Parietal cells contain most of the myosin, filamin and actin present in rat gastric glands

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The association of myosin and a filamin-like protein to the F-actin cytoskeleton of parietal cells was studied in the rat gastric mucosa. Myosin and the filamin-like protein were localized by indirect immunofluorescence microscopy while the distribution of actin was established by using FITC-phalloidin. These cytoskeletal proteins, concentrated in the parietal cells, changed their distribution in correlation with the hydrochloric acid (HCl) secretory state of the cells and the appearance of a developed intracellular canaliculus. Thus, in resting parietal cells, actin showed a patchy distribution, delimiting the poorly developed secretory canaliculi, while myosin and the filamin-like protein distributed diffusely over the cytoplasm. In secreting cells, F-actin was concentrated in the cytoplasmic projections filling the canalicular lumen, while myosin and the filamin-like protein were excluded from this region, concentrating in the adjoining cytoplasm. The present results show that myosin and the filamin-like protein change their association with the secretory membranes in relation to the development of the secretory canaliculus of parietal cells. In resting cells, both proteins associate with the endocellular secretory membranes. In secreting cells, the microvillar projections of the canalicular surface formed by these membranes bind F-actin, but exclude myosin and the filamin-like protein.

Key words: *actin, filamin, gastric glands, HCl secretory cycle, myosin, parietal cells, zymogen cells.*

INTRODUCTION

The functional role of the actin cytoskeleton in cellular dynamic activities has been clearly documented. To accomplish this role, the assembly of actin must be regulated precisely, in particular times and in discrete places within cells (Pollard and Cooper, 1986). Control of the mechanical properties of the cytoplasmic matrix is exerted by proteins, collectively known as actin-binding proteins. These proteins provide the driving force for the rearrangement of the actin cytoskeleton and may be targets for modulation by second messengers (Stendahl *et al*, 1980; Valerius *et al*, 1981; Stossel *et al*, 1985; Pollard and Cooper, 1986; Way and Weeds, 1990; Whitney *et al*, 1990; Cano *et al*, 1992; Way *et al*, 1992). Vertebrate hydrochloric acid (HCl) secreting cells, present in the gastric glandular epithelium, form part of a continuous sheet of cells joined by intercellular junctions. These cells undergo remarkable changes in shape and membrane redistribution during their secretory cycle (Vial and Orrego, 1960; Vial *et al*, 1985). Thus, they constitute an excellent model for studying the role of actin and actin-associated proteins in the translocation of cellular organelles and in cell shape changes of epithelial cells *in situ*. The intracellular machinery necessary for the

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displacement of membranes in HCl secreting cells is unknown. However, in several cell systems endocytic and exocytic processes involve interactions of membranous vesicles with cytoskeletal elements (Soroka *et al*, 1993).

In amphibian and chicken oxyntic cells, which secrete both acid and pepsinogen, the presence and distribution of myosin, actin and a protein immunologically related to filamin have been established (Vial and Garrido, 1976; Dabiké *et al*, 1986; Koenig and Dabiké, 1990). In the mammalian gastric mucosa, HCl and pepsinogen are secreted by two different cells. Previous evidence has shown that actin, myosin and other high molecular weight (HMW) actin-binding proteins are present in supernatants of homogenates of rat gastric glands (Wolosin *et al*, 1983). Besides, the secretory pole of rat parietal cells, responsible for HCl secretion, contains a meshwork of actin microfilaments that is redistributed in relation to this morphological reorganization (Vial and Garrido, 1976; Mercier *et al*, 1989). Resting parietal cells contain membranous tubules located in the vicinity of the undeveloped canaliculus. Upon stimulation the tubular membranes fuse among themselves and with the secretory plasma membrane giving rise to a tortuous, profusely branched intracellular canaliculus. The canalicular lumen is filled with cytoplasmic finger-like projections supported by actin-microfilaments. The developed canaliculus is formed by an in situ remodeling of the cytoplasmic domain occupied by the tubulovesicular system (Vial *et al*, 1985). The ultrastructural changes are inhibited by cytochalasin B (Black *et al*, 1982) and by epidermal growth factor (Gonzalez *et al*, 1981), agents that act upon the actin cytoskeleton, but not by colchicine (Kasbekar and Gordon, 1979).

Since studies on the distribution of actin, myosin and the filamin-like protein in HCl secreting cells should help understanding their role in the membrane displacements that take place during the HCl secretory cycle, the distribution of these proteins was established in resting and secreting rat parietal cells. In addition, the localization of these cytoskeletal proteins in zymogen cells was determined.

METHODS

Stomachs were obtained after laparotomy of anesthetized Sprague-Dawley rats (Na pentobarbitone 40 mg/Kg) weighing 200-250 g. The resting state of parietal cells was induced by im administration, to 24 h fasted rats, of two doses of cimetidine (100 mg/kg) given 12 h and 1 h before laparotomy. HCl secretion by parietal cells was induced by im injection of 1 mg histamine, 20 min before laparotomy. The HCl secretory state of the mucosas was determined by measuring the pH of the gastric contents with pH paper. Cytochemical detection of the gastric K^+-activated para-nitrophenyl-phosphatase (K^+-NPPase) was used to establish parietal cell ultrastructural organization (Koenig, 1984). Rat fundic cells were isolated according to the procedure reported by Dabiké and Koenig (1983).

