNA binding ability, an Aly mutant lacking the UAP56 binding domain failed to be recruited into spliced mRNA.[62] A mutation in the yeast *S. cerevisiae* UAP56 ortholog *SUB2* is defective for export of poly(A)+ RNA, confirming a role for UAP56/Sub2p in mRNA export through the coupling of the splicing and export machineries.[42,95] In addition to the Aly-UAP56 interaction, TAP itself appears to interact directly with the spliceosome via the smaller U2AF subunit U2AF35.[104] Thus, U2AF is a key player that recruits export factors to spliced mRNP complexes. Interestingly, Mex67p and Sub2p compete for binding to a domain of the Yra1p protein, a yeast counterpart of Aly,[95] suggesting that Mex67p may promote a release of the mRNP from the spliceosome and target it to the NPC for export.

It was surprising that Sub2p is essential for general mRNA export in *S. cerevisiae* since only a limited number of intron-containing genes exist in the genome of the organism (only 250 out of the ~6,000 genes in *S. cerevisiae* contain introns).[61] This indicates that mechanisms of recruiting export factors onto intronless mRNA must exist in yeast and probably also in metazoans. An interesting observation is that Yra1p associates with coding regions of the yeast genes *PMA1* and *GAL10* in a transcription-dependent manner, suggesting that Yra1p might also be recruited cotranscriptionally.[58] A 22-nucleotide element of the intronless histone H2a gene recruits the shuttling splicing factors SRp20 and 9G8 to promote export of its transcribed mRNA in higher eukaryotic cells.[39] In this case, these splicing factors may directly mediate export of the intronless mRNA or may act as recruiters of export machinery, similar to the role of U2AF and Sub2p in export of spliced mRNAs.

Interactions between mRNA Export Machineries and Nucleoporins

In contrast to the study of soluble transport factors, little progress has been made toward elucidating the events that occurs in the NPC during mRNA export. Although the importance of interactions between of export receptors and nucleoporin FG repeats is clear, the pathway of transit through the NPC and the events that occur in the pore channel are not yet well-understood. Genetic screens in yeast have implicated several nucleoporins as important participants in mRNA export (reviewed in ref. 22). Most of members of two major NPC subcomplexes, Nup84-85-120-145C-Seh1-Sec13[90] and Nup159-82-116-Nsp1,[6,36] have been shown to be essential for the export of poly(A)+ RNA. The former subcomplex is found at the central core of NPC and the latter on the cytoplasmic fibrils, suggesting that they may constitute a mRNP docking site and a terminal release site, respectively. Some mammalian homologs of these nucleoporins, including CAN/Nup214, Nup98 and p62, physically interact with TAP,[5,47,87] suggesting that the route of mRNA export in the nuclear pore is conserved among various organisms. Interestingly, TAP seems to share NPC binding sites with β family receptors, but TAP does not depend on RanGTP/GDP for these interactions.[5]

The yeast genetic screens have also identified several NPC associating soluble factors implicated in mRNA export and the studies have been extended onto their mammalian homologs. An ATP-dependent DEAD-box helicase, Dbp5p, is mainly located in the cytoplasm where it interacts with cytoplasmic fibrils of the NPC, probably by associating with the FG nucleoporin Nup159p.[38,86] Gle1p also localizes to the cytoplasmic side of the NPC, where it interacts with the FG-repeat nucleoporin Rip1p/Nup42p as well as with Dbp5p.[71,93] By these multiple interactions, Gle1p may provide a platform for mRNP disassembly and mRNA release at the cytoplasmic side of the NPC. Gle2p/Rae1 (also known as mrnp 41 in mammals) is a shuttling poly(A)+RNA associating factor.[13,52,70] It associates with the NPC by recognizing specific peptide sequence called GLEBS, which are found in nucleoporins such as Nup116p in yeast and Nup98 in mammals.[7,78] These properties of Gle2p/Rae1 meet all the prerequisites for an mRNA export receptor, suggesting that Gle2p/Rae1 may act in a second pathway of mRNA export that is redundant with the TAP-p15 pathway.

