Volume 14 Number 13 1986 Nucleic Acids Research

Transcriptional and posttranscriptional control of immunoglobulin mRNA production during B lymphocyte development

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Received 9 April 1986; Revised and Accepted 27 May 1986

ABSTRACT

A variety of cell lines representing different maturation stages of the B lymphocyte were used to analyse developmental changes in the transcriptional pattern through the μ-δ locus and the relationship between μ mRNA accumulation and transcriptional activity. As anticipated from earlier studies, we observed that RNA polymerase loading in the region between the μ_m cleavage/poly A addition site and the δ1 exon is markedly decreased in IgM^m secreting cells compared to cells bearing surface IgM or surface IgM and IgD. In several IgM secreting hybridomas, transcriptional termination mainly occurred downstream of the first μ_m exon. Thus, the predominance of μ_s-terminated transcripts in these cells would appear to be principally determined by RNA processing events, most likely by more efficient cleavage at the μ_s poly A site and/or less efficient splicing of the Cμ and μ_m exons. In two plasmacytoma lines, polymerase unloading between the μ_s and μ_m sites also contributed significantly to the high μ_s mRNA phenotype. Our results further indicate that posttranscriptional regulation is largely responsible for the greatly increased accumulation of μ mRNA in the IgM secretors. Interestingly, the sterile-μ RNA components do not seem to be subject to this posttranscriptional regulation.

INTRODUCTION

During the course of B lymphocyte maturation there are striking changes, both qualitative and quantitative, in the mRNA derived from an individual immunoglobulin (Ig) gene. In early developmental stages, for example in pre-B cells and fetal and neonatal B cells, mRNAs encoding the membrane (μ_m) and secreted (μ_s) forms of μ chain are concurrently produced from the functionally rearranged heavy chain (H) gene, with μ_m mRNA usually being the predominant species (1-6). Later, in the B cells of adult animals, the same H gene also produces substantial amounts of mRNA encoding the membrane form of the δ chain as well as the μ_m and μ_s mRNAs (5,7-10). Still later, in mature IgM secreting plasma cells, μ_s mRNA becomes the preponderant species and δ mRNA is essentially undetectable (1-6). Concomitant with these changes, the cellular content of both heavy and light chain mRNAs increases by 10 to 100-fold between the pre-B/B cell and plasma cell stages (1,4,6,11,12).

It is of considerable importance to know how these changes in mRNA production are determined. Previous studies in our laboratory with lymphoid tumor cell lines exhibiting phenotypes characteristic of different developmental stages (1,4,5,11) and by Yuan and Tucker with populations of splenic B cells stimulated toward terminal development with a polyclonal mitogen (10,12) indicated that regulation of Ig mRNA production occurs at both transcriptional and posttranscriptional levels and furthermore that the contribution of a particular type of regulation may vary over the course of development.

In the studies described in this report we have extended our survey of representative tumor lines to include a broader spectrum of phenotypes and have improved the resolution and quantitation of the transcriptional measurements by analyzing a greater number of Ig gene segments and by normalizing their activities to that of a set of housekeeping (ribosomal protein) genes. In agreement with our previous findings, we observe a dramatic decrease in the extent of RNA polymerase loading of the δ gene in IgM secreting cells compared to cells exhibiting surface IgM or surface IgM and IgD. Moreover, our present results also demonstrate that polymerases mainly exit downstream of the first μ_m exon in IgM secreting cells, suggesting that regulation at the level of RNA processing is required to ensure the high μ_s mRNA phenotype of these cells. We also conclusively show that increased polymerase loading of Ig genes in secreting cells generally makes only a minor contribution to the large increase in relative abundance of Ig mRNA.

MATERIALS AND METHODS

All cell lines except CH31, which was obtained from Dr. Christopher Penell (13), were described in earlier publications from this laboratory (1,4,5,11,14). The procedures for RNA-blot measurements of mRNA content and nuclear run-on assays of transcriptional activity were detailed previously (11,15). The total cell poly A^+ RNA used for the RNA-blot experiments was obtained from combined nuclear and cytoplasmic RNA extracts prepared by the citric acid fractionation procedure (11,16). The nitrocellulose-bound RNA was incubated with saturating amounts of DNA probe to ensure quantitative assays of RNA content (1). For transcriptional run-on measurements, 1 to $2x10^7$ nuclei were incubated for 10 min with α^{32}P-UTP. The yield of radioactive RNA was relatively uniform among the various cell types, ranging from about 1 to 2 dpm/nucleus. All hybridizations were carried out with single and double inputs of RNA; proportionality of hybridization signal to RNA input verified that the DNA sequences were in adequate excess for quantitative assays (5,15).

In order to have an internal standard which could be used to compare transcription from one cell type to another, we measured the aggregate activity of 5 ribosomal protein genes (S16, L7, L18, L30 and L32) using an approximately equimolar mixture of the cloned cDNAs (17). When normalized to equivalent inputs of radioactive RNA and conditions of autoradiographic exposure, the intensity of the ribosomal protein signal fluctuated by about ± 30%, but showed no significant variation among cells representing different developmental stages.

