# Characterization of a Fusion cDNA (RARA/myl) Transcribed from the t(15;17) Translocation Breakpoint in Acute Promyelocytic Leukemia

### KUN-SANG CHANG,<sup>1</sup>\* SANFORD A. STASS,<sup>1</sup> DA-TONG CHU,<sup>1</sup> LARRY L. DEAVEN,<sup>2</sup> JOSE M. TRUJILLO,<sup>1</sup> AND EMIL J. FREIREICH<sup>3</sup>

Hematopathology Program, Division of Laboratory Medicine,<sup>1</sup> and Adult Leukemia Research Program,<sup>3</sup> The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, and Life Sciences Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545<sup>2</sup>

Received 25 March 1991/Accepted 19 November 1991

A nonrandom chromosomal translocation breakpoint, t(15;17)(q22;q21), is found in almost all patients with acute promyelocytic leukemia (APL). Most of these breakpoints occur within the second intron of the retinoic acid receptor-alpha (RARA) gene. We screened a cDNA library of APL and have identified and sequenced a cDNA transcribed from the t(15;17) translocation breakpoint. The 5' end of cDNA p1715 consists of 503 bp of the RARA exon II sequence. A 1.76-kb cDNA without homology to any known gene available in GenBank was found truncated downstream. This cDNA sequence was assigned to chromosome 15 by dot blot hybridization of the flow cytometry-sorted chromosomes. We designate this fusion cDNA RARA/myl, which is different from myl/RARA reported by de The et al. (H. de The, C. Chomienne, M. Lanotte, L. Degos, and A. Dejean, Nature (London) 347:558-561, 1990). This result demonstrates that the two different types of hybrid mRNA can be transcribed from this breakpoint. We screened a non-APL cDNA library and identified a 2.8-kb myl cDNA. This cDNA is able to encode a polypeptide with a molecular weight of 78,450. Alternative splicing of the myl gene which resulted in myl proteins with different C terminals was found. Southern blot analysis of the genomic DNA isolated from 17 APL patients by using the myl DNA probe demonstrated that the myl gene in 12 samples was rearranged. Northern (RNA) blot analysis of RARA gene expression in two APL RNA samples showed abnormal mRNA species of 4.2 and 3.2 kb in one patient and of 4.8 and 3.8 kb in another patient; these were in addition to the normal mRNA species of 3.7 and 2.7-kb. The myl DNA probe detected a 2.6-kb abnormal mRNA in addition to the normal mRNA species of 3.2, 4.2, and 5.5 kb. Using the polymerase chain reaction, we demonstrated that both RARA/myl and myl/RARA were coexpressed in samples from three different APL patients. From this study, we conclude that the t(15;17) translocation breakpoint results in the transcription of two different fusion transcripts which are expected to be translated into fusion proteins.

Acute promyelocytic leukemia (APL) is a clonal proliferation of abnormal promyelocytes. A nonrandom chromosomal translocation breakpoint, t(15;17)(q22;q21), occurs in almost all patients with APL (39). A significant number of these patients achieve complete clinical remission after all-trans retinoic acid treatment (9, 12, 26). Since the retinoic acid receptor-alpha (RARA) gene has been localized to chromosome 17q21, in proximity to the breakpoint of APL (33), this gene was thought to play a critical role in the pathogenesis of APL. Indeed, Borrow et al. (3), de The et al. (15), and our studies (10) have shown that the RARA gene is involved in the t(15;17) chromosomal abnormality that results in transcription of abnormal mRNAs. It was thought that the majority of the breakpoints were in the first intron of the RARA gene. However, when Brand et al. (5) characterized the promoter region of the RARA gene, an additional exon was found 12 kb further upstream of exon I. Therefore, it is now clear that most APL breakpoints occur within the second intron of the RARA gene.

The retinoic acid receptor (RAR) is a member of a nuclear receptor superfamily that includes the thyroid and steroid hormone receptors. Four different forms of the human RAR have been identified, and its cDNA has been cloned and characterized (6, 22, 30, 32, 37). Retinoic acid (RA) can induce differentiation of a number of cell lines (29, 38, 44, 46), including the human leukemia cell line HL-60. RA is a morphogen, as has been demonstrated in chick limb experiments (7). Recent evidence indicates that purified RAR protein can interact directly with RA without cellular RAbinding protein (42). Therefore, RAR may be directly involved in mediating the RA concentration in the nucleus. Since the translocation breakpoint occurs within the RARA gene in APL, this may partially impair the normal function of RA and result in disruption of normal differentiation. In addition, chromosome translocations can produce potentially oncogenic fusion proteins (8, 25).

We report the characterization of a cDNA transcribed from the t(15;17) breakpoint region. This hybrid cDNA consists of exon II of the RARA gene and is truncated downstream by an unknown sequence from chromosome 15 (the *myl* gene). This hybrid cDNA (RARA/*myl*) differs from the *myl*/RARA fusion gene reported by de The et al. (15) in that the arrangement of the two genes involved in the breakpoint is reversed. Therefore, this finding demonstrates that the nonrandom translocation breakpoint in APL can result in the transcription of two different types of hybrid mRNAs, as shown by the transcription activities of the RARA and *myl* genes. We have also identified and characterized the *myl* cDNA.

<sup>\*</sup> Corresponding author.

### MATERIALS AND METHODS

Construction of a cDNA library from poly(A) RNA of an APL patient and screening for the hybrid mRNA. Total RNA was isolated from leukemic promyelocytes of an APL patient by using guanidinium isothiocyanate as described previously (11). Poly(A) RNA was selected by the method of Aviv and Leder (1). Ten micrograms of poly(A) RNA was used to synthesize cDNA by the method of Gubler and Hoffman (23); the cDNA was ligated into a lambda Zap II vector (Stratagene, La Jolla, Calif.). The library was screened by using an exon II probe (10) of RARA cDNA in accordance with established procedures (41). Dideoxy-DNA sequencing of a plasmid subclone was performed by the method of Zagursky et al. (48). DNA sequence analysis and comparison with the GenBank sequences were performed by using MicroGenie software (Beckman Instruments, Inc., Fullerton, Calif.).

Southern and Northern (RNA) blot analysis. Genomic DNA was isolated from human leukemic cells by an established method (10). Ten micrograms of total genomic DNA was restriction digested to completion and electrophoresed on a 0.75% agarose gel. DNA samples were transferred to a Nytran membrane in accordance with the manufacturer's instructions (Schleicher & Schuell, Inc., Keene, N.H.). Poly(A) RNA from patient samples was selected as described above. Two micrograms of the poly(A) RNA was denatured by glyoxalation, electrophoresed on a 1.2% agarose gel, and transferred to a Nytran membrane. The filters were hybridized to <sup>32</sup>P-labeled probes (10<sup>9</sup> cpm/µg of DNA), washed, and exposed to Kodak XAR-5 film.

Chromosomal assignment. Chromosomes from diploid human lymphoblastoid cells (GM-130A) or rodent-human hybrid cell lines (35) were sorted in an EPICS V flow cytometer and spotted onto nitrocellulose filters. Each spot contained 30,000 chromosomes, except the 9-12 spot from GM 130A, which contained 120,000 chromosomes. Chromosomes 9, 10, 11, and 12 were also sorted individually from hybrid cell lines. The chromosome-specific DNA on the filters was denatured and hybridized as previously described (14). A polymerase chain reaction (PCR)-amplified, 1.2-kb cDNA fragment isolated from the unknown portion of p1715 (nucleotides 504 to 1704 [see Fig. 2]) and not including the RARA sequence was  $^{32}P$  labeled to  $10^9$  cpm/µg of DNA and hybridized to the filters as described previously (19). Filters were washed and exposed to Kodak X-ray film. Since the Southern blot analysis described above indicated that both EcoRI and BglII digestions yield a single hybridizing band, this unknown gene was likely a single human gene. Therefore, cross-hybridization to DNA sequences from other chromosomes was not expected.

