The AMF-R tubule is a smooth ilimaquinone-sensitive subdomain of the endoplasmic reticulum

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SUMMARY

Autocrine motility factor receptor (AMF-R) is a marker for a distinct smooth membranous tubule. Ilimaquinone (IQ) is a sea sponge metabolite which induces the complete vesiculation of the Golgi apparatus and we show here that the addition of IQ to MDCK cells also results in the disruption of the AMF-R tubule. By immunofluorescence microscopy, the resultant punctate AMF-R label resembles the products of IQ-mediated vesiculation of the *trans*-Golgi network, however, the two labels can be distinguished by confocal microscopy. AMF-R tubule fragmentation occurs after nocodazole or taxol treatment of the cells demonstrating that the action of IQ on AMF-R tubules is not related to the ability of IQ to depolymerize microtubules. IQ activity is therefore not Golgi-specific. Electron microscopy of IQ-treated cells reveals that AMF-R is distributed to fenestrated networks of narrow interconnected tubules which are distinguishable from the uniform Golgi-derived vesicles and morphologically equivalent to smooth ER. Distinct fenestrations are visible in incompletely fragmented tubules which may represent intermediates in the fragmentation process. Smooth AMF-R labeled tubules exhibit continuity with rough ER cisternae and IQ selectively targets smooth and not rough ER. AMF-R tubules can be distinguished from the intermediate compartment labeled for ERGIC-53 by confocal microscopy and thus constitute a distinct IQ-sensitive subdomain of the smooth ER.

Key words: Autocrine motility factor receptor, Membrane tubule, Madin-Darby canine kidney, Ilimaquinone, Smooth endoplasmic reticulum

INTRODUCTION

Autocrine motility factor receptor (AMF-R) is a marker for a distinct tubular organelle (Benlimame et al., 1995). The AMF-R tubule is not equivalent to either endosomes or lysosomes and therefore not part of the endocytic pathway. Electron microscopic analysis of AMF-R distribution in MDCK, HeLa and NIH-3T3 cells shows that AMF-R is localized primarily to smooth tubular organelles of variable diameter (Benlimame et al., 1995; Benlimame and Nabi, 1997). In MDCK cells, significant labeling is also detected on ribosome studded tubules of the rough ER as well as transitional part rough/part smooth tubules demonstrating a relationship between smooth AMF-R tubules and the endoplasmic reticulum. However, by immunofluorescence, AMF-R tubules are clearly distinguished from rough ER tubules labeled for calnexin (Benlimame et al., 1995). Nevertheless, AMF-R tubules associate with the microtubule cytoskeleton in a similar fashion to that described for the endoplasmic reticulum (Terasaki et al., 1986; Lee et al., 1989; Benlimame et al., 1995; Nabi et al., 1997).

Disruption of microtubule integrity results in the loss of the organized cytoplasmic distribution of AMF-R tubules but does not effect their tubular morphology (Benlimame et al., 1995). Similarly, following microtubule depolymerization, the Golgi apparatus is dispersed into clusters which retain the stacked morphology and functionality of the Golgi apparatus (Rogalski et al., 1984; Thyberg and Moskalewski, 1985; Ho et al., 1989; Turner and Tartakoff, 1989; Cole et al., 1996). Subsequent vesiculation and loss of functionality of the Golgi apparatus occurs during mitosis (Lucocq et al., 1989). The dictyoceratid sea sponge metabolite ilimaquinone (IQ) also induces the breakdown of Golgi membranes to small vesicles and inhibits protein transport between Golgi stacks and protein secretion (Takizawa et al., 1993). Reformation of Golgi stacks after IQ treatment is a two step process involving NSF and p97 dependent steps, as described for Golgi reformation from mitotic Golgi fragments (Acharya et al., 1995a; Rabouille et al., 1995). The action of IQ is distinct from that of BFA in that IQ does not induce retrograde transport to the ER and does not block the transport of newly synthesized proteins from the ER to *cis*-Golgi vesicles (Takizawa et al., 1993; Veit et al., 1993). With respect to cellular organelles, to date the activity of IQ is specific to the Golgi apparatus and IQ affects neither fluid phase endocytosis nor mitochondrial or ER morphology (Takizawa et al., 1993; Veit et al., 1993). We show here that IQ also targets the AMF-R tubule demonstrating that IQ activity is not Golgi-specific. We further demonstrate that AMF-R tubules are not equivalent to the ER-Golgi intermediate compartment (ERGIC) and identify this organelle as a smooth IQ-sensitive subdomain of the ER.

MATERIALS AND METHODS

Chemicals and antibodies

IQ was the kind gift of Dr Vivek Malhotra (UCSD, San Diego, California). Paclitaxel (taxol) and nocodazole were purchased from

Sigma. Monoclonal antibody against AMF-R was used in the form of concentrated hybridoma supernatant (Nabi et al., 1990). Antibody against ERGIC-53 was kindly provided by Hans-Peter Hauri (Biozentrum, Basel, Switzerland). Antibodies to Tac (IL25) were purchased from AMAC (Westbrook, ME). Antibodies to α-tubulin were purchased from ICN (Mississauga, Ontario, Canada). Secondary antibodies conjugated to either FITC, Texas Red or 12 nm gold particles were purchased from Jackson Immunoresearch Laboratories (West Grove, PA). The fluorescent secondary antibodies were designated for use in multiple labeling studies and no interspecies cross-reactivity was detected. To detect the anti-AMF-R rat IgM, secondary antibodies specific for the μ chain of rat IgM were used.

Cells and cell culture

All cells were grown in an air-5% CO_2 incubator at constant humidity. MDCK cells transfected with a Tac-TGN38 chimeric protein (MDCK-TGG cells) (Humphrey et al., 1993; Rajasekaran et al., 1994) were obtained from Enrique Rodriguez-Boulan (Dyson Eye Institute, Cornell University Medical College, New York, New York). MDCK, MDCK-TGG and HeLa cells were grown in Dulbecco's minimum essential medium (DMEM) containing non-essential amino acids, vitamins, glutamine and a penicillin-streptomycin antibiotic mixture (Gibco, Burlington, Ontario, Canada) supplemented with 5% fetal calf serum (Immunocorp, Laval, Quebec, Canada). IQ was used at a concentration of 25 μM and added to cells in culture medium supplemented with 25 mM Hepes, pH 7.0.

