unknown, the identification of γ_c as a component of the IL-7R may explain some features of XSCID, perhaps even more completely than the knowledge that γ_c is part of the IL-2 receptor. Although IL-2 stimulates proliferation of murine thymocytes (*18*), IL-2–deficient mice have normal thymic development (*6*). In contrast, mice treated with a monoclonal antibody to IL-7 have a significant decrease in thymic cellularity (*19*). The exact sites of γ_c function in T cell development remain unclear, but γ_c is expressed on all thymic populations studied (*20*). In addition to participation in T cell development, IL-7 promotes the growth of pre-B cells (*19*). Thus, a loss of IL-7 signaling might contribute to both the T cell and B cell defects found in XSCID. Nevertheless, the involvement of γ_c in the IL-2 and IL-7 receptors may not fully explain the defects of XSCID. Indeed, it is now clear that γ_c is also a part of the IL-4 receptor and perhaps part of other cytokine receptors as well (*21*).

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25. Cells were harvested and resuspended at 10^6 cells in 100 μl of RPMI 1640, 25 mM Hepes (pH 7.4), bovine serum albumin (BSA) (5 mg/ml). ^{125}I–IL-7 (30 pM) was added to cells in the presence of 0 to 500 nM unlabeled IL-7 and incubated on ice for 2 hours. Cells were then overlayed onto 150 μl of 20% olive oil, 80% dibutylphthalate and centrifuged at 4500*g* for 4 min. Pellets and supernatants were counted. Receptor numbers and K_d's were determined with the Ligand computer program (*22*). Although it is reasonable to hypothesize that cells expressing both IL-7R and IL-2Rγ might express high- (IL-7R associated with IL-2Rγ), intermediate- (IL-7R not associated with IL-2Rγ), and low- (endogenous IL-7–binding activity) affinity receptors, the Ligand computer program could only fit the data to two binding affinities rather than three, even for the data in (H) where one might envision the existence of three different affinities. We present only the data "objectively" derived from the computer program.
26. Cells were washed twice with ice-cold medium and resuspended at 4×10^7 cells per milliliter in prewarmed (37°C) medium. At the indicated times, two 50-μl samples of the cell suspension were removed. One sample was treated with acid (*23*), the cells were centrifuged through olive oil–dibutylphthalate as in Fig. 3, and the cell pellet and supernatant were counted to determine the acid-resistant (that is, internalized) and acid-sensitive (that is, cell surface–bound + dissociated) IL-7. The other sample was immediately centrifuged through the oil layer, and the radioactivity in the supernatant was measured to determine the amount of dissociated IL-7. The acid-resistant counts at 10, 20, 40, and 60 min are expressed as a fraction of the ligand specifically bound at time 0 (percent internalized) to normalize for variations in transfection efficiency and for the higher affinity binding expressed by cells transfected with IL-7R + IL-2Rγ as compared with IL-7R alone.

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Interleukin-2 Receptor γ Chain: A Functional Component of the Interleukin-4 Receptor

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The interleukin-2 (IL-2) receptor γ chain (IL-2Rγ) is an essential component of high- and intermediate-affinity IL-2 receptors. IL-2Rγ was demonstrated to be a component of the IL-4 receptor on the basis of chemical cross-linking data, the ability of IL-2Rγ to augment IL-4 binding affinity, and the requirement for IL-2Rγ in IL-4–mediated phosphorylation of insulin receptor substrate–1. The observation that IL-2Rγ is a functional component of the IL-4 receptor, together with the finding that IL-2Rγ associates with the IL-7 receptor, begins to elucidate why deficiency of this common γ chain (γ_c) has a profound effect on lymphoid function and development, as seen in X-linked severe combined immunodeficiency.

