

## Rab Proteins as Membrane Organizers

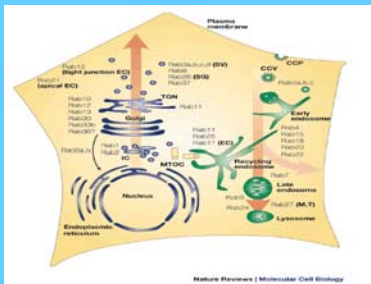
Marino Zerial and Heidi McBride  
Nature Reviews Feb 2001

Presented By Erikka Carr  
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## Regulation of Vesicular Traffic Through Rab

- Largest family of monomeric small GTPases
- GTP binding protein that plays an important role in vesicular transport
  - Distributed to distinct compartments
  - Cycling from GDP to GTP-bound conformation mediated by hydrolysis and nucleotide exchange
    - Temporal and spatial regulation on membrane transport

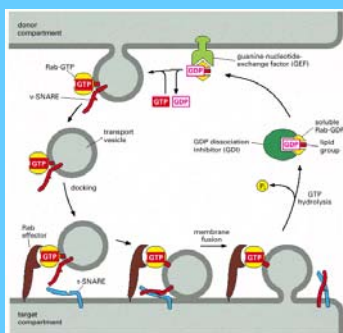
All organelles have at least one Rab associated with its membrane



## Regulation of Intracellular Transport

- Rab proteins known to function in
  - **tethering of vesicles to the target membrane**
  - vesicular budding/formation
  - Interaction of vesicles to the cytoskeleton to regulate vesicular motility

## Rab-GTP and Vesicle Tethering/Docking



## Tethering of vesicles to the Target Membrane

- Rab and its effectors are recruited to membranes to act as anchors while SNARE complex mediates membrane fusion
- Rab-effector interaction required before SNARE complex interaction can occur
- Evidence for Rab and effector recruitment on membrane tethering in ER derived vesicles to Golgi as well as Golgi to plasma membrane
- Rab5 required for clathrin coated vesicular transport from the PM to early endosomes and subsequent fusion

## Vesicle Budding

- Contradictory evidence depending on experimental system
- Rab5 required for vesicle formation *in vitro*
- Possible roles for Rab proteins in budding
  - Regulation of concentration or assembly of protein coats
  - Help incorporate cargo into vesicles
  - Active state may act as a checkpoint that ensures vesicle delivery to its appropriate target membrane

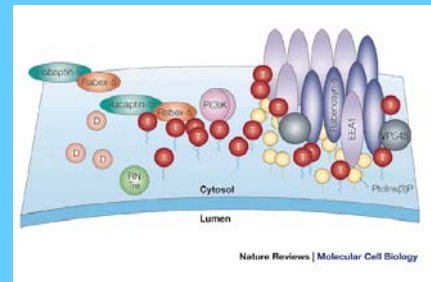
## Vesicle Motility

- Rab proteins have been shown to regulate movement of vesicles along cytoskeleton
- Human Disease Model
  - Griscelli syndrome
    - Defect in pigmentation due to accumulation of melanosomes in melanocytes
    - Mouse mutants of gene homologs have defect in pigment granule transport

## Rab5 Effectors

- Affinity column chromatography identified more than 20 effectors that bind Rab5
- Rabaptin-5 forms a complex with Rabex-5 which recruits Rab proteins
- Active Rab-GTP feeds back to recruit more Rab via rabaptin-5
  - Forms a Rab-GTP cluster that recruits other effectors

## Rab Effectors Act Cooperatively to Form Rab Domains



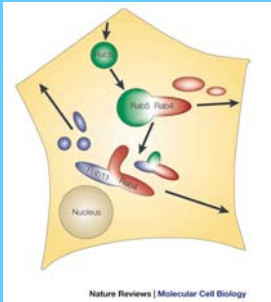
## Rab Domains

- Rab effectors act cooperatively in a cluster to form a Rab domain
- Protein-lipid interaction
  - Localized PI(3)P synthesis requires Rab-GTP
    - Recruits FYVE effectors and contributes to effector clustering
  - PI(3)P also localized in internal vesicles of multivesicular endosomes
    - PI(3)P can also be synthesized at other endocytic pathway stages with other rab proteins
- Effectors oligomerize with each other to form clusters
  - EEA1, rab5 effector has a rab5 binding domain and a FYVE finger domain

## Visualizing Rab Domains

- Expression of a constitutively activated Rab mutant, Rab5Q79L, shows Rab5 and its effector EEA1 cluster on the endosomal membrane
  - Speculation that these clusters are enriched at the interface between fusing vesicles
- GFP tagged Rab4, Rab5 and Rab11 show endosomal membrane enrichment in distinct compartments
  - Rab5 only
    - Early/sorting endosomes
  - Rab4 and Rab5
    - Early/sorting
  - Rab4 and Rab 11
    - Recycling endosomes

## Rab Proteins are Compartmentalized within Membranes of Early Endosomes



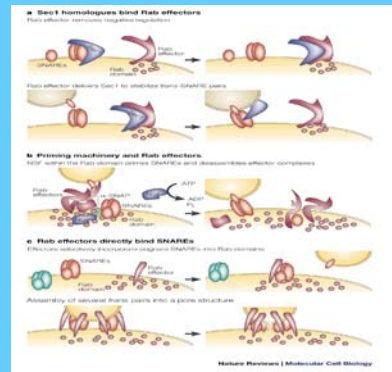
## How are These Domains linked?

- Transferrin, a ferric ion transporter was found to migrate from the Rab5 domain to Rab4 and Rab11 domains sequentially
  - Speculation of a Rab effector mosaic that interacts but maintains a stable distribution over time
- Membrane compartmentalization also found in yeast biosynthetic pathways

## Rabs Linkage to SNARE

- Selective incorporation of cis-SNARE complexes within a Rab domain is required for trans-SNARE pairing
- Rab effector and SNARE machinery cooperatively
  - Direct interaction
    - Rab effectors bind SNAREs
  - Indirect interaction
    - Sec1, a trans-SNARE inhibitor, binds Rab effector
    - Rab effectors bind priming machinery

## Rab Effector and SNARE Interactions



## Conclusion

- Rab proteins act with effectors to coordinate vesicle transport
- Rab proteins regulates membrane docking while trans-SNARE interactions pull membranes close together to induce fusion
- Rab5 effectors show cooperativity with each other to form membrane clusters called rab domains
- Rab effectors and SNAREs have been shown to directly and indirectly interact
- Authors postulate that formation of a cis-SNARE complex is required for trans-SNAREs interactions

## Questions?