Immunofluorescence

Cells were plated (25,000-50,000 cells/35 mm dish) on glass coverslips for 2 days before each experiment. Cells were fixed by the addition of precooled ($-80°C$) methanol/acetone (80%/20%, v/v) directly to the coverslips, and then placed at $-20°C$ for 15 minutes. After fixation, the cells were rinsed extensively with PBS, pH 7.4, supplemented with 0.1 mM Ca^{2+} and 1 mM Mg^{2+} (PBS/CM), and then incubated for 15 minutes with PBS/CM containing 0.5% BSA (PBS/CM/BSA) at room temperature to reduce nonspecific binding. All washings and incubations with both primary and secondary (FITC and Texas Red conjugated) antibodies were done with PBS/CM/BSA. After labeling, the coverslips were mounted in Airvol (Air Products and Chemicals Inc, Allentown, PA). Labeled cells were viewed in a Zeiss Axioskop fluorescence microscope equipped with a ×63 Plan apochromat objective and selective filters. Images were photographed using Kodak T-Max
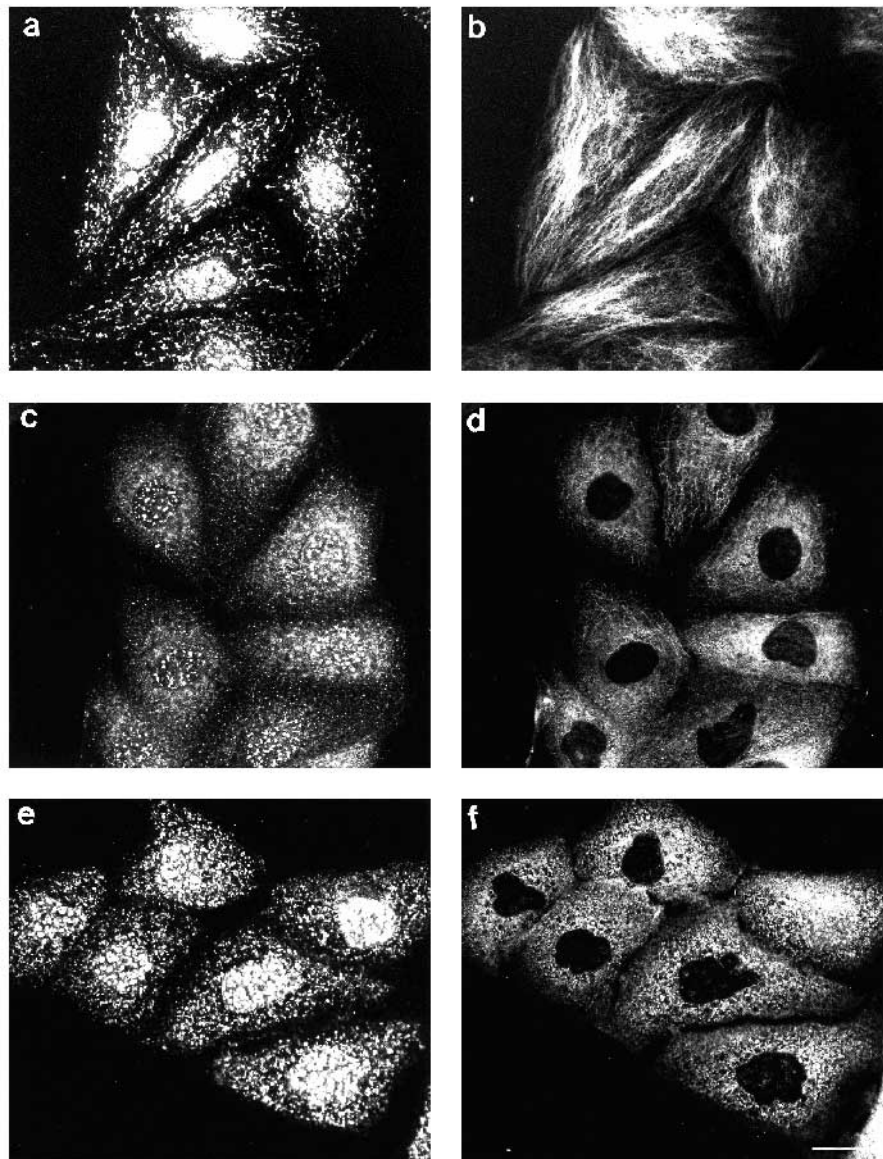
Fig. 1. Fragmentation of AMF-R tubules by ilimaquinone in MDCK cells. Untreated MDCK cells (a,b) or MDCK cells treated with 25 μM IQ for 30 (c,d) or 60 minutes (e,f) were double immunofluorescently labeled for AMF-R (a,c,e), and tubulin (b,d,f). In control cells (a,b), AMF-R tubules exhibit a linear morphology and are oriented towards the cell periphery of the cells in coordination with the microtubule cytoskeleton. In cells treated with 25 μM IQ (c,d), microtubules are depolymerized and AMF-R labeling is no longer localized to distinct tubular structures but exhibits a punctate distribution throughout the cytoplasm (c,d). Treatment with IQ for 60 minutes (e,f) results in the formation of larger AMF-R labeled structures (e). Bar, 20 μM.

400 film. Confocal analysis was performed with a Zeiss confocal microscope and printed with a Kodak XLS 8300 digital printer (Department of Cell Biology and Anatomy, McGill University, Montreal, Quebec).

Electron microscopy

Cells grown on Petri dishes were washed rapidly twice with Ringer's solution before fixing in the same solution containing 2% paraformaldehyde and 0.2% glutaraldehyde for 30 minutes at 37°C (Benlimame et al., 1995). The fixed cells were rinsed in PBS/CM, scraped from the Petri dish and collected by centrifugation. The cell pellet was post-fixed for 30 minutes with 1% osmium tetroxide in PBS/CM containing 1.5% potassium ferrocyanide, dehydrated and embedded in LR-White resin. Ultra-thin sections were blocked with 2% BSA in PBS/CM, and then incubated at room temperature with anti-AMF-R antibody followed by 12 nm gold-conjugated goat anti-rat secondary antibodies. The sections were stained with 5% uranyl acetate and visualized in a Philips 300 electron microscope.

