Molecular architecture and function of the SEA complex

Seung Joong Kim, R Pellarin, P Cimermancic, Andrej Sali group, UCSF
w/ R Algret, J Fernandez-Martinez, Y Shi,
E Cochet, BT Chait, MP Rout, and S Dokudovskaya

• From a proteomics screen, yeast Npr2 and Npr3 are regulators of TORC1 (Neklesa & Davis, 2009).
• The TORC1 (Target of Rapamycin Complex 1) signaling pathway plays a major role in the control of cell growth and response to stress.
• The SEA complex in yeast also contains Sea1-4, Sec13 (COPII and NPC) and Seh1 (NPC) (Dokudovskaya et al, 2011).
• Mammalian analogs of SEA are GATOR1 and GATOR2, corresponding to SEACIT and SEACAT in yeast (Bar-Peled et al, 2013).
• Localized at vacuolar membrane; SEA proteins are predicted to contain alpha-solenoid and beta-propeller domains present in complexes that interact and curve membranes (eg, NPC, HOPS, CORVET, NPC, COPI, COPII, clathrin/adaptin).
The SEA complex is dynamically associated with (or localized around) the vacuole membrane.

The TORC1 (Target of Rapamycin Complex 1) signaling pathway plays a major role in the control of cell growth and response to stress.

The SEA complex physically interacts with TORC1 and is an important regulator of its activity.

S. Dokudovskaya et al, “A conserved coatamer-related complex containing Sec13 and Seh1 dynamically associates with the vacuole in Saccharomyces cerevisiae”. MCP, 2011.
Integrative structure determination of the SEA complex

**Experimental data**
- **Residue-specific cross-linking**: 45 inter-molecular and 143 intra-molecular cross-links
- **Protein and domain interactions**: 23 affinity purifications
- **Stoichiometry**: 8 proteins
- **X-ray crystallography**: 2 proteins / domains

**Statistical inference and physical principles**
- **Comparative modeling**: 19 domains
- **Bioinformatics**: 8 proteins
  - Fold models
  - Excluded Volume

**Gathering Data**
- Representing and translating data into spatial restraints

**Sampling the Good Scoring Configurations**
- Random configurations
  - Initial sampling (Cross-links and selected composite restraints)
- Connected configurations
  - Refinement (Cross-links and ALL composite restraints)
- Final structures

**Analyzing and Assessing the ensemble**
- Model clustering
- Protein and domain contact frequency
- Protein and domain localization
Data: Residue-specific DSS (Lys-Lys) crosslinks

45 inter-molecular (and 143 intra-molecular) DSS crosslinks

Two subunits
1. SEA1, Npr2, Npr3
2. SEA3/Sec13, SEA4/Seh1, SEA2

The interactions between SEA2, SEA3/Sec13 and SEA4/Seh1 strongly depend on the C-terminal RING domain.

The connection between these two subunits happens via Npr3/SEA1 and N-terminal part of SEA3.

SEA3 forms a dimer with Sec13
SEA4 forms a dimer with Seh1

Yi Shi, Javier Fernandez-Martinez
Data: Residue-specific DSS (Lys-Lys) crosslinks

45 inter-molecular and 143 intra-molecular DSS (Lys-Lys) crosslinks (XLs)

RED dot: XL in "DISORDERED" region.
GREEN dot: XL in "STRUCTURED" region
BLACK dot: XL in "UNKNOWN" region.

