





Structure and Function of the Nuclear Pore Complex Cytoplasmic mRNA Export Platform

Seung Joong Kim Integrative Structural Biology Workshop, 2016

Cytoplasmic mRNA Export Platform in the Nuclear Pore Complex (NPC)



Goal: dissect the structure of the platform to gain functional insights into the mRNA export process

Integrative Calculation of the Nup82 Holo-complex Structure



Seung Joong Kim, Riccardo Pellarin, Ilan Chemmama, Andrej Sali (NCDIR collaborators)

Comprehensive Cross-linking and MS of the Native Nup82 Holocomplex



High coverage: 1131 unique cross-links

Yi Shi, Junjie Wang, Brian Chait (NCDIR collaborators)

Stoichiometry



Shape and Dimensions of the Complex SAXS analysis of components fragments

Negative Stain EM of the Native Nup82 Complex





Use negative stain class averages of tilted and un-tilted particles directly as spatial restraints

Nup82 Holo-complex Stoichiometry

Holo-complex size: 640 kDa Stoichiometry : 2:2:2:2 Consistent with Gaik et al., JCB 2015

Paula Upla, Seung Joong Kim, Wenzhu Zhang (NCDIR collaborators)

Integrative Structure of the Native Nup82 Holo-complex



Precision: 9.0 Å

Allows location and orientation of the constituent proteins and domains of the holo-complex

Asymmetric arrangement of two compositionally identical hetero-trimeric subunits

Fernandez-Martinez et al., Cell 2016, in press

The Nup82 Complex Connects to the Nup84 Complex and Forms the Scaffold for the mRNP Remodeling Platform

Nup84 complex (outer rings)



The Nup82 Complex Connects to the Nup84 Complex and Forms the Scaffold for the mRNP Remodeling Platform



Are Nup84 Complex Mutant mRNA Export Defects Related to Defective Attachment of the Nup82 Complex?

Nup84 Complex Mutants give Characteristic mRNA Export Defect Phenotypes



Deletion of Nup85 Arm Causes Defect in Nup82 Complex NPC localization: Explains mRNA Export Phenotypes Nup84 complex heat map

Fernandez-Martinez et al., Cell 2016, in press

Nup82-84 Complex Forms a Strut in the NPC that Faces into the Central Transport Channel

Nup82-84 Complex Fits Into Yeast NPC Map... ... And Into Human Cryo-EM Map





Alber et al., Nature 2007

Von Appen et al., Nature 2015

Nup82-84 Complex Strut Aligns mRNA Export Machinery at Cytoplasmic End of Central Transport Channel

Old View

Current Structure



- Old idea of RNP processing sites projecting into cytoplasm not accurate
- Instead, project inwards towards central channel
- Provides structural framework to understand the mRNP remodeling and export processes

Transport & Remodeling Separate

Transport & Remodeling Integrated

Fernandez-Martinez et al., Cell 2016, in press

Validation of the Nup82 Holo-complex Structure





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