Interleukin-2 (*1*) and IL-4 (*2*) are multifunctional cytokines. Both can act as lymphocyte growth factors, although their ranges of actions are quite different. IL-2 is the principal T cell growth factor (*1*); IL-4 also exhibits T cell growth factor activity, but its principal actions are to regulate B cell growth and immunoglobulin class switching (*2*, *3*). The structure of each cytokine consists of a bundle of four α helices in which the first and second helices and the third and fourth helices are connected by long overhand loops, resulting in a characteristic "up, up, down, down" configuration (*4*). The prototypic molecule to exhibit this structure is growth hormone. Growth hormone transduces signals by homodimerization of the growth hormone receptor (*5*), whereas for IL-2 the formation of a heterodimer of the IL-2Rβ and IL-2Rγ

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chains is essential for signaling (6). Until now, only a single chain of the IL-4 receptor has been recognized. This chain binds IL-4 with high affinity and has a long cytoplasmic domain (*7, 8*).

Humans with mutated IL-2Rγ chains have X-linked severe combined immunodeficiency (XSCID) (*9*), a disease characterized in part by a virtual absence of T cells (*10*). In contrast, mice in which the IL-2 gene was inactivated by targeted gene disruption exhibit relatively normal T cell development (*11*), which suggests that IL-2Rγ is also used by other cytokine receptors (*9*). IL-2Rγ is a functional component of both the IL-2 (*12*) and the IL-7 (*13*) receptors, and we therefore proposed that IL-2Rγ be denoted the common γ chain, γ_c (*13*). Features of the biological actions of IL-4 and knowledge that the role of γ_c was not limited to the IL-2R suggested that IL-4 signaling might be mediated not by homodimerization of the IL-4R as has been speculated (*14*), but rather by heterodimerization of the IL-4R with γ_c.

To investigate this possibility, we first used ^{125}I-labeled IL-4 in affinity labeling experiments. Cross-linking of ^{125}I–IL-4 to COS-7 cells transfected with a vector control yielded an affinity-labeled complex of 145 to 150 kD (Fig. 1A). Because IL-4 has a molecular size of 16 kD, this was consistent with constitutive expression on COS-7 cells of small amounts of a 130- to 135-kD IL-4 receptor. A weak band of 85 kD was seen that did not appear related to the IL-4R or γ_c because it was not precipitated with antibodies to either of these molecules. Transfection of the human IL-4R significantly increased the intensity of the 145- to 150-kD affinity-labeled band (Fig. 1A). Faint 70- and 85-kD bands were also seen (*7, 15*). Transfection of cells with γ_c alone yielded a pattern similar to that seen with the vector control. When cells were transfected with IL-4R and γ_c or with IL-4R and the γ_c-ΔSH2 or γ_c-ΔCT truncation mutants (*16*), new bands of sizes expected for IL-4 cross-linked either to γ_c or to the smaller mutants were observed (Fig. 1A). No such bands were found with cells transfected with the IL-4R and IL-2Rβ. These results indicate that γ_c could associate with IL-4 in the presence of the IL-4R and that the cytoplasmic domain of γ_c (truncated in γ_c-ΔSH2 and in γ_c-ΔCT) was not required for this interaction.

The presence of γ_c in the affinity-labeled bands was confirmed with an antiserum specific for γ_c (anti-γ_c) (Fig. 1B); anti-γ_c immunoprecipitated affinity-labeled γ_c and IL-4R only when both complementary DNAs (cDNAs) were cotransfected (Fig. 1B). A weak signal was obtained from cells transfected with γ_c, which indicates that the endogenous COS-7 IL-4R can also associate with human γ_c. The low intensity of the signal may reflect the low IL-4R expression on these cells and may explain why γ_c was not seen in lane 3 in Fig. 1A. No bands were immunoprecipitated by anti-γ_c when γ_c truncation mutants were cotransfected because anti-γ_c recognizes an epitope distal to the truncations.

The 145- to 150-kD affinity-labeled species was immunoprecipitated by antibodies to the IL-4R (anti–IL-4R) from all samples; the signal was strongest when the IL-4R cDNA was transfected (Fig. 1C). Bands corresponding to labeled γ_c or to truncated γ_c were also precipitated with anti–IL-4R after transfection of the IL-4R with γ_c or the γ_c truncations. Consistent with our results shown in Fig. 1, A and B, little if any γ_c was coprecipitated in the absence of the transfected IL-4R (Fig. 1C). Similar results (*17*) were obtained with the IL-4R monoclonal antibody M56 (*18*). No bands were immunoprecipitated by preimmune serum (Fig. 1D). Thus, IL-4 can be cross-linked both to the IL-4R and to γ_c; the efficiency of cross-linking to γ_c correlated with the amount of IL-4R expression. The ability of antibodies to either IL-4R or γ_c to immunoprecipitate both affinity-labeled IL-4R and γ_c suggests that γ_c is associated with the IL-4R. Similar affinity labeling and immunoprecipitation results were obtained with MLA 144 cells, which constitutively express the IL-4R and γ_c (Fig. 2, A and B).