PCR. Synthesis of cDNA and PCR amplification of total RNA were performed as previously described (17, 28), with some modifications. A 20- $\mu$ l reverse transcriptase reaction mixture contained 1 mM of deoxynucleoside triphosphates (dNTP), 20 U of RNasin (Promega Corporation, Madison, Wis.), 20 ng of primer, 5  $\mu$ g of total RNA, and 1× PCR buffer (Perkin Elmer-Cetus). The reaction was started by adding 200 U of Moloney murine leukemia virus reverse transcriptase (Gibco-BRL, Gaithersburg, Md.). After incubation at room temperature for 10 min, the reaction mixture was transferred to 42°C for 60 min, boiled for 5 min, and quick chilled on ice. An 80- $\mu$ l PCR reaction mixture was added (0.3 mM dNTP, 2.5 mM MgSO<sub>4</sub>, 0.2  $\mu$ g of each of the two primers, 50 mM KCl, 20 mM Tris-HCl, pH 8.4, and 1 mg of nuclease-free bovine serum albumin). The mixture was overlaid with mineral oil, and PCR was performed for 35 cycles in a thermal cycler (Perkin Elmer-Cetus). Each cycle was carried out at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min.

Sequencing of the PCR-amplified DNA fragments. DNA fragments were first purified by passing them through a Centricon-100 (Amicon, Beverly, Mass.) to remove primers. Approximately 0.1  $\mu$ g of the purified DNA was used in a 25-cycle PCR using a single primer (24). After PCR was performed, the single-stranded DNA was purified by using a Centricon-100 and used directly in the DNA-sequencing reaction. DNA sequencing was performed in the presence of about 0.5  $\mu$ g of single-stranded DNA and 20 ng of internal primer (R1 or R4) by using the DNA-sequencing kit obtained from the United States Biochemical Corporation (Cleveland, Ohio).

Nucleotide sequence accession number. The nucleotide sequences of p1715 and pMYL211 have been submitted to GenBank and have been assigned the accession numbers M73958 and M73840, respectively.

RESULTS

Identification and characterization of the fusion cDNA RARA/myl encoded from the t(15;17)(q22;q21) breakpoint of APL. The cDNA library constructed from poly(A) RNA isolated from the promyelocytes of an APL patient (see Fig. 7, lane 6) was screened with the RARA second-exon probe (10). Several cDNA clones were obtained. A brief restriction map of each clone was determined to further identify clones that were different from the RARA cDNA. One of the clones, p1715, had a restriction pattern different from that of RARA (Fig. 1). This clone was further characterized by DNA sequence analysis. Results revealed that the 503-bp DNA sequence on the 5' end of p1715 matched exactly the second exon of the RARA cDNA sequence. However, DNA sequence homology stopped once it passed the second exon/intron splice junction (Fig. 1B).

Figure 2 shows the nucleotide sequence of p1715, with the junction of the exon II/intron II splice site (31) indicated by an arrowhead. The 1.75 kb of the cDNA sequence truncated downstream of the RARA second exon is unrelated to the RARA gene. This cDNA sequence was compared with the GenBank sequences, and no homology to any gene sequence was identified. Intron II of the RARA gene splits the ACC codon of amino acid 60 into exon II and exon III after translocation. The first nucleotide of the amino acid codon AAT is derived from the RARA cDNA. According to our DNA sequence analysis, the hybrid mRNA can encode a polypeptide of 178 amino acids, with a predicted molecular weight of 18,500. The other two reading frames of the myl cDNA fail to translate a long polypeptide. At least 1.5 kb of the DNA sequence remains beyond the stop codon. In order to understand whether the correct reading frame of the myl mRNA is retained after the translocation, it was necessary to characterize the normal myl cDNA.

Isolation and characterization of the normal cDNA of the *myl* gene. A cDNA library was constructed from poly(A) RNA isolated from an AML blast sample without the t(15; 17) translocation and with high-level expression of *myl* mRNA. The cDNA library was screened with the *myl* cDNA probe (a 1.2-kb PCR-amplified DNA fragment of plasmid p1715 nucleotides 504 to 1704 [Fig. 2]). A 2.8-kb cDNA clone, pMYL211, was identified and completely sequenced. This cDNA clone may be close to full length, since the major





FIG. 1. Restriction map of RARA and p1715 cDNAs and DNA sequencing strategy of p1715. (A) The restriction map of RARA was derived from the report of Giguere et al. (22). pRA1 is a RARA cDNA clone of 2.1 kb. A poly(A) tail was found 850 bp upstream of the reported RARA cDNA (22). DNA sequencing of p1715 was performed by dideoxy sequencing of the plasmid DNA by using the Sequenase sequencing kit obtained from United States Biochemical. Restriction endonuclease sites indicated are abbreviated as follows: Kp, KpnI; P1, PstI; S1, SacI; Sm, SmaI; Bm, BamHI; B2, BglII; P2, PvuII. The open box region represents the gene sequence derived from chromosome 15. (B) DNA sequences of RARA and p1715 cDNAs indicating the region of sequence homology between the two sequences. Homology stops after the exonII/exonIII splice junction. The inverted triangle indicates the RARA exon II/exon III splice site, which was determined as described by the report of Leroy et al. (31).

band of the *myl* mRNA was 3.2 kb according to the Northern blot analysis (see below).

Figure 3 shows the DNA sequence and the predicted amino acid sequence of the pMYL211 cDNA. This cDNA is able to translate a polypeptide of 713 amino acids with a predicted molecular weight of 78,450. The solid vertical arrow at nucleotide 1042 indicates the fusion junction of the RARA/myl cDNA, as shown in Fig. 2. The amino acid sequences predicted from the cDNA sequences of pMYL211 and p1715 downstream of the fusion junction are identical. This demonstrates that the correct amino acid-coding frame of the myl mRNA was utilized by the fusion transcript. However, as shown in Fig. 2, a termination codon (TGA) which is not found in the myl cDNA (Fig. 3) was found at nucleotides 856 to 858 of p1715. DNA sequence comparison between pMYL211 and p1715 at this region indicates an 8-bp insert in the p1715 cDNA (nucleotide 832 to 840 [Fig. 2]) which is not present in the pMYL211 cDNA. The presence of this additional DNA fragment alters the amino acid-coding frame. To determine whether this 8-bp insert is the result of cloning artifact, a set of oligonucleotide primers (RAR1, nucleotides 441 to 460, and myID6, nucleotides 1142 to 1167 [Fig. 2]) was designed for PCR amplification by using total RNA isolated from the APL patient. The PCR-amplified DNA fragment was subcloned into pUC18 and sequenced. The result of this experiment demonstrated that the 8-bp insert is not a result of a cloning artifact, since cDNA sequences with and without the 8-bp sequence were found (data not shown). These same oligonucleotide primers were used to amplify the genomic DNA of the APL patient and of an acute myelogenous leukemia patient without the t(15;17)breakpoint. A DNA fragment of about 1.5 kb was amplified. This PCR-amplified DNA fragment was subcloned into pUC18. DNA sequences from both ends of the DNA fragment were determined. As shown in Fig. 4, line 3, the 8-bp insert was derived from the intron immediately adjacent to the intron/exon splice junction. Interestingly, a sequence that agreed with the splice donor consensus in seven of nine places was found at the 5' end of the 8-bp DNA fragment. This result suggests that alternative splicing is responsible for generating different myl mRNAs with or without the eight additional bases. This differential splicing could result in the translation of different myl proteins with considerably different C terminals.