RESULTS

Characteristics of Cell Lines.

The cell lines used for this study exhibited phenotypes representative of the range from pre-B cell to IgM secreting plasma cell (Table 1). Included in this study were an A-MuLV induced tumor line (3-1) with pre-B cell phenotype; a line (70Z/3) which exhibits a phenotype intermediate between pre-B and B cells; three lines (38C-13, WEHI231 and CH31) typical of surface-IgM bearing B cells, one of which (CH31) might have potential for IgM secretion (13); one line (X16c) with characteristics of an IgM + IgD-bearing B cell; and two tumors (104.76 and PC 3741) typical of IgM secreting plasma cells. Also included were a hybridoma of 38C-13 designated D2, which has retained both the productive (H^+) and nonproductive (H^-) alleles, another 38C hybridoma, designated C2, which contains only the H^- allele, and five hybridoma derivatives of fetal liver cells with pre-B cell genotypes (FL796, FL865, FL277, FL310 and FL233). In hybridomas, the abundance of μ mRNA and the relative proportions of μ_s and μ_m species are generally characteristic of secreting plasma cells (2-4), presumably owing to the activity of trans-acting regulatory factors supplied by the myeloma fusion partners.

Some of the cells contain Cμ or sterile-μ RNA components transcribed from the H^- and/or H^+ alleles (11,18) in addition to the μ mRNAs transcribed from the H^+ allele. The sterile-μ components usually comprise about 20 to 30% of the total μ RNA in cells with pre-B and B cell phenotypes, but only a very minor proportion (<5%) in plasmacytomas and hybridomas (11). As will be demonstrated below, the relative transcription of productive and sterile-μ RNA appears to be similar in cells with early and late stage phenotypes.

Transcription Measurements.

The transcriptional activity of the H locus was determined by the transcriptional run-on procedure (19,20). Under appropriate conditions these data can be used to estimate the extent of RNA polymerase loading of a particular

TABLE 1 Characteristics of the Cell Lines Used in this Study

Cell line	Description	Representative Phenotype	μ RNA Production
3-1	A-MuLV transformed bone marrow (μ only)	pre-B cell	mRNA (μ_m, μ_s), sterile-μ RNA
C2	Hybridoma of 38C-13, lacking H^+ allele	----	sterile-μ RNA
70Z/3	B cell lymphoma (μ,κ^*)	pre-B/B cell	mRNA ($\mu_m > \mu_s$), sterile-μ RNA
38C-13	B cell lymphoma (μ,κ)	B cell (sIgM)	mRNA ($\mu_m > \mu_s$), sterile-μ RNA
WEHI231	B cell lymphoma (μ,κ)	B cell (sIgM)	mRNA (μ_m), sterile-μ RNA
X16c	B cell lymphoma (μ,δ,κ)	B cell (sIgM, sIgD)	mRNA (μ_m, μ_s), δ RNA (δ_m)
CH31	B cell lymphoma (μ,κ)	B cell (sIgM)	mRNA ($\mu_s > \mu_m$)
D2	Hybridoma of 38C-13, with H^+ and H^- alleles	plasma cell (IgM secretor)	mRNA ($\mu_s >> \mu_m$)
FL 796,865 277,310,233	Fetal liver hybridomas (intracellular μ, no κ)	----	mRNA ($\mu_s >> \mu_m$)
104.76	Plasma cell tumor - *in vitro* line (μ,κ)	plasma cell (IgM secretor)	mRNA (μ_s)
PC 3741	Plasmacytoma - subcutaneous tumor (μ,κ)	plasma cell (IgM secretor)	mRNA (μ_s)

* No κ production unless the cells are exposed to lipopolysaccharide (1).

gene segment relative to that of another segment in the same locus or to that of segments which are part of other loci (5,12,14,15,19,20). The DNA fragments used for these studies span the region from the J_H elements to a point just upstream of the first Cδ exon, and in three cases for which a cloned V_H gene was available, include the site of transcriptional initiation for the H^+ allele (Fig. 1, top). Comparisons of Ig transcription among the different cell lines were made by normalizing to the collective activity of a set of five unlinked ribosomal protein genes. The activity of these genes did not vary significantly among cells representing different developmental stages