RESULTS
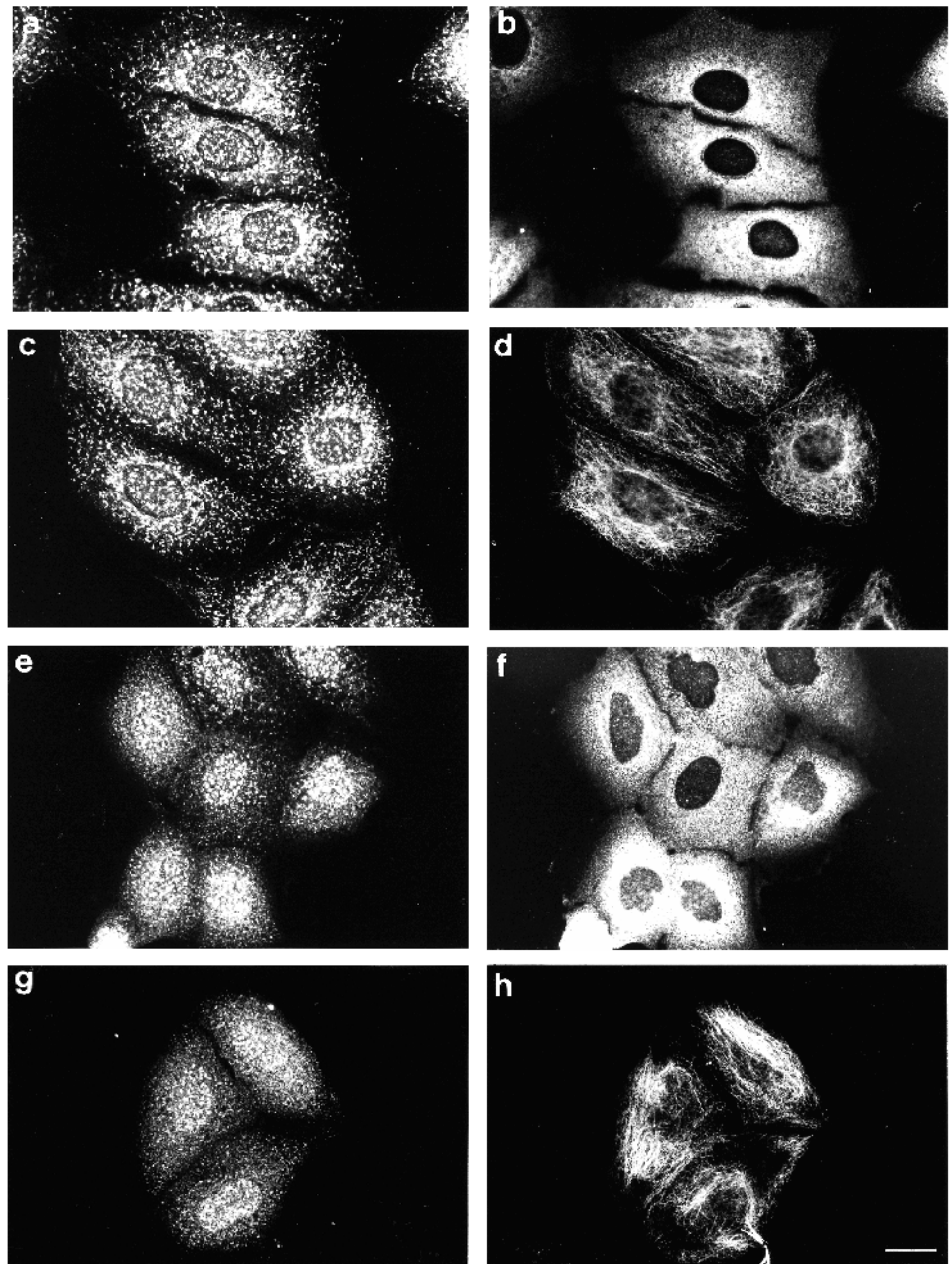
Fragmentation of AMF-R tubules by IQ

IQ has previously been described to vesiculate the Golgi apparatus and depolymerize microtubules but not to affect other cellular organelles or cytoskeletal filaments (Takizawa et al.,

1993; Veit et al., 1993). AMF-R tubules were previously characterized in MDCK cells (Benlimame et al., 1995), an IQ sensitive cell line (Takizawa et al., 1993), and we therefore tested the effect of IQ on AMF-R tubule morphology in MDCK cells (Fig. 1). In untreated cells, AMF-R tubules exhibit a linear morphology and extend to the cell periphery in alignment with the microtubule cytoskeleton (Fig. 1a,b). After treatment with IQ for 30 minutes the microtubules are extensively depolymerized and the AMF-R labeling exhibits a punctate distribution throughout the cell (Fig. 1c,d). Treatment with IQ for 60 minutes results in the formation of larger AMF-R labeled structures (Fig. 1e,f).

In order to study in parallel the effect of IQ on AMF-R tubules and the TGN, we used MDCK cells transfected with a chimeric protein construct consisting of the ectodomain of IL25 (Tac) and the transmembrane and cytoplasmic domains of TGN38 which localizes to the TGN (MDCK-TGG cells) (Humphrey et al., 1993; Rajasekaran et al., 1994). AMF-R tubules do not colocalize with the β-COP labeled Golgi apparatus in transformed MSV-MDCK cells and anti-AMF-R antibodies do not label the Golgi apparatus by post-embedding immunoelectron microscopy (Benlimame et al., 1995; Nabi et al., 1997). The distribution of AMF-R tubules and the TGN in untreated cells is quite distinct and the two labels do not colocalize (Fig. 2a,c,e). Following IQ treatment for 30 minutes at 37°C, both labels exhibit a similar
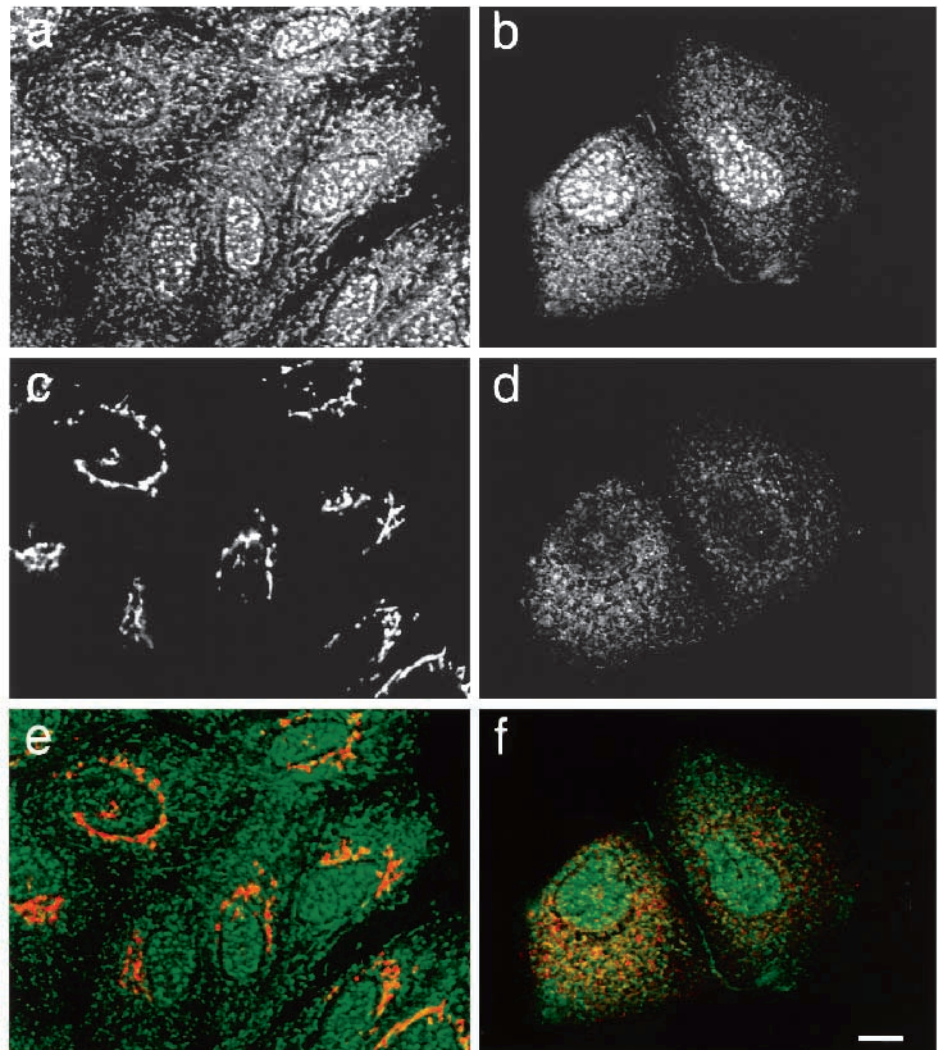
Fig. 2. IQ targets TGN membranes and AMF-R tubules. MDCK-TGG cells were untreated (a,c,e) or treated with 25 μM IQ for 30 minutes (b,d,f) and the cells were double immunofluorescently labeled for AMF-R (a,b) and with anti-Tac antibody to reveal TGN38 (c,d). Dual color merged confocal images reveal that the AMF-R (green) and TGN (red) labels can be distinguished in both control (e) and IQ treated (f) cells. Bar, 20 μM.