Immunofluorescence microscopy. Pieces of gastric mucosa were quickly immersed in embedding matrix (Lipshaw) and frozen in liquid nitrogen. 4 μm cryostat sections were stained using antimyosin or antifilamin as first antibody or FITC-labeled phalloidin (Sigma), as described (Koenig and Dabiké, 1990). The preparation and characterization of rabbit antisera against human platelet myosin heavy chain and against chicken smooth muscle filamin have been described elsewhere (Dabiké *et al*, 1986). Specific antisera were diluted 1:50. FITC-labeled goat antibodies against rabbit IgG (Sigma) were used as second antibodies. Controls consisted of substitution of the immune sera for preimmune sera, absorption of immune sera with the purified antigen and staining of the sections with FITC-labeled antibody, only.

RESULTS

Distribution of myosin and a filamin-like protein in gastric glands

Staining of frozen sections of rat gastric mucosas with antifilamin antibodies and with antibodies against the heavy chain of human platelet myosin shows that parietal cells are largely the most immunoreactive cells in the gastric glands. Figure 1a is a low mag-

nification micrograph of secreting gastric glands of the body of the stomach stained with antifilamin antibodies. Clearly, parietal cells present at the base of the glandular tubes are more immunoreactive than those at the glandular neck. At this magnification zymogen cells, dispersed among parietal cells at the base of the glandular tubes, show faintly stained apical and basolateral borders, while mucous neck cells appear unreactive. A similar result is obtained when myosin and actin are detected using antimyosin antibodies and FITC-phalloidin, respectively (data not shown). Careful examination of Figure 1a shows that neck parietal cells have a distended secretory canaliculus easily seen at the light microscopy level even at low magnification. In contrast, the canaliculi of parietal cells at the base of the glandular tubes, are difficult to visualize.

Subcellular distribution of the filamin-like protein and myosin

Mucous neck cells. These cells do not stain in sections of resting and secreting mucosas treated with antifilamin (Fig 1f) or antimyosin antibodies (Fig 2e).

Neck parietal cells. In neck parietal cells of resting and secreting mucosas the filamin-like protein and myosin concentrate in the vicinity of the expanded secretory canaliculus and show a mottled arrangement (Figs 1f and 2e). The rest of the cytoplasm stains diffusely.

Resting basal parietal cells. In parietal cells at the base of the glandular tubes of resting mucosas, the filamin-like protein (Fig 1a-d) and myosin (Fig 2a-c) distribute diffusely over the whole cell cytoplasm.

Secreting basal parietal cells. In properly sectioned secreting parietal cells at the base of the glandular tubes, the region occupied by the secretory canaliculus is readily recognized because it is clearly devoid of immunoreactivity (Figs 1e and 2d). Both proteins distribute diffusely over the rest of the cytoplasm of parietal cells (Figs 1e and 2d). Frequently, the cytoskeletal protein concentrates slightly in the contour of the canaliculus (Fig 1e).

Zymogen cells. In resting and in HCl secreting mucosas, the basolateral borders of these cells appear faintly decorated with antifilamin (Fig 1g) and antimyosin (Fig 2a) antibodies.

In addition, the filamin-like protein and myosin concentrate in the luminal pole of parietal (Fig 1c,d and 2b,c) and zymogen cells (Figs 1b and 2a). Detection of myosin in isolated parietal and zymogen cells shows that this protein is organized as a peripheral ring in both cell types (Fig 2f).

Strongly stained elements, probably corresponding to fibroblasts or isolated smooth muscle cells, are apparent among the glandular tubes (Figs 1a-c,e and 2a).

Distribution of the K^+-NPPase product at the ultrastructural level in parietal cells at the base and at the neck of the glands

Neck parietal cells. In both resting and secreting mucosas, neck parietal cells show extremely wide intracellular canaliculi (Fig 3a). The reaction product of the K^+-NPPase concentrates on the cytoplasmic projections of the canalicular surface (Fig 3a).

Secreting basal parietal cells. Secreting cells at the base of the glandular tubes show developed but narrow intracellular canaliculi filled with microvillar extensions (Fig 3b). The reaction product of the K^+-NPPase concentrates on these microvillar extensions and on the membranes of the tubulovesicular system that persist in the cytoplasm (Fig 3b). Insets show the distribution of myosin, at the light microscopy level, in neck parietal cells (Fig 3a, inset) and in basal secreting parietal cells (Fig 3b, inset). A clear correlation between the ultrastructure of the cells, the distribution of the enzyme marker of the secretory membranes and the localization of myosin is observed.

Subcellular distribution of F-actin

Mucous neck cells. In both secretory states, a fine fluorescent line delimits the apical and basolateral borders of the mucous neck cells treated with FITC-phalloidin (data not shown).

Resting basal parietal cells. After staining with FITC-phalloidin, cells at the base of glandular tubes of resting mucosas, show scattered brilliant patches (Fig 4a) or irregu-

