Intron or no intron: a matter for nuclear pore complexes

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Nuclear pore complexes (NPCs) have been shown to regulate distinct steps of the gene expression process, from transcription to mRNA export. In particular, mRNAs expressed from intron-containing genes are surveyed by a specific NPC-dependent quality control pathway ensuring that unspliced mRNAs are retained within the nucleus. In this Extra View, we summarize the different approaches that have been developed to evaluate the contribution of various NPC components to the expression of intron-containing genes. We further present the mechanistic models that could account for pre-mRNA retention at the nuclear side of NPCs. Finally, we discuss the possibility that other stages of intron-containing gene expression could be regulated by nuclear pores, in particular through the regulation of mRNA biogenesis factors by the NPC-associated SUMO-protease Ulp1.

Introduction

Nuclear pore complexes (NPCs) are large macromolecular assemblies inserted within the nuclear envelope and composed of multiple copies of proteins called nucleoporins (Nups). Subcomplexes of Nups form a structural scaffold which surrounds a central channel harboring phenylalanine-glycine (FG) repeats involved in dynamic interactions with the cargoes, e.g. proteins and ribonucleoparticles. While several decades of research have provided detailed information about the function of NPCs as gateways for the nucleo-cytoplasmic trafficking of macromolecules, an increasing number of reports have revealed that NPCs have also important roles in gene expression, genome maintenance and cell cycle progression.1 Establishment of gene expression programs requires the integration of the multiple nuclear and cytoplasmic steps of mRNA metabolism, including transcription, processing, nuclear export, translation and degradation. Besides the critical function of FG-containing nucleoporins in the translocation of mRNAs into the cytoplasm, other NPC components have also been shown to contribute directly, or indirectly, to different stages of the gene expression process.2,3 One of the central players in the connection between NPC and gene expression is the nuclear basket, a peripheral extension of the nuclear pore which protrudes toward the nucleus and forms a platform which interacts with genes, mRNAs and other regulators, such as the SUMO-deconjugating enzyme Ulp1/SENP2, both in yeast and metazoaans.4 Notably, proteins of the nuclear basket have been involved in a quality control mechanism preventing the export of misassembled or unprocessed messenger ribonucleoparticles (mRNPs), in particular pre-mRNAs harboring an unspliced intron.5 Cytoplasmic pre-mRNA leakage has been observed in a number of mutant conditions, notably in yeast cells inactivated for nuclear basket components (Nup60, Mlp1)6 or their interacting partners (Pml39, Ulp1).7,8 However, the molecular mechanisms underlying the relative contribution of these different players to the nuclear retention of intron-containing pre-mRNAs have remained elusive.

In spite of the additional costs associated with intron-containing mRNA metabolism, eukaryotic genomes benefit from the presence of introns in various
situations: intron retention modulates transcript stability and thereby gene expression levels\textsuperscript{9}; alternative splicing increases the diversity of the proteome\textsuperscript{16}; and introns can stimulate mRNA export.\textsuperscript{11} In view of the important flow of intron-containing mRNAs produced in eukaryotic cells, regulated splicing events must be precisely coordinated with mRNA export in order to prevent pre-mRNA translation into aberrant proteins. In this Extra View, we will focus on the role of nuclear pore components in the control of intron-containing gene expression, from transcription to pre-mRNA retention, mainly based on studies performed in \textit{S. cerevisiae}. For further details about the connection between gene expression and NPCs in other model organisms, the reader is invited to refer to review articles where this topic has been extensively discussed.\textsuperscript{2,3,5}

Unraveling the Multiple Roles of Nuclear Pore Components in the Expression of Intron-Containing Genes

Several complementary approaches have been used in the past to decipher the multiple contributions of nucleoporins to gene expression in budding yeast.\textsuperscript{2} Chromatin immunoprecipitation\textsuperscript{12,13,14} and microscopy observation of tagged loci\textsuperscript{15,16,17,18} have revealed the interaction of NPCs with a subset of genes. In addition, molecular analyses and reporter systems have uncovered gene expression defects in nucleoporin mutants.\textsuperscript{19,20,21,22} Finally, \textit{in situ} hybridization and dedicated assays have been used to monitor mRNA export and pre-mRNA retention, as previously reviewed.\textsuperscript{5}

