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# Regulatory elements necessary for termination of transcription within the Ig heavy chain gene locus

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## ABSTRACT

**Previous experiments have shown that the extent of δ gene transcription during B cell development is regulated primarily at the transcriptional level. We have shown that deletion of a sequence located between the μ and δ coding regions in the Ig heavy chain locus where transcriptional termination has been previously mapped abrogates the termination. Restoration of termination requires reintroduction of this segment as well as sequence elements within the μM poly (A) site which cannot be substituted by the μS poly (A) site. Recognition of the termination site by non-lymphoid cells suggests that initiation of δ transcription in mature B lymphocytes requires the activation of an anti-termination mechanism not yet developed in early B cells.**

## INTRODUCTION

Phenotypically distinct stages of B cell maturation can be characterized by the expression of the Ig genes. Pre-B cells express cytoplasmic μ heavy chains, immature cells express complete cell-surface IgM molecules, mature cells express both IgM and IgD, and secreting cells express low cell surface levels of all isotypes (Reviewed in 1). The δ gene, which encodes the constant region of the IgD heavy chain, contains no switch region and is located 3′ to the μ gene, which encodes the constant region of the IgM heavy chain (2–3). Previous work studying the regulation of the μ-δ gene locus has shown that a transcriptional mechanism is primarily responsible for the μ only phenotype found in both pre-B and immature B cells (4). Specifically, bone marrow derived B cells transcribe the δ gene at much lower levels than the μ gene. In these cells approximately 90% of all initiated polymerases terminate within the μ-δ intron whereas in mature, non-activated B cells expressing both IgM and IgD, only 40% of the polymerases terminate. Using an extensive panel of probes we have previously shown that beyond this discrete region of termination transcription remains essentially equimolar throughout the δ gene until some 2 kb 3′ of the last δM poly(A) addition site (5). Therefore, a change in the level of transcription termination 5′ to the δ gene in this location is correlated with maturation of B cells. Upon antigenic stimulation, B cells differentiate to high rate Ig secretion. High rate secretion is accompanied by increased loading of the μ gene and increased usage of the μS poly(A) site (6–8). This altered 3′ end processing is associated with a second transcriptional termination event which maps to positions more upstream than the location in non-secreting B cells (9–10).

Several groups have investigated the regulation of the 'secretion-associated' termination event. Law et al have evidence for a μM poly(A) site binding protein which is preferentially expressed in secreting cells (11). Tisch et al have identified a 1243 bp region containing the μM poly(A) site which appears to be necessary for termination in a plasmacytoma line (12). However, these studies of termination in activated B cells do not necessarily address the question of regulation of δ gene expression since termination associated with secretion occurs upstream of the site possibly involved in B cell maturation.

Because termination occurring in immature B cells has been mapped to a relatively restricted location, it was important to determine if sequences within this region of the Ig locus contain cis-acting elements which mediate this event. The results of experiments to be described below show that sequences within the μ-δ intron, containing a 162 bp unique sequence inverted repeat (USIR) can induce termination. This intronic sequence functions in an orientation dependent manner. Interestingly, these sequences are only effective when placed downstream of the μM poly(A) addition site. Furthermore, this termination motif is fundamentally different from those described in other transcriptional units in that the μM poly(A) site cannot be replaced by an alternative poly(A) site. In addition, our results shed new insights on the 'secretion-associated' termination event. In contrast to reported studies (12), we found that the μM poly(A) site and immediate downstream elements are not sufficient to induce polymerase termination and suggest that other cis-acting regulatory factors may be involved in this later stage of B cell differentiation.

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## MATERIALS AND METHODS

### Cell lines

M12.4 (13), J558L (14), Bine 4.8 (a non-producing subline of 18-81 (15) obtained from M.Wabl, University of California at San Franscisco) and RAW 264.7 (16) were maintained in RPMI supplemented with 10% FCS, glutamine, sodium pyruvate, non-essential amino acids, Hepes, and $5\times10^{-5}$ M 2-mercapto-ethanol. (Gibco, Grand Island, NY).

### Constructs

1.) *pLLn5R.* (W.Borne, P.-L.Lim and P.W.Tucker, unpublished) This 26 kb vector contains a S107 rearranged VDJ and an intact $\mu$-$\delta$ H chain gene in germ line configuration up to and including C$\delta$3. A fusion of the C$\delta$3 exon to the $\delta$M exons eliminates approximately 6 kb from the genomic sequence including the $\delta$S and $\delta$x exons. The Ig locus is cloned into the pSV2neo vector described by Mulligan et al. (17).

2.) *P$\mu$ (18).* This vector contains a rearranged Ig $\mu$ gene extending up to the *Xho I* site within the $\mu$-$\delta$ intron, the polyoma virus early region, and a modified histone gene.

3.) *P$\mu$M$\delta$M.* An approximately 5.3 Kb deletion from *Hpa I* to *Eco RV* of the Ig gene in P$\mu$ was made. Thus, this intermediate vector, called P$\mu$M-117 has deleted C$\mu$1 through C$\mu$4 and $\mu$S, leaving intact the rearranged VDJ, enhancer, the $\mu$M1 and $\mu$M2 exons and the 3′ untranslated region. Next, the 2340 bp *Bam HI* fragment containing $\delta$M1 and 2 (19) was cloned into the *Xho I* site in the 5′-3′ orientation. This construct contains, therefore, only the $\mu$M and $\delta$M exons and does not have the $\mu$-$\delta$ intervening region where termination has been observed.