Square box, template structure coverage:
RED: 100% sequence identity.
BLUE: 10~15% sequence identity.
Data: Affinity co-purification

7 protein pullouts, 16 domain deletion pullouts

```
<table>
<thead>
<tr>
<th></th>
<th>SEA1</th>
<th>SEA1</th>
<th>SEA2</th>
<th>SEA3</th>
<th>SEA4</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>S1</td>
<td>S1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>S2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>S3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>S4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPR2</td>
<td>S1</td>
<td>S1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>S2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>S3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>S4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPR3</td>
<td>S1</td>
<td>S1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>S2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>S3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>S4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPR4</td>
<td>S1</td>
<td>S1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>S2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>S3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>S4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```

Romain Algret
The SEA complex was isolated in 5-20% sucrose velocity gradients and the resulting 12 equal fractions were analyzed on 4%-12% Bis-Tris gels. Gels were stained with SYPRO Ruby (Molecular Probes) and digitized.

1:3 stoichiometry for Sea4 and Seh1

Benchmark, with known answers.
Hierarchical model representation facilitates using imprecise information
Representation of the SEA Complex

Multi-scale Representation

- Resolution = 0 (atomic resolution)
- Resolution = 1 (1 bead / 1 residue)
- Resolution = 5 (1 bead / 5 residues)
- Resolution = 10 (1 bead / 10 residues)
- Resolution = 100 (1 bead / 100 residues)

Resolution = 100
~100 residues per bead

Domain mapping (composite) restraints shown is also a “Gaussian” envelope
Sampling good scoring models

Monte Carlo sampling with simulated annealing:
• Start with a random configuration of protein centers.
• Minimize violations of input restraints by Monte Carlo with simulated annealing.
• Obtain an “ensemble” of many independently calculated models (885 refined models).

Total score =
188 Harmonic Upper Bounds for Crosslinks (17Å) +
23 Composite Restraints +
Linkers Between Beads +
Excluded Volume

885 best scoring models satisfy all restraints.
Protein Localization Probability and Volume

Calculated from the structural superposition of the ensemble of models that satisfy all input restraints

Hierarchical clustering based on the RMSD distance matrix

Protein localization probability (contoured to get 1.5 times protein volume)

Can see position of every SEA protein!
Molecular architecture of the SEA complex

- SEACAT and SEACIT sub-complexes interact via the Sec13 / Sea3 hub
- Cluster of beta-propellers from Seh1, Sec13, Sea4, and Sea2 (cf, COPI and COPII)
- RING domains of Sea2/3/4 interact with each other
- Stoichiometry of Sea4 and Seh1 is 3
- Can map mutations associated with diseases (e.g., cancer) on structure
- Can map transient protein-protein interactions to expand from this stable complex ...
- SEACAT activates TOR1; SEACIT inhibits TOR1
- Provides a starting point for X-ray, EM, more cross-linking
Contact frequency map and crosslinks

The proximities of any two residues in the topological map were measured by their relative “contact frequency”. A contact between a pair of residues is defined when their corresponding bead surfaces are less than 20 Å from each other.

Crosslinks were plotted as the red dots, and the residue contact frequency is indicated by a color ranging from white (0) to dark blue (1). Each box contains the contact frequency between the corresponding pair of the SEA complex proteins.
The TORC1 (Target of Rapamycin Complex 1) signaling pathway plays a major role in the control of cell growth and response to stress. The SEA complex physically interacts with TORC1 and is an important regulator of its activity.

A number of functional data indicated a role for the SEA complex in intracellular trafficking, amino acid biogenesis, regulation of the TORC1 pathway and autophagy.
Localization, inhibition, and activation of TORC1 depend on the SEA complex

Adapted from Panchaud et al. Cell Cycle 12, 2013.

V-ATPase

membrane-associated scaffold, needed for activation of TORC1

interacts with and inhibits TORC1

Adapted from Panchaud et al. Cell Cycle 12, 2013.
Python Modeling Interface (PMI)

Create hierarchies, rigid bodies, and flexible parts for bead representations

PMI provides repository of python classes and libraries to represent, score, sample and analyze models based on IMP.

Easy-to-use python libraries.

Cross-link restraint

Monte Carlo Sampling and Optimization

Riccardo Pellarin, Peter Cimermancic, Daniel Russel, Charles Greenberg, Elina Tjioe, Seung Joong Kim, Max Bonomi, Yannick Spill