By comparing the cDNA sequences of pMYL211 and p1715, we have also found a 144-bp DNA sequence between nucleotides 1113 and 1257 of pMYL211 (Fig. 3) which is not found in p1715. This DNA fragment encodes a short polypeptide of 48 amino acids and does not affect the open reading frame of the myl mRNA. According to the Southern blot analysis shown in Fig. 6, a single band was detected by the myl probe with BglII and HindIII digestion. This suggests that the myl gene represents a single gene in the human haploid genome. It is likely that this 144-bp additional DNA sequence is also the result of alternative splicing. Differences in the nucleotide sequences of the cDNAs of p1715 and pMYL211 were also found at the 3' ends. The polyadenylation signal AATAAA was found 16 bp upstream of the poly(A) tail of the pMYL211 cDNA. However, the 3' end of the p1715 cDNA extended 160 bp further downstream of the poly(A) tail of pMYL211. Interestingly, the last 22 nucleotides upstream of the poly(A) tail of pMYL211 (Fig. 3), including the polyadenylation signal AATAAA, did not match the 3' end sequence of p1715 (Fig. 2). It is possible that the mismatched portion of p1715 is the result of a cloning artifact. An oligonucleotide primer set was synthesized in accordance with the DNA sequences of nucleotides 1868 to 1888 and 2230 to 2250 (antisense) of p1715 (Fig. 2). PCR amplification of the genomic DNA resulted in a DNA fragment of 380 bp. Sequence analysis of the DNA fragment demonstrated that the genomic DNA fragment exactly matched the p1715 sequence. Furthermore, the 22-bp nucleotide sequence present in the myl cDNA was not found within this exon. It is possible that this 22-bp sequence is the result of alternative splicing. We are currently in the process of studying the genomic organization of the myl gene. Information obtained from this study should help us to understand the mechanism of alternative splicing occurring within the myl gene.

RARA/myl is transcribed from the t(15;17) breakpoint of APL. Since the RARA gene has been assigned to chromosome 17 (33), if the cDNA clone p1715 is a hybrid transcript encoded from the t(15;17) translocation breakpoint, the DNA fragment downstream of the RARA gene should be localized to chromosome 15. Figure 5 shows the dot blot hybridizations of the flow cytometry-sorted chromosomes. The 1.2-kb DNA sequence derived from the unknown portion of p1715 clearly hybridizes to the chromosome 15 spot. Two separate sets of dot blot filters were used in the experiment, and similar hybridization signals to chromosome 15 were observed for each. This demonstrates that the unknown cDNA truncated downstream of the RARA gene exon II is derived from chromosome 15. Therefore, the cDNA of p1715 is transcribed from the t(15;17) translocation breakpoint of the APL patient.

**Rearrangement of the** *myl* gene in acute promyelocytic leukemia. Southern blot analysis of 17 DNA samples isolated from APL patients with the t(15;17)(q22;q21) chromosomal

