The EMBO Journal vol.6 no.13 pp.3969–3977, 1987

Regulation of membrane IgM expression in secretory B cells: translational and post-translational events

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Communicated by Cesar Milstein

IgM secreting cells express little or no membrane IgM. This is not always due to absence of the relevant mRNA. To investigate the synthesis and processing of membrane (μ_m) and secreted (μ_s) polypeptides in secretory B cells, myeloma cells were transfected either with a plasmid containing an intact μ gene or with one only capable of directing μ_m (not μ_s) mRNA synthesis. Although myeloma transfectants could make abundant levels of μ_m mRNA, they did not express IgM on the cell surface. In the myeloma host, μ_m mRNA is translated some 5-fold less efficiently than μ_s mRNA. However, this translational control does not totally preclude μ_m synthesis, indicating post-translational regulatory events. No difference between μ_m and μ_s chains could be detected in their rate of assembly with light chains or in their stability, although both types of heavy chain were degraded more rapidly when synthesized in the absence of light chain, or when the hydrophobic nature of the leader sequence was destroyed by site-directed mutagenesis. However, whereas intracellular μ_s chains in IgM-secreting plasmacytoma were found to be concentrated in the Golgi, the μ_m chains were mainly located in the endoplasmic reticulum. Retention in the endoplasmic reticulum is also observed for both μ_m and μ_s when synthesized in the absence of light chain. We propose that it is the expansion of the endoplasmic reticulum that accompanies B cell to plasma cell differentiation which is in part responsible for the down-regulation of surface IgM expression. Such a mechanism may also affect the expression of other surface proteins.

Key words: membrane and secreted Ig/translation/post-translational modifications/B cell differentiation/immunolocalization

Introduction

Like all immunoglobulin (Ig) isotypes, μ heavy (H) chains are produced in two forms, one (μ_m) which possesses a hydrophobic C-terminal portion allowing its insertion in the membrane of B cells, and the other (μ_s) which has a shorter, hydrophilic C-terminal segment and is secreted by plasma cells. The two molecules are encoded by distinct mRNAs that arise by alternate processing of the primary μ gene transcript (Alt *et al.*, 1980; Early *et al.*, 1980; Rogers *et al.*, 1980). Most myelomas (the malignant counterpart of plasma cells) have a very high ratio of μ_s mRNA to μ_m mRNA, in many cases μ_m mRNA being undetectable (Alt *et al.*, 1980; Rogers *et al.*, 1980; Lamson and Koshland, 1984). This indicates that the absence of μ_m chain in these cells is due to a pretranslational event.

Several lines of evidence, however, suggest that the change in the type of IgM expressed is not solely regulated at the level of RNA processing. For instance, bacterial lipopolysaccharide (LPS)-stimulated B lymphoma (I.29 and BCL_1) cells express little surface IgM, even though they contain twice (or more) as much μ_m mRNA as unstimulated cells (Yuan and Tucker, 1982; R.Sitia *et al.*, in preparation). A similar discrepancy between μ_m mRNA and surface IgM expression has also been observed in normal and malignant human B cells (A.Rubartelli, S.Guazzi and R.Sitia, unpublished observations), and in some hybridomas [e.g. B1-8 (Neuberger and Rajewsky, 1981; Y.Argon, unpublished data)].

There is a suggestion that the regulation of IgM expression might differ from that of other isotypes. Both a human hybridoma (Diaz Espada *et al.*, 1987) and a mouse hybridoma (Neuberger and Rajewsky, 1981) have been described which secrete IgM and IgG simultaneously and yet only IgG is put on the cell membrane. A similar discrepancy between IgM and other isotypes is seen in the regulation of secretion. For example, in the case of the I.29 B cell lymphoma, while I.29μ^+ cells do not secrete IgM, even though they produce μ_s chains, I.29ϵ and I.29γ_{2a} switch variants secrete immunoglobulin efficiently (Sitia, 1985 and unpublished data; Alberini *et al.*, 1987). It is therefore clear that B cells do not rely exclusively on RNA processing mechanisms to control the pattern of IgM expression.

Analysis of possible translational and post-translational regulation of membrane IgM expression has been hampered by the fact that plasma cells make large quantities of μ_s polypeptide which can obscure the study of μ_m and may even compete with μ_m in the protein processing pathways. In order to obviate these problems, we have studied the fate of μ_m heavy chains in transfected plasmacytomas which do not synthesize μ_s.

Results

In this work, as an analogue of a B cell, we use I.29μ^+. This cell line contains μ_m and μ_s mRNAs in similar amounts (Figure 1), although it only expresses surface IgM and does not secrete. On fusion with the plasmacytoma NS1, the resulting hybrid ID43 makes practically only μ_s mRNA and secretes IgM, but makes no detectable surface immunoglobulin. This therefore exemplifies a change in IgM expression that accompanies differentiation and the altered IgM expression is largely accounted for by changes in RNA processing.

However, as discussed in the Introduction, circumstantial evidence indicates that surface IgM expression in plasma cells is also down-regulated by translational or post-translational processes. To demonstrate this we constructed a plasmid that only directs the production of μ_m mRNA. The parent plasmid, pSV-Vμ1, contains a mouse μ heavy chain gene cloned into the vector pSV2gpt. The V_H exons specify a variable region which, on combination with a mouse λ_1 light chain, creates a binding site for the hapten 4-hydroxy-3-nitrophenacetyl (NP) (Neuberger, 1983). Plasmid pSV-Vμ_m is a derivative of pSV-Vμ1 in which the μ_s polyadenylation site and part of the μ_s secreted tailpiece have been deleted (Figure 2). This plasmid should encode μ_m

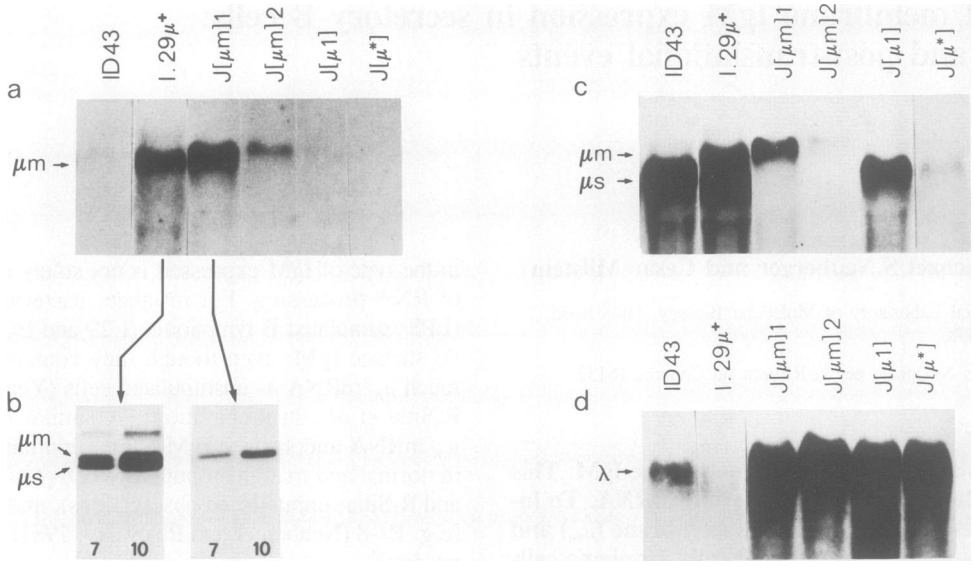

Fig. 1. Northern blot analysis μ_m mRNA in plasma cells. A Northern blot containing total cytoplasmic RNA was sequentially hybridized with probes specific for μ_m (**panel a**), total μ (**panel c**) and λ (**panel d**). J[μ_m]1 and J[μ_m]2 are two independent clones. Arrows on the left of each panel indicate the expected migration of the relevant bands. The μ chains synthesized by I.29μ^+ and J[μ_m] cells, labelled for 7 or 10 min with [^{35}S]methionine in the presence of tunicamycin are shown in **panel b**. Arrows on the left indicate the migration of unglycosylated μ_m and μ_s chains. The slower migrating band co-precipitated in I.29μ^+ cells at 10 min is probably BiP (Haas and Wabl, 1983).

