RNA Processing in Immunoglobulin Gene Expression

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DNA rearrangements are central to the expression of immunoglobulin genes and to the generation of antibody diversity (Honjo et al.; Hood et al.; Marcu et al.; Tonegawa et al.; all this volume). Since immunoglobulin mRNAs are generated from larger nuclear RNA precursors (Gilmore-Hebert and Wall 1978, 1979; Gilmore-Hebert et al. 1978; Schibler et al. 1978; Perry et al. 1979), RNA processing also plays an essential role in immunoglobulin gene expression. It is our goal to define the molecular events in immunoglobulin RNA processing and to determine whether immunoglobulin gene expression is regulated by these posttranscriptional events. Previously, we have approached these issues by studying immunoglobulin nuclear RNAs in immunoglobulin-producing plasmacytoma or myeloma cells. Several important insights emerged from these studies. First, polyadenylation precedes RNA splicing. RNA-splicing steps apparently remove intervening sequences (introns) in an ordered sequence. However, the removal of introns does not necessarily occur in a $5' \rightarrow 3'$ direction along the RNA precursor. Potential RNA-splicing sites can be effectively ignored. For example, the active MOPC-21 κ light-chain gene, in which the V region is joined to J_4, still contains three J regions in the $V_{\kappa 21} \rightarrow C_\kappa$ intron (Max et al. 1979; Sakano et al. 1979). When transcribed into nuclear RNA, these J regions (excluding the anomalous J_3) obviously contain potential splicing sites capable of combining with the C_κ region. Nonetheless, these potential splicing sites are effectively bypassed in MOPC-21 κ light-chain mRNA biogenesis.

Recently, we proposed (Rogers and Wall 1980) a mechanism for RNA splicing in which a small nuclear RNA, designated U-1 snRNA, recognizes RNA-splicing sites. Comparison of all known DNA sequences for junctures between introns and exons (Seif et al. 1979; Lerner et al. 1980; Rogers and Wall 1980) yielded the following consensus sequences, in which each nucleotide assigned is found in ≧45% of all known junctures. The underlined GT and AG, at the upstream and downstream junctures, respectively, apparently are obligatory as they are universally present.

5′–exon—AG/GTAAGTA—intron—
TTTTYTTTTTTCTTNCAG/G—exon–3′

The most abundant of the stable snRNAs of eukaryotic cells, U-1 snRNA (Reddy et al. 1974; Ro-Choi and Henning 1977), is complementary to these consensus sequences. Accordingly, we have proposed that U-1 snRNA is the recognition component of the nuclear-RNA-splicing enzyme and that it base-pairs with both ends of an intron in nuclear RNA so as to align them in exact register for cutting and splicing (Fig. 1). Although Figure 1 shows U-1 simultaneously base-paired with both ends of an intron, it is also possible that U-1 may base-pair sequentially, first with one end of an intron and then with the other. Lerner et al. (1980) have independently proposed a role for U-1 snRNA in RNA splicing. Their observation that some sera from systemic lupus erythematosus patients react with ribonucleoprotein particles containing U-1 and other snRNAs provides a promising experimental tool for studying the possible role of snRNAs in RNA splicing (Lerner and Steitz 1979; Lerner et al. 1980).

In this paper we summarize our recent findings, which provide further understanding of the signals and molecular mechanisms in RNA splicing. First, we present the results of our studies on nonproducing and variant-producing myeloma cells, which indicate that RNA-splicing defects can cause the loss of immunoglobulin production or the production of chains with internally deleted domains. Second, we summarize our results, in collaboration with Leroy Hood's laboratory (California Institute of Technology, Pasadena), on μ heavy-chain gene expression; these results reveal that the transition from membrane-bound μ (μ_m) to secreted μ (μ_s) heavy chains early in B-cell development is controlled through RNA processing (Calame et al. 1980; Early et al. 1980; Rogers et al. 1980). Finally, we propose that such developmentally regulated RNA processing generally can be used to generate membrane and secreted forms for other classes of immunoglobulin heavy chains.

RESULTS AND DISCUSSION

Immunoglobulin Light-chain Variants

To define further the elements in RNA processing, we screened a number of variant MPC-11 cell lines for mutants defective in RNA splicing. Wild-type MPC-11 cells (clone 45.6) simultaneously produce a normal κ light chain and a κ light-chain fragment containing the P (signal sequence) and C_κ regions, but lacking the V and J regions (Rose et al. 1977). Southern mapping of the C_κ regions in *Bam*HI-cleaved genomic DNA from MPC-11 cell lines and spleen revealed that wild-type 45.6 cells contain two rearranged C_κ gene segments of 3.0 kb and 7.7 kb (Choi et al. 1980) (summarized in Table 1). Spleen DNA, which has unrearranged C_κ re-

