N- and C-terminal Domains Direct Cell Type-specific Sorting of Chromogranin A to Secretory Granules*

(Received for publication, December 14, 1999, and in revised form, December 20, 1999)

Darrin J. Cowley‡§, Yancy R. Moore‡, Douglas S. Darling‡§, Paul B. M. Joyce‡, and Sven-Ulrik Gorry‡***

From the ‡Department of Molecular, Cellular and Craniofacial Biology and the §Department of Biochemistry and Molecular Biology, University of Louisville, Louisville, Kentucky 40292 and the ¶Department of Chemistry and Biochemistry and Centre for Structural and Functional Genomics, Concordia University, Montreal, Quebec H3G 1M8, Canada

Chromogranins are a family of regulated secretory proteins that are stored in secretory granules in endocrine and neuroendocrine cells and released in response to extracellular stimulation (regulated secretion). A conserved N-terminal disulfide bond is necessary for sorting of chromogranins in neuroendocrine PC12 cells. Surprisingly, this disulfide bond is not necessary for sorting of chromogranins in endocrine GH4C1 cells. To investigate the sorting mechanism in GH4C1 cells, we made several mutant forms removing highly conserved N- and C-terminal regions of bovine chromogranin A. Removing the conserved N-terminal disulfide bond and the conserved C-terminal dimerization and tetramerization domain did not affect the sorting of chromogranin A to the regulated secretory pathway. In contrast, removing the C-terminal 90 amino acids of chromogranin A caused rerouting to the constitutive secretory pathway and impaired aggregation properties as compared with wild-type chromogranin A. Since this mutant was sorted to the regulated secretory pathway in PC12 cells, these results demonstrate that chromogranins contain independent N- and C-terminal sorting domains that function in a cell type-specific manner. Moreover, this is the first evidence that low pH/calcium-induced aggregation is necessary for sorting of a chromogranin to the regulated secretory pathway of endocrine cells.

Peptide hormones and neuropeptides are stored at high concentrations in secretory granules of endocrine and neuroendocrine cells. This storage is required for the proteolytic processing of prohormones and the subsequent rapid release of active peptides in response to extracellular stimulation (for review, see Refs. 1–5). Secretory proteins that are not stored in secretory granules are secreted directly by the constitutive secretory pathway, originating in the trans-Golgi network (2) or the constitutive-like secretory pathway that originates from immature secretory granules (1). Thus, granule storage of secretory proteins appears to require two sorting steps (sorting for entry and sorting by retention; Ref. 3) that refine the final complement of stored proteins.

Several mechanisms have been proposed for sorting of secretory proteins into secretory granules. These sorting mechanisms include low pH and/or calcium-induced aggregation (6–8), receptor-mediated transport of selected secretory proteins (8, 9), and direct binding to specific lipid domains in granule membranes (10). Different sorting mechanisms appear to be responsible for sorting of different secretory proteins. Thus, pro-opiomelanocortin (POMC)† is sorted by binding to ca
tboxypeptidase E and this sorting depends on an N-terminal disulfide bridge in POMC (9). Chromogranins contain a similar N-terminal disulfide bond that is both necessary and sufficient for sorting in PC12 cells (11–14), although carboxypeptidase E does not appear to mediate this sorting (11, 15). While chromogranins aggregate at low pH and in the presence of calcium, this aggregation is neither necessary nor sufficient for sorting in PC12 cells (12, 16). In contrast, a narrowly defined aggregation domain is necessary for sorting of pro-opiomelanocortin to the regulated secretory pathway in AtT-20 cells (17). These findings suggest that individual secretory proteins contain protein-specific sorting signals that interact with different cellular sorting mechanisms.

It has long been assumed that regulated secretory proteins contain a single sorting domain that directs their sorting and storage in secretory granules (see, e.g., Ref. 18). However, we have recently found that the N-terminal disulfide bond that is necessary for sorting of chromogranin B in neuroendocrine PC12 cells (14) is not required for sorting in endocrine GH4C1 cells (11). This result suggests that, in endocrine cells, the signals and mechanisms used for sorting of regulated secretory proteins into secretory granules are different from those used in neuroendocrine cells. These endocrine-specific sorting signals remain unknown.

