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The Golgi apparatus of goblet cells in the mouse descending colon: Three-dimensional visualization using a confocal laser scanning microscope

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Abstract

The three-dimensional structure of the Golgi apparatus was studied in goblet cells in lectin-stained sections of the mouse descending colon by using a confocal laser scanning microscope. In the lower part of the crypt, the Golgi apparatus formed a dome- or globe-like structure in the supranuclear region. The wall of the dome or the globe had some holes, one of which usually faced toward the nucleus and others toward the apical cytoplasm. Mucous granules seemed to be initially released into the interior of the dome and transported toward the apical cytoplasm through the holes. In the upper part of the crypt, on the other hand, the Golgi apparatus formed a cup- or funnel-like structure with a larger opening toward the cell apex and a smaller opening toward the nucleus. A large mass of mucous granules occupied the inside of the cup to the apical cytoplasm. It is thought that the accumulation of mucous granules enlarges holes at the ceiling of the dome to form a large opening, which makes the configuration of the Golgi apparatus cup-shaped.

Introduction

The three-dimensional structure of the Golgi apparatus has been studied by using conventional and high voltage electron microscopes. In protein-secreting exocrine cells, it has been generally considered that the Golgi apparatus is in the supranuclear region: the convex side of the stacks faces toward the nucleus, and the concave side toward a group of secretory granules (Beans 1968; Noda and Ogawa 1984; Rambourg et al. 1987; Yamashina 1995). We recently proposed a new three-dimensional model of the Golgi apparatus in the mouse Brunner's gland cell, in which the Golgi apparatus is a dome-like structure with its convex cis-side facing toward the cell apex and its concave trans-side toward the nucleus (Suzaki and Kataoka 1999).

Concerning the Golgi apparatus of a goblet cell, Neutra and Leblond (1966) schematically drew a U-shaped Golgi apparatus with the convex bottom facing the nucleus and the inside of the U being occupied by mucous granules that continue to the apical cytoplasm. Attempts have been made to three-dimensionally reconstruct the Golgi apparatus of porcine tracheal goblet cells (Adler et al. 1982) and rabbit colonic goblet cells (Radwan et al. 1990) by using electron microscopy. It was mentioned in the former report that several separate Golgi apparatuses were present in the supranuclear region. More precise observation was carried out in the latter study, and changes in cell shapes as well as three-dimensional structures of the Golgi apparatus were reported. However, three-dimensional reconstruction from electron micrographs

is too time-consuming even with the aid of a high performance computer, and examination has therefore been limited to only some units of the extensive Golgi apparatus in a cell or the Golgi apparatus in a small number of cells.

Lectins make good markers of the Golgi stacks and mucous granules in cells actively secreting O-glycosylated glycoproteins, such as goblet cells and Brunner's gland cells (Roth 1984; Tsuyama et al. 1986; Ellinger and Pavelka 1988, 1992; Pavelka and Ellinger 1991; Suzaki and Kataoka 1992). Lectins specific to α -N-acetylgalactosamine (GalNAc), such as Glycine max agglutinin (SBA), Helix aspersa agglutinin (HAA), Helix pomatia agglutinin (HPA) and Maclura pomifera agglutinin (MPA), usually bind to cis-stacks of the Golgi apparatus, whereas Ulex europaeus agglutinin I (UEA I) specific to α -fucose, a terminal sugar residue, binds to trans-stacks and to mucous granules. In addition, lectins specific to α -GalNAc bind to trans-stacks and mucous granules in some cells that produce glycoproteins with α -GalNAc as a terminal sugar residue (Roth 1984; Tsuyama et al. 1986; Pavelka and Ellinger 1991; Suzaki and Kataoka 1992). The results of our preliminary electron microscopic cytochemistry on goblet cells of the ICR mouse colon agreed well with these previous results. HAA conjugated with colloidal gold bound to cis-stacks of the Golgi apparatus, and in addition, trans-stacks to mucous granules of a few goblet cells were weakly labeled.

A confocal laser scanning microscope (CLSM) is a versatile tool for stereoscopy.