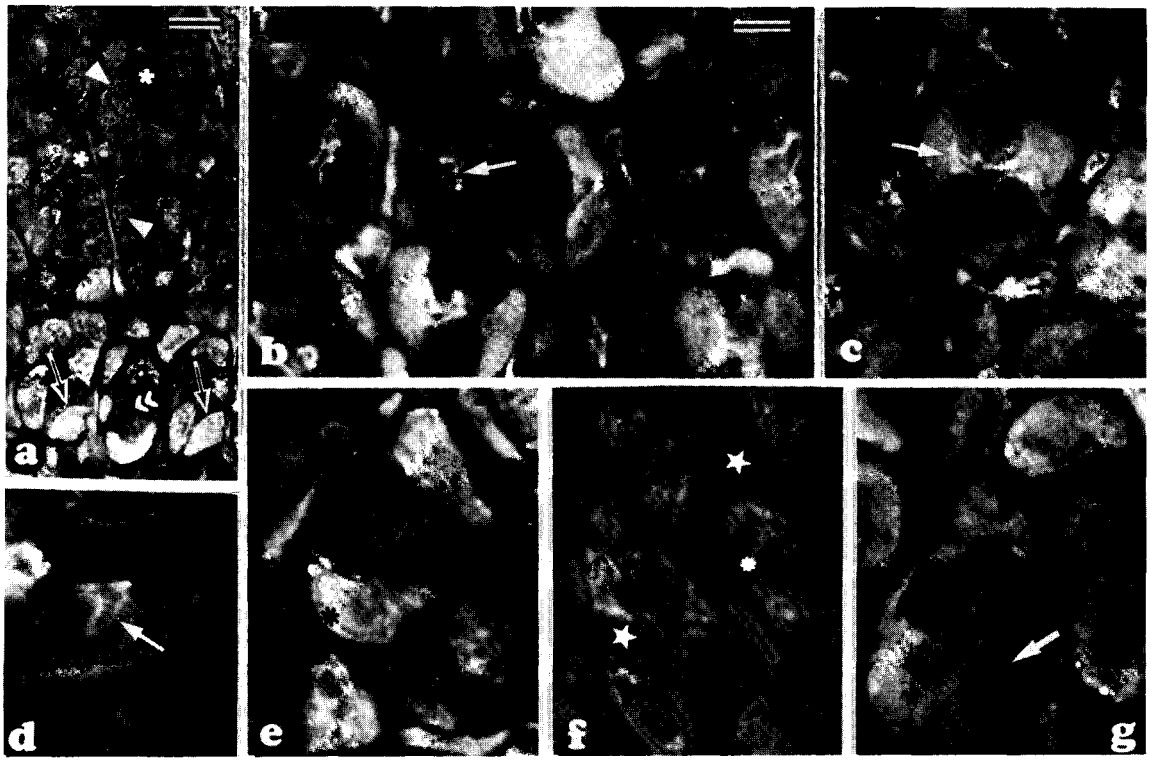

Fig 1. Frozen sections of gastric mucosa stained with antifilamin antibodies. In the low magnification micrograph (**a**), the highly developed intracellular canaliculi of neck cells are easily visualized (arrowheads), while they are not evident in the cells at the base of the glandular tubes (arrows). Zymogen cells dispersed among basal parietal cells show faintly stained apical and basolateral cell borders (double arrowhead). Mucous neck cells appear unstained (*). At higher magnification, the diffuse distribution of the filamin-like protein in the cytoplasm of parietal cells present at the base of resting glands is clearly seen (**b-d, g**). In parietal cells (**e**) at the base of the glandular tubes of secreting mucosas the staining is also distributed in the whole cytoplasm, except in the region occupied by the secretory canaliculus (*). In neck parietal cells (**f**) the region occupied by the secretory canaliculus is devoid of immunoreactivity (*) and the filamin-like protein concentrates in the canalicular border. The secretory canaliculi of cells at the base of glandular tubes of secreting mucosas (* in **e**) appear poorly developed when compared with those of neck cells (* in **f**). In parietal cells, the filamin-like protein also concentrates in an apical peripheral ring (arrows in **c** and **d**). In zymogen cells the immunoreactivity is restricted to the apical and basolateral borders (arrows in **b** and **g**). Mucous neck cells appear unstained (stars in **f**). Bars: 20 μm (**a**); 10 μm (**b**). Same magnification in **c-g** as in **b**.

lar structures that traverse the cytoplasm (Fig 4b). The rest of the cytoplasm gives a faint diffuse fluorescence.

Secreting basal parietal cells. In parietal cells at the base of the glandular tubes of secreting mucosas, the whole cytoplasm stains rather strongly with FITC-phalloidin. The distribution of the staining is not homogenous but appears arranged in a lacelike pattern (Fig 4c, arrows). A similar distribution of the staining is observed in neck parietal cells (not shown).

Careful examination of parietal cells present in resting and secreting mucosas shows that F-actin also concentrates in the peripheral ring localized at the level of the intercellular junctions (Fig 4b). This is especially evident in isolated cells (Fig 4e).

Zymogen cells. In cells of resting and secreting mucosas the luminal border decorates strongly (Fig 4b,c).

The basolateral cell borders of parietal (Fig 4a-c) and zymogen cells (Fig 4a) stain too with FITC-phalloidin. In sections through the basolateral surface of parietal cells, this cortical actin is seen organized as a fibrillar meshwork (Fig 4d).

Among the glandular tubes, fusiform immunoreactive elements probably corresponding to connective cells or isolated muscle cells are seen (Fig 4a-c).

Remodeling of the canaliculi of parietal cells

Figure 5 (a-c) corresponds to images of parietal cells stained with FITC- phalloidin.

