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Regulated Immunoglobulin (Ig) RNA Processing Does Not Require Specific *cis*-Acting Sequences: Non-Ig RNA Can Be Alternatively Processed in B Cells and Plasma Cells

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Alternative RNA processing of the heavy-chain immunoglobulin μ gene is regulated during B-cell maturation and requires competition between splice and cleavage-polyadenylation reactions that have balanced efficiencies. Studies with modified μ genes have failed to identify gene-specific sequences required for regulation. Thus, the only important feature for regulation may be the balanced competing splice and cleavage-polyadenylation reactions themselves. If this is so, then alternative RNA processing from any gene with similar competitive RNA processing pathways should also be regulated when expression is compared between B cells and plasma cells. To test this prediction, two nonimmunoglobulin genes engineered to have competing splice and cleavage-polyadenylation reactions were expressed in B cells and plasma cells. The ratios of alternative RNAs produced from both genes are different in the two cell types; like the μ gene, relatively more spliced RNA is produced in B cells than in plasma cells. Also, in a survey of μ gene expression in nine non-B-cell lines, only a T-cell line had an expression pattern similar to that of B cells; the expression patterns of all other lines resembled that of the plasma cells. Therefore, regulated μ RNA processing must be mediated by changes in general processing factors whose activity or abundance is regulated, most likely, in B cells.

The mRNAs encoding the membrane-associated (μ_m) and secreted (μ_s) forms of heavy-chain immunoglobulin M (IgM) are alternatively processed from a common primary transcript containing signals for competing splice and cleavage-polyadenylation reactions (for a review, see reference 32). If the transcript is cleaved and polyadenylated at the μ_s poly(A) site, μ_s mRNA is produced. If instead it is spliced between the Cμ4 and M1 splice junctions, μ_m mRNA is produced (see Fig. 1A). The relative usage of these two mutually exclusive RNA processing pathways, and thus the ratio of μ_s to μ_m mRNA, is regulated during B-cell maturation; splicing to form μ_m mRNA is predominant in pre-B and B cells, whereas cleavage-polyadenylation to generate μ_s mRNA is heavily favored in mature plasma cells. The balance between the efficiencies of splicing and cleavage-polyadenylation is critical to μ gene regulation. By making specific modifications to the μ gene, the efficiencies of these competing reactions can be individually or coordinately altered. These modifications change the relative amounts of cleaved and polyadenylated μ_s mRNA and spliced μ_m mRNA produced. However, as long as two RNAs can be alternatively processed from a single modified μ gene, a shift in the μ_s/μ_m mRNA ratio is seen when the same gene is expressed in B cells and plasma cells. That is, relatively more spliced RNA is made in B cells than in plasma cells.

Numerous modifications of the μ gene have been made to investigate the mechanisms responsible for regulated alternative RNA processing. These experiments have failed to identify gene-specific *cis*-acting sequences required for regulation. Instead, they have identified a number of parameters that affect the μ_s/μ_m mRNA expression ratio without affecting the regulatory shift in expression between B cells and plasma cells. The size of the Cμ4-M1 intron influences the μ_s/μ_m ratio: the shorter the intron is, the more spliced RNA and the less cleaved-polyadenylated RNA is made (12, 33, 35, 36, 41, 49). The strength of the competing poly(A) site (μ_s) can be altered by replacing it with other Ig and non-Ig poly(A) sites (31, 33, 36). Point mutations to improve the evolutionarily conserved suboptimal Cμ4 5′ splice junction greatly enhance splicing (31, 36). Sequences within exon M2 act to enhance Cμ4-M1 splicing when M1 and M2 are prejoined (52), but the M1-M2-μ_m poly(A) region can be replaced with simian virus 40 (SV40) splice and poly(A) sequences without disrupting regulation (31). In addition, the size of the Cμ4 exon affects the μ_s/μ_m ratio; μ RNA containing a smaller exon produces relatively more spliced mRNA, while that containing a larger exon produces less spliced and more cleaved and polyadenylated mRNA (33). This may suggest that the size of an exon can affect whether it is recognized as an internal exon to be spliced or as a terminal exon to be cleaved and polyadenylated (38). Thus, a number of parameters, including intron and exon size and splice junction, poly(A) site, and exon sequences, all contribute to the relative strengths of the competing splice and cleavage-polyadenylation reactions but do not affect the cell-specific changes in the polyadenylated mRNA/spliced mRNA (pA/splice mRNA) ratio.

Other Ig isotypes, IgG, IgE, and IgA, also undergo a switch from membrane-associated to secreted Ig production as B cells mature to plasma cells. The structures of the gene segments encoding these other Ig isotypes are very similar to that of μ (for a review, see reference 32). Studies with the γ gene suggest that expression of the alternative mRNAs is regulated in a way similar to that for the μ gene (5, 22). In addition, we have recently shown that the α gene also contains balanced competing splice and cleavage-polyadenylation reactions whose efficiencies can be improved or reduced by the same parameters that affect μ RNA processing (41). This strongly suggests

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that all Ig genes are subject to the same regulatory mechanisms.

