Proteolytic Processing of Sulfated Secretogranin II in the trans-Golgi Network of GH3B6 Prolactin Cells*

Laurent Muller‡, Alain Barret, Renée Picart, and Claude Tougard

From the Groupe de Biologie de la Cellule Neuroendocrine, CNRS URA 1115 and INSERM U 36, Collège de France, 11, Place Marcellin Berthelot, 75231 Paris Cedex 05 France

Secretogranin II (SgII) is a protein specific to the matrix of the secretory granules in neurons and neuroendocrine cells. We have already demonstrated the precursor-product relationship between sulfated SgII and four N-terminal derived peptides in GH3B6 prolactin cells. In this study, we have investigated the subcellular compartment in which the cleavage of SgII is initiated by taking advantage of its tyrosine sulfation in the trans-Golgi network (TGN). In order to prevent export of radiolabeled SgII from the TGN, we used brefeldin A (BFA) as well as incubation at 20 °C. BFA completely inhibited the cleavage of SgII when added immediately post-pulse. BFA added a few minutes post-pulse or after a 20 °C incubation, however, permitted the cleavage of SgII in the presence of the drug. These SgII-derived peptides generated in the presence of BFA could not be released upon stimulation of the cells by either thyroliberin, a physiological secretagogue, or KCl. These results demonstrate that SgII can be cleaved in the TGN. They also evidence that the cleavage occurs in a distal compartment of the TGN different from the sulfation site. The transfer of SgII from the sulfation site to this distal compartment of the TGN involves BFA-sensitive membrane dynamics.

Bioactive peptides and hormones secreted by neurons and neuroendocrine cells are synthesized as precursors that must undergo post-translational processing before their release. The different steps of this processing occur during the vectorial transport of the proteins from the rough endoplasmic reticulum, where they are synthesized, to the secretory granules, in which they are stored. Proteolytic processing of the precursors is one of the last of these post-translational modifications. Although it occurs mainly in the secretory granules, it can be initiated in the trans-Golgi network (TGN)† (reviewed in Ref. 1). Indeed, immunocytochemical (2–5) and biochemical studies (6–9) have evidenced the cleavage of precursors in the TGN as well as in the secretory granules. In this study, we have investigated the intracellular cleavage site of secretogranin II (SgII) in GH3B6 prolactin cells.

SgII is a member of the granin family (reviewed in Ref. 10). These proteins are specific to the dense core secretory granules of neuroendocrine cells and neurons (reviewed in Ref. 11–13). They are considered as precursors of secreted peptides (reviewed in Ref. 14). SgII is the precursor of secretoneurin (15), a peptide whose activity has recently been proposed (16). SgII and chromogranin B are expressed in normal anterior pituitary prolactin cells and in GH3B6 prolactin cells (17). Both are present in the same secretory granules with prolactin but display different intragranular localizations (18). We have demonstrated the precursor-product relationship between SgII and four sulfated fragments in prolactin cells (see Fig. 1) (19). The terminal sulfated fragment is a 21-kDa protein that is accumulated in the secretory granules and released upon stimulation by thyroliberin (TRH), a prolactin secretagogue, or KCl. The processing of SgII is fast, and more than 70% of mature SgII is cleaved 30 min after a 5-min pulse with [35S]sulfate (19).

We took advantage of the tyrosine sulfation of SgII to study the intracellular site of its proteolytic processing. Indeed, sulfation is a post-translational modification specific to the TGN (20–22). Pulse-labeling PC12 cells with [35S]sulfate has already allowed the use of sulfated SgII as a marker of intracellular transport between the TGN and the plasma membrane via the dense core secretory granules (23–25). PC12 cells, however, lack the prohormone convertases PC1 and PC2, which are involved in the processing of SgII (26, 27). Our experiments were performed in GH3B6 cells, a subclone of GH3 cells, which express PC2 but not PC1 (28). Cultivating these cells in the presence of insulin, 17β-estradiol, and epidermal growth factor increases the number of secretory granules (29), the expression of PC2 (28) and the storage of SgII-derived peptides in the granules (19). GH3B6 cells therefore provide an ideal model for studying the proteolytic processing of SgII in the TGN and secretory granules.