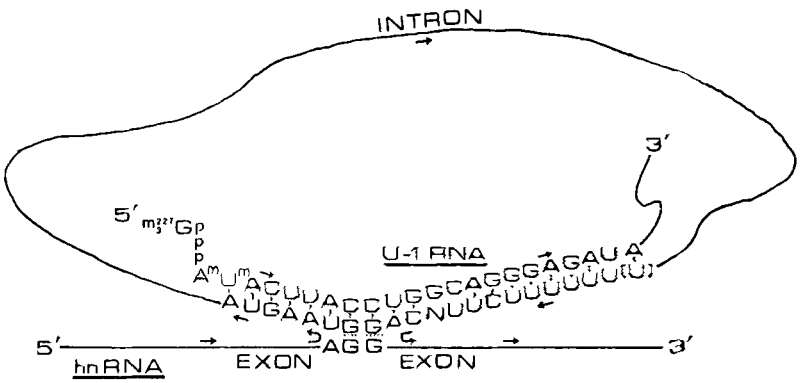

Figure 1. Model for base-pairing of heterogeneous nuclear RNA (hnRNA) to U-1 snRNA in the RNA-splicing complex. Dots indicate the predicted points of cutting and splicing. The model shows both upstream and downstream splicing sites simultaneously aligned with U-1 snRNA. Sequential splicing might alternatively occur through the binding of U-1 first with one end of an intron and then with the other end.

gions, contains only a single C_κ band at approximately 14 kb. Three nonproducing variant cell lines (4N1, 4N2, and NP.2), which synthesize only the C_κ-fragment chain but not the normal MPC-11 κ light chain, all contain only a single C_κ DNA band at 3.0 kb (Table 1). Since this is the only C_κ fragment in these nonproducing MPC-11 cells, it must be the κ-fragment-chain gene (Choi et al. 1980; Seidman and Leder 1980). The 7.7-kb *Bam*HI fragment must represent the C_κ region that encodes the normal κ light chain in MPC-11 cells, since the loss of normal light-chain expression in the three nonproducing cell lines is always correlated with the loss of this fragment.

These findings indicate that the loss of normal light-chain expression in the 4N1, 4N2, and NP.2 variant cell lines is due to the deletion of the rearranged C_κ region coding for this chain. This result is quite different from that in two other variant MPC-11 cell lines (G403 and L3NP) that no longer produce normal light chains but continue to express the C_κ fragment. The L3NP variant cells contain two rearranged C_κ regions in *Bam*HI fragments of 7.7 kb and 3.0 kb, just as in wild-type 45.6 cells. Since the gene for normal κ chains appears not to be grossly altered, the loss of κ production in the L3NP cells probably results from a defect in RNA splicing rather than in transcription. In contrast, the nonproducing G403 cells contain the C_κ-fragment gene with a C_κ region in a new *Bam*HI fragment of 5.3 kb. The loss of normal light-chain production in these cells could result from a defect either in transcription or in RNA processing.

Table 1. C_κ Regions in Genomic DNA of Spleen and MPC-11 Cell Lines

Spleen	45.6	4N1, 4N2, NP.2	L3NP	G403
14.0	—	—	—	—
—	7.7	—	7.7	5.3
—	3.0	3.0	3.0	3.0

*Bam*HI fragments (in kb) from Southern blot of genomic DNA hybridized with cloned C_κ-region cDNA probe (Choi et al. 1980). Cell lines NP.2, 4N1, 4N2, L3NP, and G403 are nonproducing variants that no longer make the normal MPC-11 κ chain.

RNA mapping studies have confirmed that the two κ mRNAs in wild-type 45.6 MPC-11 cells are generated from independent transcription units through different RNA-processing pathways (Choi et al. 1980). The 45.6 cells contain five distinct C_κ nuclear RNA species of 6.5, 5.6, 3.6, 1.2, and 0.8 kb (Fig. 2). The 1.2-kb and 0.8-kb species represent the normal κ light-chain mRNA and κ-fragment mRNA, respectively. The NP.2 cells (as well as the 4N1 and 4N2 variant cells) contain only two κ nuclear RNA species, of 3.6 kb and 0.8 kb. The processing pathway for the full-length κ mRNA is

$$6.5\text{ kb} \rightarrow 5.6\text{ kb} \xrightarrow{V \rightarrow C_\kappa \text{ splice}} 1.2\text{ kb mRNA}$$

The $V \rightarrow C_\kappa$ splice (removing 3.5 kb) may proceed through a rapidly labeled RNA intermediate of approximately 2.1 kb, which is readily detected in pulse-labeling experiments (Schibler et al. 1978; Choi et al. 1979) but not in RNA blots, since the latter technique primarily detects stable species of nuclear RNA. Accordingly, at least one additional splice removing a yet undefined intron (of <1 kb) may be involved in generating a 1.2-kb MPC-11 κ mRNA.