Fig. 3. IQ fragmentation of AMF-R tubules is microtubule-independent. MDCK cells were treated with 20 μM nocodazole for 30 minutes (a,b,e,f) or with 10 μM taxol (c,d,g,h) for 30 minutes and then incubated for a further 30 minutes in the presence of these drugs with (e,f,g,h) or without (a,b,c,d) the addition of 25 μM IQ. Cells were double immunofluorescently labeled for AMF-R (a,c,e,g) and for tubulin (b,d,f,g). Bar, 20 μM.

punctate distribution throughout the cell (Fig. 2b,d,f). Dual color merged images of the IQ treated cells (Fig. 2f, AMF-R in green and Tac-TGN38 in red) show that the AMF-R and TGN labels can be distinguished. This is particulary evident in the cell to the right which exhibits a more spread morphology. As described for the IQ-mediated breakdown of Golgi membranes, IQ does not affect AMF-R tubule morphology when cells are treated at 4°C or in the presence of azide (data not shown). IQ action on both Golgi and AMF-R tubule membranes is therefore similar.

The effect of IQ on AMF-R tubules is microtubule-independent

AMF-R tubules are microtubule-associated membranous organelles, however, disruption of microtubule integrity does not influence the tubular morphology of this organelle but rather affects their peripheral orientation and tubular extension

(Benlimame et al., 1995). IQ also depolymerizes microtubules (Veit et al., 1993) and we questioned whether the effect of IQ on AMF-R tubules is related to the effect of IQ on microtubule integrity. MDCK cells were pretreated with either 20 μM nocodazole for 30 minutes to disrupt microtubules or with 10 μM taxol for 30 minutes to stabilize microtubules and then treated with 25 μM IQ for 30 minutes in the continued presence of either nocodazole or taxol. After treatment with either nocodazole or taxol, elongated tubules were still present (Fig. 3a,c). In both the absence of microtubules in nocodazole treated cells or in the presence of stabilized microtubules in taxol treated cells, IQ still disrupted the tubular morphology of AMF-R tubules (Fig. 3e,g). As described for the Golgi apparatus (Veit et al., 1993), IQ mediated disruption of AMF-R tubules is independent of the ability of IQ to depolymerize microtubules.
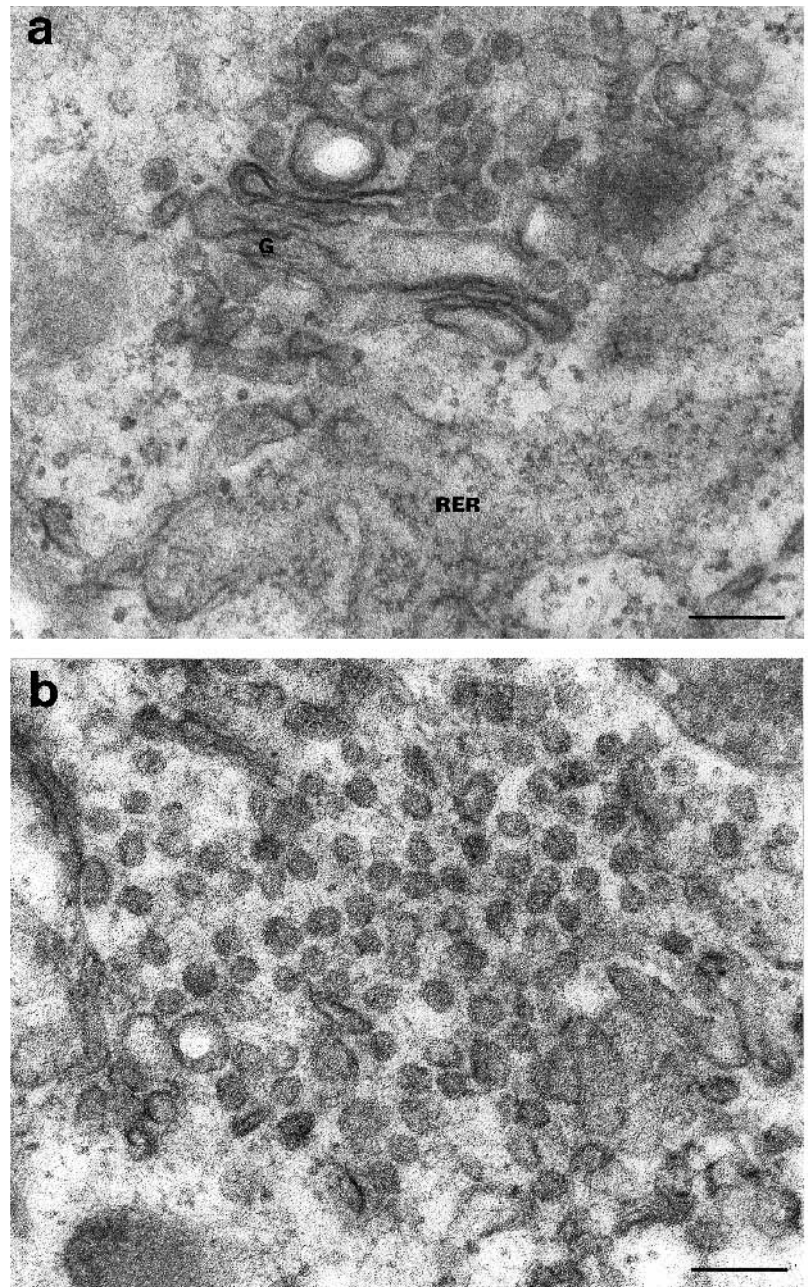
Fig. 4. IQ mediates Golgi membrane vesiculation in MDCK cells. Cells treated with 25 µM IQ for 30 minutes were visualized by electron microscopy. Intact Golgi saccules (G) could be observed in the vicinity of uniform 60-90 nm vesicles (a). Clusters of these vesicles were more frequently observed in the absence of Golgi saccules (b). RER: rough ER. Bars, 0.2 µM.
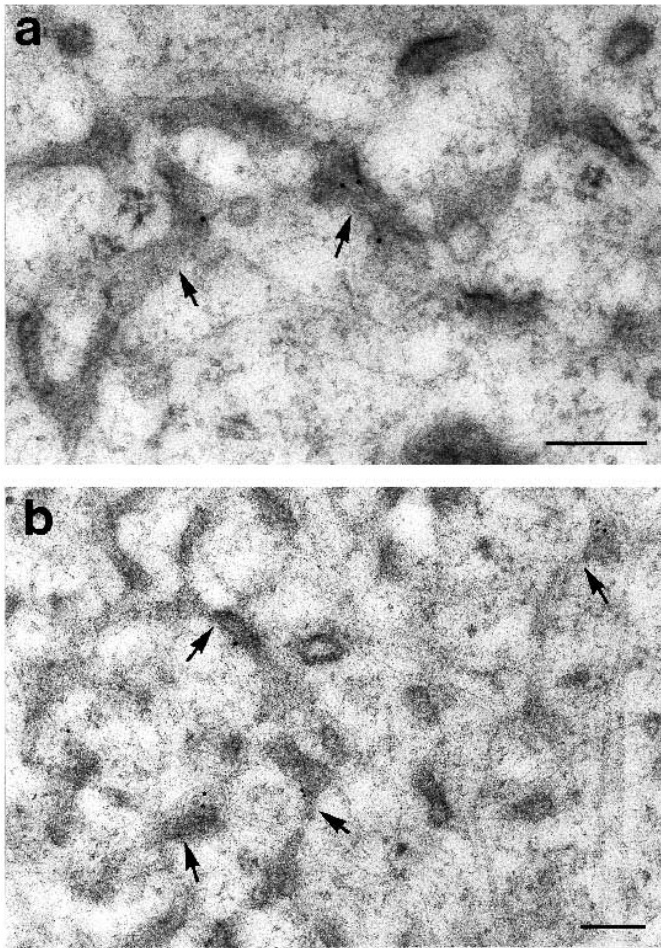
Ultrastructure of AMF-R tubules after IQ treatment

