Structural investigation of Sam68-driven transcription/splicing coupling

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The identification of 20-25,000 human genes by the human genome project came as a big surprise since the estimated number of human proteins is around 130,000. This discrepancy can only be explained if one single gene can generate many proteins. It then became clear that alternative RNA splicing is a major regulatory event in cells, allowing for the production of many messenger RNAs and proteins from a single gene. This process is highly regulated by RNA binding proteins, called splicing factors, and defects in its regulation lead to a large number of diseases, including cancer, often due to overexpression or mutations of splicing factors.

A typical example is the splicing factor Sam68, which is overexpressed in a large number of cancers and whose function in alternative splicing is strongly modulated by signaling pathways (1). However, very little is known about the molecular mechanisms that govern these regulations and modulations.

We have recently revealed the molecular basis of RNA recognition by Sam68 and proposed a model of Sam68's function in alternative splicing (2). However, several lines of indirect evidence suggest that Sam68 might provide a link between transcription and alternative splicing. Indeed, Sam68 has been shown to bind transcription factors, such as the Androgen Receptor (3), FBI-1 (4), CBP (5), SND-1 (6), Brm (7), and we have observed that Sam68 regulates splicing in cells but not in in vitro splicing assays. The molecular mechanisms of this coupling remain unknown.

This project aims at investigating the structures of Sam68 with its transcription factor partners, using X-ray crystallography and Nuclear Magnetic Resonance (NMR) and identifying small molecules that interfere with these interactions. In parallel, we have already developed an in vitro transcription/splicing coupled assay that allows us to investigate the coupling between transcription and splicing in a controlled manner. Using this assay we aim to investigate at the molecular level the contribution of Sam68 and its transcription factor partners in transcription and splicing.

(1) Bielli et al. *The RNA-binding protein Sam68 is a multifunctional player in human cancer* **Endocr. Relat. Cancer** 18, R91–R102 (2011).

(2) Feracci et al. *Structural Basis of RNA recognition and dimerization by the STAR proteins T-STAR and Sam68*, **Nat. Comms**, 10355 (2016)

(3) Rajan et al. *The RNA-binding and adaptor protein Sam68 modulates signal-dependent splicing and transcriptional activity of the androgen receptor*. **J. Pathol.** 215, 67–77 (2008).

(4) Bielli et al. *The transcription factor FBI-1 inhibits SAM68-mediated BCL-X alternative splicing and apoptosis*. **EMBO Rep** 15, 419–427 (2014).

(5) Hong et al. *Physical and functional interaction between the transcriptional cofactor CBP and the KH domain protein Sam68*. **Mol Cancer Res** 1, 48–55 (2002).

(6) Capellari et al. *The transcriptional co-activator SND1 is a novel regulator of alternative splicing in prostate cancer cells.* **Oncogene**, 33, 3794-3802 (2014)

(7) Batsché et al. *The human SWI/SNF subunit Brm is a regulator of alternative splicing*. **Nat Struct Mol Biol** 13, 22–29 (2006).