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Presence of a polyadenylated RNA fragment encoding the membrane domain for immunoglobulin α chain indicates that mRNAs for both secreted and membrane-bound α chains can be produced from the same RNA transcript.

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ABSTRACT

RNA blotting was employed to examine polyadenylated immunoglobulin α chain RNAs in a B lymphoma synthesizing membrane-bound and secretory IgA and in a hybridoma which synthesizes predominantly secretory IgA. Both cell lines were derived from the I.29 lymphoma and expressed the identical heavy chain variable region gene. In addition to the predicted mRNA precursors, four novel species of polyadenylated α RNAs were detected. (1) The presence of a RNA species which was too large to have the same 3′ end as the largest mRNA for membrane-bound α chain (αm) implied that transcription continued past the αm poly(A) site, and that such transcripts could be polyadenylated. Alternatively, transcription of this αRNA was initiated 5′ to the normal cap site. (2) Two species of RNA were detected which encoded the αm domain and the intervening sequence between the α constant (Ca) and αm domains but not the Ca domain. These RNA molecules were of sizes appropriate for their derivation by endonucleolytic cleavage of a precursor for αm mRNA at the poly(A) site of the mRNA for secreted α chains (αs). The presence of these three α RNA species suggested that alternative and successive cleavage/polyadenylation events could occur on a single transcript to produce either αm or αs mRNAs. (3) An additional novel species of RNA was detected which indicated that the order of removal of the large IVSs did not always proceed in the 5′ to 3′ direction.

INTRODUCTION

Immunoglobulin (Ig) heavy (H) chains each exist in two forms -- a smaller form present in secreted Ig and a larger form present in membrane-bound Ig, the latter of which contains an hydrophobic region near the carboxyl terminus for anchoring the Ig in the membrane (1-10). Transcripts of the expressed Ig α chain gene are processed into three predominant forms of α mRNA: two encoding the α chain present in membrane-bound IgA (αm), and one encoding the α chain present in secreted IgA (αs). The α constant region (Ca) gene in BALB/c mice has been sequenced, and the regions of the gene which encode the three α mRNAs have been mapped (8, 11) (See Fig. 1). The carboxyl terminus present in secreted α chains is encoded adjacent to the third domain of the C region, and the single membrane exon is encoded by 193 nucleotides.
located ~2.35 Kb 3' to the secreted terminus. The smaller of the two mRNAs for membrane α chains has a 3' untranslated region consisting of ~365 nucleotides, and the larger mRNA for membrane α has a 3' untranslated region consisting of 1290-1340 nucleotides (8).

Two alternative mechanisms for the generation of mRNAs encoding membrane-bound and secreted Ig H chains have been proposed: (1) alternative sites of termination of transcription, i.e. sites where RNA polymerase is unloaded from the DNA template, or (2) alternative sites of cleavage and polyadenylation of a single primary transcript which extends 3' to the membrane domain(s) (2).

Evidence has been obtained supporting the latter mechanism for the alternative generation of mRNAs for membrane-bound and secreted μ chains. Using the nuclear run-on assay to measure the rate of transcription from various regions within the μ gene, including regions located 5' and 3' to the μ membrane (μm) domains, Yuan and Tucker (12) showed that in normal B cells and in a B cell hybridoma the rate of transcription of the μm domains was approximately the same as of the Cμ gene itself whether cells were synthesizing membrane-bound or secreted IgM. Similar experiments demonstrated this for the γ2a gene also (13). Recently, Danner and Leder (14) have also provided evidence that transcription does not terminate between the 3' ends of the μs and μm mRNA precursors. Furthermore, Kemp et al. (15) detected a small polyadenylated RNA fragment which hybridized with a probe for the μm domain but not with a probe for the Cμ domain and was of a size consistent with its production by an endonucleolytic cleavage of a polyadenylated RNA transcript encoding the μ membrane domains near the 5' border of the membrane domains. Although one might expect to detect the greatest amount of this μm fragment in cells synthesizing secreted IgM, this fragment was most predominant in a T lymphoma-plasmacytoma hybrid, and much less predominant in B lymphoma-plasmacytoma hybrid and in a plasmacytoma. Other investigators have failed to find this RNA fragment in hybridoma cells (14, 16).

