

Fate of Surrogate Light Chains in B Lineage Cells

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Summary

Biosynthesis of the immunoglobulin (Ig) receptor components and their assembly were examined in cell lines representative of early stages in human B lineage development. In pro-B cells, the nascent surrogate light chain proteins form a complex that transiently associates in the endoplasmic reticulum with a spectrum of unidentified proteins (40, 60, and 98 kD) and Bip, a heat shock protein family member. Lacking companion heavy chains, the surrogate light chains in pro-B cells do not associate with either the Ig α or Ig β signal transduction units, undergo rapid degradation, and fail to reach the pro-B cell surface. In pre-B cells, by contrast, a significant portion of the surrogate light chain proteins associate with μ heavy chains, Ig α , and Ig β to form a stable receptor complex with a relatively long half-life. Early in this assembly process, Bip/GRP78, calnexin, GRP94, and a protein of \sim 17 kD differentially bind to the nascent μ heavy chains. The 17-kD intermediate is gradually replaced by the surrogate light chain protein complex, and the Ig α and Ig β chains bind progressively to the μ heavy chains during the complex and relatively inefficient process of pre-B receptor assembly. The results suggest that, in humans, heavy chain association is essential for surrogate light chain survival and transport to the cell surface as an integral receptor component.

The long-held view that the precursors of B cells cannot see antigens before they rearrange and express both their H chain and L chain genes has been challenged by the discovery that neoplastic representatives of early B-lineage cells express surrogate (ψ) L chain proteins encoded by the nonrearranging *Vpre-B* and *14.1/λ5* genes (1–14). Sharing significant homology with conventional λ L chain, the ψ L chain proteins can bind to μ H chains, which associate with transmembrane Ig α and Ig β chains to form a novel receptor complex on pre-B cells in both humans and mice (15–19). The Ig α and Ig β chains form covalently bonded heterodimers that are noncovalently associated with the μ H chain (for review see reference 20). Cross-linkage of the ψ L chain/ μ H chain/Ig α Ig β complex can initiate signal transduction in pre-B cells, albeit less efficiently than ligation of antigen receptors on mature B cells (21–24). Although the functional role of the pre-B cell receptor is still hypothetical (12, 17, 25), the analysis of λ 5-deficient mice suggests that signaling via this receptor promotes survival and differentiation of B cell precursors (26).

Studies of this differentiation pathway in humans and mice indicate that both pro-B and pre-B cells produce the ψ L chain proteins (15, 17, 19, 23, 27). Although it has been suggested that pro-B cells can express a ψ L chain/ ψ H chain receptor (9, 11, 27), we could find cell surface expression of pre-B receptor components only during a relatively late

pre-B stage, well after the onset of μ H chain expression (15, 17). The considerable discordance in reports concerning timing of the cell surface appearance of ψ L chain (10–14, 17–19, 27–30) further emphasizes the need for better understanding of how the pre-B receptor components are assembled and transported to the cell surface.

The antigen receptor assembly process in B lineage cells has been examined most extensively in mature B cells (31–40). Newly synthesized H chains and κ or λ L chains undergo glycosylation, protein folding, and oligomerization in the endoplasmic reticulum (ER).¹ Resident ER proteins involved in this assembly process include glycosylases, protein disulfide isomerase, and the molecular chaperones Bip/GRP78, calnexin, and GRP94. The μ H chains in pre-B cell lines have been shown to associate with Bip (31, 33), and it is anticipated that other chaperones will also be involved in the folding and assembly of the pre-B receptor components.

Using human cell lines representative of early stages of B lineage development, we have examined the receptor assembly process in pro-B and pre-B cells. In pro-B cells, the ψ L chain proteins were found exclusively in intracellular compartments, where they transiently associate with

¹Abbreviations used in this paper: endo H, endoglycosidase H; ER, endoplasmic reticulum.

molecules of 98, 78 (Bip), 60, and 40 kD before undergoing rapid degradation. In pre-B cells, the assembly of the pre-B receptor components was found to be a relatively elaborate process during which Bip, calnexin, and GRP94 participate in the differential binding of μ H chain to Ig α , Ig β , and, less efficiently, the surrogate L chain proteins to form a stable receptor complex that can be expressed on the cell surface.

Materials and Methods

Antibodies. The following mouse mAbs were used: SA-DA4,4 (γ 1 κ), anti-human μ H chain (41); JDC12, anti-human κ LC (Southern Biotechnology Associates, Birmingham, AL); CB3-1 (γ 1 κ) anti-human Ig β (16); HM57 (γ 1 κ) anti-human Ig α (42); SLC1 and SLC2 (γ 1 κ and μ κ , respectively), anti-human ψ L chain (17); AF8 (γ 1 κ), and anti-IP90/calnexin (40). The JH3 (γ 1 κ) anti-human Ig idiotype (43), CT4 (γ 1 κ) anti-chicken CD4 (CLC), and Cla (μ κ) anti-chicken MHC class II (44) were used as controls. An anti-Bip mAb (32) and rabbit antibodies to GRP94 (45) were also used. PE-conjugated goat anti-mouse Ig was obtained from Southern Biotechnology Associates.

