

with either Klenow or *Taq* polymerases. A third primer, complementary to a region of the DNA between the two PCR primers, was end-labeled with ^{32}P and used in the chain-termination sequencing reaction (19). The sequence of the Klenow polymerase-catalyzed amplification product displays base pair ambiguities at several positions (Fig. 6). The origin of these extra bands is attributed to the presence of δ -globin gene sequences. The δ -globin gene is closely related to β -globin, and both of the PCR primers match δ -globin at 18 out of 20 positions (2). Because of the relative nonspecificity of the Klenow-mediated amplifications, δ -globin is coamplified to at least 10% of the level of β -globin (7). However, the higher specificity of *Taq* polymerase reactions performed with 55°C annealing does not permit the primers to anneal to δ -globin and only the β -globin segment is amplified (Fig. 6).

The amplification of RNA transcripts can also be performed with *Taq* polymerase PCR. After conversion of the messenger RNA (mRNA) to first-strand cDNA with oligo(dT) primers and reverse transcriptase by standard methods (20), the resulting single-stranded cDNA can be directly amplified by PCR. With the HLA-DQ α PCR primers reported previously (7), mRNA transcripts present at about 0.01% in 100 ng of cDNA prepared from lymphoblastoid polyadenylated [poly(A) $^{+}$] RNA could be easily amplified to generate approximately 1 μg of the specific 242-bp amplification fragment.

Our data demonstrate the highly specific nature of *Taq* polymerase-mediated PCR and its effect on the efficiency and sensitivity of the reaction. The amplification of both DNA and RNA targets was readily accomplished by means of this thermostable enzyme, often with yields and purities comparable to fragments prepared from clonally isolated recombinants. This facilitates rapid sequence analysis of mutants and variants at a known locus by allowing the PCR product to be sequenced directly. Similarly, the analysis of unknown sequences could be expedited by PCR amplification of the cloned segments with vector-specific primers that flank the insertion site. The ability to amplify and manipulate a target sequence present only once in a sample of 10^5 to 10^6 cells should prove valuable in many areas of molecular biology. Clinical applications include the diagnosis of infectious diseases and of rare pathologic events such as chromosomal translocations. Moreover, the sensitivity of the procedure should enable the analysis of gene expression or rearrangement in single cells. By virtue of the exponential accumulation of literally billions of copies

derived from a single progenitor sequence, PCR based on *Taq* DNA polymerase represents a form of "cell-free molecular cloning" that can accomplish in an automated 3- to 4-hour in vitro reaction what might otherwise take days or weeks of biological growth and biochemical purification.

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13. Names and sequences of the synthetic oligonucleotides used in this report. GH20: GAAGAGC-CAAGGACAGGTAC, GH21: GGAAAATAGAC-CAATAGGCAG, KM29: GGTGGCCAATCTAC-TCCCAGG, KM38: TGGTCTCCTTAAACCTGT-CTTG, PC03: ACACAACTGTGTTCCTAGC, PC04: CAACTTCATCCACGTTCACC, RH09: AACCTCAAACAGACACC, RS40: ATTTTCCCAC-CCTTAGGCTG, RS79: CATGCCCTCTTTCACCATTC, RS80: TGGTAGCTGGATTGTAGCTG, RS81: CTGGGTAAAGGCAATAGCA, RS114: CCTCCAAATCAAGCCTCTAC, RS115: ATCCTGAGGAAGAATGGGAC, RS116: GTTGTAGTAG-CCTCACTTC, 19A: CTCCTGAGGAGAAGTCTGC, 1218: GGTGGCGACGACTCTGTGGAGCCCG, 1222: TTGACACCAGACCACTGGTAATG.
14. The "plateau" effect is not directly determined by the number of cycles or degree of amplification. Rather, it is the concentration of total PCR product, the concentration of the enzyme, and the length of extension time at 70°C that defines the conditions under which the activity of the enzyme becomes limiting. Sufficient molar excesses of deoxyribonucleotide triphosphates and primers were present in the reactions so the consumption of these reagents was not a factor.
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26. We thank T. L. Bugawan and C. Long for assistance with cloning and sequencing of PCR products; L. Goda, D. Spasic, and C. Levenson for synthesis of oligonucleotides; L. Johnson, R. Leath, D. Jones, and J. Widunas for engineering and instrument support; D. Carlin for statistical interpretation; S. Nilson and E. Ladner for graphic services; A. Wilson for the phrase "cell-free cloning"; and J. Sninsky and T. White for advice and encouragement.