The processing pathway to κ-fragment mRNA is

$$3.6\text{ kb} \xrightarrow{P \rightarrow C_\kappa \text{ splice}} 0.8\text{ kb mRNA}$$

The $P \rightarrow C_\kappa$ splice removes approximately 2 kb (Seidman and Leder 1980). An additional splice, possibly involving a 5′ leader sequence, also apparently occurs in the generation of the 0.8-kb mRNA. The 3.6-kb nuclear

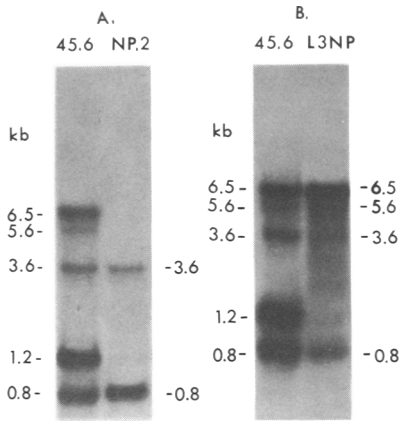

Figure 2. Light-chain RNAs in wild-type and variant MPC-11 cells. Nuclear RNAs were analyzed by means of RNA blots (Alwine et al. 1977; Choi et al. 1980). (*A*) Wild-type (45.6) nuclear RNA compared with nuclear RNA from a nonproducing variant (NP.2) that does not synthesize normal κ chains. The RNA pattern seen in the NP.2 cell line has also been seen in the 4N1, 4N2, and G403 cell lines, which also no longer produce normal κ chains. (*B*) Wild-type (45.6) nuclear RNA compared with nuclear RNA from a different nonproducing variant (L3NP) cell line. Mouse rRNAs were used to calculate the sizes shown for hybridized bands.

RNA precursor contains the germ-line MOPC-321 V region without J regions in the arrangement P—V_{321}—C_κ (Choi et al. 1980; Seidman and Leder 1980). Accordingly, this V_{321} region lacks an upstream splice site and cannot be spliced to the C_κ region. It appears that the upstream splicing site at the 3′ end of the P region is spliced to the downstream splicing site at the 5′ end of the C_κ region. It is likely that this unusual P → C_κ splice occurs at a comparatively high frequency, since the κ-fragment mRNA comprises some 20% of the κ mRNA in strain 45.6 cells.

This raises an interesting possibility regarding the mechanism of RNA splicing. First, we suppose that in MPC-11 cells the V → C_κ splice precedes the P → V splice. Second, the removal of an individual intron by splicing may occur in two steps. The initial recognition occurs at the downstream intron site. Recognition of the upsteam site would then follow as a second step. In the κ-fragment transcript, the V_{321} region lacks a functional upstream splicing site and is thus bypassed in favor of the upstream splicing site at the P region. The splicing of nonadjacent exons does not occur at a detectable frequency in normal mRNA biogenesis. However, this example shows that RNA splicing can bypass a mutant upstream splicing site provided that the RNA precursor contains a remaining intron with a functional upstream splicing site. This mode of RNA splicing may be responsible for other variant chains that have internally deleted exons or domains. This appears to be one possible explanation for those immunoglobulin heavy-chain deletion variants that have lost internal domains or the hinge region.

Nuclear RNA blots were carried out to define the molecular defect accounting for the loss of normal κ-chain production in the nonproducing L3NP and G403 variants of MPC-11 cells, which retain two rearranged C_κ regions. The defect in G403 cells appears to be at the level of transcription, since these cells only contained the κ-fragment RNA species of 3.6 kb and 0.8 kb, with no detectable κ RNA transcripts from the 5.3-kb C_κ region (E. Choi, unpubl.). In contrast, the loss of normal κ light-chain production in the variant L3NP cells appears to result from a block in RNA splicing. In addition to the 3.6-kb and 0.8-kb κ-fragment RNA species, the L3NP cells contained the 6.5-kb and 5.6-kb precursors seen in wild-type 45.6 cells (Fig. 2). This eliminates defective transcription as the cause for the loss of normal κ chains in L3NP cells. Indeed, the L3NP cells apparently carry out the 6.5-kb → 5.6-kb splicing step but then are blocked in further splicing steps. Thus, these nonproducing variant cells provide an instance in which a defective RNA-splicing step leads to the loss of immunoglobulin mRNA production. Determination of the defective nucleotide sequence involved should provide further understanding of the RNA-splicing mechanisms.

Regulation of Immunoglobulin Gene Expression through RNA Processing

These results show how studies on nuclear RNA complement gene cloning and sequencing by revealing the events in RNA processing. However, the immunoglobulin genes in these fully differentiated plasma cells are not likely to be useful candidates for studying the mechanisms controlling immunoglobulin gene expression. First of all, these myeloma cells produce a substantial fraction (10–20%) of the total cell protein synthesized as immunoglobulins. In addition, quantitative studies on MOPC-21 κ mRNA processing indicate that all (>90%) of the κ mRNA sequences transcribed into large nuclear RNA are conserved and processed into κ mRNA (Gilmore-Hebert and Wall 1979). Estimates of the transcriptional activity of this κ gene indicate that it approaches the most active estimates for rRNA genes in animal cells. The other immunoglobulin genes in myeloma cells studied to date also appear to be as actively transcribed, and their transcripts as efficiently processed, into cytoplasmic mRNA as the MOPC-21 κ mRNA (Schibler et al. 1978).