We used brefeldin A (BFA) and 20 °C incubation to block anterograde transport of sulfated proteins in the TGN. We have already described the effect of these treatments on the distribution and secretion of prolactin in GH3B6 cells (30). BFA is a fungal metabolite that blocks anterograde transport of proteins at different steps of the secretory pathway (reviewed in Ref. 31). It inhibits the formation of secretory vesicles and granules from the TGN, without preventing the exocytosis of the secretory granules stored in the cytoplasm (8, 32, 33). Incubating the cells at 20 °C accumulates secretory products in the TGN (34, 35) and thus permits the study of post-translational modifications at the exit of the Golgi apparatus (7–9, 20, 36, 37). The combination of the sulfate labeling with the BFA and 20 °C transport blocks allowed an accurate study of the steps of transport of SgII in the TGN. Our results indicate that the cleavage of SgII may occur in the TGN in a compartment distal from the sulfation site but before the packaging of SgII in the secretory granules.

* This work was supported by grants from the CNRS (URA 1115) and from the INSERM (U 36). The costs of publication of this article were defrayed in part by the payment of page charges. This article must be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ Recipient of a fellowship from the Ministère de la Recherche et de l’Enseignement Supérieur. To whom correspondence should be addressed. Present address: Dept. of Biochemistry and Molecular Biology, Louisiana State University Medical Center, 1901 Perdido St., New Orleans, LA 70112. Fax: 504-588-3370.
† The abbreviations used are: TGN, trans-Golgi network; SgII, secretogranin II; TRH, thyroliberin; PC, prohormone convertase; BFA, brefeldin A; PAGE, polyacrylamide gel electrophoresis.
Fig. 1. Schematic representation of rat secretogranin II. The dibasic potential cleavage sites (RK and KR) are represented. S corresponds to the sulfation site on tyrosine 126. Two identified SgII-derived peptides are represented: the 21-kDa N-terminal fragment (hatched bar) and secretogranin (dark bar). Hypothetical cleavage sites and sequence of cleavage are represented from Dittie and Tooze (26), Hoflehner et al. (27), and Muller and Tougaard (19). The molecular mass of the SgII-derived peptides is indicated on the left in kDa. N-ter, N-terminal; C-ter, C-terminal.

EXPERIMENTAL PROCEDURES

Cell Culture—GH3B6 cells were cultured in Ham’s F-12 medium supplemented with 15% horse serum (PAA Labor) and 2.5% fetal calf serum (Life Technologies, Inc.) at 37 °C in 5% CO2. For experiments, they were grown in 35-mm dishes for 6 days in the presence of 180 nM insulin (Sigma), 10 nM epidermal growth factor (Becton Dickinson Labware), and 1 nM 17β-estradiol (Sigma).

Metabolic Labeling—Before metabolic labeling, cells were incubated in sulfate-free Ham’s F-12 medium for 30 min. They were then pulse-labeled for 5 or 10 min at 37 °C in the presence of 200–250 μCi/dish carrier free [35S]sulfate (ICN Biomedicals, Inc.). Cells were washed three times with Ham’s F-12 on ice before further chase. The 20 °C incubations were performed by transferring the cells from ice to a 19–20 °C water bath. BFA (Epicentre Technologies) was always used at 10 μM. When added after a 20 °C incubation, BFA was always added at 20 °C, 3–5 min before the temperature shift to a 37 °C water bath. Chloroquine (Sigma) was always used at 40 μM. In some experiments, the release of SgII was stimulated with either 30 nM TRH (Calbiochem) or 30 mM KCl. All the experiments were performed with duplicate culture dishes and reproduced three times.