Most experiments with the μ gene, as well as those performed with the γ and α genes, indicate that the relative use of alternative RNA processing pathways depends on the balance between the efficiencies of competing splice and cleavage-polyadenylation reactions. It is possible that balanced competing processing reactions are sufficient for the μ gene to respond to cellular changes that occur during B-lymphocyte maturation and that Ig gene-specific sequences are not required. This model predicts that any gene with a similar combination of processing signals whose efficiencies are balanced should respond, as do the Ig genes, to changes in the cellular environment of B cells and plasma cells. We expressed both a rabbit β-globin-derived gene and a mouse major histocompatibility complex (MHC) class I D^d gene, each having a poly(A) site within an intron, in B cells and plasma cells. The ratio of pA to splice RNA shifts exactly as does the Ig mRNA, as determined by comparison of expression in these two cell types. This proves that the mechanism regulating μ_s and μ_m mRNA processing does not require Ig gene-specific sequences. Additionally, a survey of μ-gene expression in nine non-B-cell lines suggests that B cells and not plasma cells regulate μ RNA processing.

MATERIALS AND METHODS

Plasmid constructions. The rabbit β-globin-derived genes p4FpApy, p5FpApy, and pApy were constructed from p4FpA, p5FpA, and p8FpA, respectively (a kind gift from D. Chang and P. Sharp), by first replacing the *Pst*I-*Nhe*I vector fragment with a double-stranded oligonucleotide containing compatible ends and an internal *Bgl*II site. A 3.8-kb *Bam*HI fragment from polyomavirus was inserted into this *Bgl*II site; the polyomavirus fragment allows these plasmids to replicate in mouse cells (45) and is essential for a high level of expression in the mouse lymphoid cell lines. The pA+py plasmid was made by linearizing p8FpA at the *Apa*I site, making the ends blunt with the Klenow fragment of DNA polymerase I, and inserting a 900-bp blunt-ended fragment from an intron of the mouse S16 ribosomal protein gene (51). The polyomavirus fragment was cloned into this plasmid as described above.

To construct $D^d\alpha s$, we modified SV40-Igκ-D^d (46), which contains the mouse MHC class I D^d gene (42) controlled by the SV40 enhancer and the Ig κ light-chain gene promoter. First, 18 bp was inserted in two steps within the third exon to distinguish it from the endogenous gene. SV40-Igκ-D^d was linearized with *Bsm*I and ligated to a 14-bp double-stranded oligonucleotide containing compatible ends and an internal *Hin*dIII site. Then this *Hin*dIII site was filled in with the Klenow fragment of DNA polymerase I to increase the insert size to 18 bp and restore the translational reading frame. The αs poly(A) site from the mRNA encoding secreted IgA was then inserted as a 184-bp *Dpn*I-*Hin*dIII fragment (50) into the *Nco*I site within intron 3 after all the ends had been made blunt with the Klenow fragment of DNA polymerase I. The 3.8-kb *Bam*HI polyomavirus fragment was inserted at the *Cla*I site within the vector using a *Cla*I-*Bgl*II-*Cla*I oligonucleotide adapter.

Cell culture and transfections. The mouse plasmacytoma line S194 was maintained in Dulbecco's modified Eagle's medium supplemented with 10% horse serum; the mouse B-cell line M12, as well as WEHI-3 and EL-4, was maintained in RPMI medium supplemented with 10% fetal bovine serum and 50 μM 2-mercaptoethanol; 293 and P19 cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum; and L, HeLa, NIH 3T3, F9, and HepG2 cell lines were maintained as described previously (46). S194, M12, WEHI-3, and EL-4 cell lines were transfected following the DEAE-dextran protocol (14); all the other cell lines were transfected following a calcium phosphate method (46).

RNA preparation and analysis. Cytoplasmic RNA was prepared from M12, S194, EL-4, and WEHI-3 cells (40); total RNA was prepared from all other cell lines using a hot-phenol protocol (43). S1 nuclease analysis was performed with the end-labeled DNA fragments diagrammed in the figures and by following the protocol described previously (36). The *Bsu*36I-*Nhe*I β-globin probe, the *Ban*I-*Acc*I $D^d\alpha s$ probe, and the *Pst*I-*Hin*dIII μ probe that each distinguish spliced from cleaved-polyadenylated RNA were 3′ end labeled with the Klenow fragment of DNA polymerase I and [α-^{32}P]dCTP and were hybridized to RNA at 45°C (β-globin probe) or 50°C ($D^d\alpha s$ and μ probes). For all but the $D^d\alpha s$ probe, S1 digestion was done with 80 U of S1 nuclease at 37°C for 30 min; for the $D^d\alpha s$ probe, 60 U of S1 nuclease was used at 45°C for 45 min. Protected fragments were separated on 6% polyacrylamide–7 M urea gels, the gels were dried, and the fragments were quantitated with an Ambis radioanalytic imaging system.