To attempt to determine how the three Ig α chain mRNAs are produced, we have characterized the polyadenylated α RNAs present in an IgA+ B lymphoma cell line (BFO.3) which expresses membrane-bound IgA and secretes little or no IgA, and in an IgA+ hybridoma (ID150) which expresses predominantly secreted IgA (17). Both cell lines were derived from the I.29 B cell lymphoma: BFO.3, by cloning the IgA+ cells from the lymphoma (18) and ID150, by fusion of I.29 cells with the non-productive myeloma, NS.1 (19). BFO.3 cells contain slightly more of the mRNAs for membrane-bound (both combined) than secreted α
chains (Fig. 2, lane 1); ID150 cells contain ~10 times more mRNA for secreted α chain than do BFO.3 cells, and ~3 times less of the larger α_m mRNA (Fig. 2, lanes 1, 2 and 5,6). (Note that 3 μg of poly(A)_+ RNA from BFO.3 cells was loaded in lane 1, but only 1μg of poly(A)_+ RNA from ID150 cells was loaded in lane 2.)

We report here the results of RNA blotting experiments on total cell poly(A)_+ RNAs from the two cell lines. Our data support the hypothesis that a single primary RNA transcript can be cleaved and polyadenylated at alternative sites to yield either α_m or α_s mRNAs, as we detected in BFO.3 cells two species of polyadenylated RNA which hybridized with probes for the α_m domain but not with a probe for the Ca domain. This result and our additional finding that the largest polyadenylated α RNA species detected was too large to have the same 3' end as any of the α mRNAs, assuming it initiated at the normal RNA cap site, further suggests that a single RNA transcript can be cleaved and polyadenylated at more than one site.

MATERIALS AND METHODS

Abbreviations

α_m, membrane-bound α chain; α_s, secreted α chain; Ca, α constant domain; Ig, immunoglobulin; Kb, kilobases; V_H, variable region of Ig heavy chain.

Cells

BFO.3, ID150, and BCL_1 (20) cells were maintained in RPMI 1640 containing 10% fetal bovine serum (Flow Labs), 2mM glutamine (Gibco), 0.1mM nonessential amino acids (Gibco), 0.1mM sodium pyruvate (Gibco) and antibiotics (penicillin, streptomycin and kanamycin, from Gibco).

IgM.S (an IgM_+ cell line derived from the I.29 lymphoma) (21) cells were maintained by intraperitoneal passage in (I/St x BALB/c) F_1 mice. These cells are >99% IgM_+.

Extraction of total cell RNA

Cells were washed two times in STE (100 mM NaCl, 10 mM Tris-HCl pH7.4, 1 mM disodium EDTA), lysed by Dounce homogenization in STE containing 0.5% sodium dodecyl sulfate (SDS) and 0.1% diethyl pyrocarbonate, and the nucleic acids were extracted 3 times with an 1:1 mixture of phenol and chloroform. Nucleic acids were ethanol-precipitated, dissolved in 20 mM Tris-HCl pH7.4, 5 mM MgCl_2, and treated with DNase (Millipore, DPRF) (2.5 μg/ml) for 30 min. at 37°C. SDS (0.5%) and EDTA (10 mM) were added, and the nucleic acids were extracted with phenol-chloroform once, and ethanol-precipitated. Poly(A)_+ RNA
was isolated by 2 successive adsorptions to oligo(dT) cellulose (Collaborative Research, T3).

**Gel electrophoresis, RNA blotting, and hybridization**

The RNA samples and HindIII fragments of \(\lambda\) bacteriophage DNA were
denatured with dimethyl sulfoxide (DMSO) and glyoxal, and fractionated by
electrophoresis in 1% agarose gels in 10 mM sodium phosphate pH 7 (22). The
RNA and DNA fragments were blotted onto diphenylthioether paper (23), and the
blots were hybridized sequentially with nick-translated DNA fragments
containing various segments from the expressed Ig \(\alpha\) chain gene. In a few
experiments, RNA probes transcribed by SP6 polymerase from inserts cloned in
SP6 vectors were utilized (24). Hybridization was performed in 50% deionized
formamide, 900 mM NaCl, 90 mM sodium citrate, 0.6 mM disodium EDTA, 1 mg yeast
RNA per ml, 0.02% each of bovine serum albumin, Ficoll, and polyvinyl
pyrrolidine. Blots were washed in 0.1% SDS, 15 mM NaCl, 0.1 mM disodium EDTA,
1 mM sodium phosphate pH 6.8 at 50-52°C. To re-use blots, hybridized probes
were removed by incubation of the blots for 2-3 hr at 68°C in 75% formamide
(Bethesda Research Lab), 50 mM sodium phosphate pH 7, 5 mM disodium EDTA pH 7.