GCCCATCTAGGAGT	40	50	40	50	60	70	80	90	100	110	120
	GCATCTTTT	TTEGTECCCI	GAAGGCCAG	TCTGGACCTT	CCCAGGAAA	GTGCCAGCTO	CACAGAACTGO	TTGACCAAA	GACCGGCTC'	TTGAGACATCO	CCCAAC
130	140	150	160	170	180	190	200	210	220	230	240
CCACCTGGCCCCCA	SCTAGGGTGG	gggtccagg/	GACTGAGAT	ragcctgccc1	CTTTGGACA	BCAGCTCCAG	SACAGGGCGGG	TGGGCTGAC	CACCCAAACCI	CCATCTGGGCC	CAGGCC
250	260	270	280	290	300	310	320	330	340	350	740
CCATECCCCEAGEA	RECETERTO	GAAGCCCAC	AGAGECCCC	TOCCAGACTO	CTOCCTCCC	TOTEACTET	eccentree	ATCOCCACC	ACAGCAGCT	CTOCCCOAC/	VCCTCCC
CCHTGCCCCGHGGH	9999199101	OHHOLLLHLL	HOHOLLUL	I GUUMBHU I GI		TUTOHUTON	900000011000	MIGGLUNGU	HLHGCHGU		
								MetAlaSeri	Asn Ser Ser S	erCysProThr	ProGly 14
370	380	390	400	410	420	430	440	450	460	470	480
RECERBENCETEN	TREGTACCCG	STRUCTURE	ACRCCTTCT	TOTTCCCCCC1	ATECTOEST	SGACTETCCC	CRCCARRCRC1	CTRACCACT	TCCARCACC	ARCTTCCART	TARTREA
	-01	010001000			Mahl auGlud	21 vd. ev See Br		Lew The The			Com Club 52
GIVGIVHISCOUNS	DAU	VAIPPOPPO	YFMI AFNEFI	hernerrorro	AMELCEUDI M	or Arengel Li	OFF OF TWI 4		.eusinni 55.	TURANLLOVA	Ser GIV J2
RA		-MTL									
490	500	510	520	530	540	550	560	570 🕳	580	590	600
TATAGCACCATC	CCCAGCCAAT	GCAGCTGTA1	CCAAGAAAG	CCAGCCCAGAG	GCTGCCAGC/	ACTCCCAGGG(	ACCCTATTGAC	GTTGACCTO	BATGTCTCCA	ATACAACGAC/	AGCCCAG
TursCarTheBroCa	ProAl sAre	A1 - A1 - U-14	Carl val vað	a Ger ProGl	A1 = A1 = See."	The Profes	ProlleAs	Val Anni au	Annual CarA	en The The Th	AL +G1 - 92
Tyr Ser Three Coe				Laber Frudit		ini ri uni yn	when or remet	/ armspcan	-spratoer -		HIBOIN
610	620	630	640	650	660	670	-680	690	700	710	720
AAGAGGAAGTGCAG	CCAGACCCAG	TOCCCCAGO	AGGTCATCA	AGATGGAGTC1	(Gaggagggg)	Aggaggcaai	<b>JGTTGGCTCG</b>	SAGCTCCCCG	Bagcagccca	GGCCCAGCAC	CTCCAAG
I veAral veCveSe	cGl nThrGl n	CysProAral	veVal II eL	veMet G1uSer	61 uG1 uG1 v	VEGLUAL AA	oLeuAl aAro	SerSerPro	BluBloProA	roProSer The	Serlve 132
_,,,			-,	,		-,					
770	740	750	740	770	780	700	800		000		
/30	/40	730	760	//0	/80	///		810	820	630 🗢	840
GCAGTCTCACCACC	CCACCTGGAT	GGACCOCCT	ACCCCCAGGA	GCCCCGTCAT	NGGAAQTGAG	STCTTCCTOC	CCAACAGCAAC	CACGTOGCC	AGTGGCGCCG	eggaggcagg:	TAGGGAG
AlaValSerProPr	oHisLeuAso	61 vProPro	SerProAraS	erProValIle	61 vSer 61 u	Val PheLeuPi	oAsnSer Asi	hHisValAla	Ber Gl vAl aG	$1 \sqrt{61} uA1 a \overline{61}$	Ara61u 172
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050	840	870	000	000	800	810	830	870	840	ORA	840
830	000	8/0	000	870	700	710	720	730	740	730	780
AGGAACGCGTTGTG	GTGATCAGCA	GCTCGGAAG	ACTCAGATEC	CBAAAACTCG	recategaeci	CCATOGAGACI	COCCEAGCCA	CAGTCCTCGC	CABCCCACTC	CTCOCCAOCCI	CACTCCT
ArgAsnAlaLeuTr	pEnd										177
970	980	990	1000	1010	1020	1030	1040	1050	1060	1070	1080
COCCARCOCACTOC	TOOCCARTOC	ARTOTOTOC		AGGAGECTEC	ACCTOCCT	BTOOCACATA		CTTOOCCTC		COCTOAOCAO	PCTOCCA
COLLAGECCALICE	TCBCCHBTCC	AGICICIGC	BHBHBLALA	HOGHOLLILL		9199CHCHIH				LOCIONOLHO	
1090	1100	1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
CCCCCGATGCTGAG	COTCACABOR	ARCETCETR	TCACCARGA	RCRCCCTRCC	STCCACCRTR	REATCORCTA	CCTRTTRTAC	RARCACARA	GARCCATCCG	CETTERCEAT	RECETCE
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1210	1220	1230	1240	1250	1260	1270	1280	1290	1300	1310	1320
GCTTGCACCCTCA	TT004T0000			TECCEATATA	<b>STCCAAGCCA</b>	BCACTCCTBC	CATCACAGGG	CCCCTCAACC	ATCCTGCCAA	TOCCCAGGAA	CATCCTB
	IIULAILUUU	CLLCIATIC	3946119916								
	TIGLATCOOD	CCCCTATTC	30HC11001C	100004110104							
	TIBLATLEGE	CCCCTATTC	39HC 11991C								
	THUATCOOD	CCCCTATTC									
1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430	1440
1330 CCCA8CT8CAAA86	1340 IGGCATCAGCO	1350 CACECCACE	1360 GGATACBAGG	1370 GGCTGTGCGA	1390 TCCCGCAGCC	1390 BCTCCCTCCG	1400 BBBCTCCTCC	1410 CATTTATCCC	1420 AGTGGCTCAA	1430 CAACTTTTT	1440 BCCCTCC
1330 CCCABCTGCAAAGE	1340 IGGCATCAGCO	1350 CACCCACC	1360 GGATACBAGG	1370 GGCTGTGCGA	1390 TCCCGCAGCC	1390 BCTCCCTCCB	1400 BBBCTCCTCC	1410 CATTTATCCC	1420 AGTGGCTCAA	1430 CAACTTTTT	1440 BCCCTCC
1330 CCCAGCTGCAAAGG	1340 IGGCATCAGCO	1350 CACCCCACC	1360 GGATACSAGG	1370 GGCTGTGCGA	1390 TCCCGCAGCC	1390 BCTCCCTCCG	1400 BBBCTCCTCC	1410 CATTTATCCC	1420 ABTBGCTCAA	1430 CAACTTTTT	1440 BCCCTCC
1330 CCCA9CTGCAAA99	1340 1360 1360	1350 CACCCACC	1360 66ATAC9A66	1370 GGCTGTGCGA	1390 TCCCGCAGCC	1390 BCTCCCTCCG	1400 BBGCTCCTCC	1410 CATTTATCCC	1420 ABTBGCTCAA	1430 CAACTTTTTT	1440 GCCCTCC
1330 CCCABCTGCAAAGG 1450	1340 1360 1360 1360	1350 CAECCCACC 1470	1360 GGATACBAGG	1370 GGCTGTGCGA 1490	1380 TCCCGCAGCC 1500	1390 BCTCCCTCCB 1510	1400 BBBCTCCTCC	1410 CATTTATCCC 1530	1420 ABTBBCTCAA 1540	1430 CAACTTTTTT 1550	1440 BCCCTCC 1560
1330 CCCA9CTGCAAA90 1450 CCTTCTCCTCCATC	1340 IGGCATCAGCO 1460 IGCTTCCCAGO	1350 CACCCCACC 1470 CTTGACATGT	1360 GGATACBAGG 1480 CTTCCGTGGT	1370 GGCTGTGCGA 1490 GGGGGCAGGG	1380 TCCCGCAGCC 1500 BAABGCAGAG	1390 BCTCCCTCCB 1510 CCCABACTCT	1400 BBBCTCCTCC 1520 TBBABCABTT	1410 CATTTATCCC 1530 STTCCCCCTG	1420 AGTGGCTCAA 1540 GGGACTCTGT	1430 CAACTTTTTT 1550 CAGAGGCTCC	1440 BCCCTCC 1560 ATBGA86