RESULTS

Expression of β-globin-derived genes. A series of rabbit β-globin-derived genes was obtained from D. Chang and P. Sharp. These genes originate from pβG-RRE, pβ5F, and pβ8F (7) and contain the rabbit β-globin poly(A) site within the intron downstream of the human immunodeficiency virus Rev response element (Fig. 1B). The presence of the Rev response element in these genes is not relevant to the experiments presented here. The RNA processing options within a pre-mRNA from these modified β-globin genes are the same as those at the 3′ end of the μ gene (Fig. 1). p4FpApy contains wild-type splice junction sequences, while p5FpApy and pApy contain point mutations in the conserved dinucleotides at the 5′ and 3′ splice junctions, respectively (Fig. 1B). When p4FpApy was expressed in B cells and plasma cells, only spliced RNA was produced (Fig. 2A, lanes 1 and 2). That the poly(A) site was not used is consistent with the results of previous experiments with a poly(A) site placed within an intron (23, 28). p5FpApy contains a T-to-C point mutation within the 100% conserved GT dinucleotide of the 5′ splice junction which should abolish splicing (Fig. 1B). When expressed in B cells and plasma cells, p5FpApy produced only RNA that was cleaved and polyadenylated at the inserted poly(A) site (Fig. 2A, lanes 3 and 4). In addition, the amount of RNA accumulated from p5FpApy was much lower than that accumulated from p4FpApy (see below). The plasmid pApy contains an A-to-T point mutation within the 100% conserved AG dinucleotide at the 3′ splice junction (Fig. 1B). This mutation did not abolish splicing, as measured by use of the 5′ splice junction, but did weaken the reaction so that both spliced RNA and RNA that was cleaved and polyadenylated at the intronic β-globin poly(A) site were detected when pApy was expressed in B cells and plasma cells (Fig. 2A, lanes 5 and 6). The pA/splice RNA expression ratio in B cells is different from this ratio in plasma cells (Table 1): B cells have relatively more spliced RNA than plasma cells. Therefore, expression of this chimeric β-globin-derived gene is regulated like that of the Ig genes.

The amount of RNA from pApy that is cleaved and polyadenylated at the intronic poly(A) site was low (Fig. 2A, lanes 5 and 6). One possible explanation is that the poly(A) site was