With microscopic sections stained with suitable fluorochrome-labeled lectins, confocal laser scanning microscopy can provide three-dimensional images of the Golgi apparatus. Although electron microscopy can offer better resolution, the images obtained by using a CLSM give satisfactory resolution for studying the overall configuration of the Golgi apparatus in a relatively large number of cells distributed in a relatively large area of tissue. In the present study, we examined the three-dimensional configuration of the Golgi apparatus in goblet cells of the mouse descending colon by using a CLSM after staining with several α -GalNAc-specific lectins and/or UEA I.

Materials and methods

Chemicals

The lectins used were HAA, MPA, SBA and UEA I. FITC- or Texas red-labeled lectins were purchased from EY Laboratories (San Mateo, CA, USA). According to the manufacturer, HAA binds to α -GalNAc and α -N-acetylglucosamine, MPA to α -GalNAc, α -galactose and galactose(β 1,3)GalNAc, SBA to α - and β -GalNAc and GalNAc(α 1,3)galactose, and UEA I to α -fucose and fucose(α 1,2)galactose(β 1,4)N-acetylglucosamine, respectively.

Haptenic sugars used for controls were GalNAc for HAA, MPA and SBA, and fucose for UEA I.

Animals and preparation of tissues

Adult ICR male mice (2 months old), fed ad libitum, were used for this study. All procedures were performed in accordance with the Guidelines for Use of Laboratory Animals of Hiroshima University School of Medicine. Under sodium pentobarbital anesthesia, they were perfused from the left ventricle of the heart with 4% formaldehyde in 0.1 M phosphate buffer containing 2.5% sucrose (pH 7.2), and the descending colon was dissected into small pieces. The tissues were fixed by immersion in the same fixative for 6 hr at 4℃ and soaked in 0.1 M phosphate buffer (pH 7.2) overnight. They were dehydrated in a graded series of ethanol and embedded in paraffin. Sections (5 μ m or 15 μ m in thickness) were cut and mounted on a gelatin-coated glass slide. The sections were deparaffinized and hydrated for lectin histochemistry.

Lectin histochemistry

All incubations were performed at room temperature for lectin histochemistry. The sections were treated with 10 mM phosphate-buffered saline containing 0.5 mM CaCl2 (pH 7.2) (PBS) for 5 min and then with 1% bovine serum albumin in PBS for 10 min, followed by a brief wash with PBS.

The FITC- or Texas red-labeled lectins were diluted with PBS at a concentration of 0.5mg/ml. For single staining, the sections were incubated with either FITC-labeled HAA, FITC-labeled MPA, FITC-labeled SBA or FITC-labeled UEA I for 50 min. For

double staining, the sections were incubated with Texas red-labeled HAA for 50 min, washed with PBS, and subsequently incubated with FITC-labeled UEA-I for 50 min. After washing in PBS, the sections were mounted with 50% glycerine in PBS with anti-fading reagents.

For controls of the lectin staining, each FITC- or Texas red-labeled lectin solution was incubated with 0.2 M of the respective haptenic sugar for 15 min, and then this solution was applied to control sections.

Microscopy and three-dimensional reconstruction

The 5 μ m-thick sections were examined under a conventional epifluorescence microscope, Nikon Optiphot XF-EF (Nikon, Tokyo, Japan), while 15 μ m-thick sections were examined under a CLSM, Carl Zeiss LSM-410 (Jena, Germany). In the latter case, 20-27 optically sectioned serial images (0.5 μ m-steps) were collected and were reconstructed to obtain a whole configuration of the Golgi apparatus. In the sections stained with a single lectin, the three-dimensional architecture was examined using either stereo-pairs or images colored according to depth. In the double-stained sections, the three-dimensional images were examined using stereo-pairs.

