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# When cells are down on their LUC7L2, alternative splicing rewires metabolism for OXPHOS

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**Daniels et al. (2021) and Jourdain et al. (2021) identify LUC7L2 as a component of the U1 snRNP capable of reprogramming cellular metabolism through changes in alternative pre-mRNA splicing.**

Metabolic reprogramming changes how metabolic intermediates are used and how energy is generated by cells. Two pathways, glycolysis and oxidative phosphorylation (OXPHOS), produce the bulk of ATP as well as pools of metabolites for use by the cell. The balance between these pathways is altered in numerous physiological contexts, such as differentiation, but most notably in cancer where aerobic glycolysis is commonly observed. It is now appreciated that these metabolic changes are not simply an indirect result of damaged mitochondria but due to substantial reprogramming of metabolism to support proliferation (Pavlova and Thompson, 2016). Important questions remain as to how this is accomplished and regulated. In *Molecular Cell* and *Cell Reports*, Jourdain et al., 2021 (this issue) and Daniels et al., 2021 provide evidence of a key role for alternative splicing and LUC7L2 in rewiring the balance between glycolysis and OXPHOS. They collectively identify LUC7L2 and its homologs (LUC7L and LUC7L3) as components of the U1 small nuclear ribonucleoprotein (snRNP)—a critical member of the splicing machinery responsible for initial recognition of 5′ splice sites (5′ SS) (Roca et al., 2013). Loss of any one of the LUC7L homologs leads to changes in alternative splicing. However, only loss of LUC7L2 results in changes in glycolysis and metabolic reprogramming.

Jourdain et al. identified LUC7L2 when they reanalyzed data from a previous CRISPR/Cas9 whole-genome screen in K562 cells (Arroyo et al., 2016) to identify genes that, when knocked out, promote cellular viability in the presence galactose, a poor glycolytic substrate. The gene set was heavily enriched for splicing-associated factors, including components of the U1 snRNP, RNA helicases, and heterogeneous nuclear ribonucleoproteins (hnRNPs). Examination of individual gene knockouts revealed that the little-studied U1 snRNP subunit LUC7L2 had the strongest effect on metabolism, with analysis of intracellular and media metabolites pointing to phosphofructokinase (PFKM) in glycolysis and the cystine/glutamate antiporter system $X_c^-$ as major control points. When LUC7L2 is lost, Jourdain et al. identified alternative splicing events, principally exon skipping and alternative 5′ SS splice site usage, in the PFKM and SLC7A11 (a subunit of system $X_c^-$) transcripts that lead to decreased expression of functional proteins and a number of metabolic consequences, including reduced flux through glycolysis, increased glycogenesis, reduced glutamate efflux, and a metabolic shift toward OXPHOS (Figure 1).

In complementary work, Daniels et al. studied the regulated alternative splicing events, RNA-binding sites, and protein interactors of each LUC7L homolog in depth. The proteins crosslinked almost exclusively to unique, non-overlapping sites on RNAs with LUC7L2 and LUC7L3 crosslinked mostly to exons near the 5′ SS, and LUC7L crosslinked to introns. Even though the proteins interacted similarly with core splicing factors, LUC7L2 and LUC7L3 both associated with SR proteins and were enriched at AAGAAG sites, while LUC7L was found associated with hnRNP proteins and enriched at uridine-rich sequences. Consistent with the work of Jourdain et al., Daniels et al. also identified exon skipping as the major change in alternative splicing due to loss of LUC7L2 and glycolysis as a key regulated pathway. Both Daniels et al. and Jourdain et al. propose distinct but partially overlapping functions in alternative splicing for the LUC7 homologs with LUC7L2 and LUC7L3 being functionally more similar to one another than to LUC7L—which is surprising, since LUC7L and LUC7L2 are the most alike in amino acid sequence.