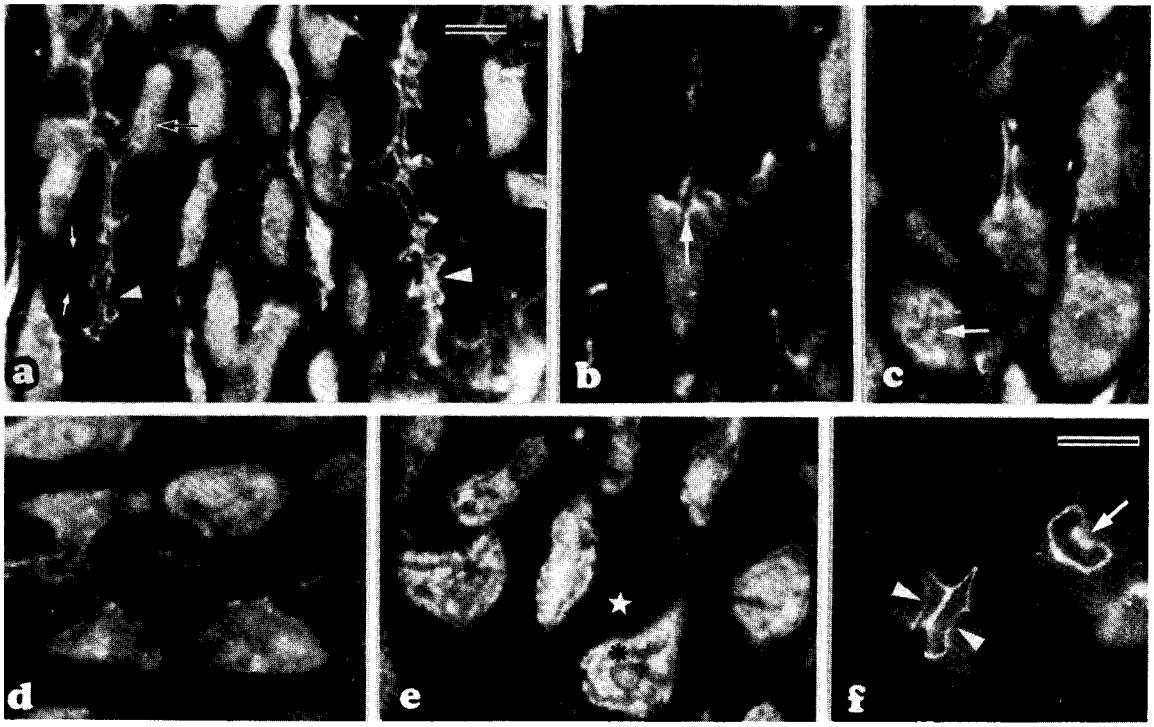

Fig 2. Frozen sections of gastric mucosa (**a-e**) and isolated cells (**f**) stained with antimyosin antibodies. In parietal cells at the base of resting glands (**a-c**), myosin is diffusely distributed. In cells at the base of the glandular tubes of secreting mucosas (**d**) and in neck parietal cells (**e**), myosin concentrates in the cytoplasm adjacent to canaliculi, while the region occupied by the developed secretory canaliculi appears unstained (*). Arrangement of myosin in an apical ring (arrows) is evidenced in sectioned mucosa (**a-c**) and in isolated parietal cells (**f**). In zymogen cells, myosin is concentrated in apical peripheral rings (arrowheads in **a** and **f**) and in basolateral cell borders (small arrows in **a**). Mucous neck cells do not stain (star in **e**). Bars: 10 μm. Same magnification in **b-e** as in **a**.

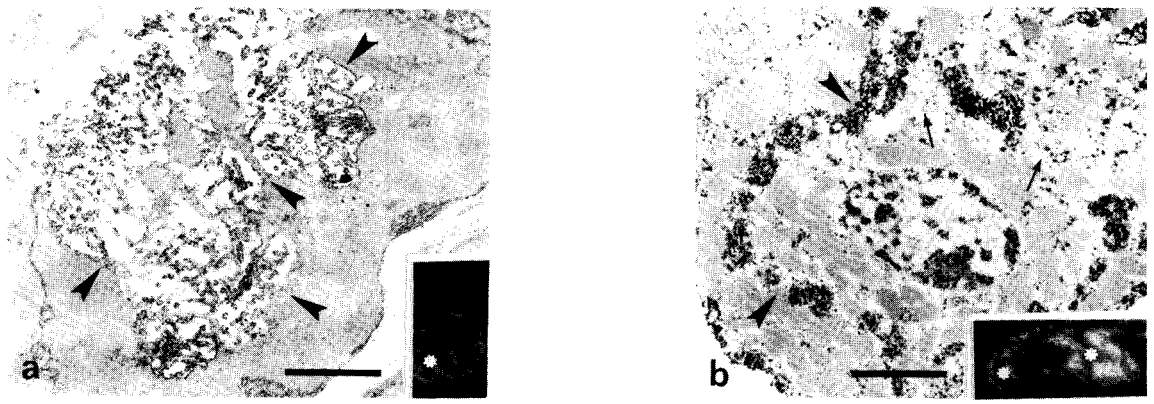

Fig 3. Electron micrographs of parietal cells, after K+-NPPase cytochemical staining of the luminal secretory membranous system. A neck parietal cell (**a**) shows an intracellular canaliculus (*) characterized by an extremely wide lumen. The K^+-NPPase reaction product draws the numerous microvilli of the canalicular surface. Mitochondria are closely associated with the canalicular secretory surface (arrowheads). Inset in **a**, distribution of myosin, detected by immunofluorescence, in a neck parietal cell. A secreting parietal cell at the base of a glandular tube (**b**) shows an intracellular canaliculus with a narrow lumen (*) filled with microvilli intensely stained with the K^+-NPPase reaction product. These microvilli are closely associated with mitochondria (arrowheads) and with some elements of the tubulovesicular system (arrows). Inset in **b**, localization of myosin, detected by immunofluorescence, in a parietal cell at the base of a glandular tube. Nucleus (n). Bars: 1 μm.

Remodeling of the secretory canaliculus is followed by staining the F-actin associated with the canalicular cytoplasmic surface. In cells with a highly developed canaliculus most of the cell volume corresponds to the microvillar extensions of the secretory surface supported by F-actin (d).