4.) *P$\mu$MUSIR$\delta$S.* The *Pst I-Pvu II* fragment containing the USIR sequences and 5′ end of C$\delta$1 (20) was cloned into Pgem4. PCR primers were then generated to convert the *Kpn I* site of Pgem4 into a *Cla I* site (using the 5′ oligo) as well as to introduce a *Cla I* site just 5′ of the C$\delta$1 exon (using the 3′ oligo). The 5′ oligo used was a 20 mer with the sequence ATTCGAGATCG-ATACCCGGG. The 3′oligo was a 22 mer with the sequence CTGCAAGATCGATAGTGAAGTT. The oligos were designed so that they had a common annealing temperature of 40°C. This 1200 bp *Cla I* fragment was then amplified by PCR and cloned into the pBluescript II KS vector (Stratagene, La Jolla, CA). The 1800 bp $\delta$S containing *Bam HI* fragment from Pcp 26 (19) was then cloned into the same pBluescript II KS vector downstream of the *Cla I* insert. A 3643 bp *Sma I* fragment containing the USIR and entire $\delta$S intron and exon sequences in the 5′-3′ orientation was cloned into the blunted *Xho I* site of P$\mu$m-117 (described in 3).

5.) *P$\mu$SUSIR+.* The 1200 bp *Cla I* fragment just described was cloned 5′-3′ into P$\mu$950 linearized with *Cla I.* The P$\mu$950 vector (21) is a derivative of P$\mu$ containing a 950 bp deletion within the $\mu$S-$\mu$M intron which contains a unique *Cla I* site.

6.) *P$\mu$MrUSIR$\delta$M.* This vector was made by cloning, in the 3′-5′ orientation, a 1550 bp *Bam HI* fragment containing the 1200 bp *Cla I* fragment described above plus 350 bp of pBR flanking sequences into a *Bcl I* site just upstream of the $\delta$m1 exon and 100 bp downstream of the Xho I site in P$\mu$M$\delta$M. This construction places the USIR region in a reversed orientation directly between the $\mu$M and $\delta$M exons. Fig. 1 shows a schematic representation of the recombinant $\mu$-$\delta$ locus in all of the vectors we have used for transfection and 'run-on' analysis.

### Transient transfection

Transient transfections were performed essentially as described by Sherman et al.(22). Briefly, $2\times10^7$ cells were resuspended in 0.3 ml of complete media. 5−10 $\mu$g of cesium chloride purified supercoiled DNA was added and electroporation was performed at 250V and 960$\mu$F. Cells were diluted into 20 ml of complete media and assayed 48 hours later.

### Selection of stable transfectants

Cells were either singularly transfected with 5 $\mu$g of linearized plasmid (pLLn5R transfectants) or doubly transfected with 10 $\mu$g linearized P$\mu$ derivatives and 1 $\mu$g linearized pSV2neoS107 [the pSV2neoS107 vector is a 7 kb *Bam HI* insert coding for a rearranged S107 kappa light chain cloned into the pSV2neo vector (17)] at 250V, 960$\mu$F. Cells were then either subjected to bulk selection in G418 (Sigma, St Louis, MO) or were subcloned under selection at limiting dilution. Concentrations of G418 for optimal selection were determined to be 400$\mu$g/ml for M12.4 and J558L transfectants and 800$\mu$g/ml for Bine 4.8 transfectants.

### S1 analysis

S1 analysis was performed as described in Maniatis (23). To determine the ratio of $\mu$S-$\mu$M mRNA, a 3′ end-labeled 467 bp *Hpa II−Hind III* probe containing part of the C$\mu$4 exon, the $\mu$S polyadenylation site, and the 3′ untranslated region from CH28-257.3 (20) was hybridized to total cytoplasmic RNA at 46°C. After hybridization and digestion with Nuclease S1 (400 u/ml), protected fragments were run on a 6% denaturing acrlyamide gel and visualized by autoradiography. A 267 nt band corresponds to $\mu$S mRNA cleaved at the appropriate site. A 75 nt band corresponds to $\mu$M mRNA resulting from splicing at the $\mu$M site. The $\delta$S probe is a 5′ end-labeled 610 bp *Bam HI−Sau 3A* fragment which encompasses the $\delta$S exon and upstream sequences. Protection by appropriately spliced RNA yields a 220 nt fragment. The $\delta$M probe is a 5′ end-labeled 540 bp *Hind II−Cla I* fragment which contains the $\delta$m1 and $\delta$m2 exons. Protection by appropriately spliced RNA yields a 210 nt fragment. The $\mu$M probe is a 5′ end labeled 435 bp *Sph I−Xba I* fragment which covers the $\mu$m2 exon and some pBR sequences. Protection by appropriately spliced RNA yields a 170 nt protected fragment.

### Labeling of probes for S1 analysis

The 3′ end of the $\mu$S probe was labeled by filling in with 10$\mu$Ci of [$^{32}$P] $\alpha$-dCTP using Klenow fragment of DNA polymerase. The 5′ ends of the $\mu$M, $\delta$S, and $\delta$M probes were end-labeled using 5 $\mu$Ci each of [$^{32}$P] $\tau$-ATP using polynucleotide kinase.

### *In vitro* 'run-on' analysis

Nuclei were prepared from transfected cells and nascent RNA was labeled for 15 minutes at 30°C as previously described (6) using 200 $\mu$Ci of [$^{32}$P] UTP (3000 Ci/mole, ICN Radiochemicals, Irvine, CA) per sample. Following labeling, RNA was extracted, hybridized and analyzed as previously described (6). In order to be able to compare between experiments, hybridization to the $\mu$M probe (No. 2) is always normalized to 1.