Antibody-producing cells undergo a series of transitions or switches in immunoglobulin gene expression during the development of the immune response (summarized in Table 2). These developmental transitions are promising candidates for examining the role of DNA rearrangements and RNA processing in immunoglobulin gene regulation. Cytoplasmic μ chains appear to be the earliest immunoglobulin chains expressed in so-called pre-B cells (Burrows et al. 1979). Membrane IgM (composed of μ_m + light chains) is the class of immunoglobulin detected next in B-cell differentiation. The membrane IgM molecules apparently function as receptors on the outer membranes of lymphocytes not stimulated by antigen (for review, see Kettman et al. 1979). IgD is subsequently detected along with IgM on

Table 2. Early Stages and Immunoglobulin Gene Products in B-cell Development

	Stem cell	→ Pre-B cell	→ B Cell	→ Plasma cell	
Cell line	—	18-81[a]	W279[b] W231[b]	MOPC-104E	MXW279[c]
Immunoglobulin	—	cytoplasmic μ, no light chains	IgM_m	IgM_m	IgM_s
Immunoglobulin mRNAs (kb)					
μ	—	2.7 2.4	2.7 2.4	(2.7) 2.4	none 2.4
κ	—	none	1.2	1.2	1.2 0.8

Immunoglobulin mRNAs were fractionated on methylmercury-hydroxide–agarose gels and screened in RNA blots (Alwine et al. 1977; Rogers et al. 1980). The 2.7-kb μ_m species in MOPC-104E is present at ~10–15% of the level of the 2.4-kb μ_s species. The 0.8-kb κ species in MXW279 is the κ-fragment mRNA of the MPC-11 myeloma used in construction of the MXW279 hybridoma.
References describing cell lines: [a] Siden et al. 1979; [b] Warner et al. 1979; [c] Raschke et al. 1979.

the cell membranes of most mouse B lymphocytes. The V-region domains of the membrane IgM and IgD molecules on the same cells appear to be identical (Fu et al. 1975). Antigen stimulation causes B lymphocytes bearing surface receptors for the specific antigen to divide, producing clonal descendants that secrete IgM (composed of μ_s + light chains), and then undergo switching to the other classes of immunoglobulin (IgG, IgA, and IgE) (for review, see Hood et al. 1978). All of these clonal descendants retain the V-region specificity, i.e., have the same V_L and V_H regions, of the membrane receptor immunoglobulin IgM in the parent lymphocyte. These "switches," in which one heavy-chain V region is subsequently expressed with different heavy-chain C regions, appear to be a fundamental aspect of the differentiation of B cells.

Fortunately, there are tumors available that appear to correspond to each of these stages in B-cell development and that facilitate analysis of the transitions in immunoglobulin gene expression. The pre-B cell is represented by Abelson-virus-transformed cells (Siden et al. 1979). The B cell is represented by B lymphomas (Warner et al. 1979), and the plasma cell is represented by plasmacytomas or myeloma tumors.

The switches between different immunoglobulin heavy-chain classes (IgM, IgG, IgA, etc.) appear to involve heavy-chain DNA rearrangements and deletions (Honjo et al.; Hood et al.; Marcu et al.; Tonegawa et al.; all this volume). We now summarize our recent finding, in collaboration with L. Hood's laboratory, that the early transition from membrane receptor IgM (μ_m heavy chains) in B cells to secreted IgM (μ_s heavy chains) occurs through developmentally regulated RNA processing (Early et al. 1980; Rogers et al. 1980). DNA rearrangements do not play a role in this key early transition in μ expression. As discussed later, such a complex mode of RNA processing may also account for the simultaneous production of IgM and IgD in B lymphocytes.

The μ mRNAs in various μ-producing cells were analyzed by RNA blots (summarized in Table 2). An example of such an RNA blot from the pre-B-cell line (18-81) is shown in Figure 3. This cell line, which synthesizes only cytoplasmic μ chains but no light chains, contains C_κ regions in the germ-line or unrearranged configuration (M. Kuehl, unpubl.). Two prominent μ mRNA species at 2.7 kb and 2.4 kb are seen in 18-81 as well as in other μ-producing cells studied (Perry and Kelley 1979; Alt et al. 1980; Rogers et al. 1980). Nucleotide sequencing of cDNA clones generated from MOPC-104E myeloma μ mRNAs (Calame et al. 1980; Rogers et al. 1980) indicate that the 2.4-kb species codes for the μ_s chain. The 2.7-kb μ mRNA is identical to the μ_s sequence up to the end of the $C_\mu 4$ domain, where it contains a different C-terminal coding region. After the $C_\mu 4$ domain, the μ_s chain has a hydrophilic C-terminal segment of 20 residues, which is required for μ_s secretion, whereas the μ_m chain has a C-terminal segment of 41 residues, which we have designated the M (membrane) segment. The 20-amino-acid-long C-terminal

