Regulation of Protein Expression in Differentiation by Subunit Assembly

HUMAN MEMBRANE AND SECRETED IgM*

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Newly synthesized IgM heavy chains are either expressed as functional IgM or catabolized, depending on the stage of differentiation of the B cell. Heavy chains of the membrane type are rapidly degraded in pre-B cells, but expressed on the cell surface as monomeric IgM in resting and secreting B cells. Secreted-type heavy chains are catabolized in pre-B and resting B cells, but secreted as pentameric IgM by secreting B cells. The differences between the heavy chains that are expressed and those that are catabolized are post-translational. Stable membrane and secreted heavy chains have been covalently assembled with light chains, terminally glycosylated, and removed from intracellular proteases by insertion into the plasma membrane or by secretion.

The carboxylic ionophores monensin and nigericin have been used here to determine the relative importance of these post-translational events in stabilizing newly synthesized heavy chains. Monensin and nigericin inhibited both the rates and extents of terminal glycosylation and of intracellular transport of these proteins, without affecting the covalent assembly processes. These ionophores did not affect the rate of catabolism of heavy chains in either a resting or a secreting B cell line. IgM heavy chains thus appear to be stabilized against intracellular proteolysis by full covalent assembly to monomeric membrane IgM and to secreted pentameric IgM. The fully assembled IgM can then be terminally glycosylated and transported to the cell surface.

In B cell differentiation, post-translational events regulate the expression of IgM heavy chains as functional immunoglobulins. All B cells synthesize both the \( \mu_m \) and \( \mu_s \) forms of IgM heavy chains (1, 2). The fate of the heavy chains, however, depends on the stage of differentiation. In pre-B cells, both \( \mu_m \) and \( \mu_s \) chains are rapidly catabolized (3, 4), and neither membrane nor secreted IgM is expressed. In resting B cells, some \( \mu_s \) chains become stable membrane IgM, while \( \mu_m \) chains are rapidly degraded (4, 5). In secreting B cells, \( \mu_m \) chains also are processed to stable IgM, and are secreted (6).

In earlier studies of the expression of membrane IgM in a cultured human B cell line, we found that \( \mu \) chains were susceptible to nonspecific intracellular proteolysis unless they were protected by post-translational events (4). Stable membrane IgM heavy chains differed from less processed unstable heavy chains in several respects, any or all of which might have contributed to specific protection from intracellular proteolysis. Stable heavy chains had been covalently assembled with light chains, and subsequently subjected to terminal glycosylation processes. Stable membrane IgM had also been removed from the site(s) of intracellular proteolysis by insertion into the plasma membrane.

In order to distinguish the relative importance of these post-translational events in regulating IgM expression, IgM synthesis has been studied in two human B cell lines. The Daudi cell line has the phenotype of a resting B cell, and expresses only membrane IgM. The RPMI 1788 cell line has the phenotype of a secreting B cell, and expresses both membrane and secreted IgM.

Both cell lines have been treated with the carboxylic ionophores monensin and nigericin, and the synthesis of membrane and secreted IgM has been followed by biosynthetic labeling procedures. Carboxylic ionophores have previously been shown to inhibit terminal glycosylation and secretion of IgM in mouse cells, without affecting the rates of covalent assembly of heavy and light chains (7). They also inhibited the terminal glycosylation and surface expression of membrane proteins in cultured hepatoma cells (8). These ionophores can therefore be used to distinguish the relative contributions of terminal glycosylation, covalent assembly, and intracellular transport in protecting newly synthesized IgM heavy chains from catabolism.

Intracellular IgM has also been subjected to mild in vitro trypsinization, to identify the maturation stage at which membrane and secreted IgM gain resistance to nonspecific proteolysis.

**EXPERIMENTAL PROCEDURES**

**Materials**—Monensin and nigericin were obtained as a gift from Dr. R. L. Hamill (Eli Lilly Corp.) and stored as a 0.1 mM stock solution in 10% dimethyl sulfoxide. \( [\text{S}] \)-Cysteine, \( [\text{H}] \)-tyrosine, and carrier-free Na\(^{22} \)I were from New England Nuclear Corp.

**Cell Culture Conditions**—Cell lines were maintained in stationary culture in RPMI 1640 with 50 \( \mu \)M 2-mercaptoethanol, penicillin/streptomycin, and 10% fetal calf serum.

