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Biochemistry

Regulated production of μ_m and μ_s mRNA requires linkage of the poly(A) addition sites and is dependent on the length of the μ_s–μ_m intron

(splicing/RNA processing/immunoglobulin)

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ABSTRACT **mRNAs encoding the membrane-associated (μ_m) and secreted (μ_s) forms of μ heavy chain are derived from transcripts of the same immunoglobulin gene by differential RNA processing. To help elucidate the mechanism that regulates the production of these two μ mRNAs during the course of B-lymphoid maturation, we produced a series of specifically modified μ-chain genes and studied their expression when transfected into cells representing either early or late developmental stages. We have established that proper regulation depends on linkage of the μ_s and μ_m poly(A) addition sites and the length of the μ_s–μ_m intron. Deletion of an 800- to 900-nucleotide segment from the central region of this intron abolishes regulation; replacement of this segment with miscellaneous DNA sequences restores it. From these results we propose a model in which regulation is principally achieved by competition between cleavage/polyadenylylation of the μ_s site and splicing of the $C_\mu 4$ and μ_m exons.**

Two discrete mRNAs encoding the membrane-associated (μ_m) and secreted (μ_s) forms of IgM heavy chain are produced from a single rearranged immunoglobulin gene. The difference between the secreted and membrane form of the IgM protein is at the carboxyl terminus of the μ chain: the membrane-associated form contains a hydrophobic anchor segment, whereas the secreted form has a short hydrophilic segment. The production of μ_m mRNA, which entails the use of a distal poly(A) addition site and a splice that eliminates the μ_s terminus, is predominant in early-stage B-lymphocytes. The production of μ_s mRNA, which entails the use of a proximal poly(A) addition site that eliminates the 3′ splice junction, is heavily favored in mature plasma cells (1, 2).

Previous studies of a variety of lymphoid tumor lines (3) and of mitogen-stimulated splenic B lymphocytes (4) revealed that the extent of RNA polymerase traversal through the μ–δ locus is foreshortened in IgM-secreting cells compared to that in surface-Ig-bearing cells. In some IgM secretors, a substantial fraction of the polymerase unloading occurs prior to the μ_m poly(A) addition site and therefore contributes significantly to the high μ_s:μ_m mRNA ratio in these cells (5). However, in many other cells with $\mu_s >> \mu_m$ phenotypes, transcription extends well into the μ_m region, indicating that the shift to high μ_s mRNA production is primarily dependent on a change in the mode of RNA processing rather than an early exit of polymerase (4–7). An analogous situation seems to hold for the regulated production of secreted and membrane-associated γ2b mRNA in plasma vs. memory cells (8, 9).

Three general models for a regulatory mechanism operating at the level of RNA processing may be envisioned. In one type of model, the unprocessed primary transcript could be cleaved and polyadenylylated at either the μ_s or the μ_m site and this choice of alternative poly(A) addition sites could determine which mature mRNA is produced. This model necessarily assumes that the conformation of a transcript that is cleaved and polyadenylylated at the μ_m site would automatically favor splicing of the $C_\mu 4$ and μ_m exons over usage of the μ_s poly(A) addition site—i.e., that a μ_m-terminated primary transcript is solely a precursor of μ_m mRNA. In another type of model, the critical choice would be between cleavage/polyadenylylation at the μ_s site and the splice between the $C_\mu 4$ and μ_m exons. Since these reactions are mutually exclusive, this choice would determine the nature of the mature mRNA. This decision could be applied to an unprocessed transcript or to a transcript that is cleaved and polyadenylylated at the μ_m site. With either of these models, specificity could be achieved by the presence or absence of factors specific for a particular site or by differences in the concentration of more universal factors. In a third model, the major determinant would be a difference in the relative stability of μ_s and μ_m mRNA or their precursors during the course of B-cell maturation. Such posttranscriptional regulation appears to be largely responsible for the overall increase in μ mRNA accumulation during development (5, 10) and conceivably could be used to discriminate between μ_s- and μ_m-terminated transcripts.

