Note added in proof: To reduce reagent costs and increase convenience, the PCR reaction can conveniently be scaled down to 20 μl with an overlay of mineral oil, and *Taq* polymerase (Perkin Elmer–Cetus Inc.), can be used according to the manufacturer's instructions. The alternate method of PCR amplification followed by sequencing of double-stranded DNA has been used to examine mutations in the β-globin gene (*8*).

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2. Method: The PCR, transcription, and sequencing reaction were performed as previously described with minor modifications (*4, 9, 10*), except that Klenow fragment was used in PCR at 50°C with 10% dimethyl sulfoxide (DMSO). In brief, a microfuge tube containing 1 μg of DNA in a volume of 100 μl was denatured at 95°C for 10 minutes (2 minutes in subsequent cycles) in the presence of the following: 50 m*M* sodium chloride, 10 m*M* tris-HCl (*p*H 7.6), 10 m*M* magnesium chloride, 10% DMSO, and 1.5 m*M* of each of the four deoxyribonucleoside triphosphates (dATP, deoxyadenosine triphosphate; dCTP, deoxycytidine triphosphate; dGTP, deoxyguanosine triphosphate; TTP, thymidine triphosphate). After microfuging, samples were then annealed at 50°C for 2 minutes, and subsequently one-half unit of Klenow fragment was added. Samples were incubated at 50°C for another 2 minutes. Twenty-six additional cycles of denaturation, annealing, and polymerization were performed.

   It is crucial to assure that the Klenow fragment added at later cycles has the same activity as that added at early cycles. To this end, fresh aliquots of Klenow fragments were removed from the −20°C freezer every seven cycles and diluted from the manufacturer's buffer to 1 U/μl with dilution buffer [10 m*M* tris (*p*H 7.5), 1 m*M* dithiothreitol (DTT), 0.1 m*M* EDTA, and 1.5 m*M* of the four deoxyribonucleoside triphosphates].

   After a final denaturation, 3 μl of the amplified material was added to 17 μl of the RNA transcription mixture: 40 m*M* tris-HCl (*p*H 7.5), 6 m*M* magnesium chloride, 2 m*M* spermidine, 10 m*M* sodium chloride, 0.5 m*M* of the four ribonucleoside triphosphates, RNAsin (1.6 U/μl), 10 m*M* DTT, 10 U of T7 RNA polymerase, and diethyl pyrocarbonate–treated H_2O. Samples were incubated for 1 hour at 37°C and the reaction was stopped with 5 m*M* EDTA.

   For sequencing, 2 μl of the transcription reaction and 1 μl of ^{32}P end-labeled (see below) reverse transcriptase primer were added to 10 μl of annealing buffer [250 m*M* KCl, 10 m*M* tris-HCl (*p*H 8.3)]. The samples were heated at 80°C for 3 minutes and then annealed for 45 minutes at 45°C (approximately 5°C below the denaturation temperature of the oligonucleotide). Microfuge tubes were labeled with A, C, G, and T. The following was added: 3.3 μl of reverse transcriptase buffer [24 m*M* tris-HCl (*p*H 8.3), 16 m*M* magnesium chloride, 8 m*M* DTT, 0.4 m*M* dATP, 0.4 m*M* dCTP, 0.8 m*M* dGTP, and 0.4 m*M* TTP] containing 5 U of AMV reverse transcriptase, 1 μl of a dideoxyribonucleoside triphosphate: 1 m*M* ddATP or 1 m*M* ddCTP or 1 m*M* ddGTP or 2 m*M* ddTTP, and finally, 2 μl of the primer RNA template solution. The sample was incubated at 50°C for 45 minutes and the reaction was stopped by adding 2.5 μl of 100% formamide with 0.3% bromophenol blue and xylene cyanol FF. Samples were boiled for 3 minutes and 3 μl was loaded onto a 100-cm sequencing gel separated by electrophoresis for about 15,000 volt-hours. Subsequently, autoradiography was performed.

