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A hydrophobic amino acid cluster inserted into the C-terminus of a recycling cell surface receptor functions as an endosomal sorting signal

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ABSTRACT

Cell surface receptors ubiquitylated after ligand stimulation are internalized and delivered to the lysosomal pathway for degradation. Ubiquitylated receptors are captured by ESCRT protein complexes that sort them to the lysosomal pathway. Hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) is a component of endosomal sorting complexes required for transport (ESCRT)-0 that recognizes ubiquitin attached to receptors, indicating that it functions as a key molecule for ubiquitin-dependent endosomal sorting. In a previous study on interleukin (IL)-2 receptor β (IL-2Rβ) and IL-4 receptor α (IL-4Rα), which are constitutively internalized without ligand stimulation, we revealed that Hrs bound to IL-2Rβ and IL-4Rα in a ubiquitin-independent manner, and identified a hydrophobic amino acid cluster in the cytoplasmic region of IL-2Rβ and IL-4Rα as the Hrs-interacting domain. However, a chimeric receptor containing the hydrophobic amino acid cluster inserted into the C-terminal of IL-2Rα was not delivered to late endosomes, but recycled back to the plasma membrane. In the present study, we explored the functional domain related to endosomal sorting in IL-2Rβ together with the hydrophobic amino acid cluster, and discovered the importance of an approximately 30-amino acid stretch following the C-terminus of the hydrophobic amino acid cluster in IL-2Rβ. Even though the amino acid stretch following the hydrophobic amino acid cluster was composed of arbitrary amino acids, such a stretch was also permissive for the sorting ability, suggesting that the hydrophobic amino acid cluster functions as an endosomal sorting signal. These findings clarify part of the molecular mechanism underlying the ubiquitin-independent endosomal sorting of cytokine receptors that are constitutively internalized without ligand stimulation.

1. Introduction

Cell surface receptors/transmembrane proteins are internalized and delivered to endosomes, from which the receptors are either recycled back to the plasma membrane or degraded via transport routes to the lysosome. These divergent fates are governed by a complex system of endosomal sorting signals in the receptors and a molecular machinery that recognizes those signals and delivers the proteins to their appropriate targets. Various endosomal sorting signals consisting of short linear arrays of amino acid residues have been identified and characterized in the cytoplasmic domains of receptors. On the other hand, ubiquitylation of receptors also serves as a signal for endosomal sorting to the lysosome. For example, ubiquitylation of epidermal growth factor (EGF) receptor is induced by EGF binding [1]. Endosomal-sorting complex required for transport (ESCRT) protein complexes recognize ubiquitylated receptors via subunits that contain a ubiquitin-interacting motif (UIM) domain, and mediate endosomal sorting from early endosomes to late endosomes [2–4]. Hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) and signal transducing adaptor molecule (STAM), which both possess a UIM domain, are the first ESCRT complexes to bind to receptors internalized from the cell surface, and are sometimes referred to as ESCRT-0 [5]. We previously found that Hrs recognizes a hydrophobic amino acid cluster in the cytoplasmic region of interleukin (IL)-2 receptor β (IL-2Rβ) and IL-4 receptor α (IL-4Rα) in a ubiquitin-independent manner [6]. Receptor mutants, in which the hydrophobic amino acids of the cluster were substituted with alanine, exhibited impaired endosomal sorting to LAMP1-positive late endosomes. These findings suggest that Hrs recognizes not only ubiquitin, but also a sorting signal consisting of an array of amino acid residues.

IL-2Rα is constitutively internalized without IL-2 binding and recycled back to the plasma membrane [7]. It is often used as an

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evaluation system for endosomal sorting signals, through the construction of chimeric receptors composed of IL-2Rα and sorting signals such as dileucine- and tyrosine-based motifs derived from the cytoplasmic regions of transmembrane proteins [8]. Chimeric IL-2Rα including the sorting signal escaped the recycling pathway and was directed to late endosomes or the lysosomal targeting pathway. We also inserted the hydrophobic amino acid cluster from IL-2Rβ into the C-terminus of IL-2Rα and evaluated the sorting ability of the chimeric receptor. However, the chimeric receptor was not delivered to late endosomes, but found on the plasma membrane [6], suggesting that another cytoplasmic region together with the hydrophobic amino acid cluster is necessary for the function as a sorting signal. In the present study, we examined the conditions under which the hydrophobic amino acid cluster inserted into the C-terminus of IL-2Rα can serve as a sorting signal to late endosomes.

