Regulated exocytosis was the first intracellular membrane fusion step that was suggested to involve both Ca\(^{2+}\) and calmodulin. In recent years, it has become clear that calmodulin is not an essential Ca\(^{2+}\) sensor for exocytosis but that it is likely to have a more regulatory role. A requirement for cytosolic Ca\(^{2+}\) in other vesicle fusion events within cells has become apparent and in certain cases, such as homotypic fusion of early endosomes and yeast vacuoles, calmodulin may be the primary Ca\(^{2+}\) sensor. A number of distinct targets for calmodulin have been identified including SNARE proteins and subunits of the vacuolar ATPase. The extent to which calmodulin regulates different intracellular fusion events through conserved SNARE-dependent or other mechanisms remains to be resolved.

Two of the first factors proposed to regulate exocytic membrane fusion were Ca\(^{2+}\) and its effector calmodulin. More recently, evidence has accrued that these factors may regulate many other intracellular fusion events. In this review, we will highlight those fusion events, for which a calcium dependence has been established and discuss the potential roles that calmodulin may play in the regulation of each membrane traffic step.

1. Sources of Ca\(^{2+}\) for intracellular fusion

For regulated exocytosis, it is well established that elevation of Ca\(^{2+}\) over basal levels (~ 100 nM) is required [1,2]. Depending on the cell type, this Ca\(^{2+}\) elevation could be due to either Ca\(^{2+}\) entry across the plasma membrane [3] or Ca\(^{2+}\) mobilization from internal stores or both [4,5]. Often localized and brief increases in cytosolic Ca\(^{2+}\) concentration are important in triggering exocytosis. Any Ca\(^{2+}\) sensor involved in regulated exocytosis will have an affinity for Ca\(^{2+}\) well above resting Ca\(^{2+}\) concentration and has been variously estimated to be in the range 1–10 \(\mu\)M [6–8] or even as high as 100 \(\mu\)M [9,10]. In synaptic neurotransmission where high Ca\(^{2+}\) is required, and for which Ca\(^{2+}\) loading of the Ca\(^{2+}\) sensor would need to occur in less than 100 \(\mu\)s, synaptic vesicles are likely to be within a few nanometers of the open Ca\(^{2+}\) channel [11,12]. Synaptotagmins are currently the overwhelming favourites as the Ca\(^{2+}\) sensors for exocytosis [13] and show Ca\(^{2+}\)-binding on- and off-rates in the physiologically relevant ranges in biochemical assays [14].

In other intracellular fusion steps where Ca\(^{2+}\) appears to be required, it is not always clear whether the Ca\(^{2+}\) requirement is for a basal level of Ca\(^{2+}\) or elevated Ca\(^{2+}\). Inhibition of fusion or transport by Ca\(^{2+}\) chelators may simply indicate that a free Ca\(^{2+}\) concentration around 100 nM is sufficient as long as this exists close to the site of membrane fusion. Relatively few estimates have been made of the free Ca\(^{2+}\) concentration required. Several intracellular fusion events are inhibited by the fast Ca\(^{2+}\) chelator BAPTA but not by the slower chelator EGTA. Specific inhibition by BAPTA is consistent with a requirement for a very fast (< 1 ms) and local Ca\(^{2+}\) elevation as in neurotransmitter release [15]. It is not expected, therefore, that differential effects of BAPTA and EGTA would be observed for processes requir-
ing basal Ca\textsuperscript{2+} concentration. In fact, EGTA is as effective as BAPTA in inhibiting regulated exocytosis due to Ca\textsuperscript{2+} entry in endocrine cells [16] and even in certain synapses [17,18]. For BAPTA-sensitive fusion pathways with much slower overall rate constants, such as endosome fusion or intra-Golgi transport, it may be that local release of Ca\textsuperscript{2+} from lumenal stores is tightly coupled with a particular step along the fusion pathway.