In parietal cells stained with antimyosin

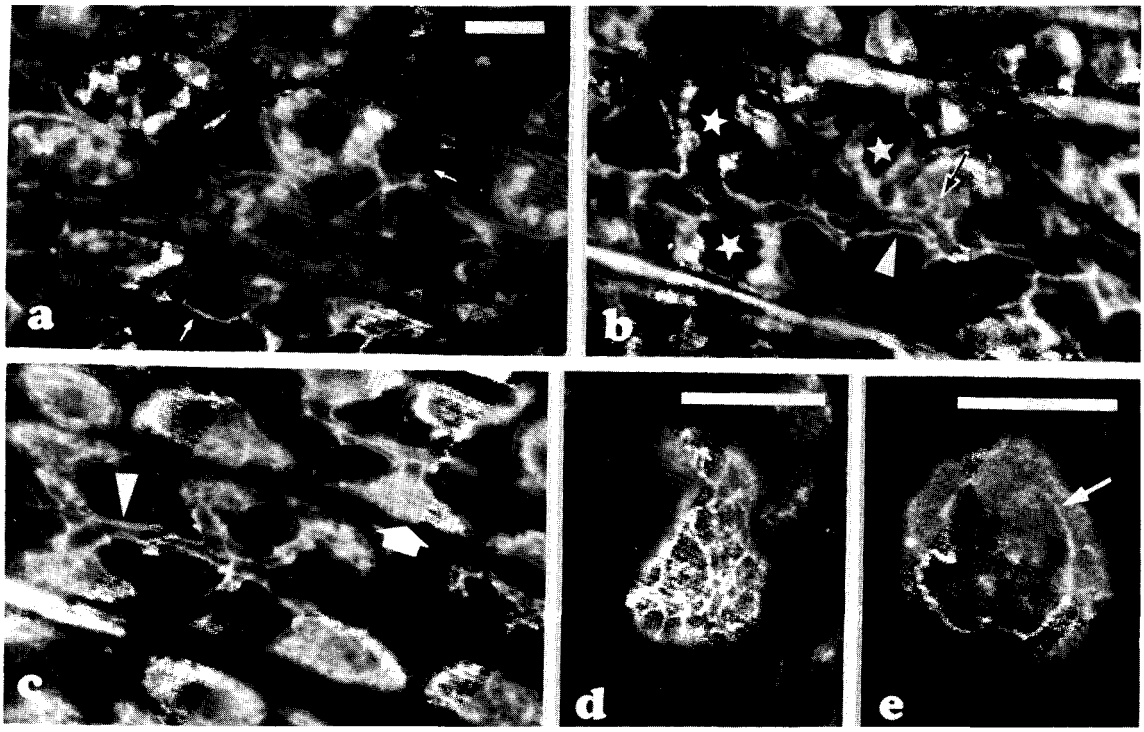

Fig 4. Frozen sections of gastric mucosa (**a-d**) and isolated parietal cell (**e**) stained with FITC-phalloidin. In resting mucosas, F-actin shows a patchy distribution in parietal cells (**a**). In adequately sectioned resting cells, the staining outlines the whole secretory canaliculus (stars in **b**). In parietal cells at the base of glandular tubes of secreting mucosas, the entire cytoplasm frequently appears strongly fluorescent (**c**) and the staining shows a lacelike pattern (arrows in **c**). F-actin of parietal cells also concentrates in apical peripheral rings (arrows in **b** and **e**). In tangential sections through the cortical cytoplasm of these cells, F-actin is seen organized as a fibrillar meshwork (**d**). In zymogen cells, actin concentrates in the basolateral borders (small arrows in **a**) and in rings in the apical periphery of the cells (arrowheads in **b** and **c**). Bars: 20 μm (**a**); 10 μm (**d, e**). Same magnification in **b-c** as in **a**.

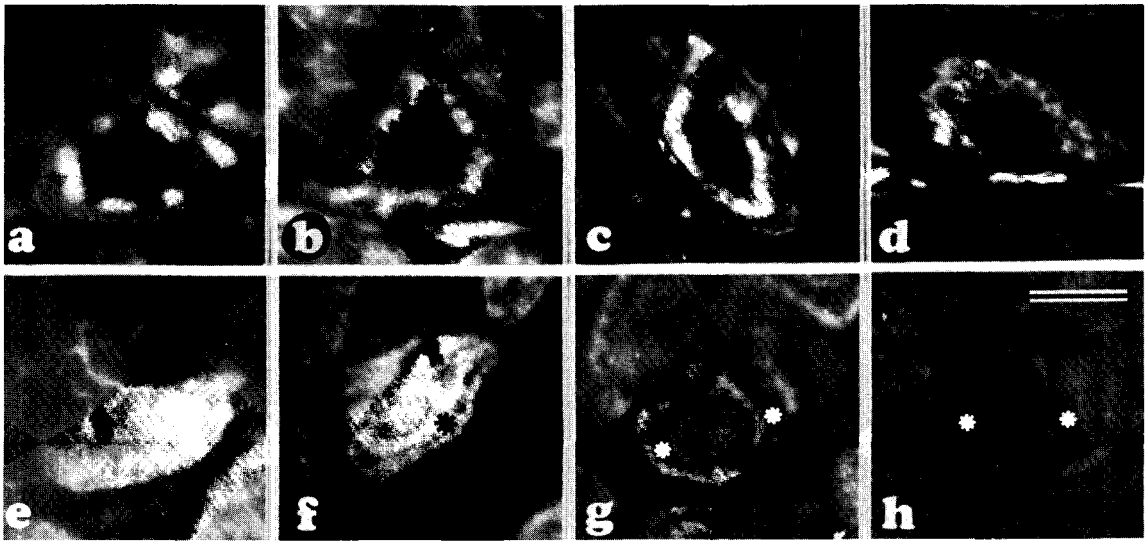

Fig 5. Remodeling of the secretory canaliculi of parietal cells, followed by staining F-actin and myosin. Parietal cells at the base of glandular tubes of resting (**a-b**) and secreting (**c-d**) gastric mucosas stained with FITC-phalloidin. The development of the intracellular canaliculus can be followed easily, because the microvillar projections that fill the lumen contain F-actin. Parietal cells at the base of the glandular tubes of resting (**e**) and secreting (**f**) gastric mucosas stained with antimyosin. Neck parietal cells (**g-h**) stained with antimyosin. The degree of development of the secretory canaliculus is clearly seen, because the region occupied by the secretory canaliculus does not stain (* in **f-h**). Bar: 10 μm. Same magnification in **a-g** as in **h**.

antibodies, canaliculi appear in negative. The changes in the organization of myosin in relation to the extension of the secretory canaliculus is evidenced (Fig 5 e-h).

