Steric Masking of a Dilysine Endoplasmic Reticulum Retention Motif during Assembly of the Human High Affinity Receptor for Immunoglobulin E

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Abstract. Signals that can cause retention in the ER have been found in the cytoplasmic domain of individual subunits of multimeric receptors destined to the cell surface. To study how ER retention motifs are masked during assembly of oligomeric receptors, we analyzed the assembly and intracellular transport of the human high-affinity receptor for immunoglobulin E expressed in COS cells. The cytoplasmic domain of the α chain contains a dilysine ER retention signal, which becomes nonfunctional after assembly with the γ chain, allowing transport out of the ER of the fully assembled receptor. Juxtaposition of the cytoplasmic domains of the α and γ subunits during assembly is responsible for this loss of ER retention. Substitution of the γ chain cytoplasmic domain with cytoplasmic domains of irrelevant proteins resulted in efficient transport out of the ER of the α chain, demonstrating that nonspecific steric hindrance by the cytoplasmic domain of the γ chain accounts for the masking of the ER retention signal present in the cytoplasmic domain of the α chain. Such a mechanism allows the ER retention machinery to discriminate between assembled and nonassembled receptors, and thus participates in quality control at the level of the ER.

S IGNALS that can cause ER retention have been characterized in the cytoplasmic domain of many proteins that are resident in the ER and in the ER-Golgi intermediate compartment. A usual feature of these signals is the presence of two lysine residues at positions -3 and -4 from the COOH-terminal end of the cytoplasmic domain (Jackson et al., 1990). These signals participate in ER retention of proteins functionally required in the ER such as the UDP-glucuronosyltransferase, which is responsible for the glucuronidation of 6-α-hydroxyl bile acids in the ER (Fournel-Gigleux et al., 1989; Nilsson et al., 1989). Although for simplicity they are referred to as retention motifs, dilysine signals actually ensure ER localization of tagged proteins by causing their continuous retrieval from post-ER compartments back to the ER (Jackson et al., 1993; Gaynor et al., 1994). The continuous retrograde transport of dilysine-tagged proteins involves the recognition of the dilysine motifs by the coatomer complex in post-ER compartments (Cosson and Letourneur, 1994; Letourneur et al., 1994).

Similar ER retention signals are also present in the cytoplasmic domain of certain transmembrane proteins destined to the cell surface. They were found in the cytoplasmic domain of individual subunits of oligomeric surface receptors, and presumably participate in quality control at the ER level by retaining unassembled chains. For example, ER retention signals are present in the COOH-terminal portion of the cytoplasmic domains of the CD3-γ and -δ chains of the T cell receptor (Letourneur and Klausner, 1992). Only the fully assembled T cell receptor is efficiently transported out of the ER and to the cell surface (for review see Klausner et al., 1990), indicating that these retention signals, as well as others, are not functional in the assembled receptor.

How cytoplasmic ER retention motifs are masked during assembly of oligomeric receptors is not known. It was shown earlier that ER retention and degradation motifs found in transmembrane domains are masked as transmembrane domains interact with each other during assembly (Bonifacino et al., 1990). No direct interaction between cytoplasmic domains has yet been observed during assembly of oligomeric receptors. On the other hand, one obvious consequence of assembly is to juxtapose the cytoplasmic domains of the various subunits of the receptor. If one considers the assembly of two chains A and B, at least three simple mechanisms could account for disruption of ER retention signals after assembly: (*a*) the cytoplasmic ER retention signal in chain A is masked sterically by the cytoplasmic domain of chain B that is juxtaposed to it during assembly; (*b*) the ER retention signal in chain A specifically interacts with an "antisignal" in the cytoplasmic domain of chain B and is made nonfunctional; or (*c*) a dominant export signal in the cytoplasmic domain of chain B can cause transport of the AB complex inde-

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pendent from the presence of a retention signal in A.

Here we analyzed the assembly and intracellular transport of the human high-affinity receptor for immunoglobulin E (FcεRI)[1] transfected into COS 1 cells. We show that the cytoplasmic domain of the α chain contains a dilysine ER retention signal, which is masked during assembly with the γ chain, thus allowing transport out of the ER and to the cell surface upon assembly. Our data indicate that nonspecific steric hindrance by the cytoplasmic domain of the γ chain accounts for the masking of the ER retention signal in the α cytoplasmic domain.

Materials and Methods

Construction and Transfection of Mutant DNAs

cDNAs coding for the mouse CD8 α and β chains and the α chain of the interleukin-2 receptor (Tac) were subcloned into a modified version of the pCDM8 expression plasmid (Seed, 1987; Bonifacino et al., 1991), using PCR, and a BglII site was introduced on the lumenal side of the transmembrane domain (Cosson et al., 1991; Hennecke and Cosson, 1993). The cDNAs coding for the human FcεRI α and γ chains were a kind gift of Dr. J. P. Kinet [#139] National Institutes of Health, Bethesda, MD; (Kochan et al., 1988; Shimizu et al., 1988; Küster et al., 1990). All mutant molecules were constructed by PCR mutagenesis (Jones and Howard, 1990). Chimeric proteins of Tac and of the FcεRI α and γ chains are denoted by a three-letter name, in which each letter represents the lumenal, transmembrane, and cytoplasmic domains in order. Chimeric proteins composed of the CD8α lumenal and transmembrane domains and of the cytoplasmic domain of chain x are denoted A-x, whereas similar constructs with CD8β are denoted B-x. COS-1 cells (American Type Culture Collection, Rockville, MD) were plated on 150-mm culture dishes and transfected with 20 μg of plasmid DNA using the calcium phosphate precipitation method (Graham and Van der Eb, 1973). After 16 h, transfected cells were trypsinized, plated on 35-mm culture dishes, and allowed to grow for an additional 24 h.

