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Balanced efficiencies of splicing and cleavage-polyadenylation are required for μs and μm mRNA regulation

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The relative abundance of the RNAs encoding the membrane (μm) and secreted (μs) forms of immunoglobulin μ heavy chain is regulated during B cell maturation by a change in the mode of RNA processing. This regulation depends on a competition between two mutually exclusive RNA processing reactions, cleavage-polyadenylation at the μs poly(A) site and splicing of the Cμ4 and M1 exons. Previously, the efficiencies of these two reactions were altered independently. When an efficient processing signal replaced the normal suboptimal signals of the μ gene, a single RNA product was produced exclusively. In this report, two efficient signals are combined in a single μ transcript and shown to restore a processing balance such that two mRNAs can once again be alternatively processed from a single RNA precursor. The ratio of the two RNAs generated from these μ genes containing balanced competing strong splice and cleavage-polyadenylation reactions display the expected developmental shift when expressed in B cells and plasma cells. Therefore, the balance between cleavage-polyadenylation and splicing efficiencies is critical to the developmentally regulated expression of μs and μm mRNA. Also shown here is that the entire μm region, including the M1 and M2 exons and the μm poly(A) site, can be replaced with SV40 splice and poly(A) sequences. Regulation is maintained in these μ genes, indicating that no specific sequences within the μm region are required.

The mRNAs encoding the membrane-bound (m) and secreted (s) forms of IgM heavy chain (μ) are produced from identical primary transcripts that are alternatively processed at their 3′ ends. The relative abundance of these two mRNAs is regulated during B cell maturation by two alternate and mutually exclusive RNA processing reactions; cleavage-polyadenylation at the μs poly(A) site to produce μs mRNA, and splicing of the Cμ4 and M1 exons to produce μm mRNA, which is subsequently polyadenylated at a downstream site (Peterson and Perry, 1989). In pre-B and B cells, splicing and utilization of the downstream poly(A) site is favored, while mature plasma cells utilize the upstream μs poly(A) site predominantly (Alt et al., 1980; Early et al., 1980).

We previously demonstrated that the Cμ4-M1 splice and not the μm poly(A) site was in competition with cleavage-polyadenylation at μs by improving the efficiency of the Cμ4-M1 splice reaction and showing that, in this case, splicing dominated over cleavage-polyadenylation at the μs site (Peterson and Perry, 1989). We increased the efficiency of the Cμ4-M1 splice reaction by mutating the evolutionarily conserved subopti-

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mal Cμ4 5′ splice junction to the consensus 5′ splice junction sequence. Splice junctions which are more similar to the consensus sequence (Shapiro and Senapathy, 1987) and therefore can basepair more strongly with U1 small nuclear RNA have been shown to be stronger, more efficient splice junctions (Eperon et al., 1986; Zhuang et al., 1987). μ precursor RNA containing the consensus splice junction was totally spliced, even in plasmacytoma cells that normally heavily favor cleavage-polyadenylation at μs, thus proving that the splice is in competition with μs cleavage-polyadenylation. In addition, contrary to a prediction of a μs vs. μm poly(A) site competition model, the μm poly(A) site could be substituted with other poly(A) sites without any effect on cleavage-polyadenylation at μs (Peterson and Perry, 1989).

We have also shown that no unique sequence feature of the μs poly(A) site is required for developmentally regulated μs/μm expression (Peterson and Perry, 1989). μ genes with other poly(A) sites substituted for the μs site showed a regulatory shift in μs/μm expression between B cells and plasmacytoma cells, as long as both spliced and cleaved-polyadenylated RNA could be processed from a single precursor transcript. With the μm poly(A) site substituted for μs, RNA was totally cleaved and polyadenylated at this first poly(A) site in both cell types. However, when the Cμ4-M1 splice was enhanced by shortening the intron (Peterson and Perry, 1986; Galli et al., 1987; Tsurushita and Korn, 1987), regulation was restored; the μs/μm expression ratio was about 10-fold higher in plasmacytoma cells than in B cells (Peterson and Perry, 1989). When the poly(A) site for the secreted form of α heavy chain (αs) replaced μs, the regulatory shift in expression was also not affected. That the developmental shift in expression ratios was observed only when both μs and μm mRNAs could be processed from the same transcript suggests that the efficiencies of the two competing processing reactions must be balanced in order for this gene to respond to developmental changes that occur during B cell maturation.