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Genomic Amplification with Transcript Sequencing

E. S. STOFLET, D. D. KOEBERL, G. SARKAR, S. S. SOMMER*

A sequencing method called genomic amplification with transcript sequencing (GAWTS) is described that is based on amplification with the polymerase chain reaction (PCR). GAWTS bypasses cloning and increases the rate of sequence acquisition by at least fivefold. The method involves the attachment of a phage promoter onto at least one of the PCR primers. The segments amplified by PCR are transcribed to further increase the signal and to provide an abundance of single-stranded template for reverse transcriptase-mediated dideoxy sequencing. An end-labeled reverse transcriptase primer complementary to the desired sequence generates the additional specificity required to generate unambiguous sequence data. GAWTS can be performed on as little as a nanogram of genomic DNA. The rate of GAWTS can be increased by coamplification and cotranscription of multiple regions as illustrated by two regions of the factor IX gene. Since GAWTS lends itself well to automation, further increases in the rate of sequence acquisition can be expected.

IN CONTRAST TO AUTOSOMAL RECESSIVE mutations, deleterious X-linked mutations are eliminated within a few generations because the affected males reproduce sparingly if at all. Thus, each family in an X-linked disease such as hemophilia B

represents an independent mutation. From the perspective of efforts to understand the

Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, MN 55905.

*To whom correspondence should be addressed.

expression, processing, and function of factor IX, this is useful, since a large number of mutations are potentially available for analysis. Recently, a rapid method of sequencing an allele in a region of known sequence was developed (1). The method involves amplification with polymerase chain reaction (PCR) and subcloning into M13 phage.

We have developed a modification of PCR, genomic amplification with transcript

sequencing (GAWTS), which should facilitate structure-function correlations and make it practical to perform direct carrier testing and prenatal diagnosis of at-risk individuals. By amplifying and sequencing 11 regions of the hemophilic factor IX gene that total 2.8 kb, it should be possible to delineate the causative mutation in the overwhelming majority of individuals as these regions contain the putative promoter, the

5' untranslated region, the amino acid coding sequences, the terminal portion of the 3' untranslated region, and the intron-exon boundaries. Once the mutation is delineated, GAWTS can be used to directly test an at-risk individual, thereby avoiding the multiple problems associated with indirect linkage analysis.

GAWTS depends on two types of sequence amplification and a total of three oligonucleotides to generate the needed specificity (Fig. 1). The protocol is described in (2).

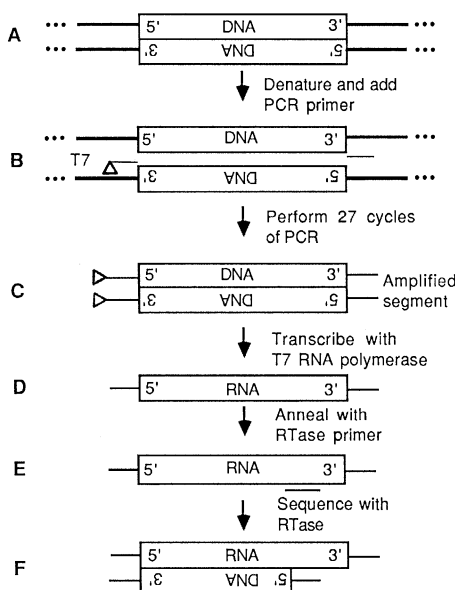
The first region chosen for amplification was part of the amino acid coding region of exon 8 of the factor IX gene. Figure 2 shows the relevant sequence and indicates the locations of the PCR primers and the reverse transcriptase primer. Primers are named by means of the numbering scheme in Yoshitake *et al.* (3) (legend to Fig. 2).