Several groups of investigators have addressed this problem by introducing intact or modified heavy chain genes into various lymphoid and nonlymphoid cells and then measuring the relative usage of the secreted and membrane-associated poly(A) addition sites (6, 7, 9, 11). These studies have helped to rule out transcriptional termination as the primary cause of μ_s vs. μ_m determination and have implicated the cleavage/polyadenylylation-recognition signal of the secreted terminus as an essential element in the regulatory process. However, it is still uncertain whether regulation depends solely on the particular properties of such signals, which are usually located within 50 nucleotides of the poly(A) addition sites (12), or whether other features of the gene structure are also important. Such information is clearly critical when evaluating the validity of the models discussed above.

To answer this question, we established a protocol in which the 3′ terminal portion of the μ-chain gene is transiently expressed in an appropriate manner when transfected into cells representing either early or late developmental stages. With this protocol we have investigated whether regulation requires linkage of the μ_s and μ_m sites and have examined the effects of sequence deletions and substitutions in the intronic region between the μ_s site and the μ_m exons. This paper describes the results of these experiments and discusses their implications for models of μ_s vs. μ_m regulation.

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Abbreviations: bp, base pair(s); kb, kilobase(s); C, constant; SV40, simian virus 40.

MATERIALS AND METHODS

Plasmid Construction. To construct pSV5C$_{\mu_{s-m}}$ (Fig. 1*A*), a 6-kilobase (kb) *Bgl* II fragment of the μ constant (C) region gene was isolated from the phage CH28-257.4 (13) and ligated into the *Bgl* II site within the *neo* gene of pSV5neo (14). The pSV5C$_{\mu_m}$ construct was made by deleting a 1290-base-pair (bp) *Hae* II–*Kpn* I fragment containing the μ_s poly(A) addition site from pSV5C$_{\mu_{s-m}}$. The pSV5C$_{\mu_s}$ construct was made by deleting a 2977-bp *Eco*RV fragment containing the μ_m region from pSV5C$_{\mu_{s-m}}$ (Fig. 1*B*). Portions of the μ_s–μ_m intron of pSV5C$_{\mu_{s-m}}$ were deleted as follows: ΔAK has 891 bp deleted between the *Acc* I and *Kpn* I sites, ΔAR has 797 bp deleted between the *Acc* I and *Eco*RV sites, ΔAN has 446 bp deleted between the *Acc* I and *Nco* I sites, and ΔNR has 351 bp deleted between the *Nco* I and *Eco*RV sites (Fig. 1*B*). The three plasmids that contain miscellaneous DNA fragments inserted into the ΔAR μ_s–μ_m intron at the *Kpn* I site are ΔAR/rpI$_{900}$, containing a 900-bp *Bam*HI–*Sma* I fragment from an intron of the S16 ribosomal protein gene (ref. 15; a gift from O. Meyuhas); ΔAR/pBR$_{910}$, containing a 910-bp *Alu* I fragment from pBR322; and ΔAR/pBR$_{403}$, containing a 403-bp *Alu* I fragment from pBR322. The μ_{s-m}/rpI$_{900}$ construct has the S16 ribosomal protein intron fragment inserted into the undeleted μ_s–μ_m intron.