The experiments presented here were performed to address the question of whether μ genes containing any combination of balanced splice and cleavage-polyadenylation signals substituted for the native μ signals could be regulated between B cells and plasma cells. Specifically, could a μ gene containing the strong consensus splice junction in competition with a strong poly(A) site be regulated? We show here that a strong poly(A) site does restore both alternate 3′ end processing and regulation to consensus 5′ splice junction containing μ genes. In addition, the μm 3′ splice junction and poly(A) site can be functionally replaced with SV40 splice and poly(A) signals, and these μ genes are also regulated. Therefore, there are no specific sequences required for regulation within the μm region. These experiments firmly establish the requirement for balanced efficiencies of splicing and cleavage-polyadenylation, however it is achieved, in μs/μm regulation. Since to date no μ gene-specific sequences have been identified to be required for regulation, it is possible that the processing balance is the critical requirement for regulation and allows the μ gene to respond to subtle developmental changes in cellular milieu, such as changes in general processing efficiencies.

Materials and methods

Plasmid construction

The construction of plasmids s-m, μs, m-m, and SP have been described in Peterson and Perry (1986 and 1989). To construct SPμs, a 2977 bp EcoR V fragment containing the μm region was deleted from SP, as was done for the μs plasmid (Peterson and Perry, 1986). The plasmid SP/rpI$_{900}$ contains the 900 bp fragment from an S16 ribosomal protein gene intron used previously to alter the Cμ4-M1 intron size (Peterson and Perry, 1986), inserted within the Cμ4-M1 intron at the Kpn I site. SP$_{m\text{-}m}$ and SP$_{L\text{-}m}$ were constructed by replacing the μs poly(A) site region of SP with the μm and SV40 late poly(A) sites, respectively, as described in Peterson and Perry (1989). SP$_m$ and SP$_L$ were derived from SP$_{m\text{-}m}$ and SP$_{L\text{-}m}$ by deleting the μm-containing EcoR V fragment described above.

Cell culture and DNA transfections

The M12 B cell line and the 3-1 Abelson murine leukemia virus-transformed pre-B cell line were maintained in RPMI 1640 medium containing 10% fetal bovine serum and 50 μM 2-mercaptoethanol. The plasmacytoma S194 and myeloma MPC11 cell lines were maintained in Dulbecco modified Eagle medium containing 10% horse serum. The DEAE-dextran transfec-

tion protocol (Grosschedl and Baltimore, 1985) was used for the transient expression assays.

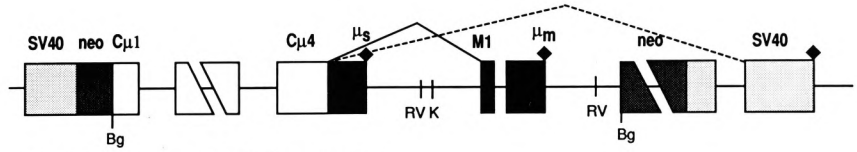
RNA preparation and analysis

Cytoplasmic RNA was prepared from transfected cells (Schibler et al., 1978); poly(A)$^+$ RNA was isolated by passing total cytoplasmic RNA over oligo(dT) columns. S1 nuclease analysis of total cytoplasmic RNA was performed as described previously, using the μs p(A)/μm 5′ splice probe, which distinguishes RNA cleaved at the μs poly(A) site from RNA spliced from Cμ4 to M1 (Peterson and Perry, 1989). The data from S1 nuclease analyses were quantitated by directly counting the dried polyacrylamide gels using an Ambis blot analyzer. Northern blot analysis of poly(A)$^+$ RNA was performed as described previously, using the 384 bp SV*neo* probe (Peterson and Perry, 1989). The filter was stripped and rehybridized with an 885 bp Pst I-BamH I fragment containing the SV40 poly(A) site region from pSV2*neo*.

Results

The chimeric μ gene employed in the current study has been used extensively in the past (Peterson and Perry, 1986 and 1989; Fig. 1, construct s-m). Transient expression assays are performed using plasmids derived from pSV5*neo* which replicates in mouse cells (Southern and Berg, 1982). The pSV5-based plasmids have a 6 kb Bgl II fragment containing most of the Cμ region inserted within the neomycin resistance gene. The chimeric μ transcripts are driven by the SV40 promoter and enhancer and contain 344 nt of the *neo* gene fused out-of-frame to the Cμ1 exon. This chimeric gene is appropriately regulated when expressed in pre-B, B, and plasma cell lines (Peterson and Perry, 1986 and 1989; Peterson et al., 1991).

