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Molecular Mechanisms that Control Expression of the B Lymphocyte Antigen Receptor Complex

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Summary

The B cell receptor for antigen (BCR) is a complex of membrane immunoglobulin (mIg) and at least two other proteins, Igα (mb-1) and Igβ (B29). This complex promotes surface expression of the BCR and acts to transduce an activation signal. We have used a system of μ heavy chain constructs transfected into murine B cell lines to probe structure-function relationships in the BCR complex. One mutant μ chain, in which two polar transmembrane residues (Tyr587, Ser588) are replaced with valine, fails to associate with Igα and Igβ and is incapable of transducing signals as a result of mIg cross-linking. This mutant is expressed on the surface at high levels when transfected into a plasmacytoma line that lacks Igα, whereas wild-type μ is retained in this cell line in the endoplasmic reticulum. Pulse-chase and immunoprecipitation analyses indicate that the mutant is more rapidly released from calnexin than the wild-type μ. Further, transfection of Igα into this Igα-negative cell line allows release of the μ chain from calnexin and surface expression of the BCR. These results identify the transmembrane residues of μ heavy chain that control binding to calnexin and Igα, and suggest that calnexin-dependent intracellular retention is an important control mechanism for expression of the BCR complex.

The B cell receptor for antigen (BCR) is a membrane-bound immunoglobulin (mIg), expressed on the surface of mature B cells as part of a complex of molecules. This complex, which has many features in common with the TCR-CD3 complex (1), includes Igα (the product of the mb-1 gene) and Igβ (the product of the B29 gene). Both mb-1 and B29 have been cloned from T cell-subtracted B cell libraries (2-4). Igα is a 32-kD phosphoprotein (5-7), and Igβ exists as two differentially processed proteins, one 39 kD and one 37 kD, that form disulfide-linked dimers with Igα (8, 9). Both Igα and Igβ have structural homologies to CD3 chains (10). Igα and Igβ are noncovalently associated with mIg, at least in part via polar interactions in the transmembrane (TM) region of the mIg molecule (11, 12). The two functions of the BCR are internalization of bound antigen for subsequent presentation, and antigen-induced cellular activation. We and others have shown that transmission of the TM activation signal is dependent on an intact BCR complex (11, 12), whereas the importance of Igα and Igβ for the antigen internalization and presentation function of the BCR is less clear (11, 13, 14).

The fully assembled BCR complex is expressed on mature, antigen responsive B cells but not on B cell progenitors or differentiated plasma cells, which are not antigen responsive. This developmentally regulated expression of the BCR is controlled by two main mechanisms. One is preferential transcription, first of the membrane-bound forms of heavy chains in mature B cells and later of the secreted forms in activated B cells and plasma cells. The other control mechanism is dependent on Igα, which is expressed only in pre-B and mature, surface Ig* B cells. This control of BCR surface expression was the first characterized function of Igα, and again has analogies to surface expression of the TCR complex in T cells (1). Thus, mIg transfected into either non-lymphoid or plasmacytoma cells, in which Igα is not synthesized, is retained intracellularly and is not expressed efficiently on the plasma membrane (15-17). The site of intracellular retention is the endoplasmic reticulum (ER) (16-18), but the specific mechanism for mIg retention in the absence of Igα and how Igα promotes surface expression of mIg are not known. Expression of the BCR complex is a model for studying the biochemical mechanisms that control the expression of multimeric membrane receptors.

We have created a number of human μ constructs with
nonconservative mutations in the TM and cytoplasmic domains (13), which we have used to define the structural requirements for the formation and function of the BCR complex (11). One such mutant, called YS:VV, in which two polar transmembrane residues, Tyr\text{Thr} and Ser, are replaced with valines, fails to associate with Ig\text{CL} and Ig\text{C} and fails to signal upon antigen binding or to efficiently present bound antigen. Despite this lack of association with the BCR complex, however, the TM mutant \( \mu \) is expressed at levels comparable to wild-type (WT) \( \mu \) or the endogenously produced Ig when transfected into a mouse mature B cell line A20 (13). Furthermore, unlike a mutation which deletes the cytoplasmic tail of mlg, thereby rendering the molecule phosphatidylinositol-linked, the transmembrane mutant YS:VV is expressed as an integral membrane protein (19). Thus, the YS:VV mutant seems to escape the normal control mechanism for the expression of mlg on the B cell surface, in that it is expressed in the absence of the other molecules of the BCR complex.