### Probes for 'run-on' analysis

1.) *Cμ* is a 700 bp *Pvu II−Pst I* fragment from the cDNA of NZB (3741), containing most of the constant region sequences of the μ chain. (P.W. Tucker and K. Marcu, unpublished data) cloned into M13mp10. 1′) *Cμ′* is the above fragment cloned into M13mp11. 2) μM is a 917 bp *Kpn I-Hind II* genomic fragment from BALB/c which contains sequences that span the μm1 and μm2 exons, subcloned from Ch28-257.3 into M13mp10. 2′) μM′ is the above fragment cloned into M13mp11. 3) *XP* is a 347 bp *Xho I−Pst I* fragment of the μ-δ intron subcloned from pδ3.9 (20) into M13mp19. 3′) *XP′* is the above fragment cloned into M13mp18. 4) *PR* is a 565 bp *Pst I−Eco RI* fragment adjacent to XP of the μ-δ intron subcloned from pδ3.9 into M13mp19. 4′) *PR′* is the above fragment cloned into M13mp18. 5) *δ-528* is a 528 bp *Bam HI-HincII* fragment about 1450 bp 5′ of the start of the δS exon subcloned from Pcp 26 (5,19) into M13mp19. 5′) *δ-528′* is the above fragment cloned into M13mp18. 6) δS-*764* is a 764 bp *Hinc II−Bam HI* fragment 525 bp 3′ to δ-528, containing the δS exon and 3′ untranslated region of δS mRNA, (5,19) subcloned from Pcp 26 into M13mp19. 6′) δS-*764′* is the above fragment cloned into M13mp18. 7) δM is a 710 bp *Hinc II−Bgl II* genomic fragment containing δm1 and δm2, subcloned from Pcp 13 (19) into M13mp10. 7′) δM′ is the above fragment cloned into M13mp11. All probes have been sequenced and do not contain repetitive regions. All probes were checked for the presence of appropriate inserts to ascertain that the inserts were not lost during phage propagation. The numbered bars below the loci in Fig. 1 show the location of the probes within the constructs.

## RESULTS

### Recapitulation of transcription termination in transfected transcription units

In order to study the cis-acting elements that mediate transcription termination within the μ-δ intron, it was necessary to utilize recombinant Ig vectors transfected into Ig heavy chain loss mutants which represent different stages of normal B cell development. To verify that these Ig negative cell lines had retained the ability to regulate termination within the Ig μ-δ intron, DNA encoding a functionally rearranged, VDJ-Cμ-CδM1M2 locus (pLLn5R, see Fig. 1) was introduced. This vector contains a 6 kb deletion within the δ gene which fuses Cδ3 to δM1, removing δX and δS. Otherwise there are no changes from the germline sequence, spacing of the μ-δ intron, or flanking regions. Note that the segment encompassed by probes No. 3 and 4 is where we had previously mapped transcriptional termination in early B cells (4). The construct was transfected into three cell lines, representative of three B cell differentiation stages. Bine 4.8, is a pre-B cell lymphoma line, derived from an Abelson virus transformation. M12.4 is a lymphoma which is the closest analogue of a mature, non-secreting B cell. J558L is a

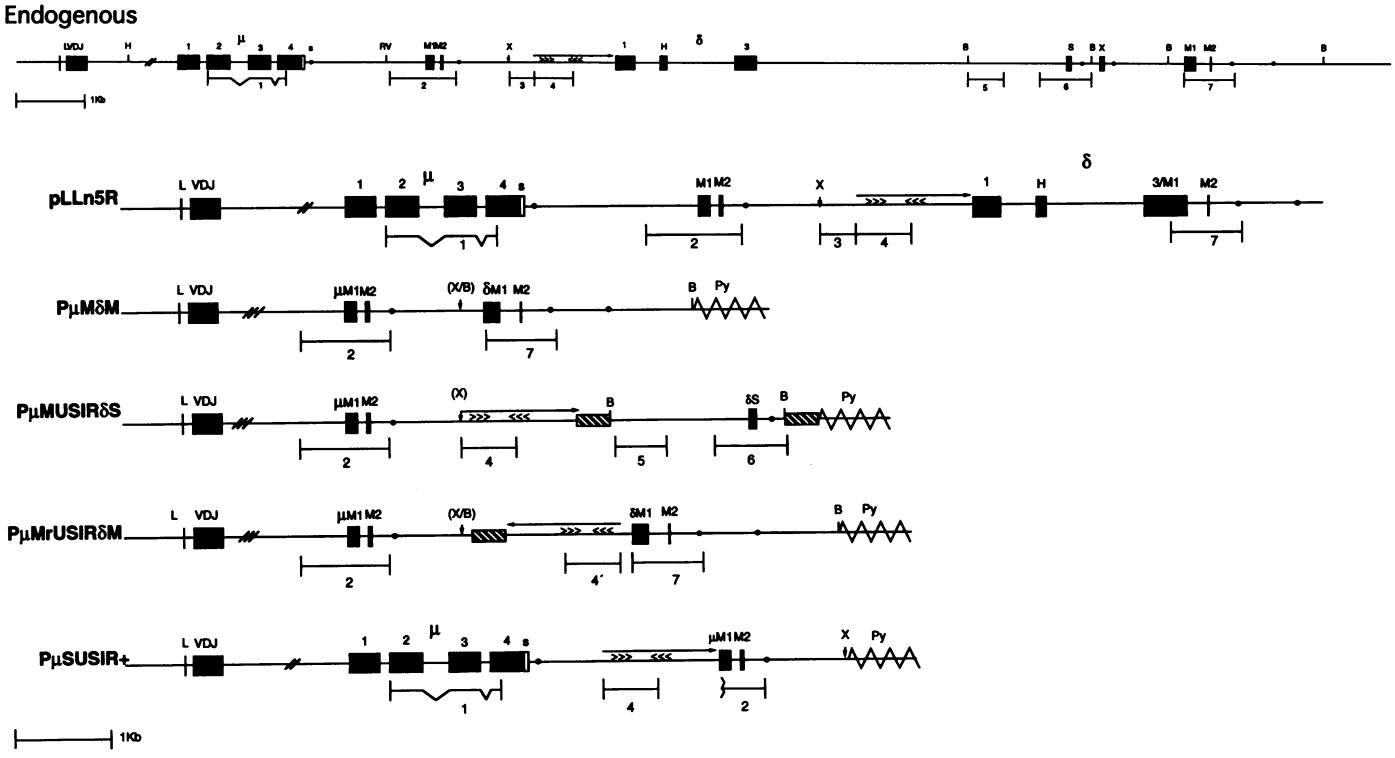


**Figure 1.** Schematic representation of the recombinant plasmids used in the 'run-on' analysis. The top panel depicts the non-mutated μ-δ locus. H, RV, X, and B indicate the positions of *Hpa* I, *Eco RV*, *Xho* I, and *Bam* HI sites, respectively. The *Bam* HI sites define the boundaries of the δS and δM regions cloned into the minigenes. Each construct is described in Materials and Methods and is drawn to scale. The horizontal arrow indicates orientation of the insert. The polyoma virus early region is denoted by Py. Hatched bars indicated pBR sequences. < < < > > > indicates the boundaries of the USIR structure. Bars and numbers below each construct indicate the single-stranded probes used in transcriptional analysis and are also described by number in Materials and Methods.