**Labeling Conditions**—For short biosynthetic labeling times, cells were preincubated at \( 2 \times 10^6 \) cells/ml for 60 min in RPMI 1640 without cysteine or tyrosine, depending on the radioactive amino acid used. The medium was supplemented with 50 \( \mu \)M 2-mercaptoethanol, penicillin/streptomycin, glutamine, and 5% dialyzed heat-inactivated fetal calf serum. \( [\text{S}] \)Cysteine or \( [\text{H}] \)tyrosine was then added at 50 \( \mu \)Ci/ml. After 30 min, the cells were washed and resuspended at \( 2 \times 10^6 \) cells/ml.
Regulation of IgM Expression in Differentiation

The expression of membrane and secreted IgM was studied in two human lymphocytic cell lines. The Daudi cell line expresses monomeric (μL2) surface IgM, but does not secrete μ chains in any form. The RPMI 1788 cell line secretes pentameric IgM, and expresses some surface monomeric IgM. Daudi cells therefore have the phenotype of resting B cells, and were used to study the processing of membrane IgM. RPMI 1788 cells have the phenotype of secreting B cells, and were used to study the processing of secreted IgM.

Both cell lines processed newly synthesized μ chains, with time, to a form that migrated more slowly in SDS-PAGE. The slower μ chain band in Daudi corresponded to the form found on the cell surface (4). Similarly, the slower μ chain bands from RPMI 1788 cells co-migrated with the μ chains in secreted pentameric IgM (Fig. 1).

The change in μ chain mobility reflected terminal processing of oligosaccharide side chains. As noted in Daudi cells (4), μ chains were terminally glycosylated in RPMI 1788 cells only after the full covalent assembly of IgM (Fig. 2). Terminal glycosylation was also detected only after polymerization in the mouse cell line WEHI 279 and the human cell line SeD (not shown). In all four cell lines, the terminally glycosylated μ chains were the only forms of μ chain found on the cell surface by vectorial iodination, or in the culture supernatants. In both the Daudi cell line and the RPMI 1788 cell line, the μ chains in stable IgM differed from unstable precursor μ chains by covalent assembly with light chains, by intracellular trafficking from the site of synthesis to the outer membrane or extracellular space, and by terminal glycosylation. In order to define which of these processes regulated catabolism of IgM precursors, and therefore determined the expression of IgM in B cell differentiation, carboxylic ionophores were used to inhibit certain post-translational modifications.

Ionophore Effects on IgM Processing—Carboxylic ionophores such as monensin and nigericin have been shown to inhibit terminal glycosylation and final expression of membrane and secreted glycoproteins (7, 8). These ionophores similarly inhibited processing and expression of both membrane and secreted human IgM. Carboxylic ionophores could therefore be used to study the relative contributions of different post-translational events in determining the rate of catabolism of newly synthesized μ chains.

As reported earlier for secreted IgM in murine plasma cells (7), low concentrations of ionophores did not affect the covalent assembly process in either cell line studied. The relative amounts of unassembled and assembled μ chains were not altered by ionophore treatment, as shown for Daudi cells in Table I.

In both Daudi and RPMI 1788 cells, μ chains were subjected to a limited number of terminal glycosylation modifications, even in the presence of ionophores. The modifications that occurred in the presence of ionophores, like those in the control incubations, occurred only after covalent assembly to monomers and pentamers. The assembled, modified μ chains could be detected on the surface of Daudi cells as membrane IgM by vectorial labeling with [3H]inosine and as secreted pentamers in the supernatant of RPMI 1788 cell cultures (not shown). While RPMI 1788 cells secreted nonglycosylated μ chains after incubation in the presence of tunicamycin (1), Daudi cells did not assemble nonglycosylated heavy and light chains and did not express surface IgM after tunicamycin treatment (4). While inhibiting co-translational glycosylation can alter protein conformation sufficiently to prevent IgM expression

10% in complete medium and harvested after the indicated chase time, or pulsed for 4 h without chasing. For a 16-h label, 20 μCi/ml [3H]lysine were added to cells at 2 × 10^5/ml in complete medium.

Ionophore treatment at the concentrations used in these studies did not affect cell viability during a 6-h experiment, and decreased viability by less than 5% during a 24-h experiment. Nigericin at 50 nm and monensin at 100 nm showed major effects on IgM processing in these cell lines and were used interchangeably.