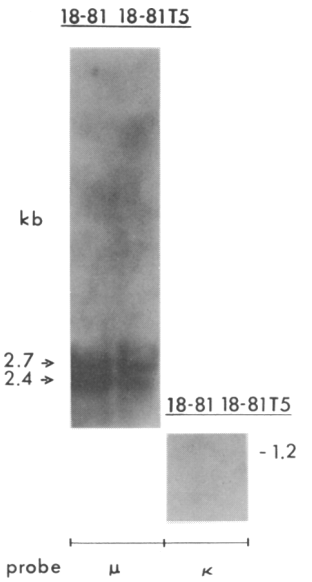

Figure 3. Immunoglobulin μ mRNAs in 18-81 Abelson-virus-transformed cells producing only cytoplasmic μ chains. Cytoplasmic RNAs from two cloned lines of 18-81 were analyzed by means of RNA blots (Alwine et al. 1977) using a cloned μ cDNA probe (Rogers et al. 1980) and κ cDNA probe (Wall et al. 1978). These cell lines, which contain unrearranged C_κ genes, do not produce detectable κ mRNA.

coding segment in the 2.4-kb μ_s mRNA is terminated by a stop codon immediately following codon 576. Then there are 128 nucleotides comprising the 3′ untranslated (3′UT$_s$) segment. This 3′UT$_s$ ends with AATAAA(N)$_{17}$TC-poly(A). The 2.7-kb μ_m mRNA sequence has 41 C-terminal codons followed by 270 nucleotides of a different 3′ untranslated sequence. From the nucleotide sequence of the μ_m cDNA clone, we have predicted the amino acid sequence of the 41-residue μ_m C-terminal segment or M segment (Rogers et al. 1980). This sequence contains a hydrophobic region with properties consistent with its being a transmembrane peptide. The 3′UT of the μ_m cDNA clone ends with an AATAAA(N)$_{17}$TC-poly(A) sequence. The μ_m mRNA is 205 nucleotides longer than the μ_s mRNA, assuming that the poly(A) tracts and the sequences preceding the C$_\mu$4 domain are identical in the two species. This difference is consistent with the observed sizes of 2.7 kb and 2.4 kb for the μ_m and μ_s mRNAs.

There is a single C$_\mu$ gene per haploid genome whose 3′ regions are not detectably rearranged in μ_s-producing myeloma cells (Calame et al. 1980; Early et al. 1980). The μ_s C-terminal sequence is contiguously encoded with the C$_\mu$4 domain, and the μ_m C-terminal sequence is encoded in two separate exons (the M exons) 1.8 kb from the end of the μ_s C-terminal sequence in DNA (Early et al. 1980). The μ_m and μ_s mRNAs appear to be generated from a single μ transcription unit by alternate patterns of RNA splicing. Comparison of the μ_s and μ_m cDNA sequences showed that there is an RNA splicing site at codons 557–558 at the 3′ end of the C$_\mu$4 domain, where the μ_m and μ_s cDNA sequences diverge. The μ_m clone has the sequence TG/AGGGGG, and the μ_s clone has the sequence TG/GTAAAC. This sequence in the μ_s cDNA clone is identical with that of the chromosomal gene. It clearly resembles the consensus sequence for an upstream RNA splicing site, AG/GTAAGT, in which the stroke divides the exon from the intron. The μ_m mRNA is generated by RNA splicing between this site and the first M exon and also between the two M exons.

Although the production of μ_m versus μ_s mRNA involves two additional RNA splices, we have proposed that the primary decision between μ_m and μ_s is made by the developmentally regulated addition of poly(A) either at the 3′UT site of the μ_s or at the 3′UT site of the M exons (Early et al. 1980; Rogers et al. 1980) (see Fig. 4). This proposal is based on the widespread experimental finding that the posttranscriptional addition of poly(A) to the 3′ ends of nuclear RNA precursors is a rapid process that precedes RNA splicing (Kinniburgh et al. 1978; Nevins and Darnell 1978; Schibler et al. 1978; Gilmore-Hebert and Wall 1979).

All immunoglobulin heavy-chain genes may have the same RNA-splicing sequence for joining heavy-chain domains and M exons. Strikingly, the immunoglobulin heavy chains that have now been sequenced at the DNA or protein level all have a glycine-lysine sequence precisely at the end of the last domain, which could be encoded by the same RNA splicing site that joins the C$_\mu$4 exon to the M exon. In the genomic γ1 (Honjo et al. 1979) and γ2b (Tucker et al. 1979) clones, as in the μ_s clone, this dipeptide has been shown to be encoded by the same nucleotide sequence (GGTAAA) that forms the upstream splice site at the 3′ end of the C$_\mu$4 exon. Because of the striking conservation of the sequence at the splicing site for μ_m at the end of the final domain of all immunoglobulin heavy chains now sequenced, we have predicted that this mode of RNA processing will be generally used to produce either secreted or membrane-bound forms of other immunoglobulin heavy chains. M. Kuehl and C. Word (in prep.) have now isolated several lines of γ2a-producing cells that make both membrane-bound and secreted IgG2a containing 68-kD and 62-kD γ2a heavy chains, respectively. RNA blots show that, in analogy to the finding for μ_m and μ_s, these cells contain two distinct species of γ2a mRNA (C. Carter and R. Wall, unpubl.).