Metabolic Labeling and Immunoprecipitation

Transfected cells were labeled for 15 min at 37°C with 1 ml of 0.25 mCi/ml [³⁵S]methionine in methionine-free DME containing 5% FCS, as described previously (Bonifacino et al., 1991). When indicated, labeled cells were chased by incubating at 37°C in DME containing unlabeled methionine and 5% FCS. At each time point, cells were scraped from the culture plate, pelleted by centrifugation, and frozen at −70°C before detergent lysis in the presence of 10 mM iodoacetamide and immunoprecipitation (Bonifacino et al., 1991). Immunoprecipitates were analyzed by SDS-PAGE under reducing conditions.

For coprecipitation experiments, cells were labeled as described above with [³⁵S]cysteine (Amersham Corp., Arlington Heights, IL) and lysed in digitonin lysis buffer (1% digitonin, 0.12% Triton X-100, 20 mM Tris, pH 7.4, 150 mM NaCl) in the presence of 10 mM iodoacetamide and protease inhibitors. After incubation for 2 h at 4°C on protein A-Sepharose beads coated with the anti-Tac antibody, beads were washed twice in lysis buffer and twice in PBS. Immunoprecipitates were analyzed by 13% SDS-PAGE under nonreducing conditions.

Immunofluorescence Microscopy

Immunofluorescence microscopy was performed as previously described (Hennecke and Cosson, 1993). Both COS-1 and CV-1 cells were used and gave identical patterns in all cases. Transfected cells were grown on 20-mm coverslips for 40 h before fixation for 10 min with 2% (vol/vol) formaldehyde in PBS. After rinsing with PBS, cells were permeabilized with 0.2% Triton X-100 for 3 min, washed with PBS, and incubated for 15 min with 0.2% BSA in PBS (PBS-BSA). Cells were then incubated for 45 min at

25°C with 50 μl of a hybridoma cell culture supernatant containing the indicated antibody. After rinsing with PBS-BSA, cells were incubated for 30 min with a 1:500 dilution of rhodamine-conjugated goat anti-mouse IgG or anti-rat IgG (Cappel Laboratories, Cochranville, PA) in PBS-BSA. Cells were then rinsed with PBS-BSA and coverslips were mounted on glass microscope slides in Mowiol (Heimer and Taylor, 1974). For surface immunofluorescence, cells were incubated at 4°C for 45 min with the first antibody, then washed and incubated for 30 min at 4°C with rhodamine-conjugated secondary antibody. Cells were then washed, fixed with paraformaldehyde, and mounted.

Antibodies used were as follows: for the Tac antigen, the mouse mAb 7G7 (Rubin et al., 1985); for CD8α, the 19.178 mAb (Hämmerling et al., 1979); for CD8β, the 35.17.2 mAb (Golstein et al., 1982); for the FcεRI α chain, the mAb 15-1 (Wang et al., 1992); for the FcεRI γ chain, the rabbit anti-peptide serum #666 (a kind gift of Dr. J. P. Kinet; Letourneur et al., 1991).

Results

To determine how cytoplasmic ER retention motifs are masked during assembly of oligomeric receptors to allow transport of the fully assembled receptors to the cell surface, we analyzed the assembly and intracellular transport of the human FcεRI. Although the FcεRI is expressed in mast cells, as a αβγ2 complex (for review see Ravetch and Kinet, 1991), it is also found as αγ2 complexes at the surface of Langerhans cells (Wang et al., 1992). In addition, it has been previously reported that cotransfection in COS cells of the human FcεRI α and γ chains results in cell surface expression of the αγ2 complex, whereas α expressed alone does not reach the cell surface (Küster et al., 1990; see Fig. 2). It was also observed that a truncated form containing only the lumenal domain of the FcεRI α chain can be transported to the cell surface in the absence of the γ chain (Blank et al., 1991). These results suggest that the transmembrane and/or cytoplasmic domains of the α chain play a crucial role in assembly and control of the intracellular transport of the FcεRI. To test this hypothesis, we constructed a chimera comprised of the extracellular domain of Tac (Leonard et al., 1984) fused with the transmembrane and cytoplasmic domains of the FcεRI α chain (Tαα; Fig. 1 A). Chimeric proteins are denoted by a three-letter name, in which each letter represents the lumenal, transmembrane, and cytoplasmic domains, in order. As observed for the wild-type FcεRI α chain, the Tαα chimera coexpressed with the FcεRI γ chain was transported to the cell surface (Fig. 2, A and B), whereas in the absence of γ it was retained in the ER as assessed by immunofluorescence (Fig. 2, C-F). Transport of the Tac antigen out of the ER can also be measured by the conversion of the protein from an endoglycosaminidase H-sensitive 44-kD to an endoglycosaminidase H-resistant 55-kD species following conversion to complex N-linked sugars in the Golgi apparatus (Bonifacino et al., 1989; Fig. 3 A). The Tαα chimera transfected alone remained in its ER form and was rapidly degraded (Fig. 3 B), whereas cotransfection with the FcεRI γ chain resulted in efficient transport out of the ER (Fig. 3 C). To characterize the domains causing ER retention and degradation of Tαα, we analyzed the intracellular transport of chimeras comprising only the transmembrane domain (TαT) or the cytoplasmic domain (TTα) of the FcεRI α chain (Fig. 1 A). TαT was retained and degraded in the ER (Fig. 3 D), whereas TTα was retained and stable in the ER (Fig. 3 E), indicating that both the transmembrane and the cytoplasmic domains of the FcεRI α chain