Based on our fluorescence microscopy observations, the maximal extent of IQ-mediated vesiculation of AMF-R tubules occurred between 30 and 45 minutes, depending on the experiment, and after 60 minutes larger clusters predominated (Fig. 1). Variability in the extent of disruption of both the Golgi apparatus and AMF-R tubules by IQ observed by immunofluorescence was confirmed by EM. After a 30 minute IQ treatment, vesiculation of the Golgi apparatus could be observed at various stages (Fig. 4). The products of Golgi vesiculation could be identified by the presence of intact Golgi saccules in the immediate vicinity of clusters of homogenous vesicles of 60-90 nm diameter (Fig. 4a). These vesicles are morphologically identical to those previously identified as Golgi-derived in NRK cells (Takizawa et al., 1993; Acharya et al., 1995a,b). Clusters of these vesicles were for the most part

observed in the absence of Golgi saccules yet could be morphologically identified as Golgi-derived (Fig. 4b). Such vesicle clusters were not observed in untreated cells.

AMF-R tubules, similar to tubular lysosomes (Heuser, 1989), are labile structures which are stabilized by Ringer's solution and previous fixation protocols included a 15 minute incubation in Ringer's solution prior to fixation (Benlimame et al., 1995). In order to eliminate possible consequences of IQ washout during a 15 minute preincubation with Ringer's solution, the cells were only rinsed twice rapidly with Ringer's solution prior to fixation. Under these conditions AMF-R labeling is localized predominantly to smooth tubules with a minimum diameter of 40 nm (Fig. 5). As previously described, the post-embedding labeling is weak but specific (Benlimame et al., 1995). The labeled tubules are similar in morphology to those described following Ringer's treatment (Benlimame et

Fig. 5. Ultrastructure of AMF-R tubules by electron microscopy. MDCK cells were rinsed twice rapidly in Ringer's solution prior to fixation and post-embedding immunogold labeling for AMF-R. Labeled smooth AMF-R tubules are indicated by arrows. Bars, 0.2 μM.

al., 1995) but extended tubular networks are not present to the same extent.

AMF-R labeling of IQ treated cells is localized to highly fenestrated networks of narrow elongated tubular membranes which are observed only in IQ treated samples (Fig. 6a,b). These tubular networks represent the majority of AMF-R labeled structures, can be distinguished from the Golgi-derived vesicles and are morphologically equivalent to smooth ER. The majority of structures labeled for AMF-R were tubular networks identical to those in Fig. 6a and regions of complete vesiculation, as seen for the Golgi (Fig. 5b), were not observed. Numerous caveolae were also observed in IQ treated cells (not shown). The tubular networks may thus represent the final product of the effect of IQ on AMF-R tubules. In both untreated and treated cells, AMF-R tubules consist of broader regions of variable diameter connected by narrower tubules. In untreated cells, the diameter of double membrane tubular connections, essentially the narrowest portion of the tubule, is 30.5±9.0 nm (range: 19-54 nm) while following IQ treatment the connecting tubules are significantly narrower measuring 18.9±6.4 nm (range: 10-38 nm) (Fig. 6a,b). The tubular networks are highly fenestrated and contain distinct membrane

bounded fenestrations both within individual tubules (arrowheads) and between tubules. As described for the Golgi, incompletely fragmented AMF-R tubules of larger diameter are also present in cells treated with IQ for 30 minutes (Fig. 6c). Both intratubular fenestrations (arrowheads) as well as larger fenestrations within the tubular network are present. Fenestrations of AMF-R tubules are never observed in untreated cells.

AMF-R tubules are a smooth ER subdomain distinct from ERGIC

As previously demonstrated for control MDCK cells (Benlimame et al., 1995), in IQ treated cells AMF-R labels rough ER tubules as well as tubules which exhibit both rough and smooth portions (Fig. 7). Of particular interest is the fact that while IQ does not affect the rough portion of the tubule, the smooth portion presents a morphology of interconnected narrow tubules morphologically identical to the tubular clusters described in Fig. 6. Direct connections between the smooth tubulated portion of the AMF-R tubule and the rough ER are evident (Fig. 7b, arrow). Smooth AMF-R tubules are therefore a subdomain of the ER and only the smooth portion of part rough/part smooth tubules is susceptible to IQ-mediated fragmentation. As previously described (Takizawa et al., 1993; Veit et al., 1993), both mitochondria (M) and rough ER tubules (RER) remained morphologically intact after IQ treatment (Figs 5, 6 and 7).

AMF-R tubules are therefore smooth tubular extensions of the rough ER. AMF-R labeling of structures similar to transitional ER elements, classically considered to be exit sites of newly synthesized proteins from the ER en route to the Golgi apparatus (Palade, 1975), suggests that AMF-R tubules might be equivalent to ERGIC. β-COP has been described as a marker for the *cis*-Golgi and ERGIC in exocrine pancreatic cells (Oprins et al., 1993) and confocal microscopy has shown that AMF-R tubules do not colocalize with β-COP in MSV transformed MSCK cells (Nabi et al., 1997). To further establish the relationship between AMF-R tubules and ERGIC, we performed double IF labeling for AMF-R and ERGIC-53, a well-defined marker for ERGIC (Schweizer et al., 1988) (Fig. 8). ERGIC-53 is predominantly localized to the perinuclear region where AMF-R tubules also accumulate in HeLa cells. While it is difficult to distinguish the two labels in this region, AMF-R labeled tubular structures are clearly defined in this region compared to the more diffuse labeling of ERGIC-53. Furthermore, AMF-R tubules extending to the periphery of the cell are unlabeled for ERGIC-53 and we have never observed an AMF-R tubule to be labeled for ERGIC-53. AMF-R tubules are therefore a smooth ER compartment distinct from ERGIC.