RNA Processing and the Nature of Eukaryotic Transcription Units

The eukaryotic cellular genes studied to date all appear to be simple transcription units that, although they may involve many RNA-splicing events, give rise to only a single mRNA product (for review, see Darnell 1978). Complex transcription units that give rise to more than one mRNA species are well documented for the DNA tumor viruses: adenovirus, SV40, and polyoma virus (for review, see Darnell 1978). Darnell (1978) has classified two alternate models of posttranscriptional RNA processing that can generate different mRNA species from the primary transcript of a complex transcription unit. In their simplest form, these involve either alternate poly(A)-addition sites or alternate RNA-splicing events. More complicated modes of com-

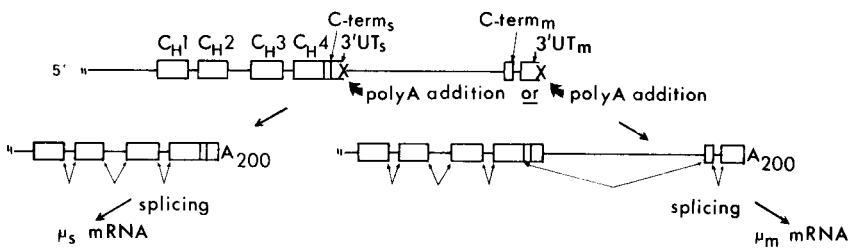

Figure 4. Model for the production of μ_m and μ_s mRNAs from a complex transcription unit having alternate poly(A)-addition sites. For simplicity, only the C-region sequences are shown in the μ primary transcript. The alternate poly(A) sites (X) and the splicing patterns generating μ_s and μ_m mRNAs are shown below the primary transcript.

plex RNA processing employing both polyadenylation and splicing options are used in the DNA-virus systems.

The transcription unit that generates either μ_s or μ_m mRNA is the first identified example of a complex transcription unit for a eukaryotic cellular gene. The μ transcription unit apparently uses alternate poly(A) sites to generate either μ_s or μ_m (Fig. 4). No example of a complex cellular transcription unit that uses alternate RNA splicing sites, or a combination of alternate poly(A) sites and splicing sites, has yet been discovered. However, the simultaneous production of μ and δ chains is a likely example (Gilbert 1978). The concomitant production of μ and δ heavy chains is a frequent, and possibly obligatory, stage in the ontogeny of B lymphocytes (Vitetta and Uhr 1975; Kearney and Abney 1978). Coexpressed μ and δ chains appear to have the same V region (Fu et al. 1975) and are encoded on the same chromosome (Herzenberg et al. 1977; Bourgois et al. 1977). Just as transcription through the μ_s polyadenylation site leads to production of μ_m mRNA, transcription beyond the μ_m polyadenylation site could lead to production of δ mRNA. This would involve a third alternate poly(A)-addition site 3′ to the δ C-region gene segment. Splicing of the V_H region to the C_δ, with the excision of the entire C_μ and M_μ gene segments from the large primary transcript, would occur through the alternate choice of RNA splicing sites. This proposed mechanism for μ and δ mRNA production involving multiple poly(A) sites and splicing sites resembles that established for late adenoviral RNA processing (for review, see Darnell 1978).

The recent discovery that the immunoglobulin C_δ region is encoded only several kilobases 3′ to the μ M exons (Marcu et al., this volume) makes this an attractive and experimentally testable hypothesis. If this model for μ plus δ production is combined with the model for the $\mu_m \rightarrow \mu_s$ developmental switch already described, one can predict that preferential poly(A) addition at the μ_s site in plasma cells and hybridomas will switch off not only μ_m synthesis but also δ synthesis. This is in accord with experiments on normal B lymphocytes (Bourgois et al. 1977; Preud'homme 1977; Kearney and Abney 1978), in which B lymphocytes expressing μ_m plus δ ceased μ_m and δ production at the same time when stimulated by lipopolysaccharide to produce large amounts of μ_s. This model for μ plus δ production would also predict that the δ gene would not be rearranged in cells simultaneously producing both chains. Rare cases of abundant production of IgD alone might still be explained by a DNA rearrangement with possible deletion of the μ gene, analogous to the events in the switching of other heavy-chain classes (Honjo and Kataoka 1978; Davis et al. 1980; Kataoka et al. 1980; cf. Honjo et al.; Hood et al.; Marcu et al.; Tonegawa et al.; all this volume).

Some single B cells simultaneously express multiple different immunoglobulin heavy-chain classes other than μ plus δ (e.g., μ plus γ). Such cells may represent transitional stages in the switching of different heavy-chain classes. On the basis of recent findings that suggest that the switching of different heavy-chain classes occurs through DNA rearrangements and deletions, it seems likely that the simultaneous production of μ along with heavy chains other than δ occurs through DNA rearrangements rather than RNA processing. In such cases, DNA rearrangements presumably generate new transcription units, each coding for one heavy-chain class.

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REFERENCES

ALT, F., A. L. M. BOTHWELL, M. KNAPP, E. SIDEN, E. MATHER, M. KOSHLAND, and D. BALTIMORE. 1980. Synthesis of secreted and membrane-bound immunoglobulin μ heavy chains is directed by mRNAs that differ at their 3′-ends. *Cell* **20:** 293.

