Three-dimensional Structure of the rSly1 N-terminal Domain Reveals a Conformational Change Induced by Binding to Syntaxin 5
Demet Arac, Irina Dulubova, Jimin Pei, Iryna Huryeva, Nick V. Grishin and Josep Rizo

Presented by Illya Tolokh,
University of Guelph
March 23, 2005

Outline
- Introduction to membrane fusion
- Experimental methods
- Results
- Discussion and conclusion
What is Membrane Fusion Needed For?

- Membrane fusion occurs in a multitude of cellular processes
- Involves both plasma membrane and organelles (in particular, the endoplasmic reticulum and the Golgi apparatus)
- Fusion is regulated by special proteins: SNAREs/SMs(?)

Overview of Vesicular Traffic in ER and Golgi

- Endoplasmic reticulum (ER): synthesis of proteins in ribosomes
- Golgi apparatus: post-translational modification and targeting of proteins

Nelson and Cox (2000)

Purves et al. (1998)
Vesicular Fusion Mechanism

- Special proteins (SNAREs) work to bring the vesicle and the cell membranes together
- “core complexes” are formed
- Two membranes fuse and vesicle contents are released inside the cell
- (5)-(7): complete fusion
- (8)-(10): “kiss-and-run” fusion
- Fission – imagine this going in the backward direction

Syntaxin: an Important SNARE Protein

- SNARE = soluble \( N \)-ethylmaleimide-sensitive factor attachment protein receptor
- Very important for eukaryotic membrane fusion!!
- Membrane proteins but also have large water-soluble region
- Example of an important SNARE protein: syntaxin
- Syntaxin is located in the ER and in the Golgi apparatus.
- SNARE domain: 60-70 amino acids
- Habc: three N-terminal regulatory domains
How do SNAREs Work?

- SNAREs from opposing membranes bind to each other’s SNARE motifs/domains (Qa, Qb, Qc, R)
- Form highly stable bundles of four alpha-helices (core complexes)
- Bring membranes together, causing them to fuse
- The complex “unbundles” through help from α-SNAP and NSF

Pratelli et. al. (2004)

SM Proteins

- SM – Sec1/Mun18-like cytosolic proteins
- 600-700 residues
- Seven SM proteins in vertebrates: munc18-1, munc18-2, munc18-3, Sly1, Vps45, Vps33a, and Vps33b
- Four SM proteins in yeast: Sec1p, Sly1p, Vps45p, and Vps33p
- SMs interact with SNAREs from the syntaxin family

Dietrich et. al. (2003)
General Structure of SM Proteins

- SM proteins exhibit homology throughout their entire structure
- SMs contain three domains, arranged in an arch shape
- Central cavity ~ 15Å

Role of SM Proteins in Membrane Fusion

- Largely unclear! Contrast with SNAREs
- Experiments shed some light on SMs’ function:
  - SM mutations => complete block of ER → Golgi traffic
  - SM over-expression => disruption of Golgi structure
- Proposed functions of SMs:
  - vesicle docking
  - assisting in core complex formation
- Mechanism of the above two proposed functions is unclear
Interaction of SMs with Syntaxins

- SMs can interact with SNAREs from the syntaxin family
- These interactions are diverse:
  - some SMs compete with core complex formation
  - some bind to core complex
  - some do not interact at all
- Diverse interactions yet unknown function!!

Interaction of Sly1 (SM) with Syntaxin 5

- Interaction studied in this paper: (rat) rSly1 + syntaxin 5
- N-terminal domain interaction: the most widespread mode of SM/SNARE syntaxin interaction
- How do we know that this interaction is important?
- Experiment: disrupt Sly1/syntaxin 5 binding => complete block in ER-to-Golgi transport, disruption of Golgi structure
- How does rat Sly1N 3D structure change upon syntaxin 5 binding?
Methods: Procedure Overview

- Use NMR (mostly NOESY-HSQC) to get the 3D structure of:
  - isolated rSly1N
  - complex between rSly1N and Syx5N (syntaxin 5 N)
- Compare:
  - isolated rSly1N ↔ (indirectly) rSly1N/Syx5N complex
- Elucidate structural changes in rSly1N upon Syx5N binding
- Sequence alignments of vertebrate and yeast SM proteins

Methods: Protein Expression and Purification

- rSly1N (residues 2-147):
  - expressed in bacteria
- $^{15}$NH$_4$Cl with or without $^{13}$C-glucose was used as a sole nitrogen/carbon source in growth medium
- Affinity-purification and gel-filtration to extract rSly1N from bacteria
- Syx5N (residues 1-33)
  - prepared synthetically
  - not labeled, so that only rSly1N conformational changes are detected
Methods: NMR Spectroscopy