DISCUSSION

IQ targets another organelle: the AMF-R tubule

The dictyoceratid sea sponge metabolite ilimaquinone induces breakdown of Golgi membranes to very small vesicles uniformly distributed throughout the cytoplasm, inhibiting protein transport between Golgi stacks and protein secretion (Takizawa et al., 1993). We show here that IQ activity is not Golgi specific and that the AMF-R tubule is another target for IQ. After IQ treatment, the tubular AMF-R labeling visualized
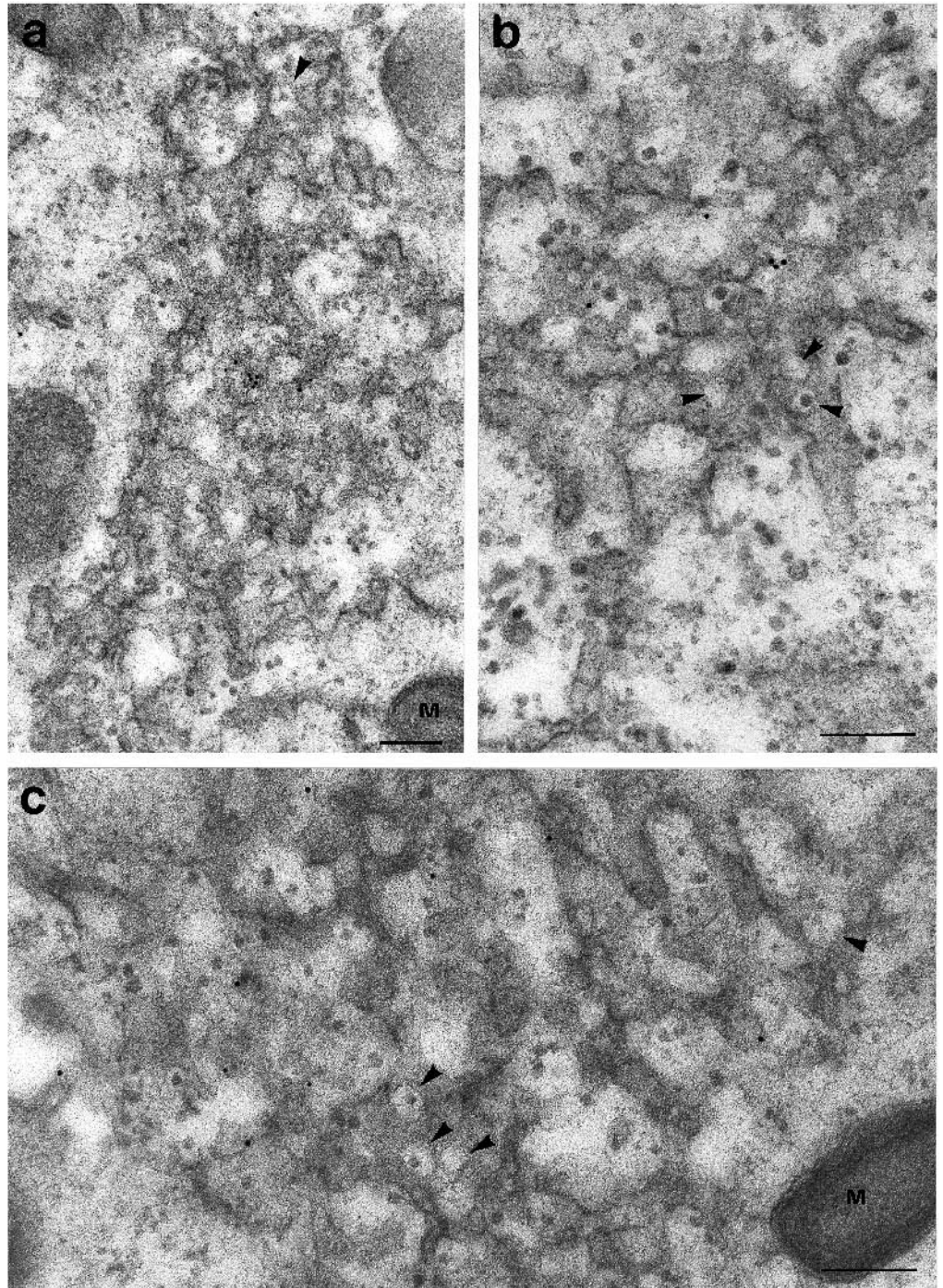
Fig. 6. AMF-R is localized to tubular networks in IQ treated cells. By postembedding immunoelectron microscopy, AMF-R is localized to networks of narrow interconnected tubules in MDCK cells treated with 25 µM IQ for 30 minutes (a,b). Incompletely fragmented AMF-R labeled tubules of larger diameter are also present in treated cells (c). Distinct membrane bounded fenestrations are present within tubules of larger diameter (arrowheads) as well as within the tubular networks. Bars, 0.2 µM.

by immunofluorescence is lost and AMF-R exhibits a punctate distribution throughout the cytoplasm. AMF-R distribution following IQ treatment resembles that of the Golgi apparatus but the products of IQ-mediated vesiculation of the TGN can be distinguished by confocal microscopy from those derived from AMF-R tubules. By EM, IQ treatment of AMF-R tubules gives rise to fenestrated networks of short narrow tubules labeled for AMF-R which can be distinguished from the uniform vesicles of 60-90 nm derived from Golgi fragmentation (Takizawa et al., 1993; Acharya et al., 1995b). The tubular networks derived from IQ-mediated fragmentation of AMF-R tubules are morphologically equivalent to smooth ER. While

we cannot be certain that the effect of IQ was complete, the majority (~80%) of identifiable Golgi derived structures in the treated cells were completely vesiculated (Fig. 5). Similarly, tubular networks (Fig. 6a) represent the majority of AMF-R labeled structures and complete vesiculation of AMF-R labeled structures was not observed. The interconnected tubular clusters may thus represent the final stage of IQ-mediated fragmentation of the AMF-R tubule.

