Molecular Characterization of the Signal Responsible for the Targeting of the Interleukin 2 Receptor β Chain toward Intracellular Degradation*

(Received for publication, February 17, 1998, and in revised form, July 31, 1998)

Agathe Subtil‡, Anna Rocca‡, and Alice Dautry-Varsat§

From Unité de Biologie des Interactions Cellulaires, URA CNRS 1960, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France

During receptor-mediated endocytosis, most growth factor receptors are transported to late endocytic compartments and degraded. This process is important to control their expression on the cell surface and requires sorting in early endocytic compartments. Little is known about the mechanisms and the signals involved. We have studied the signal involved in targeting the interleukin 2 receptor β chain (IL2Rβ), a member of the cytokine receptor superfamily, toward degradation after internalization. We show that a motif of 8 amino acids in the cytosolic tail of IL2Rβ is sufficient to target a normally recycling receptor toward degradation. Deletion of this signal strongly impairs IL2Rβ degradation. Further molecular characterization of the motif shows that it does not resemble the well documented tyrosine and dileucine families of trafficking signals.

Receptor-mediated endocytosis is a process used by cells for rapid and specific uptake of extracellular macromolecules. After their internalization from the plasma membrane, receptors travel from one organelle to another. They may recycle back to the cell surface, thus following the default pathway, or reach other compartments such as late endosomes and lysosomes, the trans-Golgi network, or other specialized compartments. Sorting may take place at several steps during this journey, to ensure that each receptor reaches its proper destination (1).

Sorting mechanisms described to date involve short amino acid sequences carried by the cytosolic region of the membrane protein to be routed. A large majority of these motifs, which target receptors to various intracellular compartments, resemble the well described internalization signals, which allow receptors to interact with clathrin-coated pits (2). They have been classified in two groups, a tyrosine-based motif and a dileucine-based motif (3).

Many internalization signals have now been described, but much less is known about the characteristics of the motifs involved in targeting to late endocytic compartments and to lysosomes. Most of our knowledge comes from studies on lysosomal membrane glycoproteins. After synthesis, these proteins are destined to late endocytic compartments after endocytosis are the growth factor receptors. In this case, degradation of the receptors plays an important role in controlling their expression and consequently the cellular response to growth factors. Little is known about the motifs involved in their sorting.

The cytokine interleukin 2 (IL2) is produced by activated helper T lymphocytes and stimulates proliferation and effector functions of a variety of cells of the immune system. High affinity IL2 receptors (Kd ~ 10–100 pM) are composed of three distinct components, the α, β, and γ chains, which are associated in a noncovalent manner (9). Both the β and γ chains, but not the α chain, belong to the cytokine receptor superfamily (10). This hematopoietic cytokine receptor family includes receptors for several cytokines, for erythropoietin, the granulocyte colony-stimulating factor, the granulocyte-macrophage colony-stimulating factor, the leukemia inhibitory factor, growth hormone, prolactin, and ciliary neurotrophic factor. Many receptor subfamily members share at least one component; thus, the receptors for IL2, -4, -7, -9, and -15 have a common γ chain, and the receptors for IL2 and IL15 share the β chain (reviewed in Ref. 11).

One of the early events following IL2 binding to high affinity receptors on the cell surface is the internalization of IL2 receptor complexes (12, 13). After endocytosis, the components of this multimolecular receptor have different intracellular fates: one of the chains, α, recycles to the plasma membrane, while the others, β and γ, are routed to late endocytic compartments (14).

We have previously shown that the interleukin 2 receptor β chain (IL2Rβ) is constitutively endocytosed and degraded when expressed in cells lacking the α or the γ chains (15). A truncated form of IL2Rβ, IL2Rβc, composed of the extracellular and transmembrane domains followed by the first 27 cytosolic amino acids out of 286, is also efficiently internalized and degraded (16). We have reported that the IL2Rβc construct carries several weak internalization signals within the transmembrane and

* This work was supported by CNRS, action Biologie Cellulaire. 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.