Extraction of Secretogranins and SDS-PAGE—Cells were scraped on ice in 20 mM Tris, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.3% Tween 20 containing 200 μM phenylmethylsulfonyl fluoride, 1 μM leupeptin, and 1 μM pepstatin. Lysates were boiled for 3 min, cooled on ice, and centrifuged 10 min at 23,000 × g. Supernatants were collected and acetone precipitated overnight at −20 °C. Media were collected and centrifuged to pellet any detached cell before trichloroacetic acid precipitation of the proteins overnight at 4 °C. Proteins were separated by SDS-PAGE on acrylamide gradients (5–20%) mini-gels (Hoeffer). Gels were stained with Coomassie Blue R-250 either before direct autoradiography or treatment for fluorography with Amplify (Amersham Corp.). Gels were exposed to either Bio-Max films (Kodak) or φ-Max films (Amersham Corp.). Quantitation of autoradiograms was performed by densitometric analysis with Agfa Arcus Plus and NIH Image.

RESULTS

Sulfation and Cleavage of SgII Are Inhibited in the Presence of BFA—To investigate the intracellular site of sulfation, we pulse-labeled GH3B6 cells with [35S]sulfate for 5 min in both the presence and the absence of 10 μg/ml BFA. In control cells, [35S]sulfate was incorporated in heparan sulfate proteoglycans, chromogranin B, and SgII (see Fig. 2A). Sulfation was completely abolished after a 30-min pretreatment of the cells with BFA (see Fig. 2A). Longer pretreatment of 120 min provided the same results (not shown). If BFA was added at the beginning of the pulse without pretreatment, sulfation was strongly inhibited and could not be increased by extending the pulse up to 240 min in the presence of the drug (see Fig. 2A).

We also investigated the effect of BFA on the cleavage of sulfated SgII by adding the drug immediately post-pulse. In control cells, sulfated SgII is sequentially cleaved from the C terminus to the N terminus (Fig. 1). The cleavage products are accumulated in the cells as 27- and 21-kDa fragments (Fig. 2B) (19). BFA completely prevented this proteolytic processing. It also inhibited the secretion of sulfated SgII (not shown), as already described in PC12 cells (32, 33).

BFA Blocks the Transport of Sulfated SgII to the Cleavage Compartment—BFA blocks anterograde transport by inhibiting the association of the ADP-ribosylation factor and the coat proteins with the membranes of the organelles involved in the secretory process (38, 39). The coat proteins associated with the Golgi apparatus (β-COP) and the TGN (γ-adaptin) are dispersed in the cytoplasm within 1 min in the presence of BFA (40, 41). We have taken advantage of the rapidity of the dispersion to study the kinetics of the inhibition of SgII proteolytic processing by BFA. Chase media were replaced by BFA containing media either 10, 20, or 30 min after a 5-min pulse (Fig. 3). Whereas BFA completely inhibited the processing of SgII when added immediately post-pulse (Fig. 3, lane 5), it had no effect 20 min or more after the pulse (Fig. 3, lane 7 and 8). If BFA was added 10 min after the pulse (Fig. 3, lane 6), two pools of sulfated SgII were present in the cell extracts: one was BFA-sensitive and not cleaved, the other was BFA-resistant and cleaved. 10 min after the pulse, the processing of SgII had not begun (Fig. 3, lane 1), and the fragments of SgII present when BFA was added at 10 min chase time were thus generated in the presence of the drug. It is worth noting that this cleaved SgII was not accumulated as 80- or 40-kDa intermediate fragments but rather as 27- and 21-kDa terminal fragments. This demonstrated that BFA inhibited the cleavage of SgII through a block of transport from the sulfation site to the cleavage compartment and not through the inhibition of the proteolytic activity itself.