If local release of Ca\textsuperscript{2+} occurs from the lumenal stores of the organelles involved in fusion, can this explain the differential effects of BAPTA and EGTA? The on-rate of Ca\textsuperscript{2+} binding to BAPTA (4 × 10\textsuperscript{8} M\textsuperscript{-1} s\textsuperscript{-1}) [19] is similar to that for calmodulin [20] so that this chelator can compete with calmodulin for initial binding of Ca\textsuperscript{2+}. In contrast, EGTA has a considerably slower on-rate (2.5 × 10\textsuperscript{8} M\textsuperscript{-1} s\textsuperscript{-1}) [19]. The differences in on-rate would only be relevant for Ca\textsuperscript{2+} gradients lasting for less than 1 ms. It has been calculated that buffering by 10 mM BAPTA would reach equilibrium in around 3 μs, whereas that due to EGTA would take around 1.2 ms [15]. From the known diffusion rate for Ca\textsuperscript{2+} in cytosol (10–13 μm\textsuperscript{-1} s\textsuperscript{-1}) [21], it is clear that such a Ca\textsuperscript{2+} gradient would dissipate within a distance of around 20 nm over 1 ms. This is a possible situation for a fast calmodulin-dependent fusion process requiring Ca\textsuperscript{2+} elevation to levels into the micromolar range (or higher as in yeast vacuole–vacuole fusion [22]). If basal Ca\textsuperscript{2+} concentration was sufficient, one would not expect a differential effect of the two chelators on a Ca\textsuperscript{2+}/calmodulin-dependent fusion process. The differential effect of the Ca\textsuperscript{2+} chelators on various intracellular transport steps (see below) indicates that the requirement must be for Ca\textsuperscript{2+} elevation due to short-lived pulses of Ca\textsuperscript{2+} release very close to the site of membrane fusion. Ca\textsuperscript{2+} release and fusion would have, therefore, to be tightly coupled both spatially and temporally. One way in which this is likely to be achieved for synaptic exocytosis is through the direct interaction of neuronal SNARE proteins with voltage-gated Ca\textsuperscript{2+} channels [23]. Perhaps similar interactions could occur with intracellular Ca\textsuperscript{2+} release channels.

In mammalian cells the best-characterized Ca\textsuperscript{2+} store is the endoplasmic reticulum (ER). Most of the calcium is sequestered by calcium-binding proteins but with free Ca\textsuperscript{2+} estimated to be as high as 0.7 mM [24]. Release of Ca\textsuperscript{2+} from the ER can be triggered by activation of Ins(1,4,5)\textsubscript{P\textsubscript{3}} or ryanodine receptors [25]. In addition to the ER, the Golgi complex has been shown to be a Ca\textsuperscript{2+} store that can sequester high levels of Ca\textsuperscript{2+}, due to binding to lumenal proteins [26], and release Ca\textsuperscript{2+} in response to IP\textsubscript{3} [27]. Endosomal vesicles must contain millimolar levels of Ca\textsuperscript{2+} at the time of pinching off from the plasma membrane. It has been suggested that the concentration of lumenal Ca\textsuperscript{2+} is rapidly reduced to around 3 μM within 20 min after endocytosis of a Ca\textsuperscript{2+}-sensitive probe [28]. Depletion of lumenal Ca\textsuperscript{2+} from the organelles involved in the fusion event inhibits homotypic fusion of yeast vacuoles [22] and endosomes [29], endosome–lysosome fusion [30] and intra-Golgi transport [31] leading to the idea that fusion requires local release of luminal Ca\textsuperscript{2+} during the process of membrane fusion. The low estimate for endosomal Ca\textsuperscript{2+} would not be compatible with luminal Ca\textsuperscript{2+} release from this organelle and recent work has questioned the values for endosomal Ca\textsuperscript{2+} and demonstrated Ca\textsuperscript{2+} concentrations as high as 400–600 μM in phagosomes [32]. Further analysis of endosomal Ca\textsuperscript{2+} and regulation of its release is required to resolve the significance of this source of Ca\textsuperscript{2+} for endosome fusion.