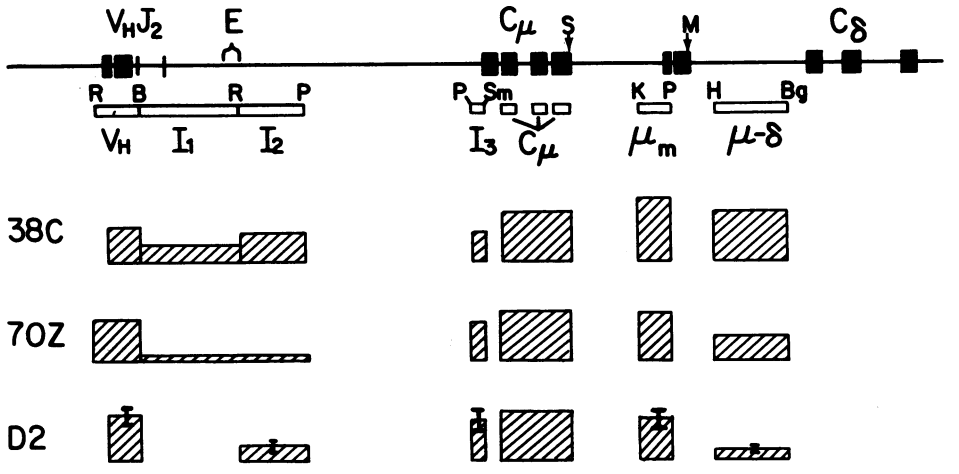

Figure 1: Relative transcription through the μ-δ locus. The upper diagram represents the H^+ locus in 38C-13 and D2 cells (VDJ_2 rearrangement); the H^+ locus in 70Z/3 cells is similar except that it is a $^2VDJ_1$ rearrangement. Exons are shown as filled boxes. E, enhancer element; S, secreted terminus; M, membrane terminus. The fragments used in the transcriptional run-on experiments and as probes for Northern blots are shown as open rectangles delimited by their restriction sites. R, Eco RI; B, Bam HI; P, Pst I; Sm, Sma I; K, Kpn I; H, Hind III; Bg, Bgl II. The shaded boxes represent the transcriptional activity, measured as described in Fig. 2 and Table 2, normalized to the Cμ value. The vertical lines on the D2 boxes illustrate the range of values for 5 separate determinations.

(see Materials and Methods), presumably because the rate of ribosome production (cell proliferation rate X ribosome content per cell) is relatively uniform.

Typical sets of transcriptional run-on data are illustrated in Fig. 2. For each set of data, the dot intensities were measured by densitometry, and the relative transcriptional activity was calculated by dividing the peak height by the number of T residues in the corresponding DNA fragment. This arithmetical adjustment simultaneously compensates for differences in length of the gene segments being monitored and for variations in the number of UMP residues incorporated per unit length of transcript. Adjusted data for three cell lines for which cloned V_H segments were available are diagrammed in Fig. 1. Except for the region monitored by I_1 and I_2, there is fairly uniform transcriptional activity across the H locus from V_H to μ_m. A slightly lower activity of the V_H region relative to the Cμ-μ_m regions is to be expected since the V_H segment monitors only productive transcription whereas the Cμ and