2. Materials and methods

2.1. Plasmids

The human Hrs expression construct pCXN2-Hrs, human IL-2Rα expression construct pMXs-IL-2RαWT, and IL-2Rα-IL-2Rβ chimera expression constructs pMXs-IL-2Rα-β269-551 and pMXs-IL-2Rα-β365-369 were described previously [6,9]. Two IL-2Rα-β269-551-derived mutants, IL-2Rα-β365-551 and IL-2Rα-β370-551, were generated by PCR-based site-directed mutagenesis using pMXs-IL-2Rα-β269-551 as a template. Five additional mutants, IL-2Rα-β365-380, IL-2Rα-β365-394, IL-2Rα-β365-412, IL-2Rα-β365-551d370-394, and IL-2Rα-β365-551d370-412, were generated by PCR-based site-directed mutagenesis using pMXs-IL-2Rα-β365-551 as a template. pMXs-IL-2RαWT-EGFP containing IL-2Rα and the C-terminal region of enhanced green fluorescent protein (EGFP) (residues 211–240) was generated by inserting the C-terminal region of EGFP at the C-terminal end of IL-2Rα. IL-2Rα-FFFHL-EGFP and IL-2Rα-LFLDLL-EGFP containing the hydrophobic amino acid cluster of IL-2Rβ (residues 365–394) and IL-4Rα (residues 410–415), respectively, were generated by PCR-based gene insertion using pMXs-IL-2RαWT-EGFP as a template. A series of HA-tagged constructs were generated by inserting the HA epitope (YPYDVPDYA) between amino acids Glu196 and Glu197 in the IL-2Rα coding region by PCR.

2.2. Cell lines

Mouse embryonic fibroblast (MEF) cells and human embryonic kidney-derived (HEK) 293T cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics.

2.3. Infection and immunofluorescence microscopy

For introduction of IL-2Rα chimeric genes into MEF cells, we used a pMXs retrovirus vector system (kindly provided by T. Kitamura, University of Tokyo, Tokyo, Japan). For immunofluorescence, infected MEF cells grown on coverslips were incubated with 10% FBS-DMEM containing 1 μg/ml of mouse anti-human IL-2Rα monoclonal antibody (H-31) [9] at 0 °C for 30 min. The cells were then washed three times with PBS, and incubated with 10% FBS-DMEM at 37 °C in a 5% CO_2 incubator for 120 min. After the incubation, the cells were fixed with 3% paraformaldehyde in PBS for 15 min at room temperature, and permeabilized with 0.1% Triton X-100 in PBS for 10 min at room temperature. Receptors labeled with H-31 were visualized with an Alexa 594-conjugated secondary antibody (Life Technologies, Gaithersburg, MD). For double-staining with LAMP1, the cells on the coverslips were subsequently incubated with 10% FBS–PBS containing 1 μg/ml of rat anti-mouse LAMP1 monoclonal antibody (1D4B; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at room temperature, washed three times with PBS, and incubated with an FITC-conjugated secondary antibody (Life Technologies). After washing with PBS, the coverslips were mounted on glass slides in glycerol containing 0.1% p-phenylenediamine. Fluorescence images were captured using a TCS SP2 (Leica Microsystems, Wetzlar, Germany) or LSM EXCITER (Carl Zeiss, Oberkochen, Germany) confocal microscope.

2.4. Immunoprecipitation and immunoblotting

Immunoprecipitation and immunoblotting were carried out as described previously [10]. Briefly, HEK293T cells (1×10^6) were cotransfected with 5 μg of each pMXs construct and 1 μg of pCXN2-Hrs using a calcium phosphate precipitation method, and lysed with NP-40 cell extraction buffer (1% NP-40, 20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM Na_3VO_4, 2.5 mM Na-pyrophosphate, 1 mM β-glycerol phosphate, 1 mM aprotinin). The lysates were immunoprecipitated with a mouse anti-human IL-2Rα monoclonal antibody (H-48) [9] immobilized on Protein G-Sepharose beads (GE Healthcare, Little Chalfont, UK) at 4 °C for 1 h. The immunoprecipitates were extensively washed with wash buffer (1% NP-40, 20 mM Tris–HCl, pH 7.5, 500 mM NaCl, 1 mM EDTA, 1 mM Na_3VO_4, 2.5 mM Na-pyrophosphate, 1 mM β-glycerol phosphate), boiled in sample buffer, separated by SDS–PAGE, and transferred onto Immobilon-P membranes (Millipore, Billerica, MA). After blocking with 5% nonfat milk, the membranes were incubated with the indicated primary antibodies at room temperature for 1 h, washed, and incubated with horseradish peroxidase-conjugated secondary antibodies (GE Healthcare or Cell Signaling Technology, Danvers, MA) at room temperature for 1 h. After thorough washing, the signals were visualized using Immobilon™ Western reagents (Millipore). The primary antibodies utilized were a rabbit anti-HA antibody (GeneTex, Irvine, CA) and a rat anti-Hrs monoclonal antibody (Imos-1) [10].

