In all the major secretory organs regulated exocytosis is a fundamental process that is used for releasing molecules in the extracellular space. Molecules destined for secretion are packaged into secretory vesicles that fuse with the plasma membrane upon the appropriate stimulus. In exocrine glands, large secretory vesicles fuse with specialized domains of the plasma membrane, which form ductal structures that are in direct continuity with the external environment and exhibit various architectures and diameters. In a recent study, we used intravital microscopy to analyze in detail the dynamics of exocytic events in the salivary glands of live rodents under conditions that cannot be reproduced in in vitro or ex vivo model systems. We found that after the opening of the fusion pore large secretory vesicles gradually collapse with their limiting membranes being completely absorbed into the apical plasma membrane canaliculi within 40–60 sec. Moreover, we observed that this controlled collapse requires the contractile activity of actin and its motor myosin II, which are recruited onto the large secretory vesicles immediately after their fusion with the plasma membrane. Here we suggest that the actomyosin complex may be required to facilitate exocytosis in those systems, such as the salivary glands, in which the full collapse of the vesicles is not energetically favorable due to a difference in membrane tension between the large secretory vesicles and the canaliculi.

Regulated exocytosis occurs in several organs with different modalities. Secretory vesicles can undergo: (1) full fusion, in which the limiting membrane of the secretory vesicle is completely absorbed into the plasma membrane (PM),3,4 (2) kiss and run, in which the fusion pore opens transiently, a quanta of the cargo molecule is released, and the vesicle detaches from the PM,5,6 and (3) compound exocytosis, where a secretory vesicle fuses with the PM and serves as a fusion site for other vesicles, generating strings of interconnected vesicles (Fig. 1A).3,7-9 Furthermore, the time a secretory vesicle spends at the PM after the fusion step varies considerably, ranging from few milliseconds, as in the case of small synaptic vesicles, up to minutes, as in the case of alveolar type II cells.3 These differences suggest that the molecular machineries regulating the exocytic steps differ significantly among the various secretory systems, possibly to accommodate their different architectures.

So far exocytosis has been extensively studied using both in vitro (i.e., cultured cells) and ex-vivo (i.e., organ cultures) experimental models. Although both systems continue to provide a plethora of important information, they suffer from two major limitations: (1) in in vitro systems, the geometry and the polarity of the secretory apparatus are not maintained, especially for exocrine models,10-13 and (2) in ex vivo systems, the lack of contribution from a series of signaling molecules provided by the vasculature and the central nervous system may have profound effects on the signaling pathways controlling...
For these reasons, it is crucial to study exocytosis in native tissue under the appropriate physiological conditions. Recently, we have described the dynamics of exocytosis in the acini of rodent salivary glands (SGs), focusing on the step that follows the fusion of large secretory vesicles with the apical PM and the opening of the fusion pore. Specifically, we used intravital microscopy, an imaging approach based on light microscopy, which provides the necessary spatial and temporal resolution to study the dynamics of subcellular organelles in live animals. The architecture of the acini and the site where exocytosis occurs have been characterized extensively. Acini are formed by 9–10 polarized acinar cells, which are tightly packed (Fig. 1B). The apical domains of the PM of two adjacent cells form narrow canaliculi with a diameter of 0.2–0.3 μm and a length of 10–15 μm. Each cell shares 2–3 canaliculi with adjacent cells, and they are all interconnected, ultimately merging into a larger duct at the base of the acinus, called the intercalated duct (Fig. 1B and reviewed in ref. 4). A single acinar cell contains 250–300 secretory vesicles (termed secretory granules (SCGs)), which have a diameter of 1–1.5 μm. After fusion with the PM, the SCGs gradually collapse within 40–60 s. The kinetics is very slow, especially when compared with other exocytic vesicles, such as synaptic or neuroendocrine vesicles, which exocytose in few milliseconds. Some studies suggest that the slow kinetics of exocytosis may reflect the nature of large cargo molecules, which are tightly packed and require longer time to disassemble once exposed to the extracellular space. However, since in SGs cargo molecules do not form dense aggregates, we favor another possibility based on the difference between the biophysical properties of the fusing membranes. Specifically, when two membranous compartments fuse there is a flow of membranes toward the area with the lowest membrane tension. Based on geometrical consideration, large SCGs should have a lower membrane tension than narrow canaliculi, and indeed we observed that after the opening of the fusion pore, membranes flow from the canaliculi into the SCGs (see Fig. 2 in ref. 4). This suggests that...
are significantly expanded (diameter 1.8–2.0 \( \mu \)m). Under this condition, SCGs should have a higher membrane tension than the expanded canaliculi and the process could possibly be brought to completion without the need for a contractile activity (Fig. 2C). This model may apply to other systems as well. For example, the recruitment of the actomyosin scaffold around the SCGs have been observed in other exocrine glands such as the pancreas and the lacrimal glands, which share a similar geometry with the SGs, and in which exocytosis proceeds with a slow kinetics as well.25,26 Actin and myosins have been observed