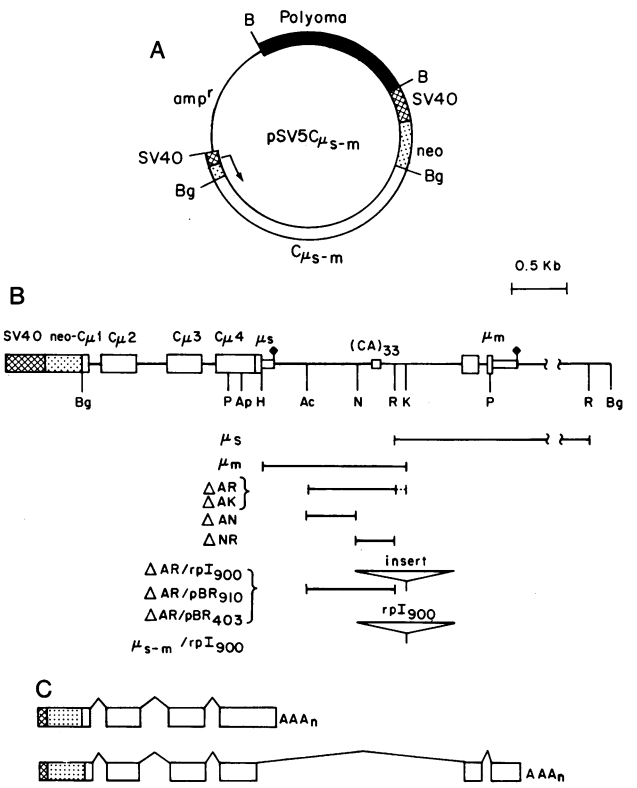

FIG. 1. Map of recombinant constructs and resulting chimeric transcripts. (*A*) Map of pSV5C$_{\mu_{s-m}}$. The 6-kb μ constant region (C$_{\mu_{s-m}}$) is denoted by the open bar; the *neo* gene sequences are stippled bars; the simian virus 40 (SV40) sequences are cross-hatched; the polyoma sequence is a filled-in bar; the pBR322-derived sequences are denoted by a thin line; direction of transcription is shown by an arrow. The plasmid pSV5C$_{\mu_{s-m}}$ is 15.5 kb. (*B*) Enlarged map of the SV40-*neo*-C$_{\mu_{s-m}}$ region. The SV40 and *neo* sequences are denoted as in *A*. Open bars indicate μ region exons (wide bars are protein encoding; narrow bars are 3′ untranslated regions); thin lines are introns and the 3′ flanking region. The individual exons are labeled above the diagram. The vertical line in C$_\mu$4 indicates the 5′ μ_m splice site; the diamonds denote the cleavage/polyadenylylation sites. The small box labeled (CA)$_{33}$ within the large intron is the alternating purine/pyrimidine tract. The constructions described in this paper are listed on the left below the map; the bars indicate sequences that have been deleted; insertions into the *Kpn* I site are indicated. (*C*) Structure of the two chimeric transcripts produced from pSV5C$_{\mu_{s-m}}$. SV40, *neo*, and C$_\mu$ sequences are represented as above. The splicing patterns for the 1.8-kb μ_s-terminated and 2.0-kb μ_m-terminated transcripts are shown. Ac, *Acc* I; Ap, *Apa* I; B, *Bam*HI; Bg, *Bgl* II; H, *Hae* II; K, *Kpn* I; N, *Nco* I; P, *Pst* I; R, *Eco*RV.

Cell Culture and DNA Transfection. The myeloma cell line MPC11 was maintained in Dulbecco's modified Eagle's medium (GIBCO) containing 10% horse serum (16). The Abelson murine leukemia virus-transformed cell line 3-1 (17) and the immature B-cell line 70Z/3 (18) were maintained in RPMI medium (GIBCO) containing 10% fetal bovine serum. The transient transfection assays were carried out by using the DEAE-dextran (Pharmacia) protocol, followed by a chloroquine diphosphate (Sigma) treatment as described by Grosschedl and Baltimore (19). Consistent results were obtained in replicate (generally ≥3) transfection experiments with the same construct and cell line.

RNA Preparation and Analysis. Transfected MPC11 cells were harvested 40 hr after transfection; transfected 3-1 and 70Z/3 cells were harvested 24 hr after transfection. Either total RNA or cytoplasmic RNA was extracted (20) and passed over oligo(dT) columns to isolate poly(A)$^+$ RNA. Transfer blot analysis of the RNA was performed on 1.5% agarose/formaldehyde gels as detailed elsewhere (21). The fragments used for S1 nuclease analysis are diagramed in Fig. 3. Twenty to 30 ng of double-strand probe was precipitated with 1 μg of poly(A)$^+$ RNA and 100 μg of nonspecific carrier RNA. For the μ_s probe, hybridization was carried out in 80% formamide/0.6 M NaCl/10 mM Pipes, pH 6.5/1 mM EDTA at 50°C for at least 6 hr. For the μ_m probe, hybridization was as above except in 90% formamide at 37°C (11). The samples were treated with S1 nuclease and analyzed as described elsewhere (21).

RESULTS

Transfected Construct Is Appropriately Regulated. For transient transfection experiments we used a derivative of the pSV5neo construct that contains the early region of polyoma virus and is therefore capable of replicating in mouse cells (14). A chimeric gene was constructed by inserting a 6-kb fragment containing the 3′ portion of the C_μ gene into the *Bgl* II site within the coding region of the *neo* gene. The resulting plasmid, termed pSV5C$_{\mu_{s-m}}$ (Fig. 1*A*), contains most of the C$_\mu$ coding exons, the two μ_m exons, five intact introns, and 2.1 kb of 3′ flanking sequence (Fig. 1*B*). Transcription from this gene is driven by the SV40 promoter and enhancer. Since the transcripts contain 322 bases of the *neo* gene fused to the first constant region exon (Fig. 1*C*), they can be readily discriminated from endogenous μ transcripts on RNA transfer blots if a probe specific for the SV40-*neo* sequences is used. Chimeric transcripts that are cleaved and polyadenylylated at the μ_s site and are properly spliced would be ≈1.8 kb, whereas those that are cleaved and polyadenylylated at the μ_m site and properly spliced would be ≈2.0 kb.