The half-life of IQ activity in culture medium is less than 60 minutes and the effect of IQ on the Golgi apparatus has been shown to be reversible (Takizawa et al., 1993). By immunofluorescence, we observe the formation of larger structures
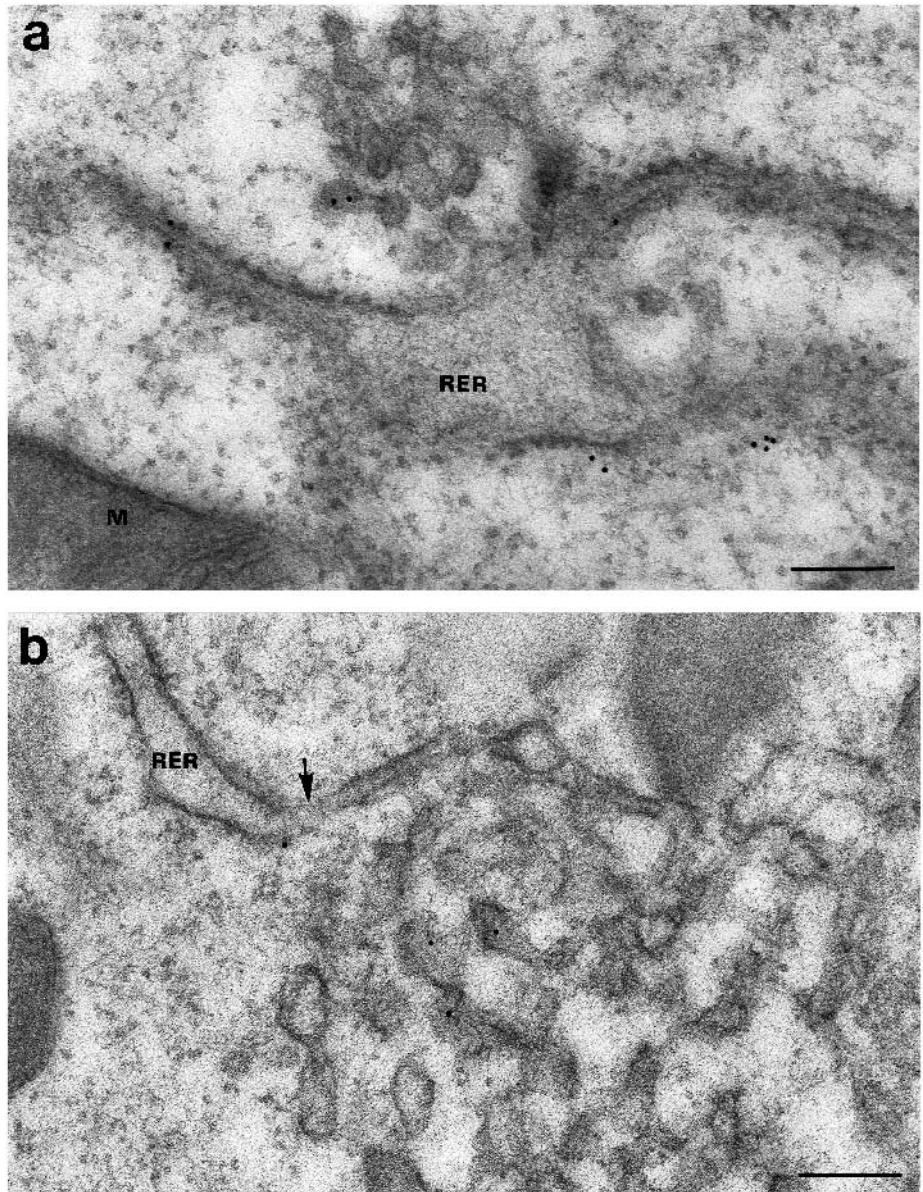
Fig. 7. IQ selectively targets smooth extensions of the ER. Only the smooth portion of AMF-R labeled part smooth/part rough ER membranes is fragmented in IQ treated cells (a,b). Connection between a ribosome studded rough ER cisterna and smooth ER networks is indicated by the arrow (b). Both rough ER cisternae (RER) and mitochondria (M) are intact in IQ-treated cells. Bars, 0.2 μM.

after 60 minutes of IQ treatment (Fig. 1e). By electron microscopy, these correspond to larger fenestrated clusters, however 4 hours after IQ treatment only unfenestrated tubules are present demonstrating that the effect of IQ is reversible (not shown). IQ also disrupts the integrity of microtubules and both the Golgi and AMF-R tubules are microtubule-associated organelles. Disruption of the microtubule cytoskeleton fragments the Golgi apparatus but does not disrupt the stacked cisternal morphology of the Golgi (Thyberg and Moskalewski, 1985). Similarly, disruption of the microtubule cytoskeleton results in a loss of the tubular extension and peripheral orientation of AMF-R tubules but not of their tubular morphology (Benlimame et al., 1995). For both organelles, the action of IQ is independent of the state of the microtubule cytoskeleton and the addition of IQ after nocodazole or taxol treatment induces a further fragmentation of both organelles to smaller structures (Fig. 3; Veit et al., 1993). The action of IQ on the two organelles is therefore similar in that it is not microtubule-dependent, is reversible, and does not occur at 4°C or in

the presence of azide (Takizawa et al., 1993) (data not shown) suggesting that the molecular target of IQ in both organelles is the same. However, while IQ clearly induces Golgi vesiculation, the tubular network of AMF-R tubules formed following IQ treatment suggests that IQ does not stimulate vesicle budding.

The AMF-R tubule is a highly labile organelle and optimal fixation requires a rapid freezing fixation method in which methanol/acetone precooled to −80°C is added rapidly to cells. As described for the tubular lysosome, also a highly labile tubular organelle (Robinson et al., 1986), the tubular morphology of the AMF-R tubule is stabilized by Ringer's solution (Heuser, 1989; Racoosin and Swanson, 1993) and a protocol based on Ringer's solution permitted visualization of the AMF-R tubule by conventional postembedding EM procedures (Benlimame et al., 1995). Pretreatment of cells with Ringer's solution for 15 minutes results in the identification of extended smooth tubular networks (Benlimame et al., 1995) which are not present to the same extent if the cells are only rinsed rapidly
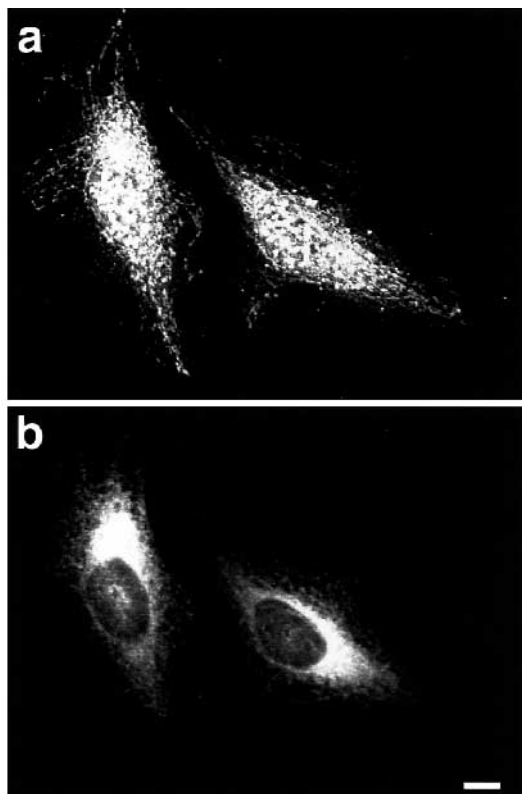
Fig. 8. AMF-R labeled tubules do not colocalize with the intermediate compartment labeled for ERGIC-53. HeLa cells were double labeled with antibodies to AMF-R (a) and to ERGIC-53 (b) and three adjacent confocal 1 μM sections summated. Distinct AMF-R labeled tubular structures can be visualized in the perinuclear region compared to the diffuse ERGIC-53 label and AMF-R tubules in the cell periphery are not labeled for ERGIC-53. Bar, 20 μM.

with Ringer's solution prior to fixation (Fig. 4). The presence of immunofluorescently labeled tubular structures in cells rapidly fixed by the addition of precooled (−80°C) methanol/acetone indicates that AMF-R tubules are present in viable cells and not a consequence of the EM fixation procedure.