3. Results

In our previous report, chimeric receptors of IL-2Rα including the hydrophobic amino acid cluster (residues 365–369: IL-2Rα-β365-369) or full-length cytoplasmic region (residues 269–551: IL-2Rα-β269-551) of IL-2Rβ were constructed and examined for their endosomal sorting ability [6]. We found that IL-2Rα-β269-551 expressed on the cell surface was delivered to LAMP1-positive late endosomes during 120 min, whereas IL-2Rα-β365-369 was located on the plasma membrane similar to wild-type IL-2Rα These findings indicated that another domain in addition to the hydrophobic amino acid cluster in the cytoplasmic region of IL-2Rβ contributes to the function of endosomal sorting to late endosomes. To define the domain of IL-2Rβ required for the function as a sorting signal together with the hydrophobic amino acid cluster, we constructed additional chimeric receptors of IL-2Rα. The full-length cytoplasmic tail of IL-2Rβ (residues 269–551), hydrophobic amino acid cluster (residues 365–369), and specific amino acid sequences (residues: 365–380; 365–394; 365–412; 365–551; 370–551; 365–551 lacking 370–394; 365–551 lacking 370–412) were inserted into the C-terminus of IL-2Rα (Fig. 1A). MEF cells transiently expressing wild-type IL-2Rα and the chimeric receptors described above were incubated at 0 °C and treated with anti-IL-2Rα antibody H-31. The cells were then incubated at 37 °C for 120 min and analyzed by confocal microscopy. IL-2Rα-β365-551, containing the hydrophobic amino acid cluster of IL-2Rβ, was detected in LAMP1-positive compartments as well as IL-2Rα-β269-551, containing the full-length cytoplasmic tail of IL-2Rβ (Fig. 1B). These