The μm 3′ splice junction can be functionally replaced by the SV40 t antigen 3′ splice junction. We previously demonstrated that when the evolutionarily conserved suboptimal Cμ4 5′ splice



PLASMID	5′ SPLICE	POLY(A)	
s-m	wild-type	μs	
μs	wild-type	μs	Δ
SP	consensus	μs	
SPμs	consensus	μs	Δ
SP/rpI900	consensus	μs	900
m-m	wild-type	μm	
SPm-m	consensus	μm	
SPL-m	consensus	SVL	
SPm	consensus	μm	Δ
SPL	consensus	SVL	Δ

Figure 1. Diagram of chimeric immunoglobulin genes. Shown at the top is the chimeric SV40-*neo*-Cμ gene from the plasmid pSV5Cμs-m (Peterson and Perry, 1986), not drawn to scale. The angled lines above the map designate the wild-type Cμ4-M1 splice (solid line) and the Cμ4-SV40 t antigen splice (dashed line). Open boxes indicate sequences common to both μs and μm mRNA, filled boxes are sequences specific to μs and μm mRNA as marked, lightly shaded boxes are SV40 sequences, medium shaded boxes are sequences of the neomycin resistance gene, boxes broken on the angle indicate sequences omitted from this diagram, lines indicate introns or noncoding sequences, the diamonds are the poly(A) sites. The restriction sites marked are Bg, Bgl II; RV, EcoR V; and K, Kpn I. Note that the neomycin gene is interrupted by Cμ sequences at the Bgl II site and the 3′ *neo* sequences are not incorporated into the mature RNAs. Below the map, the plasmids used in this study are described in tabular form. The parameters varied in this plasmid series are: (1) the sequence of the Cμ4 5′ splice junction (wild-type or consensus), (2) the μs poly(A) site [the wild-type μs site is left unchanged or replaced with either the μm or SV40 late poly(A) site], (3) the 3′ splice junction (the wild-type μm site or, when deleted, the SV40 antigen t site), and (4) the size of the Cμ4-M1 intron (made longer by 900 bp in SP/rpI$_{900}$). The Δ identifies the μm sequences deleted; the insertion into the Kpn I site is indicated.

junction was mutated to a consensus splice junction (Fig. 1, construct SP), only spliced RNA was expressed in all cell types (Peterson and Perry, 1989; Fig. 2, lane 2). It was unlikely that this effect of the SP consensus splice mutation was due to a steric inhibition of cleavage-polyadenylation at the μs poly(A) site, since only 15–17 nt surrounding the 5′ splice junction of several pre-mRNA substrates can be protected from RNase digestion in vitro by the U1 snRNP particle (Mount et al., 1983; Black et al., 1985), and the μs poly(A) site is about 190 nt downstream from the Cμ4 5′ splice junction in the μ gene. However, to ensure that the effect of the SP consensus splice site mutation was due to the improved efficiency of the splice reaction and not to a direct steric inhibition of cleavage-polyadenylation at the μs poly(A) site, the μm 3′ splice and poly(A) site were removed from SP (Fig. 1, construct SPμs). Previously, this same 3 kb EcoR V fragment containing the μm coding region, 0.6 kb upstream and 1.9 kb downstream, was deleted from the unmutated μ gene to construct the μs plasmid (Fig. 1); by Northern and S1 nuclease analyses the μs plasmid made only μs mRNA (Peterson and Perry, 1986; Fig. 2A, lane 3). This suggested that by removing the 3′ splice site and eliminating the competing splice reaction, cleavage-polyadenylation at the μs poly(A) site was the only possible processing reaction. Similar results were expected with the SPμs plasmid. As seen by S1 nuclease analysis of RNA from SPμs transiently expressed in plasmacytoma cells, removing the μm region from SP activates cleavage-polyadenylation at the μs poly(A) site, albeit at a low level (Fig. 2A; compare lanes 2 and 4). The fact that the μs poly(A) site can be used indicates that splicing complexes formed at the consensus 5′ splice do not completely interfere with cleavage-polyadenylation at this site. This is confirmed by experiments described later.