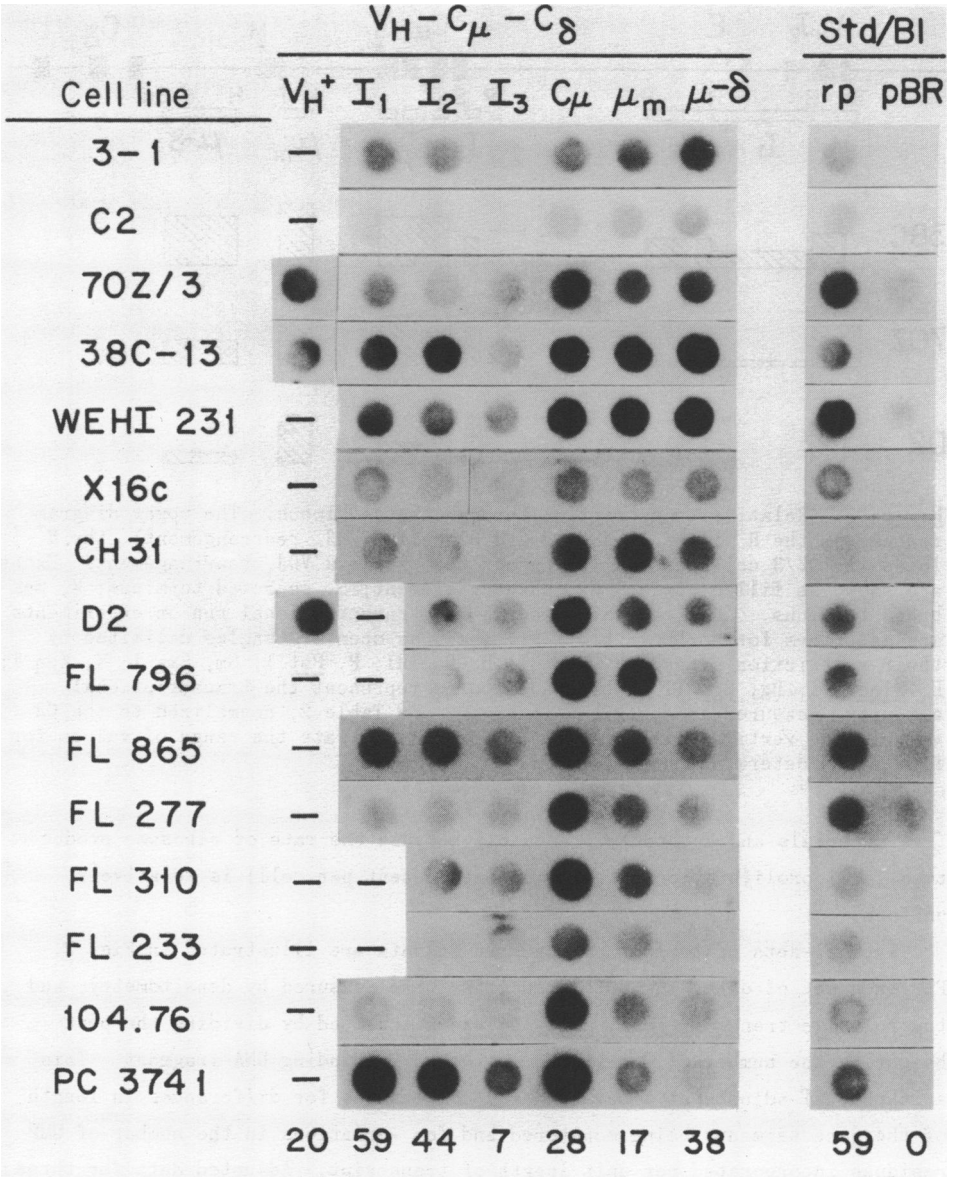

Figure 2: Transcriptional activity of the μ-δ locus in a variety of lymphoid cell lines. Nuclei from each of the cell lines described in Table 1 were incubated for 10 min with ^{32}P UTP. RNA was extracted and incubated for 72 hr with nitrocellulose strips bearing 4 μg dots of linearized DNA containing various segments of the μ-δ locus (see Fig. 1), a mixture of ribosomal protein cDNAs (rp) and a plasmid vector blank (pBR). The numbers at the bottom represent the number of thymidine residues (X 0.1) in the DNA segment; the cap site

was considered as the 5' terminus of the V_H segments. Horizontal comparisons of dot intensities may be made directly since each set is from the same autoradiograph of a single experiment. Vertical comparisons may be made only if normalized to the rp standards.

μ_m segments monitor both productive and sterile transcription. The apparently diminished activity in the I_1-I_2 region may be due to a technical artifact, or alternatively, it may reflect an anomalous transcriptional property of this region (see Discussion); in either case it does not affect the major conclusions of this paper.

Transcription through the Cμ-Cδ Region.

The relative proportions of μ_s and μ_m mRNA vary considerably among the various cell lines under study. As listed in Table 1 and illustrated by the RNA-blot analyses of Figure 3, the range extends from μ_s/μ_m ratios of 0.5 or less in B-lymphomas like 70Z/3, 38C-13, WEHI231 and X16c to ratios of 10 and greater in hybridomas and plasmacytomas like D2, the FL series and 104.76 (see also ref. 4). In the cell lines producing a substantial proportion of μ_m mRNA, the ratio of polymerase loading in the regions preceding the μ_s and μ_m poly A sites ranges from 0.9 to 2.1 (avg. 1.5), while in lines producing mainly μ_s mRNA, this ratio ranges from 0.2 to 0.9 (avg. 0.7) (Table 2). Although there is a tendency for decreased loading of the μ_m region in IgM secreting cells, this decrease does not appear to be sufficient to account for the high μ_s/μ_m mRNA ratios in these cells. For example, the polymerase loading of the μ_m region in the hybridomas is nearly the same as that in 70Z/3 and X16c cells, and yet the μ_s/μ_m mRNA ratio in hybridomas is at least 20 fold greater. It would appear, therefore, that the shift to high μ_s mRNA production in IgM secreting cells is mainly caused by a change in the mode of RNA processing rather than by an early exit of RNA polymerase from the DNA template. However, our results also indicate that in some cases, e.g. plasmacytomas PC3741 and 104.76, early exiting of polymerase can contribute significantly to the $\mu_s >> \mu_m$ mRNA phenotype.

In agreement with the results of our previous studies (5), the data of Table 2 also show that the extent of polymerase loading of the μ-δ region is markedly decreased in IgM secreting cells compared to cells with pre-B and B cell phenotypes. Indeed, polymerase loading is negligible throughout the δ locus in IgM secretors (5), indicating that very few actively transcribing polymerases reach the first Cδ exon in these cells. In the earlier studies we could not specify whether the polymerases exit before or after the μ_m poly A site because the μ_s-μ_m region was not monitored. The data presented here

TABLE 2 Relative Transcription through the Cμ-Cδ Region

Segment	Cells Producing a Substantial Proportion of μm mRNA						
	3-1	70Z/3	38C-13	WEHI231	X16c	CH31	Avg.
μ_m	1.8	0.9	1.2	1.9	0.9	2.1	1.5
μ-δ	1.5	0.6	1.2	0.8	0.6	0.6	0.9
	(2)	(1)	(3)	(2)	(3)	(1)	

Segment	Cells Producing Mainly μs mRNA								
	D2	FL796	FL233	FL310	FL277	FL865	104.76	3741	Avg.
μ_m	0.8	0.8	0.8	0.7	0.9	0.7	0.4	0.2	0.7
μ-δ	0.2	0.05	0.06	0.06	0.2	0.2	0.1	0.02	0.1
	(5)	(1)	(1)	(1)	(1)	(1)	(1)	(2)	