We have recently investigated the molecular basis for NPC-associated pre-mRNA quality control and further clarified the role of distinct nuclear pore components in unspliced mRNA retention.\textsuperscript{19} For this purpose, we took advantage of a set of LacZ-based reporter constructs\textsuperscript{24} to evaluate mRNA expression, mRNA splicing and cytoplasmic pre-mRNA leakage. These analyses have been performed in a battery of yeast mutants affecting the nuclear pore scaffold (Nup133, Nup120, Nup188), the nuclear basket (Mlp1, Pml39, Ulp1) or representative components of the mRNA biogenesis/export pathway, such as mRNA export adaptors (Yra1, Nab2, Npl3), the mRNA export receptor (Mex67) and other mRNP-associated factors previously shown to couple transcription with export (THO/TREX, TREX-2). Since previous characterization of these mutants had revealed profound defects in gene transcription that could possibly hinder pre-mRNA leakage phenotypes,\textsuperscript{20,25,26} we have used an ultra-sensitive β-galactosidase assay with improved properties (e.g., fast and amenable to medium-throughput screening) in order to distinguish mutants affecting pre-mRNA retention from those affected at another stage of the gene expression process.

Through this study, we have been able to define 2 classes of mutants: (i) mutants of the nuclear basket (\textit{mlp1Δ}, \textit{pml39Δ}), which trigger \textit{bona fide} pre-mRNA leakage, and (ii) mutants of the Nup84 NPC subcomplex (\textit{nupl20Δ}, \textit{nupl33Δ}), which exhibit decreased levels of the SUMO-protease Ulp1 at NPCs\textsuperscript{27} and primarily affect expression of the reporter constructs. Epistasis analysis further revealed that Ulp1 acts on gene expression by targeting the previously reported sumoylation of the Hpr1 subunit of the THO complex,\textsuperscript{28} a process independent from the pre-mRNA retention mediated by Mlp1/Pml39.\textsuperscript{23} In addition, this study uncovered an unexpected feature of mutants affecting the Nup84 complex, the SUMO-protease Ulp1 or the THO complex, e.g., that they have less pronounced effects on the transcription of intron-containing genes as compared to intronless genes.\textsuperscript{23}

An alternative system, in which the unspliced and the spliced versions of an unique reporter gene encode distinct fluorescent proteins, has been recently used to evaluate pre-mRNA splicing and pre-mRNA export by multicolor flow cytometry analysis of yeast cell populations.\textsuperscript{29} The use of this reporter system notably allowed to define typical expression signatures in mRNA transcription or export mutants. In particular, it suggested an increased pre-mRNA splicing in mutants primarily affecting mRNA export, a result consistent with the phenotypes that we scored using LacZ-based reporters in some mRNA export mutants (e.g., \textit{yra1} and \textit{nab2}).\textsuperscript{23} These findings likely reflect the fact that an extended nuclear retention in export mutants favors the splicing of the poorly spliced introns present in both reporters.\textsuperscript{24,29} Another advantage of this fluorescent reporter is that it could also measure cell-to-cell variations in gene expression processes. However, the design of this assay did not take into account the expression of the corresponding intronless gene and, thereby, was not expected to identify the differential requirement of Nup84/Ulp1/THO for intron-containing and intronless gene expression as displayed in our study. The combined use of both enzymatic and cytometry reporter-based assays is therefore appropriate to further refine our understanding of the multiple stages of intron-containing gene expression, from transcription, splicing to pre-mRNA retention and mRNA export.

Different Models Accounting for Intron-Containing mRNA Retention at Nuclear Pores

Our study,\textsuperscript{23} along with previous reports, has revealed that \textit{bona fide} pre-mRNA leakage is a defining feature of a subset of nuclear pore mutants, e.g. those affecting the structural component of the nuclear basket Mlp1 and its interacting partner Pml39. Biochemical analyses further demonstrated that these factors are not required for the proper formation of export-competent mRNP,\textsuperscript{23} in agreement with a later function at the export stage. However, it remains unclear whether Mlp1/Pml39 rather select fully processed/assembled mRNP and favor their commitment into the mRNA export pathway, or retain at NPCs unprocessed/ misassembled mRNP – including intron-containing mRNP, and further prevent their translocation.\textsuperscript{30}

In the “selection” model (Fig. 1A), nuclear basket-associated proteins would bind completely processed mRNP and further favor their export. Proteomic analyses of mRNP composition have indeed revealed that Mlp1 and its paralogue Mlp2 interact with a number of mRNP
Proteins that could specify the complete processing and/or packaging status of the mRNAs include: (i) the SR-proteins Gbp2 and Hrb1, which have been reported to bind spliced transcripts and to favor their interaction with the export receptor Mex67, and whose inactivation triggers pre-mRNA leakage; (ii) the poly(A)-binding protein and mRNA export adaptor Nab2, whose interaction with Mlp1 is required for optimal mRNA export. It should however be pointed that inactivation of MLP1 or PML39 is not sufficient per se to trigger bulk mRNA accumulation in the nucleus, suggesting that mRNA selection at NPCs is not a mandatory step in the mRNA export process.