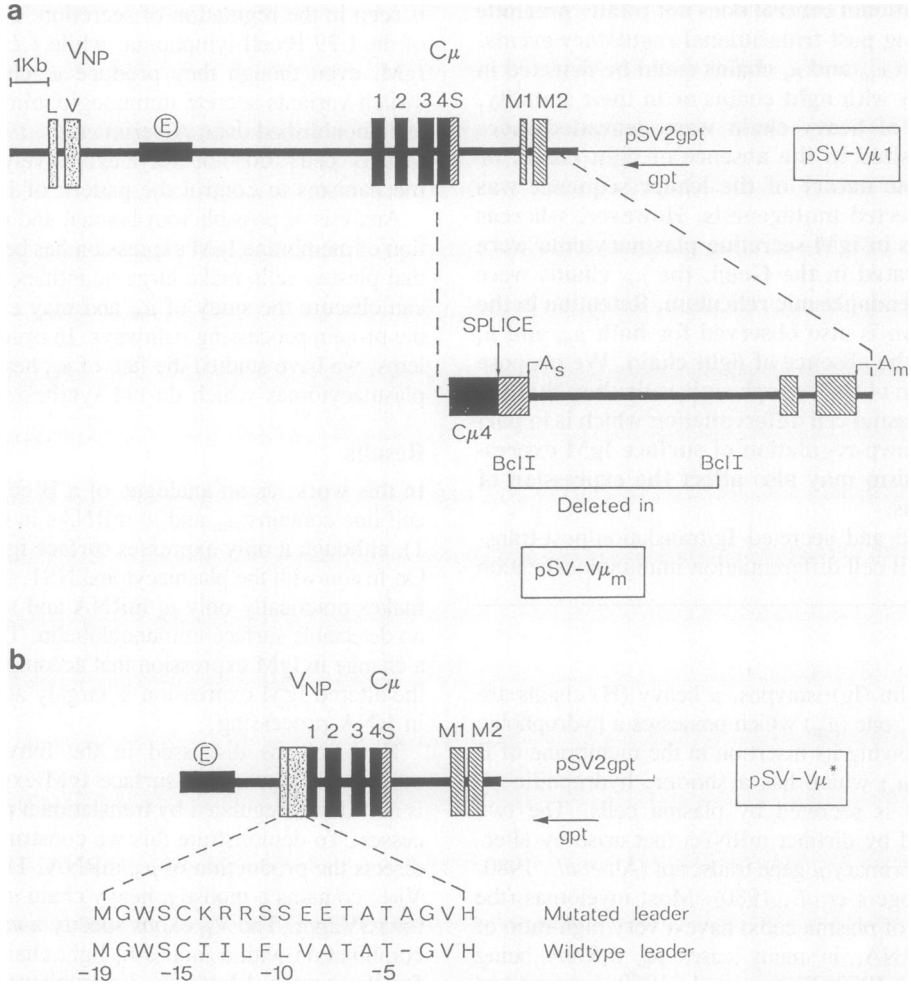

Fig. 2. pSV-Vμ1 have been described previously (Neuberger, 1983). Plasmid pSV-Vμ_m has a deletion spanning the polyadenylation for μ_s mRNA (A_s). In plasmid pSV-Vμ^*, the signal sequence has been mutated so as to destroy its hydrophilic nature and the IgH enhancer (E) has been placed upstream of the transcription unit.

Table I.

Cell line	Abbreviation	Immunofluorescence[a]			FACS[b]			Secretion[c]	μ chain localization[d]	μ chain half-life (min)[e]
		Surface μ	Cyto μ	Cyto λ	IgM	H2	Ia			
J558L[pSV-Vμ1]	(Jμ1])	0	90	95	3	55	7	+	Golgi + ER	150
NSO[pSV-Vμ1]	(N[μ1])	0	45	0	0	15	0	−	ER	60
J558L[pSV-$V\mu_m$]	(J[μ_m])	<3	>75	95	2	35	2	−	ER	135
NSO[pSV-$V\mu_m$]	(N[μ_m])	0	30	0	1	13	2	−	ER	50
J558L[pSV-Vμ*]	(J[μ*])	0	5−15[g]	95	ND[f]	ND	ND	−	Cytosol	<30
I.29μ^+		95	95	95	276	166	196	−	ER + lysosomes	120
ID43 (I.29 × NS.1)		0	95	40[h]	3	45	4	+	Golgi + ER	130
BCL_1.B3		95	>95	>95	106	176	160	±	ND	ND

[a]Formaldehyde-fixed cells were stained with fluorescent anti-μ or anti-λ before (surface) and/or after (cyto) permeabilization with 0.05% Triton X-100.
[b]Data represent mean fluorescence intensity after background subtraction.
[c]Detected by immunoprecipitation and SDS−PAGE of the supernatants of biosynthetically labelled cells.
[d]Determined by confocal immunofluorescence.
[e]Determined by pulse chase experiments (see Figures 3 and 4).
[f]Not determined.
[g]Very weak staining, limited to the cytosol.
[h]Most cells were labelled by anti-κ light chain antibodies.

polypeptides and be incapable of directing μ_s synthesis.

Plasmid pSV-$V\mu_m$ was transfected into the J558L plasmacytoma, which secretes λ_1 light chains but expresses no heavy chains of its own. Whereas pSV-Vμ1 transfectants of J558L (Jμ1 cells) make practically only μ_s mRNA, pSV-$V\mu_m$ (J[μ_m]) transfectants only make μ_m mRNA (Figure 1). As shown in Table I, very few cells (<3%) within any one cloned J[μ_m] transfectant express surface IgM, although most of the cells contain intracytoplasmic μ chains. Thus, given the results of the Northern blot analysis, it is clear that there is a translational or post-translational block to surface IgM expression in plasma cells and this lack of surface IgM in IgM-secreting plasmacytomas cannot simply be attributed to competition between μ_m and μ_s.

Differential control of the rate of translation

The lack of surface IgM expression could be due to translational or post-translational events. As regards translational control, one can ask (i) whether μ_m and μ_s mRNAs are translated equally efficiently in plasma cells and (ii) whether μ_m mRNA in plasma cells is translated as efficiently as μ_m mRNA in B cells.

Biosynthetic labelling experiments were performed using short pulses in order to compare the rates of μ polypeptide synthesis in J[μ1] and two J[μ_m] clones. By normalizing with respect to endogenous light chain production, we conclude that μ_m mRNA in J558L is translated with ~20% the efficiency of that of μ_s mRNA (Table II).