The SgII-derived Peptides Generated in the Presence of BFA Cannot Be Released by Secretagogues.—The transport step inhibited by BFA could correspond to the formation of secretory granules as evidenced by several groups (32, 33) or could occur within the TGN itself. In order to distinguish between these two possibilities, we studied the release of the proteolytic products generated in the presence of BFA by stimulating the cells with 30 nM TRH. Cells were pulse-labeled with [35S]sulfate for 5 min and chased in control medium for 5 min before the addition of BFA for 55 min. This provided the same pattern of cleavage as on Fig. 3 (lane 6); one pool of BFA-sensitive uncleaved SgII and another of BFA-resistant SgII, which was present as 27- and 21-kDa fragments. Cells were then stimu-
TRH (Fig. 4). Experiments performed with 30 mM KCl provided BFA during both chases, the maturation products that had chased with TRH. When cells were incubated in the presence of inhibit this release if it was present only during the second lated with TRH for 30 min (Fig. 4). This secretagogue released with [35S]sulfate for 5 min and chased in control conditions for 10 (lane 1, 20 (lane 2), 30 (lane 3), and 60 min (lane 4) or in the presence of 10 μg/ml BFA (lanes 5–8). BFA-treated cells were incubated in the presence of the drug immediately post-pulse (lane 5) or 10 (lane 6), 20 (lane 7), or 30 min (lane 8) after the pulse and for a total chase period of 60 min. Heat stable proteins were extracted at the end of the chase and analyzed by SDS-PAGE and fluorography. Arrowheads indicate the SgII-derived peptides. Dark bars represent the labeling period.

lated with TRH for 30 min (Fig. 4). This secretagogue released the SgII maturation products from control cells. BFA did not inhibit this release if it was present only during the second chase with TRH. When cells were incubated in the presence of BFA during both chases, the maturation products that had been generated in the presence of BFA could not be released by TRH (Fig. 4). Experiments performed with 30 mM KCl provided the same results (not shown). Some SgII fragments could therefore be generated in the presence of BFA but not released.

The Proteolytic Processing of SgII Is Strongly Slowed Down at 20 °C—Incubating cells at 20 °C accumulates proteins in the TGN and blocks the formation of secretory vesicles (34, 35). SgII was sulfated in GH3B6 cells incubated at 20 °C (not shown). When the cells were pulse-labeled at 37 °C and chased at 20 °C, SgII could be processed to the same peptides, but the kinetics of the cleavage was dramatically slowed down: the first fragments appeared between 30 and 60 min (Fig. 5). In order to determine if this inhibition resulted from the transport block or from the inhibition of the proteolytic activity, we took advantage of the results obtained with BFA, i.e. the absence of effect of the transport block on the cleavage of SgII 20 min after the pulse. Cells were first chased at 37 °C for 30 min in order to allow transport of SgII to the cleavage compartment. They were then incubated at 20 °C for 30 min (Fig. 5). The cleavage pattern observed at the end of this second chase was similar to that observed after the first 30 min chase at 37 °C. This demonstrated that SgII was not further processed during the second chase at 20 °C. The inhibition of proteolytic processing at 20 °C was thus caused by the inhibition of the proteolytic activity rather than by the transport block alone. This could possibly be related to the inhibition of PC2 at 20 °C already evidenced in vitro (42).

A 20 °C Preincubation Lowers the Effect of BFA on the Cleavage of SgII but Not on Its Release—We investigated the effect of BFA after an incubation at 20 °C. Cells were thus chased at 20 °C for 30 min, after a 5-min pulse with [35S]sulfate and prior to a 60-min incubation at 37 °C in the presence of BFA. This protocol provided the same proteolytic pattern of SgII as that already observed when BFA was added 10 min after the pulse: one pool of sulfated SgII was BFA-sensitive and uncleaved, whereas the other was BFA-resistant and accumulated as 27- and 21-kDa fragments (Fig. 6A). We also investigated the release of the fragments generated in the presence of BFA by stimulating the cells with 30 mM TRH (Fig. 6B). TRH could release the fragments generated under control conditions, even if it was added together with BFA. The fragments generated in the presence of BFA could, however, not be released upon stimulation of the cells. The same results were obtained after stimulation of secretion with 30 mM KCl (not shown). These experiments demonstrate that the transport block induced by BFA, which results in the inhibition of the cleavage of SgII, was upstream from the 20 °C accumulation site, unlike the other block, which results in the inhibition of the release of the fragments of SgII.