ALWINE, J. C., D. J. KEMP, and G. P. STARK. 1977. Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl paper and hybridization with DNA probes. *Proc. Natl. Acad. Sci.* **74:** 5350.

BOURGOIS, A., K. KITAJIMA, I. R. HUNTER, and B. A. ASKONES. 1977. Surface immunoglobulins of lipopolysaccharide-stimulated spleen cells: The behavior of IgM, IgD, and IgG. *Eur. J. Immunol.* **7:** 151.

BURROWS, P., M. LE JEUNE, and J. F. KEARNEY. 1979. Evidence that murine pre-B cells synthesize μ heavy chains but no light chains. *Nature* **280:** 838.

CALAME, K., J. ROGERS, P. EARLY, M. DAVIS, D. LIVANT, R. WALL, and L. HOOD. 1980. The mouse C_μ heavy chain immunoglobulin gene segment contains three intervening sequences which separate domains. *Nature* **284:** 452.

CHOI, E., M. KUEHL, and R. WALL. 1980. RNA splicing generates a variant light chain from an aberrantly rearranged κ gene. *Nature* **286:** 776.

CHOI, E., M. KUEHL, M. KOMAROMY, P. CLARKE, and R. WALL. 1979. Light chain genes, transcription units and RNA precursors. In *Eukaryotic gene regulation* (ed. T. Maniatis et al.), p. 421. Academic Press, New York.

DARNELL, J. E. 1978. Transcription units for mRNA production in eukaryotic cells and their DNA viruses. *Prog. Nucleic Acid Res. Mol. Biol.* **22:** 327.

DAVIS, M. M., K. CALAME, P. W. EARLY, D. L. LIVANT, R. JOHO, I. L. WEISSMAN, and L. HOOD. 1980. An immunoglobulin heavy-chain gene is formed by at least two recombinational events. *Nature* **283:** 733.

EARLY, P., J. ROGERS, M. DAVIS, K. CALAME, M. BOND, R. WALL, and L. HOOD. 1980. Two mRNAs can be produced from a single immunoglobulin μ gene by alternative RNA processing pathways. *Cell* **20:** 313.

FU, S. M., R. J. WINCHESTER, and H. G. KUNKEL. 1975. Similar idiotypic specificity for membrane IgD and IgM of human B lymphocytes. *J. Immunol.* **114:** 250.

GILBERT, W. 1978. Why genes in pieces? *Nature* **271:** 501.

GILMORE-HEBERT, M. and R. WALL. 1978. Immunoglobulin light chain messenger RNA is processed from large nuclear RNA precursors. *Proc. Natl. Acad. Sci.* **75:** 342.

———. 1979. Nuclear RNA precursors in the processing pathway to MOPC 21 κ light chain mRNA. *J. Mol. Biol.* **135:** 879.

GILMORE-HEBERT, M., K. HERCULES, M. KOMAROMY, and R. WALL. 1978. Variable and constant regions are separated in the 10 kb transcription unit coding for immunoglobulin κ light chains. *Proc. Natl. Acad. Sci.* **75:** 6044.

HERZENBERG, L. A., L. A. HERZENBERG, S. J. BLACK, M. R. LOKEN, K. OKUMURA, W. VANDER LOO, B. A. OSBORNE, D. HEWGILL, J. W. GODING, G. GUTMAN, and N. L. WARNER. 1977. Surface markers and functional relationships of cells involved in murine B-lymphocyte differentiation. *Cold Spring Harbor Symp. Quant. Biol.* **41:** 33.

HONJO, T. and T. KATAOKA. 1978. Organization of immunoglobulin heavy chain genes and allelic deletion model. *Proc. Natl. Acad. Sci.* **75:** 2140.

HONJO, T., M. OBATA, Y. YAMAWAKI-KATAOKA, T. KATAOKA, T. KAWAKAMI, N. TAKAHASHI, and Y. MANO. 1979. Cloning and complete nucleotide sequence of mouse immunoglobulin γ_1 chain gene. *Cell* **18:** 559.

HOOD, E. J., I. L. WEISSMAN, and W. B. WOOD. 1978. The antibodies produced in a humoral immune response are heterogeneous in specificity and may include all immunoglobulin classes. In *Immunology*, p. 39. Benjamin/Cummings, Menlo Park, California.

KATAOKA, T., T. KAWAKAMI, N. TAKAHASHI, and T. HONJO. 1980. Rearrangement of immunoglobulin γ_1-chain gene and mechanism for heavy chain class switch. *Proc. Natl. Acad. Sci.* **77:** 919.

KEARNEY, J. F. and E. R. ABNEY. 1978. Immunoglobulin isotype expression. *Contemp. Top. Immunobiol.* **8:** 245.

KETTMAN, J. R., J. CAMBIER, J. W. UHR, F. LIGLER, and E. S. VITETTA. 1979. The role of receptor IgM and IgD in determining triggering and introduction of tolerance in murine B cells. *Immunol. Rev.* **43:** 69.