Figure 3 shows an agarose gel after 27 cycles of the polymerase chain reaction and the subsequent transcription reaction. In sample 1, the input DNA was 40 pg of pSP6-9A, a 6.5-kb plasmid containing factor IX complementary DNA (cDNA). The total amount of the region to be amplified was approximately 1 pg. There was a discrete amplified fragment (predicted size, 209 bp), which migrated as expected relative to the size markers (Fig. 3A, lane 1). From the intensity of ethidium bromide fluorescence relative to known size standards, it is estimated that a 500,000-fold amplification had occurred.

Amplified material (25 ng) was transcribed with T7 RNA polymerase, resulting in approximately 10 µg of transcript (Fig. 3B). Ten percent of the transcribed material was then added to a reverse transcriptase-sequencing reaction. Perfect agreement with the published sequence was obtained. In sample 2, the input was 1 µg of genomic DNA from a normal individual and, in sample 3, the input was 1 µg of DNA from an individual with hemophilia B. Although spurious amplification masked the expected band, the specificity conferred by the reverse transcriptase primer allowed unambiguous sequence determination (Fig. 3C). No sequence alterations were seen in the 115 bases of sequence that lie between the reverse transcriptase primer and the 48-base polymerase chain reaction primer. This region was examined for an additional 38 males (6 with hemophilia B and 32 unaffected individuals from a variety of ethnic groups), and no sequence alternations were seen.

To test the sensitivity of GAWTS, the amount of genomic DNA was incrementally decreased. With the aid of an intensifying screen, a sequence could be discerned with

Fig. 1. Schematic of GAWTS. (A) The region of genomic DNA to be amplified is indicated by the open rectangle. Two strands with their 5' to 3' orientation are shown. The darkened regions represent flanking sequences. (B) The oligonucleotides anneal to sites just outside the sequence to be amplified. One of the oligonucleotides has a T7 promoter sequence. (C) PCR consists of repetitive cycles of denaturation, annealing with primers, and DNA polymerization. Since the number of fragments with defined ends increases much faster than the number with undefined ends, virtually all the fragments are of defined size after 27 cycles. However, since the oligonucleotides anneal to other sites in the genome, multiple spurious fragments are also amplified. The segment pictured is a specifically amplified sequence. (D) RNA is transcribed from the T7 promoter. This provides a convenient source of single-stranded nucleic acid for dideoxy sequencing. (E) Because of the complexity of the mammalian genome, the amplified and transcribed sequences contain other genomic segments whose flanking sequences cross-hybridize with the PCR primers at the stringency generated by the DNA polymerization reaction. As a result, another level of specificity is crucial to obtaining interpretable sequences. That specificity is provided by utilizing an oligonucleotide primer for reverse transcriptase, which lies in the region of interest. (F) Reverse transcriptase is used to generate sequence data by the dideoxy method.



T7 promoter
GGTACCTAATACGACTCACTATAGGGAGA E8(30884)-48D

244.....Thr Glu Gln Lys Arg Asn Val Ile Arg Ile Ile Pro His His Asn Tyr Asn Ala Ala Ile Asn Lys
30851 5'.....ACA GAG CAA AAG CGA AAT GTG ATT CGA ATT ATT CCT CAC CAC AAC TAC AAT GCA GCT ATT AAT AAG
3'.....TGT CTC GTT TTC GCT TTA CAC TAA GCT TAA TAA GGA GTG GTG TTG ATG TTA CGT CGA TAA TTA TTC

Tyr Asn His Asp Ile Ala Leu Leu Glu Leu Asp Glu Pro Leu Val Leu Asn Ser Tyr Val Thr Pro Ile Cys Ile
TAC AAC CAT GAC ATT GCC CTT CTG GAA CTG GAC GAA CCC TTA GTG CTA AAC AGC TAC GTT ACA CCT ATT TGC ATT
ATG TTG GTA CTG TAA CGG GAA GAC CTT GAC CTG CTT GGG AAT CAC GAT TTG TCG ATG CAA TGT GGA TAA ACG TAA