Intensities of Cμ, μ_m and μ-δ dots from experiments such as those illustrated in Fig. 2 were measured by densitometric scanning of autoradiograms. Exposures were chosen so that signals were in the linear portion of the dose-response curve. The peak heights were divided by the number of T residues in the corresponding DNA segment to give values which represent the transcriptional activity per unit length of segment (see text). The values for the μ_m and μ-δ segments were divided by that for the Cμ segment to give a measure of the transcriptional activity of these regions relative to that of the Cμ region. The number of determinations with different samples of nuclei are given in parentheses; when more than one determination was made, average values are listed.

indicate that polymerases frequently exit between the Pst I site (278 nucleotides upstream of the μ_m poly A site) and the 5' portion of the μ-δ segment (544 nucleotides downstream of the μ_m poly A site). Whether exiting occurs in a restricted portion of this region or is more uniformly distributed is not yet known. Since there is no precedent for abrupt transcriptional termination in higher eukaryotes, we presume that polymerase unloading is gradual, and therefore, that at least half of the polymerases exit downstream of the μ_m poly A site. Verification of this point, however, must await high resolution studies of this 800 nucleotide region. In cells typical of early developmental stages, there is also some termination in the μ-δ and δ regions, but many polymerases proceed through the entire δ locus and exit downstream of the δ_m poly A site (5). In these early-stage cells, the relative proportion

of μ and δ mRNA is governed mainly by the efficiency of the combined usage of the μ_s and μ_m cleavage/poly A sites.

Relative Levels of mRNA Accumulation and Transcription.

The steady state level of heavy chain mRNA varies by about two orders of magnitude among the various cells studied. The content of μ mRNA in the pre-B and B lymphomas is generally about 0.05 to 0.1 that in the hybridomas which in turn is about 0.1 to 0.2 that in the plasmacytomas (Fig. 3, Table 3). This is consistent with previous measurements of mRNA abundance in these and similar cell lines (1,4). The transcriptional activity, measured as polymerase loading of the Cμ segment normalized to the loading of ribosomal protein genes, is much more uniform from one cell line to the next, the maximum difference being about 4-fold (Fig. 2, Table 3). Thus, if a comparative scale is chosen so that the ratio of content to transcription of μ RNA is near unity for most pre-B and B lymphomas, this ratio is 3 to 12 for the hybridomas and 26 to 30 for the plasmacytomas (Table 3). This ratio serves as a crude index of the relative contributions of posttranscriptional and transcriptional events to the developmental increase in mRNA content, and therefore we would conclude that posttranscriptional events are of primary importance for this aspect of immunoglobulin mRNA regulation.

Transcription and Accumulation of Sterile-μ RNA.

The sterile-μ transcripts generally constitute about 20 to 30% of the total μ RNA in early stage cells, but only a negligible fraction in the mature IgM secretors (Fig. 3, Table 4). The relative proportion of total μ RNA transcription devoted to μ mRNA and sterile-μ components can be roughly estimated from the relative polymerase loading of the V_H and Cμ segments. Such estimates indicate that sterile-μ components account for about 10 to 30% of μ RNA transcription in 38C-13, 70Z/3 and D2 cells (Fig. 1, Table 4). An independent estimate of the contribution of sterile-μ components to transcription and accumulation measurements can be obtained by comparing hybridoma D2, which contains both the H^+ and H^- alleles of 38C-13, with hybridoma C2, which contains only the H^- allele, and therefore can produce only sterile-μ components. Considering the limits of error of such measurements, the two estimates of sterile-μ contribution are in reasonably good agreement. These data indicate that there is no significant difference in either transcription or accumulation of sterile-μ components between cells representative of early and late developmental stages (Table 4). Thus, the posttranscriptional mechanism responsible for the increase in μ RNA content in late stage cells can apparently discriminate between productive and sterile-μ transcripts.

