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A passionate kiss, then run: exocytosis and recycling of IgG by FcRn

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The MHC-class-I-like Fc γ receptor FcRn recycles immunoglobulin (Ig)G from most cells and transports it bidirectionally across epithelial barriers to affect systemic and mucosal immunity. Recent studies have shown that FcRn rescues IgG from intracellular lysosomal degradation by recycling it from the sorting endosome to the cell surface. Most recycling vesicles fuse completely with the plasma membrane in a classical pattern of exocytosis. Similar to the process seen for neurotransmitter release at synaptic junctions, other vesicles fuse only partially, releasing FcRn–IgG complexes to mix into

the plasma membrane in cycles of 3–4 s over prolonged periods of time.

Introduction

Immunoglobulin (Ig)G mediates the majority of humoral immunity in humans. Because a high concentration of IgG bathes most cells, it is readily internalized by nonspecific mechanisms of fluid-phase endocytosis. Unlike most other protein solutes internalized by fluid-phase endocytosis, however, IgG is rescued from degradation in the lysosome by the MHC-class-I-like Fc γ receptor FcRn [1]. FcRn binds to IgG and recycles it back to the plasma membrane and

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into the circulation. The FcRn receptor is widely expressed and the recycling pathway is thought to be constitutive [2,3]. This explains why IgG displays the longest half-life of all of the serum proteins [4]. Exactly where FcRn initially binds to IgG in the endocytic pathway, however, and how the receptor traffics IgG back to the cell surface remain a mystery. Two recent articles by Ober *et al.* [3,5] go a long way to solving these problems. The studies show that FcRn carries IgG all the way from the sorting endosome to the plasma membrane. Whereas most FcRn-containing recycling vesicles fuse completely with the plasma membrane in a classical pattern of exocytosis [6], other vesicles fuse only partially, releasing FcRn–IgG complexes to mix with contents of the plasma membrane in cycles of 3–4 s over prolonged periods of time.

FcRn is a trafficking receptor

At low pH, FcRn binds to IgG, whereas at neutral pH it lets go [7,8]. This pH-dependent ligand binding typifies FcRn and it has led to the current model in which FcRn binds to IgG after fluid-phase uptake in the acidic endosome and then releases it at neutral pH after moving back to the cell surface [9]. This model of FcRn-dependent IgG trafficking is typical of recycling membrane proteins but almost the complete opposite of the classical pathway for the pH-dependent uptake of nutrient solutes, as typified by the low-density lipoprotein (LDL) and transferrin (Tfn) receptors (for review, see Ref. [10]). One problem with the current model is that, unlike LDL receptors and Tfn receptors, almost no FcRn is detected at the cell surface [11]. In the majority of cell types, most FcRn resides in an intracellular compartment [12] – probably the sorting endosome [5].

In polarized epithelial cells, the lack of cell-surface expression of FcRn is even more of an interesting conundrum. At mucosal surfaces such as those found in the intestine and lung, and at the maternal–fetal barrier of the human placenta, FcRn mediates the transepithelial transport of IgG [13–18]. Such transport explains how humoral immunity is transferred from mother to newborn and how IgG affects immune surveillance and host defense [19]. Although FcRn transports IgG across mucosal epithelial cells, however, the small fraction of FcRn detected on the cell surface is strongly polarized to the basolateral membrane [20].

How can FcRn transport IgG all the way across epithelial barriers or even recycle it out of cells without ever reaching the cell surface? Perhaps FcRn lets go of IgG while still inside the secretory pathway or perhaps exocytosis is uniquely transient. So far, studies performed at steady state have not answered these questions.

The recycling pathway

Ober *et al.* have now examined these problems by imaging both FcRn and IgG in living cells [3,5]. In one investigation [5], they studied the initial step in the recycling pathway. The results show that FcRn binds to IgG in the sorting endosome and diverts it away from fluid-phase cargo found in the same compartment. IgG and FcRn localize together in tubular extensions and in rapidly moving vesicles that arise from the sorting endosome.