Cells. Cell lines were maintained in stationary culture in RPMI 1640 medium supplemented with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μ g/ml), and 10% FCS at 37°C in 5% CO₂. Human cell lines included the RS4;11 (46) and Nalm16 (47) pro-B cells, and the 697 (48), OB5 (59), and Nalm6 (47) pre-B cells.

Cytofluorometry. Viable cells were incubated with mAbs specific for μ H chains, ψ L chains, Ig β , or a control mAb, all of γ 1 κ isotype, and then with PE-conjugated goat antibodies to mouse Ig as a developing reagent. Stained cells were analyzed using a FACScan® flow cytometer (Becton Dickinson & Co., Mountain View, CA).

Biosynthetic Labeling. For pulse labeling and chase experiments, cells ($1-2 \times 10^8$) were preincubated in Met- and Cys-free RPMI 1640 or Iscove's medium for 2 h to deplete internal pools, and then labeled with 300–500 μ Ci of both ³⁵S-Met and ³⁵S-Cys for 2–30 min. Labeling was terminated by the addition of 100-M excess of cold Met and Cys, and the pulsed cells were incubated for various chase intervals, harvested, lysed, and subjected to analysis by immunoprecipitation. Data are presented only for experiments involving a 15-min pulse-labeling interval period, since analysis of the experiments performed at variable labeling intervals indicated that a 5-min pulse did not result in optimal labeling, and a 15-min pulse label yielded a protein-labeling pattern similar to that obtained after 30 min of labeling. For standard immunoprecipitation experiments, cells (3×10^7) were metabolically labeled with ³⁵S-Met and ³⁵S-Cys (100 μ Ci each) for 6 h.

Immunoprecipitation. Labeled cells were harvested and lysed in 1% NP-40 or 1% digitonin lysis buffer. Nuclei were sedimented at 10,000 g for 20 min, and the resulting supernatant was used for immunoprecipitation. After successive incubations with BSA and IgG-coupled Sepharose 4B beads, the precleared lysates were incubated in plastic wells coated with test or control antibodies (17). Bound materials were eluted with Laemmli's sample buffer (50) and resolved by SDS-PAGE, using 12% acrylamide in most experiments. In some experiments, the immunoadsorbed proteins were treated with endoglycosylase H (endo H; ICN Pharmaceuticals, Inc., Irvine, CA) for 18 h at 37°C, according to the manufacturer's instructions, before elution with Laemmli's buffer.

For two-dimensional gel electrophoresis analysis, immunopre-

cipitates were size separated in the first dimension on a nonreducing SDS-PAGE. The lanes were then excised, equilibrated for 45 min in SDS sample buffer containing β -ME, rotated 90°, and electrophoresed in the second dimension on SDS-PAGE under reducing conditions.

Densitometric Analysis. Analysis of the relative intensities of radiolabeled proteins was performed as a function of the pulse-chase interval using a densitometer (UltraScan XL) and software (GelScan XL; Pharmacia LKB Biotechnology, Uppsala, Sweden). Background readings of the autoradiographic films were designated zero, and the average relative intensity of the radioactivity for the protein bands compared was then plotted in arbitrary units for each time interval during the pulse-chase assay.

Results

Biosynthesis and Fate of the ψ L Chain Complex in Pro-B Cell Lines. Pro-B cell lines express the same complex of ψ L chain proteins (~22, 18, and 16 kD) that the pre-B cell lines express, although the pro-B cells have not yet undergone productive H and L chain gene rearrangements (17). The 22-kD protein is the product of the *14.1/λ5* gene, and the 16- and 18-kD proteins represent *Vpre-B* gene products (5, 8).

To examine the biosynthesis of the ψ L chain proteins and their fate in pro-B cells, we performed pulse-chase experiments by using the RS4;11 cell line, <1% of which expressed μ H chain in its cytoplasm (17), and the Nalm16 cell line, which retains its L chain and V_H genes in germline configuration and therefore expresses no μ H chain protein (47). The biosynthesis of ψ L chain, Ig α , and Ig β proteins could be detected easily in the RS4;11 cells (Fig. 1), although these were not expressed on the cell surface (Fig. 2; reference 17). When the pro-B cells were pulse labeled for 15 min and then chased over a 4-h period, the SLC1 mAb precipitated the ψ L chain triplex together with proteins of ~98, 60, and 40 kD under both reducing and nonreducing conditions (Fig. 1, A and B). The noncovalent association of the ψ L chain complex with the 98-, 60-, and 40-kD proteins was confirmed by two-dimensional gel analysis of the SLC immunoprecipitates (Fig. 1 B), although the ψ L chain association with these proteins was sufficiently avid to be detected in cell lysates prepared with NP-40 detergent as well as with the milder digitonin detergent.