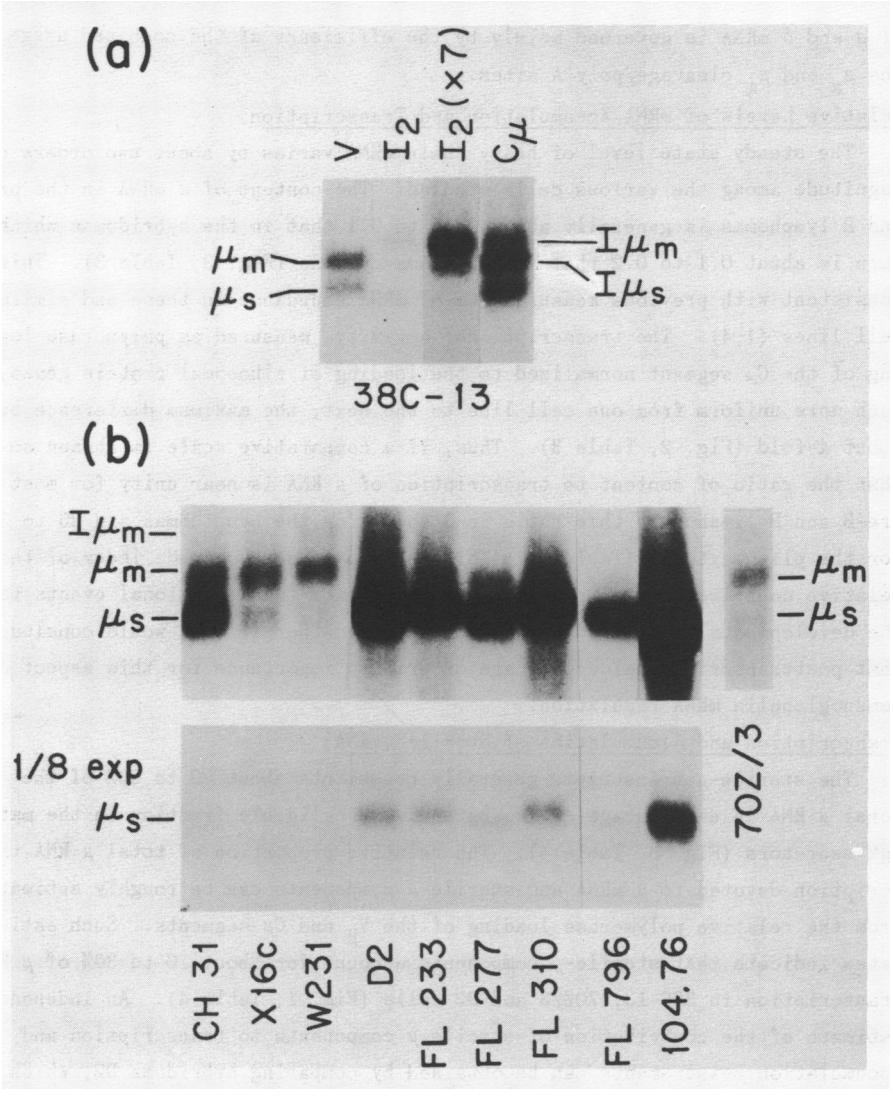

Figure 3: Content of μ RNA in various lymphoid cell lines. (a): Northern blot analysis of total cell poly A^+ RNA (5 μg) from 38C-13 cells, hybridized with V, I_2 and Cμ probes (probe specific activity ca. 300 dpm/pg, 45 min exposure of autoradiograph with an intensifier screen). The productive μ mRNAs, revealed by the V and Cμ probes, are designated μ_m and μ_s; the sterile-μ components, revealed by the I_2 and Cμ probes, are designated $I\mu_m$ and $I\mu_s$ (a 7-fold longer autoradiographic exposure is included to show these components more clearly). (b): Comparison of the content of μ RNA in various cell lines. Northern blots as in panel (a) of 0.5 μg (D2, FL233, FL277, FL310, FL796, 104.76) or 1 μg (CH31, X16c, WEHI231, 70Z/3) total cell poly A^+ RNA were incubated with the Cμ probe and autoradiographed [exposures, 4h (upper), 30 min (lower)]. The RNAs were derived from the same cell harvests used for nuclear run-on experiments of Fig. 2.

TABLE 3 Comparison Between Relative Transcriptional Activity and Content of μ RNA

Phenotype	Cell Line	μ RNA (relative)		
		Content[a]	Transcription[b]	Content/Trans.
Pre-B	3-1	0.66	1.2	0.6
Immature B	70Z/3	0.87	0.8	1.1
B	38C-13	2.15	1.6 ± 0.3	1.3
	WEHI231	0.94	1.1 ± 0.2	0.9
	X16c	1.06	0.9 ± 0.1	1.2
Late B	CH31	4.0	0.6	6.7
Hybridoma	D2	21.5	2.4 ± 0.4	9.0
	FL796	9.4	3.2	2.9
	FL233	15.8	1.3	12.2
	FL310	15.3	3.5	4.4
	FL277	11.2	0.9	12.4
	FL865		1.1	
Plasmacytoma	104.76	101	3.8	26
	3741	114	3.8	30

[a] Data from blots of poly A^+ RNA such as those shown in Fig. 3 were scanned densitometrically and the total amount of μ RNA/sample estimated from the total signal intensity in the region encompassing the μ_m, μ_s and sterile-μ components. The intensities were normalized to equivalent amounts of poly A^+ RNA/sample, probe specific activities and autoradiographic exposures, and multiplied by the amounts of poly A^+ RNA/cell (0.14 pg on average for pre-B/B cells and 0.22 pg for hybridomas and plasmacytomas) to give a measure of the μ RNA content/cell. For ease of comparison, WEHI231 and X16c cells were selected as representative standards, and all values expressed as multiples of the average μ RNA content of these two cell lines.

[b] Intensities of Cμ and rp dots were measured by densitometry and the ratio Cμ/rp tabulated. These values were then divided by the average value for WEHI231 and X16c standards. Average values and mean deviations are listed in cases where multiple determinations were made.