As a representative mature B cell we used MPC11, an IgG-secreting myeloma, in which the ratio of secreted to membrane γ2b mRNA is at least 50:1. Since there is no endogenous production of μ mRNA in MPC11 cells, transcripts from the transfected genes can be assayed with C$_\mu$ probes as well as with the SV-neo probe. As cells representative of early B-cell stages, we used 70Z/3, an immature B cell, and 3-1, an Abelson murine leukemia virus-transformed pre-B cell; in these cells the ratio of μ_m to μ_s mRNA is about 2:1 (22–24).

When pSV5C$_{\mu_{s-m}}$ was transfected into MPC11 cells, a single component of 1.8 kb, the size predicted for the μ_s-terminated chimeric transcript, was detected (Fig. 2*A*, lane 1). This component also hybridized with a C$_\mu$-specific

probe, but not with a μ_m-specific probe (data not shown). When pSV5C$_{\mu_{s-m}}$ was transfected into 70Z/3 or 3-1 cells, two components were observed: a 1.8-kb species indistinguishable from that produced in MPC11 cells and a 2.0-kb species that has the predicted size of a μ_m-terminated chimeric transcript (Fig. 2*A*, lanes 2 and 3). S1 nuclease protection analysis (see below) verified that the 1.8- and 2.0-kb components were respectively terminated at the proper μ_s and μ_m poly(A) addition sites. The ratio of membrane to secreted chimeric mRNA in 70Z/3 and 3-1 varied from 1:1 to 1:3 in various experiments. Although this ratio is not as high as that of the endogenous mRNA, it is nevertheless substantial when compared to the negligible proportion of μ_m-terminated transcript produced in the transfected myeloma cells. These results indicate that the control mechanisms that regulate the endogenous levels of secreted and membrane-associated mRNA are also effectively operating on the transcripts of the transfected chimeric genes.

Regulation Requires that μ_s and μ_m be Physically Linked. The first question addressed with this system was whether regulated production of μ_s and μ_m mRNA would still occur when the μ_s and μ_m poly(A) addition sites were located on separate transcripts. If it did, it would argue that interactions involving local cleavage/polyadenylylation signals are sufficient for the regulation and would therefore tend to rule out models that implicate other features of the gene organization. We constructed two plasmids that can produce only one or the other of the two chimeric transcripts. One construct, pSV5C$_{\mu_s}$, contains the μ_s poly(A) addition site and 1.1 kb of 3′ flanking sequence but lacks 600 bp of the intron and all μ_m coding exons. The other, pSV5C$_{\mu_m}$, lacks the μ_s poly(A) addition site but retains the $C_\mu 4$–μ_m splice junctions, including 74 bp of 5′ and 494 bp of 3′ intronic sequence, as well as the μ_m exons and 3′ flanking sequence (see Fig. 1*B*).

When pSV5C$_{\mu_s}$ and pSV5C$_{\mu_m}$ were individually transfected into MPC11 or 3-1 cells, a single transcript of the expected size containing C_μ and SV-*neo* sequences was seen in each case (Fig. 2*B*, lanes 1, 2, 4, 5, 8, and 9). Moreover, the relative usage of the two unlinked poly(A) addition sites is the same in both cell types; when pSV5C$_{\mu_s}$ and pSV5C$_{\mu_m}$ were cotransfected into MPC11 or 3-1 cells, the relative amounts of μ_s- and μ_m-terminated transcripts produced were roughly proportional to the relative amount of DNA transfected (Fig. 2*B*, lanes 3, 6, 7, 10, and 11). Thus, the regulated production of μ_s and μ_m mRNA is abolished when the μ_s and μ_m poly(A) addition sites are on separate transcripts; linkage of the two sites is apparently essential for their stage-specific usage.