- $^{15}$N-labeled rSly1N with or without Syx5(1-33) =>
  => 3D $^1$H-$^{15}$N –NOESY-HSQC => acquire initial spectra
- $^{15}$N- and $^{13}$C-labeled isolated rSly1N: a suite of pulsed field gradient enhanced NMR experiments:
  - Resonance assignments: (TOCSY)-HSQC, HNCO, HNCACB, CBCA(CO)NH, (H)C(CO)NH-TOCSY, H(C)(CO)NH-TOCSY, and HCCH-TOCSY
  - NOEs for structure determination: 2D NOESY, 3D $^1$H-$^{15}$N NOESY-HSQC, and 3D $^1$H-$^{13}$C NOESY-HSQC
- Structure calculations

Methods: Short NOESY-HSQC Overview

- 2D NOESY – coherence transfer experiment through space dipolar coupling
- Nuclei that are spatially close together will yield a signal
- Cross-peak intensity $\sim r^{-6}$
- This lets one estimate internuclear distances
- Heteronuclear interactions
  => NOESY-HSQC => 3D data
- Generates many-many distance constraints
- Characteristic patterns exist for regular secondary structures, e.g. alpha-helices and beta-sheets => helps in structure verification
Results: 3D Structure of rSly1N

- Multidimensional heteronuclear NMR experiments
- Total of 2493 experimental restraints, including 738 NOEs
- 20 structures with lowest energy were selected
- Structure validity was verified with Ramachandran plots

<table>
<thead>
<tr>
<th>Ramachandran plot statistics (Arac et. al. (2005))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residues in most-favored regions (%)</td>
</tr>
<tr>
<td>Residues in additionally allowed regions (%)</td>
</tr>
<tr>
<td>Residues in generously allowed regions (%)</td>
</tr>
<tr>
<td>Residues in non-allowed regions (%)</td>
</tr>
</tbody>
</table>

3D Structure of rSly1N

- Left (a): backbone superposition of the 20 calculated rSly1N structures with the lowest energy
- Left (b): ribbon diagram of the lowest energy rSly1N structure
- Right: diagram of the yeast Sly1p complex structure
Results: Syx5 Binding → Conformational Change in rSly1N

- 3D $^1$H-$^{15}$N HSQC-NOESY spectrum of rSly1N shows cross-peak shifts upon binding to Syx5:
  - X → L111NH/K140H
  - X → A139NH/Y109NH
  - Different patterns in general
- Numerous unexpected NOEs between two C-terminal beta strands
- This agrees with the hypothesis that Syx5 binding causes a significant conformational change in rSly1N

Usefulness of NOE Data of the Rat Complex

- The NOE data of the rSly1N/Syx5 complex is not sufficient to calculate the 3D structure
- But! NOE data comparison shows that structure of the rat rSly1N/Syx5 complex is analogous to that of the yeast Sly1p/Sed5p complex
Summary of Logic

- There is no 3D structure of the mammalian rSly1N/Syx5 complex
- There is a 3D structure of the yeast Sly1p/Sed5p complex
- NOE data says that binding modes of mammalian rSly1N/Syx5 and yeast Sly1p/Sed5p complexes are the same
- Hence, the authors compared the isolated mammalian protein structure with the yeast complex

Are There Any Possible Artefacts?

- 3D structure of a truncated protein (i.e. rSly1N) was measured
- How do we know that its conformation would be the same if it weren’t truncated?
- Measure HSQC spectrum of rSly1N with seven additional residues at the C terminus => practically identical spectra!
Structural Change #1: H5 and S5 Move Apart

- Binding of Sed5p involved multiple hydrophobic contacts between its key phenylalanine residue and helix 5 and strand 5 of Sed5p.
- Thus, h5 and s5 are further away in the complex than in the isolated structure to free up the syntaxin binding site.

Structural Change #2: Shift of S5 Relative to S4

- Syx5 binding induces the downshift of strand 5 with respect to strand 4 to form hydrophobic binding pocket for Syx5.
Change is Conserved in Sly1 Family

- Linker (between h5 and s5) is 3-4 residues longer in Sly1 family than in other 3 families
- This linker is a distinctive feature of the Sly1 family
- Key to conformational change occurring only in Sly1 family
- Longer linker => stretches/contracts more => S5 is more mobile => accommodates for Syx5 binding
- The four SM protein families diverged early in evolution

Conclusions and Further Questions

- Diverse modes of interaction between SNAREs and SMs
- Sly1 binds to short N-terminal sequences of syntaxins, instead of their SNARE motifs
- This binding causes striking conformational changes in Sly1, which is distinctly different from other mammalian SMs
- Why such divergence?
  - Different interactions => differentiation of membrane compartments?
  - Different regulatory requirements of traffic in various compartments?
- Functional role of Sly1/Syx5 interaction?
  - Further research needs to be done besides mutations/knock-outs
  - How does conformational change in Sly1 translate into its function?
References

- Dr. Ladizhansky’s lecture notes - 2005