RAB35 small GTPase activity coordinates endosome dynamics to facilitate apicosome formation in a human model of epiblast formation

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ABSTRACT

Formation of a central lumen in the developing human epiblast is a critical step that occurs during implantation. However, there is a significant knowledge gap in the mechanistic understanding by which epiblast cells initiate polarization and form a central lumen. Using a human model of epiblast formation, our previous studies have shown that apical polarization in such model is initiated by the formation of an apicosome, an intracellular membrane-bound structure with apically polarized features including microvilli and primary cilium. During apicosome formation, apically polarized vesicles accumulate and fuse at the peri-nuclear region, and gives rise to a small apicosome, which grows in size and matures overtime. Here, we comprehensively characterized endosomal trafficking dynamics during apicosome formation, and identified RAB35 as an apicosomal RAB that plays a critical role in apicosome formation by controlling early, recycling, and late endosome dynamics. Unlike previously characterized RABs that encase, but not directly associate with, apicosomal membrane, RAB35 co-localizes with apicosome membrane and membrane associated proteins. Strikingly, in the absence of RAB35, several apicosomes that are smaller in size are formed, and defective early and recycling endosome dynamics are seen. Mechanistically, RAB35 GTPase activity is critical, as, while the expression of wild-type or a constitutively active form of RAB35 leads restoration of single apicosome formation, the expression of a dominant negative form of RAB35 leads to the multi-apicosome phenotype. Together, these results show that RAB35 organizes membrane trafficking that aids in vesicle fusion to promote single apicosome formation.