   End-labeling of the reverse transcriptase primer was performed by incubating a 0.1-μg sample of oligonucleotide in a 13-μl volume containing 50 m*M* tris-HCl (*p*H 7.4), 10 m*M* $MgCl_2$, 5 m*M* DTT, 0.1 m*M* spermidine, 100 μCi [α-^{32}P]ATP (5000 Ci/mmol) and 7 U of polynucleotide kinase for 30 minutes at 37°C. The reaction was heated to 65°C for 5 minutes and 7 μl of water was added for a final concentration of 5 ng of oligonucleotide per microliter. One microliter of labeled oligonucleotide was added per sequencing reaction without removal of the unincorporated mononucleotide.
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Nuclear Factors in B Lymphoma Enhance Splicing of Mouse Membrane-Bound μ mRNA in *Xenopus* Oocytes

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Regulation of the synthesis of membrane-bound and secreted immunoglobulin μ heavy chains at the level of RNA processing is an important element for B cell development. The precursor μ RNA is either polyadenylated at the upstream poly(A) site (for the secreted form) or spliced (for the membrane-bound form) in a mutually exclusive manner. When the mouse μ gene linked to the SV40/HSV-TK hybrid promoter was microinjected into *Xenopus* oocytes, the μ messenger RNA (mRNA) was processed primarily to the secreted form. The processing pattern of μ mRNA was altered by coinjection of nuclei of mouse surface IgM-bearing B-lymphoma cells to include the synthesis of the membrane-bound form. An increase in the membrane-bound form was not observed when nuclei of IgM-secreting hybridoma cells or fibroblast cells were coinjected. Deletion of the upstream poly(A) site did not eliminate the effect of B-lymphoma nuclei suggesting that membrane-specific splicing is stimulated. Further, splicing of other μ gene introns was not affected by coinjection of B-lymphoma nuclei. These results suggest that mature B cells contain one or more trans-acting nuclear factors that stimulate splicing specific for membrane-bound μ mRNA.

EXPRESSION OF THE IMMUNOGLOBULIN heavy chain gene is under complex regulation at the level of DNA rearrangement, transcription initiation, and RNA processing (*1, 2*). In mature B cells, immunoglobulin M (IgM) molecules lie on the cell surface and serve as receptors to antigen. When a B cell differentiates into a plasma cell, the secreted form of IgM is primarily produced. The switch between the synthesis of the two forms of IgM is accomplished by producing, from a single gene, two alternative forms of μ heavy chain messenger RNA (mRNA) that differ only in their 3′ termini (*3–5*). Production of the two μ mRNAs seems to be regulated at the level of RNA processing (*2*). When precursor μ RNA is polyadenylated at the upstream poly(A) site, the resulting mature μ mRNA produces a secreted form of μ heavy chain. On the other hand, when the upstream poly(A) site is spliced out and the downstream poly(A) site is used, the membrane-bound form is produced. In mature B cells almost equal amounts of secreted and membrane-bound μ mRNAs are produced, while primarily secreted μ mRNA is produced in terminally differentiated plasma cells. Thus, the regulatory mechanism for the differential processing of μ mRNA is one of the important elements for B cell development.

Analyses of the expression of the μ gene in non-B cells showed that precursor μ RNA is processed primarily to the secreted form in the nonregulated state (*6, 7*). On the basis of studies of the expression of mutated mouse μ genes in various mouse cell lines, it has been suggested that splicing of the C4–M1 intron, specific for membrane-bound μ mRNA, is enhanced in mature B cells (*7, 8*).

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In addition, the length of the C4–M1 intron plays a role in determining the ratio between the two μ mRNAs (*8–10*). However, despite these studies, the molecular mechanism of the developmentally regulated differential processing of μ mRNA is still unclear.

The *Xenopus* oocyte microinjection system has been successfully used for the analysis of RNA processing and transcription initiation (*11–14*). Korn and Gurdon (*15, 16*) showed that developmentally inert oocyte-type 5*S* RNA genes in *Xenopus* erythrocytes could be activated by microinjecting nuclei of the erythrocytes into *Xenopus* oocytes, suggesting that factors in the oocytes required for the expression of the oocyte-type 5*S* RNA genes were able to enter the injected erythrocyte nuclei through the nuclear membrane. We have adapted this system (*15, 16*) to the search for trans-acting factors affecting differential processing of μ mRNA. In this study, we microinjected nuclei of mouse B cell lines along with the mouse μ gene and analyzed the effects on the differential processing of the μ mRNA. The results show that nuclei of mature B cells contain an activity that increases production of the membrane-bound form of μ mRNA in *Xenopus* oocytes.