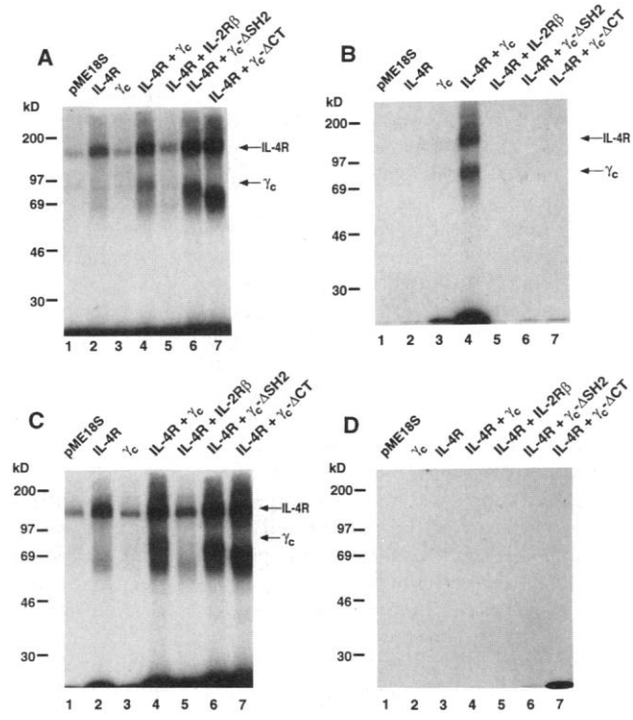


Fig. 1. COS-7 cells transfected with the use of DEAE dextran were affinity labeled (*33*) with 1 nM ^{125}I–IL-4 [labeled with Iodogen (Pierce)]. COS-7 cells were transfected with pME18S (lane 1) or with pME18S containing cDNAs encoding the IL-4R (lane 2), γ_c (lane 3), IL-4R and γ_c (lane 4), IL-4R and IL-2Rβ (lane 5), IL-4R and γ_c-ΔSH2 (lane 6), or IL-4R and γ_c-ΔCT (lane 7) (*16, 34*). After affinity labeling, cells were lysed with extraction buffer and analyzed on a 7.5% SDS-polyacrylamide gel (**A**) or were immunoprecipitated with anti-γ_c (**B**), anti–IL-4R (**C**), or preimmune serum (**D**) before analysis on SDS gels (*18*). The positions of the affinity-labeled IL-4R and γ_c are indicated.

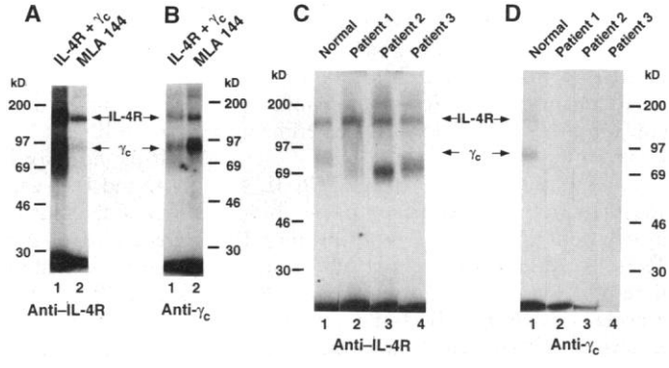
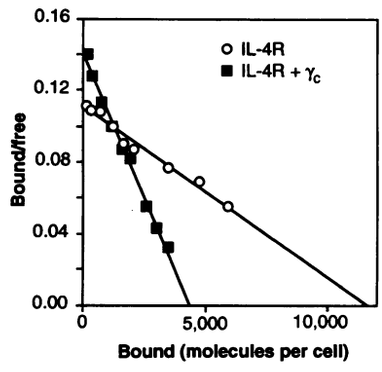