Figure 2. Role of the actomyosin complex in the gradual collapse of the secretory granules. (A) SCGs (blue) fuse with the APM (red), the fusion pore opens, and membranes flow from the APM into the SCGs (red arrows). The difference in membrane tension may not favor the flow of the membranes toward the APM that would lead to the gradual collapse of the SCGs. The contractile activity of the actomyosin complex (actin, green rods; myosin II, blue) that assembles around the SCGs may push the membranes (black arrows) and/or dilate further the fusion pore (gray arrows) to facilitate the gradual collapse. (B) In the absence of a functional actin cytoskeleton the SCGs expand forming large vacuoles (2–5 \( \mu \)m). Membranes flow into the large vacuoles, which acquire the properties of the APM. Due to the lower membrane tension of the large vacuoles, the remaining SCGs gradually collapse without the need of a functional actomyosin complex. (C) In the absence of a functional myosin II, the acinar canaliculi significantly expand in size (1.8–2.0 \( \mu \)m). The SCGs fuse with the APM and slightly expand in size (2–2.5 \( \mu \)m). Under these conditions the difference in membrane tension may be more favorable to a actomyosin-independent gradual collapse.

the collapse of the SCGs into the PM may not be an energetically favorable process. We propose that the actomyosin complex is recruited onto the surface of the SCGs to form a contractile scaffold, which provides the energy to complete this step (Fig. 2A). Although we do not know whether the force generated by the scaffold acts by pushing the membranes or by enlarging the fusion pore, we suggest that the actomyosin complex is required to reduce the size of the SCGs, thus increasing their membrane tension to a point in which the collapse becomes more energetically favorable. Notably, the disruption of the actin cytoskeleton results in a failure of the collapse of the SCGs, which grow in size forming large vacuoles, whereas the inhibition of the myosin motor activity results in an initial expansion of the SCGs followed by a gradual collapse, which is significantly delayed in a small subpopulation of vesicles.

Two interesting observations further support this model. First, in the absence of a functional actin cytoskeleton the SCGs collapse with the large vacuoles. Under this condition, SCGs (diameter 1–1.5 \( \mu \)m) have a higher membrane tension than the large vacuoles (4–5 \( \mu \)m) and their collapse is more favorable (Fig. 2B). Second, we noted that when the activity of the myosin II motor is impaired, the acinar canaliculi are significantly expanded (diameter 1.8–2.0 \( \mu \)m). Under this condition, SCGs should have a higher membrane tension than the expanded canaliculi and the process could possibly be brought to completion without the need for a contractile activity (Fig. 2C).
around cortical granules in Xenopus eggs, in Weibel-Palade bodies in endothelial cells, and in lamellar bodies in alveolar type II cells. However, in these systems the role of the actomyosin complex may be related to the dense packing of cargo molecules rather than the differences in membrane tension.

In other exocytic systems, such as endocrine glands or pre-synaptic terminals, secretory vesicles are much smaller in size (diameter 50–300 nm) and they fuse very rapidly with membrane domains that have much lower tensions. Interestingly, in cells, and in lamellar bodies in alveolar type II cells, cortical granules in Xenopus eggs, and in Weibel-Palade bodies during endocytosis. J Cell Biol 2011; 194:613-29; PMID:21844207; DOI: 10.1083/jcb.20101119.

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