We have used these mlg constructs to analyze the mechanisms that control mlg expression, by transflecting the mlg into a cell line that lacks Igo\text{C}. Our results indicate that binding to the ER chaperone, calnexin, is an important mechanism for retaining \( \mu \) heavy chains intracellularly, and one function of Ig\text{C} may be to release heavy chains from calnexin. Such regulated release from intracellular retention may be a general mechanism for controlling surface expression of multimeric receptor complexes.

**Materials and Methods**

**Ig Constructs and Cell Lines.** The Ig constructs used in these studies have been described elsewhere (13). The WT \( \mu \) consists of a rearranged V-D-J from the mouse plasmacytoma S107 and human C\( \mu \) regions. The two mutants are Tyr\text{Thr}/Ser\text{Ser} to Val/Val (YS:VV) and Tyr\text{Thr} to Phe (Y:F). The functions of these mutants transfected into the mature B lymphoma line A20 have been described previously (13). Transfectants of J558L, a murine plasmacytoma cell line, were prepared similarly by coelectroporating the WT or mutant \( \mu \) constructs with the S107 \( \kappa \) chain in a plasmid containing the neomycin resistance gene. After transfetion and selection in the presence of 6-thioguanine, the cells were grown in bulk populations (representing a large number of separate transfection events), and were also cloned by limiting dilution. No sorting to isolate higher surface expression was employed. To produce the WT/Igo\text{C} transfected of J558L, J558L WT cells were then further transfected with a vector containing the m:\beta-1 gene (20, kindly provided by Dr. Michel Nussenzweig, The Rockefeller University, New York), which codes for Ig\text{C}, as well as the His resistance gene. Histidinol and G418 resistant clones were obtained by limiting dilution. Again, no sorting to isolate higher surface expression was employed.

**Antibodies.** Affinity-purified goat anti-human \( \mu \) and goat anti-human \( \kappa \) (Southern Biotechnology Associates, Birmingham, AL) and normal goat IgG (Cappel Laboratories, Durham, NC) were coupled to CNBr-activated Sepharose CL4B (Sigma Chemical Co., St. Louis, MO) for use in preclearing or immunoprecipitation. Anti-mouse calnexin antiserum is described elsewhere (21). Anti-Igo\text{C} antiserum was the kind gift of Dr. John Cambier (National Jewish Medical Center, Denver, CO).

**Western Blotting.** 25 x 10^6 cell equivalents of each cell line were lysed with 1% dodecyl maltoside (DM; Antrace; Maumee, OH) or 1% NP-40 (Sigma Chemical Co.) in lysis buffer (150 mM NaCl, 10 mM Tris, pH 7.3, and 1 mM EDTA) in the presence of protease inhibitors (PMSF, leupeptin, and aprotonin; Sigma Chemical Co.), followed by a 20-min spin at 12,000 g to remove nuclei and membrane fragments. The lysates were precleared with goat IgG-Sepharose, immunoprecipitated with Sepharose-coupled goat anti-human \( \mu \), washed, separated by SDS-PAGE, and blotted onto PVDF membranes. The blots were then probed with either alkaline phosphatase anti-human \( \mu \) or anti-Ig\text{C} or anti-calnexin antisera in the presence of 3% BSA. Alkaline phosphatase-conjugated goat anti-rabbit IgG (Southern Biotechnology Associates) was used as a secondary antibody where necessary and blots were developed by an alkaline phosphatase reagent system (Vector Labs, Inc., Burlingame, CA). To examine the association of Ig\text{C} with transfected \( \mu \), the anti-Ig\text{C} immunoprecipitated proteins were divided into two aliquots and electrophoresed separately. One aliquot was probed for \( \mu \) heavy chain and the other for Igo\text{C}.

