Role of the Differentially Spliced Carboxyl Terminus in Thromboxane A₂ Receptor Trafficking

IDENTIFICATION OF A DISTINCT MOTIF FOR TONIC INTERNALIZATION*

The thromboxane A₂ receptor (TP) is a G protein-coupled receptor that is expressed as two alternatively spliced isoforms, α (343 residues) and β (407 residues) that share the first 328 residues. We have previously shown that TPβ, but not TPα, undergoes agonist-induced internalization in a dynamin-, GRK-, and arrestin-dependent manner. In the present report, we demonstrate that TPβ, but not TPα, also undergoes tonic internalization. Tonic internalization of TPβ was temperature- and dynamin-dependent and was inhibited by sucrose and NH₄Cl treatment but unaffected by wild-type or dominant-negative GRKs or arrestins. Truncation and site-directed mutagenesis revealed that a YXXφ motif (where X is any residue and φ is a bulky hydrophobic residue) found in the proximal portion of the carboxyl-terminal tail of TPβ was critical for tonic internalization but had no role in agonist-induced internalization. Interestingly, introduction of either a YXXφ or YXXΦ motif in the carboxyl-terminal tail of TPα induced tonic internalization of this receptor. Additional analysis revealed that tonically internalized TPβ undergoes recycling back to the cell surface suggesting that tonic internalization may play a role in maintaining an intracellular pool of TPβ. Our data demonstrate the presence of distinct signals for tonic and agonist-induced internalization of TPβ and represent the first report of a YXXφ motif involved in tonic internalization of a cell surface receptor.

Cell surface receptors provide a primary mechanism by which cells perceive their environment. Many cell surface receptors are dynamically regulated and often undergo a process of endocytic sorting (1). For some receptors (e.g. G protein-coupled and growth factor), sorting is often initiated by hormon binding, whereas for others (e.g. low density lipoprotein and transferrin), the receptors undergo continuous or tonic internalization and recycling. Recent studies have demonstrated that several GPCRs including the CXCR4, thryosin, M₂ muscarinic, and thrombin receptors also undergo tonic internalization (2–5). Although no particular motif responsible for tonic internalization of GPCRs has been identified, tyrosine-containing (YXXφ) and dileucine motifs have been shown to be determinants for a number of other receptor types (6). Various studies have demonstrated direct interaction between YXXφ motifs and the μ chain of the clathrin-associated proteins AP-1, AP-2 (Ref. 6 and references therein), and AP-3 (7, 8), allowing the efficient targeting of transmembrane proteins containing these motifs to clathrin-coated vesicles.

Thromboxane has been implicated in a number of cardiovascular, bronchial, and kidney diseases (9, 10). It is produced by the sequential metabolism of arachidonic acid by cyclooxygenase and thromboxane synthase following activation of a variety of cell types including platelets, macrophages, and vascular smooth muscle cells (11). Thromboxane is a strong activator of platelet aggregation and smooth muscle cell proliferation and mediates its effects via interaction with a specific GPCR. The thromboxane A₂ receptor (TP) is encoded by a single gene that is alternatively spliced in the carboxyl terminus resulting in two variants, TPα (343 residues) and TPβ (407 residues) that share the first 328 amino acids (12–14).

In a previous study, we demonstrated that TPβ, but not TPα, undergoes agonist-induced internalization in a variety of cell types (15). Internalization of TPβ was dynamin-, GRK-, and arrestin-dependent in HEK293 cells, suggesting the involvement of receptor phosphorylation and clathrin-coated pits in this process. Additional characterization of the role of arrestins in this process revealed that arrestin-3 coexpression promoted agonist-induced internalization of both TPα and TPβ but not of a mutant truncated after residue 328. Analysis of various carboxyl-terminal deletion mutants revealed that a region between residues 355 and 366 in TPβ was essential for agonist-promoted internalization. During the course of these studies, we observed that TPβ, but not TPα, also undergoes tonic internalization. In the present study, we characterize the mechanisms involved in tonic internalization of TPβ. These studies reveal that a YXXφ motif found in the proximal portion of the carboxyl-terminal tail of TPβ is responsible for tonic internalization.