Changes in the efficiency of translation of μ_m mRNA during B cell differentiation are difficult to establish, since a B cell line and a plasmacytoma that contain the same μ_m mRNA (i.e. with the same V_H region) are not available. However, short of that, we can compare J[μ_m] and I.29 cells. Comparison both of μ_m mRNA levels and of the rates of μ_m polypeptide synthesis in the two cell lines agrees with the suggested regulation of μ_m synthesis at the translational level. The biosynthetic labelling using short pulses (7 or 10 min) was done in the presence of tunicamycin, to allow unambiguous identification of the μ_m band in I.29μ^+ cells. The results (Table II) indicate that μ_m mRNA in J558L is translated some 5-fold less efficiently than the μ mRNA in I.29. However, as discussed above, caution must be exercised in the interpretation of this result, since the μ_m mRNAs of I.29 and of the J558L transfectants use different V_H segments. A direct comparison will be facilitated by the transfection of pSV-$V\mu_m$ into B cell lymphomas, an experiment currently in progress.

Table II. The μ_m mRNA in secretory cells is subjected to translational control

Cells	μ Protein[a]		μ mRNA[b]		Translational efficiency[c] (%)
	Area	%[d]	Area	%[d]	
Exp. 1					
J(μ1)	11.8	100	7.4	100	100
J(μ_m)1	1.4	12	3.9	52	23
Exp. 2					
I.29μ^+	11.7 ± 1.9	100	2.5 ± 0.6	100	100
J(μ_m)1	3.4 ± 0.8	31	3.4 ± 0.8	141	22
J(μ_m)2	0.4 ± 0.1	3	0.6	21	14

[a]Cells were labelled for 10 min with [^{35}S]methionine in the presence of tunicamycin; μ chain production was quantitated by densitometric tracing of polyacrylamide gels. In Exp. 1, the data were normalized with respect to the endogenous λ chain, while in Exp. 2, identical amounts of TCA-precipitable radioactivity were immunoprecipitated from each sample.
[b]mRNA content was determined by densitometric tracing of Northern blots (shown in Figure 1) hybridized with a membrane μ specific (Exp 2) or a Cμ (Exp. 1) probe.
[c]Translational efficiency was calculated according to the formula: (%μ protein/% μ mRNA) × 100.
[d]Results are expressed as per cent relative to J(μ1) in Exp. 1 and relative to I.29μ^+ in Exp. 2.

Kinetics of assembly and degradation

Translational control may thus go some way to explain the absence of surface IgM in J[μ_m], despite the presence of large amounts of the corresponding mRNA. Even so, considerable synthesis of intracellular μ_m polypeptide is detectable. We therefore performed pulse chase experiments and these showed that the synthesized material is properly assembled and not degraded faster than the secreted counterpart, although little of the synthesized μ_m polypeptide reached the cell surface.

Cells were pulsed for 5 or 10 min with L-[^{35}S]methionine and chased for different times. The immunoglobulin polypeptides in

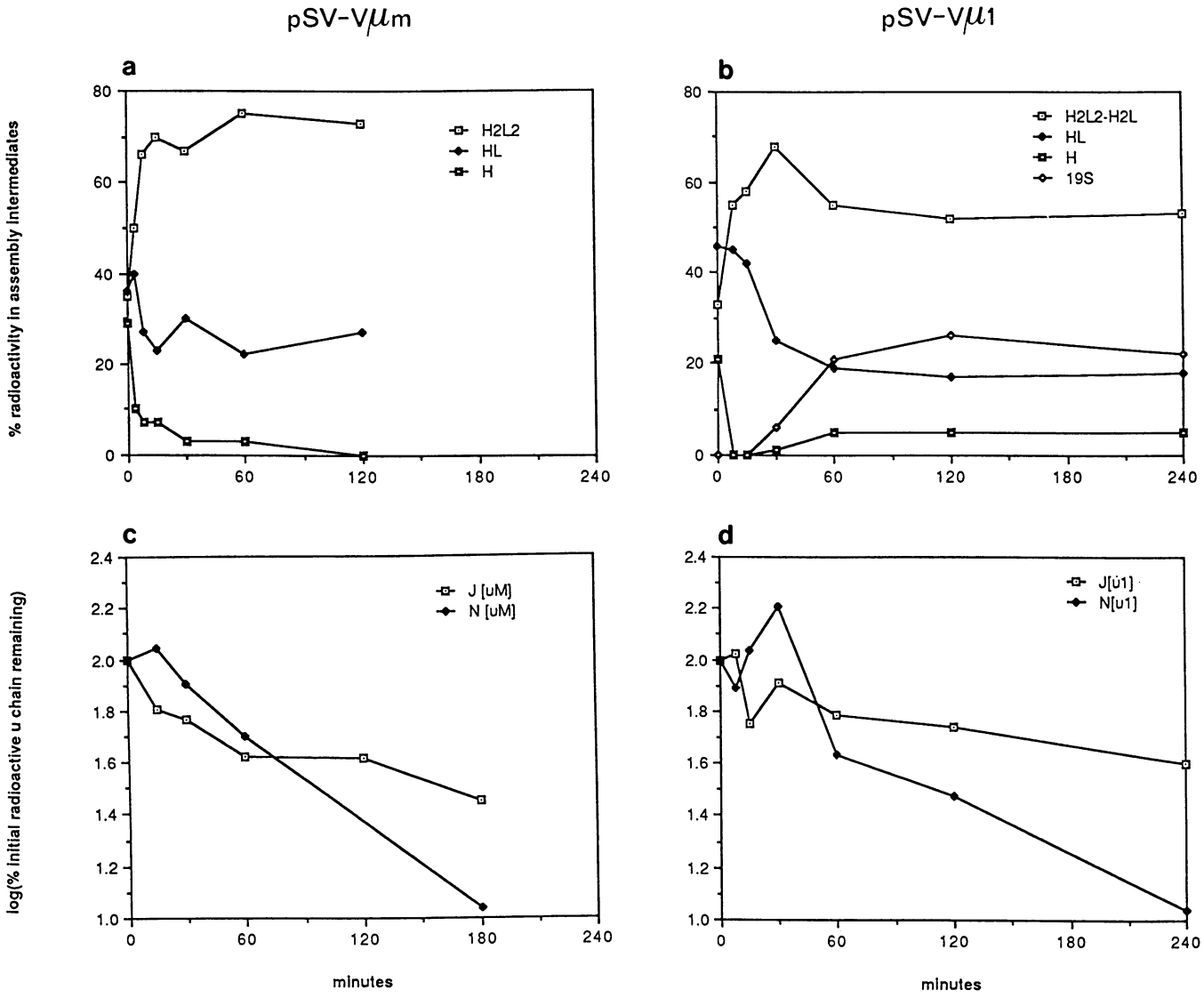

Fig. 3. Assembly of μ_m and μ_s to light chains: kinetics and its role in heavy chain stabilization. NS0 and J558L cells transfected with pSV-Vμ1 or pSV-Vμ_m were pulsed for 5 min with [^{35}S]methionine and chased for different times in the presence of excess cold methionine before lysis. The anti-μ immunoprecipitates were then resolved on reducing or unreducing SDS–polyacrylamide gels. To analyse the kinetics of assembly (**panels a** and **b**, J[μ_m] and J[μ1] cells respectively), the distribution of radioactivity amongst the different assembly intermediates [H, HL, H2L + H_2L_2 and $(H_2L_2)_n$] was determined by densitometric analysis of non-reducing gels. To determine the decay of μ_m and μ_s (**panels c** and **d** respectively) in the presence (J558L host □——□) or absence (NS0 host ◆——◆) of light chains, fluorograms of the reducing gels were scanned by densitometry and the μ chain areas expressed as the logarithm of the percent of initial radioactive μ chain (time 0) remaining in the cell lysates.