**Hybridization probes**

The DNA regions present in the hybridization probes are indicated in Fig.
1A. The 5' probe was a nick-translated 1.15 Kb BamHI-SacI fragment subcloned
into pSP64 from a recombinant \(\lambda\) bacteriophage containing the expressed \(\text{H}
chain gene from IgM\(^+\) cells from the I.29 lymphoma (D. Klein, J. Blance and J.S.,
in preparation). The I.29 heavy chain variable region (\(V_H\)) probe was a 368
bp PstI fragment subcloned from this same phage encoding the I.29 \(V_H\) region
from amino acid residue 3, including the 80 bp IVS, through amino acid number
101 (D. Klein, J. Blance and J.S., in preparation). \(J_{H3-4}\) was a 2 kb
BamHI-EcoRI fragment from BALB/c germline DNA (25). The IVS 1 probe was a 4.6
kb SstI-PstI fragment encoding the \(\alpha\) switch (Sa) region and the 3' portion of
IVS 1, cloned from the non-rearranged \(\alpha\) gene present in I.29 cells
(unpublished data). The \(C_\alpha\) probe was a 1.4 kb MspI fragment from the cDNA
clone pa(J558)\(^{13}\) (25). The IVS 2 probe was an 0.85 kb HindIII-Eco RI
fragment encoding the 5' portion of IVS 2, subcloned from a recombinant \(\lambda\)
phage (\(\lambda\)57.1) containing the the germline \(\alpha\) gene cloned from I.29 cells
(unpublished data). The \(\alpha_{m5}'\) (3 kb HindIII fragment) and \(\alpha_{m3}'\) (0.7 kb
HindIII-Eco RI fragment) probes were isolated from the plasmid pa4.5, which
contains the BALB/c genomic DNA segment encoding the \(\alpha_m\) domain (8). The
\(\alpha_{m3}'\) fragment was subcloned into pBR322. The 3' probe was a single-stranded
RNA complementary to the coding strand transcribed by Sp6 polymerase from a BamHI-SacI fragment subcloned into pSP65 from λ57.1 (unpublished data).

RESULTS

Structure of \( \alpha \) genes in BFO.3 and ID150 cells

The structure of the IgH chain genes in the IgA+ cells from the I.29 lymphoma, e.g. the BFO.3 cells and the I.29 component of ID150 hybridoma cells, has been analyzed by blotting experiments on genomic DNAs and on cloned \( \alpha \) genes, and by nucleotide sequence analyses of cloned genes (21; D. Klein, J. Blance and J. S., in preparation). These cells contain one expressed \( \alpha \) gene, produced by the joining of a variable region (\( V_H \)) gene segment with a D\(_H\) and the J\(_{H4}\) gene segments. On the non-expressed H chain chromosome the D\(_{Q52}\) gene segment (26) has recombined with the J\(_{H2}\) gene segment, and this recombined gene segment has undergone a switch recombination with the \( \gamma_3 \) gene. Thus, the non-expressed chromosome contains an \( \alpha \) gene in the germline configuration (21; J.S. and O. Kekish, unpublished data).

A map of the expressed \( \alpha \) gene present in the BFO.3 cells, with the location of the probes used in these experiments indicated above it, is given in Fig. 1A. Some of the sites for the enzymes BamHI, SacI, and EcoRI differ from those in the \( \alpha \) gene in BALB/c mice because I/St mice (in which the I.29 lymphoma arose) have the Igh\(_C\) allotype and BALB/c mice, the Igh\(_D\) allotype (27, 28). The location of the regions encoding the C\(_\alpha\) and the \( \alpha_m \) domains in the I.29 \( \alpha \) gene were determined by hybridization of nick-translated probes from BALB/c \( \alpha \) genes to restriction fragments from a cloned genomic \( \alpha \) gene from I.29 cells (data not shown).