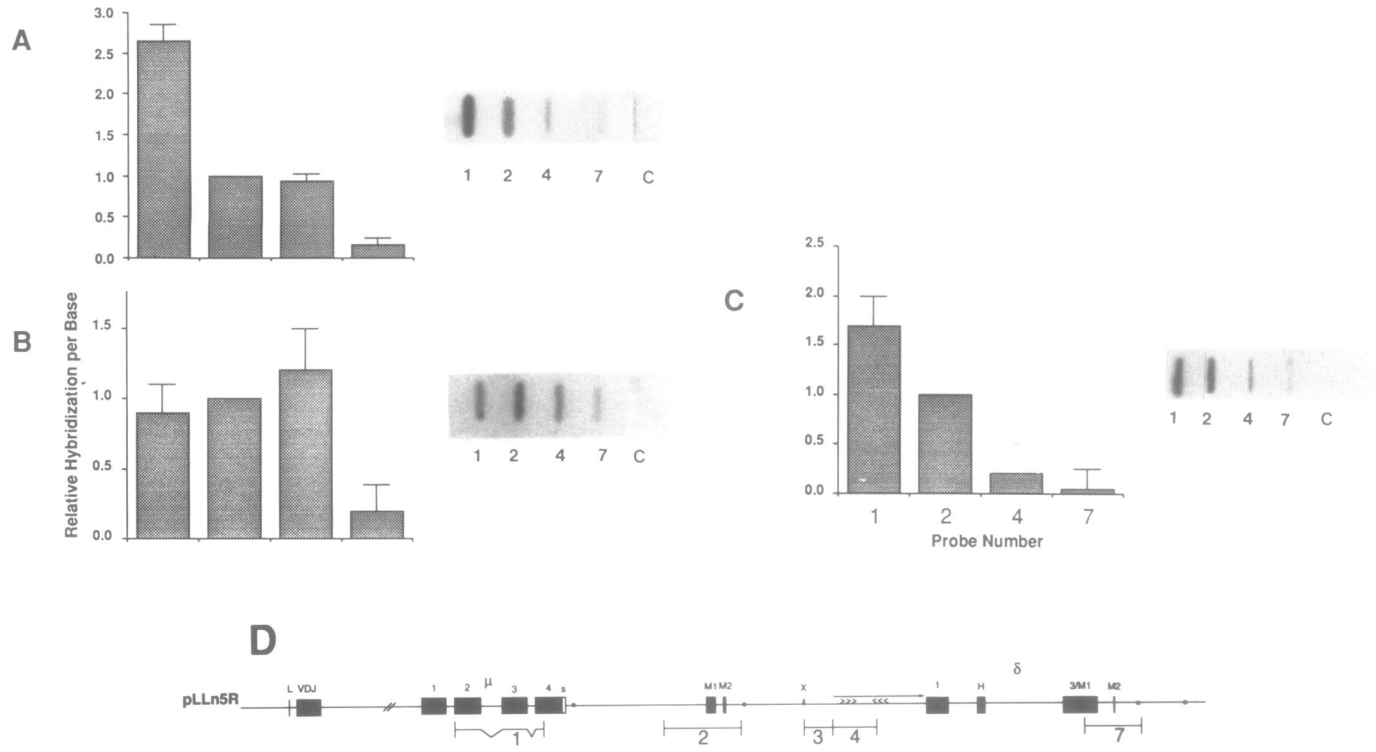


**Figure 2.** Transcription profiles of the pLLn5R transfectants. Stable transfections of the vector pLLn5R were made in **A)** Bine 4.8, **B)** M12.4 or **C)** J558L cells and analyzed by 'run-on'. Each graph is the average (+/− standard deviation) of at least three experiments. All profiles were normalized by comparing the level of hybridization to the probe #2 (shown as 1). One representative autoradiogram for each cell line is shown on the right of the transcription profile. **D)** Map of the recombinant pLLn5R locus showing the gene segments contained within the single-stranded probes used in the analysis.

plasmacytoma derived from an IgA-secreting terminally differentiated plasma cell. Stably transfected cells were selected by neomycin resistance and cloned. In order to determine if transcription termination occurs between the $\mu$ and $\delta$ exons of the transfected gene, nuclei were prepared from the transfectants and pulse-labeled with $P^{32}$ $\alpha$UTP. Fig 2 shows the extent of hybridization of nascent RNA to probes containing segments of DNA within this region.

The Bine 4.8 and M12.4 cell line each showed an approximately 80% decrease of $\delta$M transcription compared to $\mu$M. The J558L transfectant showed an approximately 95% decrease in $\delta$M transcription relative to $\mu$M. These results indicate that transcription termination does occur downstream of the $\mu$M exons and confirm previous findings utilizing normal B lymphocytes (4,10). In addition to the termination event prior to $\delta$M, transcription profiles in both Bine 4.8 and J558L, show polymerases downloading within the $\mu$M probe (No.2) relative to the C$\mu$ probe (No.1). This partial downloading of polymerases continued into the gene segment defined by probe No. 3 (see Fig. 1, data not shown) as well as probe No. 4. This pattern is reminiscent of that previously documented in both LPS stimulated B cells (9) and plasmacytomas (21,24). It is correlated with the observation that transfectants of these cells express higher levels of $\mu$S mRNA than transfectants of Ml2.4 cells (data not shown) and therefore must utilize the $\mu$S poly(A) site more extensively.