To verify that the 1.8- and 2.0-kb transcripts have been cleaved at the authentic poly(A) addition sites, we assayed the RNA from MPC11 transfectants by S1 nuclease-protection analysis with probes specific for the μ_s and μ_m termini. As seen in Fig. 3, pSV5C$_{\mu_s}$ and pSV5C$_{\mu_m}$ direct transcripts that have the same 3′ ends as the endogenous μ_s and μ_m RNAs of 70Z/3 cells. The S1 nuclease assay also demonstrated that pSV5C$_{\mu_{s-m}}$-directed transcripts are accurately cleaved and polyadenylylated at the μ_s site in MPC11 cells and that the quantity of μ_m-terminated transcripts in these cells is negligible, as was indicated by the RNA transfer blot data. These data confirm that the chimeric transcripts are indeed faithfully cleaved and polyadenylylated and, therefore, that the variable–diversity–joining (VDJ) portion of a normal heavy chain transcript is not required for accurate cleavage/polyadenylylation or for proper regulation.

Intron Deletions Affect Regulation. Since the μ_s and μ_m poly(A) addition sites must be located "in cis" for proper regulation to occur, we examined the intron between the μ_s site and the μ_m exons for the presence of specific sequences that might be involved in the regulation. For this analysis, we

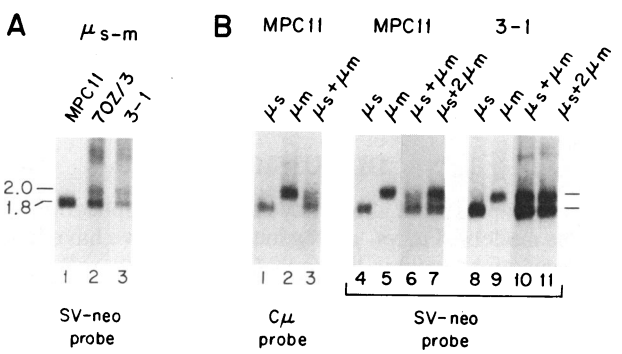

FIG. 2. RNA transfer blot analysis of the transcripts from linked and unlinked C_μ gene constructs transfected into lymphoid cell lines representing different developmental stages. (*A*) Linked construct, pSV5C$_{\mu_{s-m}}$, transfected into MPC11, 70Z/3, and 3-1 cells. One microgram of poly(A)$^+$ RNA from cells transfected with pSV5C$_{\mu_{s-m}}$ was probed with a nick-translated 384-bp fragment of SV40-*neo* sequences (SV-neo probe). The two chimeric transcripts of 2.0 kb and 1.8 kb are indicated. (*B*) Unlinked constructs, pSV5C$_{\mu_s}$ and pSV5C$_{\mu_m}$, transfected into MPC11 and 3-1 cells. One to 2 μg of poly(A)$^+$ RNA from transfected MPC11 cells was probed either with nick-translated SV-neo probe or the 6-kb *Bgl* II fragment containing C_μ sequences (C_μ probe) as indicated. RNA from transfected 3-1 cells was probed with the SV-neo probe. The transfected DNA is shown above each lane: μ_s, pSV5C$_{\mu_s}$; μ_m, pSV5C$_{\mu_m}$; $\mu_s + \mu_m$, approximately equal amounts of each plasmid; $\mu_s + 2\mu_m$, an approximately 2-fold excess of the μ_m plasmid. The total amount of DNA transfected was kept constant at ≈0.2 pg per cell. The diffuse background seen in some blots is due to transcripts that also hybridize with a pBR-amp region probe; these "read-through" transcripts were more prevalent in 70Z/3 and 3-1 transfectants than in MPC11 transfectants.