‡ These authors contributed equally to this work.

§ To whom correspondence should be addressed. Tel.: 33-1-45-68-85-74; Fax: 33-1-40-61-32-38; E-mail: adautry@pasteur.fr.

1 The abbreviations used are: MHC, major histocompatibility complex; EGF, epidermal growth factor; IL2, interleukin 2; IL2Rβ, IL2 receptor β chain; PCR, polymerase chain reaction.
cytosolic domains, acting in an additive fashion (17). Furthermore, its cytosolic tail contains a signal that is sufficient to target a recycling receptor toward degradation (16). Here we show that this signal is unique since, when it is deleted, IL2Rβ is no longer degraded. We have further characterized this sequence by mutational analysis. It does not have the characteristics of the tyrosine or dileucine families of signals.

**EXPERIMENTAL PROCEDURES**

**Cells and Monoclonal Antibodies**—K562, a human erythroleukemia cell line, was grown in suspension in RPMI 1640, 10% decomplemented fetal calf serum, supplemented with 2 mM l-glutamine. Stably transfected K562 cells were grown in the same medium supplemented with 1.5 mg/ml G418.

Monoclonal antibodies 7G7B6 (IgG2a) or 2A3A1H (IgG1) directed against the α chain of the IL2 receptor were obtained from the American Type Culture Collection (Rockville, MD) (18). Monoclonal antibodies 341 (IgG1) and 561 (IgG2a), directed against IL2Rβ, were kind gifts from Dr. R. Robb (Dupont Merck Pharmaceutical Co., Wilmington, DE) (19). Phycoerythrin-conjugated goat F(ab′)2 anti-murine IgG was obtained from Immunotech (Marseilles, France).

**Plasmids**—All β2-m and αβ2-m chimeras were generated by polymerase chain reaction (PCR) as described (16). The truncated form of the human IL2Rβ lacking cytosolic residues 22–27 (β2-m 22–27) was constructed by PCR amplification of the cDNA (16). The cDNA product was cloned into the Sac I restriction site overlapping the junction between residues 22 and 23. The fragment was then used to replace the corresponding Sac I site in the IL2Rβ expression vector using the NotI/Sac I restriction sites. The fragment coding for amino acid 28 to the last amino acid (286) of the β chain was then added using the unique Sac I site in IL2Rβ cDNA.

**Cell Transfection**—All kinetics and half-life analyses described here have been performed in stably transfected K562 cells. To generate stable transfectants, 7 × 10^6 K562 cells were washed once in Dulbecco’s modified Eagle’s medium, 4.5 g/liter glucose and resuspended in 800 µl of the same medium, with 20 µg of the plasmid of interest. Electroporation was performed using the EasyJet electroporator (Eurogentec) with a single pulse, 240 V, 1500 microfarads. Selection with 1.5 mg/ml G418 (Geneticin, Life Technologies, Inc.) was initiated 2 days after transfection, and the cells were cloned in 96-well dishes. G418-resistant clones were assayed for expression by flow cytometry using anti-α (7G7B6) or anti-β (341) antibodies. The expression levels of recombinant proteins in all clones tested were the same or less than their normal level in activated lymphocytes.

**Internalization Assays**—Internalization of the different β constructs was measured using radiolabeled anti-β antibody. Antibody 561 was radioiodinated with 125I by the chloramine T method to a specific activity of 2–10 µCi/µg. Cells (2 × 10^7) were incubated in 100 µl of RPMI-Hepes, pH 7.2, 1 mg/ml bovine serum albumin, at 37 °C, and 1–5 nM 125I-labeled antibody is represented. All experiments were done at least three times, with different clones expressing the same construct. The means ± S.E. are shown.