### 2. Calmodulin as a Ca\textsuperscript{2+} sensor

Calmodulin consists of an N-terminal and a C-terminal lobe each with two EF-hand Ca\textsuperscript{2+}/binding sites and separated by a flexible linker [33]. Calmodulin can regulate its many target proteins in either a Ca\textsuperscript{2+}-independent or Ca\textsuperscript{2+}-dependent manner. In some cases, calmodulin is prebound to its target in its Ca\textsuperscript{2+}-free state [34] so that it can rapidly modify target protein function as soon as Ca\textsuperscript{2+} is bound. Calmodulin has intrinsic properties that allow it to act as a fast Ca\textsuperscript{2+} sensor. All EF hand proteins acting as Ca\textsuperscript{2+} sensors, that have been studied have very fast on-rates (\(\sim 10^8 \text{M}^{-1} \text{s}^{-1}\)) so that Ca\textsuperscript{2+}-binding is limited only by Ca\textsuperscript{2+} diffusion [20]. Prebound calmodulin can, therefore, be activated and modify associated proteins on a millisecond time scale as demonstrated by its regulation, for example, of voltage-gated Ca\textsuperscript{2+} channels during their open state [35–37]. The Ca\textsuperscript{2+} off-rate is variable between different EF-hands over a 1000-fold range depending on the nature of the amino acid at the ninth position of the EF hand [20]. Such variability in off-rate is seen for the EF hands of calmodulin where the off-rate for the EF-hands in the N-terminal lobe (405 s\textsuperscript{-1}) is over 100 times faster than for the two in the C-terminal lobe (2.4 s\textsuperscript{-1}) [38] potentially allowing independent regulation by each lobe of calmodulin [39]. The equilibrium dissociation constant for Ca\textsuperscript{2+}/calmodulin in the absence of target proteins based on in vitro Ca\textsuperscript{2+}-binding is around 5 μM [40] but this can be modified by interactions within cells. The off-rates are substantially modified by the interaction of calmodulin with target proteins leading to slowing of Ca\textsuperscript{2+} dissociation [38] and resulting in persistent activation of calmodulin and its targets even after cytosolic Ca\textsuperscript{2+} concentrations fall back to resting levels [41]. Measurement of both cytosolic Ca\textsuperscript{2+} concentration and Ca\textsuperscript{2+}-bound calmodulin has been carried out in living cells. Ca\textsuperscript{2+} binding to calmodulin was determined by measurement of FRET between calmodulin and a calmodulin-binding peptide in the same fusion protein. This indicated that no Ca\textsuperscript{2+} was bound until cytosolic Ca\textsuperscript{2+} was elevated to about 200 nM and half-maximal occupancy of calmodulin by Ca\textsuperscript{2+} occurred at 1 μM free Ca\textsuperscript{2+} [42]. If calmodulin is to function in very rapid events (<1 ms), then much higher Ca\textsuperscript{2+} elevations are required. From the on-rate of Ca\textsuperscript{2+} binding, it can be calculated that for complete loading of calmodulin by Ca\textsuperscript{2+} within 1 ms, a Ca\textsuperscript{2+} elevation in the vicinity of
calmodulin to >10 μM is needed. In general terms, calmodulin is a Ca\(^{2+}\) sensor that can rapidly respond to Ca\(^{2+}\) when it is elevated to high micromolar levels.

3. Calmodulin in regulated exocytosis

The requirement for an elevation of cytosolic Ca\(^{2+}\) concentration for the triggering of neurotransmitter and hormone release has been known for many years [1]. A considerable emphasis has been placed on the identification of the Ca\(^{2+}\) sensor for regulated exocytosis. It now seems to be clear that the synaptic vesicle protein synaptotagmin I performs this role in neurotransmission [43,44] with other isoforms functioning in different exocytic processes [13]. Calmodulin was previously suggested as a candidate for such a sensor in exocytosis [45]. In fact, calmodulin would not be predicted to be a good candidate for a Ca\(^{2+}\) sensor in neurotransmission as the off-rate for Ca\(^{2+}\) dissociation is too slow to account for the transient nature of the response to elevated Ca\(^{2+}\) in the nerve terminal (unless an alternative Ca\(^{2+}\) sensor mediates inactivation of neurotransmitter release). A Ca\(^{2+}\) sensor involved in synaptic exocytosis might be expected to lose activity by Ca\(^{2+}\) dissociation in less than 1 ms. In addition, calmodulin does not bind Ba\(^{2+}\) [46], which could substitute for Ca\(^{2+}\) in triggering regulated exocytosis. It was subsequently established that calmodulin is not essential for membrane fusion in regulated exocytosis in most cells [2]. Calmodulin is required, however, in a few specialized cell types and may also have a more general regulatory role in exocytosis.