Ala Asp Lys Glu Tyr Thr Asn Ile Phe Leu Lys Phe Gly Ser Gly Tyr Val Ser Gly Trp Gly Arg Val.....313
GCT GAC AAG GAA TAC ACG AAC ATC TTC CTC AAA TTT GGA TCT GGC TAT GTA AGT GGC TGG GGA AGA GTC.....3'
CGA CTG TTC CTT ATG TGC TTG TAG AAG GAG TTT AAA CCT AGA CCG ATA CAT TCA CCG ACC CCT TCT CAG.....5' 31060

E8(31022)-17U E8(31048)-27U GACGTCCAQ

Fig. 2. Oligonucleotides synthesized (Synthetic Genetics, Inc.) for GAWTS of a region in the proximal part of exon 8. The PCR primers are (T7-29)E8(30884)-48D and (PST1-9)E8(31048)-27U and the reverse transcriptase primer is E8(31022)-17U. The noncomplementary bases in E8(31048)-27U may be ignored as they are not relevant to this series of experiments. Note that by replacing these bases with a different phage promoter, it should be possible to generate an amplified fragment where both strands could be selectively transcribed and sequenced. Since oligonucleotides tend to accumulate rapidly when GAWTS is used, it is helpful to have informative names. The notation used is of the form: (identifier for noncomplementary 5' bases-length) region of the gene [location of the 5' complementary base from the numbering system of Yoshitake *et al.* (3)]-total size and 5' to 3' direction of the oligonucleotide. The region of the gene can be abbreviated by U, upstream; E, exon number; I, intron number; and D, downstream. The direction of the oligonucleotide is either U, upstream, or D, downstream, relative to the direction of transcription. Thus, (T7-29)E8(30884)-48D has a T7 promoter (plus a 6-base clamping sequence) of 29 bases. It is complementary to a sequence in exon 8 that begins at base 30884. The oligonucleotide is a 48 mer, which heads downstream relative to E9 messenger RNA. E8(31022)-17U is also located in exon 8, does not have a 5' noncomplementary sequence and begins at 31022. It is a 17 mer that heads upstream. Likewise, U(-140)-16U is a 16 mer located upstream of the gene that begins at base -140 and heads further upstream of the gene.

Fig. 3. GAWTS for exon 8 of the factor IX gene with the primers pictured in Fig. 2. For lane 1, 40 pg (1 pg of sequence to be amplified) of pSP6-9A, a plasmid containing factor IX cDNA, was the input DNA. For reactions 2 and 3, 1 µg of genomic DNA from a normal and a hemophiliac individual, respectively, was the input DNA. (A) NuSieve/SeaKem agarose (FMC) gel (3%/1%) of 30% of the PCR-amplified material. (B) NuSieve/SeaKem agarose gel (3%/1%) of the transcribed material. The unlabeled lanes contain a Hae III digest of ϕ X174 as size markers. (C) Autoradiogram of a segment of the sequencing gel. From left to right, the order of the lanes is A, T, G, and C. In set 3, there appears to be an extra "A" at position 2 but review of the original autoradiograph clearly indicates that this is an artifact.