IgM Isolation—Cells were harvested and lysed in 1% Nonidet P-40 in phosphate-buffered saline, 0.2% phenylmethylsulfonyl fluoride, 30 mM iodoacetamide, and 500 μl of 1 M (pH 5.5) for 1 h at 100°C. Cell debris was then pelleted by centrifugation at 12,000 × g. IgM was isolated from the supernatant by immunoprecipitation. Culture supernatants and cell lysates were incubated at 4°C overnight with a rabbit antisera that had been prepared in these laboratories against a human IgM-k myeloma protein. The antigen-antibody complexes were then isolated by precipitation with Staphylococcus aureus (Cowman strain I) (9). Bound IgM was removed from the bacterial cells by boiling for 5 min in 2% SDS, 50 mM Tris (pH 6.8), 5% glycerol (10).

Polyacrylamide Gel Electrophoresis—Immune complexes in SDS were reduced with 5% (v/v) 2-mercaptoethanol and applied to 7.5% polyacrylamide-SDS slab gels (10). The gels were impregnated with 2.5% dithioerythritol, dried, and subjected to fluorography (11). For quantitative analysis, the fluorographs were scanned with an Ortec densitometer. Band intensity was quantitated by cutting out the area under the peak tracing and weighing the chart paper. Band intensity was then corrected for differences in band width. The reliability of this method was verified by scanning fluorographs of SDS-polyacrylamide gels that had been loaded with different amounts of radioactive IgM. A plot of band area calculated in this manner as a function of added radioactive μ chains was linear over at least a 6-fold concentration range and intercepted the origin.

Two-dimensional SDS-PAGE was performed using a modification of the method of Sommers and Traut (12), as described previously (4). Unreduced IgM samples were electrophoresed first in an SDS tube gel with a 3% polyacrylamide stacking gel and a 5% running gel, using the buffer system of Laemmli (10). The proteins in the tube gel were reduced by incubating the gel for 20-40 min at room temperature in Laemmli sample buffer with 5% 2-mercaptoethanol. The tube gels were then applied to the top of a 7.5% polyacrylamide-SDS gel with a 4% stacking gel. A 1% agarose solution in sample buffer with 2-mercaptoethanol was used to attach the tube gel to the slab gel.

To identify pentameric IgM, tube gels were made from 2% acrylamide gels, similarly inhibited processing and expression of both membrane and secreted pentameric IgM (Fig. 1).

Ionophore treatment at the concentrations used in these studies did not inhibit the terminal glycosylation of membrane and secreted glycoproteins (7, 8). These ionophores similarly inhibited processing and expression of both membrane and secreted human IgM. Carboxylic ionophores could therefore be used to study the relative contributions of different post-translational events in determining the rate of catabolism of newly synthesized μ chains.

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In Daudi cells, inhibiting terminal glycosylation of the same protein does not prevent surface expression.

Since only modified, assembled \( \mu \) chains were found on the outer membrane of Daudi cells, formation of the upper \( \mu L_2 \) band corresponded to the earliest possible appearance of membrane IgM. The appearance of the partially or fully processed \( \mu L_2 \) protein was visualized after incubation in the presence and absence of 50 nM nigericin by SDS-PAGE under nonreducing conditions, followed by fluorography (Fig. 3). Since the rate of assembly of \( \mu L_2 \) was the same in the presence and absence of ionophores (Table I), the rates of appearance of the upper \( \mu L_2 \) bands shown in Fig. 3 can be compared directly. Quantitation by densitometry (Fig. 4) indicated that formation of the upper \( \mu L_2 \) band was slowed approximately 2-fold by ionophore treatment, indicating that the final expression of membrane IgM was at least 2-fold slower in the presence of ionophores.

\[ \text{TABLE I} \]

| chase time | H | N | P | L |
|-----------|---|---|---|---|
| 0         | 27 | 26 | 41 | 6 |
| Control   | 26 | 22 | 45 | 7 |
| 1         | 14 | 7  | 18 | 24 |
| Nigericin | 12 | 6  | 27 | 25 |
| 2         | 6  | 10 | 15 | 26 |
| Nigericin | 5  | 12 | 27 | 51 |
| 3         | 8  | 23 | 69 |
| Nigericin | 5  | 26 | 70 |
| 4         | 8  | 92 |
| Nigericin | 11 | 89 |

*Includes both upper and lower band forms.