**Pulse-Chase Analysis.** 50-100 x 10^6 cells were initially incubated in methionine and cysteine-free medium with 5% dialyzed FCS (pulse medium) for 30 min, pelleted, and resuspended in fresh warmed pulse medium with 1 mCi 35S-Express label (NEN, Boston, MA) for 5-15 min at 37°C. An aliquot of 10-20 x 10^6 cells for time 0 was collected, and the remaining cells were washed and resuspended in complete medium supplemented with methionine for the chase times indicated. At each time point, 10-20 x 10^6 cells were washed, lysed, precleared, immunoprecipitated with anti-\( \mu \) antibody as above, and analyzed by 8% SDS-PAGE. Gels were run until the 21-kD marker had reached the bottom to optimize separation at 50-100 kD. To determine the proportion of labeled \( \mu \) that was bound to calnexin after chase, lysates from each time point were sequentially immunoprecipitated with anticalnexin antisera followed by affinity-mouse \( \kappa \). This allowed separation of \( \mu \) heavy chain that was bound to calnexin from heavy chain that had been released from calnexin, processed, and complexed with light chain. The amount of heavy chain in each immunoprecipitate was quantified by PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA) analysis of 10% SDS-PAGE gels, and the amount of transfected \( \mu \) heavy chain bound to calnexin was expressed as a percentage of the total heavy chain immunoprecipitated (bound plus released). For endoglycosidase H (Endo-H) analysis, cells were pulsed with [35S]methionine for 15 min and chased in supplemented complete medium as above. Cell aliquots were lysed at each time point and immunoprecipitated with anti-\( \mu \) antibody. The proteins coupled to beads were solubilized, divided into two aliquots, and treated with Endo-H or left untreated. The treated and untreated aliquots were then analyzed by 8% SDS-PAGE.

**Analysis of Intracellular Ca\text{2+} Flux.** 2 x 10^6 cells were loaded for 30 min at 37°C with 5 \mu g/ml Fura-2 (Sigma Chemical Co.). They were then washed and resuspended in balanced salt solution containing 1.8 mM CaCl2, placed in cuvettes, and allowed to equilibrate in a fluorimeter (model LS 5B; Perkin Elmer-Cetus Corp., Norwalk, CT) at 37°C until baselines were established. Excitation occurred at 339 nm and emission was measured at 510 nm. Antibody or antigen was added and the fluorometric response was measured. Triton X-100 (Sigma Chemical Co.) was added to 1% to establish a maximum signal, followed by EGTA to 100 mM to establish a minimum.

**Results**

**Expression of Transfected Ig in J558L Cells.** To study the role of Ig\text{C} in cell surface expression of the BCR, we cotrans-
Figure 1. Surface expression of transfected WT and mutant IgM in cloned J558L transfectants. Cells were labeled with FITC-conjugated goat gamma globulin as a control (open curve) or with FITC-conjugated anti-human IgM (shaded curve). Plots indicate cell number vs. log fluorescence intensity.

Figure 2. Expression of transfected IgM and Igα in J558L transfectants. J558L WT, YS:VV, and Y:F transfectants, as well as an A20 WT transfectant, were lysed, immunoprecipitated with anti-human μ, and the immunoprecipitates split into two aliquots, followed by separation by SDS-PAGE and transfer to membranes. (A) Expression of transfected IgM. Transfected μ proteins were visualized with alkaline phosphatase-conjugated anti-human IgM. Both cytoplasmic and plasma membrane forms of IgM are detected by this assay. A single band is seen in J558L WT and J558L Y:F and doublets seen in A20 WT and J558L YS:VV, indicated by arrows. (B) Expression of μ-associated Igα in A20 and J558L transfectants detected with rabbit antipeptide antiserum to Igα. Igα is not present in any of the J558L transfectants.

Figure 3. Ca\(^{2+}\) flux measured upon mlg cross-linking. A20 WT and J558L YS:VV cells were labeled with Fura-2 and assayed for induced Ca\(^{2+}\) flux upon anti-IgM cross-linking. After equilibration, 50 μg/ml goat anti-human IgM was added to the cells (arrows) and changes in intracellular Ca\(^{2+}\) were measured. Fluorescence intensity is measured over time.
Figure 4. Processing of \( \mu \) heavy chains in J558L calls. (A) Pulse-chase analysis. J558L WT and J558L YS:VV cells were labeled with \([\text{35S}]\)methionine, chased for the time indicated (min) with chase medium, and \( \mu \) heavy chains were analyzed by immunoprecipitation and SDS-PAGE. Gels were imaged with a Phosphorimager. (B) Glycosylation of \( \mu \) heavy chains. Calls were labeled and then chased for 0 min (J5581. YS:VV) and 150 min (J558L WT and J558L YS:VV), followed by lysis in 1% NP-40. The lysates were immunoprecipitated with anti-human IgM and then treated with (+) or without (-) Endo-H, TCA precipitated, and separated by 8% SDS-PAGE.