By comparing the sequence of the I.29 \( V_H \) gene (D. Klein, J. Blance and J.S., in preparation) with that of other IgH chain genes, the start site for transcription (cap site) should be located between 211 and 217 bp 3′ to the BamHI site located immediately 5′ to the \( V_H \) gene expressed in I.29 cells (Fig. 1A) (29-32).

\( \alpha \) mRNAs present in BFO.3 and ID150 cells

To examine the \( \alpha \) RNAs present in BFO.3 and ID150 cells, total cell polyadenylated RNA was analyzed by RNA blotting. The RNAs were denatured with dimethyl sulfoxide (DMSO) and glyoxal, and fractionated by electrophoresis in 1% agarose gels in 10 mM phosphate buffer (22). The two larger \( \alpha \) mRNAs (3.1 and 2.1 Kb) encode the membrane-bound \( \alpha \) chain and the smallest one, the secreted \( \alpha \) chain (8, 17). Only the largest \( \alpha \) mRNA hybridized with the 0.7 Kb HindIII-EcoRI fragment labeled \( \alpha_{m3} \) in Fig. 1A (Fig. 2, lanes 7,8), whereas
Fig. 1A
Map of expressed α gene in IgA+ cells from I.29 B lymphoma
(21; D. Klein, J. Blance and J.S., In preparation; unpublished data).
Symbols for restriction enzymes are: B = BamHI; Bg = BglII; E = EcoRI; H = HindIII; P = PstI; S = SacI. The DNA regions present in probes used to map the α RNAs are indicated by bars above the restriction map. The dashed line at the 5' end of the IVS 1 probe indicates that the 5' end of the IVS 1 probe (derived from the non-rearranged α gene) was not complementary to regions 5' to the site of Su-Sα recombination. The rectangles on the restriction map indicate regions present in the α mRNAs. The thinner rectangle indicates the location of the 3' untranslated regions in the αm mRNAs. The 3 small circles above these rectangles indicate the locations of the 3' termini of the 3 α mRNAs.

B. α RNAs detected in BFO.3 cells. The polyadenylated RNA species detected in BFO.3 cells are diagrammed below the DNA regions encoding them. The IVSs which were removed to produce each RNA species are indicated by the thinner, non-horizontal lines. Dashed lines indicate uncertainty about the regions present in the RNA.

both αm mRNAs hybridized with the 3Kb HindIII fragment labelled αm5, in Fig. 1 (8, 33) (Fig. 2, lanes 5,6). To specifically define the RNA species transcribed from the expressed α gene, the blots were hybridized with a 368bp PstI fragment encoding the expressed Vh gene segment (Fig. 3, lanes 2,3) and with a 2Kb BamHI-EcoRI fragment encoding the BALB/c germline Jα3-4 gene segments (25) (Fig. 3, lanes 4,5).
Blots of α mRNAs present in the BFO.3 lymphoma and ID150 hybridoma cells. Lanes contained 3 μg (except lanes 2 and 8 contained 1 μg) of polyadenylated total cell RNA from the indicated cells which had been denatured, electrophoresed and blotted as described in Methods. The blots were hybridized with the probes indicated below each panel in order to illustrate the specificity of the probes. The bands corresponding to the three α mRNAs are indicated. The sizes of the α RNAs transcribed from the germline α gene(s) in IgM.S cells (lane 4) are: 3.0, 2.3, 1.6, 1.25, 0.96 and 0.81 Kb (lanes 3 and 4 are from a different gel from lanes 1 and 2). Lanes 1 and 2 are a very short exposure of Fig. 3, lanes 2 and 3. Lane 9 contains end-labelled Hind III fragments of λ phage DNA which were denatured with DMSO and glyoxal, electrophoresed in a parallel lane, and blotted along with the RNAs. The fragment lengths are given in Kb.

DMSO and glyoxal, provided molecular weight markers, as it has been demonstrated that DNA fragments migrate similarly to RNA in this gel system (22). The sizes of the three α mRNAs relative to the HindIII fragments of λ DNA, 3.1, 2.1, and 1.7 kb (Fig. 2, lane 1), agree with the prediction from the structure of the I.29 VH gene and the sizes of the BALB/c and I/St Ca and αm domains (B; D. Klein, J. Blance and J.S., in preparation), assuming that these mRNAs each contain a poly(A) sequence of 100-200 nucleotides. Although the sizes of the α mRNAs we determined differ from those determined by Word et
al. (8), which were 3.4, 2.5 and 2.0 Kb, this is due to their use of different molecular weight standards (ribosomal and non-polyadenylated viral RNAs), and not due to a difference between I/St and BALB/c mice as the sizes of the α₅ RNAs in the BALB/c plasmacytoma, TEPC 15, and in I.29 cells are identical (not shown).