Fig. 1. RPMI 1788 \( \mu \) chains. RPMI 1788 cells were pulsed with \(^{[35S]}\text{cysteine} for 30 min. Lane A, cell-associated \( \mu \) chains after 0-h chase; lane B, cell-associated \( \mu \) chains after 4-h chase; lane C, \( \mu \) chains in the culture supernatant after 4-h chase; lane D, \( \mu \) chains in the culture supernatant after pulsing, and chasing for 4 h in the presence of 50 nM nigericin. IgM was isolated by immune precipitation and electrophoresed through a 7.5% acrylamide-SDS gel after reduction. Approximately equal amounts of radioactivity were applied to each lane.

Fig. 2. Two-dimensional SDS-PAGE analysis of IgM from RPMI 1788 cell lysates. RPMI 1788 cells were pulsed for 6 h with \(^{[35S]}\text{cysteine}, and IgM was isolated by immune precipitation. Cell-associated IgM was electrophoresed from left to right under nonreducing conditions on a 5% acrylamide-SDS tube gel. The tube gel was then incubated with 2-mercaptoethanol to separate the disulfide-bonded subunits, and the proteins were then electrophoresed through a 7.5% acrylamide-SDS slab gel. The electrophoretic migration of reduced \( \mu \) chains in one-dimensional SDS-PAGE is shown on the left for comparison. The two-dimensional analysis indicates that only the fully assembled (\( \mu L_2 \)) form contained appreciable amounts of both upper and lower \( \mu \) chain bands. The bands at the top of the tube gel was identified as pentameric IgM by comparison with secreted IgM that migrated as a single band on a 2% acrylamide tube gel.

Fig. 3. Daudi IgM assembly in the presence and absence of nigericin. Daudi cells were preincubated for 60 min in the presence (N) or absence (C) of 50 nM nigericin, then pulsed for 30 min with \(^{[35S]}\text{cysteine}, and chased for 0–4 h. IgM was isolated by immune precipitation and electrophoresed without reduction on a 5% acrylamide-SDS slab gel. Molecular weight markers were as follows: myosin, 200,000; \( \beta \)-galactosidase, 116,250; phosphorylase b, 92,500; bovine serum albumin, 66,200. The assignment of covalently assembled \( \mu \) and L chains was confirmed by two-dimensional SDS-PAGE (4). Arrows indicate the position of the \( \mu L_2 \) form containing high mannose chains. The \( \mu L_2 \) forms containing terminally (C) or partially glycosylated (N) \( \mu \) and L chains migrated more slowly, and can be seen above the band indicated by the arrows. The terminally and partially glycosylated \( \mu L_2 \) bands are readily resolved in this system because all four subunits have fully or partially processed oligosaccharide side chains.

Fig. 4. Rate of formation of processed \( \mu \) chains in the \( \mu L_2 \) assembly form in the presence and absence of 50 nM nigericin. Nascent IgM from Daudi cells was separated by SDS-PAGE under nonreducing conditions, as shown in Fig. 3. The \( \mu L_2 \) assembly form separated into two bands in the presence (N) and absence (C) of ionophore, corresponding to \( \mu L_2 \) with oligosaccharide side chains that had not been processed (lower band), that had been partially processed (ionophore upper band), and that had been fully processed by terminal glycosylation (control upper band). The percentage of \( \mu L_2 \) as upper band and lower band was quantitated by densitometry of a fluorograph of the gel. The percentage of total \( \mu L_2 \) radioactivity that was found in the upper band form is shown as a function of chase time.
The final expression, or secretion, of pentameric IgM from RPMI 1788 cells was quantitated directly. As shown in Fig. 5, nigericin treatment slowed μ chain secretion by a factor of 5. The polymerization state of secreted IgM was analyzed by SDS-PAGE under nonreducing conditions, on both 2% acrylamide tube gels (to detect pentameric IgM) and 5% acrylamide slab gels (to detect monomeric IgM), as described under “Experimental Procedures.” Secreted μ chains were assembled primarily to pentameric IgM in the control situation, with a small amount present as monomeric IgM. After ionophore treatment, secreted μ chains were detected exclusively as pentameric IgM, indicating that μ chains found in culture supernatants had not been released nonspecifically by cell lysis. After a 16-h chase, secretion of μ chains from ionophore-treated cells was still less than from control cells, being only about 50% of control secretion.

