OUTLOOK

Coupling pre-mRNA splicing and 3' end formation to mRNA export: alternative ways to punch the nuclear export clock

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How does a mammalian cell determine when newly synthesized mRNAs are fully processed and appropriate for nuclear export? Müller-McNicoll and colleagues (pp. 553–566) expand on mechanisms known to be mediated by nuclear export factor 1 (NXF1) by describing SR proteins as NXF1 adaptors that flag alternatively spliced and polyadenylated mRNA isoforms as cargo ready for the cytoplasm.

The highly regulated process of mRNA export is generally able to differentiate appropriately processed mRNAs that are ready for the cytoplasm from partially processed or misprocessed mRNAs that are not. Cells define export-worthy mRNAs using RNA-binding proteins as molecular signatures of earlier and successfully executed nuclear processes that begin with the cotranscriptional 5' end capping, splicing, and 3' end formation of precursors to mRNAs (pre-mRNAs). These molecular signatures are read by cellular machines, including the nuclear pore complex, through which mRNAs are transported from the nucleus to the cytoplasm. One molecular signature for mRNA export is the nuclear export factor 1 (NXF1), which serves to ensure that an RNA 5' end is capped: NXF1 is recruited to pre-mRNAs by the transcription export complex (TREX), which itself is recruited by the cap-binding complex (CBC) of CBP80 and CBP20 (Wickramasinghe and Laskey 2015).

While cellular surveillance for the presence of a 5' end cap is a good start to ensuring that an mRNA is ripe for nuclear export, 5' end capping does not indicate whether the rest of an RNA has been matured by splicing or 3' end cleavage and polyadenylation. To complicate matters further, most pre-mRNAs undergo alternative splicing and alternative 3' end formation, raising the issue of how a cell approves of more than one mRNA isoform for export to the cytoplasm. Here, Müller-McNicoll et al. (2016) ad-

dress these issues by reporting that SR proteins are also adaptors for NXF1, thereby coupling nuclear mRNA export to proper splicing and 3' end maturation.

As proof of principle, Müller-McNicoll et al. (2016) first down-regulated NXF1 in P19 mouse embryonic carcinoma cells and showed that the abundance of thousands of transcripts was decreased in cytoplasmic fractions and concomitantly increased in nuclear fractions, indicating a block in their nuclear export. The investigators next depleted individual SR protein family members (SRSF1–7) and found that depletion of each SR protein blocked the export of a specific set of largely protein-coding mRNAs, with SRSF3 depletion inhibiting the export of the largest set of mRNAs.

Müller-McNicoll et al. (2016) found that all seven SR proteins coimmunoprecipitated with NXF1 in an interaction stabilized by RNA, with some interactions showing greater sensitivity to enzyme-mediated RNA digestion than others. From this and the finding that overexpressing SRSF3 and SRSF7 (but not SRSF2) increased the recruitment of NXF1 to mRNAs, they concluded that at least some SR proteins promote mRNA export by recruiting and stabilizing the binding of NXF1 to mRNAs. The investigators also demonstrated using individual nucleotide-resolution UV cross-linking coupled to immunoprecipitation (iCLIP) that GFP-tagged SR proteins and NXF1 cobind transcript exons. Cobinding was most pronounced for SRSF3. iCLIP data also revealed that SR proteins and NXF1 cross-link to unspliced as well as spliced transcripts. Thus, SR proteins remain associated with spliced products after splicing is completed, as would be expected of proteins that couple pre-mRNA splicing and mRNA export.

iCLIP data also demonstrated that NXF1 binds to not only pre-mRNA 5' untranslated regions (UTRs)—consistent with the TREX-mediated recruitment of NXF1 to CBC-bound 5' ends—but also open translational reading

[**Keywords:** iCLIP; mRNA export; alternative 3' end processing; SR protein; NXF1; SRSF3; SRSF7]

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Article is online at <http://www.genesdev.org/cgi/doi/10.1101/gad.278937.116>.