Chromogranin A (CgA) is a regulated secretory protein that is stored in secretory granules of both endocrine and neuroendocrine cells. To identify potential signals for sorting of CgA in endocrine cells, we prepared three mutants of bovine CgA. These mutants were designed to delete separately: 1) the N-terminal disulfide bond and dimerization domain (19), 2) the C-terminal domain involved in dimerization and tetramerization (20, 21), and 3) a putative calcium-aggregation/condensation domain. The latter was based on our observation that an approximately 8-kDa peptide of CgA is necessary for calcium-

† The abbreviations used are: POMC, pro-opiomelanocortin; CgA, chromogranin A; DMEM, Dulbecco’s modified Eagle’s medium; KRB, Krebs-Ringer-Hepes; PMSF, phenylmethanesulfonyl fluoride; PAGE, polyacrylamide gel electrophoresis; SEAP, secreted alkaline phosphatase.

* This work was supported in part by United States Public Health Service Grant 1 R01 DK 53367-01, grants from the Jewish Hospital Research Foundation (Louisville, KY), and a grant-in-aid from the American Heart Association, Kentucky affiliate (to S. U. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Supported by United States Public Health Service Grant 2 T32 DE 07254-06.

** To whom correspondence should be addressed. Tel.: 502-852-8905; Fax: 502-852-4702; E-mail: sven.gorry@louisville.edu.

This paper is available on line at http://www.jbc.org 7743
induced aggregation at acidic pH (22).

We now report that the C-terminal domain of CgA is necessary for both sorting and for low pH/calcium-induced aggregation in GH4C1 cells. In contrast, it is shown that the C-terminal domain of CgA is not necessary for sorting to the regulated secretory pathway in PC12 cells. Together with previous results (11), these findings show that CgA contains two independent cell-specific sorting domains.

**EXPERIMENTAL PROCEDURES**

**Materials**—Reagents were purchased from Fisher or Sigma, unless otherwise indicated. Cell culture media and penicillin/streptomycin were from Life Technologies, Inc. Fetal bovine and gelding equine serum were from HyClone Laboratories (Logan, UT). The expression plasmids pcDNA3 was purchased from Invitrogen (Carlsbad, CA), and restriction endonucleases were from Promega (Madison, WI). The [3H]leucine and protein A-Sepharose were from Amersham Pharmacia Biotech. The site-directed mutagenesis kit, Transformer, was purchased from CLONTECH. Phospha-Light alkaline phosphatase assay kit was from Tropix/Perkin-Elmer (Bedford, MA), and rabbit anti-platelet alkaline phosphatase antiserum was from Biomedia Corp. (Foster City, CA). The horseradish peroxidase-conjugated goat anti-rabbit IgG was purchased from Sigma or Roche Molecular Biochemicals. Phorbol didecanoate was from Calbiochem (La Jolla, CA), whereas the protease inhibitors phenylmethanesulfonyl fluoride (PMSF) and N-α-tosyl-L-lysine chloromethyl ketone were from Roche Molecular Biochemicals. Supersignal chemiluminescent reagents were from Pierce, polyvinylidene difluoride membrane was from Bio-Rad, and nitrocellulose membrane was obtained from Schleicher & Schuell. BioMax film was from Eastman Kodak Co. The antisera to bovine CgA was kindly provided by Dr. David V. Cohn (University of Louisville, Louisville, KY).

**Cell Culture**—GH4C1 rat pituitary somatotrophs were cultured in medium containing 42.5% Dulbecco’s modified Eagle’s medium (DMEM), 42.5% Ham’s F-10 medium, and 15% gelding equine serum. GH4C1 cells (ATCC, Manassas, VA) were cultured in DMEM containing 10% gelding equine serum and 5% fetal bovine serum. Both cultures were supplemented with penicillin (50 units/ml) and streptomycin (50 μg/ml) and cultured at 37 °C, in a humidified atmosphere with 5% CO₂. For each experiment, GH4C1 cells were detached with phosphate-buffered saline, while PC12 cells were detached with 0.25% trypsin in DMEM. The cells were plated in culture medium at 5 × 10⁴ cells/well in six-well culture plates (5 × 10⁵ cells/cm²).