Fig. 2. (**A** and **B**) ^{125}I–IL-4 affinity labeling of COS-7 cells transfected with IL-4R and γ_c (lanes 1) or MLA 144 cells (lanes 2), then immunoprecipitated with an antibody to the IL-4R (P7) (A) or to γ_c (R878) (B). MLA 144 is a gibbon T cell line that expresses both IL-2 and IL-4 receptors. (**C** and **D**) Affinity labeling of the IL-4R and γ_c with 1 nM ^{125}I–IL-4 on the surface of EBV-transformed B cells from a normal donor (lane 1) or from three XSCID patients (lanes 2 to 4). Patient 1 has a premature stop codon that truncates 251 amino acids (*9*), including the entire transmembrane and cytoplasmic domains; the EBV line from this patient does not encode a cell surface γ_c protein nor expresses detectable γ_c mRNA. Carboxyl-terminal truncations of 81 and 62 amino acids, respectively, of γ_c are found in patients 2 and 3 (*9*). After affinity labeling, samples were immunoprecipitated with either anti–IL-4R (C) or anti-γ_c (D).

Fig. 3. Scatchard analysis of COS-7 cells transfected with 20 μg of the IL-4R or 2 μg of the IL-4R and 20 μg of γ_c plasmids followed by IL-4 binding. Only a single affinity is seen for IL-4R and γ_c, because with the use of 10-fold excess of γ_c, the binding was quantitatively shifted to the higher affinity. The ratios of the IL-4R and γ_c could be manipulated to allow detection of both classes of the receptor on the same cells (*17*). Although COS-7 cells express small amounts of endogenous IL-4Rs, their presence did not influence our ability to achieve single affinities when the human IL-4R and γ_c were overexpressed on these cells, even though the endogenous receptors exhibited high affinity. Binding assays were performed as described (*7*, *26*) and analyzed with the Ligand computer program (*35*).



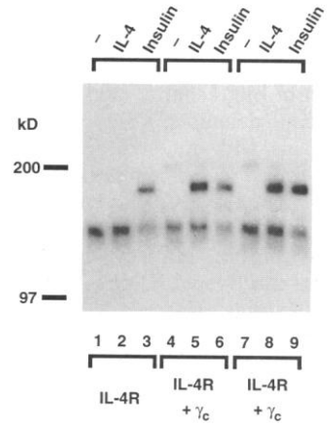


Fig. 4. Immunoblot with phosphotyrosine antibodies of an L cell clone expressing the IL-4R (lanes 1 to 3) or two clones expressing the IL-4R and γ_c (lanes 4 to 6 and 7 to 9) (*36*). Quiescent cells were not stimulated (lanes 1, 4, and 7) or were stimulated with murine IL-4 (180 pM; lanes 2, 5, and 8) or insulin (8 μM; lanes 3, 6, and 9) for 10 min, lysed, immunoprecipitated with phosphotyrosine antibody 4G10, and then immunoblotted with 4G10 (*19*).

We next used ^{125}I–IL-4 to affinity-label Epstein-Barr virus (EBV)–transformed B cells from a normal individual and three XSCID patients (*9*) who have truncated γ_c proteins. Immunoprecipitation with anti–IL-4R showed that each cell line expressed the IL-4R (Fig. 2C). The cell line derived from a normal individual also yielded an affinity-labeled band that migrated at a position expected for γ_c and was immunoprecipitated by anti-γ_c (Fig. 2D). A faint smear of 70 to 80 kD was immunoprecipitated from cells from patient 1 (Fig. 2C), but this could not be γ_c because a premature stop codon results in the deletion of the transmembrane and cytoplasmic domains, thus preventing surface γ_c expression. Anti–IL-4R immunoprecipitated affinity-labeled proteins from patients 2 and 3 that were appropriate in size for their respective truncations of γ_c (Fig. 2C). Anti-γ_c did not immunoprecipitate γ_c from any of the patient cell lines (Fig. 2D) because it recognizes an epitope distal to all three truncations.