EXPERIMENTAL PROCEDURES

Cell Culture and Expression Systems—Human embryonic kidney cells (HEK293) were maintained in Dulbecco’s modified Eagle’s Medium (DMEM), 10% fetal bovine serum (FBS), penicillin/streptomycin, 2 mM L-glutamine, and 1 mM sodium pyruvate. Cells were grown at 37 °C in a humidified incubator with 5% CO₂. Human embryonic kidney cells (HEK293) were maintained in Dulbecco's modified Eagle's Medium (DMEM), 10% fetal bovine serum (FBS), penicillin/streptomycin, 2 mM L-glutamine, and 1 mM sodium pyruvate. Cells were grown at 37 °C in a humidified incubator with 5% CO₂.

Received for publication, October 13, 2000, and in revised form, November 26, 2000
Published, JBC Papers in Press, December 8, 2000, DOI 10.1074/jbc.M009375200

Jean-Luc Parent‡§¶, Pascale Labrecque§, Moulay Driss Rochdi‡, and Jeffrey L. Benovic‖

From the Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107 and the Service de Rhumatologie, Centre de Recherche Clinique, Université de Sherbrooke, Sherbrooke, Québec, Canada

Thromboxane A₂ receptor; GRK, G protein-coupled receptor kinase; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; HEK, human embryonic kidney; DEMEM, Dulbecco's modified Eagle's medium; BSA, bovine serum albumin; TBS, Tris-buffered saline; HA, hemagglutinin; PCR, polymerase chain reaction.

This paper is available on line at http://www.jbc.org

© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 276, No. 10, Issue of March 9, pp. 7079–7085, 2001 Printed in U.S.A.
Tonic Internalization of the Thromboxane A$_2$ Receptor

A schematic representation of the carboxyl terminus of the two isoforms of the human thromboxane A$_2$ receptor is shown in Fig. 1. To investigate tonic internalization of TP$_a$ and TP$_b$, epitope-tagged receptors were transiently expressed in HEK293 cells. Previous studies have shown that the agonist binding affinities of TP$_a$ and TP$_b$ are similar (14–17). Moreover, addition of a FLAG epitope at the amino terminus of the receptors did not alter ligand affinities, nor did it affect the activation characteristics of the receptors as determined by their respective EC$_{50}$ values for agonist-promoted inositol phosphate (data not shown).

During our immunofluorescence analysis of agonist-induced internalization of thromboxane A$_2$ receptors, we noted that TP$_b$ could undergo tonic internalization (15). To further characterize this phenomenon, we performed a series of immunofluorescence studies on cells transiently expressing FLAG-tagged TP$_b$ receptors. Cells were initially incubated with the FLAG antibody at 4°C for 1 h, washed, and then incubated at different temperatures so that tonic receptor internalization could be followed. As shown in Fig. 2A, there is significant redistribution of TP$_b$ to intracellular compartments following incubation at 37°C whereas minimal internalization of TP$_a$ is observed. Quantitation of intracellular and total cell fluorescence revealed that ~60% of TP$_b$ was tonically redistributed to a subcellular compartment following a 1-h incubation at 37°C. These observations confirm that TP$_b$, but not TP$_a$, undergoes significant tonic internalization. In contrast, when the cells are incubated for 1 h at 4°C or 16°C, TP$_a$ and TP$_b$ remain entirely
Tonic Internalization of the Thromboxane A<sub>2</sub> Receptor