DISCUSSION

The foregoing experiments bear on two important features of the developmental regulation of immunoglobulin gene expression: the shift in the relative production of μ_s and μ_m mRNA that occurs when surface-Ig bearing lymphocytes mature into IgM secreting cells, and the large increase in the amount of μ mRNA per cell that accompanies this transition. In particular, our results

TABLE 4 Transcription and Content of Sterile-μ RNA

Cell line	Content[a]			Transcription[b]		
	Total	Productive	Sterile	Total	Productive	Sterile
38C-13 (lymphoma)	2.2	1.7	0.5	1.6 ± 0.3	1.2	0.4
D2 (hybridoma)	22	21	< 1	2.4 ± 0.4	2.2	0.2
C2 (hybridoma)	1.6	0	1.6	0.65 ± 0.1	0	0.6

[a] RNA content measurements as in Table 3. Apportionment into productive and sterile fractions was made by estimating the relative contributions of μ_m and μ_s mRNA and the $I\mu_s$ and $I\mu_m$ sterile components to blots hybridized with the $C\mu$ probe. Unambiguous identification of these components was made with the V_H and I_2 probes (Fig. 3a and ref. 11).

[b] Transcription measurements as in Table 3. For 38C-13 and D2 cells, the apportionment into productive and sterile fractions was based on data with V_H and $C\mu$ segments (Fig. 1).

have enabled us to evaluate the relative importance of transcriptional and posttranscriptional events in determining these developmental changes in mRNA production.

In previous studies (5), we found that the extent of RNA polymerase traversal through the μ-δ locus is foreshortened in secreting hybridomas and plasmacytomas compared to that in surface-Ig bearing lymphomas. In cells expressing surface Ig, transcription frequently extends over the entire μ-δ locus, whereas in secreting cells, almost all transcription is terminated before reaching the first δ exon. This finding was confirmed and extended by the results of the present experiments, which demonstrate that transcriptional termination in the secreting cells frequently occurs in a 800 nucleotide region downstream of the first μ_m exon. If, as discussed above, a major proportion of the polymerases exit downstream of the μ_m poly A site, these results would imply that the high μ_s/low μ_m mRNA phenotype of these cells must be primarily determined at the level of RNA processing, e.g. by preferential usage of the μ_s cleavage/poly A-addition site and/or by decreased splicing between the $C\mu$ and μ_m exons. Thus, regulation by RNA processing, which is clearly responsible for the differential production of μ and δ mRNA at earlier developmental stages (5,12), also appears to be important for μ_s vs. μ_m mRNA discrimination in mature IgM secreting cells. A similar conclusion was reached by Yuan and Tucker from studies of LPS-stimulated lymphocytes (12) and more recently by Milcarek and Hall from studies of γ gene transcription in IgG

expressing cells (21) and by Danner and Leder from a series of transfection experiments (22).

Although all of the IgM secreting hybridomas in our study exhibited substantial transcription through the μ_m region, the relative polymerase loading of this region was on average about half that in the surface-Ig bearing cells, suggesting that early transcriptional termination may generally make some, albeit minor, contribution to the high μ_s mRNA phenotype. In the two plasmacytomas studied (PC3741 and 104.76), however, this contribution is clearly more substantial. Whether this reflects a general phenotypic distinction between plasmacytomas and hybridomas or simply differences in the characteristics of these particular cell lines remains to be established.

In poly A^+ nuclear RNA from surface-Ig bearing cells, one can readily observe full-length primary transcripts terminated at the μ_m and μ_s poly A sites, whereas in poly A^+ nuclear RNA from IgM secretors, only the μ_s-terminated primary transcript is detected (11). This observation, taken together with the present results, indicates that cleavage at the μ_s poly A site is so efficient in secreting cells that it precludes any significant accumulation of μ_m terminated transcripts. Whether this cleavage normally occurs on a growing transcript prior to its reaching the μ_m poly A site or on a growing or completed transcript that has passed the μ_m site is not clear. In any case, there is negligible accumulation in IgM secretors of poly A^+ RNA fragments which extend from the μ_s cleavage site to the μ_m site (5,11,21,23). Such fragments, if actually made, would presumably be relatively unstable because they would lack a 5' terminal cap (24).

A somewhat puzzling feature of our transcriptional run-on measurements is the apparently low polymerase loading in the I_1-I_2 region of the Jμ-Cμ intron, which encompasses the enhancer element and the 5' portions of sterile-μ transcripts (25). A similar anomalously low loading of this region was noted by Yuan and Tucker (12) and presumed to be due to polymerase transit (without new initiation) during preparation of nuclei. This explanation cannot apply to our results because the I_1-I_2 values were low relative to V_H, which is further upstream. A possible technical artifact which has not yet been ruled out is that the hybridization efficiency of I_1-I_2 sequences is lower than that of the V_H or Cμ sequences because of differences in the relative proportions of AT vs. GC base pairs in these regions. [The % (A+T) in V_H, I_1, I_2, I_3, Cμ and μ_m is 52, 57, 60, 47, 47 and 47, respectively.] We intend to examine this possibility with in vitro synthesized RNA. If technical artifacts can be excluded, one might consider the possibility that there is a nonuniform polymerase dis-

tribution due to unequal rates of transit across the gene. Such nonuniform loading of polymerases is known to occur in prokaryotic and viral systems (26,27), and might become evident in run-on assays if the unequal transit rates were not maintained when the overall elongation rate is drastically reduced in isolated nuclei. Such "pausing" effects could also explain the 1.5 to 2 fold greater loading of μ_m relative to Cμ that we observe in nonsecreting cells.