Alternatively, in the “retention” model (Fig. 1B), proteins specifically associated with faulty mRNPs would be recognized by the nuclear basket and these interactions would then trigger their retention in the nucleus. In this scenario, incomplete mRNA splicing could be signaled by yet-to-be-identified intron-associated proteins. Supporting this hypothesis, Mlp1 was reported to interact with the branchpoint-binding protein Ms15, albeit in a RNA-dependent manner, while the Pml1 subunit of the Retention and Splicing (RES) complex was shown to contribute to Mlp1/Pml39-mediated pre-mRNA retention. Strikingly, the presence of the 5’ splice site motif was recently reported to trigger nuclear retention of transcripts in mammalian cells, suggesting that the presence of intron-associated factors may be a conserved feature.
recognized in retained mRNPs. Nuclear pore components would not only sense unspliced mRNAs, but also improperly packaged mRNPs, as revealed by the increased interaction of the nuclear basket with aberrant mRNPs formed in \textit{yra1} mutant cells.\textsuperscript{26}

These two models, albeit exclusive at first glance, could be reconciled if the residence time at the nuclear basket of distinct kinds of mRNPs would vary depending on their composition, and thereby on their processing. An extended or stronger interaction would stably tether to NPCs those mRNPs that are not fully mature or packaged, preventing their translocation, and providing an additional time window for processing reactions – such as splicing - or for degradation by the NPC-associated endonuclease Swt1\textsuperscript{36} (Fig. 1C). Such a model would be in agreement with recent single-molecule microscopy analyses of mRNA export in animal cells, which have detected a mRNP docking step at the nuclear side of the nuclear envelope.\textsuperscript{37}

**A Role for the Nup84 Complex and Ulp1 in Gene Transcription**

Our study has also revealed a decreased expression of LacZ reporter constructs in the absence of the Nup84 complex. Several reports had previously indicated that the Nup84 complex contributes to different stages of transcription, namely transcriptional activation by the Rap1/Gcr1/Gcr2 complex,\textsuperscript{19} repression of subtelomeric transcription,\textsuperscript{38} and transcription elongation.\textsuperscript{20} However, the underlying mechanisms were poorly documented.

In view of its well-known roles in nuclear envelope organization, mRNA export or maintenance of the SUMO-protease Ulp1 at nuclear pores,\textsuperscript{27,39} several hypotheses could account for the function of the Nup84 complex in gene expression. However, our data revealing the phenotypic similarity between Nup84 and \textit{ulp1} mutants in the intronless/intron-containing reporter assay rather favor a model in which the Nup84 complex tethers Ulp1 at NPCs, thereby regulating the sumoylation of specific targets involved in transcription. Of note, a number of reports have indicated that central players in the above-mentioned processes are regulated in a SUMO-dependent manner (Fig. 2). Sumoylation of Rap1 favors TFIID recruitment, thereby potentiating its transcriptional activation.\textsuperscript{40} Sumoylation of the Sir2 histone deacetylase disrupts its interaction with the telomeric silencing complex, further promoting transcriptional derepression at glucose-repressed genes and subtelomeres, respectively (center). THO complex sumoylation differentially affects the expression of intron-containing and intronless reporters, most likely at the transcriptional elongation stage\textsuperscript{23} (right). RNAP II, RNA polymerase II.