at the cell surface. Interestingly, the inability of TPβ to undergo tonic internalization at 16 °C is a property shared with agonist-induced internalization of TPβ (data not shown) and the β<sub>2</sub>AR (18), but quite distinct from tonic internalization of the transferrin receptor which still occurs at 16 °C (data not shown). Thus, we next determined the role of these proteins in tonic internalization of TPβ. Immunofluorescence analysis of TPβ redistribution in the presence of dominant-negative mutants of dynamin (19), GRK2 (20), and arrestin-3 (21) was performed. Coexpression of dynamin-K44A inhibited tonic internalization (Fig. 2B), whereas GRK2-K220R and arrestin-3 (201–409) had no effect (data not shown). Interestingly, coexpression of dynamin-K44A, but not GRK2-K220R or arrestin-3 (201–409), also resulted in an ~2-fold higher cell surface expression of TPβ as assessed by ELISA (data not shown). In contrast, dynamin-K44A had no effect on cell surface expression of TPα (data not shown). When cells were preincubated with inhibitors of clathrin-coated pit formation such as sucrose and NH<sub>4</sub>Cl, tonic internalization of TPβ was also suppressed (Fig. 2B). Whereas these results demonstrate that tonic and agonist-induced internalization of TPβ are both dynamin-dependent, tonically internalized TPβ is targeted to clathrin-coated pits via a mechanism independent of GRKs and arrestins.

Because the carboxyl terminus of TPβ appears critical in tonic internalization of the receptor, we next determined whether this function could be ascribed to any particular residues. Progressive deletion mutants were first used to address this question (Fig. 1). All constructs were transiently transfected in HEK293 cells using transfection conditions that yielded comparable levels of receptor expression (~1 pmol/mg protein). The removal of up to 63 residues from the carboxyl terminus (S344Stop) appeared to have no effect on tonic internalization (Fig. 3), whereas agonist-induced internalization was completely blocked (15). However, truncation of an additional 7 amino acids (L337Stop) completely abolished tonic internalization (Fig. 3). Thus, the region found between residues 338 and 344 seems to play a critical role in tonic internalization of TPβ. Comparison of this region of TPβ (EYSGLTIS) with the corresponding region of TPα (TQRSGGLQ) suggests that Tyr-339 in TPβ might be an important component of a tonic internalization motif. A role for tyrosine-based internalization motifs in tonic endocytosis of a variety of receptors has been demonstrated (1, 6, 22). To test this hypothesis, we generated a Y339A mutant TPβ and characterized tonic and agonist-induced internalization. Indeed, TPβ-Y339A did not undergo any tonic internalization (Fig. 4), suggesting a critical role for Tyr-339 in this process. Additional amino acids between residues 338 and 344 in TPβ were then individually mutated to alanine in an attempt to identify a motif for tonic internalization. The E338A, S340A, G341A, T342A, and S344A mutants of TPβ appeared to undergo normal tonic internalization, whereas I343A was completely inhibited (Fig. 4). None of these mutations affected agonist-induced internalization of TPβ (Fig. 4C), suggesting that the motif for tonic internalization is distinct from the region required for agonist-induced trafficking of TPβ (15). Thus, our data demonstrate that the YXXX1 motif plays a critical role in tonic internalization of TPβ. This sequence is closely related to the YXXφ motif identified as playing an important role in tonic internalization of the transferrin receptor, T-cell receptor (CD3), Igα/ Igβ, C1-mannose-6-phosphate receptor, polymeric Ig receptor, and TGN38 receptor (23).

To further clarify the importance of Tyr-339 in tonic internalization, we generated Q338Y and R339Y mutants in the carboxyl terminus of TPα. Interestingly, introduction of a Tyr at position 339 in TPα, creating a YXXφ motif, induced tonic