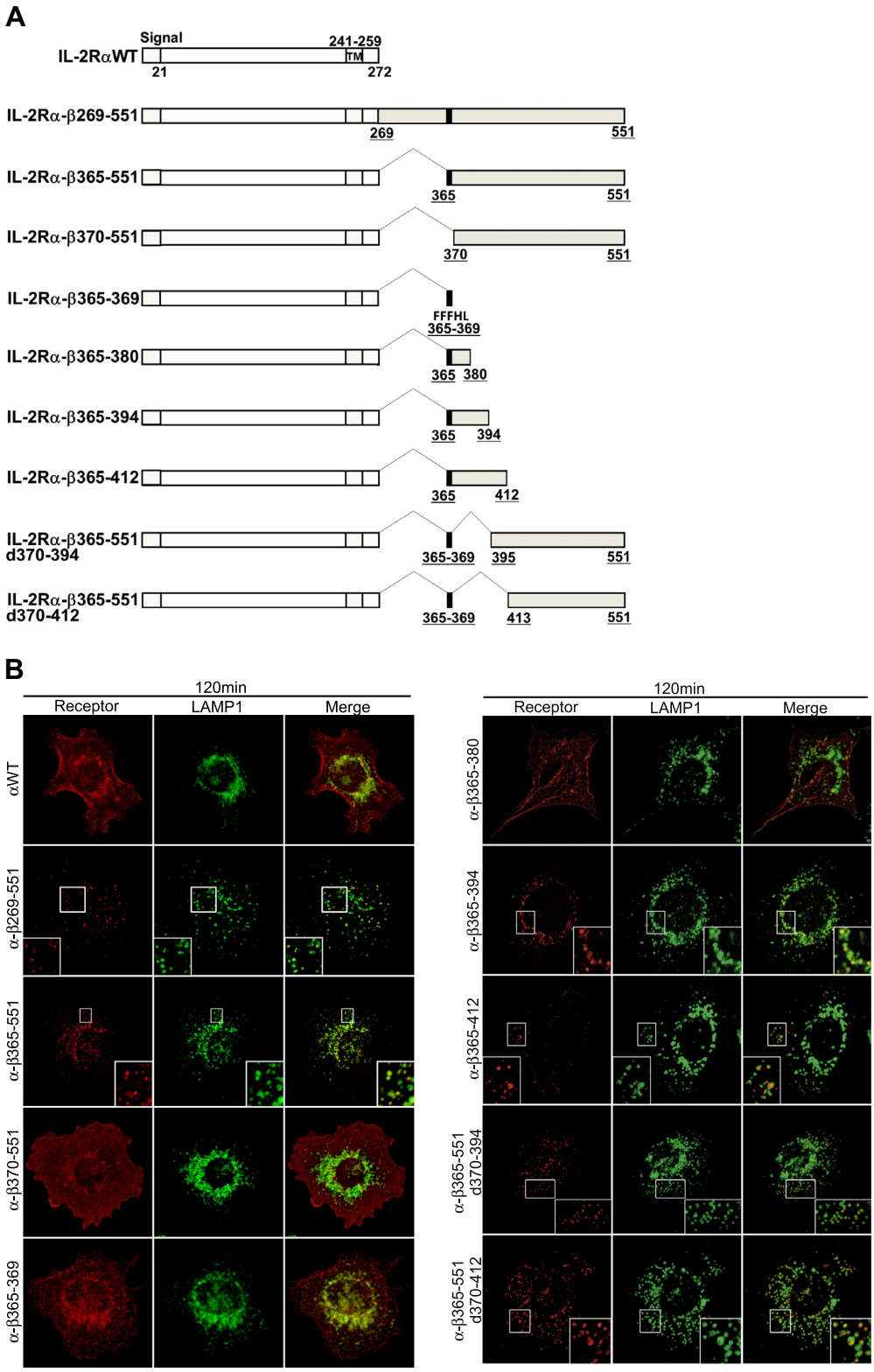

Fig. 1. Hydrophobic amino acid cluster together with another region of the cytoplasmic tail in IL-2Rβ contributes to endosomal sorting to LAMP1-positive compartments. (A) Structures of wild-type IL-2Rα (IL-2RαWT) and the IL-2Rα-IL-2Rβ (IL-2Rα-β) chimeric receptors. The signal sequence and transmembrane region (TM) are indicated. (B) Transfected MEF cells were grown on coverslips, and their cell surface receptors were labeled with H-31. After incubation for 120 min, the cells were fixed and labeled with an anti-LAMP1 antibody. The labeled cells were observed by confocal laser microscopy.

findings indicate that residues 269–364 of IL-2Rβ do not contribute to the sorting ability of the hydrophobic amino acid cluster. A large part of IL-2Rα-β370-551, lacking the hydrophobic amino acid cluster (residues 365–369), was found on the plasma membrane (Fig. 1B). Since IL-2Rα-β365-369, containing the hydrophobic amino acid cluster (residues 365–369), was observed on the plasma membrane, we extended the C-terminal amino acids beyond residue 369 of the hydrophobic amino acid cluster. IL-2Rα-β365-

380, containing 11 residues after the cluster, was detected in LAMP1-positive compartments in addition to its location on the plasma membrane. IL-2Rα-β365-394 and IL-2Rα-β365-412, containing 25 and 43 residues after the cluster, respectively, were observed in LAMP1-positive compartments (Fig. 1B). Thus, at least the first 25 residues (residues 370–394) after the cluster appear to be essential for the sorting ability of the hydrophobic amino acid cluster. To confirm the essential domain following the cluster, we examined the sorting ability of IL-2Rα-β365-551d370-412, containing the hydrophobic amino acid cluster and residues 413–551 of IL-2Rβ, but lacking residues 370–412 (Fig. 1A). Unexpectedly, IL-2Rα-β365-551d370-394 and IL-2Rα-β365-551d370-412, lacking 25 and 43 residues, respectively, after the cluster were detected in LAMP1-positive compartments (Fig. 1B). Therefore, we speculated that the stretch of about 30 residues following the cluster is essential for the sorting ability to late endosomes, and constructed chimeric receptors in which the 30 amino acid residues considered to be unrelated to endosomal sorting were inserted into the C-terminus of IL-2Rα-β365-369. Part of EGFP (residues 211–240) was inserted into the C-terminus of IL-2Rα-β365-369 (IL-2Rα-FFFHL) or IL-2Rα (Fig. 2A). Similarly, the hydrophobic amino acid cluster (LFLDLL) of IL-4Rα and residues 211–240 of EGFP were inserted into the C-terminus of IL-2Rα (Fig. 2A). MEF cells transiently expressing the chimeric receptors described above were incubated under the same conditions as the cells shown in Fig. 1. Although the major part of IL-2Rα-EGFP was detected on the plasma membrane, IL-2Rα-FFFHL-EGFP and IL-2Rα-LFLDLL-EGFP were found in LAMP1-positive compartments (Fig. 2B). These observations suggest that, for the hydrophobic amino acid cluster to serve as an endosomal sorting signal, it requires a stretch of about 30 amino acid residues after its C-terminus, composed of arbitrary amino acids.