To express the mouse μ gene in *Xenopus* oocytes, we constructed a recombinant plasmid pSVTKμ (Fig. 1) in which a 5.2-kb Bam HI–Eco RI fragment of the mouse μ gene was connected to the SV40/HSV-TK hybrid (SV/TK) promoter. The SV/TK promoter, consisting of the SV40 enhancer and the TATA box from the thymidine kinase gene of herpes simplex virus (*17*), initiates transcription efficiently and accurately in *Xenopus* oocytes (*18*). The 5.2-kb Bam HI–Eco RI fragment is known to be sufficient for cell type–specific processing of μ mRNA (*7*).

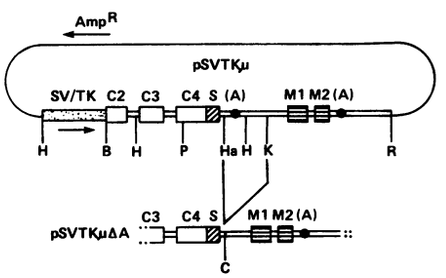

Fig. 1. Structure of μ gene recombinants (*24*). SV/TK denotes the SV40/HSV-TK hybrid promoter, which consists of the SV40 enhancer and the TATA box of the herpes simplex virus thymidine kinase gene (*17*). Amp^R, β-lactamase gene encoding ampicillin resistance. Arrows show orientation of transcription. C2 to C4, constant regions; S, 3′ exon for secreted μ mRNA; M1 and M2, 3′ exons for membrane-bound μ mRNA; (A) with a filled circle, polyadenylation site. Restriction enzyme sites: H, Hind III; B, Bam HI; P, Pst I; Ha, Hae II; K, Kpn I; R, Eco RI; C, Cla I.

For S1 nuclease analysis with probe 1 (Fig. 2A), we first used total cellular RNA of the mouse surface IgM-bearing lymphoma, WEHI-231 (*19*). Two protected fragments were observed (Fig. 2B, lane 8) representing secreted (upper band) and membrane-bound (lower band) μ mRNAs produced in WEHI-231. With the mouse IgM-secreting MxW231.1a.2 hybridoma, a fusion cell line between WEHI-231 and the MPC-11 myeloma (*20*), exclusive production of secreted μ mRNA was observed (*21*).

The pSVTKμ DNA (0.1 ng) was microinjected into the nucleus of *Xenopus* oocytes. After incubation at 19°C for 20 hours,