### Regulation of transcription termination by cis-acting elements

Since termination mapped to a region between $\mu$M and $\delta$M, a deletion construct was made to test whether or not any sequences within this region are necessary for termination. In addition, the C$\mu$ constant regions, including the $\mu$S exon and associated downstream sequences were also removed. In this way, we hoped to minimize possible effects of the termination event associated with differentiation to $\mu$S secretion. This minimal construct, P$\mu$M$\delta$M, was transiently transfected into all three cell lines (Fig. 3). As can be seen from the transcription profiles, the levels of $\delta$M and $\mu$M hybridization were equivalent in all cases. Furthermore, the results were not significantly different when a cell line containing the same construct stably integrated was analyzed (data not shown). Thus transcription termination within the $\mu$-$\delta$ intron requires cis-acting elements. However, due to the extensive deletions within this vector, regulation of termination could involve sequences within C$\mu$, C$\delta$, the $\mu$-$\delta$ intron or perhaps a combination of them all.

To narrow down the possibilities we first tested the most likely candidate: sequences within the $\mu$-$\delta$ intron. The P$\mu$MUSIR$\delta$S construct restores the $\mu$-$\delta$ intronic sequences containing the USIR element downstream of the $\mu$M poly(A) site. In order to avoid potential regulatory sequences contained within the downstream $\delta$M region, we inserted an 1800 bp $\delta$S containing fragment downstream of the 1200 bp USIR containing fragment. We chose this fragment as a 3′ indicator of termination in upstream sites because previous experiments (D. Yuan, unpublished observations) have indicated that sequences surrounding the $\delta$S exon do not induce transcriptional termination. Furthermore, termination was observed in pLLn5R (Fig. 2) despite the deletion of this region.

In all three cell lines, the transcription profile of the P$\mu$MUSIR$\delta$S transcription unit showed an approximately 80% decrease in $\delta$S transcription relative to $\mu$M (Fig. 4). The extent

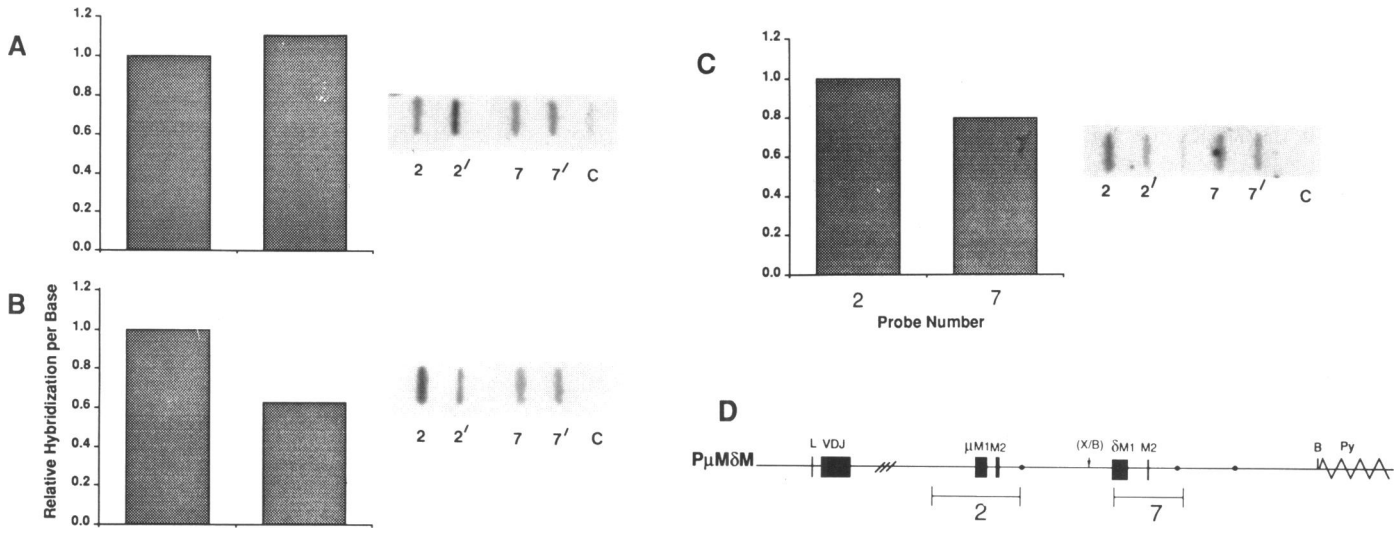


**Figure 3.** Transcriptional profiles of the PμMδM transfectants. **A)** Bine 4.8, **B)** M12.4, or **C)** J558L cells were transiently transfected with the vector PμMδM and analyzed for nascent RNA chain growth. Autoradiograms are shown to the right of each profile. **D)** Map of the recombinant PμMδM locus showing the gene segments contained within the single-stranded probes used for analysis.