**Fig. 2** Immunofluorescence analysis of TPα and TPβ distribution in HEK293 cells. A, FLAG-tagged receptors were transiently transfected in HEK293 cells. Cells were incubated with the FLAG antibody at 4 °C prior to any other treatment to detect receptors that were present initially at the cell surface. Immunofluorescence detection was performed as described under “Experimental Procedures.” Top panel, receptor distribution in cells expressing TPα (left) and TPβ (right) when incubated at 4 °C. Middle panel, after a 1-h incubation at 16 °C. Bottom panel, after a 1-h incubation at 37 °C. B, HEK293 cells, transiently transfected with TPβ, were labeled with FLAG antibody at 4 °C prior to incubation at 37 °C for 1 h. Tonic internalization of TPβ in the presence or absence of the TP receptor antagonist SQ29548, the inhibitors of clathrin-coated pit formation NH<sub>4</sub>Cl and sucrose, or dynamin-K44A was analyzed by immunofluorescence and quantitated. Data represent the percentage of intracellular immunofluorescence relative to total immunofluorescence of individual cells. Immunofluorescence was measured using the NIH Image I.62/Bf software. Results shown represent the mean ± S.E. of three independent experiments, where immunofluorescence of at least ten cells was evaluated for each experiment. Refer to “Experimental Procedures” for details.
internalization of TPα (Fig. 5). Mutation of Leu-342 to Ala in the R339Y mutant inhibited tonic internalization, demonstrating the importance of the hydrophobic residue in this process (Fig. 5). In an effort to determine the importance of the spacing between the Tyr and hydrophobic residues, we introduced a Thr between Gly-341 and Leu-342 in the R339Y mutant (R339Y-T342) to create a YXXXf motif similar to that found in TPβ. This latter addition did not affect the internalization induced by the Tyr residue in R339Y, verifying that both YX2f and YX3f can function as efficient internalization motifs. Interestingly, a Q338Y mutant (also creating a YX3f motif but one residue closer to the plasma membrane than in TPβ and TPα (R339Y-T342)) did not induce tonic internalization of TPα. This suggests that the position of the Tyr in the receptor carboxyl tail is also an important determinant in this process. As expected, none of these mutations conferred agonist-induced internalization of TPα (data not shown). Our data suggest that both the distance between the Tyr and the hydrophobic residue and the position of the YX2,3φ motif in the receptor carboxyl tail are important determinants in tonic internalization of the thromboxane receptor. It is interesting to note that a YLGI peptide sequence found in the second intracellular loop of both TP receptor isoforms is evidently not sufficient to induce tonic internalization because this is not observed for TPα. Moreover, mutation of residues within this motif in TPβ did not affect tonic internalization (data not shown).

Because tonic internalization has also been reported for other GPCRs (2–5, 24), it is important to consider the biological role of this process. It has been proposed that tonic internalization of the thrombin receptor generates an intracellular pool of receptors that is used to repopulate the cell surface with functional receptors (24). If a similar role can be attributed to tonic internalization of TPβ, we would expect that these receptors would recycle back to the cell surface following tonic internalization. To address this issue, cell surface receptors were labeled with M1 anti-FLAG antibody at 4 °C and then allowed to undergo tonic internalization for 1 h at 37 °C. The cells were washed briefly with PBS/EDTA to strip the cell surface antibody (which binds in a Ca2+-dependent manner), reincubated at 37 °C, fixed, and then receptor distribution determined by immunofluorescence. TPβ was only detected intracellularly after cell surface antibody was stripped with PBS/EDTA (Fig. 6, panel B). However, following incubation at 37 °C, there was extensive redistribution of the intracellular receptors to the cell surface (Fig. 6, panel C). This recycling was not caused by new protein synthesis or to transport of new receptors from intracellular stores because visualized receptors originated from the initial labeling of cell surface receptors with antibody. These data suggest that there is constant recycling of the tonically internalized TPβ between the cell surface and an unidentified intracellular compartment, similar to what has been observed for the thrombin receptor (24). Thus, tonic internalization of TPβ likely helps to maintain an intracellular pool of functional receptors that recycle to the cell surface to preserve agonist sensitivity.