Results

Observations by using a conventional fluorescence microscope

When we examined the colonic mucosa stained with FITC-labeled HAA, the Golgi apparatus was seen in the supranuclear region of cells lining the mucosal surface and crypts (Fig. 1A). Brightly fluorescent circular or cup-shaped profiles of the Golgi apparatuses were seen in cells located in the lower and middle parts of the crypt. In addition, the supranuclear to apical cytoplasm of a few cells had weak diffuse fluorescence. The Golgi apparatuses of goblet cells fluoresced weakly in the upper part of the crypt and on the mucosal surface, and their profiles were cup-shaped with a very large opening toward the cell apex. The supranuclear region of columnar cells showed faint fluorescence in the upper part of the crypt, and the fluorescence became brighter at the mucosal surface.

A similar result was obtained when FITC-labeled SBA or FITC-labeled MPA was applied instead of FITC-labeled HAA.

When a section was stained with FITC-labeled UEA I, mucous granules of goblet cells were seen (Fig. 1B). In the lower part of the crypt, the fluorescent granules accumulated into two masses, a supranuclear mass and an apical mass. On the other hand, in goblet cells in the middle to upper part of the crypt, the granules accumulated into a large mass occupying the supranuclear to the apical cytoplasm.

None of the control sections showed fluorescence, and background staining was virtually absent.

Observations by using a CLSM

The Golgi apparatuses stained with FITC-labeled HAA, MPA and SBA were similar in shape. When the whole configuration of the Golgi apparatus was obtained by reconstructing 20-27 optically sectioned serial images, the Golgi apparatus looked like a fluorescent dome (Fig. 2A) or a globe (Fig. 3A) in the supranuclear region in cells located in the lower part of the crypt. The wall of the dome or globe had some holes, one of which usually faced toward the nucleus. The convex face of the dome or globe, which also had some holes, faced toward the cell apex. As cells reached the middle level of the crypt, a hole at the top of the convex wall was enlarged to form a large opening toward the cell apex (Fig. 3B). In the upper part of the crypt, the Golgi apparatus looked like a funnel or a cup with a smaller opening toward the nucleus and a larger opening toward the cell apex (Fig. 2B, 3C). Some holes were also seen in the lateral wall of the cup (Fig. 2B).

By double staining with Texas red-labeled HAA and FITC-labeled UEA I, the Golgi apparatuses were seen in red (Fig. 4, 5). On the other hand, the color of mucous granules varied from green to orange depending on the relative intensities of stainings with FITC and Texas red (Fig. 4, 5). The fluorescence of both the supranuclear and apical masses of granules exhibited the same color in an individual cell. Mucous granules in cells located in the upper part of the crypt were always green (Fig. 5C).

When an image of the Golgi apparatus was reconstructed from a few optical

sections and examined from the lateral side of the cell, the Golgi apparatus appeared dome-like and a mass of mucous granules was enclosed in the interior space of the dome above the nucleus (Fig. 4). Some mucous granules were seen just passing through the holes of the red-stained dome.

The whole configuration of the Golgi apparatus was obtained by reconstructing 20-27 optically sectioned serial images of the doubly stained tissue (Fig. 5). In the cells located in the lower part of the crypt, the Golgi apparatus appeared as a red cage enclosing a mass of granules in the supranuclear region (Fig. 5A). The cage usually had an opening toward the nucleus and another toward the cell apex. The latter was enlarged as cells reached the middle part of the crypt (Fig. 5B). Almost all Golgi apparatuses seen in the lower and middle parts of the crypt enclosed a UEA I-stained mass of granules, and a Golgi apparatus without associating fluorescent granules was rarely seen.

In the upper part of the crypt, double staining with Texas red-labeled HAA and FITC-labeled UEA I revealed a mass of green mucous granules filling the red cup to the apical cytoplasm (Fig. 5C).

Discussion

Vacuolated-columnar and goblet cells are two main populations in the epithelium of the

mouse descending colon (Chang and Leblond 1971). They are formed in the lower part of the crypt and migrate toward the mucosal surface. Vacuolated cells lose the mucous granules at the upper third level of the crypt to become columnar cells.

Although both vacuolated and goblet cells secrete O-glycosylated glycoproteins, UEA I binds to mucous granules of only goblet cells in the descending colon of ICR mice (Chen et al. 1993). In the present study, almost all Golgi apparatuses associated UEA I-positive mucous granules in the lower two-thirds of the crypt. Thus, we consider that the Golgi apparatuses analyzed in the present study belong to goblet cells but not to vacuolated cells.