DISCUSSION

Actin and the actin-associated proteins myosin and the filamin-like protein are concentrated in parietal cells of the rat gastric mucosa. The distribution of F-actin in sections of resting and secreting glands, stained with FITC-phalloidin, was similar to that reported by Mercier *et al* (1989) using antiactin antibodies. In contrast with their results, immunoreactivity of myosin and of a filamin-like protein present in parietal cells was observed when antibodies against the heavy chain of platelet myosin and against chicken smooth muscle filamin were used.

The subcellular distribution of F-actin, myosin and the filamin-like protein in parietal cells, varied in relation to the extension of the secretory canaliculus. This in turn, depended on the HCl secretory state of the cells and on the location of these cells in the glandular tubes of the fundic glands.

Thus, in basal resting cells, characterized by poorly developed canaliculi, myosin and the filamin-like protein distributed diffusely. In secreting basal cells and in neck cells of resting and secreting mucosas, these proteins concentrated in the cytoplasm adjacent to the developed canaliculi, appearing extremely concentrated in neck parietal cells. Morphometric studies carried out by Helander (1976), have shown that in both fasted and nonfasted rats the secretory surface is twice as large in neck parietal cells than in the basal parietal cells. Our results showed that the intracellular canaliculus of rat parietal cells at the neck of the glands is highly developed even in fasted and cimetidine treated rats as shown by ultrastructural studies after cytochemical localization of the proton pump.

The distribution of cytoplasmic actin in parietal cells was also modified in correlation with the development of the secretory canaliculi. The images obtained after staining sections of resting and HCl secreting gastric mucosas with FITC-phalloidin reflect the ultrastructural reorganization of F-actin in parietal cells. The patchy distribution of the fluorescent material in resting cells must correspond to the staining of the subplasmalemmal actin network present beneath the smooth canalicular secretory surface. The notable staining of the whole cytoplasm of active cells is a consequence of the development of an expanded, tortuous secretory canaliculus with numerous microvillar processes supported by F-actin bundles. Concomitant actin polymerization seemed to occur, since the fluorescence pattern of secreting cells showed that the relative concentration of F-actin had significantly increased in relation to that of resting cells. In both resting and secreting parietal cells a small proportion of the F-actin was also diffusely distributed over the cytoplasm.

Therefore, in resting parietal cells F-actin is associated with myosin and the filamin-like protein in the matrix present between mitochondria and among the elements of the tubulovesicular system. In secreting cells, F-actin appears linked with myosin and the filamin-like protein in the cytoplasmic matrix localized beneath the secretory canaliculi of active cells. But, the F-actin that supports the microvillar projections of the secretory canaliculi of active cells is not associated with these proteins since both are clearly excluded from this region.

There is substantial evidence suggesting that the actin cytoskeleton plays a role in the changes in shape undergone by HCl secretory cells (Vial and Garrido, 1976; González *et al*, 1981; Black *et al*, 1982; Wolosin *et al*, 1983; Dabiké *et al*, 1986; Mercier *et al*, 1989; Koenig and Dabiké, 1990). The reorganization of actin, myosin and the filamin-like protein concomitant with the development of the intracellular canaliculus and the translocation of the secretory membranes support this view. The fact that actin and a 250 kDa protein interact in vitro to produce a gel, suggests that the cytoplasmic matrix present between the membranous elements of the tubulovesicular system is formed by short F-actin filaments crosslinked by the filamin-like protein. If this is true, modifications in the interactions between actin, the filamin-like protein and myosin could produce changes in cell matrix

consistency followed by displacement of the tubulovesicular membranes. Since association of cell surface molecules with the cytoskeleton is one of the early consequences of cellular activation of many systems (Carraway and Carraway, 1989), stimuli that induce HCl secretion might lead to the attachment of actin to the membranes of the tubulovesicular system. Approximation of the tubulovesicular membranes could be carried out by the interaction of membrane bound actin with myosin. Solation of the gelled matrix present between the membranes of the tubulovesicular system should precede approximation and fusion. Nucleation for actin filament growth at the plasma membrane mediated by severing and capping proteins (Stossel *et al*, 1985, Pollard and Cooper, 1986; Way and Weeds, 1990; Way *et al*, 1992) could also explain the development of the surface extensions proper of secreting cells.

In active parietal cells, characterized by the numerous microvillar projections that cover the canalicular surface, the distribution of myosin and the filamin-like protein is similar to that in intestinal epithelial cells that possess stationary microvilli. It has been suggested that in these cells myosin and other actin-associated proteins, such as filamin and spectrin (Mooseker, 1985) stabilize the cytoskeleton that supports the microvilli of the brush border. These proteins crosslink actin microfilaments at their base and attach them to the underlying web of intermediate filaments (Hirokawa *et al*, 1982; Mooseker, 1985). The present results show that myosin and the filamin-like protein are also localized at the base of the microvilli proper of secreting parietal cells. Mercier *et al* (1989) have shown that in active parietal cells spectrin concentrates in this region too. It can be speculated that this set of proteins helps anchor the bundles of actin microfilaments inside the microvilli to the underlying system of tonofilaments described in parietal cells (Dabiké and Koenig, 1983). At variance, it has been shown that the 80 kDa phosphoprotein ezrin is a constituent of the microvilli in both intestinal (Bretscher, 1983) and parietal secreting cells (Hanzel *et al*, 1989). Taken together, these results suggest that although the origin, function and stability of the microvilli of the brush border of intestinal cells and the microvillar projections proper of acid secreting cells are different, their cytoskeletal organization is similar.

