Evolution of the Endomembrane System
Joel Dacks, Univ. Alberta

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Questions

• What diversity exists in eukaryotic membrane-trafficking: organismal biology

• In what way does the model as derived in animal and yeast apply to eukaryotic cells in general?

• When and how did the eukaryotic membrane-trafficking machinery evolve?
Talk Outline

• How can an evolutionary approach be useful to cell biologists?
Eukaryotes are more than
In modern eukaryotes enables:
- Uptake from the environment
- Export to the environment
- Cell surface remodelling
- Intracellular targeting
- Compartmentalization
Eukaryotic Membrane Trafficking System

For the earliest eukaryotes
- Environmental sensing
- Population communication
- Communication with MROs
- Sequestration (introns, enzymes)
- Nutrient acquisition
Protein machinery in Membrane Trafficking

Bonifacino & Glick, *Cell*, 2004
Methodology

• Use of publicly available data
• Genome sequence analysis, collaboration with genome sequencing centres (JGI, TIGR) or with a few labs
• Homology searching (BLAST, HMMer, RBH,domains)
• Phylogenetic analyses (MB, PhyloBayes, RAxML)
• Molecular cell biology in collab with relevant labs
The diversity of Eukaryotes

- Arabidopsis is here
- Yeast is here
- Emiliania is here
- Trichomonas is here
- Toxoplasma and Stentor are here
- Difflugia is here
- You are here
- You are here
- Yeast is here
Use genome information to understand trafficking in these organisms.

Carlton et al. 2007, Read et al. 2013, Woo et al. 2015
A theory for the evolution of the endomembrane system
Understanding the pieces of the puzzle

Molecular evolutionary analyses of membrane-trafficking machinery to reconstruct back

Synthesize hypotheses based on robust evidence and identify patterns from multiple sets of proteins

Can they be put together?
Reconstruct back
LECA is a tractable reconstruction point
Eukaryotic diversity
Simplified view of Eukaryotic diversity
Distribution indicates history and essentiality
Distribution indicates history and essentiality
A sophisticated complement in the LECA

Comparative genomics from taxa across the span of eukaryotic diversity showed the major families of trafficking proteins present in the LECA.
Organelle or pathway-specific machinery

Understanding the history of the organelle-specific proteins:
Understand the history of the organelles
When did the organelle/pathway-specific subfamilies arise?
Adaptins

Hirst, Barlow, et al. 2011
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• What diversity exists in eukaryotic membrane-trafficking: organismal biology

• Are there pieces that we have been missing or ignoring due to the emphasis on our model cell biological systems (asymmetry?)
The asymmetry problem
Use genome information to understand trafficking in these organisms.
An extra clade of Adaptins?
AP5-A novel endosomal AP

- Characterized in Hela cells
- Localizes to LAMP1-positive endosomes
- Affects CIMPR trafficking
- NOT clathrin associated, not brefeldin sensitive
All components have similar distribution: ancient but patchy

Hirst, Barlow, et al. 2011
AP5-A novel endosomal AP

- Characterized in Hela cells
- Localizes to LAMP1-positive endosomes
- Affects CIMPR trafficking
- NOT clathrin associated, not brefeldin sensitive

- Found in high-throughput screen for Hereditary Spastic Paraplegia!
AP5-A novel endosomal AP

- Characterized in Hela cells
- Localizes to LAMP1-positive endosomes
- Affects CIMPR trafficking
- NOT clathrin associated, not brefeldin sensitive

Are there others?
4 proteins resembling APs

Large subunits

- solenoid
- Appendage

®family

© family

Medium and small subunits

Longin

MHD

SH3

100 residues

helix

strand
4 proteins forms a complex
Identified “coat” proteins

![Graph showing iBAQ ratios for different proteins](image)

- **GFP**
- **?-like (TPOON)**
- **?-like (TPLATE)**
- **?-like (TSAUCER)**
- **?-like (TCUP)**
- **XP_637150.1 (TTTRAY1)**
- **XP_637159.1 (TTTRAY2)**
- **SecG**
Predicted Secondary Structures of
*D. discoideum* proteins
TSET is Widely Distributed
FCHo/Syp1 proteins regulate clathrin
Remnants of TSET in Opisthokonts

[Temperature image]

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- **TPLATE**
- **TSAUCER**
- **TCUP**
- **TSPOON**
- **TTRAY1**
- **TTRAY2**
Phylogeny of organelle-paralogues-HTACs

TSET, AP1-5, CopI: all pre-LECA
Phylogeny of organelle-paralogues: Qa-SNAREs

Syn5(Golgi), Syn18 (ER), SynPM, SynE, Syn16 (TGN-Endosomes), and Syn 17(MAM/ER/autophagosome)

Arasaki et al.  2015
Phylogeny of organelle-paralogues: Rabs

19-23 LECA Rab clades, covering all MTS organelles, including Ran (NPC), IFT27 (Flagella) and RabTitan
Organelle-specific paralogues present in the LECA

Reconstruction of sophisticated trafficking machinery in the LECA
Also instances of unheralded trafficking machinery (TSET, AP5, ArfGAP_C2...)

- Arf/SAR
- ArfGAP (6)
- Copl
- CopII (Sec24*3)
- Clathrin/adaptins (5)

- Rab (19-23)
- RabGAP
- SNARE (6 Qa)
- SM
LECA and complexity
Back beyond the LECA to the step from the FECA
Many phylogenies show similar patterns

- **SM proteins** - Koumandou, Dacks, Coulson and Field, 2007
- **Syntaxins** - Dacks and Doolittle, 2002, 2004
- **Coats (Clathrin, COPI, COPII...NPC)** - Schledzewski et al. 1999, Singh and Gupta, 2004, ...Devos et al. 2004

**Elaborate machinery early on...**

Model of autogenous organelle evolution
Organelle Paralogy Hypothesis: A mechanism for non-endosymbiotic organelle evolution

Specificity is encoded in the combinatorial interactions

Gene duplication and co-evolution specificity machinery

Schlacht et al. 2014
Evolutionary cell biological studies of membrane trafficking machinery shows a sophisticated complement early in eukaryotic evolution.

Not only protein families but also organelle-specific paralogues. Most recently Syntaxin 17.

These have lead to discoveries of novel proteins (Rabtitan, ArfGAP_C2), complexes (TSET) and trafficking pathways (AP5) or have shed light on the origins of ‘orphan’ proteins in model cell biological systems.

Neither could have been anticipated in an application targeted for commercialization= BASIC RESEARCH.
Conclusions-II

Larger patterns of protein conservation show the mechanisms driving complexity in the LECA-OPH, PC, Neutral

Mechanisms of non-endosymbiotic organelle evolution combined with improved phylogenetic resolution are allowing for speculative, but hopefully testable models of how the membrane trafficking system arose.

New microbial diversity is allowing for new insights into the constituent contributions of cellular systems

Evolutionary cell biology is a fruitful way of discovering aspects of how cells work today, in a complementary way to focused work in model systems, and yields information about our cellular past
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