1330 CCCABCTBCAAABG 1450 CCTTCTCCTCCATG	1340 1340 1990 ATCAGOO 1460 190 TTCCCABO	1350 CACCCCACC 1470 CTTGACATGT	1360 GGATACSAGG 1480 CTTCCGTGGT	1370 GGCTGTGCGA 1490 GGGGGCA96G	1380 TCCCGCAGCC 1500 SAAGGCAGAG	1390 BCTCCCTCCG 1510 CCCAGACTCT	1400 BBBCTCCTCC 1520 TBGABCABTT	1410 CATTTATCCC 1530 STTCCCCCTG	1420 АӨТӨӨСТСАА 1540 ӨӨӨАСТСТӨТ	1430 CAACTTTTTT 1550 CAGAGGECTCC	1440 BCCCTCC 1560 ATBGABB
1330 CCCAGCTGCAAAGG 1450 CCTTCTCCTCCATG	1340 1990 ATCAGCO 1460 190 CTTCCCAGO	1350 200000000000000000000000000000000000	1360 GGATACBAGG 1480 CTTCCGTGGT	1370 GGCTGTGCGA 1490 GGGGGCAGGG	1380 TCCCGCAGCC 1500 GAAGGCAGAB	1390 BCTCCCTCCG 1510 CCCAGACTCT	1400 BBGCTCCTCC 1520 TGGABCAGTT	1410 CATTTATCCC 1530 BTTCCCCCTG	1420 AGTGGCTCAA 1540 GGGACTCTGT	1430 CAACTTTTTT 1550 CAGAGGCTCC	1440 BCCCTCC 1560 AT6GA86
1330 CCCABCTBCAAABC 1450 CCTTCTCCTCCATC	1340 IGGCATCAGCO 1460 IGCTTCCCAGO	1350 CACCCCACC 1470 CTTGACATGT	1360 GGATACBAGG 1480 CTTCCGTGGT	1370 GGCTGTGCGA 1490 GGGGGCAGGG	1380 TCCCGCAGCC 1500 SAAGGCAGAG	1390 BCTCCCTCCG 1310 CCCAGACTCT 1630	1400 BBSCTCCTCCI 1520 TGGAGCAGTTI	1410 CATTTATCCC 1530 STTCCCCCTG	1420 ABTBGCTCAA 1540 GBGACTCTBT	1430 CAACTTTTT 1550 CAGAGGETCC	1440 BCCCTCC 1560 ATBGABG
1330 CCCA9CT6CAAA66 CCTTCTCCTCCATC	1340 1340 1460 199CATCAGCO 1580	1350 CACCCCACC 1470 CTTGACATGT 1590	1360 55ATACEASG 1480 CTTCCSTGGT 1600	1370 GGCTGTGCGA 1490 GGGGGCAGGG 1610	1380 TCCCGCA9CC 1500 BAAGGCAGAG	1390 BCTCCCTCCB 1510 CCCABACTCT 1630	1400 BBCTCCTCCI 1520 TGGAGCAGTTI 1640	1410 CATTTATCCC 1530 BTTCCCCCTG 1650	1420 ABTBBCTCAA 1540 GBBACTCTBT 1660	1430 CAACTTTTTT 1550 CAGAGGGCTCC 1670	1440 BCCCTCC 1560 ATBGABG 1680
1330 CCCABCTGCAAAGG CCTTCTCCTCCAAG CCTTCTCAAGTCCAA	1340 IGGCATCAGCC 1460 IGCTTCCCAGC 1580	1350 CCACCCCACC 1470 CTTGACATGT 1590 GAAGCCTCTC	1360 66ATACBAGG 1480 CTTCCGTGGT 1600 CAATTACATT	1370 GGCTGTGCGA 1490 GGGGGCAGGG 1610 CCCCACCACCC	1380 TCCCGCAGCC 1500 BAAGGCAGAG 1620 TGTGCCCCAG	1390 BCTCCCTCCB 1510 CCCABACTCT 1630 AAABBCCCCCC	1400 BBSCTCCTCC 1520 TGGABCASTTI 1640 CATCASCCCA	1410 CATTTATCCC 1530 STTCCCCCTG 1650 STCCCAGGCG	1420 ABTGGCTCAA 1540 GGGACTCTBT 1660 CCCBTCAAGC	1430 CAACTTTTT 1550 CAGAGGETEC 1670 AGGECETEGA	1440 BCCCTCC 1560 AT6GA86 1680 BAGTGCT
1330 CCCA9CT6CAAA86 CCTTCTCCTCCATC	1340 1340 1996CATCAGCC 1460 96CTTCCCAGC 1580 96T6CCTCT60	1350 CCACCCCACC 1470 CTTGACATGT 1590 3AAGCCTCTC	1360 GGATACBAGG 1480 CTTCCGTGGT 1600 CAATTACATT	1370 GGCTGTGCGA 1490 GGGGGGCAGGG CCCACCACCC	1380 TCCCGCAGCC 1500 BAAGGCAGAB 1620 TGTGCCCCAB	1390 BCTCCCTCCB 1510 CCCABACTCT 1630 AAABBCCCCCC	1400 BBBCTCCTCC 1520 TGGABCAGTT 1640 CATCABCCCA	1410 CATTTATCCC 1530 BTTCCCCCTG 1650 BTCCCAGBCG	1420 ABTBBCTCAA 1540 GBBACTCTBT 1660 CCCBTCAABC	1430 CAACTTTTTT 1550 CAGAGGCTCC 1670 AGGCCTCTGA	1440 BCCCTCC 1560 ATBGABG 1680 GABTGCT
1330 CCCABCTGCAAAGG 1450 CCTTCTCCTCCATC 1570 CCTCTCAAGTCCAA	1340 1340 1995CATCASCC 1460 199CTTCCCASC 1580 199CTCCCTSC	1350 CCACCCACC 1470 CTTGACATGT 1590 GAAGCCTCTC	1360 GGATACBAGG 1480 CTTCCGTGGT 1600 CAATTACATT	1370 GGCTGTGCGA 1490 GGGGGCAGGG 1610 CCCACCACCC	1390 TCCCGCAGCC 1500 BAAGGCAGAG 1620 TGTGCCCCAG	1390 BCTCCCTCCG 1510 CCCAGACTCT 1630 AAABBCCCCCC	1400 BBBCTCCTCCI 1520 TBBABCABTTI 1640 CATCABCCCA	1410 CATTTATCCC 1530 STTCCCCCTG 1650 STCCCAGBCG	1420 AGTGGCTCAA 1540 GGGACTCTGT 1660 CCCGTCAAGC	1430 CAACTTTTTT 1550 CAGAGGCTCC 1670 AGGCCTCTGA	1440 GCCCTCC 1560 AT96A89 1680 GA8TGCT
1330 CCCA9CT6CAA66C CCTTCTCCTCCA76 CCTTCTCCTCCA76 1570 CCTCCA66TCCA76	1340 1990 ATCASCO 1460 1900 ATCASCO 1460 1900 ATCASCO 1580 1580 1580 1700	1350 CCACCCCACC 1470 CTTGACATGT 1590 3AAGCCTCTC 1710	1360 564TACBAGG 1480 CTTCCSTGGT 1600 CAATTACATT 1720	1370 GBCT9T8C9A 1490 GBGBGCABGB 1610 CCCACCACCC 1730	1380 TCCCGCAGCC 1500 GAAGGCAGAG 1620 TGTGCCCCAG	1390 BCTCCCTCCB 1510 CCCABACTCT 1630 AAABGCCCCC	1400 BBBCTCCTCC 1520 TBBABCABTTI 1640 CATCABCCCA 1760	1410 CATTTATCCC 1530 BTTCCCCCTG 1650 BTCCCAGGCG 1770	1420 АВТВВСТСАА 1540 ВВБАСТСТВТ 1660 СССЭТСААВС 1780	1430 CAACTTTTTT 1550 CAGAGECTCC 1670 AGECCTCTGA	1440 BCCCTCC 1560 ATBGABG 1680 BABTGCT 1800
1330 CCCA9CTGCAAAGG CCTTCTCCCCCAAG CCTTCTCAAGTCCAA CCTTCTCAAGTCCAA ACCCTTCTCTGT	1340 1340 1340 1460 1460 1560 1560 1700 1700	1350 CCACCCACC 1470 CTTGACATGT 1590 SAAGCCTCTC	1360 56414028439 1480 CTTCC61681 1600 CAATTACATT 1720	1370 GGCTGTGCGA 1490 GGGGGCAGGG 1610 CCCACCACCACCC 1730	1380 TCCCSCAGCC 1500 BAAGGCAGAG 1620 TGTGCCCCAG	1390 BCTCCCTCCG 1510 CCCABACTCT 1630 AAABBCCCCCC 1750 CATRCTCTTA	1400 BBBCTCCTCC 1520 TBBABCABTTI 1640 CATCABCCCA 1760 CABCCCTBC	1410 CATTTATCCC 1530 GTTCCCCCTG 1650 GTCCCAGGCG 1770 ACAGAGTAGC	1420 ABTBGCTCAA 1540 GBBACTCTBT 1660 CCCBTCAABC 1780 ACTCATTAAT	1430 CAACTTTTTT 1550 CAGAGGECTCC 1670 AGGECTCTGA 1790	1440 GCCCTCC 1560 ATGGA8G 1680 GAGTGCT 1800 GSAGTGA