It was surprising that the mRNA expressed from the μs and SPμs plasmids were so different. Unlike the μs plasmid, very little μs mRNA is made from SPμs; instead, mostly spliced RNA is produced. The S1 nuclease probe used here protects RNA spliced at the Cμ4 5′ splice junction, but does not provide any information regarding the 3′ splice junction. To identify the nature of the spliced RNA from SPμs, poly(A)$^+$ RNA was analyzed by Northern blotting. Figure 2B clearly shows that SPμs produces

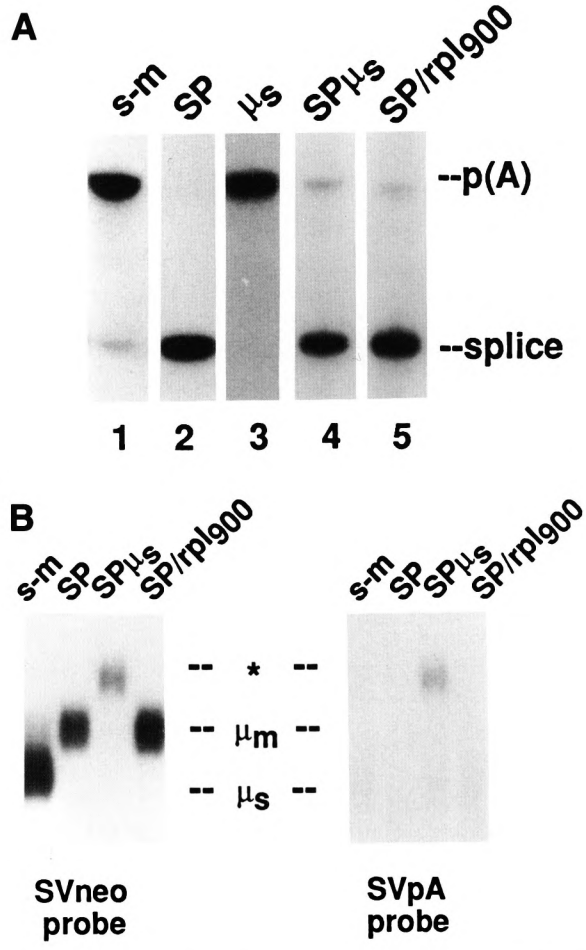


Figure 2. mRNA Expression patterns from improved splice site-containing genes in plasmacytoma S194 cells. **A.** S1 nuclease analysis of RNA from S194 plasmacytoma cells transfected with the constructs shown above each lane. The probe has been described previously (Peterson and Perry, 1989) and differentiates RNA cleaved at the μs poly(A) site (pA) and RNA spliced from Cμ4 to M1 (splice). **B.** Northern blot of poly(A)$^+$ RNA from S194 plasmacytoma cells transfected with the constructs shown above each lane. The panel on the left was hybridized with a probe specific to the SV*neo* 5′ end of the RNA (SVneo probe); the panel on the right is the same blot hybridized with a probe specific for the 3′ end of the SV40 RNA (SVpA probe). The position of μs and μm mRNA are indicated; * is the RNA spliced from Cμ4 to SV40 t. A darker exposure of the SVneo probe panel reveals a low level of μs RNA in the SPμs and SP/rpI$_{900}$ lanes, consistent with the S1 nuclease analysis.

a transcript that is longer than either the μs or μm transcripts (identified by * in that figure). This transcript is the size predicted for an RNA spliced to the SV40 t antigen 3′ splice junction located downstream from the *neo* gene (Fig. 1).

If this SV40 t antigen 3′ splice site is being used, then this longer transcript should contain sequences from the SV40 splice and poly(A) region. Indeed, when this blot was reprobed with a fragment from the SV40 splice/poly(A) region, only the larger transcript hybridized (Fig. 2B, SVpA probe). Thus, the μm 3′ splice junction can be functionally replaced by the SV40 t antigen 3′ splice junction.

The size of the Cμ4-M1 intron has a small but measurable effect on expression from SP. The size of the Cμ4-M1 intron has been shown to affect the μs/μm expression ratio; the smaller the intron, the more spliced μm mRNA produced (Peterson and Perry, 1986; Galli et al., 1987; Tsurushita and Korn, 1987). Conversely, when the intron was lengthened, μm production decreased (Peterson and Perry, 1986; Tsurushita and Korn, 1987). This effect is most likely due to the time the cleavage-polyadenylation machinery has to act on the primary transcript before the 3′ splice junction is transcribed. Since a stable committed splice complex requires both a 5′ splice junction and a branch point sequence, generally located near the 3′ splice junction (Sharp, 1987), the splice reaction cannot compete with cleavage-polyadenylation until the 3′ end of the intron is transcribed. In an attempt to decrease the efficiency of SP splicing and thereby increase μs mRNA expression, the Cμ4-M1 intron was lengthened by 900 nt in the SP/rpI$_{900}$ plasmid (Fig. 1). This construct was transiently expressed in plasmacytoma cells, and the RNA was analyzed by both S1 nuclease protection and Northern blot analyses (Figs. 2A; lane 5, and 2B). Lengthening the Cμ4-M1 intron by 900 nt activates a low level of cleavage-polyadenylation at the μs poly(A) site (Fig. 2A; compare lanes 2 and 5). The μs/μm expression ratios of SP/rpI$_{900}$ and SPμs are similar (Fig. 2A, lanes 4 and 5). In the latter case, the reduced splice efficiency may be due to either the substituted 3′ splice junction or to the increased intron size; the wild-type Cμ4-M1 intron is 1863 nt, while the Cμ4-SV40 t antigen intron is 2783 nt—an increase of 920 nt.