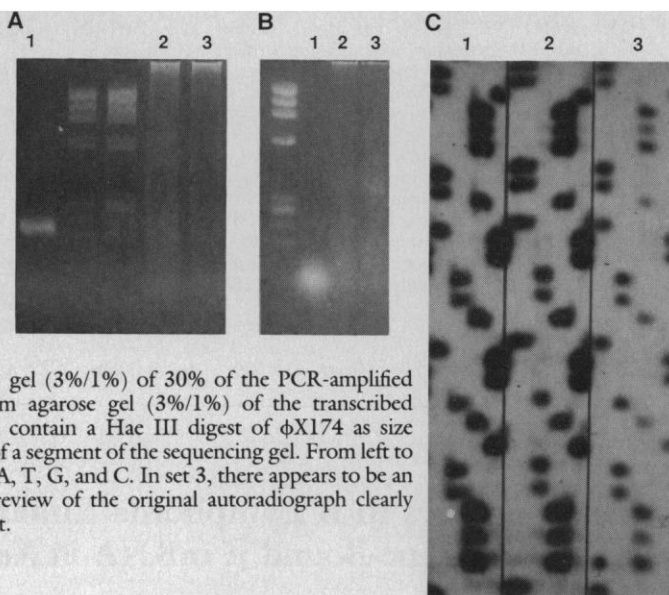
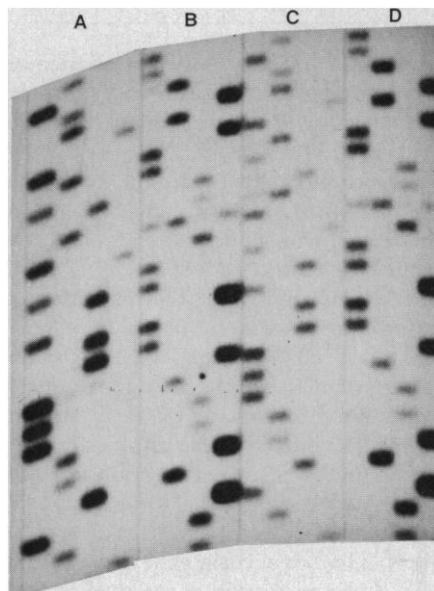


Fig. 4. GAWTS with simultaneous amplification and transcription of a 331-bp region (region I) in the amino acid-coding segment of exon 8 and a 250-bp region (region II), which begins 1.2 kb downstream in exon 8. PCR and transcription reactions were performed on 1 µg of DNA with: (i) primers specific for region I, (ii) primers specific for region II, and (iii) both sets of primers. Sequencing was performed as follows: (A) template from PCR/transcription reaction (i) with reverse transcriptase primer specific for region I, (B) template with PCR/transcription reaction (ii) with reverse transcriptase primer specific for region II, (C) template from PCR/transcription reaction (iii) with reverse transcriptase primer specific for region I, and (D) template from PCR/transcription reaction (iii) with reverse transcriptase primer for region II. The order of the lanes is A, T, G, and C.



1 ng of input DNA (the amount of DNA contained in 150 diploid cells). At this level, PCR is possible in a crude cell lysate (4).

As a test of the generality of the procedure, an attempt was made to amplify four additional regions of the factor IX gene: (i) a 332-bp sequence that includes the putative promoter region, exon 1, and the splice donor junction of intron 1; (ii) a 315-bp region that includes exon 6 and the flanking splice junctions; (iii) a 331-bp region in the amino acid-coding region of exon 8; and (iv) a 250-bp region that contains the distal 3' untranslated region of exon 8. In three of the four regions, the amplified regions had a band of expected size that was discernible above the background of nonspecific amplification and transcription on an agarose gel. Although the intensity of the signal varied, the four regions all produced unambiguous sequence data. Unlike previous methods

that involved cloning of single molecules from a mixture, the error rate of GAWTS is relatively unaffected by the fidelity of polymerization because the sequence obtained is the dominant sequence in the population.

No point mutations or new polymorphisms were found in the normal and hemophilic individuals analyzed by GAWTS for the regions mentioned above. However, the previously documented polymorphism in amino acid 148 in exon 6 was detected.

To determine whether more than one region could be simultaneously amplified with PCR and transcribed, we used the 331-bp region in the amino acid-coding region of exon 8 and the 250-bp region in the distal 3' untranslated region of exon 8. Both sequences could be obtained with appropriate reverse transcriptase primer (Fig. 4). Simultaneous amplification also was suc-

cessful for a second pair of regions, which suggested that the procedure can further enhance the rate of sequence acquisition while decreasing the cost of sample processing. The oligonucleotides used above were synthesized by phosphoramidite chemistry (5) and subsequently gel purified. Purification is not always necessary because crude (T7-29)E8(30884)-48D, a 48 mer, gave an acceptable sequence despite the fact that gel staining indicated that less than 50% of the molecules were of the desired length.

GAWTS substantially reduces the time required to sequence an allele as eight samples can be amplified, transcribed, and loaded onto a sequencing gel in an 8- to 9-hour day. Thus, in a span of less than 2 years, the rate of detection of changes in genomic sequence has increased by a factor of about 100. As a result, an array of experiments are now feasible in a diversity of fields.