Acidification of Luminal pH Is Necessary for the Cleavage of SgII—The acidification of luminal pH in the TGN and the secretory granules is necessary for the proteolytic processing of some peptide precursors and pro-hormones (reviewed in Refs. 1 and 43). We investigated the involvement of the acidification of luminal pH in the processing of SgII with 40 μM chloroquine, a lysosomotropic agent that blocks intra-organelle acidification. Chloroquine had no effect on the sulfation of SgII, but it completely inhibited the proteolytic processing of sulfated SgII (Fig. 7A). We also investigated the secretion of sulfated SgII in the presence of chloroquine. Uncleaved sulfated SgII could be released in the medium, and this release could be stimulated by TRH (Fig. 7B). This indicates that the inhibition of SgII cleav-
In this study we investigated the proteolytic processing of endogenous SgII in GH3B6 prolactin cells. Recent biochemical studies have provided evidence for the cleavage of precursors in the TGN as well as in the secretory granules (7–9). Using BFA and 20 °C incubations, we were able to demonstrate that sulfated SgII is cleaved in the TGN of GH3B6 cells. Sulfation is a post-translational modification specific to the TGN (20–22), and sulfated SgII has been used as a marker for the transport between the TGN and the plasma membrane in PC12 cells (23–25). Unlike GH3B6 cells, this cell line does not process SgII to smaller peptides. Sulfated SgII in GH3B6 cells therefore provides a unique model for studying in detail the proteolytic processing in the TGN. Our experiments indeed permitted to evidence two BFA-sensitive steps in the delivery of SgII from its sulfation site in the TGN to the secretory granules: sulfated SgII is first transported to a distal compartment of the TGN where its cleavage is initiated and then packaged in the secretory granules.

Biochemical studies and subcellular fractionation have demonstrated that sulfation is a post-translational modification specific to the TGN (20, 23). BFA has also been used to demonstrate that sulfation occurred in the TGN of epithelial (21) and neuroendocrine cells (22, 33). Our experiments are in agreement with these studies: pretreatment of the cells with BFA completely abolished sulfation, and treatment at the beginning of the pulse also strongly inhibited sulfation. These results are relevant of the absence of redistribution of the sulfotransferase to the rough endoplasmic reticulum together with the cis-, medial, and trans-Golgi (reviewed in Ref. 31). In such a hypothesis, the inhibition of sulfation observed results from the lack of delivery of substrate to the TGN, as already proposed by others (21, 22), even though we cannot exclude that the sulfotransferase activity itself is inhibited by the BFA treatment.

We used BFA to study the site of the initiation of the proteolytic processing of SgII. BFA completely abolished the cleavage of SgII in GH3B6 cells when it was added immediately post-pulse. This demonstrates that the cleavage compartment of SgII is different from the sulfation compartment. This observation is in agreement with the lack of direct sulfation of any SgII proteolytic fragment that we have already described (19). When BFA was added a few minutes after the pulse, the fragments of 27 and 21 kDa were, however, generated. This demonstrates that the cleavage of SgII may occur in the presence of BFA and that BFA blocks the delivery of sulfated SgII to the cleavage site without inhibiting the proteolytic activity itself. Two lines of evidence indicate that this transport block occurs before the formation of the secretory granules. First, incubating the cells at 20 °C as briefly as 30 min permitted the cleavage of sulfated SgII in the presence of BFA. Second, the 27- and 21-kDa sulfated fragments generated in the presence of BFA could not be released in the medium when the cells were stimulated by 30 nm TRH or 30 nm KCl, even though BFA had no effect on the release of the stored granules. This indicates that the SgII fragments generated in the presence of BFA were not present in secretory granules, not even in immature secretory granules, which have the same exocytotic potency as the mature granules (44, 45). This is in contrast to results obtained in β-cells in which BFA inhibited the cleavage of proinsulin accumulated in the TGN at 20 °C (8). This difference could be relevant of the cell types. Indeed, immunocytochemical studies have also demonstrated that the cleavage of proinsulin occurred only in the secretory granules of β-cells (2), whereas that of pro-opiomelanocortin took place in the TGN of pituitary corticotropes (4)