the lysates were then immunoprecipitated and resolved on SDS polyacrylamide gels to assess both assembly and degradation (Figures 3 and 4). Assembly for both μ_m and μ_s chains is very rapid, and follows similar kinetics (Figure 3a and b): the fraction of free or non-covalently bound μ chain, which represented ~30% of the total in a 5-min pulse of J[μ_m] cells became negligible after a 15-min chase. Approximately 25% of the intracellular IgM of a J[μ_m] clone migrated in the HL peak, even after a 120-min chase (Figure 3a). The kinetics of μ_s–λ assembly was similar to that of μ_m–λ (Figure 3b), a plateau being reached at ~60 min of chase. In J[μ1] cells, ~20% of the intracellular μ was constituted of polymeric molecules (mostly 19S). As expected, only 19S molecules $(\mu_2\lambda_2)_5$ could be isolated from the supernatant of J[μ1] cells.

In comparison with the J558L transfectants, intracellular μ chains in I.29μ^+ cells did not show major differences in the kinetics of decay (Figure 4, panel e). The increase in the intensity of the μ chain band noted at the 30-min chase point was probably due to residual intracellular [^{35}S]methionine and intermediates after the chase initiation, and was consistently more marked in J558L transfectants than in I.29μ^+ cells. The kinetics in I.29μ^+ cells stimulated with bacterial lipopolysaccharide was almost identical to that in J[μ1] (data not shown).

It is notable that incorrectly assembled chains, or chains which do not contain the hydrophobic leader, are degraded faster. For instance, a very fast decay was observed for the μ chain in J558L[pSV-Vμ*] (J[μ*]) (Figure 4c and e), where the μ signal sequence has been mutated to encode hydrophilic residues (see Figure 1). [Confocal immunofluorescence studies (not shown) confirmed that the μ chain was localized in the cytosol rather than in the endoplasmic reticulum of J[μ*] cells, where it could not assemble with light chains or heavy chain binding protein (BiP).]

The turnover of μ chains was also influenced by assembly with

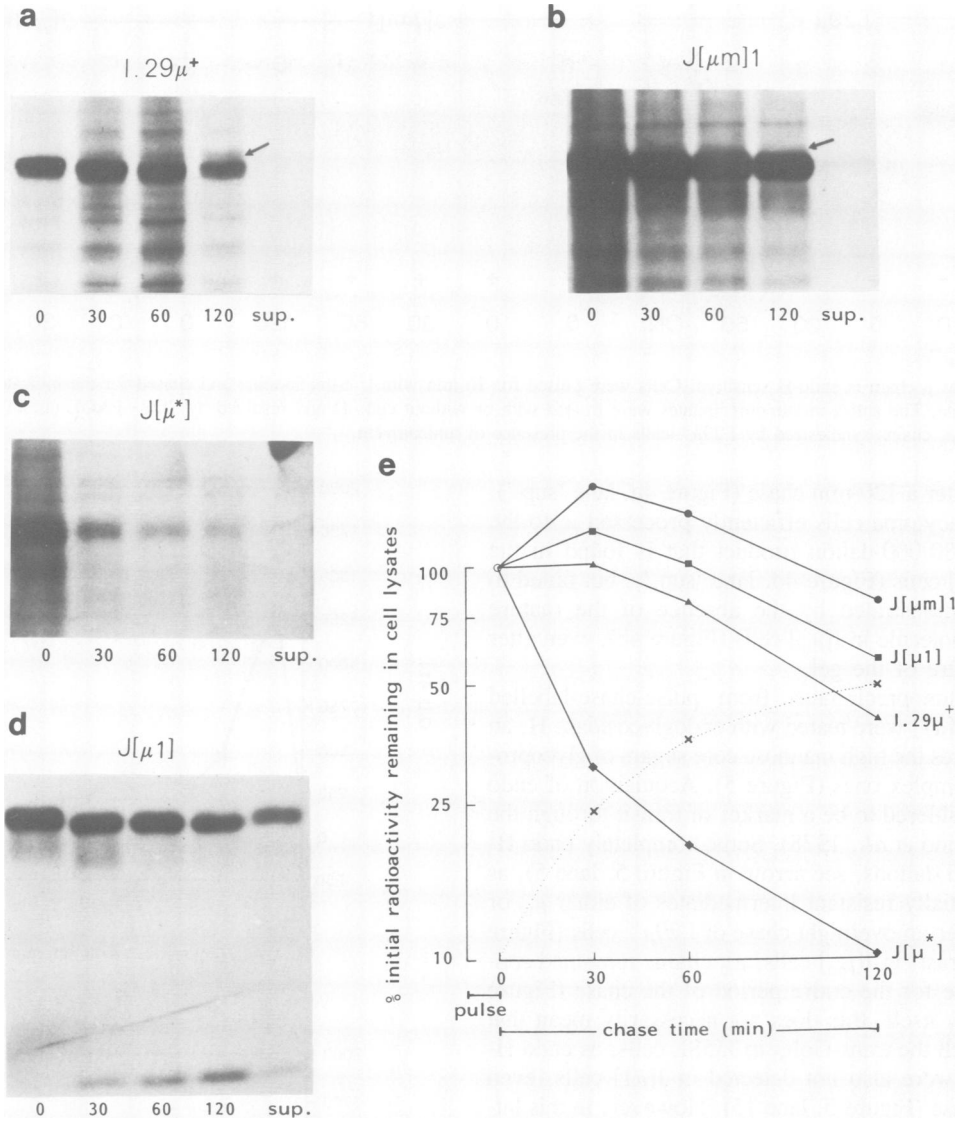

Fig. 4. Processing and stability of μ chains. I.29μ^+ (**panel a**), J[μ_m] (**panel b**), J[μ^*] (**panel c**) and J[μ1] (**panel d**) were pulsed for 10 min with [^{35}S]-methionine and chased in the presence of excess cold methionine before lysis and immunoprecipitation with anti-μ. The supernatants of cells chased for 120 min were also immunoprecipitated. The fluorograms are of SDS–PAGE (8% in **a** and **b**, 10% in **c** and **d**). The arrow on **lane 4** (**panels a** and **b**) indicates the expected migration of fully glycosylated, 82 000-dalton μ_m molecules (Sidman, 1981; Rubartelli *et al.*, 1983; Sitia *et al.*, 1984). **Panel e**: kinetics of decay of intracellular μ chain were determined by densitometric analysis. The areas of individual μ chain bands were calculated from two different experiments (one of which is shown in panels a–d) and expressed as the percentage of initial radioactive μ chains (time 0) remaining in the cell lysates. The dotted line (×···×) shows the amount of radioactive λ chain in J[μ1] co-precipitated by anti-μ. Results are expressed as per cent of the amount co-precipitated from culture supernatants where all molecules are fully assembled into 19S pentamers..

light chains. As shown in Figure 3c, the half-life of μ_m was indeed shorter (~50 min) in the NSO transfectant, N[μ_m] (which contains no light chains) than in J[μ_m] (135 min). Similar results were obtained when J[μ1] cells (half-life ~150 min) were compared to N[μ1] (~60 min) (Figure 3d). The latter did not secrete heavy chains.