A

B

FcεRI α chain

FIST|QQQVTFLLKIKRTRKGFRLLNPHPKPNPKNN

     t4 t3   t2 t1   t0

LLYC|RLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ   FcεRI γ chain

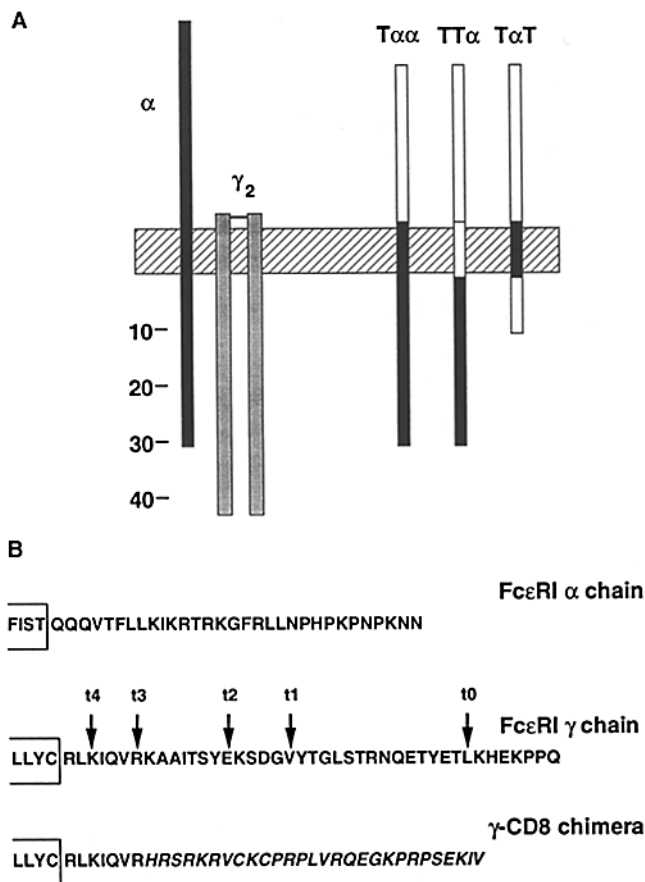γ-CD8 chimera

LLYC|RLKIQVRHRSRKRVCKCPRPLVRQEGKPRPSEKIV

Figure 1. (*A*) Schematic representation of the human FcεRI (*left*) and of the chimeras between Tac and the FcεRI α chain (*right*). The FcεRI α chain is in black, the FcεRI γ chain in grey, and the Tac in white. (*B*) Sequences of the cytoplasmic domains of the FcεRI α and γ chains. The positions of the various truncations of the FcεRI γ chain are indicated by arrows. The sequence of the cytoplasmic domain of the γ-CD8 chimera is also indicated. Transmembrane domains are boxed.

can cause ER retention. It is likely that the presence of a charged amino acid residue in the transmembrane domain of α is a major determinant of transmembrane-directed ER retention and degradation, as described in other systems (Bonifacino et al., 1989, 1991).

To define more precisely the sequence in the cytoplasmic domain of FcεRI α chain that causes ER retention, we constructed chimeras where the last eight amino acids of the FcεRI α cytoplasmic domain, PKPNPKNN, were added in frame at the end of the cytoplasmic domain of Tac (Tac-PKPNPKNN). As shown in Fig. 3 *F*, this was sufficient to induce ER retention of Tac (17% of the Tac chimera was transported out of the ER after 2 h of chase versus 55% for wild-type Tac). Substitution of the lysine residue at position -3 with a leucine residue partially restored transport out of the ER (49% transport), as did substitution of the lysine at position -7 (30% transport). Substitution of the two lysine residues resulted in efficient transport of the chimera out of the ER (Fig. 3 *G*; 63% transport). Transport could also be restored by mutating the two lysine residues to arginine (Fig.

3 *H*; 52% transport) or by adding four serine residues at the COOH-terminal end (Fig. 3 *I*; 44% transport). Thus the cytoplasmic domain of the FcεRI α chain contains a typical dilysine ER retention motif at its COOH terminus, though the two critical lysine residues are located at positions -3 and -7 instead of -3 and -4. The fact that the retention caused by the last eight amino acid residues of FcεRI (Tac-PKPNPKNN; Fig. 3 *F*) is less efficient than that observed with the full-length cytoplasmic domain of FcεRI (TTα; Fig. 3 *E*) suggests that other residues in the cytoplasmic domain of FcεRI contribute to a lesser degree to its efficient retention in the ER.

Assembly with the γ chain is an essential event for the intracellular transport of the FcεRI α chain. Previous studies suggested that interaction between transmembrane domains of individual chains directs assembly of the related IgG receptor (FcγRIIIA; Kurosaki et al., 1991). When transfected cells were solubilized in digitonin instead of Triton X-100, the γ chain coprecipitated with the Tαα but only slightly with TαT and not with TTα (Fig. 4). Deletion of the cytoplasmic domain of Tαα also resulted in a marked decrease in coprecipitation of the γ chain (TααΔCyt, Fig. 4), as did deletion of the γ cytoplasmic domain (γΔCyt, Fig. 4). Together, these results demonstrate that both the transmembrane and the cytoplasmic domains of the α and γ chains of the FcεRI are necessary for stable assembly of the two chains. We also analyzed the effect of successive deletions of the cytoplasmic domain of the γ chain on assembly with Tαα (see Fig. 1 *B*). We found that the 20 amino acids proximal to the transmembrane domain are sufficient to get efficient assembly with Tαα (γt1; 131% of control). Truncation of five additional amino acids resulted in decreased association (γt2; 29% of control), and further truncations (γt3, γt4) decreased the assembly to >5% of the control (data not shown). The limitations of this assay should be emphasized: some weak interactions might be destroyed even in digitonin lysis buffer.