Large polyadenylated α RNAs present in BF0.3 and ID150 cells

The polyadenylated RNA species detected in total cell RNA from BF0.3 and ID150 cells by probes encoding regions from the expressed α gene are listed in Table I, and the blots used to define these RNA molecules are shown in Fig. 3. The largest RNA molecule detected (~17 Kb) in BF0.3 cells was a minor species, showing as a very faint band in lanes hybridized with the Ca, α₅5', and α₃3', probes (Fig. 3, lanes 8,11,13). Hybridization of the 17 Kb RNA to the JH₃-4 probe was also seen, but only in the original autoradiograph. Although the 17 Kb RNA was not detected by the VH probe (lane 2) or by the probes encoding the large intervening sequence (IVS 1) (lane 6) or IVS 2 (lane 10), we assume this is due to the poorer detection of large RNAs on these blots. The 17 Kb RNA is surprisingly large since it is predicted from the structure of the rearranged α gene in BF0.3 cells that a non-spliced poly(A)+ RNA precursor whose 3' end is co-terminal with the 3' end of the largest α mRNA would be 13.5 Kb long. In fact, a predominant α RNA of 13.5 Kb is detected in BF0.3 cells by the VH, JH₃-4, IVS 1, Ca, IVS 2, α₅5', and α₃3', probes (Table I; Fig. 3); this 13.5 Kb RNA probably corresponds to the unspliced poly(A)+ RNA whose 3' end is co-terminal with the largest α mRNA.

We have been unable to determine whether the 17 kb RNA is initiated at a...
## TABLE I

**Total Cell Poly(A)***⁺ α RNA Species:

<table>
<thead>
<tr>
<th>Measured Size (Kb)</th>
<th>Predicted Size (Kb)</th>
<th>VH</th>
<th>JH3-4</th>
<th>IVS1</th>
<th>Ca</th>
<th>IVS 2</th>
<th>αm5'</th>
<th>αm3'</th>
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<tr>
<td>17</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13.5</td>
<td>13.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>?</td>
<td>12.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.7</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>9.5</td>
<td>9.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.6</td>
<td>5.6</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.65</td>
<td>4.6</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>2.9</td>
<td>?</td>
<td>-</td>
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<td>+</td>
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<td>-</td>
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**mRNAs:**

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<tr>
<th>Measured Size (Kb)</th>
<th>Predicted Size (Kb)</th>
<th>VH</th>
<th>JH3-4</th>
<th>IVS1</th>
<th>Ca</th>
<th>IVS 2</th>
<th>αm5'</th>
<th>αm3'</th>
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<tr>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1.70</td>
<td>1.7</td>
<td>+</td>
<td>+</td>
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**Regions Present in ID 150 Cell RNAs:**

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<th>Measured Size (Kb)</th>
<th>Predicted Size (Kb)</th>
<th>VH</th>
<th>JH3-4</th>
<th>IVS1</th>
<th>Ca</th>
<th>IVS 2</th>
<th>αm5'</th>
<th>αm3'</th>
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<td>13.5</td>
<td>+</td>
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<td>+</td>
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<td>?</td>
<td>?</td>
</tr>
<tr>
<td>9.5</td>
<td>9.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.6</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.9</td>
<td>?</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

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a) Assuming poly(A) sequence 200 bp in length, from sequencing or mapping of α genes (8; 11; D. Klein and J.S., in preparation; unpublished data).

b) The 13.5 Kb RNA in ID150 cells could be barely discerned in autoradiographs of blots hybridized with VH, IVS and αm3' probes, but not in photographs of these blots.

? Indicates probes which should hybridize with the RNA species although no definite signal was detected.

