mRNA nuclear export and human disease

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Export of mRNA from the nucleus is a central process in eukaryotic gene expression that has been implicated in several human diseases. Much of our understanding of how an mRNA is transported to the cytoplasm is derived from studies using yeast and fly models. We present here different mechanisms by which aberrant nuclear retention of mRNA can cause human disease. Emerging evidence that implicates the mRNA export factor GLE1 in two lethal motor neuron disorders is discussed and we highlight surprising links to regulatory mechanisms that were first observed many years ago in yeast. These examples illustrate how model organisms have aided in our elucidation of complex human disorders through analysis of basic cellular processes.

In eukaryotic cells, gene expression requires efficient and proper transport of mRNAs from the nucleus to the cytoplasm. Studies performed in model organisms have provided the groundwork for our understanding of mRNA nuclear export and have highlighted many mRNA maturation events that influence the export of messages to the cytoplasm. Here we briefly discuss the nuclear mRNA processing steps that are integrated with mRNA export and, through discussion of three disorders – osteogenesis imperfecta (OI) type I, myotonic dystrophy (DM) type 1 and lethal congenital contracture syndrome 1 (LCCS1) – describe how defects in the export pathway can result in disease. We emphasize the roles that model organisms, particularly the yeast *Saccharomyces cerevisiae* and the fly *Drosophila melanogaster*, have played in our comprehension of the export process relative to these disorders. Given the ease of experimental utility and the wealth of knowledge of the mRNA export pathway that *S. cerevisiae* and *Drosophila* have provided, we envisage their continued importance in elucidating the molecular pathology and therapeutic development of such disorders.

Model organisms have provided the framework for our understanding of mRNA export

Extensive work in model organisms has illustrated that, in addition to specific messenger ribonucleoprotein particle (mRNP) receptor complexes and the nuclear pore components with which they interact, mRNA export events are also highly coupled to nuclear mRNA maturation steps. The importance of intact splicing and 3’ processing signals, and modifications, such as 5’ capping, in mRNA export were initially discovered by assessing the subcellular localization of mutant pre-mRNA transcripts transformed into yeast or mammalian cells, or injected into Xenopus oocytes (Chang and Sharp, 1989; Eckner et al., 1991; Hamm and Mattaj, 1990; Legrain and Rosbash, 1989). Larger scale investigation of components required for mRNA export began in yeast, where temperature-sensitive libraries were used to identify factors that, when mutated, resulted in aberrant accumulation of polyadenylated [poly(A)] RNA in the nucleus (Amberg et al., 1992; Kadowaki et al., 1992). Furthermore, studies in yeast and metazoans have revealed that disruption of factors involved in nuclear mRNA processing events, including splicing, 3’ processing and surveillance, triggers mRNA nuclear retention (Brody and Silver, 2000; Farny et al., 2008; Gatfield et al., 2001; Hammell et al., 2002; Hieronymus et al., 2004; Strasser and Hurt, 2001).

The importance of the mRNA export pathway to cellular function is exemplified by both its conservation and adaptation between organisms. Many factors required for export in lower eukaryotes have since been shown to be similarly required for mRNA export in human cells (Guzik et al., 2001; Kapadia et al., 2006; van Deursen et al., 1996; Watkins et al., 1998; Williams et al., 2005). A recent genome-wide screen for export factors found that the complexity of the fly mRNA export pathway reflects the degree of transcript structure of the organism (Farny et al., 2008). The fact that an organism with a more involved transcript maturation program requires a more complicated mRNA export network suggests that higher eukaryotic systems model the organization of the human mRNA export process more completely than do simpler eukaryotes.

The physical and functional coupling of the export process to nuclear mRNA processing contributes to its efficiency and fidelity (reviewed by Bentley, 2005; Luna et al., 2008; Reed, 2003; Reed and Cheng, 2005; Saguet et al., 2005; Sommer and Nehrbass, 2005). Heterodimers of Mex67 (*Drosophila* NXF1, *sbr/human TAP*) and Mtr2 (*Drosophila* p15/*human NXT1*), the proteins responsible for ferrying the majority of mRNAs across the pore, have an inherently low affinity for mRNA (Braun et al., 1999). These heterodimers interface with transcripts through interactions with adaptor proteins [such as Yra1 (*Drosophila/human Aly*), see below] that coat mRNPs. Thus, one mechanism to ensure export of only fully mature mRNAs is to recruit Mex67/Mtr2 to RNA through adaptor proteins responsible for completion of distinct maturation steps (Fig. 1).