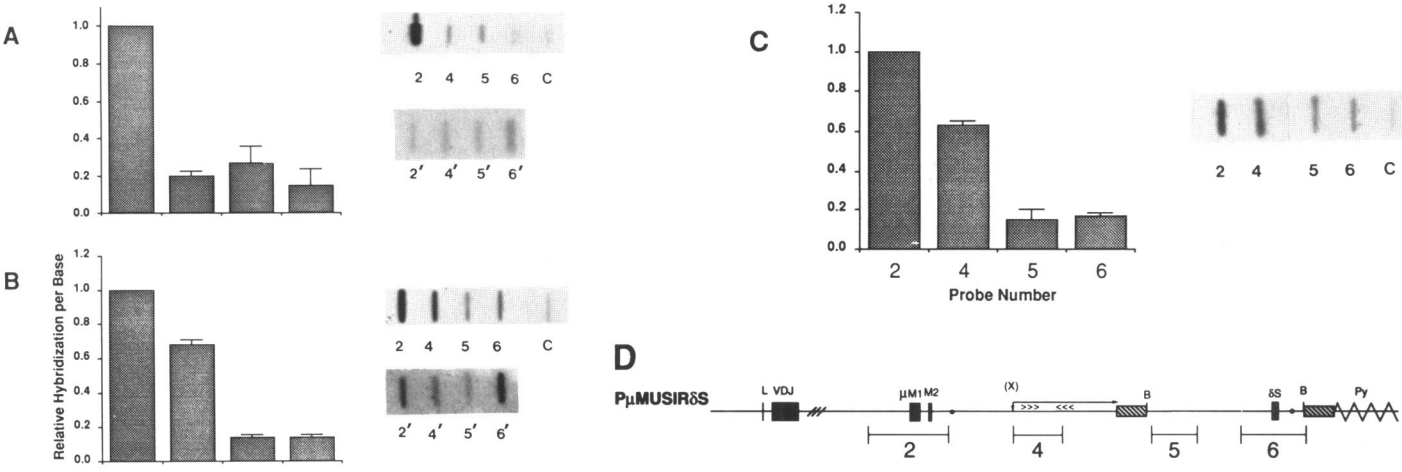


**Figure 4.** Transcription profiles of the PμMUSIRδS transfectants. Either **A)** Bine 4.8, **B)** M12.4, or **C)** J558L cells were transiently transfected with the PμmUSIRδS vector. Each graph is the average of at least three experiments (normalized as in Fig. 2). **D)** Map of the recombinant PμMUSIRδS locus showing gene segments contained within the single-stranded probes used for analysis. Representative autoradiograms are shown to the right of the transcription profile for each cell line.

**Table I.**

| Construct | Probe No. | Distance* | %Termination in Transfectants** | | | |
|---|---|---|---|---|---|---|
| | | | Bine 4.8 | Ml2.4 | J558L | RAW 246.7 |
| pLLn5R | 2 vs 7 | 4,360 | 80 | 75 | 95 | ND |
| PμMδM | 2 vs 7 | 1,030 | 0 | 30(30) | 15 | 0 |
| PμMUSIRδS | 2 vs 5 | 2,300 | 70 | 82 | 90 | 80 |
| | 2 vs 6 | 3,350 | 83 | 82 | 80 | 90 |
| PμMrUSIRμM | 2 vs 7 | 2,550 | 10 | 20 | ND | ND |
| PμSUSIR+ | 1 vs 2 | 2,300 | 0 | 0(0.3) | 0 | ND |

*The distance in bp between the 3′ end of the upstream probe and the 5′ end of the downstream probe.
**Percent termination = hybridization to the 3′ probe (relative to the μ-δ intron) minus hybridization to the 5′ probe divided by hybridization to the 3′ probe times 100. 0 indicates a value of less than 0 in the numerator. ND = not done. Numbers in brackets indicate termination found within a stable cell line containing the same construct.

of termination occurring in transfectants of each construct determined from the relevant probes is presented in Table I. These results indicate that the μM poly(A) site plus approximately 1200 bp spanning the USIR containing intronic sequences (from Pst I site up to the Cδ1 exon, see Fig 1) are sufficient for transcription termination. It should be noted that ample anti-sense transcription of the δS region can be observed. These anti-sense transcripts probably initiated within the Polyoma locus. Their presence confirms that the lack of sense transcription of δS is not a result of an aberrant deletion or rearrangement of this region in the transfected gene.

In order to test the requirement for directionality of the inserted sequences, We reversed the orientation of the 1200 bp USIR segment. Transfections of this vector, PμMrUSIRδM, into either Bine 4.8 (Fig. 5) or M12.4 cells (data not shown) demonstrated that termination was alleviated. Therefore the ability of the USIR sequence to induce termination is orientation dependent. Furthermore, this construct rules out the potential artifact that the termination seen with the correct orientation was due to the spacing between the regions covered by the 5′ and 3′ probes. Note that the distances between the 5′ and 3′ probes used to assess termination in these two constructs differs by only 250 bp (Table I).

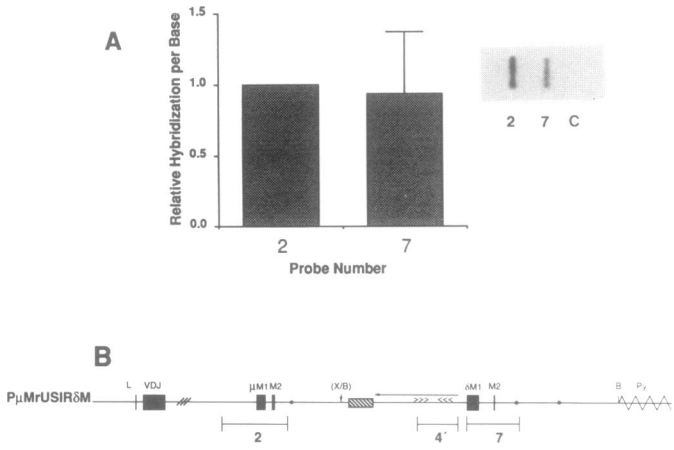


**Figure 5.** Transcription profile of the PμMrUSIRδM transient transfectants in Bine **A** 4.8 cells. The graph shown is the average of three experiments. One representative autoradiogram is shown to the right of the figure. **B)** Map of the recombinant PμMrUSIRδM vector showing gene segments contained within the single-stranded probes used in the analysis.

## Poly(A) site requirement for transcriptional termination

The termination motif in the β-globin gene has been shown to consist of a functional poly(A) site as well as an orientation dependent region of dyad symmetry located 900 bp downstream of the poly(A) site (25). In this system, the adenovirus E1A gene poly(A) site was able to substitute for the endogenous β-globin site. The ability to interchange these sites indicates a requirement merely for a functional poly(A) site, not a specific one (26). Similarly to β-globin, the USIR region of dyad symmetry is located downstream of a poly(A) site. In order to test whether another poly(A) site could substitute for the μM poly(A) site, the μS site was functionally substituted for the μM site. The entire 1200 bp USIR containing sequence was cloned downstream of the μS poly(A) site at a distance approximately equivalent to the distance from the μM poly(A) site in the endogenous location. As seen in Fig. 6, there was no evidence of transcription termination within the downstream μM probes in any cell line tested. It was important to verify that the utilization of the μS poly(A) site had not been hampered by this insertion. To do this, S1 protection assays were performed on cytoplasmic RNA from the J558L and Ml2.4 cell lines transfected with this construct (Fig. 7). Comparison of the relative μS vs μM abundance indicate that the J558L cells continue to express much greater amounts of μS mRNA and demonstrates that cleavage and processing at the μS site was not inhibited in these transfectants.