---

Site located 5' to the VH promoter or contains sequences transcribed from the region 3' to the α polyadenylation sites. Both the 5' (SacI-BamHI) and 3' (BamHI-SacI) probes (see Fig. 1A) hybridized with several RNA species in BFO.3 cells, none of which clearly corresponded to the 17kb RNA, nor any other α RNA precursor (data not shown).
Although an unspliced RNA 12.5 Kb in length, which is co-terminal with the smaller α<sub>m</sub> mRNA, should be present in BFO.3 cells, we were unable to definitively detect this RNA, probably because it was obscured by the more predominant 13.5 Kb RNA. However, in the blot hybridized with the α<sub>m5</sub>' probe, the 13.5 Kb RNA does appear to have a slightly faster migrating shadow band which could correspond to the 12.5 Kb α<sub>m</sub> RNA (lane 11), whereas when the identical blot was hybridized with the α<sub>m3</sub>' probe, which should not detect the 12.5 Kb RNA, the 13.5 Kb band appears to be more narrow and to have a more distinct lower edge (lane 13).

The size and hybridization properties of the next largest species of α RNA detected in BFO.3 cells (10.7 Kb) indicate that it could be derived from the 13.5 Kb α<sub>m</sub> precursor RNA by splicing out the 2.35 Kb IVS between the Ca and α<sub>m</sub> domains (labeled IVS 2 in Fig. 1) before splicing out the largest IVS (IVS 1). This hypothesis is consistent with the fact that the 10.7 Kb RNA was not detected by the IVS 2 probe, and was barely detected by the α<sub>m5</sub>' probe (lane 11) which mostly encodes IVS 2 (See Fig. 1), but was quite predominant when the RNA was hybridized with the α<sub>m3</sub>' probe (lane 13). The 10.7 Kb RNA species was also faintly visible in blots hybridized with the V<sub>H</sub>, J<sub>H3-4</sub>, IVS 1 and Ca probes at levels which appear consistent with this explanation. The difference in size between the 13.5 and 10.7 Kb RNAs (2.8 Kb) is larger than that predicted (2.35 Kb) from the size of the IVS 2 in the BALB/c α gene, which was determined by mapping cloned DNAs (8). It is possible that other small splices within the V<sub>H</sub> domain (80 nucleotides) (D. Klein, J. Blance and J.S., in preparation) and Ca domain (441 nucleotides) (11) have occurred which would result in a length of 10.7 Kb.

The predominant 9.5 Kb RNA species present in BFO.3 and IDI50 cells is of the size and has the pattern of hybridization predicted for a RNA molecule which is the unspliced polyadenylated precursor for α<sub>s</sub> mRNA, as it hybridizes with the V<sub>H</sub>, J<sub>H3-4</sub>, IVS 1, and Ca probes but not with the IVS 2 and the two α<sub>m</sub> probes (Table I) (Fig. 3).

The next two smaller α RNA molecules, 5.6 and 4.65 Kb, have the properties expected if they were derived by splicing out the large IVS 1 (7.4 Kb) and the three small IVSs within the V<sub>H</sub> and Ca domains (521 bases), but not IVS 2, from the 13.5 Kb and 12.5 Kb α<sub>m</sub> RNA precursors, respectively (Table I). Only the larger of these RNAs hybridizes with the α<sub>m3</sub>' probe (lane 13), and both RNAs hybridize with the V<sub>H</sub>, Ca, and α<sub>m5</sub>' probes. (The faint 4.65 Kb band seen in lane 13, was due to incomplete removal of the previously hybridized α<sub>m5</sub>', probe). The 5.6 and 4.65 Kb RNA species were not detected
by the J_{H3-4} probes, presumably because of the combined effect of their low abundance and the fact that much of the region complementary to the J_{H3-4} probe has been spliced out; they also did not hybridize with the IVS 1 probe. These $\alpha_m$ intermediates were difficult to detect in ID150 cell RNA (Fig. 3, lanes 9,12,14).