Export of improperly processed transcripts is further prevented through surveillance mechanisms that retain aberrant messages at the site of transcription for subsequent degradation. How the improperly processed transcripts are recognized and retained remains largely unknown. However, studies in yeast, fly and human cells have demonstrated that mutation of some export and nuclear processing factors (Bousquet-Antonelli et al., 2000; Burkard and

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COMMENTARY

Defects in mRNA export can result in human disease

mRNA nuclear export defects can result from two sources: mutations in pre-mRNA sequences and mutations in export or processing factors. Here, we discuss examples of both types and relay how our knowledge of the export pathway gleaned from work in model organisms has facilitated our understanding of the pathology of these disorders (Fig. 2).

Osteogenesis imperfecta type I: mRNA mutation results in transcript retention and disease

OI, which affects 1 in 10,000 people, encompasses a group of inherited diseases resulting in increased bone fragility caused by mutation in one of the two genes encoding collagen, COLIA1 and COLIA2 (reviewed by Roughley et al., 2003). A particular patient cohort suffering from OI type I possess a splice site mutation in COLIA1, which results in a decrease in collagen production (Johnson et al., 2000; Stover et al., 1993). This mutation causes improper splicing and retention of intron 26 of COLIA1 (COLIA1\(\rightarrow\)COLIA1\(^{26}\)) pre-mRNA, which results in sequestration of the transcript in nuclear RNA processing domains – known as splicing speckles – and thus renders it unavailable for translation in the cytoplasm (Johnson et al., 2000). Prolonged localization to the speckle suggests that the aberrant mRNA is stalled at a particular stage of nuclear processing.

As mentioned previously, splicing is intimately coupled with message nuclear export. Mutations in particular splicing sequences can result in stalled spliceosomal complexes and ultimately transcript retention (Chang and Sharp, 1989; Legrain and Rosbash, 1989). Additionally, splicing factors such as Sub2 (yeast, human/fly UAP56), are required for export and in some cases, specifically to recruit export adaptor proteins such as Yra1 (yeast, human/fly Aly) (Jensen et al., 2001; Strasser and Hurt, 2001). Thus, mutation of a splice site sequence in the COLIA1 pre-mRNA could affect subsequent recruitment of export adaptors and transport out of the splicing domain en route to nuclear export (Fig. 2A).

Interestingly, examples such as OI type I, in which an abnormally spliced transcript has been shown to be retained in the nucleus, are rare. Efficient retention of such aberrant messages at transcription sites and their subsequent destruction by nuclear degradation mechanisms may prevent their detection. The escape of COLIA1\(^{26}\) from its site of transcription to splicing speckles suggests that it has bypassed some initial surveillance mechanisms and thus is not degraded efficiently (Johnson et al., 2000).

More severe forms of OI can result from mis-sense mutations in COLIA1 and COLIA2, which generate dominant negative collagen fibril proteins. Since these mutations do not alter the nuclear processing of the message, the cell exports the aberrant transcripts and toxic protein products are translated (reviewed by Roughley et al., 2003). Conversely, the aberrant splicing event that produces COLIA1\(^{26}\) also causes a frameshift and the encoding of downstream toxic protein products are translated (reviewed by Roughley et al., 2003). Thus in the specific case of OI type I discussed above, a combination of transcript nuclear retention and rapid cytoplasmic message decay is likely to lessen the severity of the disorder by only allowing translation of COLIA1 generated from the wild-type allele.