![Fig. 3. Tonic internalization of different carboxyl tail truncation mutants of TPβ.](image-url)
The YXXΦ motif is one of the most extensively characterized motifs within cytosolic domains involved in the targeting of integral membrane proteins. Tyrosine-based sorting signals conforming to the YXXΦ motif have been shown to interact directly with the m1, m2, and m3 subunits of the adaptor complexes AP-1, AP-2, and AP-3, respectively (reviewed in Ref. 7). The critical tyrosine does not need to be phosphorylated and, in fact, the interaction of YXXΦ and Φ may actually be reduced by phosphorylation (25, 26). The AP-1 complex associates with the trans-Golgi network and directs the transport of lysosomal enzymes to endosomes, whereas the AP-2 complex associates with the plasma membrane and directs the trafficking of cell surface proteins via clathrin-coated pits. AP-3 is involved in the delivery of proteins to lysosomes and lysosome-related organelles (27). Recent studies also suggest that there is a fourth adaptor-related protein complex, AP-4, that is associated with nonclathrin-coated vesicles in the region of the trans-Golgi network (27, 28). The μ4 subunit of this complex specifically interacts with a tyrosine-based sorting signal, suggesting that AP-4 is also involved in the recognition and sorting of proteins with tyrosine-based motifs (27).

Ohno et al. (7) investigated the selectivity for interaction of tyrosine-based sorting signals with μ1, μ2, μ3A, and μ3B subunits via screening of a combinatorial XXXXXXΦ library using the yeast two-hybrid system. Their results revealed that there was no absolute requirement for the presence of specific residues at any of the Χ or Φ positions. This contrasted with the critical tyrosine residue that could not be substituted by any other residue without a dramatic decrease in sorting activity (6, 7, 29–32) and binding affinity for μ subunits (7, 25, 26, 33,
It was shown that each \( \mu \) subunit exhibits a preference for certain XXXY motifs; however, there was also considerable overlap in specificity (7). Although these studies did not characterize \( \mu \) interaction with XXXY motifs, the YXX\( \phi \) motif found in TP\( \beta \) displays a serine at position Y\(-3\) and a glutamic acid at Y\(-1\), analogous to one of the specific sequences that binds to \( \mu_2 \) (SFETYQPL) (7). Similarly, the asialoglycoprotein receptor has a threonine and a glutamic acid at Y\(-3\) and Y\(-1\) positions, respectively. A serine or threonine are also found at Y\(-3\) of the XXXY motif of the CI mannose-6-phosphate receptor, EGF receptor, and CTLA-4. Similar to TP\( \beta \), the CI mannose-6-phosphate receptor, poly\( \mathrm{Ig} \) receptor and HIV gp41 have a serine at position Y\(+1\) whereas the CD mannose-6-phosphate receptor and furin have a glycine at position Y\(+2\). On the other hand, the \( \mu_3A \) subunit preference for position Y\(-1\) is also a glutamic acid (7). Amino acid differences in or around the signal may possibly confer preferences for targeting to particular cell compartments. A glycine preceding the Tyr in a YXX\( \phi \) motif enhances targeting of lamp-1 and acid phosphatase to lysosomes (6 and references therein). The position of the motif within the cytoplasmic domain may also influence its activity (6). Indeed, displacement of the YXX\( \phi \) motif in lamp-1 by a single residue with respect to the transmembrane domain was reported to disrupt lysosomal targeting (35), similar to our observations in the TP\( \alpha \) mutants. Similar findings were also reported when a Tyr was inserted in the cytoplasmic terminus of influenza virus hemagglutinin to generate an artificial internalization signal. In these studies, internalization was dependent on the position of the Tyr relative to the cell membrane indicating that the structural environment of the Tyr was important (22).