**Cloning and Mutagenesis**—The expression plasmids for wild-type bovine CgA, CgAΔCC (missing the disulfide bond), and secreted alkaline phosphatase (SEAP) were described previously (11, 23). CgA341 and CgA387 were prepared from wild-type CgA by site-directed mutagenesis with primers from Genosys Biotechnologies Inc. (The Woodlands, TX). The selection primer (5'-AACTTGGGATCTGGATGATG-3') and the mutagenesis primer for CgA341 (5'-GAGGAAGATGAGGCTCCGGACG-3') changed the BstBI site of pcDNA3 to a unique ClaI site, whereas the mutagenesis primer for CgA341 (5'-AGGAAAGATGAGGCTCCGGACG-3') added a stop codon at position 342 of the mature protein. In CgA341, amino acids 339 and 341 were changed from glutamic acid and aspartic acid to glutamine and glutamic acid, respectively, during DNA manipulation. The mutagenesis primer for CgA387 (5'-GCCGCGTACCGCCGATCCCAGAAGAAGA-3') added a stop codon at position 388. Both mutagenic primers also added a BamHI restriction endonuclease site for screening. The identity of the CgA mutants was confirmed by DNA sequencing. All clones were tested for expression by transient transfection of GH4C1 cells. Expression and secretion of CgA, CgAΔCC, CgA341, and CgA387 were confirmed by immunoblotting of secretion media, while SEAP expression and secretion were confirmed by alkaline phosphatase activity.

**Transient Expression of CgA in GH4C1 or PC12 Cells**—Transfections of GH4C1 and PC12 cells were performed as described previously (11). The pcDNSA/CgA, pcDNSA/CgAΔCC, pcDNSA/CgA341, pcDNSA/CgA387, pcDNSA/SEAP plasmids (and pcDNSA as a control) were used to transiently transfect GH4C1 or PC12 cells in separate experiments. Prior to secretion or fractionation experiments, the cells were treated with 5 mM sodium butyrate for 2 h.

**Secretion Experiments**—These were performed as described previously (11) with minor modifications. Briefly, GH4C1 and PC12 cells cultured in six-well plates were washed with 1 ml of Krebs-Ringer-Hepes buffer (KRH) (129 mM NaCl, 10 mM Hepes, 5 mM NaHCO₃, 4.8 mM KCl, 2.8 mM glucose, 1.2 mM KH₂PO₄, 1.2 mM MgCl₂, and 1 mM CaCl₂ (pH 7.4)) for 15 and then 30 min. Stimulated secretion of GH4C1 cells was measured in 1 ml of KRH buffer containing a secretagogue mixture defined below. Unstimulated secretion was measured in the absence of secretagogues.

For experiments using cycloheximide treatment, GH4C1 cells were hormone-treated as described (11). CgA and CgA341 were transiently expressed in hormone-treated GH4C1 cells. The cells were treated for 4 × 30 min with 100 μg/ml cycloheximide prior to secretion experiments. Stimulated and unstimulated secretion media were then collected for 30 min in the presence of cycloheximide. Secretion was stimulated in 1 ml of KRH buffer supplemented with 50 mM KCl, 100 nM phorbol 12-myristate 13-acetate, and 1 μM Bay K8644. Unstimulated cells received 50 mM NaCl and 100 mM 4-α-phorbol 12,13-diacetate.

Secretion of CgA, CgA341, and SEAP transiently expressed in PC12 cells was measured for 30 min in either 1 ml of KRH (unstimulated) or in low salt KRH (NaCl reduced to 79 mM, no CaCl₂) supplemented with 50 mM KCl and 2 mM BaCl₂ (stimulated). For GH4C1 and PC12 cells, the secretion media were collected and centrifuged at 16,000 × g for 5 min at 4 °C to remove cell debris. The supernatants were transferred to new tubes, and Tween 20 and PMSF were added to final concentrations of 0.3% and 1 mM, respectively. EDTA was added to the GH4C1 medium to a concentration of 5 mM. The media containing secreted CgA and CgA mutants were heated at 100 °C for 10 min and centrifuged to remove denatured proteins. Soluble proteins in the supernatants were trichloroacetic acid-precipitated and analyzed by immunoblotting.

To quantitate SEAP secretion, aliquots of secretion medium were diluted in KRH (1:50 for GH4C1 cells or 1:10 for PC12 cells) and alkaline phosphatase activity was determined with the Phospha-Light alkaline phosphatase assay kit and quantitated using a Berthold LB 9501 luminometer (Wallac, Inc., Gaithersburg, MD).