Myotonic dystrophy type I: toxic RNA causes nuclear retention and disease

Another human disease that results from aberrant nuclear retention of a particular mRNA is the severe muscle wasting disorder,
myotonic dystrophy (DM) type I. Patients suffering from this disorder experience skeletal muscle myotonia and degeneration, which ultimately can be fatal. The most common form of DM in adults, DM type I, is an autosomal dominant disease caused by an abnormal CUG expansion in the 3' untranslated region (UTR) of the DM protein kinase mRNA (DMPK$_{CUG^+}$) (reviewed by Cho and Tapscott, 2007). Toxic CUG repeats within DMPK$_{CUG^+}$ result in nuclear RNAs that are resistant to export. However, unlike OI type 1, which results from diminished collagen production because of the decrease in exported COL1A1, DM1 does not result from decreased DMPK protein levels. Instead, the toxic RNA causes disease in trans via misregulation of splicing factors, including the muscle-blind protein family 1 (MBNL1) and CUG-binding protein 1 (CUGBP1) (Fig. 2B) (Charlet et al., 2002; Mankodi et al., 2002; Philips et al., 1998; Savkur et al., 2001) (reviewed by Wheeler and Thornton, 2007).

Normally, DMPK is transcribed at the periphery and then transported into splicing speckles before being escorted out of the nucleus as part of a mature mRNA. However, the presence of expanded CUG repeats prevents entry of the RNA into the speckle and instead, the messages accumulate in distinct foci at the periphery of the splicing domains (Holt et al., 2007; Smith et al., 2007). These foci sequester factors, such as MBNL1, which preferentially associate with the aggregates and prevent their normal nuclear activities, which in this case is the splicing of specific muscle-related transcripts (Dansithong et al., 2005). There are many factors that must associate with transcripts for export to occur and it is likely that inappropriate protein interactions with the 3'UTR repeats prevent the transcript from associating with factors of the export pathway. Interestingly, fusion of DMPK$_{CUG^+}$ to a viral post-transcriptional element that stimulates nucleocytoplasmic transport through a protein export pathway results in a decrease of nuclear foci and allows myoblast differentiation. Removal of the toxic foci from the nuclear compartment might at least partially diminish the disease effect (Mastroyiannopoulos et al., 2005).

Both mouse and fly disease models of DM1 have been developed and they exhibit many of the physiological and cellular phenotypes characteristic of DM1 patients, including muscle wasting, intranuclear RNA foci and splicing defects (de Haro et al., 2006; Garcia-Lopez et al., 2008; Orego et al., 2008). A recent genetic screen in one of these Drosophila mutants identified that exacerbation of the diseased phenotype was caused by defects in the nuclear export factor Aly (Garcia-Lopez et al., 2008). As a member of the conserved REF family of mRNA export factors that interact directly with both RNA and NXF1, Aly functions as an adaptor for the recruitment of export factors to maturing mRNPs (Stutz et al., 2000) (Fig. 1). Thus, the decrease in Aly function in Fig. 2. Defects in mRNA export can result in disease via different mechanisms. Export adaptors or transporters and mRNA processing factors (blue) influence transcript retention (black) and export (grey). (A) Mutation of a particular mRNA can cause improper processing and its consequent inability to be recognized by the export machinery. Tissues specifically requiring expression of the encoded protein are adversely affected (OI type 1). (B) Abnormal sequence expansion in an mRNA can result in transcript nuclear retention and sequestering of trans factors (red). Tissues that are particularly sensitive to the activity of the trans factors (DM1) and/or to the levels of the encoded protein are adversely affected. The effect of sequestration of trans factors on localization of their target transcripts is largely unknown (hatched grey). (C,D) Mutation of a specific export factor (LCCS1/LAAHD) may inhibit a subset of (C) or bulk (D) message export. (C) Tissues sensitive to the levels of the proteins encoded by the retained transcripts are affected. (D) Mutation of an export factor required for bulk mRNA export could result in global inefficiency of transcript export. Tissues requiring particularly tight temporal regulation of gene expression for their differentiation, such as neurons, may be particularly susceptible to this mutation.
the DM1 study might result in a decreased capacity to export \(DMPK_{CUG}^{6}\) transcripts. Given the genetic tractability of Drosophila and the fact that many of the processes shown to be important in mRNA nuclear export are likely to be conserved in humans, the development of these disease models is a powerful tool for the study and treatment of DM1.