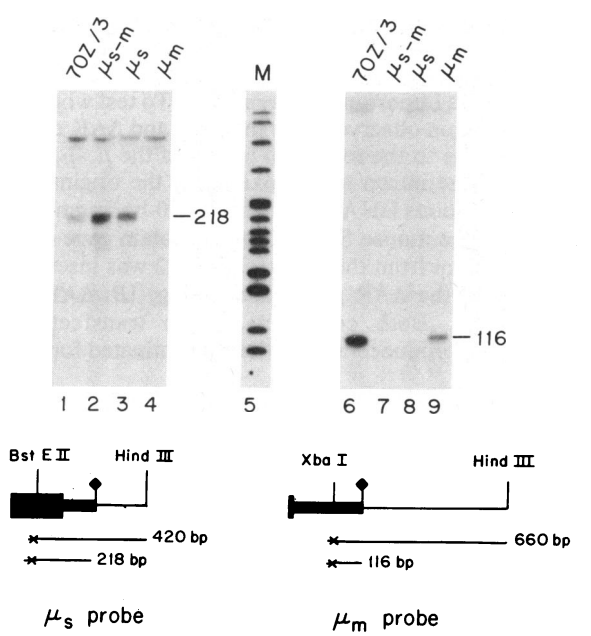

FIG. 3. Authenticity of the 3′ ends of transcripts from transfected genes demonstrated by S1 nuclease-protection analysis. Two micrograms of poly(A)$^+$ RNA from untransfected 70Z/3 cells and 1 μg of poly(A)$^+$ RNA from transfected MPC11 cells were analyzed with μ_s-specific (*Left*) and μ_m-specific (*Right*) probes. The probes and the predicted protected fragments are diagramed at the bottom: large solid bars are coding portions of exons, smaller solid bars are 3′ untranslated regions, thin lines are introns or 3′ flanking DNA, and solid diamonds are the cleavage/polyadenylylation sites. Each lane is labeled with the specific RNA used in the reaction: 70Z/3, untransfected 70Z/3; μ_{s-m}, MPC11 transfected with pSV5C$_{\mu_{s-m}}$; μ_s, MPC11 transfected with pSV5C$_{\mu_s}$; μ_m, MPC11 transfected with pSV5C$_{\mu_m}$; M, *Msp* I-digested pBR322 marker fragments. The lengths of the protected fragments in nucleotides are indicated.

constructed deletions that remove 797 or 891 bp from the central region of this 1858-bp intron. These constructs, ΔAR and ΔAK, respectively (Fig. 1*B*), retain 311 bp of sequence 3′ of the conserved AATAAA motif associated with the μ_s poly(A) addition site. Since deletion analyses of this and other poly(A) addition sites have indicated that only 30–50 bp of sequence 3′ of the AATAAA motif are required for proper function (6, 12, 25–27), the ΔAR and ΔAK deletions should not directly inhibit the μ_s cleavage/polyadenylylation reaction. Nevertheless, when ΔAR or ΔAK was transfected into MPC11 cells, the μ_s transcript was no longer exclusively produced. Instead, regulation was disturbed, resulting in nearly equivalent production of μ_s and μ_m chimeric transcripts. This was observed by RNA transfer blot analysis (Fig. 4, lanes 2, 3, 6, and 7) and quantitative S1 nuclease-protection assays (Table 1).

A comparison between the mouse and human sequences of the μ_s–μ_m intron has revealed an alternating purine/pyrimidine stretch as the only major conserved feature (28, 29). Since this stretch [$(CA)_{33}$] was removed by the ΔAR and ΔAK deletions (see Fig. 1*B*), it was suspected that this sequence might be involved in the regulation. To examine this possibility, we constructed two smaller deletions: ΔAN, which removes 446 bp from the intron but leaves the CA repeat intact, and ΔNR, which removes 350 bp that include the CA repeat. There was little difference between the transcripts produced from ΔAN and ΔNR when they were transfected into MPC11 cells (Fig. 4, lanes 5 and 8; Table 1), indicating that the $(CA)_{33}$ sequence is not the critical regulatory element. Some deregulation was observed with these deletions, although the proportion of μ_m-terminated transcripts was substantially less than that observed with ΔAR or ΔAK. This could either mean that no specific regulatory sequence is present within this region or that each of the two smaller deletions encompasses a regulatory sequence that can partially compensate for the other.

Intron Size Is Important for Regulation. To test whether the loss of regulation observed with the ΔAR and ΔAK deletions was simply due to the reduction in size of the μ_s–μ_m intron, we restored the intron to approximately the original length with miscellaneous DNA sequences. A 900-bp fragment from an intron of the mouse S16 ribosomal protein gene (15) or a 910-bp fragment from the plasmid pBR322 was inserted into the middle of the ΔAR μ_s–μ_m intron (Fig. 1*B*; $\Delta AR/rpI_{900}$, $\Delta AR/pBR_{910}$). Both constructs, when transfected into MPC11 cells, produced mainly the μ_s-terminated form of the chimeric transcript, as is routinely seen with the undeleted $pSV5C_{\mu_{s-m}}$ construct (Fig. 4, lanes 1, 4, 9, and 10; Table 1). Regulation was thus effectively restored by these miscellaneous sequence substitutions. A 403-bp pBR322 sequence inserted at the same site of ΔAR ($\Delta AR/pBR_{403}$) also increased the usage of the μ_s poly(A) addition site (Fig. 4, lane 11; Table 1) but not as effectively as the larger insertions. With the construct μ_{s-m}/rpI_{900}, which has an additional 900 bp inserted into the normal intron, essentially all transcripts are μ_s-terminated (Table 1).