The more efficient poly(A) sites compete with the consensus splice junction. The μm and SVL poly(A) sites, when substituted for the μs poly(A) site, directed the expression of 100% cleaved and polyadenylated RNA in both B cells and plasmacytoma cells (Peterson and Perry, 1989; Fig. 1, construct m-m, and Fig. 3, lane 3). This suggests that these poly(A) sites are preferentially used because they are stronger. Conversely, when the Cμ4 5′ splice junction was mutated to a consensus 5′ splice junction, 100% spliced RNA was produced in both cell types (Peterson and Perry, 1989; Fig. 1, construct SP, and Fig. 3, lane 2). In both cases, the stronger processing signals abolished the shift in μs/μm expression normally observed during B cell development. To determine whether a processing balance could be achieved if the stronger μm and SVL poly(A) sites were paired with the strong consensus 5′ splice junction, these strong processing reactions were placed in competition in a single transcript. Two μ genes, SP$_{m\text{-}m}$ and SP$_{L\text{-}m}$, were prepared by replacing the μs poly(A) site of SP with the μm or SVL poly(A) sites (Fig. 1). The transient expression of SP$_{m\text{-}m}$ and SP$_{L\text{-}m}$ in plasmacytoma cells was assayed by S1 nuclease protection and compared to the expression of the wild-type (s-m), SP, and m-m constructs (Fig. 3, lanes 1–5). In contrast to the expression pattern observed when a strong pro-

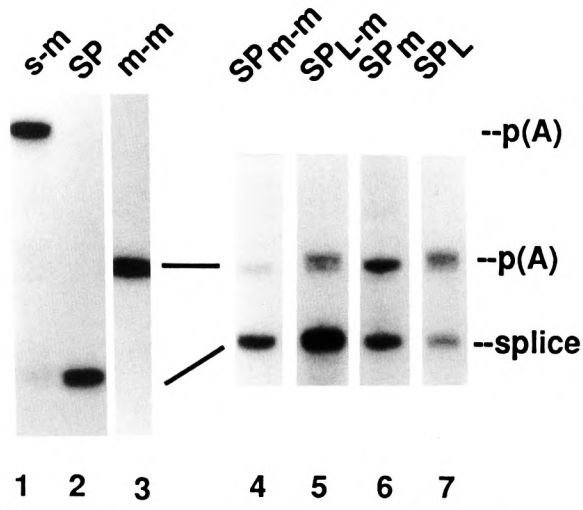


Figure 3. Balanced RNA processing efficiencies. S1 nuclease analysis of RNA from S194 plasmacytoma cells transfected with the constructs shown above each lane. The probe is the same as used in Figure 2, which, in addition to distinguishing μs RNA (top pA) from μm RNA (splice), also identifies RNA that has been cleaved and polyadenylated at substituted poly(A) sites (bottom pA). These bands represent the extent of homology between the probe and the mRNA. The double band seen with the SP$_{L\text{-}m}$ and SP$_L$ is a result of partial homology between the probe and the substituted sequences at the cloning junction; both of these bands are from RNA cleaved at the SV40 late poly(A) site.

cessing signal was in competition with the native weak signal (SP and m-m), both spliced and cleaved-polyadenylated mRNA were produced from the μ genes containing competing strong processing reactions. This result also confirms that cleavage-polyadenylation at a poly(A) site located 160 to 210 nt downstream from the Cμ4 consensus 5′ splice junction can occur and is not blocked by snRNP interactions at the splice junction.