In both diseases discussed above, we have proposed that aberrant interactions between the mutant transcripts and specific export factors result in the inability of the messages to be transported to the cytoplasm. Thus, it will be interesting to define differences in the composition of those RNA-binding proteins (RBPs) associating with both wild-type (\(COL1A1\) and \(DMPK\)) and mutant (\(COL1A1^{126}\) and \(DMPK_{CUG}^{6}\)) transcripts to uncover nuclear retention mechanisms. In particular, this would provide insight into ways to promote \(DMPK_{CUG}^{6}\) export and thus alleviate the resultant spliceopathies.

**Lethal congenital contracture syndrome: mutation of an export factor results in disease**

Recently, mutations in the mRNA export factor \(GLE1\) were linked to two motor neuron diseases: lethal congenital contracture syndrome 1 (LCCS1) and lethal arthrogryposis with anterior horn cell disease (LAAHD) (Nousiainen et al., 2008). LCCS1 and LAAHD are rare autosomal recessive disorders and they result in fatal reductions in anterior horn motor neuron development, ventral spinal cord wasting and skeletal muscle atrophy. In these diseases, a single nucleotide substitution in intron 3 of \(GLE1\) results in a mis-splicing event and consequently, inclusion of nine extra nucleotides in the mRNA and a three amino acid insertion into the protein sequence (Nousiainen et al., 2008).

The requirement for \(GLE1\) in mRNA export was initially described in yeast (Gle1) (Del Priore et al., 1996; Murphy and Wente, 1996) and later in human cells (Watkins et al., 1998). Although it shuttles between the nucleus and the cytoplasm, Gle1 predominantly localizes to the cytoplasmic face of the nuclear pore complex through interactions with the nuclear pore proteins Nup155 (yeast Nup170/Nup157) and CG1 (yeast Nup42/Rip1) (reviewed by Kendirgi et al., 2005). A genetic screen for modulators of Gle1 activity led to the discovery of the enzymes required for production of the soluble inositol hexakisphosphate (\(InsP_6\)) and thus sparked the hypothesis that \(InsP_6\) acts as a positive regulator of Gle1-mediated RNA export in yeast (York et al., 1999). Later it was shown that mRNA export activity in human cells also responds to nuclear \(InsP_6\) levels (Feng et al., 2001). Onl recently, the direct binding of \(InsP_6\) to Gle1 was found to stimulate the RNA helicase activity of the shuttling DEAD-box mRNA export factor Dbp5 (Alcazar-Roman et al., 2006; Weirich et al., 2006). A model was proposed that at the cytoplasmic face of the nuclear pore, \(InsP_6\)-bound Gle1 stimulates Dbp5 molecules that had been deposited previously onto the maturing mRNP within the nucleus. Activated Dbp5 then catalyzes the release of particular mRNP proteins required for export, such as Mex67, from the transcript (Lund and Guthrie, 2005). These newly freed export factors are recycled back into the nucleus for the next round of export (Fig. 1). Whether the LCCS1-linked mutation of \(GLE1\) directly affects the ability of the protein to stimulate Dbp5 activity and thus mRNA export remains to be shown. However, the disease-linked insertion does interrupt the coiled-coil domain in \(GLE1\), which might result in altered protein-protein interactions with shuttling mRNP components (Nousiainen et al., 2008).

Most interestingly, the causative mutations of two additional neonatal congenital contractural syndromes (LCCS2 and LCCS3) have been recently attributed to defects in enzymes that modulate the phosphatidylinositol-3-kinase/Akt signaling cascade, a pathway intimately connected with the production of soluble inositol phosphate molecules such as \(InsP_6\) (Narkis et al., 2007a; Narkis et al., 2007b). Upon first identification of these two mutations (in \(ERBB3\) and \(PIPK1\)), respectively), researchers speculated that improper regulation of phosphatidylinositol bisphosphate (\(PIP2\)) and phosphatidylinositol trisphosphate (\(PIP3\)) levels, which are critical in the regulation of vesicular trafficking and cell growth and survival, could cause the pleiotropic symptoms of LCCS2 and LCCS3. Although such disruptions are still likely to be important in the pathology of these diseases, the combination of the newly identified LCCS1 mutation with our understanding of the role of the phosphorylated inositol signaling pathway in mRNA export, suggests an interesting role for mRNA export in these disorders and a relationship between inositol signaling, export and neuronal survival.