The other major conclusion drawn from these studies is that the markedly elevated content of μ mRNA which is characteristic of IgM secreting plasmacytomas and hybridomas is largely determined by events at the posttranscriptional level. Transcriptional run-on measurements, standardized to the collective activity of a set of ribosomal protein genes, indicated that the rate of μ RNA transcription increases by only 2 to 5 fold under conditions in which the μ mRNA content per cell increases by 10 to 100 fold. This finding is in general agreement with our earlier measurements which were standardized to equivalent inputs of labeled RNA rather than to a set of housekeeping genes (5). A similar result was obtained by other workers with different sets of lymphoid cell lines (12,28).

It is interesting to compare the findings from studies of lymphoid tumor lines with those from studies of mitogen-stimulated splenic B lymphocytes (6,10,12). In B lymphocytes, the cellular content of μ mRNA increases 200 to 800 fold after LPS stimulation, as judged by quantitative Northern blot analyses. Since the amount of poly A^+ mRNA per cell increases 8 to 10 fold under these conditions, the specific increase in μ mRNA is about 25 to 80 fold, a range similar to that observed when comparing lymphomas with hybridomas and plasmacytomas. Transcriptional run-on measurements have indicated that μ gene transcription in stimulated lymphocytes is preferentially increased by about 8-10 fold over that in unstimulated lymphocytes when standardized to equivalent inputs of labeled RNA (12). These results also support the view that posttranscriptional events are involved in determining the final μ mRNA level, although they would seem to indicate that an increase in transcription rate may make a larger contribution than is apparent from studies of tumor lines. It may be enlightening to examine the activities of a common group of non-immunoglobulin genes in these two systems, including those encoding various housekeeping functions, in order to determine whether this difference is reflective of the transcriptional status of the heavy chain locus or of the other genes to which it is compared. In any case, our results demonstrate that transcriptional and posttranscriptional mechanisms can be coordinated to

define a property characteristic of the mature B lymphocyte, namely the high abundance of immunoglobulin mRNA.

We have not yet established the basis of the posttranscriptional regulation. It presumably involves differences in the efficiency of mRNA processing or in the turnover of cytoplasmic RNA or in a combination of these processes (29). In plasmacytomas and hybridomas, the levels of μ mRNA in both nucleus and cytoplasm are elevated compared to the respective levels in pre-B and B cell tumors (1,11). The increase in nuclear μ RNA (about 6 to 10 fold) is not generally as large as the increase in cytoplasmic μ mRNA, and yet is greater than can be accounted for by the observed increase in transcription rate. This would seem to suggest that the turnover rates of both nuclear and cytoplasmic μ RNA may be lower in Ig secreting cells. Our finding that this posttranscriptional regulation is directed at productive μ mRNA, but not at sterile-μ components, is noteworthy because the productive and sterile-μ components, being derived from identical sets of 3' exons and alternative 5' exons, have structurally distinctive 5' ends and different translational capacities (25). If there are developmentally regulated differences in μ mRNA stability, the specificity for such differences may reside in the 5' terminal portion of the μ mRNA molecule.

A final comment concerns the generality of the multilevel regulation exhibited by the B lymphocyte. Studies of a large variety of developmentally regulated eukaryotic genes, including those encoding the immunoglobulins, indicate that transcriptional control is largely responsible for tissue-specific differences in gene expression. This type of control is usually correlated with the extent of chromatin condensation and methylation status of the gene, and is believed to be mediated by an interplay between cis-acting regulatory elements, e.g. enhancers, activators and promoters, and trans-acting factors which bind to these elements. In contrast, developmental or physiologically induced changes in the expression of housekeeping genes are often associated with posttranscriptional regulation (30-33). Conceivably, these genes lack the regulatory elements that can respond to tissue-specific transcriptional factors. Within a particular tissue or cell lineage, the expression of tissue-specific genes is subject to both transcriptional and posttranscriptional regulation to varying degrees. Globin genes (20) and several liver-specific genes (32,34) are primarily regulated at the transcriptional level, whereas the expression of many other tissue-specific genes, including several that are responsive to hormone regulation (19,35-37) and those which encode muscle (38,39) and other liver-specific proteins (32,40)

also utilize posttranscriptional events for both qualitative and quantitative regulation. The immunoglobulin genes clearly belong to this latter category.

ACKNOWLEDGEMENTS

This research was supported by a grant from the National Institutes of Health and an appropriation from the Commonwealth of Pennsylvania.

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