1330 CCCA9CT9CAAA86 CCTTCTCCTCCAT6 CCTCCAA6TCCAT6 CCTCCAA6TCCAT6 1450 ACCCTTCTCTCCAT6	1340 1990 ATCASCO 1460 1900 ATCASCO 1380 1380 1380 1380 1380 1380 1380 1380	1350 CCACCCCACC 1470 CTTGACATGT 1590 3AAGCCTCTC 1710 CCAACACCCC	1360 56ATACBA36 1480 CTTCCST68T 1600 CCATTACATT 1720 TGCCC566CCC	1370 666CT918C6A 1490 666696CA666 1610 CCCACCACCC 1730 CTGA6CT6CC	1380 TCCC9CA9CC 1500 BAAGGCAGA9 1620 TGTGCCCCAG TGTGCCCCAG	1390 BCTCCCTCCB 1510 CCCABACTCT 1630 AAABBCCCCC 1750 CATGCTCTTA	1400 BBBCTCCTCC 1520 TBBBBCABTTI 1640 CATCABCCCA 1760 CABBCCCTBC	1410 CATTTATCCC 1530 STTCCCCTG 5TCCCAGGCG 5TCCCAGGCG	1420 AGTGGCTCAA 1540 GGGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT	1430 CAACTTTTTT 1550 CAGAGGCTCC 1670 AGGCCTCTGA 1790 TCTTGGTTAA	1440 BCCCTCC 1560 ATBGABG BABTGCT 1800 GGAATGA
1330 CCCA9CTGCA4A66 CCTTCTCCCCCA46 CCTTCTCAA6TCCA4 CCTTCTCAA6TCCA4 ACCCTTCTCTGT4	1340 1340 1996ATCAGCC 1460 1967TCCCAGC 1560 1560 1700 1700	1350 CCACCCCACC 1470 CTTGACATGT 34490 CTTGACATGT 34490 CTTGACATGT 34490 CTTGACACCCC	1360 5641405469 1480 CTTCC61681 1600 CAATTACATT 1720 T6CCC66CCC	1370 GGCTGTGCGA 1490 GGGGGCAGGGG 1610 CCCACCACCACCC : 1730 :CTGAGCTGCC	1380 TCCCSCAGCC 1500 BAAGGCAGAG 1620 TGTGCCCCAG 1740 TCCTCCAGCC	1390 BCTCCCTCCB 1510 CCCABACTCT 1630 AAABBCCCCCC 1750 CATBCTCTTA	1400 BBBCTCCTCC 1520 TBBABCABTTI A1640 CATCABCCCA 1760 CABBCCCTBC	1410 CATTTATCCC 1530 9TTCCCCCTG 9TTCCCCAGGCG 9TCCCAGGCG 1770 ACAGAGTAGC	1420 АВТЭВСТСАА 1540 СССВТСАТСТВТ 1660 СССВТСААВС АСТСАТТААТ	1430 CAACTTTTTT 1550 CAGAGGECTCC AGGECCTCTGA 1670 AGGECCTCTGA TCTTGGTTAA	1440 BCCCTCC 1540 ATBGABB 1480 BAGTGCT 1800 GGAATGA
1330 CCCA9CT9CAAA86 CCTTCTCCTCCAT6 CCTCTCAA6TCCAT6 ACCCTTCTCTCTGT/	1340 IG9CATCAGCC 1460 IG9CATCAGCC 1380 IGTGCCTCTGC 1700 IACCTTGCAGC	1350 CCACCCCACC 1470 CTTGACATGT 1590 SAAGCCTCTC 1710 CCAACACCCC	1360 56ATACBA36 1480 CTTCCST68T 1600 CAATTACATT 1720 TGCCC56CCC	1370 GGCT919CGA 1490 GGGGGCA9GG 1610 CCCACCACCC 1730 CTGAGCT9CC	1380 TCCC9CA9CC 1500 BAAGGCAGA9 1620 TGTGCCCCA6 TGTGCCCCA6	1390 BCTCCCTCCG 1510 CCCASACTCT 1630 AAABBCCCCC 1750 CATBCTCTTA	1400 BBBCTCCTCC 1520 TBBBBCABTTI 1640 CATCABCCCA 1760 CABBCCCTBC	1410 CATTTATCCC 1530 STTCCCCCTG 1650 STCCCAGGCG 1770 ACAGAGTAGC	1420 AGTGGCTCAA 1540 GGGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT	1430 CAACTTTTTT 1550 CAGAGGCTCC 1670 AGGCCTCTGA 1790 TCTTGGTTAA	1440 BCCCTCC 1550 ATBGABG 1680 GAGTGCT 1800 GGAATGA
1330 CCCAGCTGCAAAGG CCTTCTCCCCCCAAG CCTTCTCAAGTCCAA CCTTCTCAAGTCCAA ACCCTTCTCTGTA 1690 ACCCTTCTCTGTA	1340 1340 1460 1460 1580 1580 1580 1700 1700 1620	1350 CEACCCCACC 1470 CTTGACATGT 3000 3000 3000 3000 3000 3000 3000 3	1360 56ATAC8A36 1480 CTTCC6T68T 1600 CAATTACATT 1720 T6CCC566CCC 1840	1370 GGCTGTGCGA 1490 GGGGGGCAGGG 1610 CCCACCACCC 1730 CCGAGCTGCC 1850	1380 TCCC9CA9CC 5AAGGCA6A9 1500 1420 TGTGCCCCA9 TGTGCCCCA9 1740 TCCTCCA9CC 1860	1370 BCTCCCTCCG 1510 CCCABACTCT 1630 AAABBCCCCCC 1750 CATBCTCTTA 1870	1400 BBBCTCCTCC 1520 TBGABCABTTI 1640 CATCABCCCA 1760 CABGCCCTBC 1880	1410 CATTTATCCC 1530 9TTCCCCCTG 1450 9TCCCAGGCG 1770 ACAGAGTAGC 1870	1420 AGTGGCTCAA 1540 GGGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT 1900	1430 CAACTTTTTT 1550 CAGAGGCTCC AGGCCTCTGA 1670 TCTTGGTTAA 1790	1440 BCCCTCC 1560 ATBGABB 1680 GABTGCT 18900 GGAATGA 1920
1330 CCCA9CT9CAAA96 CCTTCTCCTCCAT6 CCTCTCAA6TCCA4 ACCCTTCTCTCTGT/ ACCCTTCTCTGT/ 1670 ACCA9CGAATGAAT	1340 1990	1350 CCACCCCACC 1470 CTTBACATGT 1590 30ABGCCTCTC 1710 CCAACACCCCC 1830 IGGACCTCTG	1360 56ATACBA36 1480 CTTCCST69T 1600 CAATTACATT 1720 TGCCC566CCC 1840 56CAGG6A6A	1370 GGCT9T9CGA 1490 GGGGGCA99GG 1610 CCCACCACCC 1730 CTGAGCTGCC 1850 CCT96GTCTT	1380 TCCC9CA9CC 1500 BAAGGCAGA9 1620 TGTGCCCCA9 TGTGCCCCA9 TCTCCA9CCA9 TCCTCCA9CCA9 TCCTCCA9CCA9	1390 BCTCCCTCCB 1510 CCCABACTCT 1630 AAABBCCCCCC 1750 CATBCTCTTA 1870 GABGBGAAABB	1400 BBBCTCCTCC 1520 TBBABCABTTI 1640 CATCABCCCA CAGBCCCTBC 1880 CTAAGBCATB	1410 CATTTATCCC 31530 STTCCCCCTG 1650 STCCCAGGCG 1770 ACAGAGTAGC 1890 SCTGAGATTC	1420 АВТЭВСТСАА 986АСТСТЭТ 1660 000000000000000000000000000000000	1430 CAACTTTTTT 1550 CABABBCTCC 1670 ABBCCTCTBA 1790 TCTTGBTTAA 1910 TCCABBCCTC	1440 BCCCTCC 1560 ATBGABB 1680 GAGTGCT 1800 GGAATGA 1920 TTTGCCC
1330 CCCAGCTGCAAAGG CCTTCTCCCCCAAG CCTTCTCCAAGTCCAA ACCCTTCTCTCTGT/ ACCACGAATGAAT	1340 1340 1360 1460 1350 1350 1350 1350 13700 1620 1820 1820 1820	1350 CEACCECACC 1470 CTTGACATGT 3000 3000 3000 3000 3000 3000 3000 3	1360 5664TAC8A36 1480 CTTCC6T68T 1600 CAATTACATT 1720 TGCCC566CCC 1840 56CAG98A3A	1370 GGCTGTGCGA 1490 GGGGGGCAGGGG 1610 CCCACCACCCC 1730 CCCACCACCC 1850 CCTGGGTCTT	1380 TCCC9CA9CC BAAGGCAGAG TGTGCCCCAG TGTGCCCCAG TCTCCAGCC 1860 CTCTGGCTGA	1370 BCTCCCTCCG 1510 CCCABACTCT AAABGCCCCCC 1750 CATGCTCTTA 1870 GAGGGGAAGG	1400 BBBCTCCTCC 1520 TBGABCABTTI 21540 CATCABCCCAB CAGBCCCTBC 1880 CTAAGBCATB	1410 CATTTATCCC 1530 STTCCCCCTG 1450 STCCCAGGCG ACAGAGTAGC 1770 ACAGAGTAGC	1420 AGTGGCTCAA GGGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT 1900 AAGCCACCAT	1430 CAACTTTTTT 1550 CAGAGGCTCC AGGCCTCTGA 1670 AGGCCTCTGA TCTTGGTTAA 1910 TCCAGGCCTC	1440 BCCCTCC 1560 ATBGABB 1680 GABTGCT 1900 GGAATGA 1920 TTTGCCC