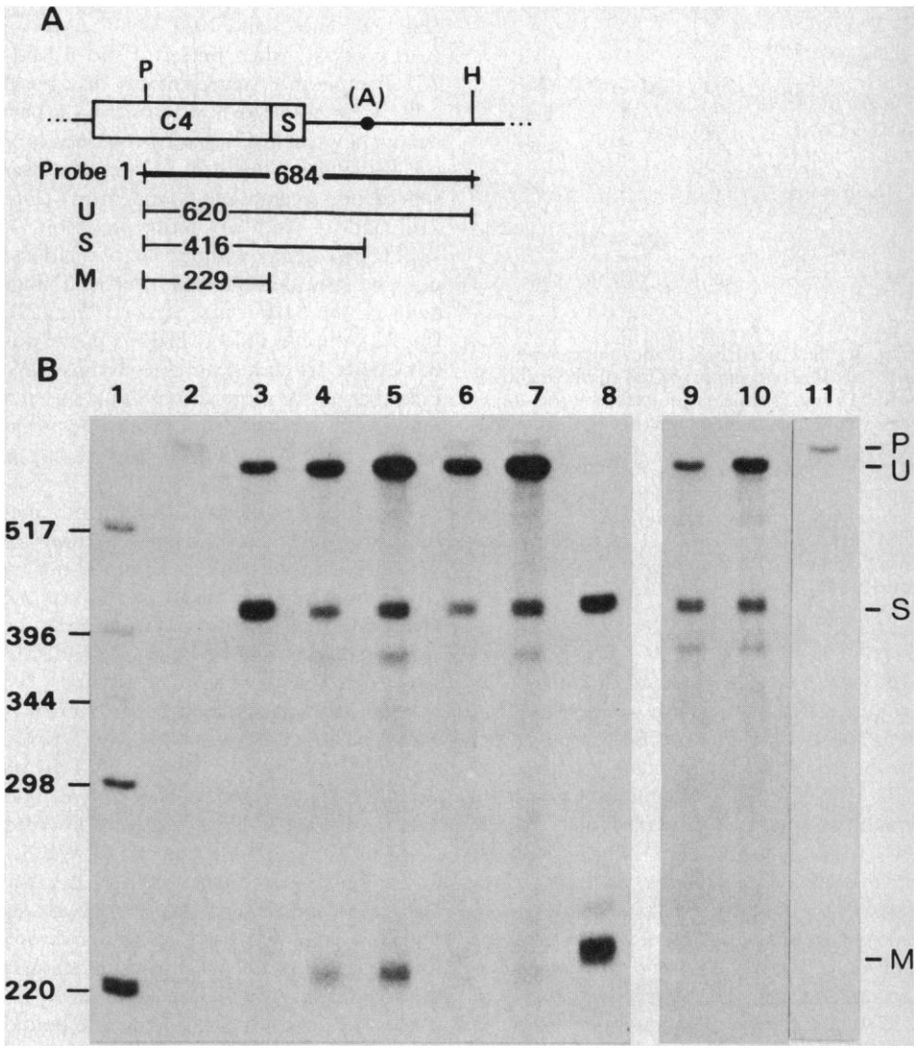

Fig. 2. Processing of μ mRNA in *Xenopus* oocytes. (**A**) Structure of probe 1 and protected subfragments. Numbers show lengths in nucleotides. Uniformly labeled single-stranded DNA probe 1 has short stretches of M13 sequences at both ends. U, S, and M denote fragments protected by unprocessed, secreted, and membrane-bound forms of μ mRNA, respectively. Other symbols are described in the legend to Fig. 1. (**B**) S1 nuclease analysis of RNAs from injected oocytes. Mouse lymphoma WEHI-231 (*19*) and hybridoma MxW231.1a.2 (*20*) were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 50 μ*M* 2-mercaptoethanol. Mouse fibroblast L cell was maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Nuclei for microinjection were prepared from 10^7 exponentially growing cells by the lysolecithin method of Gurdon (*22*) with the modification that 10 m*M* Pipes (*p*H 7.0) and 1 m*M* $MgCl_2$ were added to the SuNaSp buffer (*22*); samples used for microinjection showed 90 to 95% cell lysis. Each oocyte was injected with 0.1 ng of plasmid DNA and/or several hundred nuclei. Microinjection and extraction of total oocyte RNA were as described (*11, 25*). S1 nuclease mapping with uniformly labeled single-stranded DNA probe was described (*7*). Lane 1 shows positions and lengths (in nucleotides) of end-labeled Hinf I–digested pBR322 DNA fragments. Oocyte RNA used for the analysis: lane 2, uninjected; lane 3, injected with pSVTKμ alone; lanes 4 and 5, pSVTKμ plus nuclei of WEHI-231; lanes 6 and 7, pSVTKμ plus nuclei of MxW231.1a.2; lanes 9 and 10, pSVTKμ plus nuclei of L cells; lane 11, nuclei of WEHI-231 alone. In lane 8, total cellular RNA of WEHI-231 was used. Nuclei samples were diluted to 50% (lanes 4, 6, and 9) or 25% (lanes 5, 7, and 10) of the original concentration. P, position of undigested probe 1; U, S, and M are as described in (A). Because of gel curvature, the band shown as M in lane 8 appears a little higher than the counterparts in lanes 4 and 5.