In O-glycosylated glycoproteins, sugar chains are linked to hydroxyl groups of serine or threonine residues of the core protein by α -GalNAc (Neutra and Forstner 1987). It has generally been agreed that lectins specific to α -GalNAc bind to cis-stacks of the Golgi apparatus in cells actively secreting O-glycosylated glycoproteins (Roth 1984; Tsuyama et al. 1986; Pavelka and Ellinger 1991; Suzaki and Kataoka 1992). Our preliminary study also agreed with this in goblet cells of the mouse descending colon. Ellinger and Pavelka (1992), who studied lectin binding of goblet cells in the rat proximal and distal colon, reported that dilated cisterns of the rough endoplasmic reticulum along with cis-Golgi stacks bound lectins specific to α -GalNAc. The similar findings were also obtained in the rat proximal colon by Tsuyama et al. (1984). In the mouse descending colon, however, such dilated cisterns have not been found by

electron microscopic examination.

It has been well established that UEA I, a lectin specific to a terminal sugar residue, α-fucose, binds to trans-stacks and mucous granules (Tsuyama et al. 1986; Ellinger and Pavelka 1988, 1992; Pavelka and Ellinger 1991; Suzaki and Kataoka 1992).

From these facts, the two fluorescent structures analyzed in the present study are considered to be cis-stacks of the Golgi apparatus, and trans-stacks to mucous granules, respectively, in goblet cells.

The Golgi apparatus in goblet cells was reported to be U-or cup-shaped (Neutra and Leblond 1966). Radwan et al. (1990) studied the three-dimensional organization of goblet cells in the rabbit sigmoid colon. According to their report, goblet cells change their shapes along the longitudinal axis of the crypt, and so is the Golgi apparatus. The histology of the rabbit sigmoid colon is different from those of the mouse descending colon, though. In the mouse descending colon, goblet cells are rather slender in the lower part of the crypt, and they swell to become typical goblet-shaped with increasing mucous granules in the middle to upper part of the crypt (Chang and Leblond 1971, Chen and Kataoka 1993). The present study has shown that Golgi apparatuses of goblet cells look like a dome or globe with some holes in the lower part of the crypt and change their configuration to cup-shaped with an increasing number of mucous granules in the middle to upper part of the crypt.

The lower part of the crypt is a site of cell proliferation and differentiation in the

mouse descending colon, and undifferentiated and immature cells reside in this area (Chang and Leblond 1971). However, cells having a large dome-like Golgi apparatus, shown in the present study, are not considered immature but young goblet cells engaging in active synthesis of O-glycosylated glycoproteins, since the Golgi apparatus is intensely labeled with the lectins specific to α -GalNAc, the linkage to the core protein. Electron microscopically, most goblet cells in the lower part of the crypt contain abundant profiles of the rough endoplasmic reticulum and a large Golgi apparatus that is often seen dome-like.

The intensity of staining with the FITC-labeled lectins specific to α -GalNAc was weaker in goblet cells located in the upper half of the crypt in the present study. This suggests that the activity of O-glycosylated glycoprotein synthesis decreased in these cells. The upper opening of the Golgi cup seems to be further enlarged by accumulation of mucous granules. It is also possible that the opening is artificially enlarged by tissue preparation, since mucous granules tend to swell by hydration during tissue preparation. We consider that such artificial change does not alter the basic architecture of the Golgi apparatus but influence the size of its holes as well as the size of mucous granules.

In conclusion, the present study suggests that (1) the Golgi apparatus is fundamentally a dome-like structure in the supranuclear region of the goblet cell, (2) mucous granules are first released into the interior of the dome and then transported

toward the apical cytoplasm through holes in the dome, and (3) the Golgi apparatus changes its configuration to U- or cup-shaped with the accumulation of mucous granules.