As shown above, when the μm 3′ splice junction and poly(A) site were removed from the μ gene containing the consensus Cμ4 5′ splice junction, splicing between this consensus 5′ splice and the SV40 t antigen 3′ splice junction was observed. A low level of cleavage-polyadenylation at the μs poly(A) site was detected, suggesting that the splicing efficiency had been decreased. To explore the effect that decreasing the splice efficiency—by deleting the μm region—would have on the processing from the genes with both a strong 5′ splice junction and a strong poly(A) site, the plasmids SP_m and SP_L were produced from $SP_{m\text{-}m}$ and $SP_{L\text{-}m}$. SP_m and SP_L are missing the same μm fragment as the μs and SPμs constructs (Fig. 1). The strong poly(A) sites in SP_m and SP_L were found to compete effectively with Cμ4-SV40 t antigen splicing, as indicated by the expression of both spliced and cleaved-polyadenylated RNA from these constructs in plasmacytoma cells (Fig. 3, lanes 6, 7). In fact, the poly(A)/splice expression ratio was higher than for $SP_{m\text{-}m}$ and $SP_{L\text{-}m}$, which splice Cμ4 to M1. This is consistent with Cμ4-SV40 t antigen splicing being less efficient than Cμ4-M1 splicing.

Expression from all μ genes with balanced splice and cleavage-polyadenylation reactions is regulated. To address whether expression from the μ genes containing competing strong splice and cleavage-polyadenylation reactions is developmentally regulated, $SP_{m\text{-}m}$, $SP_{L\text{-}m}$, SP_m, and SP_L were expressed in both B cells and plasmacytoma cells. The S1 analysis of RNA from transfected B cell and plasmacytoma cells in Fig. 4 shows that in every case, the poly(A) vs. spliced RNA expression pattern was regulated. That is, more spliced "μm" mRNA was produced in the B cells than in the plasma cells. A quantitative assessment of these data (Table 1) indicates that these constructs with balanced strong processing signals are regulated, in most cases,

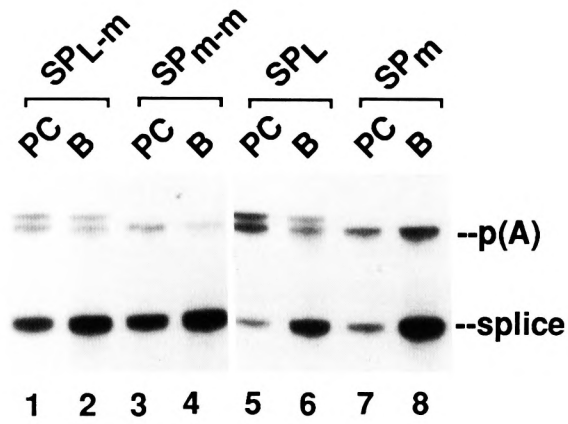


Figure 4. Regulated production of RNA from μ genes containing balanced processing reactions. S1 nuclease analysis of RNA from S194 plasmacytoma cells (PC) or M12 B cells (B) transfected with the constructs shown above each lane. The protected bands are labeled: RNA cleaved and polyadenylated at the substituted site (pA) and RNA spliced at the Cμ4 5′ splice junction (splice).

as well as the wild-type construct. The shift in the polyadenylated to spliced RNA [p(A)/splice] expression ratio between plasma cells and B cells is 7-fold for the wild-type μ gene (s-m) and varies between 4- and 13-fold for the μ genes containing competing strong processing reactions. To show that this regulatory shift in p(A)/splice expression ratios between B cells and plasma cells observed here is not specific to the two cell lines used, the $SP_{L\text{-}m}$ and SP_L constructs were expressed in two additional cell lines. The Abelson pre-B cell line 3–1 produced more spliced RNA than did the myeloma cell line MPC11 (data not shown), thus confirming the generality of this result.

A summary of each construct with respect to its 5′ splice junction, poly(A) site, and 3′ splice junction; a quantitation of the p(A)/splice expression ratio in plasmacytoma cells; and an assessment of its ability to be regulated between B cells and plasma cells are presented in Table 2. The definition of strong and weak sites is based on expression patterns when the sites are within the μ gene (Peterson and Perry, 1989): the wild-type μs poly(A) site is considered weak, while the μm and SVL poly(A) sites are strong. The 5′ splice site is the main determinant of splice strength in this system: wild-type is weak, and consensus is strong. The μ gene contains a weak poly(A) site in competition with a weak 5′ splice junction and has a p(A)/splice expression ratio

Table 1. Expression of constructs containing poly(A) site and splice junction alterations in B and plasmacytoma cells.

Expression ratios are derived from densitometric scans of autoradiograms or direct counting of S1 nuclease-protected fragments in gels and are the average ± standard deviation of at least five independent determinations.