To determine if the ψ L chain proteins were associated with known molecular chaperones, we examined three ER-based proteins—Bip/GRP78, calnexin, and GRP94—that are involved in the assembly of B cell receptors (39). No μ H chain was detected in immunoprecipitates of the ψ L chain proteins in the RS4;11 pro-B cells. The Bip/GRP78 protein was precipitated by an anti-Bip antibody, together with an ~100-kD protein (Fig. 1 A) and the ψ L chain proteins in trace amounts requiring longer radiographic exposure for detection (not shown). The ψ L chain proteins were not detected in immunoprecipitates of calnexin or GRP94, even after relatively long radiographic exposure, nor were the 90-kD calnexin or gp94 molecules found in the anti- ψ L chain precipitates (Fig. 1). The ψ L chain-associated proteins of 98, 60, and 40 kD thus appear

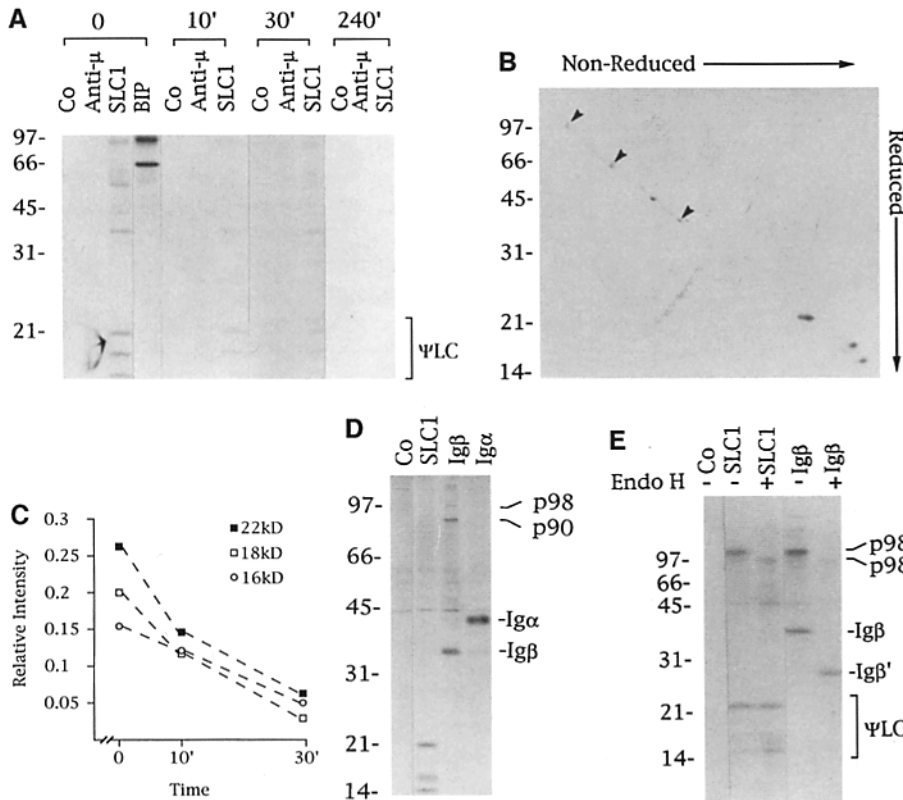


Figure 1. Biosynthetic analysis of the ψ L chains, Ig α , Ig β , and candidate molecular chaperones in the RS4;11 pro-B cell line. (A) Cells were pulse labeled for 15 min with 35 S-Met and 35 S-Cys and then chased for the indicated time intervals. Aliquots of cell lysates (1% digitonin) were incubated with anti- μ , SLC1 anti- ψ L chain, anti-Bip, or control (Co) mAbs, and the immunoprecipitates were electrophoresed in 12% SDS-PAGE. (B) SLC1 immunoprecipitates (15-min pulse label) were resolved in SDS-PAGE under nonreducing conditions in the first dimension and under reducing conditions in the second dimension. Associated proteins of \sim 98, 60, and 40 kD in the digitonin lysate of the pro-B cell line are indicated by arrows; actin (\sim 45 kD) is also seen in this 13-d autoradiographic exposure. (C) The relative amounts of labeled 22-, 18-, and 16-kD ψ L chain proteins were estimated at each time point by densitometric scanning of a representative autoradiograph (1-wk exposure), and the relative intensities are indicated. (D) To examine Ig α and Ig β protein expression in the RS4;11 line, cells were metabolically labeled (15-min pulse), lysed in 1% digitonin lysis buffer, and immunoprecipitated with SLC1, anti-Ig β , anti-Ig α , or control mAbs. Note the lack of receptor component assembly, and Ig β association with 90- and 98-kD proteins. (E) Endo H treatment confirms that the ψ L

chain proteins are not glycosylated, whereas the p98-kD protein associated with ψ L chains of Ig β and the Ig β protein are both endo H sensitive. Prime symbols indicate endo H-modified p98 and Ig β proteins of \sim 90 and 28 kD, respectively.

to be unrelated to the above three chaperones. In addition, the 60-kD molecule was found to be nonreactive with an antibody to HSP60 (data not shown).