Links between mRNA Quality Control and Nuclear Export

As previously mentioned, the loading of the exon-exon junction complex (EJC) onto spliced mRNAs serves as a marker for export-competent mRNPs, thus playing a role in mRNA quality control. The release of various EJC components from RNPs takes place at different times during the export process. Whereas two splicing factors, SRm160 and DEK, leave the mRNA inside nucleus,[64] other members of the EJC, including the export adapter Aly, remain to bound to the mRNA during its export to the cytoplasm.[49,56,64] Aly as well as TAP then dissociate from the complex when the mRNA reaches the cytoplasm and they shuttle back to

the nucleus for the next round of export. Although no direct confirmatory evidence has reported, an attractive hypothesis is that the helicase activity of Dbp5, perhaps in concert with activities of Dbp5-associating factors at the cytoplasmic fibrils of NPC, mediates the release of the export machinery from the exported RNA.

The remaining members of the EJC—RNPS1, Y14, hUpf3 and Magoh—seem not to be inert piggy-back factors, but rather they appear to have active functions as determiners of the cytoplasmic fate of an mRNA. In particular, nuclear deposition of these factors appears to direct the specific interaction of an mRNP with cytoplasmic machineries involved in such processes as translation and mRNA degradation (see Fig. 2). One example of this is the involvement of EJC components in nonsense-mediated mRNA decay (NMD), a process by which mRNAs containing a premature termination codon are targeted for degradation. The molecular mechanisms of NMD were originally studied in the yeast *S. cerevisiae,*[19] but a conserved mechanism has been shown to exist in humans. An shuttling human EJC component, hUpf3, is a homolog of the yeast Upf3p protein, which is an essential factor of the NMD pathway. As part of the EJC, hUpf3 interacts with two other components: RNPS1,[105] which was originally identified as a general splicing activator, and a novel protein called Y14.[49] These interactions are important for the recruitment of hUpf3 into the complex.[51,64] The role of hUpf3 may be to recruit two cytoplasmic NMD components, hUpf1 and hUpf2, onto the RNA after it has been exported to cytoplasm.[51,63,64] It is hypothesized that an actively translating ribosome would displace this reorganized EJC; if the ribosome dissociates from the template at a nonsense mutation, however, the remaining EJC, along with the translation release factors eRF1 and eRF3, may serve as a tag for sorting the mRNA to a degradation pathway. In the yeast *S. cerevisiae*, a specific sequence element called DSE (downstream sequence element,[84]) functions like the EEJ, since most transcripts in this organisms are not spliced. In this case, termination codons located upstream of the DSE would be regarded as nonsense mutations. The DSE appears to be recognized by a shuttling hnRNP protein called Hrp1p.[29] The Hrp1p-DSE association is thought to trigger the deposition of other yeast NMD factors such as several *UPF* and *MOF* gene products[18,19] onto the RNA, forming a complex which has similar function to the EJC.

Some mRNAs require further transport to specific sites in the cytoplasm before being translated. A well-known example occurs in *Drosophila* embryogenesis, where transcripts of genes essential for oogenesis, such as *oskar* and *bicoid*, are synthesized in nurse cells and transported to the posterior pole to allow exclusive expression of their gene products at this site. The *Drosophila* EJC component Y14 colocalizes with *oskar* mRNA at the posterior pole; functional Y14, as well as the Mago Nashi protein, a *Drosophila* counterpart of the human Magoh protein, are essential for this asymmetric localization of *oskar* and *bicoid* Mrna.[33,74] Magoh was isolated as a Y14 associating factor and it binds to Y14 and TAP as part of the EJC.[48,55,102] The mechanism of selective translocation of specific mRNAs and the anchoring of these RNAs at their destinations is still unclear, but the Y14-Magoh complex, probably along with other additional associating factors such as the *Drosophila* protein Tsunagi,[68] may act as a 'pass' for further transport in the cytoplasm.

Conclusion

The discovery of the TAP-mediated mRNA export system has been a major leap forward in our understanding of mRNA export. It is also a perfect example of how physiological cellular functions are connected and organized: the export reaction is integrated into a system of total mRNA metabolism that also includes mRNA biogenesis and degradation. The splicing event increases the diversity of gene information dramatically. At the same time, however, it has the risk of producing aberrant, deleterious peptides that may cause fatal cellular defects. By incorporating mechanisms of quality control, such as NMD, the system seems to have been

