

Introduction

- ♦ Loss of exocyst Sec5, Sec6 and Sec15 in fly epithelia results in DE-Cad/Arm accumulating in enlarged Rab11 recycling endosome (RE).
- ♦ Rab11 interact with Sec15 and Arm interacts with Sec10, as Sec15 and Sec10 are located on RE.
- ♦ The exocyst complex is important for recognition and tethering between vesicle and PM for polarized transport to apical and basal membranes.

DE-Cad/Arm accumulate intracellularly in *sec5*

- ♦ Crb-Sdt, Baz-Par6-aPKC, basolateral Dlg, Scrib and Fas3 was unaffected in *Sec5*, but DE-Cad, Arm and D α -cat accumulated in apical cytoplasm and around nucleus in punctate structures.

DE-Cad and Arm accumulate in enlarged RE in *Sec5*

- ♦ Golgi marker Lva, EE marker Rab5 and LE marker HRS were normal, but Rab11 was distributed in enlarged punctate structures with higher fluorescence. Some of these large RE were formed by coalescence of smaller punctate structures. Additional RE marker was also found in higher levels.
- ♦ DE-Cad and Arm colocalized with RE in WT cells. In *sec5* DE-Cad and Arm was excessively enriched in RE and colocalized with Nuf and Rab11. Even when distribution of Arm or DE-Cad was normal, Rab11 and Nuf accumulated. Overall the RE is enlarged in *sec5* and DE-Cad accumulation is a consequence of Rab11 and Nuf accumulation in RE.

Sec5 is associated with RE and lateral cortex

- ♦ In WT cells, Sec5 was found in both punctate structures colocalizing with mostly Rab11 and at cell cortex where it colocalized with DE-Cad/Arm/AJ. Therefore Sec5 regulates size of RE and distribution of DE-Cad.
- ♦ In *sec6*, Sec5, DE-Cad and Arm accumulated in apical punctate structures and was reduced laterally. RE was also enlarged and Sec5 structures colocalized with Rab11. Therefore in WT Sec5 may bind RE before going to cell cortex and both Sec5 and Sec6 regulate the RE.
- ♦ Sec15 interact with Rab11-GTP (Rab11 is GTPase). Therefore Sec15 is an effector of Rab11 and

Sec5 may bind Sec15 via Sec10 as Sec5 co-immunoprecipitate with Sec15 and Rab11 and Sec15 punctate structures colocalized with Sec5 and Rab11.

- ◆ In *sec15*, Sec5 was diffused into cytoplasm with DE-Cad accumulated in large RE. Since Sec5 did not accumulate in RE, this suggest Sec15 link Sec5 and RE, but Sec6 is optional. But Sec5, Sec6 and Sec15 is all required to regulate DE-Cad and size of RE.

Sec5 is required for PM DE-Cad delivery

- ◆ Without cell permeabilization and endocytosis (keep at 4°C), DE-Cad and Arm was detected on lateral PM below AJ because SJ prevent Ab from binding AJ DE-Cad.
- ◆ In *sec5*, *sec6* or *sec15*, lateral DE-Cad was strongly reduced showing reduced DE-Cad PM delivery but Fas3 was unaffected. Therefore Sec5, Sec6 and Sec15 is necessary for lateral delivery of DE-Cad to PM where Sec5 and Arm is localized.

Sec5 recycle lateral DE-Cad to AJ

- ◆ Transcytosis assay: incubate with DCAD2 Ab at 25°C for 30 minutes to allow endocytosis. Since SJ prevent Ab from reaching AJ naturally, any Ab appearing at AJ or above SJ must be result of DE-Cad transcytosis.
- ◆ In WT, DE-Cad was found in AJ with Arm and Dlg, showing lateral membrane DE-Cad was recycled to AJ and ZA above SJ respectively.
- ◆ In *sec5*, DE-Cad was not detected at AJ and Ab was found within the enlarged RE, showing DE-Cad was internalized but could not be recycled to AJ. Therefore Ssec5 is necessary for recycling of DE-Cad from RE to apical AJ.

Arm binds Sec10

- ◆ Arm interacted with Sec10, but not Sec8, Exo84 or Sec6 in GST pull-down. Arm, Sec5, Sec15 and D α -Cat coimmunoprecipitated. Also Sec5 coimmunoprecipitated with β -Cat and both along with α -Cat coimmunoprecipitated with Sec8. Thus Arm and β -Cat exist in a complex with exocyst.
- ◆ In *arm*, DE-Cad level was reduced and absent from lateral membrane and AJ but was accumulated in intracellular punctate structures and RE where it colocalized with Rab11
- ◆ Overall suggest Arm with the exocyst complex regulate delivery of DE-Cad from RE to lateral

membrane and AJ.