Pulse-chase analysis revealed a time-dependent decrease in the amounts of proteins coprecipitated by the anti- ψ L chain antibody (Fig. 1, A and C). The intensity of the ψ L chain bands decreased during the chase interval at a rate indicative of a half-life of $<$ 30 min in the pro-B cells (Fig. 1, A and C), and the ψ L chain proteins were not detected in the cell supernatant (17; data not shown), suggesting that these proteins undergo intracellular degradation. Absence of ψ L chain glycosylation precluded the use of endo H sensitivity as a marker for their possible transit from the ER through the Golgi compartments. The ψ L chain-associated p98 molecules were entirely endo H sensitive (Fig. 1 E), however, suggesting that the ψ L chain/p98 complex does not reach the late (*trans*) Golgi compartments. The same pattern of ψ L chain synthesis, protein associations, and relatively short half-life was observed in pulse-chase analysis of the Nalm16 pro-B cell line (data not shown). These data suggest that the newly synthesized ψ L chain proteins in human pro-B cell lines are destined to undergo intracellular degradation.

The ψ L chain proteins produced by the pro-B cells did not associate with Ig α or Ig β chains, although these proteins were produced in relative abundance by both the RS4;11 and Nalm16 pro-B cell line (Fig. 1, D and E). Neither Ig α nor Ig β immunoprecipitates contained the ψ L

chain proteins, and, conversely, the Ig α and Ig β chains were not detected in the ψ L chain immunoprecipitates. The newly synthesized Ig α and Ig β chains were also precipitated independently, with limited association with each other and no detectable association with ψ L chain proteins. Ig α could not be detected in Ig β immunoprecipitates (Fig. 1, D and E), whereas the presence of multiple minor bands in the Ig α immunoprecipitates made it difficult to exclude

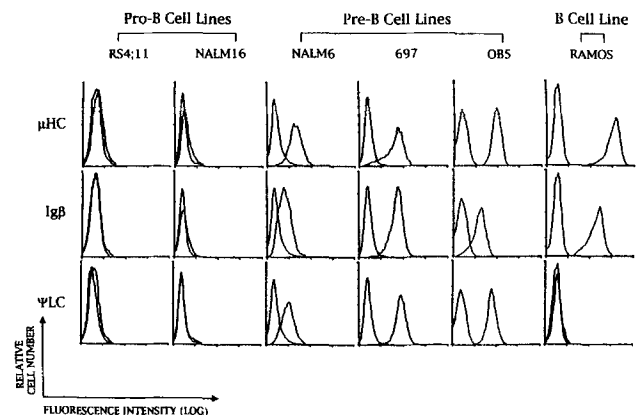


Figure 2. Analysis of the cell surface expression of receptor components on the pro-B and pre-B cell lines. Viable cells of the representative cell lines were examined by immunofluorescence for the expression of μ H, Ig β , and ψ L chains as described in Materials and Methods. Note the absence of all three components on the pro-B cells and their presence on the pre-B cell lines.

the coprecipitation of Ig β in trace amounts (Fig. 1 D). Neither Bip nor calnexin were found in association with Ig α and Ig β in the pro-B cell lines, but 98- and 90-kD proteins were coprecipitated with the Ig β molecule. Treatment with endo H indicated that both the Ig β chains and the associated 98-kD protein were endo H sensitive (Fig. 1 E), thus suggesting their confinement to the ER, *cis*- or *medial*-Golgi region. Immunofluorescence analysis of the pro-B cells with an anti-Ig β antibody confirmed that the Ig β chains do not reach the cell surface (Fig. 2). Lacking the ability to produce μ H chain, the pro-B cells thus failed to assemble their ψ L chains, Ig α , and Ig β , or to express these as components of cell surface receptors.

Biosynthesis and Assembly of Receptors in Pre-B Cell Lines. Biosynthesis and assembly of the ψ L chain/ μ H chain/Ig α β pre-B receptor complex were examined in three representative pre-B cell lines, Nalm6, OB5, and 697. When Nalm6 pre-B cells were pulse labeled for 15 min with [³⁵S]Met and [³⁵S]Cys and the labeled proteins were chased for variable time intervals, analysis of the anti- ψ L chain immunoprecipitates revealed the assembly of newly synthesized μ H chain and ψ L chain proteins, together with the 43-kD Ig α and 36-kD Ig β molecules (Fig. 3). Ig α and Ig β chains, the identities of which were verified in independent immunoprecipitations (see Fig. 1, D and E, and Fig. 5 C), were also seen in μ H chain immunoprecipitates of digitonin lysates of the pre-B cells. Although these experiments indicate that assembly of the pre-B receptor complex is initiated promptly, only a limited fraction of the available pools of receptor components appeared to undergo physical association with each other over the 4-h chase interval. The relative amounts of radiolabeled Ig α and Ig β in the anti- μ precipitates appeared to increase gradually, suggesting the sustained assembly of these Ig receptor components during this period of time.