**RESULTS**

**The IL2Rβ Cytosolic Domain Contains a Unique Sorting Signal to Target the Receptor toward Degradation after Endocytosis**—After internalization, IL2Rβ is targeted to late endocytic compartments and is degraded. We have previously described a 10-amino acid sequence, in its cytosolic domain, which functions as a sorting signal for the receptor (16). IL2Rβ cytosolic domain is 286 amino acids long and could contain more than one sorting signal. We constructed a mutated receptor in which an essential part of the signal we have characterized (amino acids 22–27) was deleted (β22–27). To analyze the fate of this receptor during endocytosis, we measured its half-life on the cell surface when protein synthesis was inhibited. We have previously shown that, under these conditions, the loss of IL2Rβ from the cell surface can be equated with degradation as measured in a pulse-chase labeling experiment (16). The half-life of β22–27 on the cell surface was very long, more than 8 h, compared with that of IL2Rβ, 3 h (Fig. 1A). This difference was not due to a defect in internalization since both proteins were internalized with similar kinetics (Fig. 1B). This result indicates that β22–27 is no longer sorted to late endocytic compartments, but is recycled back to the cell surface after internalization. Thus, IL2Rβ cytosolic domain contains a unique sorting signal that targets the receptor toward degradation after endocytosis.

**The Rate of Endocytosis of β22–27 Is Not the Limiting Factor for Its Degradation**—We have shown that IL2Rβ sorting signal functions in β22–7, a truncated form with only the first 27 cytosolic amino acids of IL2Rβ. We decided to better define IL2Rβ sorting signal by inserting point mutations in β7 and measuring the half-life on the cell surface of these mutant proteins.
Tyrosine-based Internalization Motifs—Most sorting signals also promote endocytosis. Although the IL2R described to date resemble internalization motifs, and indeed the cell surface was not accelerated compared with that of Ser-25, deletion of Ser-21 or Lys-22 did not modify β27 half-life. These results suggest that amino acids 20–23 are not essential for the IL2Rβ sorting signal function.

Mutation of Pro-23 to a tyrosine or an alanine increased the half-life of β27 to 165 and 250 min, respectively. This was not only due to a decrease in internalization compared with β27 since β27S25, which was internalized with similar kinetics as β27F23Y and β27F23A, had a 125-min half-life. This result shows that Pro-23 is an important amino acid in IL2Rβ sorting signal. Its replacement by another aromatic amino acid impairs the signal only slightly, but replacement by an alanine increased the half-life of β27 2-fold.

As reported above, mutation of Pro-24 to an alanine or a proline increased β27 half-life to 180 and 200 min, respectively, a delay that could be entirely due to a defect in internalization, since β27F24A and β27F24P are internalized about 3-fold slower than β27. The half-life of β27F24Y is slightly longer than that of β27, despite an accelerated rate of entry.

Deletion or substitution of Ser-25 by a glycine had no effect on β27 half-life. Thus, Ser-25 is not an essential amino acid in IL2Rβ sorting signal. On the contrary, deletion or mutation of Gln-26 of β27 had a dramatic effect; the half-life of both constructs increased to about 400 min, while their internalization was faster than that of β27S25. These results show that Gln-26 is important for the IL2Rβ sorting signal function.

Finally, we investigated the role of Leu-27 in this signal. The dramatic effect of its deletion (β27L27P, half-life was more than 12 h) suggested that this residue played an essential role in IL2Rβ sorting signal (16). Replacement of Leu-27 by an isoleucine slightly increased β27 half-life, while replacement by a proline and a threonine increased it about 2-fold. The internalization rates of β27L27P and β27L27T were lower than that of β27, but similar to that of β27S25, and therefore do not account for the half-life increase. These results suggest that Leu-27 plays an essential role in IL2Rβ sorting signal.