**Polyadenylated $\alpha_m$ RNA fragment**

The next largest $\alpha$ RNA species detected in BFO.3 cells was a 4.0 Kb poly(A)+ RNA molecule which hybridized with the IVS 2, $\alpha_m$5', and $\alpha_m$3' probes (Fig. 3, lanes 10,11,13), but not with the $V_H$, J_{H3-4}, Ca or IVS 1 probes (Table I). The size of the 4.0 Kb RNA species is slightly larger than the size predicted (3.9Kb) if this molecule were derived from the 13.5 or 5.6 Kb $\alpha_m$ RNAs by cleavage at the polyadenylation site used to terminate the $\alpha_s$ mRNA (8). This $\alpha_m$ RNA fragment was not detected in ID150 cells (Fig. 3, lanes 12, 14). Previously we have shown that the germline $\alpha$ gene(s) are transcribed in IgM+ cells derived from the I.29 lymphoma (35). However, the germline $\alpha$ gene transcripts detected IgM+ cells with the Ca probe (Fig. 1, lane 4) or with the $\alpha_m$ probes (not shown) are smaller than the $\alpha_m$ RNAs detected in BFO.3 cells. Thus, the 4.0 Kb $\alpha_m$ RNA is produced from the rearranged expressed $\alpha$ gene, and not the germline $\alpha$ gene.

The next smaller RNA (3.8 Kb) was only detected by the IVS 2 probe and not with the Ca nor $\alpha_m$ probes, although it appears too long to simply consist of sequences from IVS 2, which should be 2.3-2.8 Kb long. Its absence from the blot hybridized with the $\alpha_m$3' probe can be explained by it being obscured by the very predominant 3.1 Kb mRNAs for $\alpha_m$, and this may also explain its absence from the blot hybridized with the $\alpha_m$5', probe. Coleclough and Wood (36) have provided evidence that excised introns migrate as doublet bands in agarose gels, and that the lariat forms of excised introns migrate as if they were 7-8% larger than the same sequences in linear form. Thus, it appears possible that the 3.8 Kb RNA may be the linear form of a RNA fragment whose 5' end is at the poly(A) site of $\alpha_s$ mRNA and whose 3' end is at the poly(A) site of the larger $\alpha_m$ mRNA, and that the 4.0 Kb RNA (approximately 5% larger) may be the lariat form of the identical RNA segment.

Two small species of poly(A)+ RNAs, 2.9 and 1.7 Kb in length, were detected in both BFO.3 and ID150 cells with the IVS 1 probe. The 1.7 Kb RNA co-migrates with the $\alpha_s$ mRNA, and was probably detected due to hybridization of the abundant $\alpha_s$ mRNA with a small portion of the 5' region of the Ca domain which may be present in the IVS 1 probe. The 2.9 Kb RNA does not co-migrate with any other $\alpha$ RNA. It is likely that the 2.9 Kb RNA is not
transcribed from the α gene because the IVS 1 probe contains DNA sequences which are highly reiterated in the mouse genome (not shown). Furthermore, both IgE+ cells from the I.29 lymphoma and BCL1 cells (IgM+, IgD+)(20) also contain a 2.9 Kb poly(A)+ RNA which hybridizes with the IVS 1 probe, although neither of these cells contain transcripts that hybridize with the Ca probe (data not shown). Finally, if the 2.9 Kb RNA were produced by stepwise processing of IVS 1, it should not be polyadenylated.

DISCUSSION

Removal of the large IVSs may sometimes occur in the 3' to 5' direction.

The results of our RNA blotting studies indicate that the order of removal of the two large IVSs from the precursors for α mRNAs is generally in the 5' to 3' direction, but apparently not exclusively, since the 10.7 Kb α RNA molecule is best explained by the removal of IVS 2 from transcripts which still contain IVS 1. As the amount of the 10.7 Kb RNA molecule present in BFO.3 cells appears to be approximately 25% of the amount of the unspliced 13.5 Kb αm RNA precursor (Fig. 3, lane 12), and approximately 10% of the amount of the 5.6 Kb αm RNA (derived by removal of IVS 1 and retention of IVS 2), this is a minor pathway. In a similar study of the precursors for Ig μ mRNA, the removal of the two large IVSs appeared to always occur in the 5' to 3' direction (16), although it has also been shown that IVSs are not always removed from the processors for vitellogenin, ovomucoid and β globin mRNAs exclusively in the 5' to 3' direction (37-40). Polyadenylation may occur more than once on a single RNA transcript.