We previously showed that the hydrophobic amino acid cluster is essential for the association between Hrs and IL-2Rβ. To evaluate the binding ability of Hrs to the chimeric receptors with or without the hydrophobic amino acid cluster, we performed coimmunoprecipitation assays. Lysates of HEK293T cells transiently expressing the chimeric receptors of IL-2Rα along with Hrs were immunoprecipitated with an anti-IL-2Rα antibody and immunoblotted with

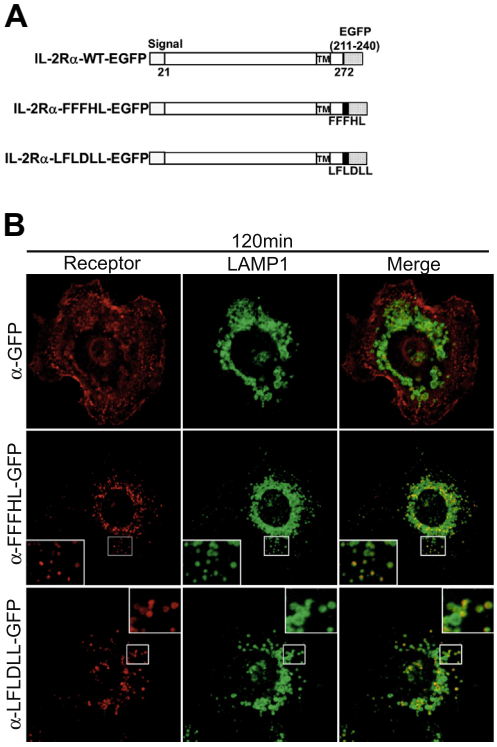

Fig. 2. Hydrophobic amino acid cluster is the essence of the sorting signal to LAMP1-positive endosomes. (A) Structures of the IL-2Rα chimeric receptors. All chimeric receptors contain an EGFP fragment (residues 211–240) at the C-terminus as an arbitrary peptide. FFFHL and LFLDLL are the hydrophobic amino acid clusters of IL-2Rβ and IL-4Rα, respectively. (B) Transfected MEF cells were grown on coverslips, and their cell surface receptors were labeled with H-31. After incubation for 120 min, the cells were fixed and labeled and with an anti-LAMP1 antibody. The labeled cells were observed by confocal laser microscopy.