In addition to GAWTS, alternate methods of rapidly accessing genomic sequencing information have been developed (6). The methods involve amplification with PCR under conditions where a specific band is visible on agarose gels; this is achieved with either *Taq* polymerase or Klenow fragment at high temperatures such as described herein. The amplified fragment is centrifuged or ethanol precipitated, or both, to eliminate salt and excess deoxyribonucleoside triphosphates. Then the linear double-stranded DNA template is sequenced by the use of a nested oligonucleotide primer.

GAWTS and PCR with double-stranded DNA sequencing are both rapid methods and only further experience can determine whether one method is superior. However, GAWTS may well have certain advantages that stem from the use of a phage promoter. Most importantly, automation should be achieved more readily because centrifugation and ethanol precipitation are not necessary. In addition, sensitivity should be greater because of the added amplification due to transcription. Finally, the success rate of GAWTS may be higher because the transcription reaction can compensate for sub-optimal PCR and the single-stranded template generated has a higher probability of being successfully sequenced than a linear double-stranded template.

The automation of GAWTS will further enhance the research and clinical applications of the technique. With relatively minor modifications of an automated PCR instrument (4) and an automated sequencer (7), it should be possible to generate a fully automated system. As the sophistication of the component instruments increases, it is conceivable that the rate of genomic information retrieval could be increased by additional orders of magnitude.

Note added in proof: To reduce reagent costs and increase convenience, the PCR reaction can conveniently be scaled down to 20 μ l 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 μ g of DNA in a volume of 100 μ l was denatured at 95°C for 10 minutes (2 minutes in subsequent cycles) in the presence of the following: 50 mM sodium chloride, 10 mM tris-HCl (pH 7.6), 10 mM magnesium chloride, 10% DMSO, and 1.5 mM 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/ μ l with dilution buffer [10 mM tris (pH 7.5), 1 mM dithiothreitol (DTT), 0.1 mM EDTA, and 1.5 mM of the four deoxyribonucleoside triphosphates].

After a final denaturation, 3 μ l of the amplified material was added to 17 μ l of the RNA transcription mixture: 40 mM tris-HCl (pH 7.5), 6 mM magnesium chloride, 2 mM spermidine, 10 mM sodium chloride, 0.5 mM of the four ribonucleoside triphosphates, RNasin (1.6 U/ μ l), 10 mM DTT, 10 U of T7 RNA polymerase, and diethyl pyrocarbonate-treated H₂O. Samples were incubated for 1 hour at 37°C and the reaction was stopped with 5 mM EDTA.

For sequencing, 2 μ l of the transcription reaction and 1 μ l of ³²P end-labeled (see below) reverse transcriptase primer were added to 10 μ l of annealing buffer [250 mM KCl, 10 mM tris-HCl (pH 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 μ l of reverse transcriptase buffer [24 mM tris-HCl (pH 8.3), 16 mM magnesium chloride, 8 mM DTT, 0.4 mM dATP, 0.4 mM dCTP, 0.8 mM dGTP, and 0.4 mM TTP] containing 5 U of AMV reverse transcriptase, 1 μ l of a dideoxyribonucleoside triphosphate: 1 mM ddATP or 1 mM ddCTP or 1 mM ddGTP or 2 mM ddTTP, and finally, 2 μ l 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 μ l of 100% formamide with 0.3% bromophenol blue and xylene cyanol FF. Samples were boiled for 3 minutes and 3 μ l 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- μ g sample of oligonucleotide in a 13- μ l volume containing 50

mM tris-HCl (pH 7.4), 10 mM MgCl₂, 5 mM DTT, 0.1 mM spermidine, 100 μ Ci [α -³²P]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 μ l 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

NAOYA TSURUSHITA,* LISA HO,† LAURENCE JAY KORN‡

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).

Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305.

*Present address: Institute for Virus Research, Kyoto University, Kyoto 606, Japan.

†Present address: Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, CA 94305.

‡To whom correspondence should be addressed at Protein Design Labs, Inc., Palo Alto, CA 94304.