Construct	p(A)/splice B cells	p(A)/splice plasmacytoma cells	Regulation[a] PC/B cells
s-m[b]	1.5 ± 0.2	11 ± 4	7
SP_{L-m}	0.10 ± 0.04	0.4 ± 0.1	4
SP_{m-m}	0.04 ± 0.01	0.2 ± 0.04	5
SP_L	0.20 ± 0.09	2.2 ± 0.4	11
SP_m	0.08 ± 0.04	1.0 ± 0.1	13

[a] The estimated standard deviation on the PC/B cell regulation ratio is less than 50% in each case.
[b] s-m wild-type expression data from Peterson and Perry (1989) included for comparison.

of 11:1 in plasma cells. The combination of two strong processing reactions results in expression ratios varying between 2:1 and 1:5. Although these ratios are considerably less than the wild-type ratio, they nevertheless fall within a range amenable to regulation between B cells and plasma cells.

Discussion

The experiments presented here demonstrate that the regulated production of μs and μm mRNA during B cell development depends on the finely tuned efficiencies of the competing μs cleavage-polyadenylation and Cμ4-M1 splice reactions. The native μ gene has balanced suboptimal cleavage-polyadenylation and splice signals. Experimentally, the cleavage-polyadenylation efficiency could be improved by changing the sequence of the poly(A) site. Likewise, the splice efficiency could be improved by changing the sequence of the 5′ splice junction and either improved or diminished by changing the size of the intron. When a strong processing signal was placed in competition with either of the suboptimal processing signals found in the native μ gene, the processing balance and developmental regulation was lost. However, when a strong splice and a strong cleavage-polyadenylation reaction were combined, the processing reactions were balanced, and regulation was restored. Suboptimal processing signals are found in a number of genes that are regulated by alternative RNA processing, and their suboptimal nature has been shown to be an important part of the regulatory mechanism in several cases (Mullen et al., 1991; Hoshijima et al., 1991). Although the μ gene has competing suboptimal processing signals, they are not required; as long as a balance is maintained, expression of μs/μm-type RNAs is regulated between B cells and plasma cells.

To date, no μ gene-specific sequences have been shown to be required for the developmental shift in μs/μm expression. Sequences surrounding the μs poly(A) site, the μm 3′ splice junction, and the μm poly(A) site and the sequence of the conserved suboptimal Cμ4 5′ splice junction can be altered without specifically interfering with regulation. Although a stem-loop structure at the 3′ M1 splice junction was reported to inhibit in vitro splicing (Watakabe et al., 1989), this region can be substituted with the SV40 t antigen 3′ splice junction and therefore is not required for regulation. This structure could, however, contribute to the overall efficiency of the Cμ4-M1 splice. Expression from the SP_m and SP_L plasmids, which contain the least amount of μ gene sequence of any μ genes constructed, is regulated. If μ-specific sequences are required in any way for this regulation, they must be within the Cμ

Table 2. Summary of processing reactions, expression, and regulation of μ gene constructs.

Expression ratios from plasmacytoma cells are derived from densitometric scans of autoradiograms or direct counting of S1 nuclease-protected fragments in gels (see Table 1). Data for SVL-m are reported in Peterson and Perry (1989).

Construct	5′ splice	Poly(A)	3′ splice	Strength ratio p(A)/splice	Expression p(A)/splice	Regulated
m-m	wild-type	μm	wild-type	strong/weak	>100/1	−
SVL-m	wild-type	SVL	wild-type	strong/weak	>100/1	−
s-m	wild-type	μs	wild-type	weak/weak	11/1	+
SP_L	consensus	SVL	SV40 t	strong/strong	2/1	+
SP_m	consensus	μm	SV40 t	strong/strong	1/1	+
SP_{L-m}	consensus	SVL	wild-type	strong/strong	1/3	+
SP_{m-m}	consensus	μm	wild-type	strong/strong	1/5	+
SP	consensus	μs	wild-type	weak/strong	<1/100	−

sequences surrounding or upstream from the Cμ4 5′ splice junction, as these are the only μ sequences remaining in the SP_m and SP_L constructs. It seems more likely that there are no specific cis-acting sites within the μ gene that regulate mRNA processing. Rather, the balance of the two competing reactions may be the critical parameter that allows this gene to respond to subtle changes in general processing efficiencies during B cell maturation. It is interesting that all of the genes encoding the other immunoglobulin isotypes, except δ, produce regulated secreted and membrane forms of mRNA and have a 3′ end structure similar to the μ gene (Word et al., 1983; Brown and Morrison, 1989). The introns analogous to Cμ4-M1 vary in size from 1360 to 2350 nt, and each has a poly(A) site in a position similar to the μs poly(A) site. The competing processing reactions of these other immunoglobulin genes may also be balanced to respond to the same cellular changes during B cell maturation as the μ gene.