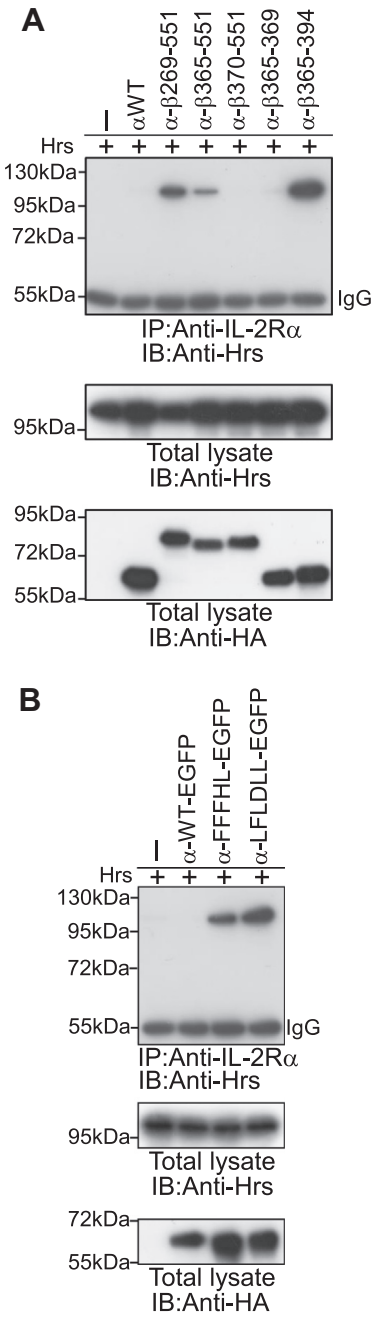

Fig. 3. Hrs interacts with the hydrophobic amino acid cluster itself. (A and B) HEK293T cells were cotransfected with HA-tagged chimeric IL-2Rα expression constructs and a wild-type Hrs expression construct. Aliquots of the cell lysates (800 μg) were immunoprecipitated with an anti-IL-2Rα monoclonal antibody and immunoblotted with an anti-Hrs monoclonal antibody (top panel). Aliquots of the lysates (10 μg) were immunoblotted with an anti-Hrs antibody (middle panel) or an anti-HA antibody (lower panel). WT: wild-type; IP: immunoprecipitation; IB: immunoblotting.

an anti-Hrs antibody. Hrs was clearly coimmunoprecipitated with IL-2Rα-β365-551 as well as IL-2Rα-β269-551, both of which contain the hydrophobic amino acid cluster (residues 365–369) of IL-2Rβ (Fig. 3A), but not IL-2Rα-β370-551 lacking the hydrophobic amino acid cluster. Although IL-2Rα-β365-369 was hardly associated with Hrs, despite containing the hydrophobic amino acid cluster, Hrs binding was detected in the lysates of HEK293T cells expressing IL-2Rα-β365-394, IL-2Rα-FFFHL-EGFP, and IL-2Rα-LFLDLL-EGFP (Fig. 3A and B). These results indicated that the 30 amino acids following the hydrophobic amino acid cluster are needed for Hrs binding to the chimeric receptors. Consequently, these 30 amino acid residues may contribute to the conformational stability of the hydrophobic amino acid cluster that is recognized by Hrs.

4. Discussion

It is well-known that ubiquitylation of various cell surface receptors serves as a sorting signal to lysosomes. EGF receptor and interferon-α receptor activated by ligand binding are ubiquitylated in the lysine residues of their cytoplasmic regions, internalized, and delivered to lysosomes [1,11]. In contrast, IL-2Rβ is constitutively internalized without ligand binding and delivered to late endosomes/lysosomes, and mutant IL-2Rβ lacking all of the lysine residues in the cytoplasmic region is also sorted to late endosomes/lysosomes, indicating that the endosomal sorting of IL-2Rβ differs from that of EGF receptor, and is independent of ubiquitylation [12]. Therefore, we previously explored the machinery for the endosomal sorting of IL-2Rβ, and identified Hrs as a functional molecule involved in the ubiquitin-independent endosomal sorting of IL-2Rβ, despite Hrs being a component of the sorting machinery for ubiquitylated cargo proteins [12]. Intensive analyses indicated that Hrs recognized an array of amino acid residues, referred to as a hydrophobic amino acid cluster, in the cytoplasmic region of IL-2Rβ and IL-4Rα [6]. The results of the present study indicate that these hydrophobic amino acid clusters inserted into the C-terminus of IL-2Rα function as an endosomal sorting signal for delivery to late endosomes.

Two major endosomal sorting signals, tyrosine-based (Yxxϕ) and dileucine-based ([D/E]xxxL[L/I]) motifs, are also involved in the delivery of membrane proteins to lysosomes [8]. Yxxϕ motifs are found in cell surface membrane proteins and proteins localized in endosomal–lysosomal organelles, suggesting multiple roles of these motifs [13]. The Yxxϕ motif in transferrin receptor is essential for its rapid internalization from the plasma membrane [14]. On the other hand, [D/E]xxxL[L/I] motifs are found in type I, type II, and multispanning transmembrane proteins, which are distributed from the plasma membrane to late endosomes, lysosomes, and specialized antigen-processing compartments. The [D/E]xxxL[L/I] motif in CD3γ participates in its rapid internalization and lysosomal targeting [8]. Both motifs in membrane proteins are recognized by adaptor protein (AP) complexes, which collaborate with clathrin, and the membrane proteins are pinched off from the plasma membrane into clathrin-coated vesicles. Meanwhile, the hydrophobic amino acid clusters in IL-2Rβ and IL-4Rα endocytosed into endosomes are recognized by Hrs, which typically recognizes ubiquitylated cytoplasmic domains of cell surface receptors, such as EGF receptor and interferon-α receptor. IL-2Rα and IL-2Rβ are constitutively internalized without ligand stimulation. The former is recycled back to the plasma membrane, while the latter is delivered to late endosomes/lysosomes. Thus, it may be reasonable that receptors constitutively internalized without ligand stimulation and delivered to late endosomes/lysosomes are recognized by Hrs through its linear arrays of amino acid residues, like the hydrophobic amino acid clusters in this study. Therefore, Hrs may play two roles in the endosomal sorting of cargo proteins, in which Hrs not only recognizes cargo proteins ubiquitylated by ligand stimulation, but also recognizes non-ubiquitylated cargo proteins containing the hydrophobic amino acid cluster, which are constitutively internalized without ligand stimulation.