Calmodulin has one well-established role in exocytosis in synapses. Here Ca\(^{2+}\) entry leads to the activation by calmodulin of calmodulin-dependent kinase II, phosphorylation of synapsins and release of synaptic vesicles immobilized by the cytoskeleton [47]. This provides a mechanism to allow recruitment of additional synaptic vesicles into the releasable pool. The synapsins are largely neuronal-specific proteins and so this mechanism does not occur in non-neuronal cells. Alternative presynaptic roles of calmodulin in neurotransmitter release have also been suggested based on the use of calmodulin antagonists in mammalian CNS neurons [48] and from the analysis of *Drosophila* expressing calmodulin with a mutation in the first EF-hand [49]. The question of whether or not calmodulin has a general role, particularly in later steps of exocytosis, has long been debated. Early experiments using anti-calmodulin antibodies introduced into adrenal chromaffin cells or sea urchin eggs [50,51] as well as calmodulin antagonists in intact cells apparently implicated calmodulin in regulated exocytosis. Further studies established that direct activation of exocytosis by Ca\(^{2+}\) addition to permeabilised chromaffin cells, which would have lost their cytosolic calmodulin by leakage, was not blocked by calmodulin antagonists [52], suggesting that the effects originally observed were indirect. Nevertheless, a possible regulatory role for calmodulin was subsequently established in permeabilised cell assays with adrenal chromaffin cells based on the stimulatory effect of added calmodulin [53,54]. Characterisation of this effect using stage-specific assays in digitonin-permeabilised chromaffin cells established that calmodulin stimulated exocytosis when added during the Ca\(^{2+}\)-triggering step but not during MgATP-dependent priming [53]. Similar data were subsequently obtained using cracked PC12 cells [55]. These results suggested that calmodulin could affect late stages in exocytosis. This conclusion was supported by the finding that addition of calmodulin via the patch pipette during whole-cell recording of membrane capacitance increased the initial rate of exocytosis [56]. An alternative role for calmodulin has been shown in *Paramecium* where a calmodulin mutant showed a complete loss of exocytosis of trichocysts in a temperature-sensitive manner [57]. This was shown to be due to a defect in their docking to the plasma membrane. It is unclear whether calmodulin is involved in exocytosis in other cell types as other data are based entirely on the use of calmodulin antagonists that could have indirect effects [58].

What is the mechanism by which calmodulin modulates regulated exocytosis? Unlike the situation in yeast vacuole fusion [22], the function of calmodulin in exocytosis is unlikely to be via interaction with the vacuolar ATPase for which there is no evidence for an involvement in regulated exocytosis. Indeed, the V-ATPase inhibitor that blocked vacuole–vacuole fusion, dicyclohexylcarbodiimide (DCCD), had no effect on regulated exocytosis in chromaffin cells [59]. It has been suggested that neurotransmitter could be released through the mediophore that was identified to be the proteolipid subunit of the V-ATPase [60]. This would be, however, a non-exocytotic mechanism for neurotransmitter release. Other possible targets have been identified. Calmodulin binds directly to the small GTPase Rab3, that is involved in exocytosis as an inhibitory regulator [61] and leads to its dissociation from membranes [62]. This is a low-affinity interaction, however, and its significance is still unclear. Calmodulin has also been found to bind the exocytotic Ca\(^{2+}\) sensor, synaptotagmin I but the consequences of this interaction for synaptotagmin function are unknown [63,64]. Alternatively, calmodulin may function through interactions with the SNARE proteins. Calmodulin has been found to co-immunoprecipitate with the SNARE complex from brain extracts [65], and more recently to bind directly to VAMP [66]. The binding site for calmodulin on VAMP has been examined and found to be close to the transmembrane domain (residues 77–90). Injection of the VAMP (77–90) peptide into chromaffin cells inhibited exocytosis, confirming that this is a functionally important domain [67]. In further experiments using PC12 cells, mutation of residues in VAMP involved in calmodulin-binding reduced the ability of transfected tetanus toxin-resistant VAMP to recover exocytosis in tetanus toxin-treated cells. These data may suggest that the VAMP/calmodulin interaction is important for exocytosis [67]. One caveat that must be added,
however, is that the calmodulin-binding domain of VAMP is also involved in phospholipid interactions [66], which may also have been disrupted. If the effects are due solely to disruption of VAMP/calmodulin interaction, then an important question is whether this interaction is merely regulatory or a key aspect of SNARE-driven fusion. A surprising result in this paper was the finding that a calmodulin antagonist, ophiobolin, completely abolished exocytosis. This is at odds with previous work using other calmodulin antagonists that suggested that calmodulin is not essential for regulated exocytosis. The significance of an earlier observation that calmodulin inhibits SNARE complex assembly in vitro is also unclear [66] but these studies have established a potential target for regulation of exocytosis by calmodulin.