In J[μ1] cells (Figure 4d), [^{35}S]λ chains were co-precipitated by anti-μ only after a 30-min chase and steadily increased thereafter (see dotted line in Figure 4e). Similar results were obtained in J[μ_m] cells and with very similar kinetics. These data remain puzzling, but may be explained by different pool sizes for μ and λ. It was thus likely that early in the chase, the newly synthesized (radioactive) μ assembled with pre-existing cold λ chains (Vassalli *et al.*, 1971). In agreement with this interpretation, labelled μ chains were precipitated by anti-λ or hapten-Sepharose very early in the chase. In addition, the μ band co-precipitated by anti-λ showed a kinetics of decay similar to that obtained by anti-μ precipitation (not shown).

μ_m and μ_s accumulate in different compartments of secretory cells

The biosynthetic labelling experiments described above did not disclose a difference in the assembly or degradation of μ_s and μ_m chains. Therefore, in order to further our understanding of the post-translational processes regulating IgM expression, we investigated the glycosylation status and intracellular localization of μ_m and μ_s chains in plasmacytoma (J558L) and B cell lymphoma (I.29μ^+). Whilst mature μ_m chains in I.29μ^+ cells became identifiable after 60 min of chase (see arrow, Figure 4a), no detectable secreted μ_s was present in the supernatants of

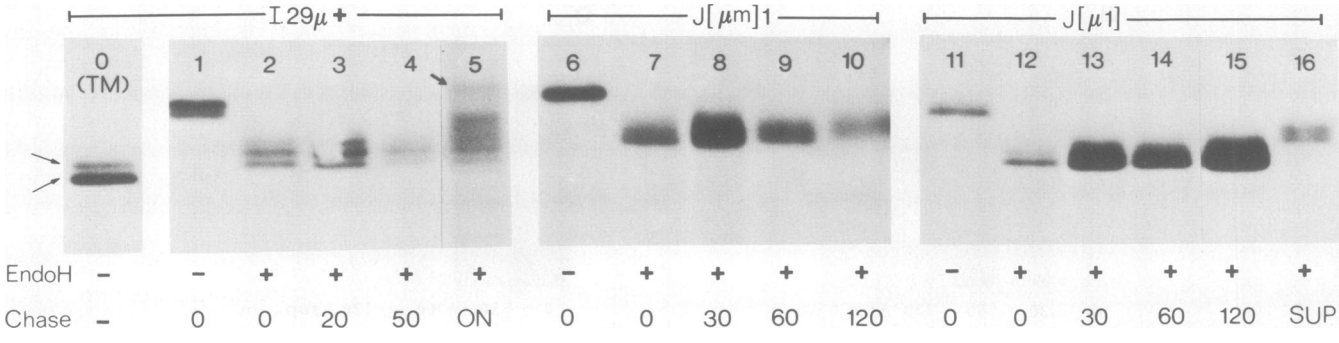

Fig. 5. Most intracellular μ chain is endo-H sensitive. Cells were pulsed for 10 min with [^{35}S]methionine and chased for the indicated times in the presence of excess cold methionine. The anti-μ immunoprecipitates were treated with or without endo-H and resolved by SDS–PAGE (10%) after reduction. Arrows in **lane 0** indicate μ_m and μ_s chains synthesized by I.29μ^+ cells in the presence of tunicamycin.

I.29μ^+ cells even after a 120-min chase (Figure 4a, lane 'sup.'). By contrast, plasmacytoma cells efficiently processed μ_s to the fully glycosylated 80 000-dalton product that is found in the supernatant of J[μ1] cells (Figure 4d, lane 'sup.'), but failed to process μ_m, as demonstrated by the absence of the mature 82 000-dalton μ_m molecule in J[μ_m] cells (Figure 4b), even after a prolonged exposure of the gel.

The anti-μ immunoprecipitates from pulse-chase-labelled I.29μ^+, J[μ_m] and J[μ1] were teated with endoglycosidase H, an enzyme which cleaves the high mannose core sugars of glycoproteins but not the complex ones (Figure 5). Acquisition of endo H-resistance is considered to be a marker of transit through the trans-Golgi (Tarentino *et al.*, 1978). Some completely endo H-resistant μ_m (82 000 daltons, see arrow in Figure 5, lane 5), as well as several partially resistant intermediates of either μ_m or μ_s, were present after an overnight chase of I.29μ^+ cells (Figure 5, lane 5). By contrast, in J[μ_m] cells, μ_m chains remained sensitive to the enzyme for the entire period of the chase (Figure 5, lanes 8–10). By itself, this does not necessarily mean that μ_m is unable to reach the trans-Golgi in J558L cells, as endo H-resistant μ_s chains were also not detected in J[μ1] cells, even after a 120-min chase (Figure 5, lane 15). However, in this latter case, endo H-resistant μ_s chains were readily detectable in the supernatant (Figure 5, lane 16). Notice that after 120 min of chase, 25% of the intracellular IgM in J[μ1] was in the 19S form (Figure 3d) and was not terminally glycosylated (Figure 5). This supports the claims of Parkhouse (1973) and Tartakoff and Vassalli (1979) that polymerization precedes terminal glycosylation.

When cells of the J558L transfectants were disrupted by nitrogen cavitation and the postnuclear supernatant fractionated on a discontinuous sucrose gradient, μ_m and μ_s chains accumulated in different fractions. As shown in Figure 6, while μ_s chains distributed in the upper fractions of the gradient containing the less dense Golgi apparatus, μ_m was found in the lower fractions; these fractions correspond to the endoplasmic reticulum (Bole *et al.*, 1986).

Differences in intracellular localization of μ_m and μ_s were also disclosed by immunolocalization experiments with the high-resolution confocal microscope. As shown in Figure 7b, the μ_m chain was found to accumulate in the rough endoplasmic reticulum of J[μ_m] cells. By contrast, the bulk of μ_s chains were concentrated in the Golgi apparatus of J[μ1] (Figure 7c). Identification of the subcellular compartments was confirmed by co-staining with either 3,3′-dihexyloxacarbocyanine iodide or anti-endoplasmin antibodies [which are specific for the endoplasmic reticulum (Terasaki *et al.*, 1984; Koch *et al.*, 1986)], or wheat