In both cell lines, ionophore treatment promoted the intracellular accumulation of IgM precursors with unprocessed high mannose oligosaccharide side chains. Newly synthesized μ chains are stabilized against intracellular proteolysis by post-translational events (4). If carboxylic ionophores inhibit the stabilizing events, then ionophore treatment should increase the rates of intracellular μ chain catabolism. The kinetics of μ chain catabolism was therefore determined in the presence and absence of 50 nM nigericin or 100 nM monensin for both Daudi and RPMI 1788 cell lines, to determine where in protein processing newly synthesized μ chains become stable.

Catabolism of Daudi μ chains was not enhanced by ionophore treatment, after either a 30-min (Fig. 6) or a 16-h pulse with [35S]cysteine. As shown in Table I, ionophore treatment also did not alter the relative abundance of IgM precursor forms. Since in Daudi cells only about 20% of newly synthesized μ chains were expressed as stable membrane IgM (4), the disappearance of incompletely assembled IgM largely reflected catabolic processes. The lack of change in the rates of disappearance of the intermediate assembly forms indicated that ionophores did not affect μ chain catabolism nonspecifically. Such nonspecific effects would be expected if ionophores slowed transport of proteins to a catabolic compartment. Ionophore treatment neither promoted nor inhibited catabolism of μ chains in Daudi cells.

Since μ chain secretion was inhibited by ionophore treatment of RPMI 1788 cells (Fig. 5), it was important to determine the fate of the unsecreted μ chains. Cell-associated μ chains were therefore quantitated in the presence and absence of ionophores to determine if the unsecreted proteins were catabolized. Cell-associated μ chains accumulated when secretion was inhibited, indicating that blocking IgM secretion did not promote μ chain degradation in these cells (Fig. 7A).

Total μ chains, as the sum of cell-associated and secreted μ chains, were also quantitated in the presence and absence of ionophores. This quantitation involves the direct comparison of immune precipitations from different solutions. Cell lysates contain a constant amount of IgM, with variable amounts of radioactive IgM. Culture supernatants, in contrast, contain variable amounts of IgM, all of which is radioactive. The two solutions are furthermore very different in their protein and detergent compositions. Direct comparison of immune precipitates from cell lysates with immune precipitates from culture supernatants therefore necessarily introduces quantitative errors. Within the limitations of this quantitation, total μ chain turnover was not markedly affected by incubation in the presence of 50 nM nigericin (Fig. 7B). The processes inhibited by ionophores did not, therefore, appear to regulate the in vivo proteolysis of nascent IgM in these cells.
Trypsinization in Vitro—In an attempt to define the stage at which μ chains become resistant to proteolysis, cell lysates that had been biosynthetically labeled for 3.5 h were subjected to mild in vitro trypsinization before immune precipitation. As shown in Fig. 8, the differently assembled forms of Daudi μ chains had different susceptibilities to trypsin digestion, even after solubilization with Nonidet P-40. Fig. 8C shows that both the upper and lower μ chains in the fully assembled μL2 form were enhanced relative to all other μ chain forms by trypsin treatment. The other μ chain bands visualized in gel C were composed chiefly of intact μ chains disulfide-bonded to light chains and to μ chain fragments (not shown). Relative protein abundances in gels B and C can be seen by comparing lanes 0 and 2h (+) in gel A, indicating that the fully assembled monomers were most resistant to trypsin treatment, whether they had been terminally glycosylated or not.

RPMI 1788 μ chains also showed different susceptibilities to in vitro proteolysis, depending on the extent of covalent assembly (not shown). The fully assembled monomers and pentamers were most resistant to trypsin treatment. Again, this resistance to in vitro proteolysis was independent of terminal glycosylation events.

Cell-associated IgM was also subjected to mild trypsinization after a 16-h chase in the presence and absence of carboxylic ionophores. This IgM was fully assembled μL2 monomers in Daudi cells, or monomers and pentamers in RPMI 1788 cells. In the presence of ionophores, cell-associated IgM contained appreciable amounts of μ chains with unprocessed high mannose oligosaccharide side chains. The μ chains isolated after a 16-h chase in vivo were resistant in all cases to mild in vitro trypsinization. These results indicate that the forms of IgM that were resistant to in vivo proteolysis and could still be detected after a 16-h chase period were also resistant to mild in vitro proteolysis. Stable intracellular IgM had therefore not simply been removed from a catabolic site by post-translational processes, but had been stabilized by covalent assembly to monomeric or pentameric IgM. This stabilization preceeded terminal glycosylation events, and trafficking to the outer membrane or extracellular space.