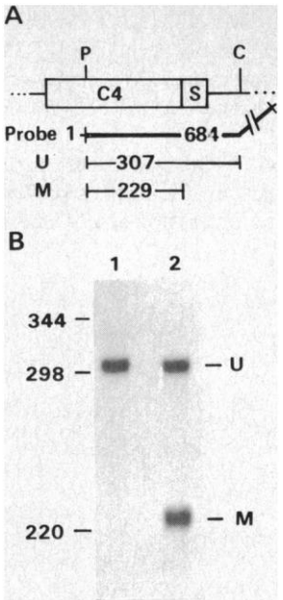

Fig. 3. Effect of deletion of the upstream poly(A) site. (**A**) Probe 1 and protected subfragments. U and M show fragments protected by the unprocessed and membrane-bound forms, respectively. Other symbols are described in the legend to Fig. 1. (**B**) Processing of the μ mRNA lacking the upstream poly(A) site. Oocyte RNAs used: lane 1, injected with pSVTKμΔA; lane 2, pSVTKμΔA plus WEHI-231 nuclei (50% dilution). Numbers on the left show positions and nucleotide lengths of Hinf I–digested pBR322 DNA fragments.

16 oocytes were pooled and the total RNA was extracted (*11*). When the oocyte RNA was analyzed by S1 nuclease mapping with probe 1, two major bands were observed (Fig. 2B, lane 3). No significant protected fragment was observed with uninjected oocyte RNA (Fig. 2B, lane 2). The upper band in Fig. 2B, lane 3 (shown as "U"), corresponds to μ mRNA that is neither polyadenylated at the upstream poly(A) site nor spliced between the C4 and M1 exons (unprocessed form). The lower band (shown as "S") corresponds to the μ mRNA that is polyadenylated at the upstream poly(A) site (secreted form). Most of the unprocessed form was polyadenylated at the downstream poly(A) site (*21*). In repeated experiments, these two bands were always observed as major bands, although their ratio varied in each experiment.

To study whether mouse B cells contain trans-acting factors that affect processing of μ mRNA, nuclei of mouse cell lines were prepared by the lysolecithin method (*22*) and coinjected with 0.1-ng pSVTKμ into *Xenopus* oocytes. In typical experiments, several hundred nuclei were injected into one oocyte. When nuclei of the mouse hybridoma MxW231.1a.2 (representing plasma cells) were coinjected, two major bands corresponding to the unprocessed and secreted forms were observed by S1 nuclease analysis of the oocyte RNA (Fig. 2B, lanes 6 and 7). Although in this particular experiment the ratio of the secreted to unprocessed form was lower than that observed after microinjection of the plasmid alone (Fig. 2B, lane 3), this difference in the ratio was only occasionally observed. A similar result was obtained when nuclei of the mouse fibroblast L cells were coinjected with pSVTKμ (Fig. 2B, lanes 9 and 10).

In contrast, when nuclei of the WEHI-231 lymphoma (representing mature B cells) were coinjected, we observed a new band (shown as "M") that corresponds to μ mRNA where the C4–M1 intron was spliced out (membrane-bound form) (Fig. 2B, lanes 4 and 5). Since injection of WEHI-231 nuclei without the plasmid did not give any detectable μ protected fragment by the S1 nuclease analysis (Fig. 2B, lane 11), the effect of WEHI-231 nuclei was not due to its endogenous μ mRNAs. We infer that one or more factors in the injected nuclei diffused into the oocyte nucleus and affected the processing of the mouse μ mRNA.

The increase in the level of the membrane-bound form caused by coinjection of WEHI-231 nuclei could be accomplished by either inhibition of polyadenylation at the upstream poly(A) site or stimulation of splicing of the C4–M1 intron. To distinguish between these two possibilities, we have analyzed the expression of a mutant μ gene lacking the upstream poly(A) site (pSVTKμΔA, Fig. 1). When pSVTKμΔA alone was injected into the oocytes, no splicing of the C4–M1 intron was observed (Fig. 3B, lane 1). When nuclei of WEHI-231 were coinjected with pSVTKμΔA, the membrane-bound form of μ mRNA was observed together with the unprocessed form (Fig. 3B, lane 2). These results suggest that the effect of WEHI-231 nuclei to increase the level of the membrane-bound form of μ mRNA is accomplished by stimulating splicing of the C4–M1 intron.