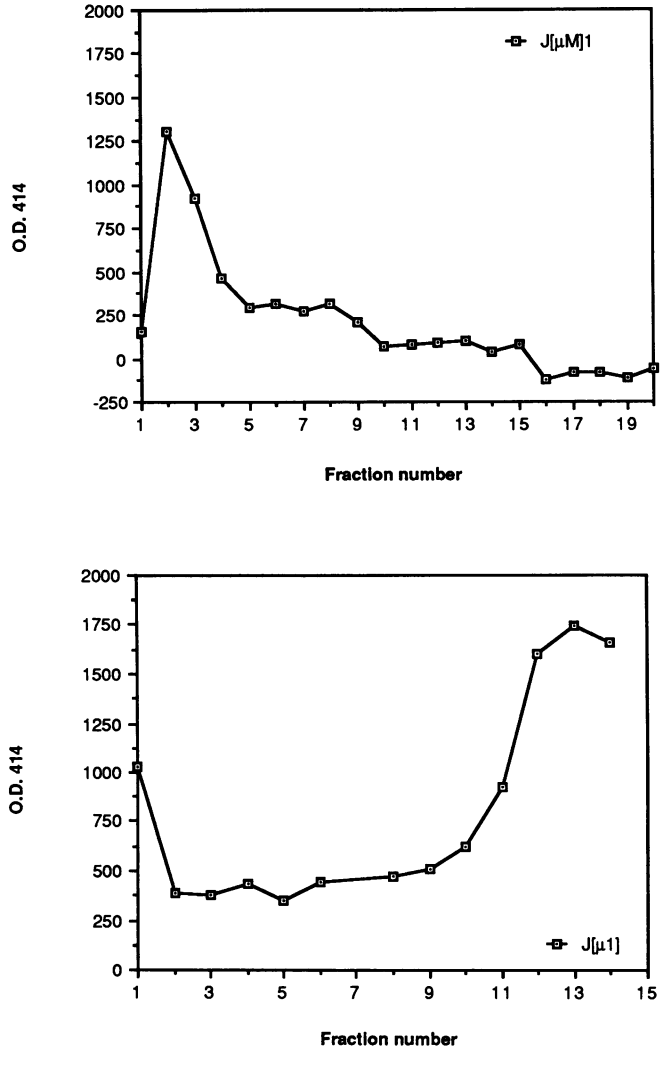

Fig. 6. Different intracellular localization of μ_m and μ_s. Cells (5×10^7) of J[μ_m] (**upper panel**) or J[μ1] (**lower panel**) were disrupted and fractionated over a sucrose gradient. Individual fractions were assayed for the presence of IgM molecules by ELISA. Twenty fractions were collected for J[μ_m] cells while the first 14 fractions out of a total of 15 collected from J[μ1] cells were tested.

germ agglutinin [specific for the Golgi apparatus (Munro and Pelham, 1987)]. The staining of I.29μ^+ cells with anti-μ (Figure 7a) was most characteristically limited to vesicles surrounding the nucleus in a discontinuous network or scattered in the

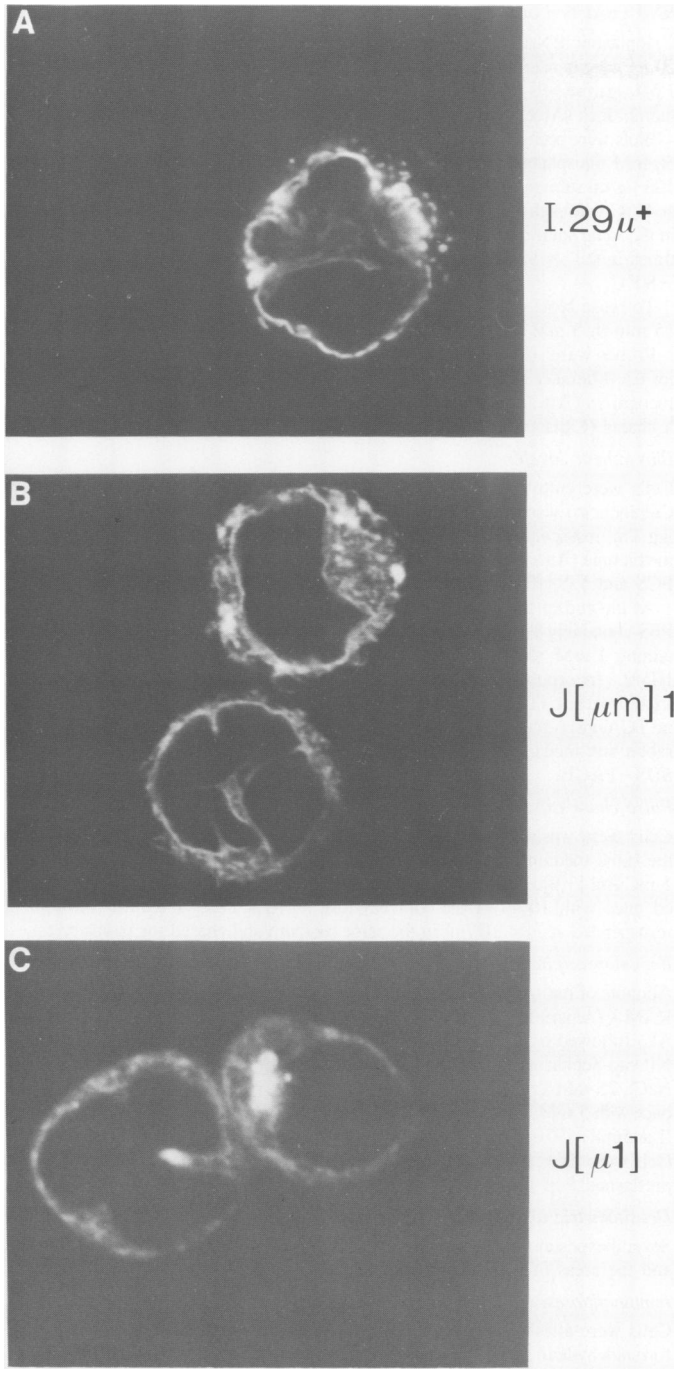

Fig. 7. Immunolocalization of μ chains by confocal fluorescent microscopy. I.29μ^+ (**A**), J[μ_m] (**B**) and J[μ1] (**C**) cells were fixed, permeabilized, stained with fluorescent anti-μ and analysed in a scanning confocal fluorescence microscope (White *et al.*, 1987).

cytoplasm. Most of these vesicles were co-stained by anti-endoplasmin, suggesting that they represented vesiculation of the endoplasmic reticulum. In the absence of λ chains (the NSO transfectants) μ_s chains no longer accumulated in the Golgi, but were retained in the endoplasmic reticulum (Table I). Thus, the migration of μ_s chains to the Golgi apparatus requires assembly with light chains, although this event is not sufficient for efficient transfer of μ_m to the Golgi of secretory B cells.

Discussion

Plasma cells are terminally differentiated, short-lived cells, which are devoted to secreting antibodies. They lack surface immunoglobulin and other surface proteins such as class II antigens and Fc and complement receptors. It is known that the main mechanism which prevents membrane IgM from being expressed on the cell surface of plasma cells is the progressive disappearance of μ_m mRNA (Early *et al.*, 1980; Milstein *et al.*, 1981; Lamson and Koshland, 1984). Here we show that this is not the only factor involved. The translational and post-translational controlling mechanisms disclosed here from the study of cell lines could play a significant role, particularly at the intermediate stages between B cell blasts and plasma cells; however, such an extension to untransformed cells remains to be established.