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References

[1] P. Soubeyran, K. Kowanetz, I. Szymkiewicz, W.Y. Langdon, I. Dikic, Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors, Nature 416 (2002) 183–187.
[2] D.J. Katzmann, M. Babst, S.D. Emr, Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I, Cell 106 (2001) 145–155.
[3] M. Babst, D.J. Katzmann, E.J. Estepa-Sabal, T. Meerloo, S.D. Emr, Escrt-III: an endosome-associated heterooligomeric protein complex required for mvb sorting, Dev. Cell 3 (2002) 271–282.
[4] M. Babst, D.J. Katzmann, W.B. Snyder, B. Wendland, S.D. Emr, Endosome-associated complex, ESCRT-II, recruits transport machinery for protein sorting at the multivesicularbody, Dev. Cell 3 (2002) 283–289.
[5] C. Raiborg, H. Stenmark, The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins, Nature 458 (2009) 445–452.
[6] Y. Amano, Y. Yamashita, K. Kojima, K. Yoshino, N. Tanaka, K. Sugamura, T. Takeshita, Hrs recognizes a hydrophobic amino acid cluster in cytokine receptors during ubiquitin-independent endosomal sorting, J. Biol. Chem. 286 (2011) 15458–15472.
[7] H. Radhakrishna, J.G. Donaldson, ADP-ribosylation factor 6 regulates a novel plasma membrane recycling pathway, J. Cell Biol. 139 (1997) 49–61.
[8] F. Letourneur, R.D. Klausner, A novel di-leucine motif and a tyrosine-based motif independently mediate lysosomal targeting and endocytosis of CD3 chains, Cell 69 (1992) 1143–1157.
[9] Y. Tanaka, T. Inoi, H. Tozawa, K. Sugamura, Y. Hinuma, New monoclonal antibodies that define multiple epitopes and a human-specific marker on the interleukin 2 receptor molecules of primates, Microbiol. Immunol. 30 (1986) 373–388.
[10] H. Asao, Y. Sasaki, T. Arita, N. Tanaka, K. Endo, H. Kasai, T. Takeshita, Y. Endo, T. Fujita, K. Sugamura, Hrs is associated with STAM, a signal-transducing adaptor molecule. Its suppressive effect on cytokine-induced cell growth, J. Biol. Chem. 272 (1997) 32785–32791.
[11] K.G. Kumar, W. Tang, A.K. Ravindranath, W.A. Clark, E. Croze, S.Y. Fuchs, SCF(HOS) ubiquitin ligase mediates the ligand-induced down-regulation of the interferon-alpha receptor, EMBO J. 22 (2003) 5480–5490.
[12] Y. Yamashita, K. Kojima, T. Tsukahara, H. Agawa, K. Yamada, Y. Amano, N. Kurotori, N. Tanaka, K. Sugamura, T. Takeshita, Ubiquitin-independent binding of Hrs mediates endosomal sorting of the interleukin-2 receptor beta-chain, J. Cell Sci. 121 (2008) 1727–1738.
[13] J.S. Bonifacino, L.M. Traub, Signals for sorting of transmembrane proteins to endosomes and lysosomes, Annu. Rev. Biochem. 72 (2003) 395–447.
[14] J.F. Collawn, M. Stangel, L.A. Kuhn, V. Esekogwu, S.Q. Jing, I.S. Trowbridge, J.A. Tainer, Transferrin receptor internalization sequence YXRF implicates a tight turn as the structural recognition motif for endocytosis, Cell 63 (1990) 1061–1072.