To exclude the possibility that coinjection of WEHI-231 nuclei causes nonspecific stimulation of splicing in *Xenopus* oocytes, we analyzed splicing of the C2–C3 intron in pSVTKμ. An aliquot of the RNA samples used for the experiment in Fig. 2 was analyzed by S1 nuclease mapping with probe 2 (Fig. 4A). With the WEHI-231 cellular RNA, most of the μ RNA was the spliced form ("Sp"; Fig. 4B, lane 1). When only pSVTKμ was injected, no splicing between the C2 and C3 exons was observed ("Un"; Fig. 4B, lane 3) as was seen with the C4–M1 intron (Fig. 3B, lane 1). Low efficiency in splicing in *Xenopus* oocytes was also observed with the SV40 early and late gene transcripts (*18, 23*). When nuclei of WEHI-231 or MxW231.1a.2 were coinjected, no significant increase in the splicing of the C2–C3 intron was detected (Fig. 4B, lanes 4 and 5). We obtained a similar result with splicing of the C3–C4 intron; the splicing was hardly observed by injecting pSVTKμ alone or with coinjection of WEHI-231 or MxW231.1a.2 nuclei (*21*). Thus, the ability of WEHI-231 nuclei to stimulate splicing is specific for the C4–M1 intron.

In this report, we have shown that nuclei of the mouse surface-IgM–bearing lymphoma, WEHI-231, contain one or more factors that stimulate splicing of the C4–M1 intron to increase the level of the membrane-bound form of μ mRNA in *Xenopus* oocytes (Figs. 2 and 3). This activity was not detected with nuclei of the IgM-secreting MxW231.1a.2 hybridoma or the fibroblast L cells (Fig. 2). It will be interesting to

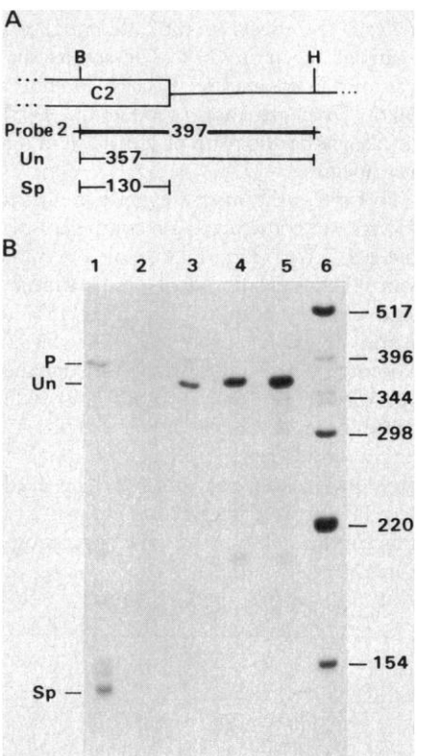

Fig. 4. Splicing of the C2–C3 intron. Un and Sp denote fragments protected by the unspliced and spliced μ mRNA, respectively. (**A**) Structure of probe 2 and protected subfragments. The probe has short stretches of M13 sequences at both ends. Symbols are described in the legend to Fig. 1. (**B**) S1 nuclease analysis of splicing of the C2–C3 intron. An aliquot of the oocyte RNAs used for the experiment in Fig. 2 was used. In lane 1, the WEHI-231 RNA was used for the analysis. Oocyte RNAs used: lane 2, uninjected; lane 3, injected with pSVTKμ alone; lane 4, pSVTKμ plus WEHI-231 nuclei (25% dilution); lane 5, pSVTKμ plus MxW231.1a.2 nuclei (25% dilution). Lane 6, end-labeled Hinf I–digested pBR322 DNA. P shows the position of undigested probe 2.

analyze the presence of the splicing stimulation activity in various B cell lines at different developmental stages with the microinjection system described here as well as to investigate whether this activity in WEHI-231 nuclei also affects differential processing of other heavy chain genes.