Our results disclosed two levels of post-transcriptional control of IgM membrane expression, quite apart from the well-established shift in the production of μ_m and μ_s mRNAs. The first level of control observed was a reduced translational efficiency of μ_m RNA in secretory cells. The absence of μ_s transcripts in these cells excluded the possibility that the reduced translation of μ_m mRNAs in secretory cells was due to competition for the ribosomes with the more abundant μ_s mRNA. The molecular mechanism of the lower efficiency of translation remains obscure. Factors inhibiting the translation of specific mRNAs have been demonstrated in several systems, including the heat shock response (Kruger and Benecke, 1981; Ballinger and Pardue, 1983), the differentiation of the surf clam *Spisula solidissima* (Rosenthal *et al.*, 1980), muscle cells (Endo and Nadal-Ginard, 1987) and fibroblasts (Farmer *et al.*, 1978). Since μ_s transcripts are translated efficiently by J[μ1] cells, the poor translational efficiency of μ_m mRNA in J558L suggests the involvement of the 3′ end of the μ_m mRNA.

Assembly and intracellular degradation of IgM

In spite of this translational control, J[μ_m] cells synthesized considerable amounts of μ_m polypeptide, which was assembled into μ_2L_2 molecules with the same kinetics as μ_s in J[μ1] or as μ_m in B cells. Assembly and/or degradation were thus not the reason why J[μ_m] did not express surface IgM. In J[μ_m] cells, μ_m chains were not processed to their final 81 000−82 000 mol. wt form, which could, by contrast, be detected in I.29μ^+ cells (Figure 4a).

The μ_m and μ_s polypeptides exhibited similar kinetics of assembly with light chains. Vassalli *et al.* (1971) have suggested that newly synthesized heavy chains assemble with preformed light chains while they are still on the polysome. Alternatively, as recently suggested by Bole *et al.* (1986), newly synthesized heavy chains could first bind to BiP and then to the light chain. Our results show that anti-λ antibodies precipitated at least 80% of the μ chain present in the cell, and that the kinetics of H−L assembly was very fast. The important role of light chain in the stabilization and traffic of heavy chains is emphasized by the results with the NSO transfectants. In the absence of light chains, the half-lives of both μ_m amd μ_s are reduced by 50% or more. More importantly, in N[μ1] cells, the μ_s chain was no longer concentrated in the Golgi, but was found in the endoplasmic reticulum. Similarly, anti-μ antibodies stained the endoplasmic reticulum of J[μ_m] cells. Therefore it also appears that assembled membrane IgM in secreting cells cannot proceed from the endoplasmic reticulum to the Golgi apparatus, where terminal glycosylation takes place. By contrast, IgM was concentrated in the Golgi of J[μ1] cells, emphasizing the different fate of IgM destined for secretion.

Where does the degradation of intracellular IgM occur? Excluding the very short-lived cytoplasmic μ chains that lack a signal sequence, the shortest half-lives were those of isolated μ_m and

μ_s chains in NSO cells (which lacks light chains). As immunolocalization experiments showed that little staining was detected outside the endoplasmic reticulum of N[μ_m] and N[μ1] cells, it follows that degradation occurs either in the endoplasmic reticulum itself, or is so rapid in another compartment that we cannot detect it by staining.

Why is membrane IgM retained in the endoplasmic reticulum of secretory B cells? Analogously to the proposed role of the invariant chain for class II antigens (Kvist *et al.*, 1982; Kaufman *et al.*, 1984) it is possible that membrane IgM needs a specific carrier molecule, lost during lymphocyte–plasma cell differentiation. We prefer an alternative explanation for this slow degradation of assembled membrane IgM which therefore never reaches the cell surface. It is possible that membrane IgM does not reach the Golgi of J[μ_m] cells because its transit time through the very expanded endoplasmic reticulum is longer than its half-life. On the other hand, μ_s chains are located in the lumen of the endoplasmic reticulum, where the transit time is not determined by the surface area of the membrane. Retention in the endoplasmic reticulum may also be affected by interaction with BiP (Hendershot *et al.*, 1987; Munro and Pelham, 1987) or other proteins. Indeed, this must be the reason why in the absence of light chain (NSO transfectants) the μ_s does not reach the Golgi, whereas they are secreted when correctly assembled with light chains. So, if the assembled μ_m retains residual binding to BiP or to another integral protein of the endoplasmic reticulum, the transit through the organelle will be further delayed in a more specific manner. This model predicts that other membrane-bound molecules could suffer analogous regulation in myeloma cells, as a consequence of the expanded endoplasmic reticulum and its constituent proteins. This should be kept in mind when transfecting genes into secretory cell lines. Indeed, certain myelomas and hybridomas are found not to express class II on the surface, despite the levels of α, β and invariant chain mRNA being similar to that in B cells (Venkitaraman *et al.*, 1987). Some antigens are expressed well on the surface of plasma cells [e.g. plasma cell antigen PC-1 (Anderson *et al.*, 1984)] and the post-translational regulation obviously does not inhibit the surface expression of all membrane proteins. However, the expression of several cell surface proteins is down-regulated during B cell to plasma cell differentiation (e.g. Fc receptors, C3 receptor) and it would be of interest to discover whether this is also partly due to post-translational events.

Materials and methods

Plasmid construction

The plasmids used are described in Figure 2. pSV-Vμ1 was described previously (Neuberger, 1983). Plasmid pSV-Vμ_m was constructed by digesting a sample of pSV-Vμ1 that had been prepared from a *dam*$^-$ *Escherichia coli* host with *BclI* and religation of the vector. Plasmid pSV-Vμ^* (M.S.Neuberger, unpublished) was constructed by site-directed mutagenesis.

Cell lines and transfection

J558L (Oi *et al.*, 1983) and NSO (Galfre and Milstein, 1981) myeloma cells were maintained in Dulbecco's minimum essential medium (DMEM) containing 10% fetal calf serum (FCS), and transfected by spheroplast fusion (Neuberger *et al.*, 1984) or electroporation (Neumann *et al.*, 1982). For electroporation, $1-2.5 \times 10^7$ cells were resuspended in 0.5 ml of PBS containing 10 μg of linearized plasmid DNA. After four pulses of 2.5 kV/cm, cells were kept for 15 min on ice and cultured for 24 h before the addition of selective medium (containing 5 μg/ml mycophenolic acid, 250 μg/ml xanthine and 15 μg/ml hypoxanthine). Positive wells were cloned by limiting dilution in selective medium. Individual clones were randomly picked, analysed and subcloned when necessary.

I.29μ^+ (Stavnezer *et al.*, 1985), ID43 (Tada and Hämmerling, 1980) and BCL$_1$-B3 (Chen *et al.*, 1986) were maintained in RPMI 1640 containing 10% FCS and 5×10^{-5} M 2ME.

RNA extraction and Northern blotting

Cytoplasmic RNA was prepared as described by Neuberger (1983). Aliquots of 20 μg were denatured with glyoxal and dimethyl sulphoxide, electrophoresed onto 1.5% agarose gels (Sitia *et al.*, 1985) and blotted and UV cross-linked to Hybond-N membranes (Amersham) following the suppliers' recommendations.

Blots were prehybridized for at least 4 h at 42°C in 50% formamide, 6 × SSC, 50 mM phosphates, pH 7, 0.5% SDS, 1 × Denhardt's, 5% dextran sulphate, 100 μg/ml salmon sperm DNA and 100 μg/ml yeast RNA, hybridized overnight at 42°C with nick-translated probes ($>10^6$ c.p.m./ml, sp. act. $>10^8$ c.p.m./μg), in the same solution, washed once in 2 × SSPE at room temperature and several times in 0.1 × SSPE, 0.1% SDS at 50°C, and exposed to Fuji X-ray film at −80°C.