**Pulse-Chase Experiments**—Transiently transfected GH4C1 cells in six well plates were pulse-labeled (2 h, 37 °C) with 50 μCi/well [3H]leucine (specific activity 154 Ci/mMol) in 1 ml/well KRH. Following the 2-h pulse, cells were washed briefly with 1 ml of KRH containing 2 mM leucine and then chased for seven consecutive 30-min intervals at 37 °C in 1 ml of KRH containing 2 mM leucine. The medium was replaced with fresh medium after each 30-min interval. Following the collection of basal secretion media for 3.5 h, the cells were incubated for 30 min in 1 ml of KRH containing 2 mM leucine, 50 mM KCl, 100 mM phorbol 12-myristate 13-acetate, and 1 μM Bay K8644 and secretion medium was collected. The secretagogue mix was used to ensure optimal stimulated secretion of stored proteins (24). Immediately after each 30-min secretion period, samples were collected and centrifuged at 16,000 × g for 2 min at 4 °C and the supernatants were transferred to new microcentrifuge tubes and frozen until immunoprecipitated. CgA immunoreactivity was immunoprecipitated in 0.1 ml sodium phosphate buffer (pH 7.4) using CgA antibody that had been bound to protein A-Sepharose. Only a negligible amount of CgA was not immunoprecipitated by this procedure, as revealed by a second immunoprecipitation with the supernatant fractions.

**Separation of Soluble and Membrane Fractions**—GH4C1 cells transiently transfected as described above were sliced from the plates into 1 ml of KRH containing 1 mM PMSF, 1 mM N-α-tosyl-L-lysine chloromethyl ketone, and 5 mM EDTA. The cells from duplicate wells were combined and lysed by scraping and repeated passage through a 29-gauge hypodermic needle. Each sample was transferred to a 1.5-ml microcentrifuge tube, frozen, and thawed twice followed by centrifugation at 200 × g for 5 min at 4 °C. The supernatant was collected and centrifuged again at 16,000 × g for 30 min at 4 °C. The resulting pellet was resuspended in 50 μl of the above buffer with or without 1% Triton X-100 and recentrifuged. After this centrifugation, the supernatant (soluble fraction) was transferred to a new tube and the pellet remaining was washed with 100 μl of KRH containing the protease inhibitors and recentrifuged. After washing, the pellet (membrane fraction) and the soluble fraction from above were analyzed by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and immunoblotting.

**Sucrose Gradient Separation**—Transiently transfected GH4C1 cells from three 35-mm culture dishes were lysed and resuspended above, in 750 μl of ice cold 0.3 M sucrose, 20 mM Heps (pH 7.4). The cell lysate was centrifuged at 500 × g for 5 min, and the supernatant fraction was layered on top of 750 μl of ice-cold 0.6 M sucrose, 20 mM Heps (pH 7.4) and centrifuged at 16,000 × g for 30 min at 4 °C. The 0.3 M and 0.6 M sucrose fractions and the pellet were adjusted to 1 ml total volume and 0.3% Tween 20. Total protein was precipitated with trichloroacetic acid in 0.5–0.45 M sucrose and analyzed by immunoblotting.
RESULTS

Chromogranins are highly conserved in both their N- and C-terminal regions. The N-terminal region is involved in sorting in PC12 cells but not in GH4C1 cells (11). To determine what regions of CgA were necessary for sorting in GH4C1 cells, we prepared three CgA mutants: CgAΔCC (missing the conserved disulfide bond), CgA387 (missing the conserved C-terminal peptide that is involved in dimerization and tetramerization), and CgA341 (lacking the putative aggregation domain) (Fig. 1). These proteins were expressed in endocrine GH4C1 cells.

Pulse-chase experiments were used to test the sorting of wild-type CgA and the three CgA mutants in GH4C1 cells. The cells were pulse-labeled for 2 h, after which chase media were collected every 30 min until the addition of secretagogues to stimulate granule release at 3.5 h of chase (Fig. 2). Wild-type CgA, CgAΔCC, and CgA387 each exhibited basal secretion that decreased over the first 2 h of chase incubation, consistent with the release of some non-stored CgA. Upon the addition of secretagogues, secretion of these three forms of CgA was strongly stimulated, consistent with granule storage (Fig. 2, compare the 3.5- and 4.0-h time points). In contrast, the CgA341 deletion mutant was completely secreted without stimulation and did not exhibit stimulated secretion (Fig. 2). These results suggested that CgA341 was secreted only constitutively in GH4C1 cells. Therefore, the secretion and subcellular localization of this mutant were further analyzed.