In the cross-linking experiments in COS-7 cells, the presence of γ_c modestly increased the intensity of the affinity-labeled bands (Fig. 1A). Consistent with this observation, Scatchard analysis of ^{125}I–IL-4 binding to COS-7 cells transfected with the IL-4R and γ_c (Fig. 3) revealed that the presence of γ_c increased the binding affinity for IL-4 approximately 2.5- to 3-fold (dissociation constant of 266 pM without γ_c and 79 pM with γ_c for the experiment shown in Fig. 3).

To investigate the role of γ_c in IL-4 signaling, we examined the tyrosine phosphorylation of insulin receptor substrate–1 (IRS-1), a protein that is phosphorylated in response to IL-4 or insulin and is an essential mediator of IL-4–induced mitogenesis in 32D myeloid progenitor cells (*19*). Tyrosine phosphorylation of IRS-1 was evaluated in murine L cells, which constitutively express the IL-4R but lack endogenous γ_c (*17*), and in L cells transfected with γ_c (Fig. 4). As expected, IRS-1 was tyrosine-phosphorylated in response to insulin in each cell line (Fig. 4). IL-4 induced IRS-1 tyrosine phosphorylation in the two clones that expressed γ_c but not in cells that lacked γ_c. Thus, γ_c expression is essential for at least some IL-4–mediated signaling events. Because IL-4 binding to the IL-4R is easily detected in the absence of γ_c, it is interesting to speculate that some IL-4–induced signals might not require γ_c, whereas others, such as IRS-1 phosphorylation, require γ_c. This would be in keeping with the suggestion that there are two IL-4 signaling pathways (*20*).

The ability of human γ_c to functionally associate with the murine IL-4R (Fig. 4) and the ability of murine γ_c to associate with human IL-2Rβ in 32D and BAF/B03 cells (*6*, *21*) and with the human IL-4R in CT.h4S cells (*22*) are consistent with the high conservation of human and murine γ_c (70% identity) (*23*) that we observed. In contrast, human and murine IL-4Rs are less well conserved, with 51% amino acid sequence identity (*7*), and will only bind IL-4 derived from the same species.

The common γ chain confers a larger increase on IL-2Rβ binding affinity than on either IL-4R or IL-7R binding affinity (increases of 70 times, 2.5 to 3 times, and 5 to 10 times, respectively). The affinity with which IL-2 binds to IL-2Rβ is very low, but the presence of IL-2Rα on activated lymphocytes may complement this to provide a ligand-binding unit equivalent to the IL-4R and the IL-7R.

It is noteworthy that although IL-2, IL-4, and IL-7 each use γ_c, each cytokine induces different signals. A possible explanation is that although γ_c participates in signaling, signaling specificity is conferred by IL-2Rβ, IL-4R, and IL-7R. This is in contrast to three structurally related cytokines (IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor) that induce similar signals on their respective target cells. In this case, the shared common chain (β_c) (*24*) has a long cytoplasmic domain and is likely the key signal transducer for each of these cytokines (*25*). In fact, if the cytoplasmic domain of the IL-4R is replaced by that of IL-2Rβ, IL-4 then induces an IL-2–specific signal (*26*). Thus, heterodimerization involving β_c or γ_c, taken together with homodimerization of the growth hormone receptor or of receptor tyrosine kinases (*27*), illustrate different mechanisms by which dimerization can mediate cytokine- and growth factor–induced signaling.

Certain activation events, such as B7 expression on B cells and T cell proliferation, can be induced by IL-2 or IL-4 (*28*). Conversely, IL-4 inhibits IL-2 binding to some cell lines (*29*) and IL-2–mediated growth (*30*). Depending on the amount used, IL-4 can either augment or inhibit IL-2–mediated generation of natural killer cells (*31*). These findings may be explained by the differential recruitment of γ_c into one system and its concomitant sequestration from the other.

In addition to the role of γ_c in the IL-2, IL-4, and IL-7 receptors, it is likely that γ_c is a component of the IL-13R, which shares a common component with the IL-4R (*32*). Furthermore, because IL-9 (a potent T cell growth factor) is closely related to IL-7 (*14*), we hypothesize that it might also use γ_c. This potentially wide use of γ_c could explain how defective expression of γ_c results in the XSCID phenotype.