The cytoskeletal ring formed by F-actin, myosin, and the filamin-like protein in both parietal and zymogen cells has been previously described in amphibian and avian oxyntic cells (Dabiké *et al*, 1986; Koenig and Dabiké, 1990) and in non-glandular polarized epithelial cells (Bretscher and Weber, 1978; Hirokawa *et al*, 1983; Klotz *et al*, 1986; Mooseker, 1985; Owaribe and Matsudaira, 1982). A structural role for this ring in preserving and stabilizing the cellular pattern of simple epithelia has been suggested (Volk and Geiger, 1986). In addition, the concentration of cytoskeletal proteins in the luminal region seems to constitute a structural framework for the luminal pole of the cells (Koenig and Dabiké, 1990; Tsukada and Phillips, 1993).

Thus, our observations suggest that there may be two subsets of actin, myosin and the filamin-like protein in parietal cells. The first forming part of a fixed cytoskeletal ring present at the level of the intercellular junctions; the other taking part in the cyclical translocation of membranes and/or in the stabilization of the cytoskeleton of the microvillar projections at the secretory surface.

On the other hand, during the last few years growing evidence points to the participation of a single headed myosin known as myosin-I in the movement of membranes (Warrick and Spudich, 1987; Pollard *et al*, 1991). At present, there is contradictory evidence related to the cross-reactivity of antibodies against muscle and nonmuscle myosins (collectively known as myosin II) and myosin I (Carboni *et al*, 1988; Drenckhahn and Dermietzel, 1988; Carraway and Carraway, 1989; Mooseker and Coleman, 1989; Pollard *et al*, 1991). This might be due to the presence of multiple myosin I isoforms (Pollard *et al*, 1991). Although there is one report of noncross-reactivity between brush border myosin I and polyclonal antibodies against human platelet myosin (Mooseker and Coleman, 1989), there is a possibility that at least part of the

immunoreactivity of parietal cells could be ascribed to the presence of myosin I in these cells.

The protein that cross-reacts with anti-filamin antibodies seems to correspond to the gelling HMW actin-binding protein described in nonmuscle cells. Antibodies against filamin cross-react with the HMW actin-binding protein of nonmuscle cells. In addition, homogenate supernatants of rat gastric glands and actin gels, contain a polypeptide with a molecular weight equivalent to that of the HMW actin-binding protein (our own unpublished results). A similar actin gelling protein is also diffusely distributed in the cytoplasm of amphibian (Dabiké *et al*, 1986) and avian (Koenig and Dabiké, 1990) oxyntic cells. Probably one of the roles of this protein is to cross-link and stabilize actin filaments giving strength to the cellular matrix (Cano *et al*, 1992).

Finally, F-actin, myosin, and the filamin-like protein present in the basolateral cell borders of parietal and zymogen cells probably form part of a thin cortical layer that has also been described in other cells (Bray *et al*, 1986; Carraway and Carraway, 1989). The function of this peripheral actin network is not clear at present.

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REFERENCES

BLACK JA, FORTE TM, FORTE JG (1982) The effects of microfilament disrupting agents on HCl secretion and ultrastructure of piglet gastric oxyntic cells. Gastroenterology 83: 595-604

BRAY D, HEATH J, MOSS D (1986) The membrane-associated "cortex" of animal cells: Its structure and mechanical properties. J Cell Sci, Suppl 4: 71-78

BRETSCHER A (1983) Purification of an 80,000-dalton protein that is a component of the isolated microvillus cytoskeleton, and its localization in nonmuscle cells. J Cell Biol 97: 425-432

BRETSCHER A, WEBER K (1978) Localization of actin and microfilament-associated proteins in the microvilli and terminal web of intestinal brush-border by immunofluorescence microscopy. J Cell Biol 79: 839-845

CANO M, CASSIMERIS L, FECHHEIMER M, ZIGMOND SH (1992) Mechanism responsible for F-actin stabilization after lysis of polymorphonuclear leukocytes. J Cell Biol 116: 1123-1134

CARBONI JM, CONZELMAN KA, ADAMS R, KAISER DA, POLLARD T, MOOSEKER MS (1988) Structural and immunological characterization of the myosin-like 110-kD subunit of the intestinal microvillar 110K-calmodulin complex: Evidence for discrete myosin head and calmodulin-binding domains. J Cell Biol 107: 1749-1757

CARRAWAY KL, CARRAWAY CAC (1989) Membrane-cytoskeleton interactions in animal cells. Biochim Biophys Acta 988: 147-171

DABIKE M, KOENIG CS (1983) Intermediate filaments of the cytoskeleton in glandular cells of the rat gastric mucosa: Immunofluorescence and electron microscopy study. Anat Rec 207: 297-308

DABIKE M, MUNIZAGA A, KOENIG CS (1986) Filamin and myosin are present in the secretory pole of amphibian oxyntic cells. An immunofluorescence study. Eur J Cell Biol 40: 185-194

DRENCKHAHN D, DERMIETZEL R (1988) Organization of the actin filament cytoskeleton in the intestinal brush border: A quantitative and qualitative immunoelectron microscope study. J Cell Biol 107: 1037-1048

GONZALEZ A, GARRIDO J, VIAL JD (1981) Epidermal growth factor inhibits cytoskeleton-related changes in the surface of parietal cells. J Cell Biol 88: 108-114