Our results identified a distinct motif (YYX\( \phi \)) in the carboxyl terminus of a G protein-coupled receptor responsible for tonic internalization. Both the distance between the Tyr and the hydrophobic residue and the position of the YYX\( \phi \) motif in the receptor carboxyl tail appear to be important determinants in this process. In addition, secondary signals that function in concert with the YYX\( \phi \) motif may influence the destination of the receptor. Further analysis of sequence and contextual requirements for the function of the YYX\( \phi \) signal will be necessary to fully understand its specificity. Importantly, two adjacent sequences in the carboxyl terminus of TP\( \beta \), the YYX\( \phi \) motif and the region between residues 355 and 366, seem to distinguish between tonic and agonist-induced internalization, respectively. Molecular analysis of the interaction of these sequences with their recognition molecules will provide additional clues as to their role in receptor trafficking. For example, it will be interesting to determine whether YYX\( \phi \) and AP-2 directly interact and, if so, which AP-2 subunit contributes to such interaction. Comparatively, the region between residues 355 and 366 may dictate, at least in part, the interaction of the receptor with arrestins (15). This would suggest...
Tonic Internalization of the Thromboxane A<sub>2</sub> Receptor

7085

tight regulation of the factors involved. The \( Y_X \phi \) motif could be continuously available for interaction with proteins involved in tonic internalization whereas ligand occupancy of the receptor might expose the adjacent sequence to proteins involved in agonist-induced internalization.

In summary, our results demonstrate that the alternative splicing of the carboxyl terminus of the thromboxane A<sub>2</sub> receptor generates isoforms that show distinct trafficking characteristics. We had previously shown that TP<sub>b</sub> but not TP<sub>a</sub> could undergo agonist-induced internalization (15). In the present report, we demonstrate that only TP<sub>b</sub> is capable of undergoing tonic internalization. Tonic internalization was attributed to a \( Y_X \phi \) motif, which is distinct from the sequence required for agonist-promoted trafficking and is the first such motif identified for tonic internalization of a GPCR. These findings raise important questions concerning how trafficking differences between TP<sub>a</sub> and TP<sub>b</sub> might contribute to mechanistic differences in the desensitization, resensitization, and/or degradation of these receptors as well as in their overall cellular physiology. Similar studies on other GPCRs will help to further characterize the signals, proteins, and cellular compartments involved in these processes.