The use of sulfate labeling in the TGN together with the BFA and 20 °C transport blocks has therefore permitted demonstration of the cleavage of endogenous SgII in the TGN and definition of the two steps in the transport of SgII from its sulfation site to the secretory granules (Fig. 8). The first step occurs in the TGN itself and corresponds to the delivery of sulfated SgII to the cleavage site, which is a distal compartment of the TGN. The second step corresponds to the packaging of SgII in the secretory granules. The distinction between these two steps was only possible under conditions that accumulate the pro-

**FIG. 6. Sulfated SgII accumulated at 20 °C can be cleaved but not released in the presence of BFA.** Cells were pulse-labeled with [35S]sulfate for 5 min at 37 °C and chased for 30 min at 20 °C. A, they were then chased for 60 min in the presence (BFA) or the absence (C) of 10 μg/ml BFA at 37 °C (Chase I). Heat stable proteins were extracted from the cells at the end of this chase. B, cells were further chased for 30 min at 37 °C (Chase II) in the presence of 30 nm TRH and in the presence (BFA) or the absence (C) of 10 μg/ml BFA. Media of chase II were collected and trichloroacetic acid precipitated. Proteins were analyzed by SDS-PAGE and autoradiography. Arrowheads indicate the SgII-derived peptides.

**FIG. 7. Chloroquine inhibits the cleavage of SgII but not its sulfation nor its release.** A, cells were preincubated for 60 min, pulse-labeled with [35S]sulfate for 10 min, and chased in the continuous presence or absence of 40 μM chloroquine. Heat stable proteins were extracted either at the end of the pulse or at the end of a 30- or 60-min chase incubation. B, cells were pretreated and pulse-labeled as in A. They were first chased in the presence or the absence of chloroquine for 30 min and then in the presence or the absence of chloroquine and 30 nm TRH for 30 min. Media of this second chase were collected and trichloroacetic acid precipitated. Proteins were analyzed by SDS-PAGE and autoradiography. Arrowheads indicate the SgII-derived peptides.

**DISCUSSION**

In this study we investigated the proteolytic processing of endogenous SgII in GH3B6 prolactin cells. Recent biochemical studies have provided evidence for the cleavage of precursors in the TGN as well as in the secretory granules (7–9). Using BFA and 20 °C incubations, we were able to demonstrate that sulfated SgII is cleaved in the TGN of GH3B6 cells. Sulfation is a post-translational modification specific to the TGN (20–22), and sulfated SgII has been used as a marker for the transport between the TGN and the plasma membrane in PC12 cells (23–25). Unlike GH3B6 cells, this cell line does not process SgII to smaller peptides. Sulfated SgII in GH3B6 cells therefore provides a unique model for studying in detail the proteolytic processing in the TGN. Our experiments indeed permitted to evidence two BFA-sensitive steps in the delivery of SgII from its sulfation site in the TGN to the secretory granules: sulfated SgII is first transported to a distal compartment of the TGN where its cleavage is initiated and then packaged in the secretory granules.

Biochemical studies and subcellular fractionation have demonstrated that sulfation is a post-translational modification specific to the TGN (20, 23). BFA has also been used to demonstrate that sulfation occurred in the TGN of epithelial (21) and neuroendocrine cells (22, 33). Our experiments are in agreement with these studies: pretreatment of the cells with BFA completely abolished sulfation, and treatment at the beginning of the pulse also strongly inhibited sulfation. These results are relevant of the absence of redistribution of the sulfotransferase to the rough endoplasmic reticulum together with the cis-, medial, and trans-Golgi (reviewed in Ref. 31). In such a hypothesis, the inhibition of sulfation observed results from the lack of delivery of substrate to the TGN, as already proposed by others (21, 22), even though we cannot exclude that the sulfotransferase activity itself is inhibited by the BFA treatment.