Remarkably, the central part of the sorting endosome displays little mobility and seems to mature into the late endosome and lysosome over time. These results are consistent with both the vesicular and the maturation models of membrane transport [10], and with much older studies of the Tfn receptor, which also recycles rapidly between the endosome and plasma membrane [21]. In fact, FcRn and Tfn receptors co-localize in the tubular extensions emanating from the sorting endosome and they might share some (or perhaps all) elements of the endomembrane system in the recycling pathway.

The exocytosis pathway

In the most recent article [3], Ober *et al.* examined the mechanism of FcRn exocytosis: the final step in sorting IgG to the plasma membrane. They used total internal reflection fluorescence microscopy and single-molecule detection in two colors so that the exocytosis of IgG and FcRn could be measured together in real time. The data show that FcRn can carry IgG all the way to the cell surface. These results are important because they exclude the idea that FcRn and IgG dissociate while still in secretory vesicles.

Remarkably, the time course for FcRn exocytosis shows evidence of two pathways. It is now known that different cells can use different mechanisms of exocytosis for different purposes. In the classical pathway of exocytosis (Box 1; see Figure 1a in Box 1), the secretory vesicle fuses completely with the plasma membrane and delivers all of its cargo to the cell surface in one large bolus (the all-or-nothing pathway) [6]. In cells that carefully regulate the volume and rate of secretion required for neurotransmitter, hormone and cytokine release, for example, a second pathway has been described [22–26] (Box 1; see Figure 1b,c in Box 1). In this pathway, the secretory vesicles form only transient fusion pores, and remain structurally distinct from the plasma membrane and poised for internalization by endocytosis (the ‘kiss-and-run’ pathway). These vesicles release all or only a fraction of cargo at each fusion event.

The new studies by Ober *et al.* show that, in human endothelial cells, a rapidly transient pattern of FcRn exocytosis occurs most of the time (Figure 1a). The authors interpret this pattern as being ‘complete fusion’ because it is consistent with classical concepts of exocytosis. A second pattern of FcRn and IgG exocytosis displays longer duration and a distinct periodicity. It is termed ‘prolonged release’ and has been interpreted to be a novel version of the kiss-and-run pathway (Figure 1b).

In the prolonged-release pathway, the fusion pore of the vesicle carrying the FcRn–IgG complex seems to open and close in repetitive cycles, each of several seconds, for up to 3 min. Remarkably, IgG is visualized migrating out of this fusion site onto the plasma membrane, sometimes circling back to return to exactly the original site of exocytosis. The data suggest that the FcRn–IgG complex is delivered to ‘hotspots’ on the plasma membrane that rapidly reverse direction and undergo endocytosis – consistent with the kiss-and-run model for exocytosis recently defined, using similar technology, for secretory vesicles in neuroendocrine cells [27–30]. Thus, FcRn might move to the cell

Box 1. Mechanisms of exocytosis

In the classical pathway of exocytosis, as originally proposed by Heuser and Reese for synaptic vesicles [6], the secretory vesicle fuses completely with the plasma membrane for release of cargo in one bolus [Figure 1a(i–iv)]. Recycling of membrane components into the cell by clathrin-mediated endocytosis occurs at another location. The internalized vesicle eventually matures into another synaptic vesicle that is poised for the next fusion event (not depicted in Figure 1). In some neurons and neuroendocrine cells that closely regulate the volume of secretion, exocytosis by secretory vesicles occurs by partial fusion with the plasma membrane (Figure 1b). In this pathway, termed kiss-and-run, the secretory vesicle remains structurally distinct from

the plasma membrane, releases all or only a fraction of its contents for action in the extracellular space [Figure 1b(i–ii)] and is rapidly internalized [Figure 1b(iii)] – poised for another fusion event if needed. In a version of this pathway, recently studied in dopamine neurons by Staal *et al.* [24], the secretory vesicle fuses to and is released from the plasma membrane in rapid succession [i.e. flickers; Figure 1c(i–v)] before it is completely endocytosed and recycled for another round of exocytosis [Figure 1c(vi)]. In some neuroendocrine cells, both the classical pathway and a version of the kiss-and-run (also termed ‘cavity recapture’ or ‘cavcapture’) pathway operate to deliver soluble and membrane-associated cargo to the cell surface [3,25–30].