A dome-like configuration of the Golgi apparatus is not limited to goblet cells. A similar dome-like Golgi apparatus has been demonstrated in Brunner's gland cells of the mouse duodenum, in which the holes in the Golgi dome were much smaller, probably because of the smaller size of secretory granules (Suzaki and Kataoka 1999). We assume that the Golgi apparatus is basically dome-like in many types of protein-secreting exocrine cells, in which holes of the Golgi dome may change their size to give different configulation of the apparatus depending on different secretory states of a cell or on different types of cells. We briefly reported that this might be the case of surface mucous cells, mucous neck cells and chief cells in the gastric fundic mucosa (Kataoka et al. 1999).

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Legends for Figures

Fig. 1 Conventional fluorescence micrographs of the mouse descending colon. **(A)** After staining with FITC-labeled HAA, the Golgi apparatuses of the cells in the lower half of the crypts are brightly fluorescent and have circular to cup-shaped profiles. The whole cytoplasm of some cells shows weak fluorescence (arrow). The fluorescence of the Golgi apparatuses is rather weak in cells in the upper part of the crypt and on the mucosal surface. They are large cup-shaped in goblet cells and dot-like in columnar cells. **(B)** Mucous granules of goblet cells are stained with FITC-labeled UEA I. They are separated into two groups, a supranuclear mass and an apical mass in a cell in the lower part of the crypt (small arrows) . As cells reach the middle level of the crypt, the space separating the two masses become obscure (large arrow). Bar =20 μ m

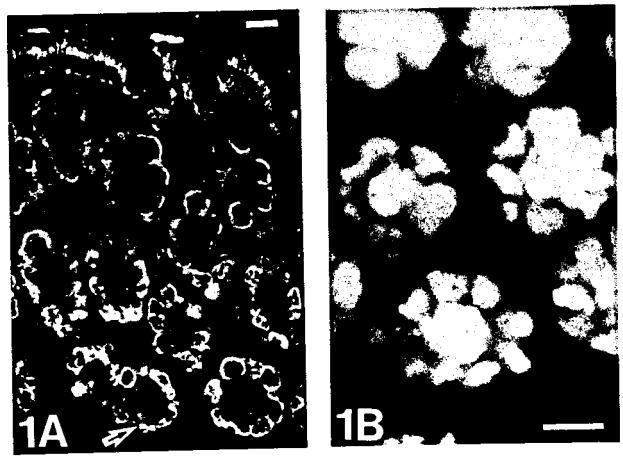
Fig. 2 The whole configuration of the Golgi apparatus was reconstructed from 22 (**A**) and 27 (**B**) optical sections after staining with FITC-labeled SBA (**A**) and FITC-labeled MPA (**B**), respectively. **(A)** In the lower part of the crypt, the Golgi apparatus is dome-like with a large opening facing toward the nucleus (n), and there are some holes (arrows) in the ceiling of the dome. **(B)** The lateral view of two Golgi apparatuses in the upper part of the crypt. They are cup-shaped with an opening toward the nucleus (n) and another large opening toward the apical cytoplasm (a). Bar = 5 μ m

Fig. 3A-C Stereo-pairs of Golgi apparatuses reconstructed from 23 (**A**), 21 (**B**), and 24 (**C**) optical sections after the staining with FITC-labeled SBA (**A**), FITC-labeled HAA (**B**), and FITC-labeled HAA (**C**), respectively. The longitudinal axis of the crypt roughly corresponds to the y-axis of these pictures. In the lower part of the crypt, the Golgi apparatus looks like a globe with some holes (**A**). As cells reach the middle level, a hole in the ceiling of the dome is enlarged (arrows) (**B**). In the upper part of the crypt, a cup-shaped Golgi apparatus has a small opening toward the nucleus (arrowheads) and a large opening toward the apical cytoplasm (a) that is swollen by an accumulation of mucous granules(**C**). n nucleus. Bar = 10 μ m

Fig. 4 A lateral view of goblet cells was reconstructed from 7 optical sections in the lower part of the crypt after staining with Texas red-labeled HAA and FITC-labeled UEA I. The Golgi apparatus is seen as a red dome over the nucleus (n). Mucous granules are either accumulated inside the dome, just passing through the wall of the dome (arrow), or accumulated in the apical cytoplasm (a). Bar = 2.5 μ m