Having defined the requirement for stable α/γ assembly, we then analyzed the effect of successive deletions of the cytoplasmic domain of the γ chain on its ability to allow transport of the Tαα chimera (Fig. 5). Pulse–chase analysis revealed that the γt0 and γt1 constructs were even more efficient than the γ chain itself at causing transport of Tαα out of the ER (Fig. 5, *A–C*). This might be due to the removal of the lysine residue located at position -4 from the COOH terminus of γ that could retard exit from the ER (Jackson et al., 1990). The γt2 mutant (15 amino acids) was less efficient than γt0 and γt1 at promoting transport of the Tαα chimera, as the processed form only appeared after 2 h of chase (Fig. 5 *D*). The γt3 mutant (7 amino acids) was unable to promote transport of Tαα, although it did stabilize a fraction of Tαα in the ER (Fig. 5 *E*, compare with Fig. 3 *B*). When cotransfected with the γt4 mutant (3 amino acids) Tαα was retained and degraded in the ER as efficiently as Tαα transfected alone (Fig. 5 *F*). In these experiments, as the unassembled α transmembrane domain specifies ER degradation, the amount of Tαα escaping ER degradation provides an estimate of the amount of Tαα associated with various γ mutants. When Tαα interacts with the γ chain, the transmembrane determinant for ER degradation is masked, and Tαα is stabilized (Bonifacino et al., 1990). Although by coprecipitation no association between γt3 and Tαα was ob-
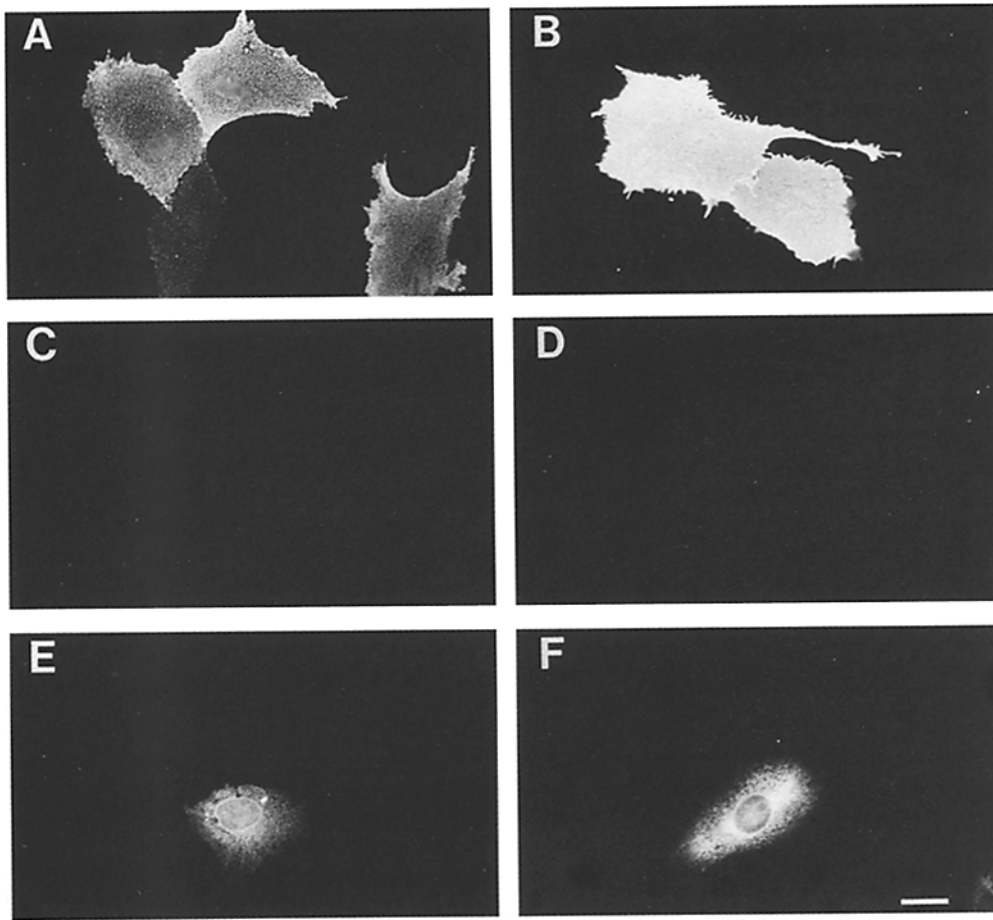
Figure 2. Immunofluorescence microscopy of cells expressing FcεRI and Tac chimeras. COS-1 cells expressing FcεRI α and γ (*A*), FcεRI α alone (*C*), Tαα and FcεRI γ (*B*), or Tαα alone (*D*) were stained at 4°C with an anti-FcεRIα antibody (*A* and *C*) or an anti-Tac antibody (*B* and *D*). The first antibody was visualized using a rhodamine-conjugated anti-IgG, and the cells were fixed and mounted as described in Materials and Methods. Cells expressing FcεRI α (*E*) or Tαα (*F*) were fixed and permeabilized before incubation with the first antibody. Bar, 5 μm.

served, the fact that γt3 stabilizes a significant fraction of Tαα suggests that it must be weakly associated with it. The lack of transport of the Tαα/γt3 complex suggests that certain features of the cytoplasmic domain of γ, which are destroyed in γt3, are necessary to allow transport of the Tαα/γ complex out of the ER.