4. Ca\(^{2+}\)/calmodulin in the secretory pathway

A recent survey has established the Ca\(^{2+}\) requirement for a number of intracellular transport steps (Fig. 1); some of which may reflect vesicle formation rather than consumption [68]. Certain transport steps have been shown to require both Ca\(^{2+}\) and calmodulin. A well-established cell-free assay for intra-Golgi transport that measures glycosylation of VSV G protein [69] is inhibited by BAPTA (IC\(_{50}\) ~ 0.8 mM) but not by EGTA [31]. This effect could be rescued by re-isolating the membranes and incubating with fresh cytosol, except if a calcium ionophore was included in the BAPTA incubation. This indicates that lumenal Ca\(^{2+}\) is required for transport. The assay reflects a complex transport event that requires cargo selection and coat assembly, vesicle budding, vesicle docking and fusion. Kinetic analysis of the acquisition of resistance to inhibitors of the transport assay showed that resistance to BAPTA is acquired subsequent to inhibition by GTP\(_{S}\) and inhibition by antibodies recognizing SBP56 a cytosolic protein required for intra-Golgi transport. The authors argue that this reflects a requirement for Ca\(^{2+}\) late in the pathway, possibly at the fusion stage. Two calmodulin inhibitors (W7 and trifluoperazine) also inhibited the transport assay. Following incubation with these inhibitors and re-isolation of the membranes, re-complementation of the transport assay required fresh cytosol together with purified calmodulin.

It appears that the requirement for Ca\(^{2+}\) may be conserved for vesicle consumption along the secretory pathway. ER-derived vesicles from mammalian cells and from yeast require cytosolic calcium for vesicle fusion [70,71]. In particular, the Ca\(^{2+}\) requirement in the yeast system was unambiguously determined at the post-docking stage [71]. It is currently unclear whether this requirement for cytosolic Ca\(^{2+}\) is necessary to maintain lumenal Ca\(^{2+}\) concentrations through active transport mechanisms.

5. Endocytic pathway

In vitro assays that reconstitute the homotypic fusion of early endosomes [72] have shown a requirement for luminal vesicular Ca\(^{2+}\) based on BAPTA inhibition and corresponding failure of EGTA to inhibit [29,73]. Furthermore, EGTA could be delivered to vesicle interiors as an AM-ester membrane permeable form (EGTA-AM), which then confers inhibitory activity to EGTA [29,73]. Two studies have also shown a role for calmodulin in regulation of early endosome fusion [73,74]. Endosome fusion is inhibited by the calmodulin antagonists calmidazolium, W7 [73] and W13 [74]. Supplementation of the in vitro assay with calmodulin stimulates endosome fusion [73] and reverses W13 inhibition [74]. In distinction to the Golgi transport assay, these assays do not require the formation of vesicular intermediates. Inhibition must therefore occur at the level of docking or fusion.

Colombo et al. [74] further suggest that CaM-dependent kinase II may be a factor in endosome fusion based on the use of antagonists. Mills et al. [73] have shown that calmodulin binds to the IQ domain of EEA1, a protein that...
is required for endosome fusion by virtue of promoting vesicle association through a tethering reaction [75,76]. Calmodulin overlays also revealed calmodulin interaction with syntaxin 13, an endosomal SNARE protein implicated in early endosome fusion [76,77], which might itself engage in a low-affinity interaction with EEA1 [77]. Calmodulin-binding to EEA1 and syntaxin 13 were both Ca\textsuperscript{2+}-dependent [73]. Clearly, calmodulin has the potential to exert complex effects on the endosomal fusion machinery.

One interesting observation is the capacity of calmodulin to displace EEA1 binding to PtdIns3P containing liposomes [73]. EEA1 localizes to endosomes through interaction between PtdIns3P and its FYVE domain, which neighbours the calmodulin-binding IQ domain in the C-terminal region of the protein [78]. As well as an active role in fusion, calmodulin could therefore play a role in recycling of tethering molecules by promoting the release of EEA1.

Assays of fusion events later in the endocytic pathway have been less well studied than early endosome fusion. Recently, Pryor et al. [30] have developed an in vitro assay of late endosome–lysosome fusion, which they have shown to be regulated by Ca\textsuperscript{2+}/calmodulin using similar approaches to those described above. Fusion is inhibited by BAPTA, EGTA-AM and by calmodulin antagonists. It is not clear how a requirement for lumenal calcium in this assay squares with the low estimate of Ca\textsuperscript{2+} concentration in late endosomes obtained by Gerasimenko et al. [28], particularly if this characteristic is conserved for late endosome homotypic fusion.