Analysis of IL2Rβ Sorting Signal Using Chimeric Proteins—In our previous study, we had shown that a 10-amino acid sequence in IL2Rβ was sufficient to mediate sorting of a recycling receptor, α1, toward degradation (16). The α1 chimera was constructed by inserting the transferrin receptor internalization motif at the carboxyl-end of the IL2 receptor α chain cytosolic domain. We have shown that this chimera was efficiently internalized and recycled back to the cell surface. In contrast, α1β18–27, in which amino acids 18–27 from IL2Rβ had been added to the cytosolic domain of α1, was sorted toward degradation after internalization. We constructed a shorter chimera, α1β20–27. Its half-life on the cell surface was the same as that of α1β18–27, 200 min, showing that IL2Rβ sorting signal can be limited to 8 amino acids (Fig. 4). Studies

![Fig. 2. Endocytosis (A) and cell surface half-life (B) of different β27 mutants in K562 cells. Experiments were performed as described in Fig. 1. The results are the means ± S.E. of at least three (A) or four (B) independent experiments using different clones for each construct.](image-url)
on the β27 mutants presented here suggested that, within these 8 residues, amino acids between Phe-23 and Leu-27 were important for sorting. We prepared a chimera between a Y and these 5 amino acids, aYb23–27. While aYb23–27 was as efficiently internalized as the aYb20–27 chimera, its half-life on the cell surface was 350 min, indicating that its sorting was impaired (Fig. 4). As shown in Fig. 4, only 4 amino acids separate 23FFSQL27 from the YTRF internalization signal in aYb23–27, while in aYb20–27 there is a 7-amino acid spacing between the two signals. A minimal spacing between these two motifs might be necessary for the sorting signal to function. To test this hypothesis, we studied the trafficking of a chimera with a three-alanine spacer between the internalization and lysosomal sorting signals, aYAAAβ23–27. This chimera was internalized with the same kinetics as aYb20–27 but was poorly degraded, showing that the difference in spacing between the two signals is not sufficient to account for the prolonged half-life of the aYβ23–27 chimera.

FIG. 3. Comparison of the internalization rates and the cell surface half-lives of different β27 mutants in K562 cells. Experiments were performed as described in Fig. 1. The internalization rates (A) or half-life values (B) are shown. The results are the means ± S.E. of at least three (A) or four (B) independent experiments using different clones for each construct. The carboxyl-terminal sequences of these mutants, starting with the 20th (Pro) cytosolic amino acid, are shown on the left.

FIG. 4. Endocytosis and cell surface half-life of aYβ20–27, aYβ23–27, and aYAAAβ23–27 in K562 cells. A, kinetics of internalization in cells stably transfected with aYβ20–27 (○), aYβ23–27 (●), or aYAAAβ23–27 (▲). Cells were labeled with anti-IL2Rα mAb at 4 °C, then incubated for the indicated times at 37 °C. Appearance of anti-IL2Rα mAb from the cell surface upon internalization was assessed by cytofluorimetry, as described under “Experimental Procedures.” B, half-life of aYβ20–27 (○), aYβ23–27 (●), or aYAAAβ23–27 (▲). Cell surface expression was assessed by cytofluorimetry on cells treated for different times with 50 μM cycloheximide. The results are the means ± S.E. of at least three (A) or four (B) independent experiments.

The IL2Rβ chain is constitutively internalized and targeted to late endocytic compartments, where it is degraded (14, 16). We had previously shown that amino acids 18–27 of the IL2Rβ cytosolic domain are sufficient to target a recycling receptor toward degradation. Here, we show that this signal is unique in the IL2Rβ, and that it is comprised within amino acids 20–27. We further characterize this sorting signal by mutational analysis. Several sorting signals have been described since the identification of the low density lipoprotein receptor internalization motif 12 years ago (28). Most of them fall into two categories: the tyrosine- and the dileucine-based motifs. These families have been first described and best characterized in the case of the internalization signals, but are also encountered as signals for sorting to the trans-Golgi network, to late endocytic compartments, to MHC class-II compartments, or for polarized