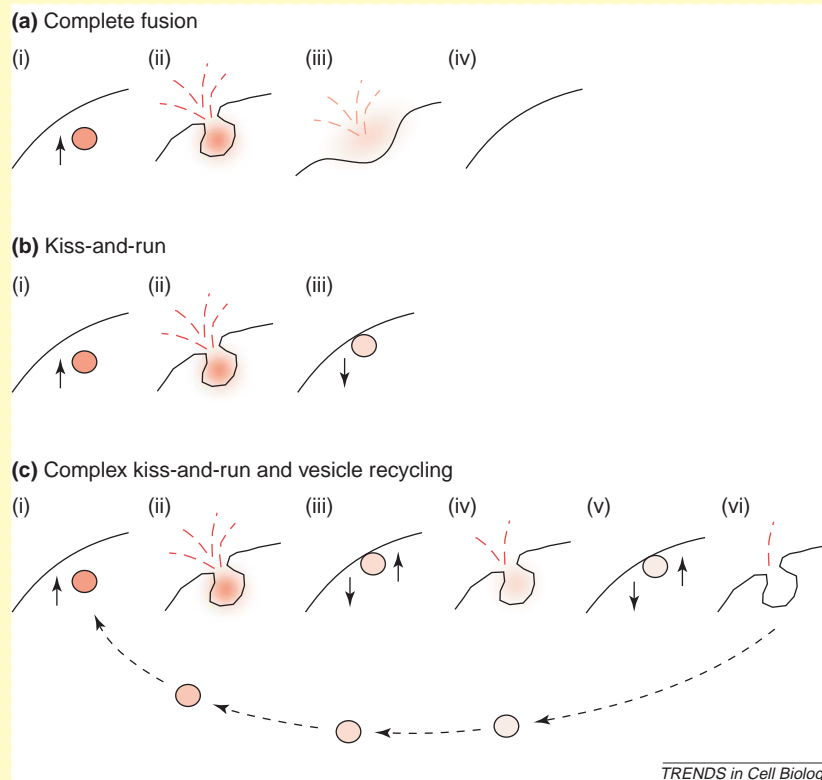


Figure 1. Pathways of exocytosis. (a)(i–iv) The complete-fusion pathway, (b)(i–iii) the kiss-and-run pathway and (c)(i–vi) the complex kiss-and-run and vesicle-recycling pathway.

surface in a vesicle that enables some vesicle components to mix with the plasma membrane while still maintaining its basic structure and being poised for rapid internalization. Prolonged-release exocytosis, however, cannot be exactly like the kiss-and-run fusion events observed in neuronal cells, which exhibit a fast rate of fusion (flickering at a frequency of 4000 Hz) [24].

The idea is interesting and consistent with current models of exocytosis of non-membrane-associated proteins in secretory vesicles from neuroendocrine cells [25–30]. Such a mechanism for transient exocytosis with incomplete fusion could explain why so little FcRn is detected on the cell surface at steady state. However, the frequencies of these events have not been quantified and the specificity has not been tested. This lack of clarity makes it hard to know whether the prolonged-release pattern of FcRn exocytosis is physiological or whether it represents a fraction of failed (or sputtering) fusion events in the classical pathway (or vice versa).