Relatively small amounts of the nascent 22-kD λ 5/14.1 gene product were detected in the anti- μ and anti-Bip immunoprecipitates at early time points in the pulse-chase analysis, regardless of whether NP-40 or digitonin was used as the detergent for cell lysis. Thus, whereas the Vpre-B

16/18-kD molecules were produced in relative abundance, these were undetectable in either anti- μ or anti-Bip immunoprecipitates at early time points in the pulse-chase analysis (Fig. 3). Instead, an intermediate-sized protein of \sim 17 kD was observed to associate transiently with the μ H chain and Bip complex of proteins. This 17-kD intermediate was later replaced by the 16- and 18-kD Vpre-B molecules, concurrently with a noticeable increase in the μ H chain-associated 22-kD λ 5 protein. The ψ L chain pattern thus became similar in the anti- μ and anti- ψ L chain lanes between 2 and 4 h after pulse labeling. Notably, the 17-kD protein species was not seen in the anti- ψ L chain precipitates at any time during the pulse-chase analysis (Fig. 3).

Calnexin, detected in the pre-B cells by the IP90 antibody, formed a transient complex with μ H chain and a 34-kD protein, but the μ H chain complex with this ER-based chaperone did not include the 17-kD protein, ψ L chains, Bip, or the Ig α and Ig β molecules (Fig. 3 A; data not shown). On the other hand, Bip formed an association with μ H chain and the 17-kD protein, but this complex did not include ψ L chains, calnexin, or Ig α and Ig β . The GRP94 immunoprecipitates (not shown) revealed association of this protein with the μ H chain and Ig β in trace amounts but no ψ L chain association, even after prolonged radiographic exposure. These association patterns of the pre-B receptor components, which were not altered by permeabilization of the pre-B cells with digitonin before Na ¹²⁵I radiolabeling (data not shown), suggest differential association of the molecular chaperones with μ H chain during the pre-B receptor assembly process.

A similar pattern of pre-B receptor assembly was observed for the OB5 pre-B cell line. In pulse-chase experiments conducted with the OB5 pre-B cell line (Fig. 4), an intermediate protein species of \sim 17 kD was also found to be transiently associated with μ H chain and Bip, and the replacement of this protein by the conventional 18- and

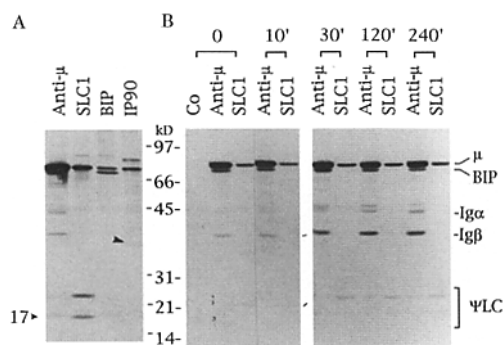


Figure 3. Analysis of receptor assembly in the Nalm6 pre-B cell line. Cells were pulse labeled for 15 min with [³⁵S]Met and [³⁵S]Cys, and then lysed in (A) 1% NP-40 or (B) 1% digitonin lysis buffer after variable chase intervals. Cell lysates were immunoprecipitated with anti- μ , SLC1, anti-Bip, or anti-IP90 antibodies. Bound materials were analyzed by SDS-PAGE under reducing conditions; autoradiographic exposure time, 3 wk.

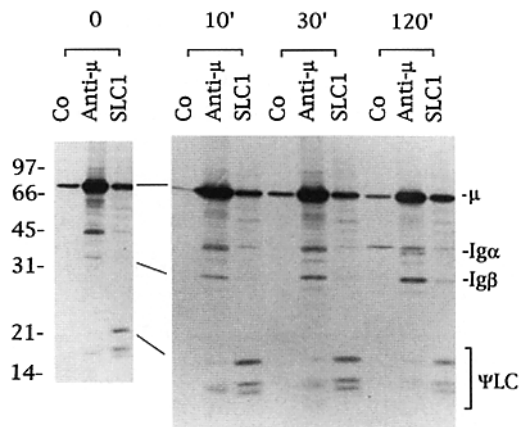


Figure 4. Analysis of receptor assembly in the OB5 pre-B cell line. Cells were pulse labeled for 15 min with ³⁵S-Met and ³⁵S-Cys; chased for 0, 10, 30, 120 min; and then lysed in 1% digitonin lysis buffer. Cell lysates were immunoprecipitated with anti- μ , SLC1, or control antibodies. Bound materials were analyzed by SDS-PAGE under reducing conditions; autoradiographic exposure time, 1 mo. Note that the \sim 17-kD protein, which binds to μ H chain (see also Bip immunoprecipitate in Fig. 3 A), is replaced by ψ L chain during the pulse-chase analysis (see also Fig. 3 B).

16-kD Vpre-B proteins together with the 22-kD $\lambda 5/14.1$ protein was cumulative over time. The ψ L chain proteins in the OB5 pre-B cells were never found in association with Bip, calnexin, or GRP94, whereas both GRP94 and μ H chain could be coprecipitated by the anti-Ig β antibody (not shown).