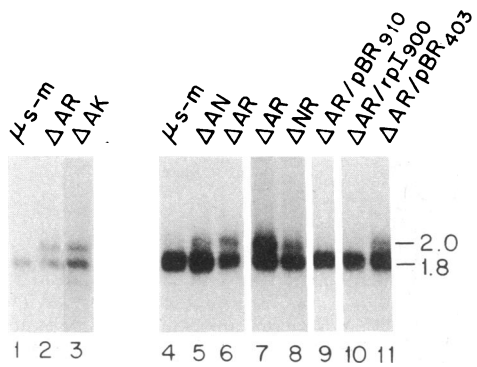

FIG. 4. Effects of deletions and intron substitutions on the regulated expression of chimeric C_μ genes in MPC11 cells: transfer blot analysis of 1.5–2 μg of poly(A)$^+$ RNA from MPC11 cells transfected with the indicated constructs. Blots were hybridized with the nick-translated SV-neo probe. The autoradiograms of the experiments in lanes 1 and 2 were exposed for a shorter time than those for the duplicate experiments in lanes 4, 6, and 7. Lanes 6 and 7 are the results from two independent transfection experiments. The 2.0-kb and 1.8-kb chimeric transcripts are indicated.

Table 1. Relative utilization of μ_m and μ_s sites in transcripts of transfected genes in MPC11 cells

Plasmid construction	Intron length, nucleotides	μ_m:μ_s ratio
ΔAK	967	0.74
ΔAR	1061	0.64 ± 0.33
ΔAN	1412	0.18
$\Delta AR/pBR_{403}$	1464	0.19 ± 0.04
ΔNR	1507	0.22
$pSV5C_{\mu_{s-m}}$	1858	0.05 ± 0.04
$\Delta AR/rpI_{900}$	1961	0.125 ± 0.005
μ_{s-m}/rpI_{900}	2758	0

One or two RNA samples from the replicate transfection experiments used for the transfer blots were subjected to quantitative S1 nuclease analysis. Various exposures of autoradiographs such as those shown in Fig. 3 were densitometrically scanned and the ratios were calculated by using the untransfected 70Z/3 cells as an internal standard; the ratio of μ_m-terminated to μ_s-terminated transcripts in 70Z/3 cells was determined to be 2.0 by RNA transfer blot analysis.

The foregoing results indicated that there is an inverse correlation between the length of the intron and the relative usage of the μ_m poly(A) addition site in MPC11 cells. This trend was also seen in transfected 3-1 cells (Fig. 5). The construct with the shortest intron, ΔAR, has the highest ratio of μ_m- to μ_s-terminated transcripts. The construct with the intermediate intron length, ΔAN, has a μ_m:μ_s ratio that is lower than ΔAR but higher than $pSV5C_{\mu_{s-m}}$ and $\Delta AR/rpI_{900}$. The construct μ_{s-m}/rpI_{900}, which has the longest intron, has the lowest μ_m:μ_s ratio.

DISCUSSION

The results presented above have important implications for various models of μ_s vs. μ_m regulation. First, we have found that regulation requires linkage of the μ_s and μ_m cleavage/polyadenylylation sites. When two plasmids, each containing one of the sites flanked by at least 1 kb of normal sequence, are cotransfected into an appropriate recipient cell, the usage of the μ_s and μ_m poly(A) addition sites is not regulated. This result, in agreement with the recent findings of Danner and Leder (6), demonstrates that Ig-secreting cells are perfectly capable of processing and accumulating substantial quanti-