To reuse blots, hybridized probes were removed by incubation at 100°C for 15 min in 5 mM Tris–HCl, pH 7.4, 1 mM EDTA and 0.1 × Denhardt's.

Probes were: (i) a 1.3-kb *BglII–HindIII* fragment from pμ (3471)[9], specific for Cμ (Marcu *et al.*, 1980); (ii) a 0.9-kb *BamHI–EcoRI* fragment from pJSS-916 recognizing Mμ (Yuan and Tucker, 1984); and (iii) pSV-HSVλ1, specific for λ chains (Cattaneo and Neuberger, 1987).

Biosynthetic labelling

Cells were cultured for 45 min in the presence of 4 μg/ml tunicamycin (Tm, Calbiochem) washed twice in ice-cold methionine-free MEM (Gibco) containing Tm, resuspended at 10^7/ml in the same medium containing 250 μCi/ml [^{35}S]-methionine (Amersham, sp. act >800 Ci/ml) glutamine, antibiotics, 2% dialysed FCS and 5×10^{-5} M 2ME and cultured for 7 or 10 min at 37°C.

At the end of the labelling period, cells were washed with 10 ml of ice-cold PBS containing 10 mM NaN_3, and lysed in phosphate buffered saline (PBS) containing 1 mM phenylmethylsulphonyl fluoride, 1 μg/ml aprotinin and 0.25% NP40. Incorporation of [^{35}S]methionine into protein was measured by trichloroacetic (TCA) acid precipitation. Aliquots containing identical amounts of TCA insoluble radioactivity were immunoprecipitated with affinity-purified rabbit anti-mouse μ (RAM μ) or goat anti-mouse λ (GAM λ) and analysed by SDS–PAGE.

Pulse chase experiments

Cells were washed twice in methionine-free MEM, kept for 30 min on ice in the same medium and pulsed for 5 min (Figure 3) or 10 min (Figure 4) with 1 mCi/ml [^{35}S]methionine at 10^7/ml. At the end of the pulse, cells were washed once with 10 ml of ice-cold DMEM + 10% FCS + 2 mM methionine, resuspended at 1×10^6/ml in the same medium and chased for indicated times.

Immunoprecipitation and gel electrophoresis

Aliquots of radiolabelled samples were immunoprecipitated with affinity-purified RAM λ (Alberini *et al.*, 1987) or GAM λ (Southern Biotechnology, Birmingham, AL) followed by Protein A Sepharose (Pharmacia, Uppsala, Sweden), or with NP-cap-Sepharose (Argon and Milstein, 1984), washed three times with 0.5 M NaCl, 25 mM Tris, pH 8.0, 0.25% NP40, 1 mM phenylmethylsulphonyl fluoride, once with 5 mM Tris, pH 7.4, eluted and resolved by SDS–PAGE under reducing (Laemmli, 1970) or non-reducing (Ziegler and Hengartner, 1977) conditions. Gels were fixed and impregnated wtih Amplify (Amersham) before exposure to preflashed Fuji film at −80°C.

Densitometric analysis

Several exposures of the same gels were scanned by a Joyce–Lobel densitometer, and the areas of individual peaks calculated and averaged.

Immunofluorescence

Cells were attached to poly-L-lysine-coated multi-well slides, fixed with 3.5% formaldehyde in PBS (20 min at room temperature) and washed with PBS. Membrane proteins were analysed by staining with fluorescent antibodies before permeabilization with 0.05% Triton X-100 in PBS (10 min at room temperature), which was essential for intracellular staining. Fluorescein and/or rhodamine conjugated anti-γ and anti-μ were purchased from Cappel, anti-λ from Nordic, wheatgerm agglutinin from Sigma. Rabbit anti-endoplasmin (Koch *et al.*, 1986) was a kind gift of Dr G.Koch, and was used in indirect immunofluorescence with FITC goat anti-rabbit IgG (Sigma).

Stained slides were mounted in 90% glycerol, 10% PBS containing 1 mg/ml paraphenylenediamine and analysed by either conventional immunofluorescence or by the 'MRC-lasersharp' confocal microscope (White *et al.*, 1987). Images of cells focused about midway through the nucleus were generated in a video monitor, which was photographed using Ilford FP4 film.

FACS analysis

Cells were stained in PBS + 10 mM NaN_3 + 1% FCS with fluorescent GAMμ (fluorescent GAMγ was used as a control) or monoclonal antibodies against MHC class I or class II antigens, followed by either fluorescent goat anti-rat Ig or GAMγ. Rat anti mouse class II (NIMR4) (Andrew and Parkhouse, 1986) was a kind gift of Dr R.M.E.Parkhouse (NIMR, Mill Hill); mouse monoclonals K.24.17 (anti I.A d.j.g.), K24.199 (anti I-A$^{d.f.j.v.w3,w.13,w16}$), K9.18 (anti H-2^d) and K.22.53

(anti H-2^j) (Klein *et al.*, 1983) were kind gifts of Dr S.Kimura (University of London).

Subcellular fractionation

Cells (5×10^7) were washed several times with PBS, resuspended in 10% sucrose, 100 mM Tris, pH 7.4, 10 mM $MgCl_2$, 1 mM phenylmethylsulphonylfluoride and disrupted by nitrogen cavitation at 800 p.s.i. for 15 min. The postnuclear supernatant was layered onto a discontinuous gradient containing 1 ml of 2 M, 3.5 ml of 1.3 M, 3.5 ml of 1.0 M and 2.5 ml of 0.6 M sucrose in 5 mM Hepes, pH 7.3 (Bole *et al.*, 1986). After spinning for 2 h at 38 000 r.p.m. in a SW40Ti Beckman rotor, gradients were collected in 15 or 20 fractions. Fraction 1 corresponds to the bottom of the gradient.

Enzyme-linked immunoassay

Aliquots from each fraction were diluted with one or three volumes of PBS + 0.5% NP40 and dispensed into individual wells of a Falcon 3912 plate which had been previously coated with 5 μg/ml purified RAMμ. After 60 min at room temperature, the plates were washed, incubated for 60 min with peroxidase-conjugated rabbit anti-mouse Ig (Dako P260), washed and developed with 2,2'-azido-bis(3-ethylbenzthiozolinesulphonic acid) (Sigma A 1888) at 0.55 mg/ml in 0.1 M citrate buffer, pH 4.3, containing 0.001% H_2O_2. Plates were scanned at 414 nm in an automated 'Titerscan' (Flow Laboratories).

Acknowledgements

We are grateful to Drs G.Butcher, S.Kimura, J.O.Mason, R.M.E.Parkhouse and G.T.Williams for antisera and reagents, Dr D.Gilmore for assistance with the FACS, Drs F.Calabi, A.Cattaneo, N.R.Ling and H.Pelham for helpful discussions and Miss J.Firth for her efficient secretarial assistance. Special thanks are due to Dr B.Amos and Dr J.White for their invaluable help with the confocal microscope. R.S. thanks the European Molecular Biology Organization for a long-term fellowship. This work has been in part supported through a grant from Consiglo Nazionale delle Ricerche, Progetto Finalizzato Ingegneria Genetica e Basi Molecolari delle Malattie Ereditare to R.S.

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Received on August 13, 1987; revised on September 30, 1987