Assembly of μ, Chains in Daudi Cells—The significant difference between μ chain processing in the Daudi resting B cells and the RPMI 1788 secreting B cells was therefore the formation of pentameric IgM in the latter cell line. This pentamerization could be controlled either at the level of formation of precursor monomeric μL2 containing μ chains, or at the level of polymerization of these monomers. To distinguish between these possibilities, Daudi μ chains that had been pulsed for 4 h with [3H]tyrosine were separated in a two-dimensional SDS-PAGE system as in Fig. 2. The μ chains from the μL and the μL2 assembly forms were eluted and subjected to carboxypeptidase digestion, as described under “Experimental Procedures.” In the absence of assembly with light chain, both in a pre-B cell line where light chain was not expressed and in the Daudi cell line when assembly was inhibited by treatment with tunicamycin, μ chains were detected as free μ chains and as μL dimers (4). It would therefore be expected that if μ chains did not assemble with light chains in the Daudi cell line, they would be found as free μ and μL2 chains, and would not be detected in the μL2 form. Carboxypeptidase analysis indicated that approximately 30% of the μ chains in the μL form were μL2 chains. Approximately 20% in the μL2 form were μL2 chains. There was therefore no indication of a failure of μ chains to assemble to μL2 monomers in Daudi cells. The formation of pentameric IgM appeared to be blocked in these cells at the level of polymerization of μL2 monomers.

In further attempts to define the differences in polymerization of μ chains from Daudi cells and from RPMI 1788 cells, μ chain synthesis was compared quantitatively in the two cell lines. After a short (5, 10, or 15 min) pulse, when catabolism should have little effect on detectable protein, RPMI 1788 cells incorporated 19 ± 2 (n = 3) times more [35S]cysteine into μ chains than did Daudi cells. The former cell line also incorporated much more label into light chains than did the latter cell line. Greater concentrations of subunits would be expected to enhance the rates of assembly to μL2. The polymerization rate varies with the concentration of the μL2 monomer, to a power greater than 1 and possibly as large as 5. Small changes in monomer concentrations should therefore have marked effects on the polymerization process. The enzyme responsible for monomer formation has also been found in greater concentrations in secretory B cell lines than in resting B cell lines (16). Relative concentration effects could therefore explain why, even though resting B cells such as the Daudi cell line have small amounts of detectable J chain (17) and of sulfhydryl oxidase activity (18), no pentamerization of IgM can be detected.

DISCUSSION

Carboxylic ionophores interfere with the expression of membrane and secreted IgM, as with other membrane and secreted proteins, by inhibiting the rate and extent of certain post-translational modifications. Carboxylic ionophores did...
not affect covalent assembly of IgM heavy and light chains, nor did they affect the in vivo disappearance of IgM intermediate assembly forms. Since in Daudi cells only about 20% of newly synthesized μ chains was expressed as stable membrane IgM (4), the disappearance of incompletely assembled IgM primarily reflected catabolic processes. The observation that the rates of disappearance of the intermediate assembly forms were unaffected by the presence of carboxylic ionophores in this cell line (Table I) indicated that ionophores did not inhibit μ chain catabolism nonspecifically. In fact, in both cell lines studied, carboxylic ionophores did not affect μ chain catabolism at all. These inhibitors also did not affect the formation of IgM forms in vitro that were resistant to in vitro trypsinization.

Carboxylic ionophores did inhibit three important post-translational events. These agents inhibited both the rate (Figs. 3 and 4) and extent (Figs. 1 and 3) of terminal glycosylation processes. They also inhibited the rate of transport to the final plasma membrane or extracellular compartment (Fig. 5). Since the ionophores did not promote the intracellular catabolism of μ chains, these results indicate that IgM was stabilized against catabolism at a stage before that inhibited by ionophores. The commitment to express membrane or secreted IgM occurred before terminal glycosylation events, and was made early in the intracellular transport process.