There is one other important observation that could explain why IgG transport might require such a mechanism of kiss-and-run exocytosis, a mechanism typically thought to occur in cell types in which secretion is tightly regulated. After exocytosis, IgG is visualized migrating for several seconds on the plasma membrane, with the same kinetics as FcRn. These data have, reasonably, been interpreted to indicate that FcRn can hold onto IgG for at least several seconds on the cell surface, possibly owing to $\text{Na}^+ - \text{H}^+$ exchange that can induce an acidic micro-environment immediately adjacent to the plasma membrane (see Ref. [20] for initial proposal of this idea). Such pH-induced stability of IgG binding to FcRn at the cell surface would enable the efficient recovery of IgG from the luminal environment, in which the concentration of IgG is much lower than in serum. It might also explain how the FcRn–IgG complex can operate at mucosal surfaces as an antigen-recognition site for immune surveillance [19]. For example, IgG might capture cognate antigen from

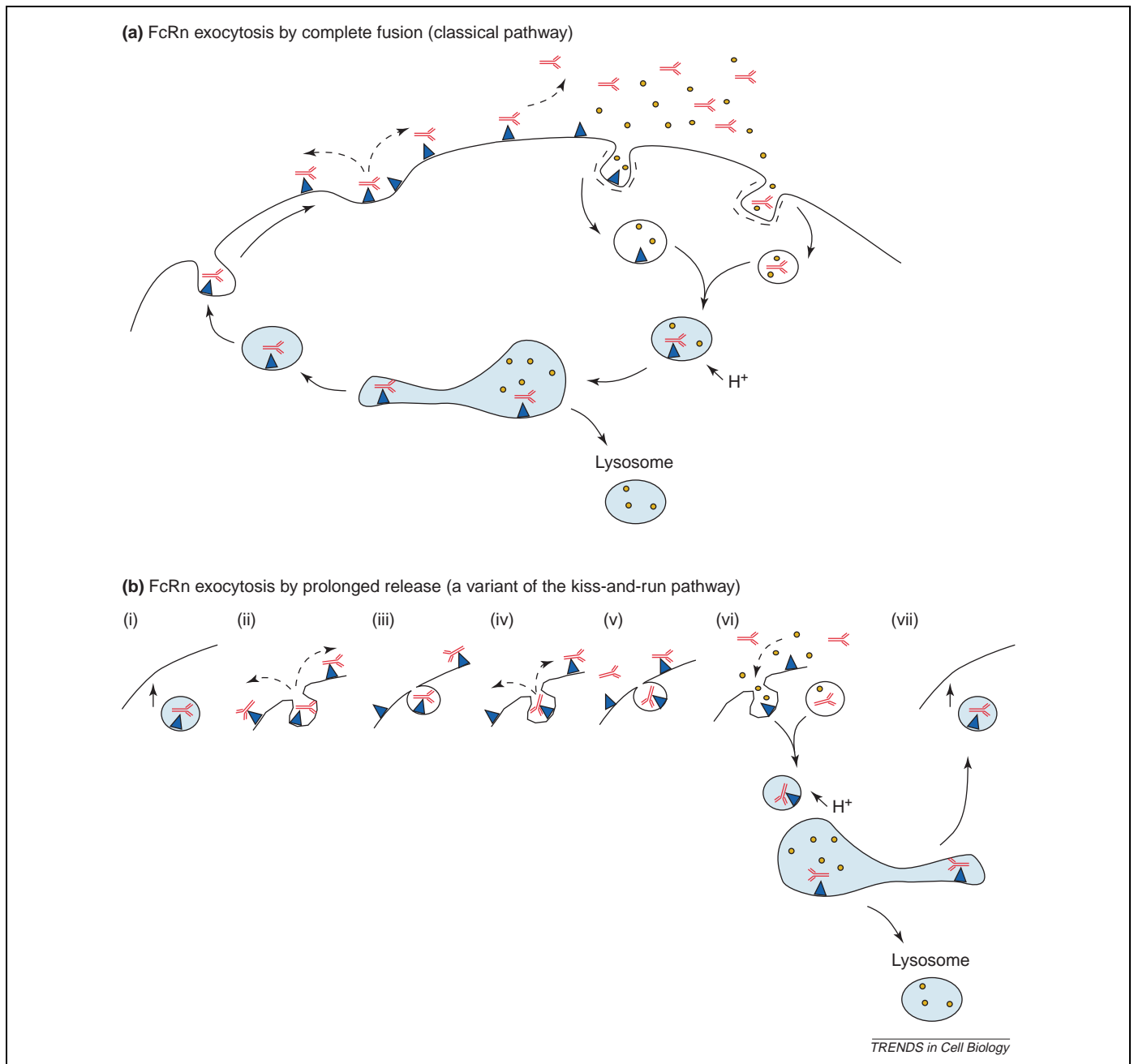


Figure 1. Mechanisms of IgG exocytosis and recycling by the Fc γ receptor FcRn. **(a,b)** In endothelial cells, IgG (red) is thought to follow nonspecific mechanisms of uptake and transport to the recycling endosome [10]. IgG binds to FcRn (blue triangles) after the endosome acidifies, and the IgG-FcRn complex is sorted away from other fluid-phase solutes in the recycling endosome [5]. The central portion of the recycling endosome that retains fluid-phase solutes (yellow spheres) matures into the lysosome. The IgG-FcRn complex is transferred to extensions of the recycling endosome that bud and mature into secretory vesicles. The secretory vesicles can then fuse with the plasma membrane through two forms of exocytosis, as proposed by Ober *et al.* [3]. In the classical form ('complete fusion'), the secretory vesicles fuse completely with the plasma membrane to release all of their cargo at once (a). IgG might dissociate from FcRn on the plasma membrane at neutral pH or it might remain bound if the immediate microenvironment maintains a low pH, presumably by Na⁺-H⁺ exchange, as occurs at mucosal surfaces in the intestine. In the so-called 'prolonged-release' pathway, the secretory vesicles follow a complex form of kiss-and-run