The secretion of total, immunoreactive wild-type CgA and CgA341 were compared in hormone treated cells (Fig. 3). Hormone treatment stimulates prolactin granule formation in GH4C1 cells (27, 28). We used the protein synthesis inhibitor cycloheximide to create a block of basal protein secretion (23, 29). Under these conditions only proteins that were stored in secretory granules prior to cycloheximide treatment would be available for stimulated secretion (23, 29). Indeed, secretion of wild-type CgA was stimulated 4.7-fold whereas secretion of CgA341 was only stimulated 2.0-fold under these conditions (Fig. 3). These findings suggested that, unlike wild-type CgA, which was sorted to the regulated secretory pathway, CgA341 was preferentially secreted by the constitutive or constitutive-like secretory pathway in GH4C1 cells.

Regulated secretory proteins are stored in dense core secretory granules, while constitutive secretory proteins are located in lighter Golgi and transport vesicles. To determine if wild-type CgA and CgA341 are located in different membrane compartments, a membrane pellet was prepared from transiently
transfected GH4C1 cells. The membrane pellet was resuspended to gently disrupt the membrane, and the extent of CgA release was determined by immunoblotting. Wild-type CgA was efficiently retained in the membrane fraction (less than 20% released), while CgA341 was readily released from the membrane fraction (75% released) (Fig. 4). The differential release of the two forms of CgA suggested that they are located in different membrane compartments. The near complete extraction of both forms of CgA with the addition of Triton X-100 showed that the proteins are enclosed in a membrane compartment, as opposed to forming an insoluble aggregate (Fig. 4). CgAΔCC and CgA387 were released similarly to wild-type CgA (not shown), consistent with their co-localization in secretory granules with wild-type CgA.

To further examine the subcellular localization of these proteins, a 0.3 and 0.6 M sucrose step gradient was used. Postnuclear supernatants were prepared in 0.3 M sucrose and then centrifuged on a 0.6 M sucrose cushion. The migration of CgA from 0.3 to 0.6 M sucrose was determined as an indicator of granule localization. The data in each experiment were normalized by dividing all values by the mean of the unstimulated samples (unstimulated secretion = 1, data not shown for clarity). Relative-stimulated secretion (open bars) = CgA in medium from stimulated cells/mean CgA in medium from unstimulated cells. Data from three to eight independent experiments are shown as mean ± S.E. (n = 6). CgA341 and SEAP are different from wild-type (p < 0.05), but are not different from each other. In separate experiments, media samples were used for aggregation experiments. Percentage of aggregation = (protein in pellet/protein in supernatant) × 100%. Net aggregation (filled bars) = % aggregation in the presence of calcium − % aggregation in the absence of calcium. CgA and SEAP aggregation data from three independent experiments are shown as mean ± S.E. (n = 6). CgA341 and SEAP are different from wild-type (p < 0.001), but are not different from each other. The data for wild-type include previously reported data that are shown for comparison (11).

GH4C1 cells require a C-terminal domain for sorting of CgA (this report). PC12 cells use an N-terminal sorting domain in chromogranin (11–14). To complete these data, we tested the sorting of CgA341 in PC12 cells. Sorting was assayed by immunoblotting of secretion medium collected in the presence or absence of secretagogues. Secretion of wild-type CgA and CgA341 were stimulated 47- and 65-fold, respectively. In con-
is sorted to immature secretory granules but retention of the hormone in mature granules is enhanced after conversion of non-aggregating proinsulin to aggregating insulin (32). Together with the present data, these reports suggest that aggregation plays a cell-specific role in sorting and retention of regulated secretory proteins.

Comparison of the sorting of total CgA and newly synthesized CgA suggests that CgA341 partially enters secretory granules (2–2.8-fold stimulation of total protein; see Figs. 3 and 6) but that this protein is not stored in mature granules (no stimulated secretion after 3.5-h chase period, Fig. 2). Instead, CgA341 appears to be secreted by the constitutive-like secretory pathway. This is consistent with the poor aggregation of CgA341 since aggregation is thought to play a role in sorting-by-retention in maturing secretory granules in endocrine cells (1, 3, 4, 6).