After pulse labeling of the 697 pre-B cells, the anti- ψ L chain antibodies precipitated the ψ L chain triplex of 16-, 18-, and 22-kD proteins together with easily detectable amounts of μ H chain (Fig. 5 A) in a temporal pattern indicating relatively prompt initiation of the association. However, at early time points in the pulse-chase analysis, the relative intensity of the 22-kD protein band coprecipitated with the anti- μ antibody was weaker than the 18- and 16-kD bands; conversely, the intensity of the 22-kD $\lambda 5$ band appeared much stronger in the anti- ψ L chain immunoprecipitates. As was observed for the other two pre-B cell lines, an increase in the amount of μ -associated 22-kD $\lambda 5$ protein was evident in anti- μ precipitates over the 2-h chase interval, during which there was no perceptible degradation of the ψ L chain proteins (Fig. 5, A and B, and data not shown).

Ig α and Ig β chains formed an association with both the μ H and ψ L chain proteins in digitonin lysates of 697 cells, as in the other pre-B cell lines. When the 697 pre-B cells were pulse labeled for 10 min, chased, and then harvested and lysed immediately, the anti-Ig β antibody precipitated Ig β (36 kD) together with μ H chain and the 98-kD glycoprotein (Fig. 5 C), whereas the anti-Ig α antibody precipitated Ig α and μ H chains; limited amounts of Ig β could be detected in the anti- α immunoprecipitates, and vice versa, with longer radiographic exposure. Although a majority of the Ig α and Ig β pools appeared not to be associated with each other or the μ H chains, pulse-chase analysis of individual Ig α and Ig β immunoprecipitates indicated a parallel increase in the amounts of associated μ H chain with time (Fig. 5 D). The relative amount of Ig β present in the anti-Ig α precipitates appeared to increase more slowly over the chase interval (Fig. 5 D), and a similar increase in Ig α was observed in the Ig β immunoprecipitates over the same time period (not shown). Remarkably, neither the anti-Ig α nor the anti-Ig β immunoprecipitates contained detectable amounts of the ψ L chain proteins during the entire 90-min chase interval.

To discriminate more precisely the composition of the μ H chain/Ig $\alpha\beta$ complex, the protein components were examined by two-dimensional gel electrophoresis after pulse labeling of the 697 cells. After a 15-min labeling period, a minor fraction of the Ig β pool appeared to be disulfide bonded to Ig α , whereas most of the Ig α chains in the anti-Ig α immunoprecipitates remained on the diagonal of two-dimensional electrophoretic gels, indicating that they had not formed covalent bonds (Fig. 6). The noncovalently associated μ chains in the anti-Ig β complex were resolved as disulfide-linked homodimers. The ψ L chain proteins were notably absent in the anti-Ig β immunoprecipitates (Fig. 6) and in comparable anti-Ig α immunoprecipitates (not shown).