The simplest interpretation of these results is that while transmembrane and membrane-proximal domains drive assembly of Tαα and the γ chain, it is the juxtaposition of the cytoplasmic domains of the α and γ chains that is responsible for masking the cytoplasmic ER retention motif present in the cytoplasmic domain of α. However the fact that the
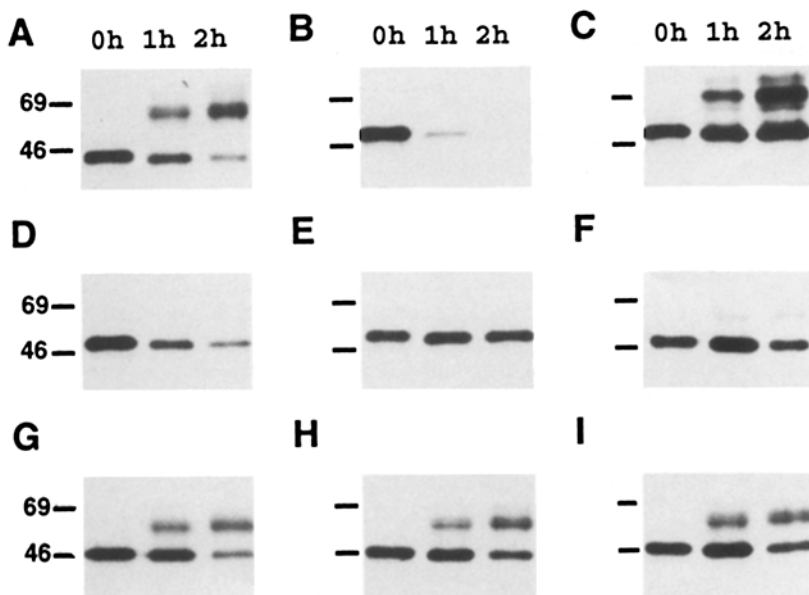

Figure 3. ER retention signals are in the transmembrane and cytoplasmic domains of the FcεRI α chain. COS-1 cells were transfected with Tac (*A*), Tαα (*B*), Tαα and FcεRI γ (*C*), TαT (*D*), TTα (*E*), Tac-PKPNPKNN (*F*), Tac-PLPNPLNN (*G*), Tac-PRPNPRNN (*H*), or Tac-PKPNPKNNSSSS (*I*). For these last four chimeras, the indicated sequences were added to the cytoplasmic domain of Tac. Transfected cells were metabolically labeled for 15 min at 37°C with [^{35}S]methionine, after which they were placed in regular culture medium at 37°C for 0, 1, or 2 h, as indicated. Labeled proteins were immunoprecipitated using an anti-Tac antibody and analyzed under reducing conditions on a 9% acrylamide gel. The positions of molecular weight markers (expressed as $10^{-3} \times M_r$) are indicated.
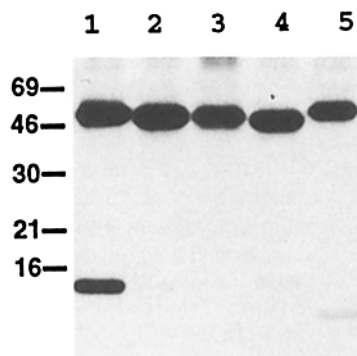
Figure 4. Role of transmembrane and cytoplasmic domains in the interactions between the FcεRI α and γ chains. COS-1 cells transfected with Tαα and FcεRI γ (lane 1), TαT and FcεRI γ (lane 2), TTα and FcεRI γ (lane 3), TαΔcyt and FcεRI γ (lane 4), or Tαα and FcεRI γΔcyt (lane 5) were labeled with [^{35}S]cysteine, immunoprecipitated in digitonin buffer with an anti-Tac antibody, and analyzed on 13% acrylamide gels under nonreducing conditions. The total levels of FcεRI γ, as determined by immunoprecipitation in parallel with an antibody directed against the cytoplasmic domain of FcεRI γ, were similar for the various transfections (data not shown). The positions of molecular weight markers (expressed as $10^3 \times M_r$) are indicated.
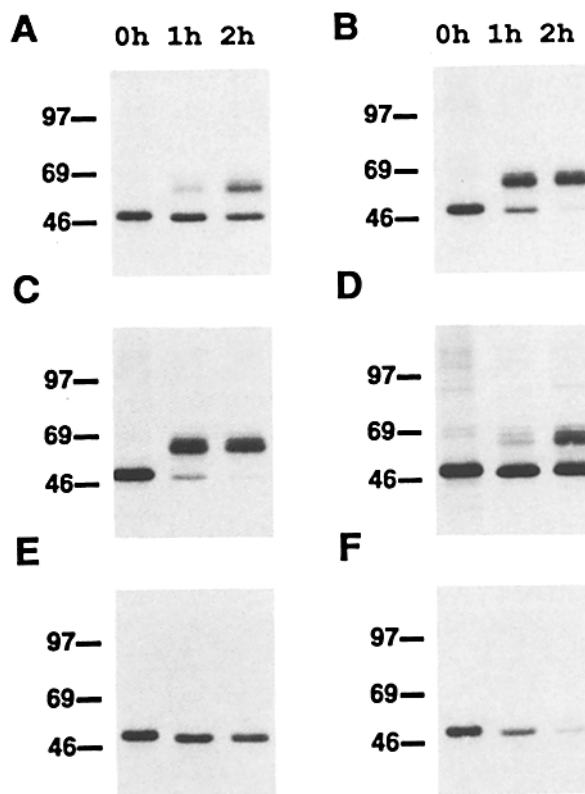

Figure 5. Role of the cytoplasmic domain of FcεRI γ in assembly with FcεRI α and intracellular transport. COS-1 cells were cotransfected with Tαα and FcεRI γ (A), FcεRI γt0 (B), FcεRI γt1 (C), FcεRI γt2 (D), FcεRI γt3 (E), or FcεRI γt4 (F). Transfected cells were metabolically labeled for 15 min at 37°C with [^{35}S]methionine, after which they were placed in regular culture medium at 37°C for 0, 1, or 2 h, as indicated. Labeled proteins were immunoprecipitated using an anti-Tac antibody and analyzed under reducing conditions on a 9% acrylamide gel. The positions of molecular weight markers (expressed as $10^{-3} \times M_r$) are indicated.