1330 CCCARCTRCAAARSC CCTTCTCCTCCAAC CCTCTCAAGTCCAA ACCCTTCTCTCTCAA ACCCTTCTCTCTGT/ ATCAACGAATGAAT	1340 1340 1990 ATCASCO 1460 1900 ATCCCASC 1580 1580 1580 1580 1700 1820 1820 1820 1820	1350 CCACCCACC 1470 CTTBACATGT 1590 SAABCCTCTC 1710 CCAACACCCC 1830 IGGACCTCTG	1360 564TACBA36 1480 CTTCCGT69T 1600 CAATTACATT 1720 TGCCCG9CCC 1840 56CAG96A3A	1370 666CT9196C9A 1490 6666696CA9696 1610 CCC6A6CC6CC 1730 CCT6A6CT6CC 1850 CCT696TCTT	1380 TCCC9CA9CC 1500 BAAGGCAGA9 1620 TGTGCCCCA6 TCTCCAGCC 1860 CTCT96CTGA	1390 BCTCCCTCCB 1510 CCCAGACTCT 1630 AAABBCCCCCC 1750 CATBCTCTTA 1870 GAGGGGAAAGG	1400 BBBCTCCTCC 1520 TGGABCABTTI 1640 CATCABCCCA 1760 CAGBCCCTBC 1880 CTAAGBCATB	1410 CATTTATCCC 31530 3TTCCCCCTG 31650 STCCCAGGCG 4000 ACAGAGTAGC 31870 BCTGAGATTC	1420 AGTGGCTCAA GBGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT 1900 AAGCCACCAT	1430 CAACTTTTTT 1550 CABABBCTCC 1670 ABBCCTCTBA 1790 TCTTGBTTAA 1910 TCCABBCCTC	1440 BCCCTCC 1560 ATBGABB 1680 GAGTGCT 1800 GGAATGA 1920 TTTGCCC
1330 CCCAGCTGCAAAGG CCTTCTCCCCCAAG CCTTCTCCCCCAAG CCTCTCAAGTCCAA ACCCTTCTCTCTGT/ ATCAACGAATGAAT	1340 1340 1340 1360 1460 1350 1350 1350 1350 1320 1820 1820 1940	1350 CEACCECACC 1470 CTTGACATGT 3000 3000 3000 3000 3000 3000 3000 3	1360 56ATACBAGG 1480 CTTCCGT68T 1600 CAATTACATT 1720 TGCCC56CCC 1840 56CAGGBAGA	1370 GGCTGTGCGA 1490 GGGGGGCAGGGG 1610 CCCACCACCCC 1730 CTGAGCTGCC 1850 CCTGGGTCTT 1870	1380 TCCC9CA9CC 5AAGGCA6A9 1500 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCA9 T0TGCCCCA9 T0TGCCCA9 T0TGCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCA9 T0TGCCCCA9 T0TGCCCA9 T0TGCCCA9 T0TGCCCA9 T0TGCCCA9 T0TGCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCA9 T0TGCCCCCCA9 T0TGCCCCCA9 T0TGCCCCCCCA9 T0TGCCCCCCCCCCCCCCA9 T0TGCCCCCCCCCCCCCCCCA9 T0TGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	1370 BCTCCCTCCG 1510 CCCABBACTCT 1630 AAABBCCCCCC 1750 CATBCTCTTA 1870 BABBGBBAABB	1400 BBBCTCCTCC 1520 TBGABCABTTI 1640 CATCABCCCA 1760 CAGBCCCTBC 1880 CTAAGBCATB 2000	1410 CATTTATCCC 1530 9TTCCCCCT9 1450 9TCCCA69659 1770 ACA6A6TA6C 1890 9CT6A6ATA6C 2010	1420 AGTGGCTCAA 1540 GGGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT 1900 AAGCCACCAT	1430 CAACTTTTTT 1550 CAGAGGCTCC AGGCCTCTGA 1670 AGGCCTCTGA TCTTGGTTAA 1910 TCCAGGCCTC 2030	1440 BCCCTCC 1560 ATBGA88 6ABTGCT 1800 GGAATGA 1920 TTTGCCC 2040
1330 CCCA9CT9CAAA960 CCTTCTCCTCCAT6 CCTTCTCCTCCAT6 CCTCTCAA9GTCCA4 ACCCTTCTCTCTGT/ ATCA9C904TG94 1930	1340 1340 1960ATCASCC 1460 1960TTCCCASC 1580 90TSCCTTSCASC 1520 1620 1620 1620 1620	1350 CCACCCACC CTTGACCACCACC CTTGACATGT 1590 SAAGCCTCTC CCAACACCCCC 1830 IGGACCTCTG	1360 BGATACBAGG 1480 CTTCCGTGGT 1600 CAATTACATT 1720 TGCCCGGCCCC 1840 GGCAGGGAGA	1370 GGCT919CGA 1490 GGGGGCA9999 1610 CCCACCACCC 1730 CCT6AGCT9CC 1950 CCT99GTCTT 1970	1380 TCCC9CA9CC 1500 T6T6CCCCA6 T6T6CCCCA6 TCCTCCA6CC TCCT69CT6A	1390 BCTCCCTCCB 1510 CCCAGACTCT 1630 AAABBCCCCCC 1750 CATBCTCTTA 1870 GAGGGGAAGG	1400 BBBCTCCTCC 1520 TBBABCABTTI 1640 CATCABCCCA 1760 CABBCCCTBC 1880 CTAABBCATB	1410 CATTTATCCC 311530 BTTCCCCCTG BTTCCCCCTG BTCCCAGGCG ACAGAGITAGC ACAGAGITAGC BCTGAGATTC 2010	1420 AGTGGCTCAA GBGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT 1900 AAGCCACCAT 2020	1430 CAACTTTTTT 1550 CABABBCTCC 1670 ABBCCTCTBA 1790 TCTTBBTTAA 1910 TCCABBCCTC 2030	1440 BCCCTCC 1560 ATBGABB 1680 GAGTGCT 1800 GGAATGA 1920 TTTGCCC 2040
1330 CCCA9CT9CA4A96 CCTTCTCCCCCATC CCTCTCA9GTCCA4 ACCCTTCTCTCTGT/ ACCCTTCTCTTGT/ ATCA9C59A7G9A1 1930 AG6A969999CTTC	1340 1340 1360 1460 1350 1350 1350 1350 1320 1820 1820 1820 1820 1940 1940	1350 CEACCECACC 1470 CTTGACATGT 3AAGCCTCTC 1710 CCAACACCCC 1830 TGGACCTCTG	1360 56617628436 1480 CTTCC676867 CAATTACATT 1720 TGCCC566CCC 1840 56CAGG66436 1960 TGTGTTCT64	1370 GGCTGTGCGA 1490 GGGGGGCAGGG 1610 CCCACCACCCC 1730 CTGAGCTGCC 1850 CCTGGGTCTT 1970 GCCCTGGCC	1380 TCCC9CA9CC 5AAGGCA6A9 T9T9CCCCA9 T9T9CCCCA9 T0T9CCCCA9 T0T9CCCCA9 T0T9CCCCA9 T0T9CCCCA9 T0T9CCCCA9 T0T9CCCCA9 T0T9CCCCCA9 T0T9CCCCCA9 T0T9CCCCCA9 T0T9CCCCCA9 T0T9CCCCCA9 T0T9CCCCCA9 T0T9CCCCCA9 T0T9CCCCCA9 T0T9CCCCA9 T0T9CCCCCA9 T0T9CCCCCA9 T0T9CCCCCA9 T0T9CCCCCA9 T0T9CCCCA9 T0T9CCCCA9 T0T9CCCCA9 T0T9CCCCA9 T0T9CCCA9 T0T9CCCA9 T0T9CCCA9 T0T9CCCA9 T0T9CCCA9 T0T9CC	1370 BCTCCCTCCG 1510 CCCABACTCT 1630 AAABGCCCCCC 1750 CATGCTCTTA 1870 GAGGGGAAGG 1970 CCTTCCATGC	1400 BBBCTCCTCC 1520 TBGABCABTTI 2440 CATCABCCCA CAGBCCCTBC 1880 CTAAGBCATB 2000 CCCGACCCCC	1410 CATTTATCCC 1530 9TTCCCCCT9 9TTCCCCA66C9 9TCCCA66C9 ACA6A8TA6C 1890 9CT6A6ATA6C 2010 ACCCCAA6CC	1420 AGTGGCTCAA GGGACTCTGT 1640 CCCGTCAAGC ACTCATTAAT 1900 AAGCCACCAT 2020 TCTCCACTAG	1430 CAACTTTTTT 1550 CAGAGGCTCC AGGCCTCTGA 1670 AGGCCTCTGA TCTTGGTTAA 1910 TCCAGGCCTC 2030 GCCTCTGCCA	1440 BCCCTCC 1560 ATBGA89 BABTGCT 1800 GGAATGA 1920 TTTGCCC 2040 GGATCTA