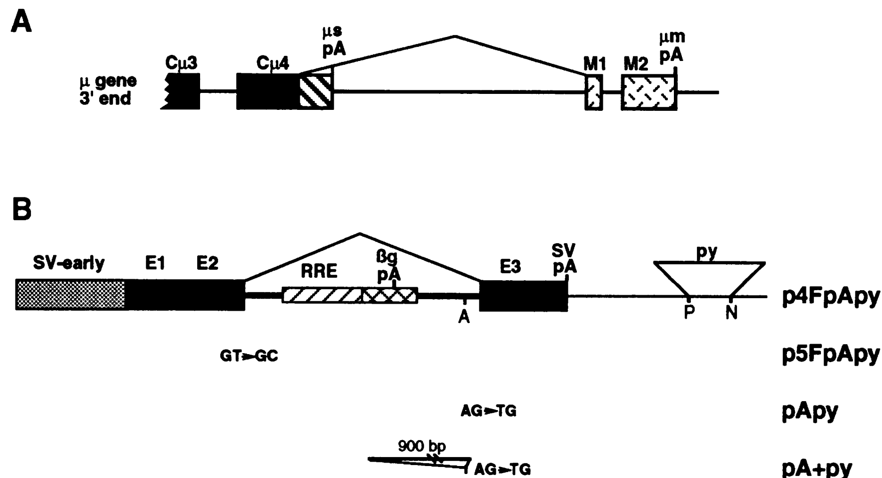

FIG. 1. (A) Diagram of μ gene 3'-end structure. Solid boxes, exons common to μ_s and μ_m mRNAs; hatched box, μ_s-specific exon; crosshatched boxes, μ_m-specific exons; pA, poly(A) sites as labeled; angled line above the map, Cμ4-M1 splice. Regulation of the alternative processing reactions requires balanced efficiencies of the competing μ_s cleavage-polyadenylation and Cμ4-M1 splice reactions. (B) Diagram of β-globin-derived genes. Stippled box, SV40 promoter and enhancer; solid boxes, rabbit β-globin exons 1 and 2 prejoined and exon 3 as labeled; thick lines, rabbit β-globin second-intron sequences; hatched box, human immunodeficiency virus Rev response element (RRE); crosshatched box, rabbit β-globin poly(A) site (βg pA); SVpA, SV40 late poly(A) site at the end of exon 3; thin lines, vector sequences; py, the 3.8-kb fragment of polyomavirus which replaces the *Pst*I-*Nhe*I (P and N, respectively) fragment of the vector sequence; angled line above the map, E2-E3 splice. p4FpApy has wild-type sequences at the splice junctions; p5FpApy has a point mutation at the 5' splice junction; pApy has a point mutation at the 3' splice junction; pA+py is pApy with a 900-bp insertion at the *Apa*I (A) site within the intron. These plasmids are derived from pβG-RRE, pβ5F, and pβ8F (7).

not used efficiently in the intronic location. In an attempt to decrease the splice efficiency further and possibly increase the use of the intronic poly(A) site, the intron was expanded by 900 nucleotides with a fragment from a mouse ribosomal protein gene intron, to create the plasmid pA+py (Fig. 1B). Lengthening the intron had the predicted effect: the pA/splice expression ratio increased about 10-fold compared with that for pApy (Fig. 2A, compare lanes 7 and 8 with lanes 5 and 6; Table 1). The regulation index, which is the change in pA/splice RNA expression ratio when the same gene is expressed in plasma cells and B cells, is 8 and 12 for pApy and pA+py, respectively (Table 1). The regulation index is about 7 for the wild-type μ gene (36) but ranges from 3 to 22 for modified μ and μα genes (31, 33, 36, 41). Thus, the regulation indices for these β-globin-derived genes compare favorably with the regulated changes established for Ig gene expression.

RNA from p5FpApy accumulated to a much lower level than did RNA from p4FpApy; in the S1 nuclease protection analyses whose results are shown in Fig. 2A (compare lanes 1 and 2 with lanes 3 and 4), similar amounts of RNA were assayed, but there was at least 20-fold less p5FpApy RNA. As an intron is required for optimal RNA accumulation from the β-globin gene (6) as well as from other genes (e.g., see references 8 and 15), it is likely that the RNA that is cleaved and polyadenylated at the inserted poly(A) site does not accumulate well because it lacks an upstream spliceable intron. It is probable that this lack of an intron at least contributes to the differences in RNA accumulation between p4FpApy and p5FpApy, as well as to the low pA/splice RNA ratio in the pApy and pA+py genes. Nevertheless, since B cells and plasma cells do not regulate the stability or the nuclear-cytoplasmic transport of μ_s versus μ_m mRNA (12, 35, 37, 56), there is no reason to believe that they would modulate the relative amounts of the polyadenylated-unspliced and spliced β-globin RNA by either of these mechanisms. Thus, the shift in the pA/splice expression ratio more likely reflects the regulated choice of alternative RNA processing pathways.

Further characterization of β-globin RNA. The point mutations at the 3' splice junctions of the pApy and pA+py constructs did not eliminate splicing but instead reduced splicing efficiency. As determined by using an S1 probe that spanned the 3' splice junction, neither pApy nor pA+py RNA was found to be spliced to the mutant splice junction; rather, they were spliced to the first AG dinucleotide downstream from the mutated 3' splice junction (data not shown). This had been seen before with 3' splice site mutations in the rabbit β-globin gene (2) and is consistent with the idea that a 3' splice site is located by a scanning mechanism that starts from the branch point-polypyrimidine tract and proceeds in the 3' direction to the first AG (44). Northern (RNA) blot analysis indicated that the major RNAs from all β-globin-derived genes have the sizes predicted for spliced RNA, RNA that is cleaved and polyadenylated at the intronic poly(A) site, and full-length read-through RNA (data not shown). Both S1 mapping and Northern analysis indicated that some of the pA+py RNA is processed at cryptic sites within the 900-bp inserted sequence. The 5' ends of the β-globin-derived RNAs were mapped with a probe that spanned the predicted transcriptional initiation site, and all RNAs were shown to be correctly initiated (data not shown).