membrane-proximal cytoplasmic sequence is required both for assembly and masking of the ER retention signal hampers direct assessment of this hypothesis. To overcome this problem, we devised a system to test directly the effect of juxtaposition of various cytoplasmic domains on their ability to cause ER retention. For this, we constructed a chimera composed of the extracellular and transmembrane domains of CD8β fused to the cytoplasmic domain of the FcεRI α chain (B-α) and a chimera composed of the extracellular and transmembrane domains of CD8α fused to the cytoplasmic domain of various FcεRIγ mutants (A-γ; Fig. 6 A). As shown in previous studies (Letourneur et al., 1990; Hennecke and Cosson, 1993), assembly and transport of the CD8 heterodimers is independent of their cytoplasmic domains. Mutants of CD8α and β with deleted cytoplasmic domains (A-ΔCyt and B-ΔCyt) assemble efficiently, as assessed by coprecipitation of A-ΔCyt with anti-CD8β antibodies, and are transported out of the ER, as shown by conversion into Golgi-processed higher molecular weight species (Fig. 6 B). As expected in this system, mutations in cytoplasmic domains do not have any effects on the assembly. When B-α is cotransfected with A-ΔCyt, the cytoplasmic domain of FcεRIα causes retention of the complex in the ER (Fig. 6 C). When B-α is cotransfected with A-γt0, the complex is efficiently transported out of the ER (Fig. 6 D), demonstrating that juxtaposition of the cytoplasmic domain of the FcεRI α chain with that of the γ chain is sufficient to promote transport out of the ER.

We analyzed in this system the effect on transport of successive deletions of the cytoplasmic domain of the γ chain, and found results qualitatively identical to those obtained previously (Table I): cotransfection of B-α with A-γ results in transport of the complex out of the ER, and transport is even more efficient with A-γt0 and A-γt1, decreases for

Table I. Masking of the ER Retention Motif of FcεRI α Chain by Various Cytoplasmic Domains

	Cytoplasmic domain length (amino acids)	Percent transport at 2 h
A-ΔCyt	3	4 ± 4
A-γt3	7	12 ± 1
A-γt2	15	31 ± 2
A-γt1	20	69 ± 12
A-γt0	35	51 ± 14
A-γ	42	33 ± 2
A-CD8	28	70 ± 6
A-CD4	38	30 ± 7
A-HLA	30	63 ± 4
A-Tac	12	10 ± 3
A-α	31	<5

Pulse–chase analysis of cells transfected with B-α and the indicated A-x construct was performed as indicated in Fig. 6. The percentage of A-x transported out of the ER after a 2-h chase compared with the total A-x at time 0 was determined by densitometric scanning of the gels. The mean and standard deviation from at least three experiments are indicated. The length of the cytoplasmic domain of the various A-x constructs is indicated.

A-γt2, and is very reduced with further truncations of the cytoplasmic domain (A-γt3; A-ΔCyt). As observed previously, efficient transport of the complex is only detected when the cytoplasmic domain of the γ chain is longer than 15–amino acid residues.

This effect could be due to the presence of a specific motif in the cytoplasmic domain of the FcεRI γ chain or to the requirement for a minimum length for efficient steric masking of the cytoplasmic domain of FcεRI α. To discriminate between these two possibilities, we constructed chimeras of γ with the cytoplasmic domain of the mouse CD8α chain (γ-CD8; Fig. 1 B). These chimeras retain 7 amino acids of the cytoplasmic domain of the γ chain, so that they can still assemble with Tαα. When cotransfected with Tαα, they promote efficient transport of Tαα out of the ER (Fig. 7 B). In the CD8 system, cotransfection of B-α with A-CD8 (that is, wild-type CD8α) also allowed efficient transport of the complex out of the ER (Table I and Fig. 7 C). Identical results were obtained in this system with chimeras using the cytoplasmic domain of human CD4 (A-CD4; 37 amino acids) or of the HLA-B27 MHC class I molecule (A-MHC; 30 amino acids) (Table I). In contrast, a chimera with a short cytoplasmic domain derived from the Tac molecule (A-Tac; 12 amino acids) did not cause efficient transport of B-α (Table I). Together, these results demonstrate that transport of assembled complexes results from the steric masking of the cytoplasmic domain of the FcεRI α chain, and is observed when any cytoplasmic domain longer than 15–20 amino acids is juxtaposed to it.