We previously reported that μ mRNA transcribed from the Moloney murine sarcoma virus long terminal repeat promoter was processed exclusively to the secreted form in mouse fibroblast cell lines (*7*). Analyses of the expression of mutated mouse μ genes in various mouse cell lines suggested that splicing of the C4–M1 intron is positively regulated in mature cells (*7*). These results are consistent with our present observation using the *Xenopus* oocytes microinjection system and strengthen the conclusion that mature B cells contain one or more nuclear factors that specifically stimulate splicing of the C4–M1 intron.

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The CD14 Monocyte Differentiation Antigen Maps to a Region Encoding Growth Factors and Receptors

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CD14 is a myelomonocytic differentiation antigen expressed by monocytes, macrophages, and activated granulocytes and is detectable with the monoclonal antibodies MO2, MY4, and LeuM3. Analyses of complementary DNA and genomic clones of CD14 show that it has a novel structure and that it maps to chromosome 5 within a region containing other genes encoding growth factors and receptors; it may therefore represent a new receptor important for myeloid differentiation. In addition, the *CD14* gene is included in the "critical" region that is frequently deleted in certain myeloid leukemias.

DIFFERENTIATION OF MYELOMONOCYTIC cells from pluripotent stem cells to mature, functioning monocytes/macrophages and granulocytes is accompanied by a variety of changes including the expression of new cell surface antigens (*1*). One such antigen, CD14, recognized by a number of monoclonal antibodies (mAbs) including MO2, MY4, and LeuM3 (*2, 3*), is a 55-kD glycoprotein expressed by monocytes, macrophages (*4, 5*), and activated granulocytes (*5, 6*). Although no biological function has yet been ascribed to this molecule, its restricted expression on mature cells suggests an important effector function. A complementary DNA (cDNA) clone encoding CD14 was isolated by means of a novel method that includes cell surface screening with monoclonal antibodies of COS 7 cells transfected with expressible cDNA clones. In addition, the *CD14* gene was isolated, characterized, and found to be located on chromosome 5 in a region containing a number of growth factor and receptor genes.

A cDNA library was constructed in the Okayama/Berg eukaryotic expression vector, pCD (*7*), with messenger RNA (mRNA) isolated from M4-AML (myelomonocytic) cells, which express high levels of CD14 (*2*). A cDNA clone encoding CD14 was isolated

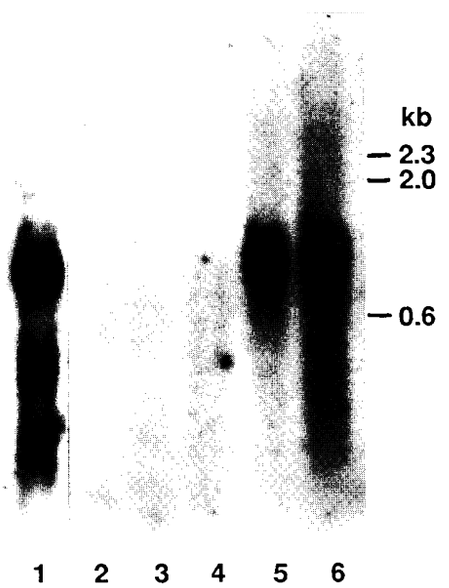

Fig. 1. Northern blot analysis of pCD-CD14 transcripts in hematopoietic cells. Total RNA was isolated from cells at various stages of differentiation on cesium chloride gradients (*16*) and polyadenylated RNA was isolated by oligo(dT) affinity chromatography (*17*). Total RNA (20 μg) (lanes 4 to 6) or polyadenylated RNA (1 μg) (lanes 1 to 3) was separated by electrophoresis in denaturing agarose gels, transferred to nitrocellulose, and hybridized with ^{32}P-labeled nick-translated CD14 cDNA (*18*). The migration of λ DNA digested with Hind III is noted. (Lane 1) EF(M4-AML); (lane 2) GM3103 (B cell line); (lane 3) HSB2 (T cell line); (lane 4) lymphocytes; (lane 5) monocytes; and (lane 6) granulocytes.

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