Expression of a modified mouse MHC class I D^d gene. A regulated change in the pA/splice RNA expression ratio for both Ig and modified β-globin genes was observed when these genes were expressed in B cells and plasma cells. To ensure that this regulation is truly independent of specific sequences and is not due to fortuitous sequence similarities between these genes, as well as to eliminate any potential complications due to low RNA accumulation, a mouse MHC class I D^d gene was modified to have the same alternative RNA processing options as the μ gene (Fig. 3A). An 18-bp insertion into the third exon distinguishes the transfected gene from the endogenous gene which is expressed in the M12 B cells and S194 plasma cells (Fig. 3B, lanes 1 and 4). The αs poly(A) site was placed into the third intron of the D^d gene. Although the αs

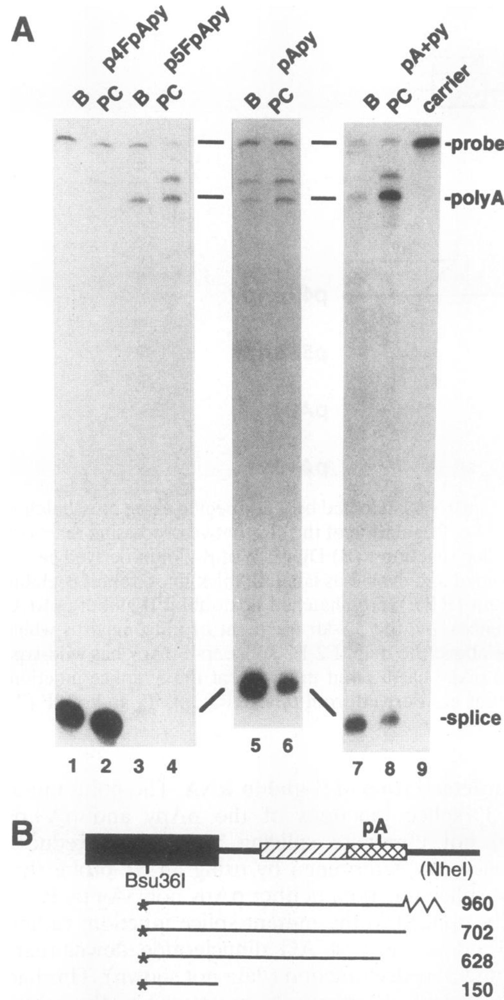

FIG. 2. Expression of β-globin-derived genes in B cells and plasma cells. (A) S1 nuclease protection analysis of RNA from M12 B cells (lanes B) and S194 plasmacytoma cells (lanes PC) transfected with the plasmids shown above each pair of lanes. For the S194 samples, 100 μg of RNA was hybridized with the probe; for the M12 samples, a nonspecific carrier was added so that the total RNA in the hybridization was 100 μg. The amounts of M12 RNA assayed were 40 μg (lane 1), 50 μg (lane 3), 25 μg (lane 5), and 80 μg (lane 7). Lane 9 contains 100 μg of carrier RNA. The positions of the probe, the fragment protected by RNA that is cleaved and polyadenylated at the intronic poly(A) site, and the fragment protected by RNA spliced at the 5′ splice junction of the β-globin intron are identified on the right. The faint band in lane 3 that is larger than the band protected by spliced RNA is likely to be background, as it is not observed consistently in other experiments. (B) Diagram of the S1 probe and the protected fragments. The restriction sites used are indicated and the symbols are as in Fig. 1.

poly(A) site is from the regulated Ig α gene, we previously established that sequences within the secreted poly(A) sites are not specifically required for regulation because both the μ_m and SV40 late poly(A) sites could substitute for the μ_s poly(A) site (36). The resulting gene, D^{d}αs, when expressed in B cells and plasma cells, generated both spliced RNA and RNA that is cleaved and polyadenylated at the inserted poly(A) site (Fig. 3B, lanes 2 and 3; Table 1). Just like the Ig and β-globin-derived genes, the pA/splice ratio shifts between cell types, so that there is relatively more spliced RNA in the B cells. The

TABLE 1. Regulated expression of non-Ig genes in M12 B cells and S194 plasma cells

Plasmid	pA/splice[a]		Regulation index[b]
	B	PC	
pApy	0.019 ± 0.002	0.16 ± 0.02	8
pA+py	0.15 ± 0.01	1.9 ± 0.2	12
D^{d}αs	0.50 ± 0.04	7.9 ± 0.5	16

[a] The pA/splice expression ratios are derived from quantitating S1 nuclease-protected fragments with an Ambis radioanalytic imaging system and are the averages ± the standard deviations for at least six independent experiments. B, B cell; PC, plasma cell.

[b] Change in the pA/splice RNA expression ratio when the same plasmid is expressed in plasma cells and B cells, calculated by dividing the pA/splice ratio for plasma cells by the pA/splice ratio for B cells. The estimated standard deviation for each ratio is less than 13%.

regulation index of 16 compares favorably with the indices for the μ and β-globin-derived genes (Table 1). This confirms that regulated RNA processing of the Ig genes does not depend on Ig gene-specific sequences.