We used BFA to study the site of the initiation of the proteolytic processing of SgII. BFA completely abolished the cleavage of SgII in GH3B6 cells when it was added immediately post-pulse. This demonstrates that the cleavage compartment of SgII is different from the sulfation compartment. This observation is in agreement with the lack of direct sulfation of any SgII proteolytic fragment that we have already described (19). When BFA was added a few minutes after the pulse, the fragments of 27 and 21 kDa were, however, generated. This demonstrates that the cleavage of SgII may occur in the presence of BFA and that BFA blocks the delivery of sulfated SgII to the cleavage site without inhibiting the proteolytic activity itself. Two lines of evidence indicate that this transport block occurs before the formation of the secretory granules. First, incubating the cells at 20 °C as briefly as 30 min permitted the cleavage of sulfated SgII in the presence of BFA. Second, the 27- and 21-kDa sulfated fragments generated in the presence of BFA could not be released in the medium when the cells were stimulated by 30 nm TRH or 30 nm KCl, even though BFA had no effect on the release of the stored granules. This indicates that the SgII fragments generated in the presence of BFA were not present in secretory granules, not even in immature secretory granules, which have the same exocytotic potency as the mature granules (44, 45). This is in contrast to results obtained in β-cells in which BFA inhibited the cleavage of proinsulin accumulated in the TGN at 20 °C (8). This difference could be relevant of the cell types. Indeed, immunocytochemical studies have also demonstrated that the cleavage of proinsulin occurred only in the secretory granules of β-cells (2), whereas that of pro-opiomelanocortin took place in the TGN of pituitary corticotropes (4).
A schematic representation of the transport steps of SgII from the sulfation site to the secretory granules. BFA inhibits the membrane dynamics involved in the formation of the network of the TGN and thus the transfer of sulfated SgII to a distal compartment of the TGN where it is cleaved. 20 °C incubation and BFA both inhibit the fission of the secretory granules from the TGN.