It is formally possible that the J558L YS:VV transfectants are revertants that express Ig\( \alpha \) (22) and that this is responsible for surface expression of the transfected, mutant IgM. To demonstrate that no J558L transfectant expressed Ig\( \alpha \) inappropriately, aliquots of immunoprecipitated \( \mu \) and \( \mu \)-associated proteins were probed for Ig\( \alpha \) expression by immunoblot using an anti-Ig\( \alpha \) antiserum. As shown in Fig. 2 B, no \( \mu \)-associated Ig\( \alpha \) was detectable in any J558L line. The WT transfectant of the Ig\( \alpha \)-expressing line A20 was used as a positive control and showed expression of Ig\( \alpha \). A second approach for assessing the integrity of the BCR complex in J558L cells was based on previous work with A20 transfectants, showing that association with Ig\( \alpha \) is required for mlg to transduce ligand-induced signals, such as an increase in intracellular \( \text{Ca}^{2+} \) (11, 12). Thus, we tested J558L YS:VV for the ability to trigger a calcium flux upon cross-linking with anti-\( \mu \) antibody. Fura-2-labeled A20 WT and J558L YS:VV cells were treated with 50 \( \mu \)g/ml anti-\( \mu \) and changes in intracellular calcium were assayed with a spectrofluorimeter. This concentration of antibody induced an increase in intracellular calcium in A20 WT, but no such signal was seen in J558L YS:VV (Fig. 3). Therefore, both biochemical and functional assays establish that the YS:VV mutant is expressed on the surface of J558L cells in the absence of Ig\( \alpha \).

Intracellular Processing of Transfected \( \mu \) in J558L. To further characterize differences in the intracellular processing of the retained WT and the expressed mutant Ig YS:VV and define the site(s) of retention of the WT \( \mu \) in the J558L cells, we used a pulse–chase technique. In these experiments, cells were metabolically labeled for a 15-min pulse, followed by a chase for 0–240 min in methionine-supplemented chase medium. In this way, it is possible to follow the cohort of Ig molecules that is synthesized during the short pulse period, looking for changes in apparent molecular weights indicative of processing and glycosylation. After the chase, cells were lysed and IgM immunoprecipitated, followed by SDS-PAGE under conditions designed to emphasize separation in the 50–100-kD range. As seen in Fig. 4 A, a higher \( M_r \) form of YS:VV IgM appeared by 150 min, consistent with processing on the way to the plasma membrane. This processing did not take place in the case of the WT IgM, in which the higher \( M_r \) form did not appear. In both cases, there was a core, glycosylated form, the presence of which decreased slowly over time, as well as a smaller form which may represent unglycosylated peptide that decreased much more quickly (within 30–60 min). The pattern of processing of the WT \( \mu \) suggests that this protein is retained in the ER.

To further define the possible site of intracellular retention of WT IgM, the pulse–chase strategy was used, followed by treatment of the immunoprecipitates with Endo-H. The glycosylation of ER resident proteins is typically Endo-H sensitive, whereas passage through the Golgi stack matures the sugar residues and renders the molecule Endo-H resistant. Fig. 4 B shows Endo-H analysis of WT and mutant Ig molecules in J558L transfectants. Directly after a 15-min pulse, almost all of the YS:VV \( \mu \) was Endo-H sensitive, consistent with ER localization. After a chase of 150 min, a large fraction of the YS:VV material became Endo-H resistant, indicating movement out of the ER and through the Golgi. This contrasts with WT, which remained entirely Endo-H sensi-

Figure 5. Association of calnexin with transfected \( \mu \). Cells were lysed with 1% DM lysis medium (DM) or 1% NP-40 lysis medium (NP) and lysates immunoprecipitated with anti-IgM Sepharose beads. DM beads were washed with DM lysis medium and NP beads were washed with a high stringency wash buffer containing 1% NP-40, 0.1% SDS, and 0.1% deoxycholate in Tris-saline. Proteins were separated by 10% SDS-PAGE followed by immunoblotting with an anticalnexin antiserum. No calnexin was detected after immunoprecipitation of cell lysates with preimmune goat IgG (not shown).
tive even at 150 min. Again, this indicates that the WT molecule has been retained within the ER.