Expression of the μ gene in non-B-cell lines. Since no Ig gene-specific sequences are required for alternative RNA processing regulation of the μ gene, the *trans*-acting mediators of regulation in B cells and plasma cells are not likely to be site-specific RNA-binding proteins. Instead, the results of our experiments imply that the activity or concentration of general splicing or cleavage-polyadenylation machinery components must change during B-cell maturation. In an effort to identify the nonregulated default processing pattern of the μ RNA and thereby determine whether the B-cell or the plasma cell pattern is regulated, the μ gene was expressed in a variety of cell lines from tissue types that normally do not synthesize μ RNA. Previous studies of Ig gene expression in non-B-cell lines have provided inconsistent results; however, no more than two cell lines were examined in any single study, and different constructs and expression protocols were utilized (27, 39, 48). We expressed the chimeric SV40-*neo*-Cμ gene (Fig. 4A) used in most of our previous studies (31, 33, 35, 36, 41) in non-B-cell lines, analyzed the μ_s/μ_m ratio by S1 nuclease protection, and compared this ratio with the B-cell and plasma cell ratios (Fig. 4B; Table 2). The μ_s/μ_m expression ratios for most non-B-cell lines resembled that of a plasma cell; in some cases the ratios were much higher. (Fig. 4B, lanes 4 and 7). The only cell line tested that resembled a B cell is the EL-4 T-cell line (Fig. 4B, lane 9). This cell line also has a low level of endogenous μ expression from the unrearranged μ locus (16); the endogenous μ_s/μ_m ratio in EL-4 is 0.7, which is also more similar to a B-cell expression pattern than the ratios of most non-B-cell lines are (Fig. 4B, lane 10).

DISCUSSION

Alternative RNA processing regulation of the μ gene requires competing splice and cleavage-polyadenylation reactions with balanced efficiencies. Then, factors within the nuclei of a B cell and a plasma cell can modulate the relative uses of the alternative processing pathways. I have now shown that RNA from two non-Ig genes modified to have competing splice and cleavage-polyadenylation reactions is alternatively processed and that the relative amounts of spliced and cleaved-polyadenylated RNAs are regulated, just like the μ gene, when expression is compared between B cells and plasma cells. This is the first demonstration that two different cell types, when presented with heterologous RNAs containing a specific ar-

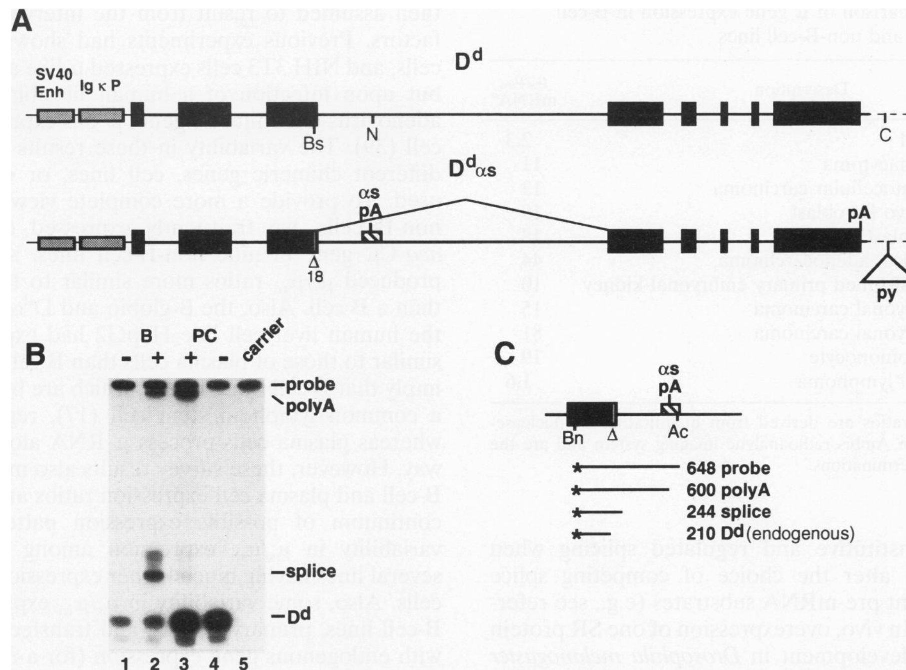

FIG. 3. Structure and expression of an MHC D^d-derived gene in B cells and plasma cells. (A) Diagram of the SV40-Igκ-D^d gene (46) (top) and the $D^d\alpha s$ gene (bottom). Stippled boxes, SV40 enhancer and Ig κ promoter; solid boxes, D^d exons; thick lines, D^d introns; thin lines and dotted lines, vector sequences; Bs, *Bsm*I restriction site into which 18 bp was inserted to distinguish the transfected gene from the endogenous gene; triangle and narrow open box in $D^d\alpha s$, inserted sequence; C, *Cla*I site in the vector where the 3.8-kb polyomavirus fragment (py) was inserted; hatched box, αs poly(A) site, inserted at the *Nco*I (N) site within the largest intron of D^d; angled line above the $D^d\alpha s$ map, the E3-E4 splice that competes with cleavage-polyadenylation at the αs poly(A) site. pA, poly(A) site. (B) S1 nuclease protection analysis of RNA from M12 B cells (lanes B) and S194 plasmacytoma cells (lanes PC) untransfected (−) or transfected with $D^d\alpha s$ (+). M12 RNA (12 μg) plus 38 μg of HepG2 carrier RNA, 50 μg of S194 RNA, and 50 μg of HepG2 carrier RNA was analyzed. The positions of the probe and protected fragments are indicated. (C) Diagram of the S1 probe and the protected fragments; Bn and Ac, *Ban*I and *Acc*I restriction sites, respectively.

