Poster I-38

Structural and Evolutionary analysis of Importin-alpha and its interaction with the nuclear localization sequence peptides.

Authors:
Agnes Alessandra Sekijima Takeda (*Dept. de Física e Biofísica, Instituto de Biociências, UNESP, Botucatu-SP, Brasil*)
Antônio Sergio Kimus Braz (*Laboratório de Biotecnologia e Genética Molecular (BIOGEM), Dept. de Genética, Instituto de Biociênc*)
Bostjan Kobe (*Dept .Biochemistry and Molecular Biology, University of Queensland, Brisbane,QLD, Australia*)
Marcos Roberto de Mattos Fontes (*Dept. de Física e Biofísica, Instituto de Biociências, UNESP, Botucatu-SP, Brasil*)

Short Abstract: Importin-alpha isoforms display cargo preferences in vitro and in vivo. In this study we present a phylogenetic analysis of Importin-alpha family, showing the independent duplication events in Metazoa and Viridiplantae. We also present structural sequence conservation and molecular dynamics studies of Importin-alpha and NLS interaction.

Long Abstract:

Nuclear proteins are synthesized in the cytoplasm and need to be imported into the nucleus. This import process is directed by special signals known as nuclear localization sequences (NLS) recognized by the nuclear import receptor Importin-alpha (ImpA). The NLSs are characterized by one or two clusters of basic amino acids (monopartide and bipartide NLSs). Crystal structures of native mammalian ImpA, their complexes with monopartite NLS peptide from SV40 and with the bipartite NLS peptides from nucleoplasmin, RB protein, and N1/N2 protein have been solved by us [1-3].Several ImpA isoforms have been described displaying cargo preferences in vitro and in vivo. While yeast has single isoform, invertebrates have three and mammals have until six. Isoform-selective NLS studies would provide an excellent tool for analyze the physiological consequences of the substrate specificities and lead to the development of new ligands that can distinguish between isoforms. The potential applications include drugs (anti-inflammatory, anti-cancer, and anti-fungal), gene therapy, drug delivery, and diagnostics.

In this work we present a phylogenetic analysis of ImpA family using several and representative Eukarya protein sequences. In the phylogenetic tree it can be observe five ImpA groups representing the Eukarya kingdoms. Most of these groups have single ImpA copy while others have many paralogues .Its remarkable the independent duplication events in Metazoa and Viridiplantae.

We also present results of structural sequence conservation. The analysis was made by ImpA sequences alignment and the ImpA2 from M. musculus crystallographic structure (pdb 1EJY) creating a structure with conservation levels along the protein surface among the organisms. The most conserved region was a central groove corresponding to the NLSs binding site.

Molecular Dynamics (MD) studies were performed with ImpA and ImpA monopartite NLS

peptide SV40/ bipartite nucleoplasmin NLS peptide complexes crystallographic data to verify the stability of the native N-terminally truncated ImpA native structure and the interactions between the ImpA and NLSs peptides. Different equilibrium states can be observed after 5ns resulting on different structures. The MD of complex ImpA;/NLS peptides led to a final structure close to the crystallographic structure, by contrast MD of the N-terminally truncated ImpA whose structure had significant changes.
Support by FAPESP, CNPq, CAPES/PRODOC and FUNDUNESP

[1] Kobe (1999) Nature Struct Biol 6, 388
[2] Fontes et al (2000) J. Mol. Biol. 297, 1183
[3] Fontes et al (2003) J Biol Chem. 278, 27981