The difference between the processing environment of the B cell and the plasma cell could be due to the activity or concentration of general splice and/or cleavage-polyadenylation factors. By expressing μ genes that contain tandem weak and strong poly(A) sites without a competing splice reaction in B cells and plasma cells, we demonstrated previously a 50 to 100% increase in general cleavage-polyadenylation efficiency in plasma cells as compared to B cells (Peterson et al., 1991). This most likely contributes to the regulation of μs and μm mRNA and may reflect a change in concentration of a cleavage-polyadenylation machinery component (Takagaki et al., 1989; Gilmartin and Nevins, 1989). In contrast, we were unable to detect differences in splicing efficiency between B cells and plasma cells using both a μ gene and an E1A gene that contain tandem weak and strong 5′ splice junctions, in the absence of a competing cleavage-polyadenylation reaction. However, the existence of such differences have not been conclusively ruled out. Indeed, in other systems, the concentration of general splice factors and the relative strengths of splice sites have been implicated in the regulation of alternative splicing. For example, the concentration of the general splice factor ASF/SF2 affects the processing of competing splice reactions (Ge and Manley, 1990; Krainer et al., 1990). This factor is probably not involved in the regulation of μs/μm processing, since 293 cells—the cell line shown to have a higher level of ASF activity (Ge and Manley, 1990)—processes μ mRNA with a μs/μm ratio similar to plasma cells, which favor the cleaved and polyadenylated μs mRNA (M. Peterson, unpublished). Neurons produce a *c-src* mRNA containing an extra neuron-specific intron. The preferred model to explain the specific inclusion of this intron is based on the relative strengths of two splice reactions that may be sensitive to subtle differences in general splicing factor concentrations between neurons and other cells (Black, 1991).

The ultimate proof that regulation is dependent on changes in general processing factors to which the μ gene is able to respond will be to construct a non-Ig gene with the same competing and balanced processing options and show that it can be differentially processed in B cells and plasma cells. This is not a trivial task, as few natural genes exist with a 3′ end structure similar to the μ gene. Such experiments are in progress (M. Peterson, unpublished).

The μ gene, with competing splice and cleavage-polyadenylation reactions, provides a sensitive assay for parameters affecting the efficiency of both of these events. Based on the p(A)/splice ratios of the set of μ constructs presented here (Tables 1 and 2), the poly(A) site strengths could be ordered SVL > μm > μs, the difference between SVL and μm being only two-fold. It is not obvious from the sequence of the SVL and μm poly(A) sites why SVL is stronger; both poly(A) sites contain the conserved AAUAAA hexanucleotide and have several T-rich and GT-rich downstream sequences. Yet the μ gene, with the competing splice reaction, is able to detect subtle differences in cleavage-polyadenylation efficiency and therefore would be a sensitive system with which to examine the basis for efficiency differences among poly(A) sites.

Parameters affecting splice efficiency have also been identified by studying the processing of altered μ genes. A 5′ splice junction sequence that is closer to the consensus sequence is a stronger splice junction (Eperon et al., 1986; Zhuang et al., 1987). We confirmed this using the μ gene (Peterson and Perry, 1989). The processing of altered μ genes has also shown that the intron size affects splice efficiency. When the intron is shortened, the splice efficiency increases (Peterson and Perry, 1986, 1989; Galli

et al., 1987; Tsurushita and Korn, 1987). Conversely, when the intron is lengthened, the splice efficiency decreases, whether in the presence of the wild-type or consensus 5′ splice junction (Peterson and Perry, 1986; Tsurushita and Korn, 1987; this paper). I have shown here that by increasing the intron length by 900 nt, an increase of 50%, splicing of the consensus Cμ4 5′ splice junction to M1 was reduced sufficiently to activate a low level of cleavage-polyadenylation at the suboptimal wild-type μs poly(A) site. It is perhaps surprising that a 50% increase in intron length has such a minor effect on splicing in the presence of the consensus 5′ splice junction. This seems to suggest that the intron size plays a minor role, as compared to the 5′ splice junction sequence, in determining the overall splice efficiency. A similar low level of μs mRNA expression was observed when the μm region was deleted from SP; SPμs has an intron length similar to the expanded intron of SP/rpI$_{900}$ but has the SV40 t antigen 3′ splice junction instead of the μm 3′ splice. This might imply that these two 3′ splice junctions are of similar strength, or alternatively that the 3′ splice junction also plays a minor role in determining overall splice efficiency in the presence of a consensus 5′ splice junction.

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