Fig. 5**A-C** The pictures were reconstructed from 21 (**A**), 22 (**B**) and 25 (**C**) optical sections after staining with Texas red-labeled HAA and FITC-labeled UEA I. The Golgi apparatus looks like a red cage in the lower part of the crypt (**A**) and at a level just above it (**B**). The cage has an opening toward the nucleus (n) and another toward the

apical cytoplasm (arrows). Although the color of mucous granules varies from green to orange, they exhibited almost the same color in a cell (arrowheads). The Fig. **5C** shows the upper part of two longitudinally oriented crypts. The cup-shaped Golgi apparatus has a large opening toward the apical cytoplasm, which contains accumulated mucous granules. Bar = 10 μ m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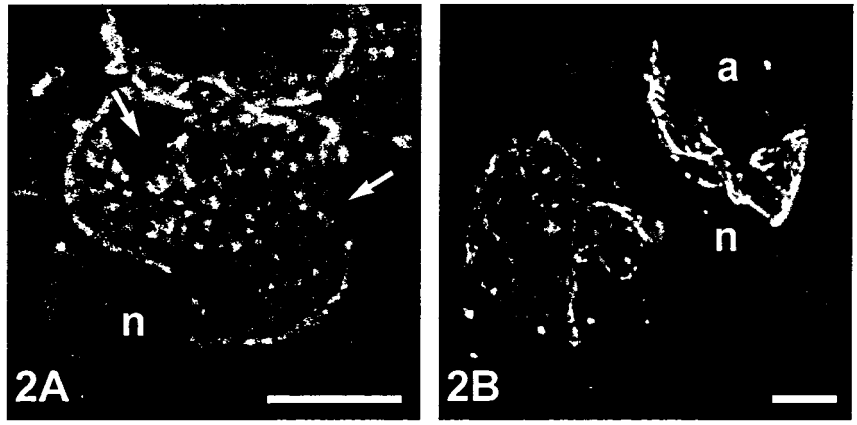
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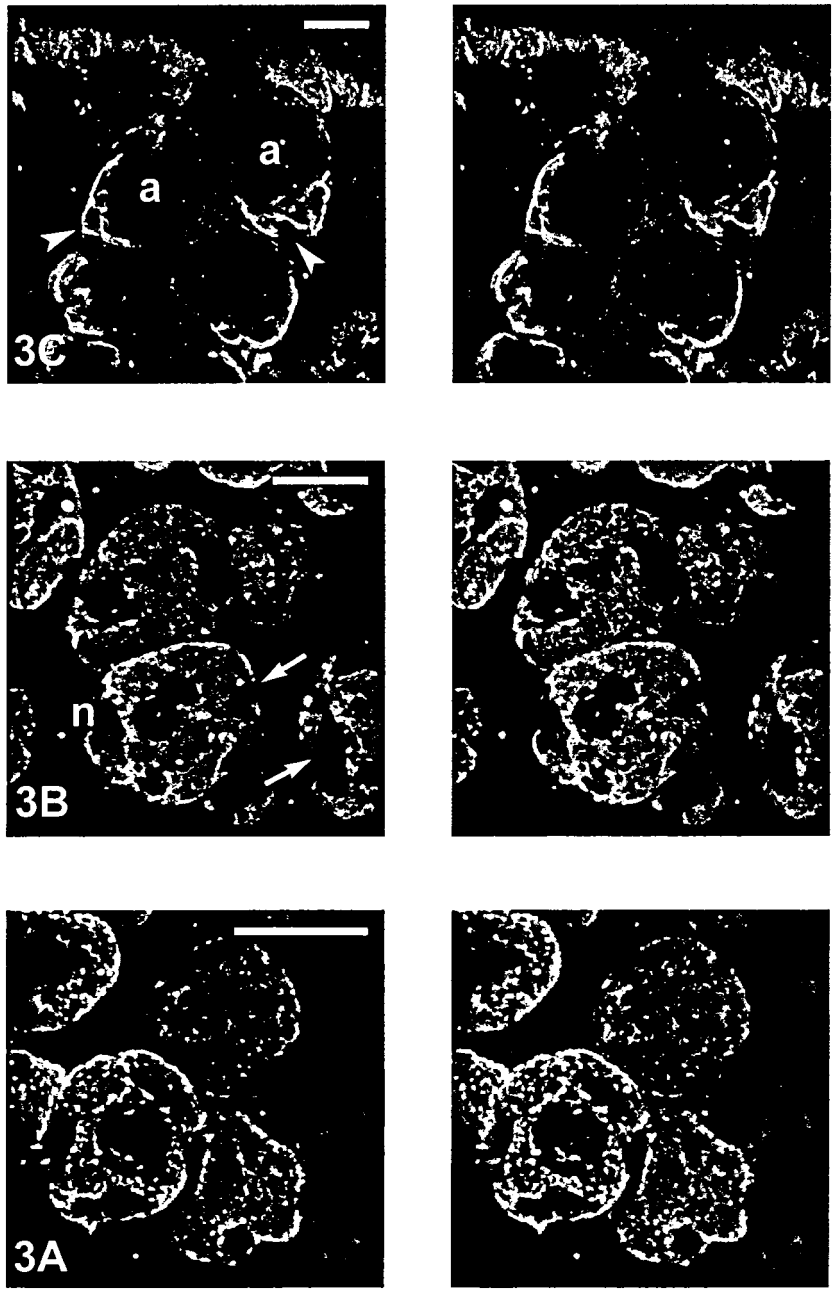
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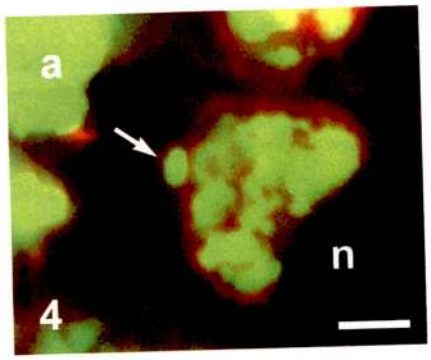
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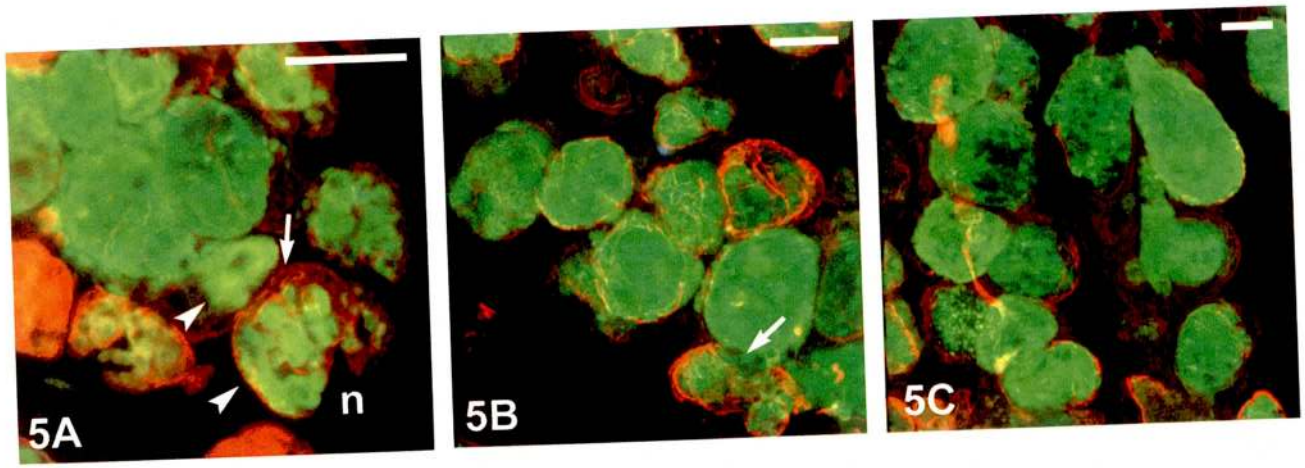
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