DISCUSSION

The IL2Rβ chain is constitutively internalized and targeted to late endocytic compartments, where it is degraded (14, 16). We had previously shown that amino acids 18–27 of the IL2Rβ cytosolic domain are sufficient to target a recycling receptor toward degradation. Here, we show that this signal is unique in the IL2Rβ, and that it is comprised within amino acids 20–27. We further characterize this sorting signal by mutational analysis. Several sorting signals have been described since the identification of the low density lipoprotein receptor internalization motif 12 years ago (28). Most of them fall into two categories: the tyrosine- and the dileucine-based motifs. These families have been first described and best characterized in the case of the internalization signals, but are also encountered as signals for sorting to the trans-Golgi network, to late endocytic compartments, to MHC class-II compartments, or for polarized
sorting (for reviews, see Refs. 3, 4, and 26). An important characteristic of these signals is that, in addition to a specific sorting step, they also promote internalization from the plasma membrane. In some cases, several signals are found in the same receptor and can have an additive effect. Another important feature is that these signals do not consist of a fixed amino acid sequence, but are relatively flexible. Some positions in the signal, such as the tyrosine itself, or one leucine, tolerate only very limited change, while others are less restricted. Our study allows to draw a comparison between the IL2Rβ sorting signal and the other well documented trafficking signals.

The IL2Rβ Sorting Signal Functions as Other Trafficking Signals—On one hand, the IL2Rβ sorting signal shares many properties with other trafficking motifs. It is also a short amino acid sequence, which functions in different protein surroundings (here the IL2Rβ or α2), and which tolerates limited change in its composition. Leu-27 is clearly an essential residue in this signal, since even a conservative substitution, by an isoleucine, slightly increased the half-life of β2L721, a defect that was further enhanced by substitution by a proline or a threonine, and since deletion of Leu-27 totally impaired sorting (16). Deletion or mutation of Gln-26 increased the half-life of β27 more than 3-fold, showing that Gln-26 is important for the signal to function. Serine 25, which could be deleted or replaced by a glycine without any consequence on the half-life, is not a crucial residue. The same seems to be true for Ser-21 and Lys-22, whose deletion did not affect sorting, and for Pro-20, which could be replaced by a leucine (16). Phe-23 and Phe-24 could each be replaced by another aromatic residue, a tyrosine, with only a small increase in the half-life. Replacement by an alanine further impaired sorting, especially in β22P23A, whose half-life was twice as long as that of β27, suggesting that these aromatic residues play a role in the signal function. In summary, our results show the importance of the FFSQL motif for lysosomal targeting of IL2Rβ, residues Phe-23, Gln-26, and Leu-27 being critical within this motif.

Another feature of IL2Rβ sorting signal common to other trafficking motifs is that many of these signals are not extremely efficient, but perform their function in an iterative manner. We measured that half of the cell surface IL2Rβ gets internalized in about 20 min, while the half-life of the receptor on the cell surface is about 180 min. Cycloheximide, used in the half-life measurement, does not slow receptor entry (data not shown). These results imply that the IL2Rβ recycles several times between the cell surface and endosomes before it gets sorted to late endocytic compartments. The same is true for the lysosomal proteins that are delivered to the plasma membrane before being sorted to lysosomes, and for the epidermal growth factor (EGF) receptor (29, 30).

We had previously shown that IL2Rβ sorting signal is part of an α-helix (16). Again, several other trafficking motifs have also been located to structured domains of receptors, either in an α-helix or in β-turns (8, 31–35). Another point in common is that in none of these examples is it known whether the structure is important for the signal to function. In vitro studies, on the recognition of internalization motifs placed in different protein environments, suggest that the surrounding amino acids, which will determine the overall structure, are not important for the signal recognition (27). Here we found that replacement of Ser-25 by a glycine did not affect sorting. Secondary structure predictions using AGADIR program (36), allowing to detect the α-helix tendencies of peptides, showed that the α-helix is very likely disrupted in the β27S25G mutant. This result therefore suggests that IL2Rβ sorting signal structure is not important for its function, as long as it is accessible to the cell machinery that recognizes it. In this respect, it is interesting to note that IL2Rβ sorting signal is more efficient at the COOH-terminal end of β27 (half-life ~ 130 min) than in IL2Rβ cytosolic tail (half-life ~ 180 min). A carboxyl-terminal location is also found in most, if not all, lysosomal proteins, and it has been suggested that this location promotes signal recognition and subsequently enzyme transport to the lysosomes (37).