The ability of IQ to fragment AMF-R tubules might be related to the labile nature of this organelle. It has been suggested that the structure of certain organelles might represent an equilibrium between homotypic fusion and periplasmic fusion, the fusion of internal membranes resulting in organelle vesiculation (Rothman and Warren, 1994). The AMF-R tubule exhibits a highly fluid morphology, contrary to the regular nature of rough ER tubules, that may permit the interaction of internal membranes necessary for periplasmic fusion (Benlimame et al., 1995; Benlimame and Nabi, 1997). In the presence of IQ AMF-R labeled tubules are narrower and present numerous fenestrations whose membranous limitations can be visualized in 80 nm ultrathin sections (Fig. 6). The disruption of AMF-R tubules by IQ as visualized by fluorescence microscopy (Figs 1, 2) would thus appear to be a consequence of the progressive fenestration of AMF-R tubules generating a fine meshwork of narrow interconnected tubules. These fenestrated networks resembles structures predicted by periplasmic fusion (Rothman and Warren, 1994). The selective effect

of IQ on Golgi and AMF-R tubules may be due to the ability of those organelles to fenestrate (Rambourg and Clermont, 1990; Weidman et al., 1993) and the ability of IQ, by an as yet unknown mechanism, to favor this process.

The AMF-R tubule is a smooth subdomain of the ER

Quantitative postembedding labeling of MDCK cells reveals the predominant labeling of smooth tubules with a lesser labeling of rough ribosome-studded tubules (Benlimame et al., 1995). AMF-R tubules do not colocalize with the calnexin labeled rough ER, however, rare tubules can be identified which are labeled for both calnexin and AMF-R. Part smooth/part rough tubules, morphologically equivalent to transitional ER, are labeled for AMF-R and support the identity of the AMF-R tubule as a smooth ER-associated organelle. In MSV transformed MDCK cells, both AMF-R tubules and the calnexin labeled rough ER selectively associate with a subdomain of microtubules enriched in stabilized microtubules (Nabi et al., 1997). Evidence that AMF-R labeled tubules are equivalent to smooth ER is supported by the morphology of AMF-R tubules in IQ treated cells. The intermingled tubules observed after IQ treatment are smaller and narrower than those in untreated cells and resemble the smooth ER of hepatocytes (Fawcett, 1981). IQ does not affect rough ER and of particular interest is the selective fragmentation of the smooth portion of labeled tubules which extend from intact rough ER cisternae (Fig. 7). These images resemble the interface between smooth and rough ER in the hepatocyte in vivo (Fawcett, 1981) and following assembly from purified rat liver microsomes in the presence of ATP and GTP (Lavoie et al., 1996).

Transitional ER has been proposed to be the site of exit of newly synthesized proteins en route to the Golgi (Palade, 1975). The presence of marker proteins, ERGIC-53, p58 and rab2 have identified ERGIC as an organelle (compartment) distinct from the ER (Schweizer et al., 1988; Chavrier et al., 1990; Saraste and Svensson, 1991). However, some data support the idea that the ERGIC is continuous with the rough ER, and thus can be considered a smooth subdomain of the ER (Saraste and Svensson, 1991; Hauri and Schweizer, 1992; Krijnse-Locker et al., 1994). Degradation of newly synthesized proteins that are misfolded or fail to assemble into the appropriate oligomers takes place in the ER or in a related compartment, possibly the ERGIC (Bonifacino and Lippincott-Schwartz, 1991; Hauri and Schweizer, 1992; Klausner et al., 1992). Unassembled Rubella virus E1 glycoprotein is arrested in a smooth membranous tubular structure contiguous with the ER proximal to and distinct from ERGIC (Hobman et al., 1992). In thymic epithelial cells derived from transgenic mice deficient for the transporter associated with antigen presentation (TAP), misfolded major histocompatibility complex class I molecules accumulate in a degradative compartment consisting of an expanded network of tubular and fenestrated membranes which contains ERGIC-53; the morphology of this expanded compartment in plastic resins is remarkably similar to that of AMF-R tubules (Raposo et al., 1995).

Double immunofluorescence labeling of HeLa cells with antibodies to AMF-R and ERGIC-53 clearly demonstrate the presence of AMF-R tubules that do not contain ERGIC 53 indicating that the smooth ER-compartment labeled for AMF-R is not equivalent to ERGIC (Fig. 7). β-COP has been localized to ERGIC as well as to the cis-Golgi (Oprins et al., 1993; Krijnse-

Locker et al., 1994). β-COP does not label peripheral AMF-R tubules in MDCK cells and confocal studies of transformed MDCK cells showed that β-COP does not colocalize with AMF-R tubules (Benlimame et al., 1995; Nabi et al., 1997). Distinction between AMF-R tubules and ERGIC is further indicated by the sensitivity of AMF-R tubules to IQ. IQ blocks intraGolgi transport but not the delivery of newly synthesized Golgi proteins to *cis*-Golgi compartments. In the presence of IQ, newly synthesized VSV G protein does not acquire endo H sensitivity and thus does not reach medial Golgi compartments; it does however exhibit a slightly modified molecular mass consistent with partial glycosylation of the protein in *cis*-Golgi elements (Takizawa et al., 1993). This apparent functionality of ERGIC in the presence of IQ and the demonstrated IQ-sensitivity of AMF-R tubules discounts identity between these two organelles. We cannot, however, exclude the possibility that the AMF-R tubule can interact with other ER-associated subdomains or organelles, including ERGIC.

The endoplasmic reticulum is a continuous membrane network linked to the nuclear envelope and is composed of sub-compartments morphologically identified as the rough ER, studded with ribosomes, and the smooth ER, which is devoid of ribosomes (Sitia and Meldolesi, 1992; Vertel et al., 1992). Transitional ER morphologically represents sites of interaction between rough and smooth subdomains of the ER, however, morphological similarity does not necessarily reflect functional identity. Distinct smooth ER subdomains have been proposed to be involved in the secretory pathway (Hobman et al., 1992) but they have also been shown to interact with organelles other than the Golgi apparatus and to be implicated in cellular functions other than ER to Golgi transport. The distinctive architecture of hippocampal neurons permitted the demonstration of the existence in axons and dendrites of smooth ER membranes distinct from ERGIC which are localized exclusively to the cell body (Krijnse-Locker et al., 1995). Autophagic vacuoles derive from ER membranes (Dunn, 1990a,b) and the transition of rough ER tubules into smooth ER tubules which subsequently fuse with lysosomes via a non-autophagic pathway has been described (Noda and Farquhar, 1992). Smooth ER has been implicated in calcium storage in a variety of cell types (Pozzan et al., 1994; Golovina and Blaustein, 1997). The expansion of smooth ER in specific cell types demonstrates the existence of specialized smooth ER compartments that can be exploited for specialized functions such as lipid biosynthesis or detoxification (Jones and Fawcett, 1966; Stäubli et al., 1969; Orci et al., 1984; Pathak et al., 1986). Induction of smooth ER in liver hepatocytes by phenobarbitol permitted the identification of epoxide hydrolase as a marker for liver smooth ER (Galteau et al., 1985). While AMF-R labels ribosome-studded tubules in MDCK cells, minimal overlap with the calnexin labeled rough ER is detected by immunofluorescence double labeling (Benlimame et al., 1995) and in NIH-3T3 and HeLa cells, AMF-R is localized almost exclusively to smooth tubules and labeling of rough ER is negligible (Benlimame and Nabi, unpublished results). AMF-R does not label a continuous network by immunofluorescence labeling suggesting that the AMF-R tubule is a subdomain of the smooth ER. The presence of AMF-R identifies a role for this subdomain of the ER in cell motility, perhaps as an internal pool of membrane which can be mobilized to the plasma membrane at the leading edge following a motile stimulus.

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