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17. S. M. Russell *et al.*, unpublished data.
18. The γ_c rabbit antiserum, R878, raised to a peptide corresponding to the eight COOH-terminal amino acids of γ_c (NH_2-Cys-Tyr-Thr-Leu-Lys-Pro-Glu-Thr-COOH) that had been coupled to keyhole lympet hemocyanin, was purified with the peptide coupled to EAH-Sepharose beads (Pharmacia). The rabbit antiserum to the IL-4R (P7) was generated with recombinant soluble human IL-4R and can neutralize IL-4 binding. M56 monoclonal antibody to human IL-4R was prepared by immunizing mice with soluble IL-4R; it cannot neutralize IL-4 binding.
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37. We thank S. Gillis and P. Beckmann for providing the P7 and M56 antibodies to the IL-4R, G. Tosato for the normal EBV cell lines, and J. H. Pierce, A. M. Weissman, and J. P. Siegel for helpful discussions.

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Active Oxygen Species in the Induction of Plant Systemic Acquired Resistance by Salicylic Acid

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A complementary DNA encoding a salicylic acid (SA)–binding protein has been cloned. Its properties suggest involvement in SA-mediated induction of systemic acquired resistance (SAR) in plants. The sequence of the protein is similar to that of catalases and the protein exhibits catalase activity. Salicylic acid specifically inhibited the catalase activity in vitro and induced an increase in H_2O_2 concentrations in vivo. H_2O_2 or compounds, such as SA, that inhibit catalases or enhance the generation of H_2O_2, induced expression of defense-related genes associated with SAR. Thus, the action of SA in SAR is likely mediated by elevated amounts of H_2O_2.

Infection of plants, particularly by necrotizing pathogens, leads to enhanced resistance to subsequent attacks by the same or even unrelated pathogens (*1*). This phenomenon is referred to as systemic acquired resistance (SAR). Because SAR provides long-term (weeks to months) protection throughout the plant (systemic) against a broad range of unrelated pathogens (*2*), its modulation through chemical or genetic engineering means holds considerable promise for reducing crop loss.

Development of SAR correlates with the systemic expression of a number of plant defense-related genes, including five or more families of pathogenesis-related (PR) genes (*3*). Some of the PR proteins inhibit pathogen growth in vitro (*4*); others confer partial resistance to fungal infection when constitutively produced in transgenic tobacco plants (*5*).

Evidence indicates that salicylic acid (SA) is a natural signal molecule for the activation of plant defense responses, including SAR. Application of exogenous SA or its derivative, acetylsalicylic acid (aspirin), induces PR gene expression and enhances resistance to plant diseases (*6*). Increases in the amount of endogenous SA are correlated with expression of PR genes and development of SAR in infected tobacco and cucumber plants (*7*). Also, in transgenic tobacco plants harboring the bacterial *nah*G gene encoding salicylate hydroxylase, which converts SA to catechol, induction of SAR by inoculation with tobacco mosaic virus is blocked (*8*).

We are interested in identifying cellular elements that directly interact with SA in order to understand the mechanism of SA action. We purified a soluble 240- to 280-kD SA-binding protein (SABP) from tobacco leaves (*9*, *10*). This protein is a complex composed of a 57-kD subunit and perhaps one or more additional polypeptides. It has an apparent K_d (dissociation constant) of 14 μM for SA—the concentration of SA found following infection ranges from 2 to 15 μM. Furthermore, the ability of a large number of SA analogs to compete with [^{14}C]SA for binding to the SABP strictly correlates with their ability to induce PR genes and resistance. These results suggest SABP mediates the defense response induced by SA.

Here, we have isolated and characterized the SABP cDNA. A clone (λCK1) encoding the 57-kD subunit of SABP was identified by screening a tobacco cDNA library with an SABP-specific monoclonal antibody (mAb; 3B6) (*11*). The protein encoded by the cloned cDNA reacted with four other SABP-specific mAbs. The amino acid sequence deduced from the cDNA sequence revealed high similarity (60 to 90% identical amino acids) to catalases of other organisms, with highest similarity to the plant catalases (Fig. 1A).

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