In summary, the results obtained in the three representa-

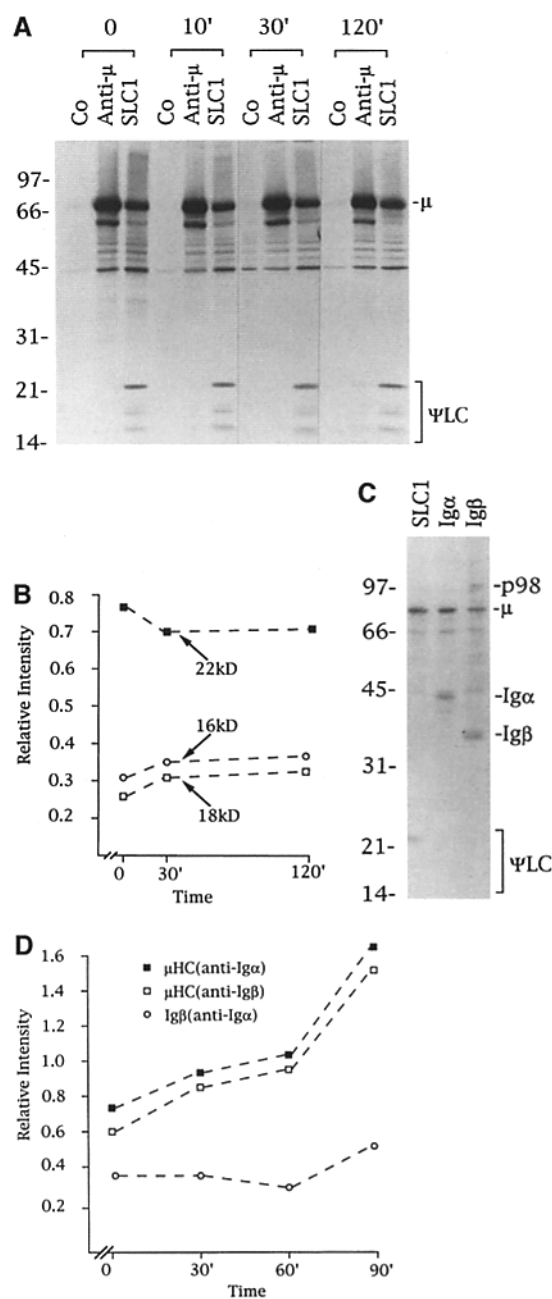


Figure 5. Analysis of receptor assembly in the 697 pre-B cell line. (A) Cells were pulse labeled for 15 min with ^{35}S -Met and ^{35}S -Cys; chased for 0, 10, 30, and 120 min; and then lysed in 1% NP-40 lysis buffer. Cell lysates were incubated with the anti- μ and SLC1 mAbs. Bound materials were electrophoretically separated on SDS-PAGE. (B) Densitometric analysis of radiolabeled bands performed as in Fig. 1 C indicates that the ψ L chains made by 697 pre-B cells are relatively stable. The same pattern was observed for ψ L chain kinetics in the Nalm6 and OB5 cell lines. (C) Both Ig α and Ig β bind to nascent μ H chains, but the majority of these proteins are precipitated independently of each other (15-min pulse label, 10-d autoradiographic exposure). Note the p98 association with Ig β , but not with Ig α , and the absence of detectable ψ L chains in the anti- α and anti- β immunoprecipitates. (D) Densitometric analysis of anti-Ig α and anti-Ig β precipitates indicates that the Ig α and Ig β chains associate with μ H chain in parallel fashion over the pulse-chase interval.

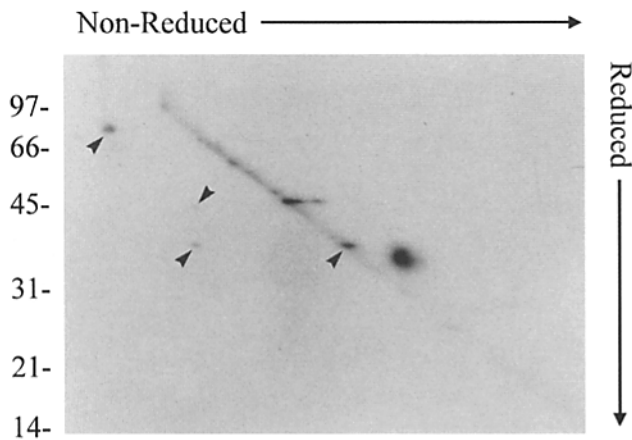


Figure 6. Two-dimensional gel analysis of the newly synthesized Ig α /Ig β / μ H chain complex in 697 pre-B cells. Digitonin lysates of metabolically labeled 697 cells prepared as in Fig. 5 and anti-Ig β immunoprecipitates were examined after a 30-min chase by two-dimensional gel analysis performed as in Fig. 1 C; autoradiographic exposure time, 1 mo. From left to right, the arrows indicate μ H chains, Ig α and Ig β chains released from the diagonal under reducing conditions, and a relatively large pool of free Ig β chains located on the diagonal. (The large spot above the diagonal is a radiographic artifact and was not seen in other experiments.) Note the lack of detectable ψ L chain products in the μ H chain/Ig α /Ig β complex.

tive pre-B cell lines suggest that the assembly of the ψ L chain/ μ H chain/Ig $\alpha\beta$ receptor occurs via convergent pathways that involve a variety of different chaperones to bring together the receptor components and that limitations exist in completion of the receptor assembly process.

Discussion

This analysis of human cell lines representative of early B lineage stages indicates that surrogate L chain (ψ L chain) proteins have very different fates in μ^- pro-B and μ^+ pre-B cell lines. These results are concordant with prior evidence indicating that, in humans, the pre-B cells can express ψ L chain on their cell surface and pro-B cells do not (15, 17), but they contrast with evidence obtained in mice suggesting that ψ L chain receptors may be expressed on both pro- and pre-B cells (7, 18, 19, 21, 27, 30).

In the human pro-B cell representatives RS4;11 and Nalm16, the *Vpre-B* and *14.1/λ5* gene products form a tripartite ψ L chain protein complex that associates with other proteins of ~98, 60, and 40 kD. Although the identity of these proteins is presently unknown, the 98-kD molecule was shown to be a glycoprotein, possibly ER based, with a protein core size of ~90 kD. Like the κ or λ L chains in B cells (51), the ψ L chain proteins in pro-B cells were also transiently associated with another ER-based protein, Bip, a glucose-regulated protein of 78 kD, but not with calnexin, which preferentially associates with glycosylated proteins (52, 53). GRP94 was also expressed in the pro-B lines, but, like calnexin, this chaperone was not associated with the ψ L chain proteins. The newly synthesized ψ L chain proteins in these pro-B cells were rapidly degraded and did not associate with their Ig α or Ig β chain neighbors,

which also showed little affinity for each other in the μ chain-negative pro-B cells. Like the ψ L chain proteins, the Ig β chains formed an association with a 98-kD glycoprotein that was endo H sensitive, suggesting confinement of this complex to the ER or early Golgi compartments. The p98 molecule that binds to Ig β and the p98 that associates with ψ L chain may be the same protein, since endo H treatment yielded a 90-kD product in each case. Because the Ig β and ψ L chain proteins do not associate with each other in pro-B cells, the ER-based p98 molecules may be separately involved in their cellular processing.