**REFERENCES**

1. Halban, P. A., and Irminger, J.-C. (1994) Biochem. J. **299**, 1–18.
2. Orci, L., Ravazzola, M., Storch, M. J., Anderson, R. W. G., Vassali, J. D., and Verrelet, A. (1987) Cell **48**, 865–868.
3. Fisher, J. M., Susin, W., Newcomb, R., and Scheller, R. H. (1988) Cell **54**, 813–822.
4. Schnabel, E., Mains, R. E., and Farquhar, M. G. (1989) Mol. Endocrinol. **3**, 1223–1235.
5. Bourdais, J., Devilliers, G., Girard, R., Morel, A., Benedetti, L., and Cohen, P. (1990) Biochem. Biophys. Res. Commun. **170**, 1263–1272.
6. Lepage-Lezin, A., Joseph-Bravo, P., Devilliers, G., Fischer-Colbrie, R., Launay, J.-M., Gomez, S., and Cohen, P. (1991) J. Biol. Chem. **266**, 1679–1688.
7. Xu, H., and Shields, D. (1993) **Cell Biol.** **122**, 1169–1184.
8. Huang, X. F., and Arvan, P. (1994) J. Biol. Chem. **269**, 20838–20844.
9. Zhou, A., and Mains, R. E. (1993) **Neurosci.** **51**, 1–4.
10. Tougaard, C., Nasciuti, L. E., Picart, R., Tixier-Vidal, A., and Hutten, W. B. (1989) J. Histochem. Cytochem. **39**, 1329–1336.
11. Ozawa, H., Picart, R., Barrett, A., and Tougaard, C. (1994) J. Histochem. Cytochem. **42**, 1097–1107.
12. Muller, L., and Tougaard, C. (1995) Mol. Cell. Endocrinol. **112**, 101–112.
13. Baeuerle, P. A., and Hutten, W. B. (1987) J. Cell Biol. **105**, 2655–2664.
14. Spiro, R. C., Freeze, H. H., Sampath, D., and Garcia, J. A. (1991) J. Cell Biol. **115**, 1463–1473.
15. Rosà, P., Mantovani, S., Rosboch, R., and Hutten, W. B. (1992) **J. Biol. Chem.** **267**, 12227–12232.
16. Tooze, S. A., and Hutten, W. B. (1990) **Cell** **60**, 837–847.
17. Miller, S. G., and Moore, H. P. H. (1991) **Cell Biol.** **112**, 39–54.
18. Grimes, M., and Kelly, B. R. (1992) **J. Cell Biol.** **117**, 539–549.
19. Dittrich, A. S., and Tooze, S. A. (1995) **Biochem. Biol. Chem.** **260**, 77440–77447.
20. Hoflehner, J., Eder, U., Laslop, A., Seidah, N. G., Fischer-Colbrie, R., and Hutten, W. B. (1995) **FEBS Lett.** **360**, 294–298.
21. Seidah, N. G., Gaspar, L., Mion, P., Marcinkiewicz, M., Mbikay, M., and Christen, M. (1996) **DNA Cell Biol.** **9**, 415–424.
22. Scammell, J. G., Burrage, T. G., and Dannies, P. S. (1986) **Endocrinology** **119**, 1543–1548.
23. Nasciuti, L. E., Picart, R., Rosenbaum, E., Tixier-Vidal, A., and Tougaard, C. (1992) **Biol. Cell.** **75**, 25–35.
24. Klausner, R. D., Donaldson, J. G., and Lippincott-Schwartz, J. (1992) **J. Cell Biol.** **116**, 1071–1080.
25. Miller, S. G., Carnell, L., and Moore, H. P. H. (1992) **J. Cell Biol.** **118**, 267–283.
26. Rosà, P., Barr, F. A., Stinchcombe, J. C., Binacchi, C., and Hutten, W. B. (1992) **Eur. J. Cell Biol.** **59**, 265–274.
27. Saraste, J., and Kuismanen, E. (1984) **Cell** **38**, 535–549.
28. Griffiths, G., Pfeiffer, S., Simons, K., and Matlin, K. (1985) **J. Cell Biol.** **101**, 949–964.
29. Kuliawat, R., and Arvan, P. (1992) **J. Cell Biol.** **118**, 521–529.
30. Milgram, S. L., Epper, B. A., and Mains, R. E. (1994) **J. Cell Biol.** **124**, 33–41.
31. Donaldson, J. G., Finazzi, D., and Klausner, R. D. (1992) **Nature** **360**, 350–352.
32. Helms, J. B., and Rothman, J. E. (1992) **Nature** **360**, 352–354.
33. Donaldson, J. G., Pinniszt-Schwarz, J., Bloom, G. S., Kreis, T. E., and Klausner, R. D. (1990) **J. Cell Biol.** **111**, 2295–2306.
34. Robinson, M. S., and Kreis, T. E. (1992) **Cell** **69**, 129–138.
35. Xu, H., and Shields, D. (1994) **J. Biol. Chem.** **269**, 22875–22881.
36. Anderson, R. G. W., and Orci, L. (1988) **J. Cell Biol.** **106**, 539–543.
37. Arvan, P., Kuliawat, R., Prabakaran, D., Zavacki, A.-M., Elahi, D., Wang, S., and Pilkey, D. (1991) **J. Biol. Chem.** **266**, 1471–1474.
38. Tooze, S. A., Platemark, T., Toze, J., and Hutten, W. B. (1991) **J. Cell Biol.** **115**, 1491–1503.
39. Chanat, E., and Hutten, W. B. (1991) **J. Cell Biol.** **115**, 1505–1519.
40. Colomer, V., Kirsch, G. A., and Rinderle, M. J. (1990) **J. Biol. Chem.** **267**, 48–55.
41. Farquhar, M. G., and Palade, G. (1981) **J. Cell Biol.** **91**, 77–103 (suppl.)
42. Tougaard, C., Picart, R., and Tixier-Vidal, A. (1980) Am. J. Anat. **158**, 471–490.
43. Rambourg, A., Clermont, Y., Chretien, M., and Pilkey, D. (1990) **J. Biol. Chem.** **265**, 1077–1087.
44. Rambourg, A., and Clermont, Y. (1990) **Eur. J. Cell Biol.** **51**, 189–200.
45. Ladinsky, M. S., Kremer, J. R., Furchiotti, P. S., McIntosh, J. R., and Howell, K. E. (1994) **J. Cell Biol.** **127**, 29–38.