

## Molecular model of TriBP1, a protein involved in nuclear import

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### Abstract

The transport of proteins and ribonucleoproteins between the nucleus and the cytoplasm of eukaryotic cells is an important activity that integrates the functions of protein and RNA synthesis and DNA replication. Both protein and nucleoprotein complexes are actively transported through the nuclear pore complex (NPC) that is embedded in the nuclear envelope, an energy and signal dependent process mediated by protein receptors<sup>1</sup>.

A major breakthrough in the study of nuclear import was the development of efficient *in vitro* systems<sup>2</sup>. Subsequently, macromolecular nuclear import has been well established for proteins that carry a short basic nuclear localisation signal (NLS), although there are various classes of NLS operating under different criteria. For example, in the best characterised class, the NLS is first recognised by importin  $\alpha/\beta$ , a soluble heterodimeric receptor. The importin  $\alpha$  subunit is essential for binding as it acts as an adapter by binding to the NLS peptide and, via its IBB domain, to importin  $\beta$ , which targets the protein to the NPC. Lastly, this complex is translocated into the nucleus by an energy-dependent mechanism aided by NTF2<sup>3</sup>.

The nuclear import of U small nuclear RNPs (snRNPs) does not follow the classical NLS model. The U snRNPs are essential components of the splicing machinery, and their genesis is a complex sequence of events in both the cytoplasmic and nuclear compartments<sup>4</sup>. The m<sup>7</sup>G cap or the cap-binding complex (CBC) are essential for nuclear export of the primary U snRNAs transcripts to the cytoplasm.

The nuclear import of U snRNPs requires Ran and Importin  $\beta$  and does not need importin  $\alpha$ . Importin  $\beta$  may collaborate with one or more adapter proteins that are similar to  $\beta$ , but which bind to U snRNP nuclear import signals instead of to the NLS. One of these factors that interact with the 5'-end cap structure of the U snRNPs has been identified and is referred to as snurportin 1<sup>6</sup>. Snurportin 1 is involved in m<sup>3</sup>G-cap dependent U snRNP nuclear import *in vivo* and *in vitro*. Nevertheless, the authors were unable to reconstitute U snRNP nuclear import in recombinant assays, thus other factors are likely to play a role in the nuclear import of U snRNPs.

We have focused on the identification of factors that can mediate the nuclear import of U snRNPs by interaction with the TMG nucleoside. In previous work, we prepared TMG nucleoside that demonstrated to be essentially similar to the m<sup>3</sup>G cap of U snRNAs<sup>7</sup>. Afterwards, we purified cytoplasmic HeLa proteins that bind to the TMG nucleoside by using TMG-affinity columns. The two major TMG-binding proteins were micro-sequenced and named

TriBP1 and TriBP2 (*Trimethylguanosine-Binding Protein 1 and 2*). TriBP1 binds m<sup>3</sup>G-capped U1 snRNPs specifically, in UV cross-link assays. TMG-binding proteins purified fraction, consisting in two major proteins (TriBP1 and 2), stimulates nuclear import in both U snRNP and protein-NLS *in vitro* import systems, mimicking the role of NTF2 in *in vitro* BSA-NLS import recombinant assays.

Comparative modelling studies were employed to shed light on the biological function of these proteins<sup>8</sup>. Modeller was used to construct a model of TriBP1 based on the known structure of the MM29898 protein from *Mus musculus* solved at 2.3 Angstroms resolution (PDB code 2a3q). The TriBP1 and MM29898 share 75% identity overall and the model reveals a structure formed mainly from alpha helices. Further structural analysis will be performed in order to understand the biological properties and roles of TriBP1 and TriBP2.

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