**Can tissue-specific effects result from mutations in a core export factor?**

As all human cells require mRNA export for proper gene expression, the mechanism underlying tissue-specific defects due to mutation of general mRNA export factors is not obvious. One explanation is that general export factors are not as general after all (Fig. 2C). Studies in yeast and Drosophila have revealed that some factors that are seemingly required for general poly(A) RNA localization to the cytoplasm are actually regulating the export of only a subset of the transcriptome (Hieronymus and Silver, 2003; Rehwinkel et al., 2004). Perhaps \(GLE1\) selectively affects the export of specific mRNAs. Expression array data comparing neural precursor cells derived from LCCS1 post mortem fetuses with those of controls revealed misregulation of specific mRNA species, some of which are associated with synapse and nerve development (Pakkasjarvi et al., 2007). Thus, it would be interesting to determine whether mutation of \(GLE1\) causes mislocalization of these transcripts.

Particular tissues might also be more sensitive to the temporal regulation of gene expression for their proper differentiation (Fig. 2D). RNA in situ hybridization revealed particularly high \(Gle1\) expression in mouse embryo ventral cells and somites: the two cell types from which motor neurons and skeletal muscle/bone, respectively, are derived (Nousiainen et al., 2008). Additionally, a study of the expression of mouse 2-kinase, responsible for production of \(InsP_6\), revealed a similar expression pattern (Verbsky et al., 2005). Enrichment of these factors in such tissues suggests particular requirements of these cells for their function. Interestingly, small bristles (\(sbr\)) (Drosophila NXXF1) mutant Drosophila embryos exhibit defects in the differentiation of neurons, muscle and bristles (Korey et al., 2001). Investigators suggested that these affected tissues require extremely tight temporal control over their differentiation process and thus are more sensitive to the diminished rate of mRNA export resulting from the \(sbr\) mutation. Additionally, researchers recently suggested that spinal muscular atrophy results from specific neuronal...
sensitivity to levels of the spliceosome assembly component survival motor neuron (SMN) protein (Zhang et al., 2008). These data further support the idea that specific tissues have differing requirements for factors involved in ‘general’ mRNA metabolism (McKee et al., 2005).

Use of model organisms has enabled our dissection of the mRNA export pathway and preliminary comprehension of the molecular pathologies of several human diseases that result from aberrant transcript retention and export factor mutation. Complex levels of coupling between mRNA processing steps as well as transcript surveillance mechanisms, aid in the fidelity of the export process, thereby protecting the cell from production of toxic proteins while maintaining an expedient gene expression regime. As InsPs, and its derivatives have additionally been implicated in a range of nuclear processes, including regulation of transcription, DNA repair and chromatin remodeling (reviewed by Alcazar-Roman and Wente, 2008; York, 2006), it is now important to determine how the spatial and temporal regulation of inositol phosphate signaling coordinate mRNA export with these other nuclear events.

In addition to those described here, other human disorders have been linked to mutation of export-associated factors, although the affect of their associated mutations on the fidelity of mRNA export remains largely uncharacterized (Abu-Baker and Rouleau, 2007; Nakamura, 2005). Developing models of diseases such as LCCS1 in Drosophila and other organisms that possess organ systems and signaling properties akin to humans, will be paramount to further our understanding of the relationship between mRNA export and disease. Additionally, using these models to aid in understanding the specificity that export factors have for transcripts and the tissue-specific needs for export components will be essential. Such models may also enable discovery of small molecules that regulate the mRNA export process via modulation of interactions between specific factors and transcripts.

ACKNOWLEDGEMENTS

We thank A. E. McKee, N. G. Farny and M. J. Moore for their helpful comments and input in writing this manuscript. We apologize to our colleagues whose work we were unable to discuss because of space restrictions.

COMPETING INTERESTS

The authors declare no competing financial interests.

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