REFERENCES
1. Mukherjee, S., Ghosh, R. N., and Maxfield, F. R. (1997) Physiol. Rev. 77, 759–803
2. Signoret, N., Oldridge, J., Pelchen-Matthews, A., Klaase, P. J., Tran, T., Brass, L. F., Rosenkilde, M. M., Schwartz, T. W., Holmes, W., Dallas, W., Luther, M. A., Wells, T. N. C., Hoxie, J. A., and Marsh, M. (1997) J. Cell Biol. 139, 651–664
3. Baratti-Elbaz, C., Ghinea, N., Lahuma, O., Looofelt, H., Pichon, and Milgrom, L. F., Rosenkilde, M. M., Schwartz, T. W., Holmes, W., Dallas, W., Luther, M. A., Wells, T. N. C., Hoxie, J. A., and Marsh, M. (1997) J. Cell Biol. 139, 651–664
4. Roseberry, A. G., and Hosey, M. M. (1999) J. Biol. Chem. 274, 3816–3820
5. Shapiro, M. J., Trejo, J., Zeng, D., and Coughlin, S. R. (1996) J. Biol. Chem. 271, 12473–12478
6. Marks, M. S., Ohno, H., Kirchhausen, T., and Bonifacino, J. S. (1997) Trends Cell Biol. 7, 124–128
7. Ohno, H., Aguilar, R. C., Yeh, D., Taura, T., Saito, T., and Bonifacino, J. S. (1999) J. Biol. Chem. 274, 29515–29521
8. Le Borgne, R., Alconada, A., Bauer, U., and Hoflack, B. (1998) J. Biol. Chem. 273, 29451–29461
9. Halushka, P. V., Allan, C. J., and Davis-Bruno, K. L. (1995) J. Lipid Med. Cell Signal. 12, 361–378
10. Sparney, R. F., Middleton, J. P., Raymond, J. R., and Coffman, T. M. (1994) Am. J. Physiol. 267, F467–F478
11. Smith, W. L. (1992) Am. J. Physiol. 263, F181–F191
12. Morinelli, T. A., and Halushka, P. V. (1991) Trends Cardiovasc. Med. 4, 178–183
13. Hirata, M., Hayashi, Y., Ushikubi, F., Yokota, Y., Kageyama, R., Nakanishi, S., and Narumiya, S. (1991) Nature 349, 617–620
14. Raychowdhury, M. R., Yakuwa, M., Collins, L. J., McGraal, S. H., Kent, K. C., and Ware, J. A. (1994) J. Biol. Chem. 269, 19256–19261; Correction (1995) J. Biol. Chem. 270, 7011
15. Parent, J.-L., Labreque, P., Orsini, M. J., and Benovic, J. L. (1999) J. Biol. Chem. 274, 8941–8948
16. Hirata, T., Ushikubi, F., Kakizuka, A., Okuma, M., and Narumiya, S. (1996) J. Clin. Invest. 97, 949–956
17. Habib, A., Vezza, R., Creminon, C., Maclouf, J., and FitzGerald, G. A. (1997) J. Biol. Chem. 272, 7191–7200
18. Cao, T. T., Mays, R. W., and von Zastrow, M. (1998) J. Biol. Chem. 273, 24592–24602
19. Damke, H., Baba, T., Warnock, D. E., and Schmid, S. L. (1994) J. Cell Biol. 127, 915–934
20. Kong, G., Penn, R., and Benovic, J. L. (1994) J. Biol. Chem. 269, 13084–13087
21. Orsini, M. J., and Benovic, J. L. (1998) J. Biol. Chem. 273, 34616–34622
22. Trowbridge, I. S., and Collawn, J. F. (1993) Annu. Rev. Cell Biol. 9, 129–161
23. Mellman, I. (1996) Annu. Rev. Cell Dev. Biol. 12, 575–625
24. Shapiro, M. J., and Coughlin, S. R. (1998) J. Biol. Chem. 273, 29009–29014
25. Ohno, H., Fournier, M. C., Poy, G., and Bonifacino, J. S. (1996) J. Biol. Chem. 271, 29009–29015
26. Shiratori, T., Miyatake, S., Ohno, H., Nkaseko, C., Isono, K., Bonifacino, J. S., and Saito, T. (1997) Immunity 6, 583–589
27. Hirst, J., Bright, N. A., Roux, S., and Robinson, M. S. (1999) Mol. Biol. Cell 10, 2677–2702
28. Dell’Angelica, E. C., Mullins, C., and Bonifacino, J. S. (1999) J. Biol. Chem. 274, 7276–7285
29. Canfield, W. M., Johnson, K. F., Ye, R. D., Gregory, W., and Kornfield, S. (1991) J. Biol. Chem. 266, 5682–5688
30. Collawn, J. F., Kuhn, L. A., Liu, L.-F. S., Tainer, J. A., and Trowbridge, I. S. (1991) EMBO J. 10, 3247–3253
31. Jadot, M., Canfield, W. M., Gregory, W., and Kornfield, S. (1992) J. Biol. Chem. 267, 11069–11077
32. Naim, H. Y., and Roth, M. G. (1994) J. Biol. Chem. 269, 3928–3933
33. Ohno, H., Stewart, J., Fournier, M. C., Bossart, H., Rhee, I., Miyatake, S., Saito, T., Gallusser, A., Kirchhausen, T., and Bonifacino, J. S. (1995) Science 269, 1872–1875
34. Boll, W., Ohno, H., Songyang, Z., Rapoport, L. C., Bonifacino, J. S., and Kirchhausen, T. (1996) EMBO J. 15, 5789–5795
35. Rohrer, J., Schweizer, A., Russel, D., and Kornfeld, S. (1996) J. Cell Biol. 132, 577–584