6. Yeast vacuolar fusion

An in vitro assay of homotypic yeast vacuole fusion has been established that allows the powerful combination of biochemistry and yeast genetics to be applied to the dissection of the fusion pathway [79]. As with early endosome fusion no intermediate vesicle is required. Peters et al. [22,80] have identified three factors downstream of trans-SNARE formation necessary for vacuolar fusion, release of Ca\textsuperscript{2+} from the vesicle lumen, calmodulin and protein phosphatase 1. Calmodulin antagonists inhibit vacuolar fusion, as do affinity-purified antibodies against calmodulin. As reported for in vitro-Golgi transport, inhibition could be overcome by re-isolation of the membranes and incubation with fresh cytosol together with additional calmodulin. Vacuoles prepared from a yeast strain carrying a temperature-sensitive mutant of calmodulin become fusion incompetent at the restrictive temperature [22].

Inclusion in the assay of the Ca\textsuperscript{2+} ionophore ionomycin or the Ca\textsuperscript{2+}-ATPase inhibitors thapsigargin and cyclopiazonic acid inhibit vacuole fusion, indicating that the luminal Ca\textsuperscript{2+} pool must be actively maintained to support fusion [22]. As with the systems described above, BAPTA but not EGTA, inhibited vacuole fusion (the requirement for Ca\textsuperscript{2+} transport into the lumen might predict that EGTA should also be inhibitory). Vacuolar Ca\textsuperscript{2+} concentration has been estimated as 2 mM [81] and therefore local Ca\textsuperscript{2+} fluxes as large as those that trigger regulated exocytosis in mammalian cells could be generated.

The yeast vacuole fusion assay has been subjected to more rigorous kinetic dissection than other in vitro assays of fusion. Analysis of the loss of susceptibility to the delayed application of inhibitors, as successive stages of the fusion pathway are completed, placed the requirement for both luminal Ca\textsuperscript{2+} and calmodulin after sensitivity to rab-GDI is lost. The authors propose that this places the requirement downstream of the completed docking reaction [22]. Data that calmodulin may be the Ca\textsuperscript{2+} sensor relevant to vacuole fusion was obtained by showing that calmodulin mutants deficient in Ca\textsuperscript{2+} binding do not support vacuole fusion, despite the fact that other functions of calmodulin which do not require Ca\textsuperscript{2+} remain intact and that uptake and release of vacuolar Ca\textsuperscript{2+} are unaffected.

A search for calmodulin-binding partners on the vacuolar membrane using chemical cross-linking identified the V0 sector of the vacuolar-ATPase [82] which is made up from Vma6, Vph1 and the proteolipids Vma3 (six copies), Vma11 and Vma16 [20]. Intriguingly, these authors found that reconstituted V0 proteolipids could respond to Ca\textsuperscript{2+}/calmodulin by forming a channel permeable to entrapped choline; furthermore, trans-complexes of V0 were shown to form, subsequent to SNARE-dependent docking of vacuoles. The authors offer the challenging speculation that this trans-V0 complex evolves under the influence of Ca\textsuperscript{2+}/calmodulin to become the fusion pore that dilates by radial expansion and dissociation of the proteolipid ring.

The universality of this proposed fusion mechanism can be called into question, SNARE deletions in yeast are often lethal but yeast can grow in the absence of Vma3 in acidic media [83], neither has the distribution of V0 throughout the cell been established. It may be that other proteins/proteolipids can fulfill this function; the authors point to Got1p and Stt2p that are small hydrophobic membrane proteins that are known to genetically interact with the Golgi SNARE Sed5p [84].

7. Conclusions

Available evidence suggests that many intracellular fusion events, in addition to requiring Rab and SNARE proteins for vesicle targeting, require the subsequent release of luminal Ca\textsuperscript{2+} and the active participation of calmodulin. This would suggest a conserved mechanism of action such as promotion of the trans-V0 complex as suggested from studies of yeast vacuole fusion, the regulation of tethering factors, or perhaps regulation of a vesicle-specific family of proteins such as the SNAREs. It is clear that calmodulin can have a multitude of further targets on individual vesicles and
so it remains to be established exactly how calmodulin regulates each of the membrane traffic steps in which it has been implicated. One issue that seems to be clear is that while calmodulin may be essential for certain intracellular traffic steps, and possibly act as the Ca\(^{2+}\) sensor for them, it is not an essential Ca\(^{2+}\)-sensor for regulated exocytosis.

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