**Role of Calnexin in Intracellular Retention of μ Heavy Chains.**

Given the ER retention of the WT IgM, it was important to look for a possible retention or chaperone molecule. One recently described candidate is calnexin (IP90, P88; 23, 24), a 90-kD molecule that is involved in ER retention of monomeric proteins until they reach their mature configuration (25). There is also evidence that calnexin may be involved in the ER retention of multimeric complexes, mediating retention until all members of the complex have been assembled (21, 23, 26). With this in mind, we used an antiserum to calnexin to probe for calnexin association with both the WT and mutant (YS:W) μ. Since the mutant is released from the ER, we hypothesized that this release was on the basis of failure of the mutant μ to bind calnexin. Fig. 5 shows that this was not the case. Immunoblot analysis of calnexin associated with WT and YS:VV μ heavy chain demonstrated that at least some of the μ molecules in each case were associated with calnexin. This was true both under more stringent and relatively less stringent lysis and wash conditions; i.e., in cells that were lysed with 1% NP-40 or the weaker nonionic detergent DM.

It was possible that although both mutant and WT μ bound to calnexin initially, the kinetics of release from calnexin of each molecule was different. To test this, we used pulse-chase labeling followed by sequential immunoprecipitation, first with anticalnexin antiserum to isolate μ protein associated with calnexin, and then with anti-mouse κ to separate heavy chain that had been released from calnexin and assembled with the transfected light chain. Fig. 6 shows that essentially all (>90%) of the WT μ chain was bound to calnexin at all time points examined. By contrast, the mutant μ (YS:VV) was released from calnexin over time, with 78% of the μ bound at time 0 and only 33% bound by 3 h. Fig. 6 shows the result from one experiment; in a second experiment, >93% of the WT μ was calnexin associated at both 0 and 3 h, whereas 80% of the YS:VV μ was calnexin associated at time 0 and 48% by 3 h.

**Role of Igα in the Release of μ Heavy Chains from Calnexin.**

If the failure of the WT μ chain to be released from calnexin is indeed due to the absence of Igα, then coexpressing Igα should lead to release of μ and surface expression of the BCR. To formally test this, we transfected J558L WT cells with Igα. Fig. 7A shows flow cytometric analysis of J558L WT and J558L WT/Igα cells demonstrating that in J558L WT/Igα double transfectants mlgM was expressed at levels equivalent to J558L YS:VV. To examine the release of μ from calnexin, J558L WT/Igα transfectants were pulsed with [35S]methionine for 5 min, chased for 3 h, and the μ heavy chain both bound to and released from calnexin was analyzed by SDS-PAGE. As shown in Fig. 7B, after 3 h of chase, in the J558L WT cells all the μ remained calnexin associated, whereas in the J558L WT/Igα double transfectants a significant fraction of the μ protein was released. Quantitation of the autoradiogram shown in Fig. 7B revealed that 100% of the μ was calnexin bound in J558L WT cells, whereas 36% of the total μ protein was released over the same time in J558L WT/Igα cells. Thus, coexpression of Igα releases the WT μ protein from calnexin and permits it to be expressed on the cell surface.

**Discussion**

The expression of Ig in B cell lines provides a model for analyzing the control of surface expression and function of integral membrane protein receptor complexes. We have used WT and mutant Ig molecules to characterize the importance of the TM region in mediating two key aspects of the mole-

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**Figure 6.** Release of μ from calnexin over time. (A) Cells were labeled with [35S]methionine for 5 min followed by a chase for the time indicated. DM lysates were sequentially immunoprecipitated with anticalnexin antiserum and anti-κ, separating heavy chain bound to calnexin (B) from released heavy chain (R). IgM heavy chain is indicated (μ), as well as an band migrating at the correct Mr to be calnexin. (B) Total incorporation was measured using the PhosphorImager and the amount of immunoprecipitated heavy chain bound to calnexin was expressed as a percent of the total immunoprecipitated μ heavy chain.
be noted that the TM regions of human and mouse/~ differ

cule's behavior. Previously, we have shown that the TM re-

data, however, indicate that the COOH-proximal polar re-

completely changes the behavior of mlgM. This mutant is

A nonconservative dipeptide substitution in the TM region

expressed at high levels on the cell surface without the re-

quirement of complex assembly. Further, our data suggest

requirement for complex assembly before the molecule can be

expressed on the cell surface. In the absence of assembly of

the complex, synthesized WT mlgM is retained in the ER.

A nonconservative dipeptide substitution in the TM region

completely changes the behavior of mlgM. This mutant is

expressed at high levels on the cell surface without the re-

quirement of complex assembly. Further, our data suggest

that association with and release from calnexin are key deter-

minants of surface expression of IgM. When IgG is

provided to complete assembly of the complex, WT IgM is released

from calnexin and expressed on the cell surface.