CgA contains two dimerization domains that have been proposed to play a role in binding to the regulated secretory pathway: an N-terminal dimerization domain that includes the disulfide bond (19) and a C-terminal dimerization/tetramerization domain (20, 21). The data for CgAΔCC and CgA387 now show that loss of either dimerization domain does not prevent sorting of CgA to secretory granules in GH4C1 cells (Fig. 2). Additionally, the C-terminal dimerization domain is not necessary for sorting of CgA in PC12 cells (Fig. 7). We cannot formally exclude that some of the CgA341 is sorted by forming heterotypic N-terminally linked dimers with endogenous CgA (13, 19). However, the very low expression levels of endogenous CgA (see control lanes of Fig. 7A) makes this an unlikely explanation for the bulk sorting of overexpressed CgA341 in PC12 cells. Thus, the physiological function of these dimerization domains remains unclear.

The N-terminal domain of CgA has been proposed to play a role in binding to granule membranes, but it is not clear how. We have previously noted that the N-terminal sorting domains of several regulated secretory proteins share similar secondary structures characterized by a hydrophobic domain that overlaps with an amphipathic α-helix (18). While it has been proposed that this structure interacts directly with lipid domains in the granule membrane (10), recent data suggest that this domain is not necessary for membrane binding of CgA in epithelial cells.2

As an alternative to direct membrane binding, CgA may bind to membrane-associated proteins. It has recently been shown that POMC binds to membrane-bound carboxypeptidase E (9). POMC exhibits an N-terminal hydrophobic domain similar to the one found in CgA (18). In addition, this domain of POMC contains a disulfide bond that is necessary for sorting of POMC and binding to carboxypeptidase E (9). While it is clear that CgA does not bind to carboxypeptidase E (11, 33), CgA may bind to other receptor-like proteins that are included in granule membranes.

The physiological significance of two independent sorting signals in CgA is not clear. It is possible that this is an adaptation to the widespread expression of chromogranins in most endocrine and neuroendocrine cells. Thus, the protein is equipped to enter secretory granules in cells that may exhibit different sorting mechanisms (4). It is of interest that, in a comparison of CgA sequences ranging from frog to man, the N- and C-terminal domains are the most highly conserved (34). An alternative, but not mutually exclusive, model is that the presence of multiple sorting signals is related to the physiological functions of CgA. The protein is a precursor for several biologically active peptides that play a role in the regulation of

2 U. Kühn and S.-U. Gorr, unpublished results.
endocrine secretion. These peptides are located in the N-terminal region, central region, and the C-terminal region of CgA (35). The presence of multiple sorting signals may ensure that N- and C-terminal fragments of CgA are correctly sorted after cell type-specific proteolytic processing of CgA. As an example, processing of CgA by furin is thought to produce parastatin and CgA347 (35). This shortened form of CgA is similar to the CgA341 tested in this report. Since furin processing takes place in the trans-Golgi network, the processed protein would contain the N-terminal but not the C-terminal sorting domain. Thus, this processing could determine the further routing and processing of CgA in the secretory pathway in different cell types. This proposed mechanism is similar to the processing of egg-laying hormones in neurons of Aplysia and Lymnaea. In these cells, cleavage by furin or furin-like enzymes determines the intracellular localization of the processing products in individual cell types (36, 37). However, unlike sorting of egg-laying hormones, which are sorted to different subcellular locations in individual cell types, the two sorting signals in CgA are used to reach equivalent secretory granules in different cell types.

Acknowledgments—We thank Dr. David V. Cohn for kindly providing the bovine CgA antisera. We thank Drs. Ulrike Kuhn and Renu Jain for helpful discussions and critical reading of the manuscript.