Discussion

In this work, we analyzed the structural constraints that control the assembly and surface expression of the human FcεRI expressed into COS cells. We show that the transmembrane domains of the α and γ chains drive the assembly of the α/γ complex. The cytoplasmic domains of the two chains are
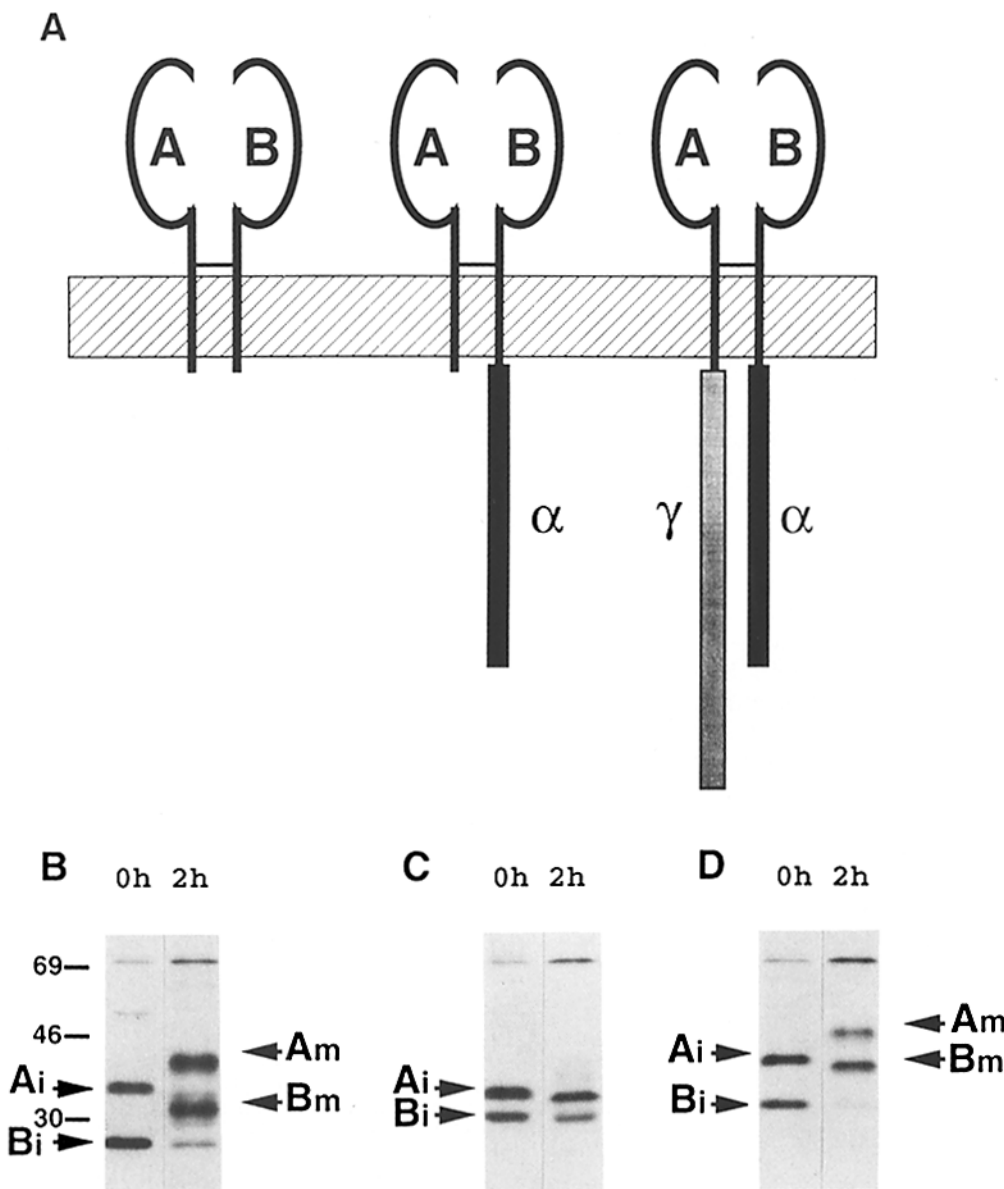

Figure 6. The ER retention signal in FcεRI α cytoplasmic domain is masked by juxtaposition with the FcεRI γ cytoplasmic domain during assembly. (*A*) Chimeras using the lumenal and transmembrane domains of CD8α (A-x) or CD8β (B-x) were constructed with either no cytoplasmic domains (Δcyt), the cytoplasmic domain of FcεRI α chain (α), or the cytoplasmic domain of FcεRI γ (γ). COS-1 cells were cotransfected with A-Δcyt and B-Δcyt (*B*), A-Δcyt and B-α (*C*), or A-γ and B-α (*D*). Transfected cells were metabolically labeled for 15 min at 37°C with [³⁵S]methionine, after which they were placed in regular culture medium at 37°C for 0 or 2 h, as indicated. Labeled proteins were immunoprecipitated using an anti-CD8β antibody and analyzed under reducing conditions on an 11% acrylamide gel. The positions of molecular weight markers (expressed as $10^{-3} \times M_r$) are indicated as well as the positions of the mature (*m*) and immature (*i*) CD8α (*A*) and CD8β (*B*) species.

A

Tαα

γ-CD8

CD8

CD8   α

B

	0h	2h
97—		
69—		
46—		

C

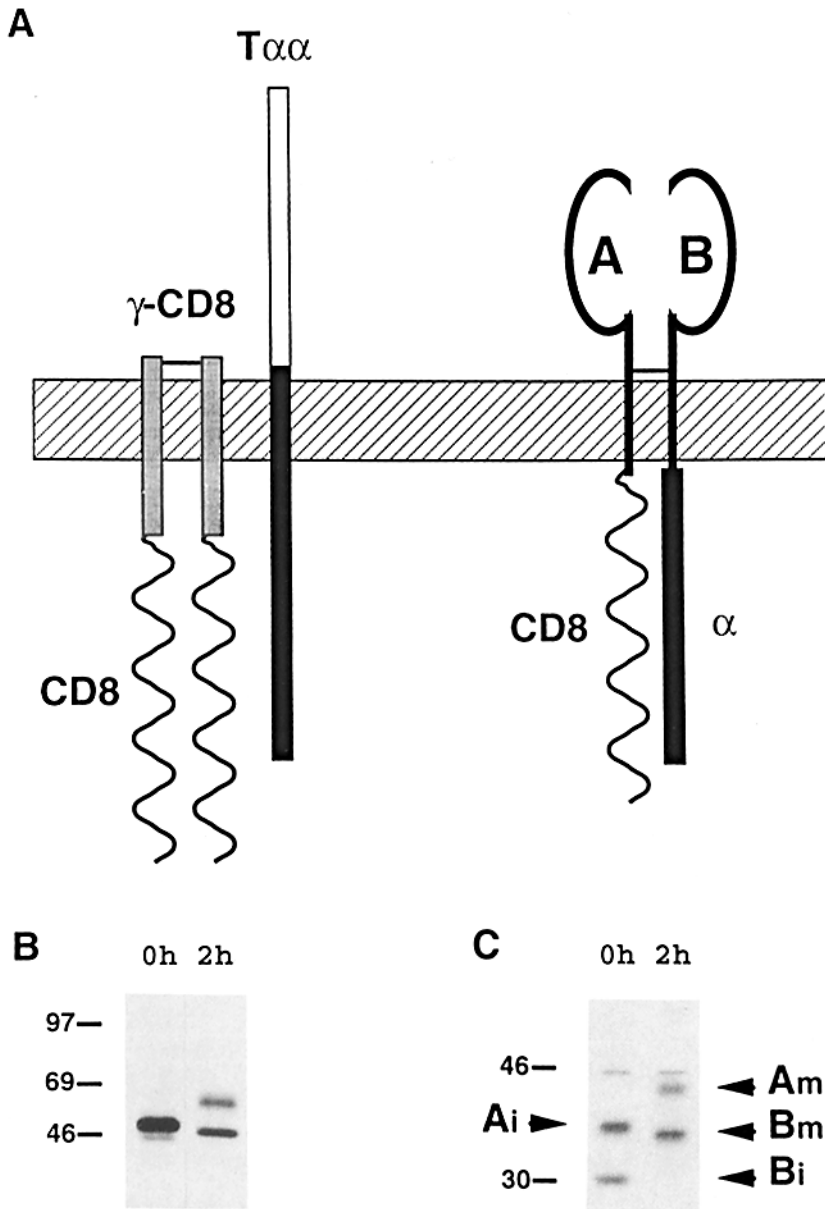|	0h	2h	
46—			◄ Am
Ai ►			◄ Bm
30—			◄ Bi