rangement of RNA processing signals, are able to process the foreign RNA in the same way as a regulated endogenous RNA. This provides convincing in vivo evidence for RNA processing regulation based primarily on the organization and relative strengths of competing processing signals within the primary transcript. Therefore, regulated processing in B cells and plasma cells does not require Ig-specific sequences and must be mediated by changes in the level or activity of nonspecific rather than specific processing factors. This idea has been proposed for other genes (e.g., see references 13, 24, and 55), and factors that could potentially mediate processing regulation have been identified. SR proteins, which seem to be

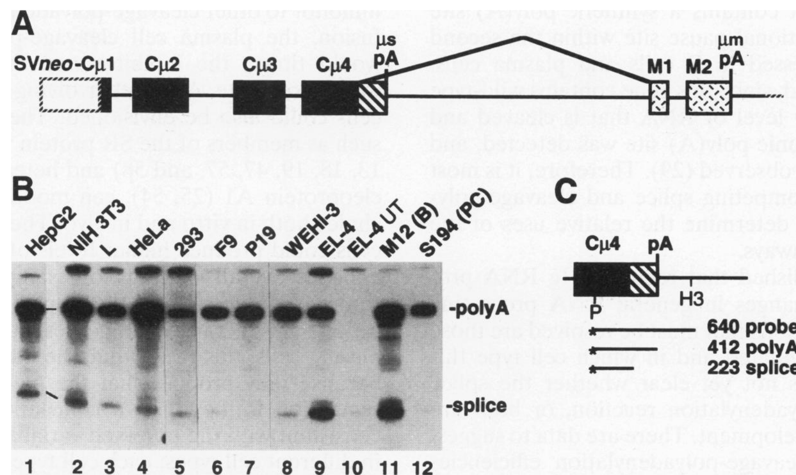

FIG. 4. Expression of the μ gene in non-B-cell lines. (A) Diagram of the chimeric SV40-*neo*C-μ gene (35). The box with the dotted border indicates SV40-*neo* sequences; other symbols are as described in the legend to Fig. 1A. (B) S1 nuclease protection analysis of RNA from the cell lines indicated above each lane transfected with the μ plasmid. The positions of the fragments protected by μ_s (polyA) and μ_m (splice) mRNA are indicated. (C) Diagram of the S1 probe and the protected fragments. P and H3, *Pst*I and *Hin*dIII restriction sites, respectively.

TABLE 2. Comparison of μ gene expression in B-cell and non-B-cell lines

Cell line	Description	μ_s/μ_m mRNA[a]
M12	Mouse B cell	2.3
S194	Mouse plasmacytoma	11
HepG2	Human hepatocellular carcinoma	13
NIH 3T3	Mouse embryo fibroblast	18
L	Mouse fibroblast	18
HeLa	Human cervical adenocarcinoma	44
293	Human transformed primary embryonal kidney	10
F9	Mouse embryonal carcinoma	15
P19	Mouse embryonal carcinoma	81
WEHI-3	Mouse myelomonocyte	19
EL-4	Mouse T-cell lymphoma	1.6

[a] The μ_s/μ_m expression ratios are derived from quantitating S1 nuclease-protected fragments with an Ambis radioanalytic imaging system and are the averages of at least two determinations.

involved in both constitutive and regulated splicing when assayed in vitro, can alter the choice of competing splice reactions from different pre-mRNA substrates (e.g., see references 11, 18, and 58). In vivo, overexpression of one SR protein can adversely affect development in *Drosophila melanogaster* (19). Also, the concentration of heterogeneous nuclear ribonucleoprotein A1 can modulate alternative splice site selection both in vitro (25) and in vivo (54).