One of the more surprising findings of this study was the detection of a 4.0 Kb poly(A)+ RNA, which by its size and hybridization properties appears to contain IVS 2, the entire αm domain, and to terminate at the polyadenylation site of the larger αm RNA. Kemp et al (15) detected a μm RNA fragment which differed from the αm fragment detected here in that the μm fragment did not contain the IVS located between the μs terminus and the μm domains, and appeared to be generated by cleavage near the splice acceptor site at the 5' side of the μm domains. Although we have not mapped the ends of the αm RNA fragment, it seems likely from its size and by the fact that it hybridizes strongly with the Hind III-Eco RI fragment from the 5' side of IVS 2 and with the αm5' probe that it contains most or all of the IVS between the αs and αm domains. The existence of the αm RNA species indicates that poly(A)+ RNA transcripts which have the entire αm domain and which have a 3' end which is co-terminal with the larger αm mRNA can be cleaved to produce an
αₘ mRNA. But, as these experiments measure the steady state level of the RNA molecules in these cells, they do not allow us to determine to what extent this pathway is used to produce αₙ mRNA in BFO.3 cells, i.e. whether it is the major or a minor pathway. However, the studies of Yuan and Tucker (12) and Danner and Leder (14) which show that termination of transcription does not occur between the polyadenylation sites for υₙ and υₘ mRNAs, indicate that generation of υₙ mRNA by cleavage/polyadenylation of a RNA precursor terminating at the υₘ poly(A) site may be the major, or only, pathway of υₙ mRNA production.

The finding of poly(A)+ RNA transcripts from the expressed α gene which were too large (~17 Kb) to be co-terminal with the largest αₘ mRNA, assuming that transcription initiated at the cap site, suggested that transcription can continue well beyond (~3.5 Kb) the most 3' υₘ polyadenylation site, and that such extended RNA transcripts can be polyadenylated. As Manley (41) has shown that the 3' ends of RNA transcripts can be polyadenylated, whether or not they are preceded by an AAUAAA sequence, it is possible that the 17 Kb RNA terminates at the site of unloading of RNA polymerase from the DNA template. It is likely that such RNA transcripts would be subsequently cleaved and polyadenylated at sites appropriate for the production of functional α mRNAs, as the presence of the 3.8 and 4.0 Kb αₘ poly(A)+ RNA fragments in BFO.3 cells suggests that polyadenylated α RNA transcripts can be cleaved at internal polyadenylation sites.

The finding of the 17 Kb RNA and the 4.0 Kb αₘ RNA fragment indicates that the site of cleavage/polyadenylation does not necessarily determine which processing steps will subsequently occur, because more than one cleavage/polyadenylation event can occur on the same RNA transcript. As we detected only a small quantity of the 13.5 Kb αₘ RNA precursor in ID150 hybridoma cells, it is possible that the first cleavage/polyadenylation event on the nascent α RNA transcript occurs more frequently at the αₙ terminus in these cells because the enzyme(s) responsible for cleavage/polyadenylation has a higher level of activity in cells differentiated to synthesize large amounts of secretory IgA (42), and the αₙ terminus is transcribed prior to the αₘ termini. Because we did not detect the 4.0 Kb αₘ RNA fragment in ID150 hybridoma cells, our data do not permit us to exclude the alternative hypothesis, that most RNA polymerase molecules terminate transcription 5' to the αₘ polyadenylation sites in IgA-secreting cells. However, by this hypothesis the regulation of production of mRNAs for membrane-bound and secreted Igs would differ in IgM⁺ and IgA⁺ cells, and thus it appears more
likely that our inability to detect the 4.0 Kb $\alpha_m$ RNA fragment was because it forms a smaller portion of the $\alpha$ RNA in IgA secreting cells than in the BFO.3 lymphoma cells, perhaps due to more rapid RNA processing and/or to greater accumulation of $\alpha$ mRNA in Ig-secreting cells. Supporting this possibility is the fact that the precursors for both $\alpha_s$ and $\alpha_m$ mRNAs form less distinct bands and appear to be a lower proportion of the total cell poly(A)$^+$ RNA in ID150 cells than in BFO.3 cells (Fig. 3, lanes 8, 9). Furthermore, other investigators (14, 16) have also failed to detect RNA transcribed from the $\mu_m$ domain in hybridoma or plasmacytoma cells by a steady-state assay (S1 nuclease), although it has been demonstrated that transcription does continue past the $\mu_m$ domain in B cells which produce mostly, or only, secreted IgM (12, 14).

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