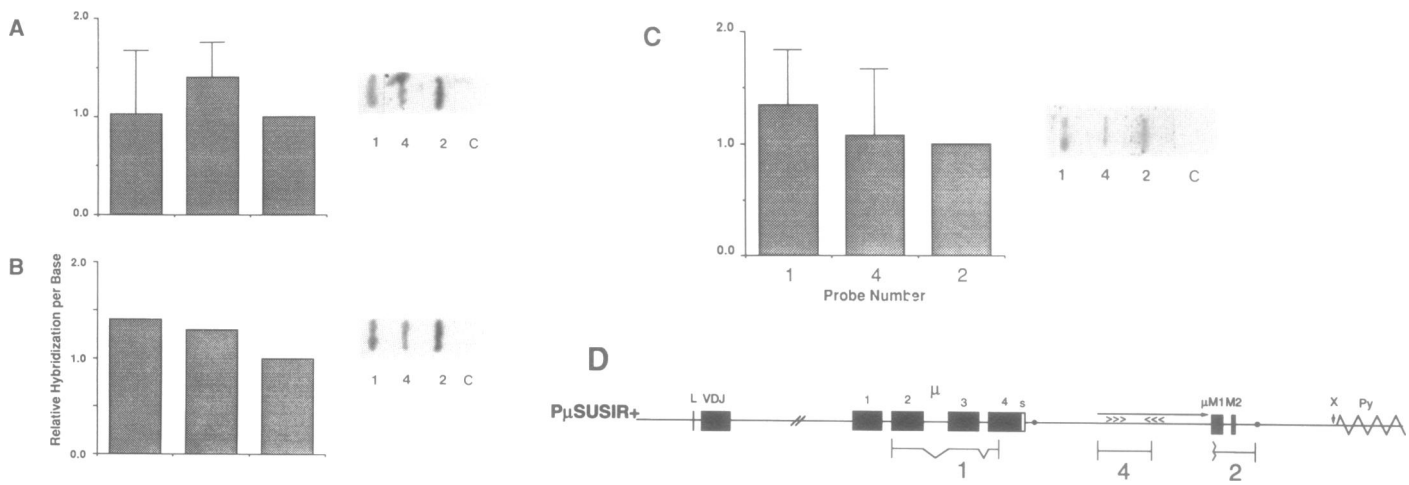


**Figure 6.**. Transcription profile of the transient transfections of the PμSUSIR+ vector. Transient transfections of the PμSUSIR+ vector were performed in either **A)** Bine 4.8, **B)** M12.4, or **C)** J558L cells. The transcription profiles are representative of at least two experiments. One representative autoradiogram is shown to the right of each cell line. **D)** Map of the recombinant PμSUSIR+ locus showing single-stranded probes used in the analysis.

Previous data have shown that the μS site is intrinsically weaker than the μM site, and that it is only used by virtue of its temporal transcription advantage over μM in mature cells where processing factors are limiting (21, 27−28). However, the μS site is able to compete efficiently in actively secreting cells where the putative processing factors are more abundant. If processing is linked to termination, it is possible that in transient transfections, in which many copies of the transcription unit are present per cell, processing and/or termination factors are limiting. To eliminate this possibility, stable transfections of these constructs were also analyzed (data not shown) The transcription profiles were found to be similar to that for transient transfections; therefore, gene copy number is probably not a factor. The results indicate that the μM site is an essential part of the termination motif in conjunction with sequences from the μ-δ intron.

### Tissue specificity of the termination motif

To determine whether or not the termination motif is lymphoid specific, transfectants of a non-lymphoid cell line were analyzed. The cell line chosen was RAW 264.7, a macrophage line. Fig. 8A shows that the transcription profile of PμMδM transfected into this line is similar to the B cell lines tested in that there was no evidence of transcriptional termination within the δM exons. Thus, the μM poly(A) site in conjunction with it's immediate downstream sequences does not induce termination. However, when this cell line was transiently transfected with the PμMUSIRδS construct (Fig. 8B), polymerase downloading was apparent in the downstream probes. Thus the termination motif is not specific for lymphoid cells and may contain an intrinsic pol II termination site which must be bypassed in more differentiated B cells.

## DISCUSSION

These experiments show that the Ig μ-δ locus contains an intrinsic pol II termination motif. The motif is bimodal, consisting of all or part of a 1200 bp intronic gene segment and regions surrounding the μM poly(A) site. The motif functions in an orientation dependent manner. Neither Cμ or Cδ constant regions nor sequences flanking them are required.

The 1200 bp USIR containing portion is similar to other termination motifs previously described (25−26, 29−31) in that it contains a region of dyad symmetry and functions in an orientation-dependent fashion. However, whether the 162 bp inverted repeat constitutes the critical element required for termination remains to be determined. It is clear that this segment cannot function when located downstream of an alternative functional poly(A) site. The location 3′ of the μM exons suggests that processing at the μM site is required for recognition of the termination region. And indeed, in all constructs we have tested, appropriate processing at all transcribed poly(A) sites has been demonstrated (data not shown). However, the fact that the μS site is unable to substitute for the μM site even in a plasmacytoma line where it is used extensively, shows that termination at the USIR sequence downstream of μM is not due to destabilization of the polymerase shortly after cleavage of the nascent transcript. Rather, it appears that sequences within the μM site itself may be important.

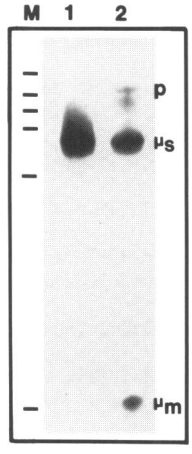


**Figure 7.** S1 Analysis of RNA from transfectants of the PμSUSIR+ vector. RNA was extracted from either J558L (Lane 1) or M12.4 (Lane 2) transfectants and hybridized with the a 467 nt *Hpa*II-*Hind*III 3′ end labeled probe. After Nuclease S1 digestion protected DNA was analyzed on a denaturing gel. M indicates positions of *Hinf*I digested pBR fragments run in parallel. The μS and μM bands correspond to fragments migrating at 267 nt and 75 nt, respectively.