The IL2Rβ Sorting Signal Is Different from Other Trafficking Motifs—On the other hand, the IL2Rβ sorting signal differs in several ways from the trafficking motifs described so far. First, although it includes a FFSQL sequence, reminiscent of tyrosine-based sequences, we show here that IL2Rβ sorting signal does not share the requirements of this category of signals. Mutation of Phe-24 to a tyrosine increased the endocytic rate of the mutant, without decreasing its half-life. Interestingly, the replacement of the tyrosine in the LAMP1 motif by alanine was shown to impair targeting to lysosomes (38). In our case, mutation to an alanine increased the half-life only slightly, a difference that could be entirely due to the 3-fold decrease on the endocytic rates of this mutant.

Second, the IL2Rβ sorting signal is not by itself an internalization motif (16). The observation that β22L27, in which it is deleted, is internalized with similar kinetics as IL2Rβ also shows that this sequence is not critical for the receptor internalization. However, we have shown that β27 internalization is promoted by several sequences in its cytosolic and transmembrane domains, and this sorting signal, together with other sequences, may also be involved in β24 internalization (17).

Third, transmembrane proteins often contain several signals, which seem to have redundant roles in targeting (39–41). This redundancy has also been illustrated in the sorting toward degradation of the EGF receptor and of the γ and δ chains of the T cell receptor (6, 30, 42). Although IL2Rβ cytosolic domain is quite large, it contains a unique signal for sorting to degradation after endocytosis, since the receptor is no longer degraded when this signal is deleted in β22L27. This last characteristic seems to be shared by some other cytokine receptors, the γ chain of the IL2R, the gp130 chain of the IL6 receptor (43, 44).

A novel lysosomal targeting signal has been recently characterized within the cytosolic tail of P-selectin (45, 46). It shares some features with the IL2Rβ sorting signal; it does not belong to the tyrosine- or dileucine-based families, it does not function as an internalization motif, and it appears to be the unique degradation signal. This sorting signal (KCP1L4) shares no obvious sequence homology with that of IL2Rβ (FFSQL).

What Might Recognize the IL2Rβ Sorting Signal?—Very little is known about the cellular machinery responsible for targeting membrane proteins to late endocytic compartments and lysosomes. Lysosomal proteins might be sorted to lysosomes directly from the trans-Golgi network by interaction of their sorting motif with the μ1 chain of AP-1 adaptors (47, 48). The EGF receptor has been the principal model to study plasma membrane receptor degradation after endocytosis. Identification of a protein that interacts with its lysosomal targeting code has been reported, but it might be specific for this receptor (49). COPI proteins, known for their role in intra-Golgi transport, might also be involved in endosome function, but the protein(s) that interact specifically with sorting signal remain to be identified (50–52).

Where Does Signal Recognition Take Place?—The location where the signals for sorting to late endocytic compartments are recognized is also unknown. It is generally accepted that membrane proteins, even internalized by different pathways, reach a common endosome (53). In this compartment, sorting physically occurs; membrane proteins carrying trafficking signals are targeted according to this information toward different compartments, while signal-free proteins are trafficked to the
revising endosome by a default mechanism, and then back to the cell surface (54). Sorting signals might be recognized in the sorting endosome, but not necessarily. Recognition could also take place earlier in the endocytic pathway, even before internalization proceeds. This model is supported by our observation that the endocytic rate is not, to some extent, a limiting factor for degradation, in agreement with another report (55). For example, \( \beta_{27} \), which was internalized 2-fold faster than \( \beta_{27}-\text{AS25} \), was not degraded faster. If the signal recognition took place after internalization, one could expect that a faster rate of entry, which results in a higher intracellular distribution at equilibrium, would favor signal recognition and therefore degradation. However, if sorting signals were recognized at the plasma membrane, this step would be favored in slowly internalized receptor, a positive effect that could compensate for the negative effect of slow internalization.