REFERENCES
1. Arvan, P., and Castle, D. (1998) Biochem. J. 332, 593–610
2. Kelly, R. B. (1985) Science 230, 25–32
3. Shennan, K. I. (1996) Curr. Opin. Cell Biol. 7, 496–500
4. Thiele, C., Gerdes, H. H., and Huttner, W. B. (1997) Curr. Biol. 7, R496–R500
5. Zhou, A., Webb, G., Zhu, X., and Steiner, D. F. (1999) EMBO J. 18, 1059–1070
6. Gorr, S. U., Kumarasamy, R., Dean, W. L., and Cohn, D. V. (1987) Bone Miner. 2, 251–255
7. Gerdes, H. H., Rosa, P., Phillips, E., Basuerle, P. A., Frank, R., Argos, P., and Huttner, W. B. (1989) J. Biol. Chem. 264, 12009–12015
8. Rindler, M. J. (1998) J. Biol. Chem. 273, 31180–31185
9. Cool, D. R., Normant, E., Shen, F., Chen, H. C., Pannell, L., Zhang, Y., and Loh, Y. P. (1997) Cell 88, 73–83
10. Tiele, C., and Huttner, W. B. (1998) Semin. Cell Biol. 9, 511–516
11. Gorr, S. U., Zhang, D. Y., Cowley, D. J., Kuliawat, R., and Arvan, P. (1999) Am. J. Physiol. 277, C121–C131
12. Grombik, M. M., Kromer, A., Salm, T., Huttner, W. B., and Gerdes, H. H. (1999) EMBO J. 18, 1059–1070
13. Kromer, A., Grombik, M. M., Huttner, W. B., and Gerdes, H. H. (1998) J. Cell Biol. 140, 1331–1346
14. Chanat, E., Weiss, U., Huttner, W. B., and Tooze, S. A. (1993) EMBO J. 12, 2159–2168
15. Normant, E., and Loh, Y. P. (1998) Endocrinology 139, 2137–2145
16. Chanat, E., Weiss, U., and Huttner, W. B. (1994) FEBS Lett. 351, 225–230
17. Canaff, L., Brechler, V., Reudelhuber, T. L., and Thibault, G. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9483–9487
18. Gorr, S. U., and Darling, D. S. (1995) FEBS Lett. 361, 8–12
19. Tiele, C., and Huttner, W. B. (1998) J. Biol. Chem. 273, 1223–1231
20. Yoo, S. H., and Lewis, M. S. (1993) Biochemistry 32, 6816–6822
21. Yoo, S. H., and Lewis, M. S. (1996) J. Biol. Chem. 271, 17041–17046
22. Gorr, S. U., Shini, J., and Cohn, D. V. (1989) Am. J. Physiol. 257, E247–E254
23. Gorr, S. U., and Moore, Y. R. (1999) Biochim. Biophys. Res. Commun. 257, 543–548
24. Hinkle, P. M., Scammell, J. G., and Shanshala, E. D. I. (1992) Endocrinology 130, 3503–3511
25. Laemmli, U. K. (1970) Nature 227, 680–685
26. Burnette, W. N. (1981) Anal. Biochem. 112, 195–203
27. Scammell, J. G., Burrage, T. G., and Dannies, P. S. (1986) Endocrinology 119, 1543–1548
28. Gorr, S.-U. (1996) J. Biol. Chem. 271, 3575–3580
29. Sachs, E., and Jamieson, J. D. (1992) Am. J. Physiol. 262, G257–G266
30. Demaurex, N., Furuya, W., D’Souza, S., Bonifacino, J. S., and Grinstein, S. (1998) J. Biol. Chem. 273, 2044–2051
31. Parmer, R. J., Xi, X. P., Wu, H. J., Helman, L. J., and Petz, L. N. (1993) J. Clin. Invest. 92, 1042–1054
32. Kuliawat, R., and Arvan, P. (1994) J. Cell Biol. 126, 77–86
33. Cool, D. R., and Loh, Y. P. (1998) Mol. Cell. Endocrinol. 139, 7–13
34. Turquier, V., Vaudry, H., Jegou, S., and Anouar, Y. (1999) Endocrinology 140, 4104–4112
35. Cohn, D. V., Fasciotto, B. H., Reese, B. K., and Zhang, J. X. (1995) J. Nutr. 125, Suppl., 2015S–2019S
36. Jung, L. J., Kreiner, T., and Scheller, R. H. (1993) J. Cell Biol. 121, 11–21
37. Klumperman, J., Spijker, S., van Minsen, J., Sharp-Baker, H., Smut, A. B., and Geraerts, W. P. (1996) J. Neurosci. 16, 7800–7949