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frames (ORFs) and 3' UTRs. SR proteins were also found to bind throughout transcripts, exhibiting more binding to ORFs than to 5' UTRs and 3' UTRs. Analysis of the 60 nucleotides (nt) centered at NXF1-binding sites revealed that while ~50% of all NXF1-binding sites in the first exon and all transcript regions were equally cobound by SR proteins, NXF1-binding sites in last exons were most often cobound by SRSF3 and SRSF4. Additionally, SRSF3 binding to the 60 nt centered at a NXF1-binding site in last exons was detected when no binding was detected for any other SR protein, suggesting that SRSF3 preferentially recruits NXF1 to last exons and/or 3' UTRs. Deducing binding motifs from the iCLIP data revealed that the only similarity between the NXF1- and SRSF-binding motifs was evident for NXF1 and SRSF3 within 3' UTRs.

SR proteins are well documented to regulate splicing (Howard and Sanford 2015). As evidence for their roles in coupling alternative splicing to mRNA export in a way that promotes the nuclear export of specific splicing isoforms, Müller-McNicoll et al. (2016) showed that depleting SR proteins, especially SRSF3 and SRSF1, resulted in widespread alteration of splicing patterns and changes in the relative abundance of splicing isoforms in the cytoplasm. iCLIP data revealed significant interaction between SR proteins and alternative exons that were excluded from mature mRNAs upon SR protein depletion, consistent with SR protein function in alternative splicing.

A large proportion of protein-coding mRNAs undergo alternative 3' end formation either through using alternative polyadenylation (APA) signals or through alternative splicing within the last exon (Tian and Manley 2013). Müller-McNicoll et al. (2016) detected changes in 3' UTR length upon SR protein depletion, particularly when SRSF3 or SRSF7 was depleted. Intriguingly, SRSF3 and SRSF7 exhibited antagonistic effects on 3' UTR lengths: SRSF3 depletion induced 3' UTR shortening, whereas SRSF7 depletion promoted 3' UTR lengthening. With a focus on SRSF3, the investigators showed that SRSF3 depletion also resulted in reduced cytoplasmic

abundance of the shortened 3' UTR mRNA isoforms. iCLIP data demonstrating widespread binding of SRSF3 to target transcripts within last exons and 3' UTRs at sites cobound with NXF1, together with the finding that SRSF3 promotes the export of those long 3' UTR mRNA isoforms that it generates, indicate that SRSF3 couples APA and nuclear export by recruiting NXF1 to last exons and/or 3' UTRs.

What we have learned from the Müller-McNicoll et al. (2016) study of mouse P19 cells will undoubtedly be applicable to other mammalian species, tissues, and cell types. This is partly because the roles of SR proteins in regulating constitutive and alternative splicing as well as other aspects of mRNA processing are evolutionarily conserved (Howard and Sanford 2015).

Acknowledgments

We thank Max Popp for comments on the manuscript. The Maquat laboratory is supported by National Institutes of Health R37 GM074593 and R01 GM059614 to L.E.M. R.A.E. derived partial salary support from National Institutes of Health P30 AR061307.

References

- Howard JM, Sanford JR. 2015. The RNAissance family: SR proteins as multifaceted regulators of gene expression. *Wiley Interdiscip Rev RNA* **6**: 93–110.
- Müller-McNicoll M, Botti V, de Jesus Domingues AM, Brandl H, Schwich OD, Steiner MC, Curk T, Poser I, Zarnack K, Neugebauer KM. 2016. SR proteins are NXF1 adaptors that link alternative RNA processing to mRNA export. *Genes Dev* (this issue). doi: 10.1101/gad.276477.115.
- Tian B, Manley JL. 2013. Alternative cleavage and polyadenylation: the long and short of it. *Trends Biochem Sci* **38**: 312–320.
- Wickramasinghe VO, Laskey RA. 2015. Control of mammalian gene expression by selective mRNA export. *Nat Rev Mol Cell Biol* **16**: 431–442.

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Genes Dev. 2016, **30**:

Access the most recent version at doi:[10.1101/gad.278937.116](https://doi.org/10.1101/gad.278937.116)

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Michaela Müller-McNicoll, Valentina Botti, Antonio M. de Jesus Domingues, et al.
Genes Dev. March , 2016 30: 553-566

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