The identification and characterization of the molecular signals that target membrane proteins toward degradation compartments is only at its beginning. Our detailed study on the IL2R\( \beta \) chain degradation signal may help to identify similar signals in other receptors. It may also open the way to the identification of the cellular components that interact with this signal.

Acknowledgments—We are grateful to Dr. M. Delepine for computer simulation, and we thank Drs. C. Bennerot, P. Cosson, and T. Kono for providing plasmids NT, T-XO, and \( \beta_{27} \)-KCR\( \beta \), respectively, and Dr. R. Robb for the gift of antibodies 341 and 561. We are grateful to Dr. T. E. McGraw for critical reading of the manuscript.

REFERENCES

1. Mellmann, I. (1996) Annu. Rev. Cell Dev. Biol. 12, 575–625
2. Kirchhausen, T., Bonifacino, J. S., and Riezman, H. (1997) Curr. Opin. Cell Biol. 9, 488–495
3. Marks, M. S., Ohno, H., Kirchhausen, T., and Bonifacino, J. S. (1997) Trends Cell Biol. 7, 124–128
4. Sandoval, I. V., and Bakke, O. (1994) Trends Cell Biol 4, 292–297
5. Shin, J., Dunbrack Jr, R. L., Lee, S., and Strominger, J. L. (1991) J. Biol. Chem. 266, 10658–10665
6. Letourneur, F., and Klausner, R. D. (1992) Cell 69, 1143–1157
7. Marks, M. S., Roche, P. A., van Dioselaar, E., Woodruff, L., Peters, P. J., and Bonifacino, J. S. (1995) J. Cell Biol. 131, 351–369
8. Motta, A., Bremnes, B., Morelli, M. A. C., Frank, R. W., Saviano, G., and Bakke, O. (1995) J. Biol. Chem. 270, 27165–27171
9. Minami, Y., Kono, T., Miyaizaki, T., and Taniguchi, T. (1993) Annu. Rev. Immunol. 11, 245–267
10. Bazan, J. F. (1990) Eur. J. Biochem. 525–530
11. Robin, L. A., Kaitman, C. C., Fritx, M. E., Biddekin, W. E., Boutin, B., Yarchoan, R., and Nelson, D. L. (1988) J. Immunol. 135, 3172–3177
12. Voss, S. D., Leary, T. P., Sondel, P. M., and Robb, R. J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2428–2432
13. Duprez, V., and Dautry-Varsat, A. (1986) J. Biol. Chem. 261, 15450–15454
14. Marks, M. S., Woodruff, L., Ohno, H., and Bonifacino, J. S. (1996) J. Cell Biol. 135, 341–354
15. Hémard, A., and Dautry-Varsat, A. (1990) Eur. J. Immunol. 20, 2629–2635
16. Davis, C. G., van Driel, I. R., Riezman, H., Brown, M. S., and Goldstein, J. L. (1987) J. Biol. Chem. 262, 4075–4082
17. McGraw, T. E., and Maxfield, F. R. (1990) Cell Regul. 1, 369–377
18. Canfield, W. M., Johnson, K. F., Ye, R. D., Gregory, W., and Kornfeld, S. (1991) J. Biol. Chem. 266, 5682–5688
19. Towbridge, I. S., and Hopkins, C. R. (1993) Annu. Rev. Cell Biol. 9, 129–161
20. Boll, W., Ohno, H., Songyang, Z., Rapseport, I., Castley, L. C., Bonifacino, J. S., and Kirchhausen, T. (1996) EMBO J. 15, 5789–5795