HANZEL DK, URUSHIDANI T, USINGER WR, SMOLKA A, FORTE JG (1989) Immunological localization of an 80-kD phosphoprotein to the apical membrane of gastric parietal cells. Am J Physiol 256: G1082-G1089

HANZEL D, REGGIO H, BRETSCHER A, FORTE JG, MANGEAT P (1991) The secretion-stimulated 80 K phosphoprotein of parietal cells is ezrin, and has properties of a membrane cytoskeletal linker in the induced apical microvilli. EMBO J 10: 2363-2373

HELANDER HF (1976) Stereological changes in rat parietal cells after vagotomy and antrectomy. Gastroenterology 71: 1010-1018

HIROKAWA N, TILNEY LG, FUJIWARA K, HEUSER JE (1982) Organization of actin, myosin, and intermediate filaments in the brush border of intestinal epithelial cells. J Cell Biol 94: 425-443

HIROKAWA N, KELLER TCS, CHASAN R, MOOSEKER MS (1983) Mechanism of brush border contractility studied by the quick-freeze, deep-etch method. J Cell Biol 96: 1325-1336

KASBEKAR DK, GORDON GS (1979) Effects of colchicine and vinblastine on in vitro gastric secretion. Am J Physiol 236: E550-E555

KLOTZ C, BORDES N, LAINE MC, SANDOZ D, BORNENS M (1986) Myosin at the apical pole of ciliated epithelial cells as revealed by a monoclonal antibody. J Cell Biol 103: 613-619

KOENIG CS (1984) Redistribution of gastric K-NPPase in vertebrate oxyntic cells in relation to hydrochloric acid secretion: A cytochemical study. Anat Rec 210: 583-596

KOENIG CS, DABIKE M (1990) Redistribution of membranes and cytoskeletal proteins in chicken oxyntic cells during the HCl secretory cycle: Ultrastructural and immunofluorescence study. Anat Rec 228: 111-122

MERCIER F, REGGIO H, DEVILLIERS G, BATAILLE D, MANGEAT P (1989) Membrane-cytoskeleton dynamics in rat parietal cells: Mobilization of actin and spectrin upon stimulation of gastric acid secretion. J Cell Biol 108: 441-453

MOOSEKER MS (1985) Organization, chemistry and assembly of the cytoskeletal apparatus of the intestinal brush border. Annu Rev Cell Biol 1: 209-241

MOOSEKER M, COLEMAN T R (1989) The 110-kD

protein-calmodulin complex of the intestinal microvillus (brush border myosin I) is a mechano-enzyme. J Cell Biol 108: 2395-2400

OWARIBE K, MATSUDAIRA H (1982) Isolation and characterization of circumferential microfilament bundles from retinal pigmented epithelial cells. J Cell Biol 95: 310-315

POLLARD TD, COOPER JA (1986) Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. Annu Rev Biochem 55: 987-1035

POLLARD TD, DOBERSTEIN SK, ZOT HG (1991) Myosin-I. Annu Rev Physiol 53: 653-681

SOROKA CJ, CHEW CS, HANZEL DK, SMOLKA A, MODLIN IM, GOLDENRING JR (1993) Characterization of membrane and cytoskeletal compartments in cultured parietal cells: immunofluorescence and confocal microscopic examination. Eur J Cell Biol 60: 76-87

STENDAHL OI, HARTWIG JH, BROTSCHI EA, STOSSEL, TP (1980) Distribution of actin-binding protein and myosin during locomotion and phagocytosis. J Cell Biol 84: 215-224

STOSSEL TP, CHAPONNIER C, EZZELL RM, HARTWIG JH, JANMEY PA, KWIATKOWSKI DJ, LIND SE, SMITH DB, SOUTHWICK SF, YIN HL, ZANER KS (1985) Nonmuscle actin-binding proteins. Annu Rev Cell Biol 1: 353-402

TSUKADA N, PHILLIPS MJ (1993) Bile canalicular contraction is coincident with reorganization of pericanalicular filaments and co-localization of actin and myosin II. J Histochem Cytochem 41: 353-363

VALERIUS NH, STENDAHL O, HARTWIG JH, STOSSEL TP (1981) Distribution of actin binding protein and myosin in polymorphonuclear leukocytes during locomotion and phagocytosis. Cell 24: 195-202

VIAL JD, ORREGO H (1960) Electron microscopic observations on the fine structure of parietal cells. J Biophys Biochem Cytol. 7: 367-372

VIAL JD, GARRIDO J (1976) Actin-like filaments and membrane rearrangement in oxyntic cells. Proc Natl Acad Sci USA 73: 4032-4036

VIAL JD, GARRIDO J, GONZALEZ A (1985) The early changes of parietal cell structure in the course of secretory activity in the rat. Am J Anat 172: 291-306

VOLK T, GEIGER B (1986) A-CAM: A 135-kD receptor of intercellular adhaerens junctions. II. Antibody-mediated modulation of junction formation. J Cell Biol 103: 1451-1464

WARRICK HM, SPUDICH JA (1987) Myosin structure and function in cell motility. Annu Rev Cell Biol 3: 379-421

WAY M, WEEDS A (1990) Cytoskeletal ups and downs. Nature: 344: 292- 294

WAY M, POPE B, WEEDS AG (1992) Are the conserved sequences in segment 1 of gelsolin important for binding actin. J Cell Biol 116: 1135-1143

WHITNEY AB, OKAMOTO CT, HANZEL DK, FORTE JG (1990) Epidermal growth factor (EGF) stimulates phosphorylation of an 80 kD protein at the apical surface parietal cells. FASEB J 4: A488

WOLOSIN JM, OKAMOTO C, FORTE T, FORTE J (1983) Actin and associated proteins in gastric epithelial cells. Biochim Biophys Acta 761: 171-182.