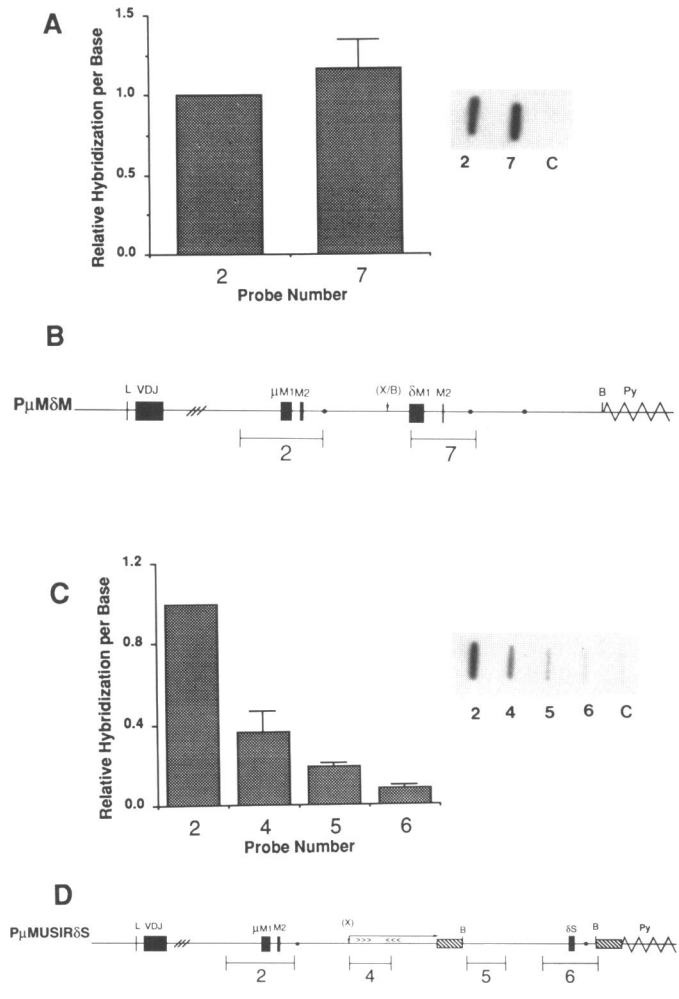


**Figure 8.**. Transcription profiles from non-lymphoid transfectants. The RAW 264.7 macrophage line was transiently transfected with **A)** the Pμmδm vector or **C)** the PμmUSIRδs vector. **B)** The map of the Pμmδm recombinant locus. **D)** The map of the PμmUSIRδs recombinant locus. Each graph is the average of two experiments. One representative autoradiogram is shown to the right of each figure.

It is clear from transfectants of Pμmδm constructs that in the absence of the 1200 bp sequence, the μM poly(A) site plus immediate 3′ sequences are not sufficient for the induction of termination. Surprisingly, these results are in direct contrast to those reported by Tisch et al.(12). They showed that a 1243 bp segment, including the μM poly(A) site and immediate downstream sequences, which has been left undisturbed in our PμMδM construct, is able to direct polymerase downloading within J558L, but not M12.4 cells. Furthermore, they conclude that sequences further downstream which include the USIR segment are not necessary. The reason for the disparate results from the two laboratories is not clear. However, the termination induced by the 1243 bp segment appears to be secretion associated in that it occurs only in cells which utilize μS extensively. Another conclusion made by these authors which appears to be in conflict with our results is that sequences which include the USIR segment are not necessary for termination in M12.4 cells. It should be noted that they have only altered the 870 bp region defined by Xho I- Eco RI sites which constitutes only the upstream portion of the 1200 bp fragment we have analyzed. Whether the difference resides in sequences 3′ of the Eco RI sites is now being investigated.

We are convinced that the sequence elements which encode the maturational termination event, are recognized by all cell lines tested, including a macrophage line. This indicates that there is no tissue specificity to this motif. We suggest that termination in this region is controlled by a default mechanism, resulting in pol II downloading at this motif whenever it is transversed. Any alleviation of termination, such as that occurring in mature B cells would require the activation of an anti-termination mechanism. Termination occurring in early B cells in this region must therefore be attributed to the immaturity or absence of this anti-termination mechanism. The orientation dependence of the termination motif suggests that the anti-termination mechanism may involve a single stranded nucleic acid-protein interaction that alleviates some inhibitory secondary structure and allows pol II to progress through to the δ gene. Alternatively, a polymerase modification factor may be required. However, this is a less likely mechanism since recognition of this motif does not appear to require appropriate promoter initiation. Sterile transcripts of the Ig locus in bone-marrow derived B cells (4) show termination within the μ-δ intron.

Finally it is important to note that although we have attempted to utilize tumor cell lines which represent various stages of B cell differentiation, the correlation is not complete. For example, despite the fact that Bine 4.8 cells have many pre-B cell characteristics, they express large amounts of μs mRNA upon transfection. While this finding is consistent with the μM/μS ratio previously reported for Abelson virus transformed pre-B cells (32), it is clearly not the case for normal pre-B cells (4). Secondly, in M12.4 cells, which are the presumed transformed counterpart of mature B cells, polymerases terminate extensively downstream of the USIR sequence when transfected with the intact μ-δ gene, pLLn5R (Fig. 2). This termination is unlikely to be totally secretion-associated since M12.4 cells utilize the μS poly(A) site to a much lesser extent than Bine 4.8 or J558L cells (Fig. 8). Therefore, it is possible that the anti-termination factor(s) is lost in the process of transformation. Indeed, there are almost no tumor cell lines available which transcribe the δ gene extensively in the absence of a μ gene deletion event (12, 33, and unpublished observations). Thus the complete understanding of the mode of regulation of μ vs δ expression may require the use of non-transformed cells obtained from mice containing mutated transgenes.

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