21. Davis, C. G., Lehrman, M. A., Russell, D. W., Anderson, R. G., Brown, M. S., and Goldstein, J. L. (1986) Cell 45, 15–24
22. Hurnikier, W., and Geuze, H. J. (1996) BioEssays 18, 379–389
23. Kornilova, E., Sorkina, T., Beguinot, L., and Sorkin, A. (1996) J. Biol. Chem. 271, 30340–30346
24. Bansal, A., and Giersch, L. M. (1991) Cell 67, 1195–1201
25. Eberle, W., Sander, C., Klaus, W., Schmidt, B., von Figura, K., and Peters, C. (1991) Cell 67, 1203–1209
26. Wilde, A., Dempsey, C., and Banting, G. (1994) J. Biol. Chem. 269, 7131–7136
27. Grote, E., Hao, J. C., Bennett, M. K., and Kelly, R. B. (1995) Cell 81, 591–598
28. Malliababrenn, A., Jiménez, M. A., Rico, M., and Alarcon, B. (1995) EMBO J. 14, 2257–2266
29. Munoz, and Serano. (1994) Nat. Struct. Biol. 1, 399–409
30. Ohno, H., Fournier, M.-C., Poy, G., and Bonifacino, J. S. (1996) J. Biol. Chem. 271, 29009–29015
31. Honing, S., and Hunziker, W. (1995) J. Cell Biol. 125, 321–332
32. Chang, C.-P., Lazar, C. S., Walsh, B. J., Komuro, M., Collawn, J. F., Kuhn, L. A., Tainer, J. A., Towbridge, I. S., Farquhar, M. G., Rosenfeld, M. G., Wiley, H. S., and Gill, G. G. (1993) J. Biol. Chem. 268, 19312–19320
33. Johnson, K. F., Chan, W., and Kornfeld, S. (1980) Proc. Natl. Acad. Sci. U. S. A. 87, 10010–10014
34. Ponnambalam, S., Rabouille, C., Luzio, J. P., Nilsson, T., and Warren, G. (1994) J. Cell Biol. 125, 253–268
35. Ogresko, L. K., Chang, C.-P., Will, B. H., Burke, P. M., Gill, G. N., and Wiley, H. S. (1995) J. Biol. Chem. 270, 4325–4333
36. Morelon, E., and Dautry-Varsat, A. (1998) J. Biol. Chem. 273, 22044–22051
37. Dittrich, E., Haft, C. R., Muys, L., Heinrich, P. C., and Graeve, L. (1996) J. Biol. Chem. 271, 5487–5494
38. Green, S. A., Setiali, H., McEver, R. P., and Kelly, R. B. (1994) J. Cell Biol. 124, 435–448
39. Blagoveshchenskaya, A. D., Narcott, J. P., and Cutler, D. F. (1998) J. Biol. Chem. 273, 2729–2737
40. Ohno, H., Stewart, J., Fournier, M.-C., Bosshart, H., Rhee, I., Miyatake, S., Saito, T., Gallusser, A., Kirchhausen, T., and Bonifacino, J. S. (1995) Science 269, 1872–1875
41. Scheckman, R., and Mellman, I. (1997) Cell 90, 197–200
42. Aniento, F., Gu, F., Parton, R. G., and Grunenberg, J. (1996) J. Cell Biol. 133, 29–41
43. Whitney, J. A., Gomez, M., Sheff, D., Kreis, T. E., and Mellman, I. (1995) Cell 83, 753–755
44. Hansen, S. H., Sandvig, K., and van Deurs, B. (1993) J. Cell Biol. 123, 89–97
45. Grunenberg, J., and Maxfield, F. R. (1995) Curr. Opin. Cell Biol. 7, 552–563
46. Zwart, D. E., Brewer, C. B., Lazarovits, J., Henis, Y. I., and Roth, M. G. (1996) J. Biol. Chem. 271, 907–917
47. Wiley, H. S., and Cunningham, D. D. (1982) J. Biol. Chem. 257, 4222–4229