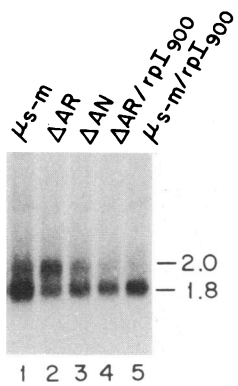

FIG. 5. Effects of deletions and intron substitutions on the regulated expression of chimeric C_μ genes in 3-1 cells: transfer blot analysis of 2 μg of poly(A)$^+$ RNA from 3-1 cells transfected with the indicated constructs. The blot was hybridized with nick-translated SV-neo probe. The 2.0-kb and 1.8-kb chimeric transcripts are indicated.

ties of μ_m-terminated RNA provided that this RNA is derived from a transcript that does not contain a functional μ_s poly(A) addition site. Thus, Ig secretors are unlikely to favor μ_s mRNA production by selective destabilization of μ_m mRNA or by strong inhibition of the μ_m-specific processing reactions. Furthermore, we did not observe preferential usage of the unlinked μ_m poly(A) addition site over the unlinked μ_s poly(A) addition site in cotransfected cells that were producing mainly μ_m mRNA from their endogenous genes. Preferential usage would be expected if, as postulated by Blattner and Tucker (30), the two cleavage/polyadenylylation recognition signals had markedly different affinities for a common processing factor that was rate-limiting in such cells.

Second, the striking effects of intron length perturbation are difficult to reconcile with models in which regulation depends solely on alternative recognition of the localized cleavage/polyadenylylation signals by trans-acting factors. A premise of such models is that μ_m-terminated transcripts assume a conformation that favors splicing of the $C_\mu 4$ and μ_m exons over cleavage at the μ_s poly(A) addition site. It is doubtful that such a specific conformation would be preserved in transcripts from the deletion-bearing constructs, which invariably yield the highest ratios of μ_m:μ_s RNA. The fact that accessibility of the μ_s site is not affected by miscellaneous sequence replacements also suggests that a highly specific conformation of this region is not critical for regulation.

A model involving competition between the μ_s cleavage/polyadenylylation reaction and the μ_m splicing reaction is more compatible with our results. Since these two reactions are mutually exclusive, a change in either one would affect the other. For example, the splicing reaction could occur at a constant rate in all B-cell developmental stages while the efficiency of cleavage/polyadenylylation changed. In early cells, if the rate of cleavage/polyadenylylation at μ_s were slow, then the splice to μ_m could efficiently compete and a significant amount of mature μ_m mRNA could be made. If the efficiency of usage of the μ_s poly(A) addition site were increased in a mature plasma cell, then the splice to μ_m would not be able to compete and very little μ_m mRNA would be made. This same scenario can be developed for the case in which the cleavage/polyadenylylation rate is constant and the splicing efficiency varies over B-cell development.

Our results could be interpreted in light of this model if the splicing efficiency increased as the intron length decreased. In previous studies of mRNA splicing, the requirements for specific sequences at the splice boundaries and a minimum intron length have been identified (e.g., refs. 31–33). Although a systematic variation in splicing efficiency that correlates with the length of the intron has not been reported, this could simply reflect the inadequacy of the assay that is generally used—namely, a measurement of the amount of spliced vs. unspliced precursor. Perhaps, if a competing reaction such as cleavage/polyadenylylation were introduced, an inverse correlation between splicing efficiency and intron length might be detected. Even if splicing efficiency, *per se*, were not affected, decreasing the intron length could indirectly favor the splice reaction by decreasing the time interval between the synthesis of the μ_s cleavage/polyadenylylation signal and the 3′ splice junction. The splicing reaction would presumably become an effective competitor only after formation of the "spliceosome" complex (34–36), which is apparently dependent on interactions with the 3′ splice junction (37, 38).

The results of Danner and Leder (6) are also consistent with a model based on competition between cleavage and splicing. When the μ_s cleavage/polyadenylylation signal was progressively weakened by a series of 3′ deletions, the amount of μ_s mRNA decreased and the amount of μ_m mRNA increased. This could be viewed as the reciprocal of our intron deletion experiments—i.e., when the competing μ_s cleavage/polyadenylylation reaction was attenuated, there was a compensatory increase in the product of the splicing reaction. Since, in both of these experiments, effects on regulation are seen by artificially altering one of two competing reactions, the actual reaction that is regulated *in vivo* cannot be specified. Nonetheless, these results raise the possibility that regulation of μ_s vs. μ_m mRNA production can occur by means of a competition between these two reactions, one of which could be regulated during B-cell development.

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