KINNIBURGH, A. J., J. E. MERTZ, and J. ROSS. 1978. The precursor of mouse β-globin messenger RNA contains two intervening RNA sequences. *Cell* **14:** 681.

LERNER, M. R. and J. A. STEITZ. 1979. Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci.* **76:** 5495.

LERNER, M., J. BOYLE, S. MOUNT, S. WOLIN, and J. A. STEITZ. 1980. Are snRNAs involved in splicing? *Nature* **283:** 220.

MAX, E. E., J. G. SEIDMAN, and P. LEDER. 1979. Sequences of five potential recombination sites encoded close to an immunoglobulin κ constant region gene. *Proc. Natl. Acad. Sci.* **76:** 3450.

NEVINS, J. and J. E. DARNELL. 1978. Steps in the processing of Ad2 mRNA: Poly(A)$^+$ nuclear sequences are conserved and poly(A) addition precedes splicing. *Cell* **15:** 1477.

PERRY, R. P. and D. E. KELLEY. 1979. Immunoglobulin messenger RNAs in murine cell lines that have characteristics of immature B lymphocytes. *Cell* **18:** 1333.

PERRY, R. P., D. E. KELLEY, and V. SCHIBLER. 1979. Comparison of mRNA precursors in plasmacytomas producing closely related κ chains. *Proc. Natl. Acad. Sci.* **76:** 3678.

PREUD'HOMME, J. L. 1977. Loss of surface IgD by human B lymphocytes during polyclonal activation. *Eur. J. Immunol.* **7:** 191.

RASCHKE W. C., E. L. MATHER, and M. E. KOSHLAND. 1979. Assembly and secretion of pentametric IgM in a fusion between a nonsecreting B cell lymphoma and an IgG-secreting plasmacytoma. *Proc. Natl. Acad. Sci.* **76:** 3469.

REDDY, R., T. S. RO-CHOI, D. HENNING, and H. BUSCH. 1974. Primary sequence of U-1 nuclear ribonucleic acid of Novikoff hepatoma ascites cells. *J. Biol. Chem.* **249:** 6486.

RO-CHOI, T. S. and D. HENNING. 1977. Sequence of 5′-oligonucleotide of U1 RNA from Novikoff hepatoma cells. *J. Biol. Chem.* **252:** 3814.

ROGERS, J. and R. WALL. 1980. A mechanism for RNA splicing. *Proc. Natl. Acad. Sci.* **77:** 1877.

ROGERS, J., P. EARLY, C. CARTER, K. CALAME, M. BOND, L. HOOD, and R. WALL. 1980. Two mRNAs with different 3′ ends encode membrane-bound and secreted forms of immunoglobulin μ chain. *Cell* **20:** 303.

ROSE, S. M., W. M. KUEHL, and G. P. SMITH. 1977. Cloned MPC-11 myeloma cells express two kappa genes: A gene for a complete light chain and a gene for a constant region polypeptide. *Cell* **12:** 453.

SAKANO, H., K. HÜPPI, G. HEINRICH, and S. TONEGAWA. 1979. Sequences at the somatic recombination sites of immunoglobulin light-chain genes. *Nature* **280:** 288.

SCHIBLER, V., K. B. MARCU, and R. P. PERRY. 1978. The synthesis and processing of the messenger RNAs specifying heavy and light chain immunoglobulins in MPC-11 cells. *Cell* **15:** 1495.

SEIDMAN, J. G. and P. LEDER. 1980. A mutant immunoglobulin light chain formed by abnormal DNA and RNA splicing events. *Nature* **286:** 779.

SEIF, I., G. KHOURY, and R. DHAR. 1979. BKV splice sequences based on analysis of preferred donor and acceptor sites. *Nucleic Acids Res.* **6:** 3387.

SIDEN, E. J., D. BALTIMORE, D. CLARK, and N. E. ROSENBERG. 1979. Immunoglobulin synthesis by lymphoid cells transformed *in vitro* by Abelson murine leukemia virus. *Cell* **16:** 389.

TUCKER, P. W., K. B. MARCU, M. NEWELL, J. RICHARDS, and F. BLATTNER. 1979. Sequence of the cloned gene for the constant region of murine γ_{2b} immunoglobulin heavy chain. *Science* **206:** 1303.

VITETTA, E. S. and J. W. UHR. 1975. Immunoglobulin receptors revisited. *Science* **189:** 964.

WALL, R., M. GILMORE-HEBERT, R. HIGUCHI, M. KOMAROMY, G. PADDOCK, W. SALSER, and J. STROMMER. 1978. Recombinant DNA clones constructed from immunoglobulin *kappa* light chain messenger RNA. *Nucleic Acids Res.* **5:** 3113.

WARNER, N. L., J. F. LEARY, and S. MCLAUGHLIN. 1979. Analysis of murine B cell lymphomas as models of B cell differentiation arrest. In *B lymphocytes in the immune response* (ed. M. Cooper et al.), p. 371. Elsevier/North-Holland, Amsterdam.

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