fusion, as recently defined in neurons and neuroendocrine cells [24,27-30]. In the case of FcRn, the secretory vesicles containing the IgG-FcRn complex fuse only partially with the plasma membrane in repetitive cycles, each of 3-4 s (b)(i-v). (vi-vii) The IgG-FcRn complex can move out of these vesicles to migrate in the plane of the membrane and then return to the exact site of exocytosis, presumably for internalization by clathrin-mediated mechanisms of recycling through the endosome.

luminal secretions without letting go of FcRn, then rapidly recycle into the cell for transcytosis and delivery of the immune complex to dendritic cells in the subepithelial space and the induction of mucosal immunity.

Concluding remarks

In their recent articles, Ober *et al.* examine the mechanism of FcRn recycling from the endosome to the cell

surface in real time. They find that FcRn carries IgG all the way from the recycling endosome to the cell surface and that exocytosis occurs by two pathways. One pathway is consistent with classical models of exocytosis of membrane receptors because the vesicle that contains FcRn fuses completely with the plasma membrane, thus delivering FcRn to the cell surface. The second pathway is consistent with a form of kiss-and-run exocytosis – the

secretory vesicle remains structurally intact, fusing only partially with the plasma membrane in repetitive cycles, although FcRn can still migrate out of the vesicle to mix with other cell-surface components.

Many important questions, however, remain unanswered. What is the physiological significance of the prolonged-release pattern of exocytosis? Does the exocytic vesicle carrying FcRn remain structurally intact, as proposed? Does the secretory vesicle return to the endocytic compartment? Is the prolonged-release pathway of exocytosis used by epithelial cells at mucosal surfaces in the transcytotic pathway? Are the pathways of FcRn exocytosis and recycling constitutive or regulated and, in polarized cells, does one form of exocytosis predominate on apical or basolateral membranes, which would explain how FcRn operates in bidirectional transcytosis? The regulated metabolism of IgG by FcRn is crucial to IgG function. We look forward to further studies of this interesting and currently unique IgG-trafficking receptor.

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