Figure 7. Steric masking of the ER retention signal in FcεRI α cytoplasmic domain. (*A*) Chimeras using the cytoplasmic domain of CD8α (CD8) associated with the extracellular and transmembrane domains of CD8α (A-CD8) or of the FcεRI γ chain (γ-CD8) were constructed. COS-1 cells were cotransfected with Tαα and γ-CD8 (*B*) or with A-CD8 and B-α (*C*). Transfected cells were metabolically labeled for 15 min at 37°C with [³⁵S]methionine, after which they were placed in regular culture medium at 37°C for 0 or 2 h, as indicated. Labeled proteins were immunoprecipitated using an anti-Tac antibody (*B*) or an anti-CD8β antibody (*C*) and analyzed under reducing conditions on acrylamide gels. The positions of molecular weight markers (expressed as $10^{-3} \times M_r$) are indicated, as well as the positions of the mature (*m*) and immature (*i*) CD8α (*A*) and CD8β (*B*) species.

also necessary to stabilize the interaction, and no interaction is observed when the cytoplasmic domain of the γ chain is shorter than seven-amino acid residues. When unassembled, the transmembrane domain of the α chain caused ER retention and rapid degradation. Upon assembly with the γ chain, this transmembrane retention/degradation signal is masked. This finding is similar to previous observations on the T cell receptor, where it was shown that transmembrane domains can act as ER retention and degradation signals as long as they are not assembled with another transmembrane domain (Bonifacino et al., 1990).

A signal that causes ER retention of the unassembled α chain is also present in its cytoplasmic domain, as substitution of Tac cytoplasmic domain by that of the α chain resulted in efficient ER retention of the chimera. This signal presents the characteristics of a typical dilysine retention motif (Jackson et al. 1990), though the two critical lysine

residues are found at positions -3 and -7 from the COOH-terminal end. This retention motif is masked upon assembly with the γ chain. When most of the cytoplasmic domain of the γ chain is deleted, it is still able to assemble with α, albeit weakly, and to mask the transmembrane ER degradation signal of the α chain, but not its cytoplasmic ER retention signal. This result suggests that it is the juxtaposition of the cytoplasmic domains of the α chain and the γ chain during the assembly of the FcεRI that releases ER retention by the cytoplasmic domain of the α chain.

The fact that the membrane-proximal cytoplasmic sequence is required both for assembly and masking of the ER retention signal hampers the direct assessment of the role of the cytoplasmic tail of the γ chain in this process. To overcome this problem, we devised a system based on the construction of artificial disulfide-linked dimers in which dimerization is independent of the cytoplasmic domains. For this

we constructed chimeras composed of CD8β extracellular and transmembrane domains fused to various cytoplasmic domains (B-x) and similar chimeras using CD8α extracellular and transmembrane domains (A-y), and followed the fate of the dimers of B-x and A-y. The cytoplasmic domain of α hooked to CD8β can cause ER retention of the dimers, when no cytoplasmic domain is attached to CD8α. Fusing the cytoplasmic domain of the FcεRI γ chain to CD8α abolishes ER retention. Successive truncations of the cytoplasmic domain of the γ chain in this system had the same effect on ER retention as observed previously: when the cytoplasmic domain of the γ chain is shorter than 15 amino acids, it cannot abolish ER retention anymore.

We also made use of this system to analyze the features of the γ cytoplasmic domain that are necessary to abolish ER retention by the cytoplasmic domain of the FcεRI α chain. We found that in this system any cytoplasmic domain longer than 20 amino acids and that does not itself contain an ER retention motif abolishes retention when it is juxtaposed to the cytoplasmic domain of the α chain of FcεRI. To confirm these results in the FcεRI model, we constructed a chimera of the FcεRI γ chain where only 7 amino acids of the cytoplasmic domain of γ remain, fused to the CD8α cytoplasmic domain. This γ-CD8 chimera is able to assemble with Tαα and to cause transport out of the ER and to the cell surface.

Together, these results indicate that the dilysine ER retention signal contained in the cytoplasmic domain of the FcεRI α chain is masked during assembly due to its juxtaposition with the cytoplasmic domain of the γ chain. The effect is apparently purely steric, as any cytoplasmic domain longer than 15 amino acids can hide the ER retention motif of α when it is juxtaposed to it.

One should not conclude that any protein having a sufficiently long cytoplasmic tail could nonspecifically mask the ER retention motif of the α chain and thus allow transport out of the ER of aberrant FcεRI complexes. Only a protein that can efficiently assemble with the FcεRI α chain could affect its fate. Moreover several retention signals are usually present in a given transmembrane protein (in this case, transmembrane as well as cytoplasmic retention motifs), and all of them must be masked before transport to the cell surface can take place. Our results illustrate the requirement for redundant mechanisms of intracellular retention in order to control efficiently the intracellular transport of membrane receptors.

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