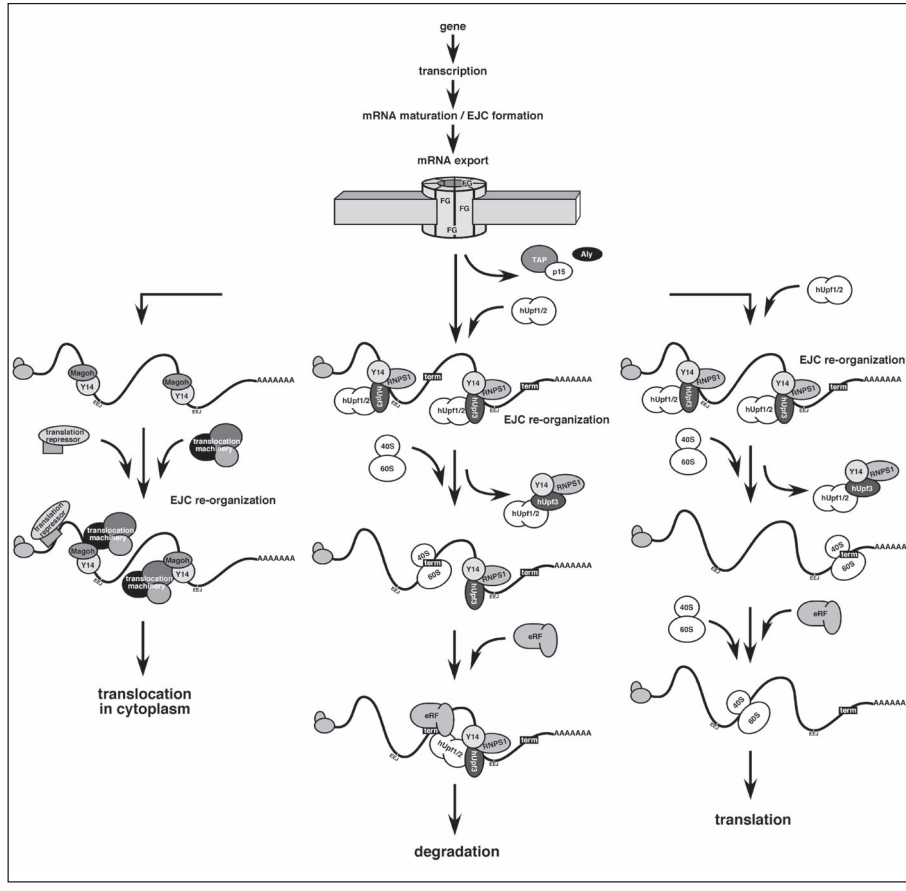

Figure 2. The nuclear history of mRNA determines the fate of mRNA in the cytoplasm. The fate of mRNA in the cytoplasm may be imprinted as protein complex during nuclear processing events. Nonsense-mediated mRNA decay (NMD) is a system for leading mRNAs carrying nonsense termination codons into a degradation pathway. The exon-exon junction complex (EJC) organized during splicing events acts as molecular memory of splicing and a NMD factor hUpf3 deposited into the EJC may have a major role. After being exported to cytoplasm, cytoplasmic NMD components hUpf1/2 may be recruited to the complex by function of hUpf3. Normally, these reorganized 'marks' should be checked and displaced by actively translating ribosomes in the cytoplasm and the mRNA can be used for following rounds of translation (translation). However, if the ribosome is released before reaching the legitimate termination codon by nonsense mutation, the remaining EJC may become a 'RNA decay' tag to prevent from synthesizing immature peptides (degradation). EJC can also function as a transit pass for being transferred to the final destination in the cytoplasm. For example, oogenesis requires strictly regulated timing and localization of gene expression. After nuclear export, transcripts of Drosophila genes osker and bicoid, both essential for oogenesis, are further transported to posterior pole without being translated during the transport. In this case, EJC components Mago Nashi and Y14 are critical, probably for deposition of the translocation machinery onto mRNA (translocation in cytoplasm).

developed to satisfy the requirements of both diversity and accuracy. Although we now have an elegant model of the export system, several unanswered questions still persist. First, the mechanism of export of intronless mRNA is still not clear. Although the TAP system may be

used for this class of mRNAs, distinct factors may be involved in the recruitment of export factors to the mRNP. Another question is the extent to which the RanGTPase system is involved in cellular mRNA export. It is conceivable that such a critical cellular function as mRNA export may require a back up system for emergencies, such in cases of environmental stress. In addition, some mRNAs may employ the RanGTPase system to find a niche for their export, using specific adapters that can regulate the export efficiency or gene expression, as is the case for *c-fos* mRNA. The answers to these questions should be provided by extensive studies on the mRNA export combined with those on other physiological functions and the findings by these research might give us another different view of the export process.

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