Instead of a genetic screen, the work of Daniels et al. was motivated by the role of LUC7L2 in cancer: loss-of-function mutations in LUC7L2 are associated with splicing defects in myeloid neoplasms (Hershberger et al., 2020). Rewiring of cellular metabolism is a common feature of cancer cells so that they can meet the high energy demands of proliferation within their environment (Pavlova and Thompson, 2016). Based on the work of Daniels et al. and Jourdain et al., dysregulation of glycolysis via alternative splicing may also disrupt normal hematopoiesis and result in metabolic rewiring that causes or supports cancer cell growth. Many other splicing factors have been implicated in blood cell cancers (Dvinge et al., 2016), and Jourdain et al. note that myelodysplastic syndrome-associated mutations in *SRSF2* give rise to the same splicing changes in *PFKM* as does

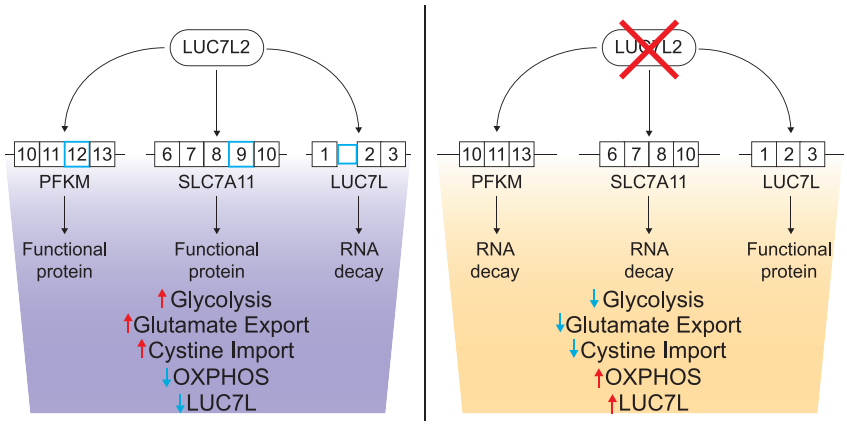


**Figure 1. LUC7L2-dependent changes in alternative splicing**

When LUC7L2 is present, it promotes exon inclusion in the PFKM and SLC7A11 transcripts from which functional proteins can be produced that support glycolysis and antagonize OXPHOS. In the absence of LUC7L2, exon skipping leads to pre-mature termination codon (PTC)-containing mRNAs that are likely degraded, decreased protein production, and metabolic rewiring away from glycolysis and toward OXPHOS. The presence of LUC7L2 also decreases expression of its homolog, LUC7L, by production of PTC-containing transcripts.

loss of LUC7L2. It is possible that mutations in different splicing factors share common mechanisms for reconfiguring metabolism to support cancer.

Another interesting consequence of these findings is that not only have they confirmed that LUC7L proteins can be components of the human U1 snRNP, but they are *variable* components. This implies that human U1, and potentially other snRNPs, is modular with subunit compositions adapted for particular types of splicing, cellular states, or tissues. By analogy to purchasing a car, the textbook U1 snRNP composed of the snRNA, U1-70K, U1-C, U1-A, and Sm proteins may be the "base model." Customizing the snRNP with upgrade packages such as LUC7L2 (the "sport model" involved in glycolysis regulation) or LUC7L3 (the "luxury model" found in brain tissue) may contribute to the specificity of alternative splicing programs. How customization is achieved is not yet known; however, both Daniels et al. and Jourdain et al. found that LUC7L2 controls splicing of LUC7L, suggesting that protein homologs found in these different upgrade packages can regulate one another.

Finally, many mysteries remain about how metabolism and splicing (and post-transcriptional gene regulation in general) are integrated. Based on previously published proteomics data showing that LUC7L2 is post-translationally modified by phosphorylation and hydroxylation, Jourdain et al. speculate that LUC7L2 and other splicing factors may be regulated by nutrients. Small molecules capable of modulating the splicing of specific transcripts are currently a major focus of research for the splicing field (Bates et al., 2017) and one such molecule, risdiplam, was recently FDA-approved for treatment of spinal muscular atrophy. Whether or not cellular metabolites can act as naturally-occurring splicing modulators is unknown; however, ATP-binding by the regulatory C-terminal cassette of the spliceosome helicase Brr2 (Absmeier et al., 2016) and inositol hexakisphosphate ($IP_6$)-binding by the spliceosome scaffold protein Prp8 (Plaschka et al., 2019) suggest that direct allosteric regulation of the spliceosome by metabolites is possible.

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