RAB3A and RAB27A Interact with Rabphilin 3A to Promote Cortical Granule Exocytosis in Mouse Eggs

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Cortical granule exocytosis, or the “slow block to polyspermy,” is an important event that occurs shortly after sperm-egg fusion to prevent entry of more than one sperm into the egg, which is not compatible with further development. Proteins in the Rab family, particularly RAB3A and RAB27A. are well known to participate in exocytic events in secretory cells and there is evidence for the involvement of these Rabs in cortical granule exocytosis (CGE) in mouse and sea urchin eggs; however, the role of RAB3A in particular needs some clarification because it has also been demonstrated to affect spindle migration during oocyte maturation. Using fluorescently tagged proteins, dominant negative proteins, and Trim-away to specifically deplete proteins, we show that both RABs 3A and 27A are required for this process. RAB3A is primarily expressed in the cytoplasm, with some cortical localization. RAB27A is localized both at the plasma membrane (PM) and in the cytoplasm, whereas Rabphilin 3A (RPH3A) is localized exclusively in the cortex opposite the meiotic spindle. All 3 proteins are rapidly removed from the PM following a Ca^{2+} stimulus. Total RAB3A and RAB27A protein remains the same after egg activation, suggesting that these proteins are recycled rather than lost during the process of exocytosis. Depletion of both RAB3A and RAB27A using Trim-away does not affect cortical granule localization at the PM but prevents the cleavage of ZP2 following egg activation. In addition, a dominant negative form of RAB27A potently blocks exocytosis without affecting cortical granule placement at the PM. Cortical granules become dispersed in response to a Ca^{2+} stimulus in eggs expressing dominant negative RAB27, suggesting that RAB27A functions at a very late stage in exocytosis. Depletion of RAB3A and/or inhibition of RAB27A prevents placement of RPH3A at the PM. However, a dominant negative form of RPH3A does not block CGE, suggesting that more than one RAB effector is important for CGE. These results demonstrate that RAB3A and RAB27A are both required for cortical granule exocytosis and work through RPH3A, though not exclusively.