Previous studies from other laboratories have also implica-

ted the TM region of ~ in intracellular retention (27), but the

constructs used differ significantly from ours in two

respects. First, Williams et al. (27) used murine ~ constructs,

whereas we have used human /3 because its expression can

be followed unambiguously, and hybrids between transfected

and endogenous Ig heavy chains are not seen (13). It should

be noted that the TM regions of human and mouse ~ differ

only in a single nonpolar residue at position 572. Second,

Williams et al. (27) concluded that the NH2-terminal polar

patch of the TM region (TTAST, beginning at residue 572)

is involved in retention, using a construct with many more

TM residues altered than those employed in our studies. Our

data, however, indicate that the COOH-proximal polar re-

region (YSTTVT, beginning at residue 587) is key, both in

controlling association with ~G and IgG and in controlling

specific intracellular retention. Our analysis of a nonconserv-

ative dipeptide mutation in the TTAST region (TTAST to

TTAVV) demonstrates intact signaling and binding to ~G

and IgG (11). The reasons for the differences in our results

and those of Williams et al. (27) are not clear, but may relate
to the use of different constructs.

The striking expression of mlgM in the absence of associated

I spacer in J558L cells has given us a tool for studying how sur-

face expression of this protein is controlled. Calnexin has been

implicated in the ER retention of immature proteins as they

are prepared for secretion or surface expression. Monomeric

proteins are bound to calnexin during or soon after synthesis

and then released when correctly processed and folded (25),

whereas misfolded or aberrantly glycosylated proteins are not

released. More recently, calnexin association has also been

demonstrated as a potential control mechanism for multi-

meric complex assembly, for both MHC class I (28) and the

TCR-CD3 complex (26). In these cases, it is hypothesized

that single chains of a multimeric complex are retained in

the ER by calnexin until all molecules of the complex are

assembled, at which time the complex is released for trans-

port to the Golgi and on to the surface. The studies described

here support a similar role for calnexin in the expression of

the BCR complex. Thus, we postulate that newly synthet-

ized WT ~ heavy chains are bound to calnexin and retained

in the ER (Fig. 8). In this model, ~G, possibly associated

with IgG, releases ~ from its calnexin-binding site, allowing

subsequent processing and surface expression. In the absence

of ~G, as in the J558L transfectants, the WT ~ remains cal-

nexin bound. In contrast, the YS:W mutant binds only tran-

siently to calnexin and is spontaneously released without the

requirement for ~G, resulting in surface expression. Cotrans-

fection of WT ~ and ~G formally shows that ~G is required

for the release of the ~ heavy chain from calnexin. Note that

the YS:W mutation does not abolish the association between

calnexin and the ~ protein, since the mutant transiently binds
to calnexin.

Given the wide variety of proteins that calnexin chaperones,
it seems impossible that there is any consensus sequence motif

for calnexin binding. Rather, it is more likely that binding
to calnexin is mediated by carbohydrate moieties. This is con-

sistent with the release of monomers as they achieve mature

conformation and glycosylation. The YS:VW mutation may

alter the glycosylation of the ~ chain, thereby reducing the

avidity of its association with calnexin and allowing "spontane-
ous" release from the chaperone. In WT ~ proteins, calnexin-binding carbohydrate moieties may only be detached

calnexin by the binding of the BCR complex compo-

Figure 7. Effect of ~G on calnexin release and surface expression of WT mlgM. (A) Flow cytometric analysis of mlgM in J558L transfectants: from

left to right, control (J558L WT cells stained with FITG-gamma globulin), J558L WT (stained with FITG-GAH3), and J558L WT/lg (FITG-

GAH3). (B) Release of transfected ~ from calnexin. J558L WT and J558L WT/IgG transfectants were labeled with [35S]methionine, chased for 3 h,

and lysates sequentially immunoprecipitated as in Fig. 6. The ~ bound to calnexin (B) and released (R) after 3 h are shown.
Figure 8. Model for the control of mIg expression. In mature, Igα-expressing B cells (represented by the lymphoma line A20), IgM is retained in the ER until the BCR complex is assembled, including Igα. When the complex is assembled, IgM is released for expression on the cell surface. In differentiated B cells that no longer express Igα (represented by the plasmacytoma line J558L), WT IgM is retained in the ER by calnexin over time. Expression is restored by transfection of J558L with Igα. The mutant YS:VV, on the other hand, does not require assembly with Igα for release to the surface.

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