![Figure 2. Possible functions of the Nup84 complex and Ulp1 in gene transcription. The sumoylated components of the transcription machinery which could mediate the roles of the Nup84 complex and Ulp1 in gene expression are represented. While Ulp1 was shown to target both Ssn6 and the THO complex for desumoylation,\textsuperscript{21,28} its activity toward Rap1 and Sir2 has not been investigated. The different boxes illustrate the positive role of sumoylated Rap1 in transcriptional activation (left), as well as the function of sumoylated Ssn6 and desumoylated Sir2 in transcriptional repression at glucose-repressed genes and subtelomeres, respectively (center). THO complex sumoylation differentially affects the expression of intron-containing and intronless reporters, most likely at the transcriptional elongation stage\textsuperscript{23} (right). RNAP II, RNA polymerase II.](image)
subtelomeres. Sumoylation of the Hpr1 subunit of the THO complex regulates mRNP assembly, possibly impacting on transcriptional elongation. Epistasis analyses further support the fact that some of the contributions of Ulp1 to gene expression depend on Hpr1 sumoylation. Additional analyses of non-sumoylatable mutants of these different targets, in combination with Nup84 complex or Ulp1 inactivation, could indicate to which extent SUMO-dependent processes actually connect the NPC to gene transcription. In this respect, an elegant study recently reported that Ulp1-mediated desumoylation of the transcriptional repressor Ssn6 contributes to derepression of the GAL locus. In the future, systematic identification of Ulp1 targets within the transcription and mRNA processing machineries will certainly shed a new light on the intimate relationships between gene expression and nuclear pores.

A Differential Requirement of the THO Complex for Intron-Containing and Intronless Gene Expression

Beyond an alteration of transcription, our study revealed a lower impact of Nup84 complex, ulp1 and THO complex mutants on the expression of distinct intron-containing reporters as compared to their intronless counterpart. The THO complex is a conserved multiprotein assembly, which is recruited onto transcribed chromatin and mRNAs, and contributes to mRNP assembly, stability and export. Improper mRNP formation in theo mutants is believed to trigger the accumulation of mRNA:DNA hybrids (or R-loops), which are detrimental for transcription elongation, interfere with replication and further enhance genetic instability.

Previous studies have indicated that the THO complex is mainly required for the expression of highly expressed genes, suggesting that a complete mRNP packaging machinery is critical in case of elevated levels of mRNA production. Consistently, a reduction in the rates of transcription initiation or elongation has been reported to suppress the recombination and expression defects associated with THO inactivation. Our observation that introns can also alleviate the phenotypes of theo mutants could thus be easily explained if the presence of introns led to lowered transcription rates. However, this hypothesis is not currently supported by our data: first, theo phenotypes have been suppressed in the context of strong decreases in transcription initiation, achieved by either mutating the essential initiation factor TFIIH or modifying the promoter, whereas our chromatin immunoprecipitation assays have revealed that the presence of the intron does not lead to a detectable decrease in transcription initiation. Second, theo mutant gene expression defects were previously suppressed by treatment with mycophenolic acid (MPA), an inhibitor of transcription elongation; in contrast, similar MPA treatment of theo cells did not alleviate the expression defects of the LacZ intronless reporter as an intron did (our unpublished results).

Another mechanism may explain how introns alleviate the transcriptional defects of theo mutants: alternative mRNP assembly pathways may contribute to the packaging of intron-containing mRNAs, in particular upon THO complex deficiencies. Consistent with this hypothesis, an earlier report suggested differences in mRNP composition between mRNAs expressed from intron-containing and intronless genes in wt cells. Candidate factors that may take over mRNP assembly at intron-containing mRNAs in case of improper function of the THO complex include: (i) the SR-protein Npl3, which has been identified in association with the spliceosome and can function as an adaptor for the mRNA export receptor Mex67; (ii) the splicing helicase Sub2, which can interact with the mRNA export adaptor Yra1; and (iii) the spliceosome-associated Prp19-complex, whose inactivation is lethal in the context of yeast theo mutants. Gene-specific analyses of mRNP composition should further allow to determine how introns can impact on mRNA biogenesis and export, in normal and challenged situations.

Conclusion

Multiple reports have revealed that distinct nuclear pore components can modulate gene expression, either directly, through interactions between Nups and genes/mRNPs, or indirectly, by targeting the sumoylation of the transcription machinery. Of note, each of these different mechanisms connecting NPCs and gene expression appears individually dispensable for cell viability. However, they may be collectively required for cell fitness, as suggested by the synthetic lethality triggered by the combined inactivation of the Mlp1/Pml39 pathway, involved in mRNA quality control, and Ulp1, which is important for optimal mRNA synthesis.

Some of these studies have notably shown that intron-containing and intronless genes do not similarly require the function of NPCs components. The importance of the nuclear pore complex in the control of intron-containing mRNA export is expected to be even more crucial in metazoans in view of their highly complex exon-intron gene organization. In the future, the combination of dedicated biochemical and microscopy-based analyses in distant species should further unravel the differences between intron-containing and intronless mRNA metabolism, from transcription to mRNP assembly and export.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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