The most likely event regulating IgM expression was the full covalent assembly of μ, to μ5Lp and of μ, to (μ2Lp)3. This covalent assembly both stabilized newly synthesized μ chains against in vitro trypsinization and allowed terminal glycosylation to proceed. Our earlier work with Daudi cells indicated that blocking covalent assembly of heavy and light chains by inhibiting preliminary glycosylation events promoted rapid catabolism of both μ, and μ, chains. In the absence of covalent assembly of heavy and light chains, membrane IgM was not expressed and μ, chains were not stabilized against intracellular proteolysis. In pre-B cells, such as the Raji and Jomsh 4 cell lines, the absence of light chain synthesis similarly prevented the assembly of μ chains to monomeric IgM. Newly synthesized μ chains in these cells are not stabilized against intracellular proteolysis and are rapidly catabolized (3, 4). In these pre-B cells, as in Daudi and RPMI 1788 cells, all newly synthesized μ chains have been subjected to preliminary glycosylation events (1, 3). Preliminary glycosylation in the absence of assembly to IgM monomers does not protect μ chains from intracellular proteolysis, either in pre-B cells or in cells such as Daudi where only a fraction of newly synthesized μ chains assemble to IgM.

Similar results have been observed for the acetylcholine receptor, correlating the assembly and catabolism of subunits when preliminary glycosylation was inhibited by tunicamycin treatment (19). The nonglycosylated subunits failed to assemble normally, and were rapidly catabolized. In bone marrow cells from patients with β-thalassemia, also, excess production of the hemoglobin α-subunit promoted rapid catabolism of the free subunit compared to the normally stable assembled hemoglobin (20). In both cases, the assembly processes were noncovalent, indicating that disulfide bond formation per se is not an essential stabilizing event. Assembly processes appear to protect the subunits of at least three different multimeric proteins from intracellular proteolysis, to allow normal protein expression.

In differentiation, the molecular events that promote expression of IgM would be as follows. In differentiation from a pre-B cell to a resting B cell that expresses membrane IgM, light chain protein is synthesized. The expression of a light chain with suitable affinity for the heavy chain already present would then promote assembly to μ5Lp monomers, which can be expressed on the cell surface.

In differentiation from a resting B cell to a secreting B cell, the ability to form pentameric IgM would regulate the switch from catabolism of μ, chains to their secretion. Pentamer formation is controlled in differentiation by the increased synthesis of J chain (6) and of sulfhydryl oxidases (18). In both cases, μ chains would be stabilized against nonspecific proteolysis by assembly processes. These assembly processes depend on the synthesis of proteins other than IgM heavy chains. Other factors of quantitative importance in regulating IgM expression include changes in the rates of synthesis and assembly of heavy and light chains, and changes in the degradative capacity of a normal B cell with differentiation.

Fully assembled IgM may normally be subject to a positive recognition step, since in both cell lines studied the fully assembled form was the only form of IgM with significant amounts of terminally glycosylated μ chains. In both cell lines studied, a simple kinetic competition between catabolism and processing would not explain the failure to detect terminally glycosylated, incompletely assembled IgM after a 6-h chase period. Also, in RPMI 1788 cells, both incompletely and terminally glycosylated pentameric IgM was detected in cell lysates. Since in this cell line, approximately 100% of newly synthesized μ chains becomes stable IgM (Fig. 7), the high mannose form of pentameric IgM was most probably a precursor of the terminally glycosylated pentameric IgM. This finding would indicate that pentamerization preceded terminal glycosylation.

The regulation of IgM expression through covalent assembly could explain why secreted IgM is subject to a more complex assembly process than is membrane IgM. Resting B cells express a surface antigen receptor in the form of membrane IgM. These cells are stimulated to differentiate to express other surface immunoglobulin isotypes and to secrete IgM only after an appropriate antigen signal is transmitted through membrane IgM occupancy (reviewed in Ref. 21). The expression of membrane IgM must therefore precede and be independent of the expression of secreted IgM. The cell could readily distinguish membrane and secreted IgM precursors if the μ5Lp form of the μ, chain can be terminally glycosylated and transported to the cell surface while the same form of the μ, chain requires further disulfide bonding before it can be processed and secreted. Formation of secreted pentameric IgM could then readily be promoted in differentiation by increased concentrations of precursor monomeric IgM, J chain, and polymerizing enzymes.

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