Although the results of experiments with artificially derived constructs have suggested that poly(A) sites within introns are not used efficiently (1, 23, 28), this cannot be a general rule. First, the μ gene and others that naturally contain a poly(A) site within an intron (e.g., see references 3, 26, and 53) produce pre-mRNA that can be either spliced or cleaved and polyadenylated. Second, as demonstrated here, by adjusting the relative strengths of the splice and/or cleavage-polyadenylation reactions, genes that express a precursor RNA that is alternatively spliced or cleaved-polyadenylated can be created. Only spliced RNA was made from a rabbit β-globin-derived gene with a poly(A) site in the intron; the intronic poly(A) site was not recognized until the splice junctions were mutated. In addition, to produce two alternatively processed RNAs from the D^d gene, the relatively weak αs poly(A) site had to be used instead of the stronger μ_m poly(A) site (30). Another rabbit β-globin-derived gene that contains a synthetic poly(A) site and the α globin transcriptional pause site within the second intron (9) also was expressed in B cells and plasma cells. Spliced RNA predominated, since this gene contains wild-type splice junctions, but a low level of RNA that is cleaved and polyadenylated at the intronic poly(A) site was detected, and cell-specific regulation was observed (29). Therefore, it is most likely the efficiencies of competing splice and cleavage-polyadenylation reactions that determine the relative uses of the alternative processing pathways.

Since it has been established that regulated Ig RNA processing is mediated by changes in general RNA processing components, the next questions that must be resolved are those of which reaction(s) is regulated and in which cell type this reaction is regulated. It is not yet clear whether the splice reaction, the cleavage-polyadenylation reaction, or both are regulated during B-cell development. There are data to suggest that both splicing and cleavage-polyadenylation efficiencies may be affected during B-cell maturation, but the evidence is not conclusive (21, 33, 34, 52). One way to address the second question is to determine the default, or nonregulated, processing pattern that is seen in most cell types. The other pattern is then assumed to result from the intervention of *trans*-acting factors. Previous experiments had shown that COS cells, L cells, and NIH 3T3 cells expressed μ like a plasma cell (27, 48), but upon infection of a human fibroblast cell line with an adenovirus-Cμ chimeric gene, μ was expressed more as in a B cell (39). The variability in these results could be due to the different chimeric genes, cell lines, or expression protocols used. To provide a more complete view of Ig expression in non-B cells, we transiently expressed our chimeric SV40-*neo*-Cμ gene in nine non-B-cell lines. All but a T-cell line produced μ_s/μ_m ratios more similar to that of a plasma cell than a B cell. Also, the β-globin and D^dαs genes expressed in the human liver cell line HepG2 had expression ratios more similar to those of plasma cells than B cells (29). These results imply that B cells and T cells, which are believed to arise from a common lymphoid stem cell (17), regulate μ processing, whereas plasma cells process μ RNA along the default pathway. However, these survey results also might suggest that the B-cell and plasma cell expression ratios are two points along a continuum of possible expression patterns, since there is variability in μ_s/μ_m expression among the cell lines, with several lines having much higher expression ratios than plasma cells. Also, some variability in μ_s/μ_m expression exists among B-cell lines, primary B cells, and transfected genes compared with endogenous gene expression (for a review, see reference 32).

Another observation that should be considered in formulating models of μ processing regulation is that when B cells are fused to plasma cells, the plasma cell phenotype is dominant (e.g., see references 10 and 20). While the most straightforward interpretation of this result is that plasma cells produce a regulatory factor responsible for the processing shift, this is not consistent with the data from the non-B-cell-line survey, which suggest that the B-cell environment is regulating. One model that could account for both observations proposes that B cells produce limiting amounts of a cleavage-polyadenylation component, lowering overall cleavage-polyadenylation efficiency. Upon fusion with a plasma cell, this component would no longer be limiting and the plasma cell phenotype would dominate. This idea has been suggested previously (4). Another model consistent with these observations proposes that B cells produce a factor that lowers cleavage-polyadenylation efficiency, perhaps by competitive inhibition or by titration of a required factor; the important feature would be the ratio of the inhibitor to other cleavage-polyadenylation components. Upon fusion, the plasma cell cleavage-polyadenylation machinery would titrate the inhibitor and the plasma cell phenotype would dominate. A model involving splicing enhancement in B cells could also be envisioned. The relative levels of factors, such as members of the SR protein family (e.g., see references 13, 18, 19, 47, 57, and 58) and heterogeneous nuclear ribonucleoprotein A1 (25, 54), can modulate alternative splice site choice both in vitro and in vivo. Therefore, it is possible that B cells could produce higher levels of a splice factor that could enhance overall splicing, for example, by enhancing spliceosome assembly. Again, the important feature would be the ratio of this factor to others present in the cell; upon fusion to plasma cells, this ratio would be altered. All of these models, because they propose that the parameter that is critical for regulation is the relative concentration of factors, are also consistent with the observed variability in μ_s/μ_m mRNA ratios in different cell types; each cell type and perhaps each cell line would have its own characteristic concentration of factors. In addition, these models are not mutually exclusive. Confirmation of any of these models must await identification of processing regulators in lymphocytes.

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