Immunofluorescence analysis of viable pro-B cells confirmed that neither the ψ L chain proteins nor the Ig β chains reach the cell surface at this stage of human B-lineage differentiation (17). In the absence of potential μ H chain partners, the fate of the ψ L chain proteins is thus very different in pro-B cells than in the μ H chain-positive pre-B cells. Although ψ L chains were not expressed on the surface of these human pro-B cell lines, identification of a 98-, 78- (Bip), 60-, and 40-kD complex of ψ L chain-associated proteins is reminiscent of the complex of proteins that have been reported to be associated with the ψ L chain on murine pro-B cell lines (18, 22, 30).

A very different picture was revealed by the analysis of three representative pre-B cell lines, all of which express cell surface receptors composed of ψ L chains, μ H chains, and Ig α/β . In contrast with the rapid ψ L chain degradation observed in pro-B cells, the nascent ψ L chain proteins in these pre-B cells were relatively stable. The proteins with which the newly synthesized ψ L chain associated were also different from those with which the ψ L chain associated in pro-B cells. The μ H chains in pre-B cells were found to be transiently associated with Bip, calnexin, and GRP94 as in mature B cells (37, 39). Newly synthesized ψ L chain proteins in the pre-B cell lines had a relatively long life span (>4 h), and they were not found to be associated with the companion 98- and 40-kD proteins observed in the pro-B cell lines. Since the ψ L chain proteins associate with each other in the absence of μ H chain, it was anticipated that the three ψ L chain proteins would bind rapidly and simultaneously to the nascent μ H chain, but this was not the case in the three pre-B lines. In two of these, an intermediate-sized protein of ~17-kD could be seen to bind to the μ H chain initially and was replaced later with the 16-, 18-, and 22-kD proteins. This transitional 17-kD protein was not recognized by the anti- ψ L chain antibodies and was not identified as a component of the mature ψ L chain/ μ H chain/Ig $\alpha\beta$ receptor complex on the cell surface. This component could represent the human counterpart of a murine 16.5-kD protein or the *Vpre-B3* gene products of similar size that have been found to be transiently associated with the H chains in murine pre-B cells (54, 55). Whatever its final identity, the 17-kD intermediate adds an additional level of complexity to the ψ L chain receptor assembly process.

The Ig α and Ig β chains are signal-transduction components of both pre-B and B cell receptors (16, 20, 56), and they are essential for the pre-B to B cell transition (57).

These receptor components are covalently bonded heterodimers that associate noncovalently with the μ H chain, so it was anticipated that Ig α /Ig β heterodimer formation would precede their association with the μ H chain. Although our analysis suggests that the Ig α and Ig β proteins may associate with the μ H chain in pre-B cells as disulfide-linked heterodimers, many of the Ig α and Ig β chains in pre-B lines were found to be unassociated with each other. The association of Ig α and Ig β chains with μ H chain occurred in parallel as a gradual cumulative process in the pre-B cells. Covalent association of these chains could thus be a prerequisite to their μ H chain coupling, although the Ig α and Ig β chains may associate with different ER-based proteins during the receptor assembly process.

Even though the Ig α /Ig β heterodimers progressively associated with μ H chain homodimers in pre-B cells, this complex was apparently deficient in companion ψ L chain proteins over the initial 2-h interval of pulse-chase analysis. Although the receptors that reach the pre-B cell surface are composed of ψ L chain proteins together with μ H chain and Ig α / β dimers, it is conceivable that the μ H chain/Ig α / β conjugates could function as signal-transducing units (58) within the ER compartment. However, studies of λ 5 gene-deleted mice clearly suggest that the ψ L chain components of the pre-B receptors are essential for efficient progression of pre-B cell and B cell development (26). The initial identification of pre-B cells in mice (59) and humans (60) was based on the observation that most of their μ H chains reside within the ER network, and relatively few of these reach the cell surface. Earlier studies indicated that

most of the μ H chains are catabolized within the pre-B cell (61), and the results of the present analysis suggest that another reason for this outcome is the relative inefficiency of the receptor assembly process in pre-B cells. Elucidation of the precise basis for this inefficiency requires further analysis, but the present results indicate that, during the assembly of pre-B receptors, each receptor component follows a distinctive route in the receptor assembly pathway. Although the *Vpre-B* and *14.1/λ5* gene products apparently bind avidly to each other, the ψ L chains differ from conventional κ and λ L chains in that their composite association with μ H chains proceeds at a relatively slow tempo that involves potentially rate-limiting intermediate interactions.

In conclusion, these results suggest that, in human pro-B cells, the ψ L chain proteins associate with Bip and other ER proteins, but these interactions lead to degradation rather than receptor assembly with Ig α , Ig β , and the surrogate heavy chain candidates reported in mouse pro-B cells (18, 22, 30). In pre-B cells, however, the μ H chains, ψ L chain, and Ig α and Ig β chains are all produced, and a panoply of ER-based chaperones may be involved in bringing these together to form a receptor complex. The fully assembled pre-B receptor is transported to the cell surface, where its interaction with presently unidentified ligand(s) may transduce signals that allow the pre-B cell to survive and progress along the differentiative pathway. On achieving productive V_L - J_L rearrangement, the κ or λ L chain products then displace the ψ L chain complex (17), and a B cell is born.

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