1330 CCCARCTRCAAARSC CCTTCTCCTCCATC CCTCTCAARGTCCAA ACCCTTCTCTCTTGT/ ATCAACGAATGAAT ATCAACGAATGAAT	1340 1340 1960ATCASCC 1460 1960TTCCCASC 1580 1580 1580 1580 1580 1620 1820 1820 1820 1820 1820 1820 1840 1940	1350 CCACCCCACC 1470 CTTGACATGT 1590 GAAGCCTCTC 1590 CCAAGCACCCCC 1830 rGGACCTCTCG 1950 rGCACTCTCC	1360 1480 1480 CTTCCGTGGT 1600 CAATTACATT 1720 1840 GGCAGGGAGA 1960 TGTGTTCTGA	1370 GGCTGTGCGA 1490 GGGGGCAGGG 1490 CCCACCACCA CCACCACCACCC CCACCACCACCC CCACCA	1380 TCCC9CA9CC 8AA99CA9A9C 1500 TGT9CCCCA9 TCCTCCA9CCA9 TCCTCCA9CCA9 CTCT99CT6A CTCT99CT6A	1390 BCTCCCTCCB 1510 CCCABACTCT 1630 AAABBCCCCCC CATBCTCTTA 1870 GAGGBGAAGB CATBCTCTTA 1970 CCTTCCATBC	1400 BBBCTCCTCC 1520 TBBABCABTTI 1640 CATCABCCCA CAGBCCCTBC CAGBCCCTBC CTAABBCATB 2000 CCCCGACCCCC	1410 CATTTATCCC 311530 3TTCCCCCTG 3TTCCCCAGGCG 3TTCCCCAGGCG ACAGAGATAGC 31890 3CTGAGATTC 2010 ACCCCAAGCC	1420 AGTGGCTCAA GBGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT 1900 AAGCCACCAT TCTCCACTAG	1430 CAACTTTTTT 1550 CABABBCTCC 1670 ABBCCTCTBA TCTTGBTTAA 1910 TCCABBCCTC 2030 BCCTCTBCCA	1440 GCCCTCC 1560 ATGGA8G 1680 GAGTGCT 1800 GGAATGA 1920 TTTGCCC 2040 GGATCTA
1330 CCCA9CT9CA4A96 CCTTCTCCCCCA46 CCTTCTCCCCCA46 CCTCCCA46TCCA4 ACCCTTCTCTTG74 ACCCTTCTCTTG74 ATCAACGAATGAAT AAGAAAGAAAGAAACTTG	1340 1340 1360 1460 1350 1350 1350 1350 1350 1350 1350 135	1350 CCACCCCACC 1470 CTTGACATGT 1590 3AAGCCTCTC 1710 CCAACACCCCC 1830 rGGACCTCTG	1360 GGATACSAGG 1480 CTTCCSTGGT 1600 CAATTACATT 1720 TGCCCGGCCC 1840 GGCAGGGAGA 1960 TGTGTTCTGA	1370 GGCTGTGCGA 1490 GGGGGCAGGGG 1610 CCCACCACCACCC 1730 CTGAGCTGCC 1850 CCTGGGTCTT 1970 GTCCCTGGCC	1380 TCCC9CA9CC 1500 GAAGGCAGA9 1620 TGTGCCCCA9 TGTGCCCCA9 TCCTCGACCCA9 1860 CTCTG9CTGA 1980 AATAGCACA9	1390 9CTCCCTCC9 1510 CCCASACTCT 1630 AAABBCCCCC 1750 CATGCTCTTA 1870 GAGGGGAAGG 1990 CCTTCCATGC	1400 BBBCTCCTCC 1520 TBBABCABTH A440 CATCABCCCA CACBBCCCTBC CAAGBCCCTBC 1980 CTAAGBCATB 2000 CCCGACCCCC	1410 CATTTATCCC 1530 BTTCCCCCTB 1650 BTCCCAGBCB ACAGAGTABC 1770 ACAGAGTABC 1870 BCTGAGATTC 2010 ACCCCAAGCC	1420 AGTGGCTCAA 1540 GGGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT 1900 AAGCCACCAT 2020 TCTCCACTAG	1430 CAACTITITI 1550 CAGAGECTCC AGGECTCTGA 1670 TCTTGGTTAA 1910 TCCAGGECCTC 2030 GCCTCTGCCA	1440 BCCCTCC 1560 ATBGA89 GABTGCT 1800 GBAATGA 1920 TTTGCCC 2040 GBATCTA
1330 CCCA9CTGCAAAGG CCTTCTCCCCCATG CCTTCTCAAGTCCAA ACCCTTCTCTGTA ACCCTTCTCTGTA ATCAACGAATGAAT AAGAAAGAAACTTG 2050	1340 1340 1340 1460 1460 1580 1580 1580 1580 1580 1580 1580 1580 1580 1620 1620 1620 1620 1620 1620 1640 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1700 1650 1650 1650 1700 1650	1350 CCACCCCACC 1470 CTTGACATGT 1590 GAAGCCTCTC 1590 CCAACACCCC 1970 1950 IGCACCTCTCC 2070	1360 564TACBA99 1480 CTTCC9T99T CAATTACATT T6CCC99CCC 1840 56CA959A9A 1960 T979TTCT9A 2080	1370 GGCTGTGCGA 1490 GGGGGCAGGGG 1410 CCCACCACCC CCACCACCACCC CCACCACCACCC CCACCA	1380 TCCC9CA9CC BAAGGCAGA9 TBT9CCCCA9 TCTCCA9CCA9 TCCTCCA9CCA9 CTCTG9CTGA CTCTG9CTGA A1980 AATA9CACA9 2100	1390 BCTCCCTCCB 1510 CCCAGACTCT 1630 AAABGCCCCC CATGCTCTTA 1870 GAGGGGAAGG CATGCTCTTA 1970 CCTTCCATGC 2110	1400 BBBCTCCTCC 1520 TBBABCASTTI 1640 CATCABCCCA CAGBCCCTBC 1880 CTAABBCATB 2000 CCCCBACCCCC 2120	1410 CATTTATCCC 31530 3TTCCCCCTG 3TTCCCCAGGCG 4050 3TTCCCCAGGCG 4050 3TTCCCCAGGCG 4050 3TTCCCCAGGCG 4050 3TTCCCCAGGCG 4050 3TTCCCCCAGCC 4050 3150 3150 3150 3150 3150 3150 3150 3	1420 AGTGGCTCAA GGGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT 1900 AAGCCACCAT 12020 TCTCCACTAG 2140	1430 CAACTITITI 1550 CAGAGGECTCC 1670 AGGECTCTGA TCTTGGTTAA 1910 TCCAGGECCTC 2030 GCCTCTGCCA 2150	1440 GCCCTCC 1560 ATGGABG 1680 GAGTGCT 1800 GGAATGA 1920 TTTGCCC 2040 GGATCTA 2140
1330 CCCA9CT9CA4686 CCTTCTCCTCCA46 CCTTCTCCTCCA46 CCTCCCA66TCCA4 ACCCTTCTCTTGT/ ACCCTTCTCTTGT/ ATCAACGAATGAAT AAGAAAGAAAGAAACTT( AGCCCATGACCA47	1340 1340 1360 1460 1350 1350 1350 1350 1350 1350 1350 135	1350 CCACCCCACC 1470 CTTGACATGT 1590 3AAGCCTCTC 1710 CCAACACCCCC 1830 FGGACCTCTG 1950 FGGACCTCTCC 2070	1360 5664TACSASS 1480 CTTCCSTGST 1600 CAATTACATT 1720 TGCCCSGCCC 1840 560CAGSSASA 1960 TGTGTTCTGA 2080 CTTGSCASA	1370 GGCTGTGCGA 1490 GGGGGCAGGGG 1610 CCCACCACCACCC 1730 CTGAGCTGCC 1850 CCTGGGTCTTT 1970 GTCCCTGGCC 2090	1380 TCCC9CA9CC 1500 SAABGCAGA9 1620 TGTGCCCCA6 TGTGCCCCA6 TCTCGGCTCA6 CTCTGGCTCA6 CTCTGGCTGA AATAGCACA6 2100	1390 9CTCCCTCC9 1510 CCCASACTCT 1630 AAABBCCCCC 1750 CATBCTCTTA 1870 9A0969AABB CCTTCCATBC	1400 BBBCTCCTCC 1520 TBBABCABTH A440 CATCABCCCA CATCABCCCA 1760 CAAGBCCCTBC 1880 CTAAGBCATBC 2000 CCCGACCCCC 2120	1410 CATTTATCCC 1530 BTTCCCCCTB 1450 BTCCCAGBCB ACAGAGTABC 1770 ACAGAGTABC BCTGAGATTC ACCCCAGGCC 2010 ACCCCCAGCC 2130	1420 AGTGGCTCAA 1540 GGGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT 1900 AAGCCACCAT 2020 TCTCCACTAG 22140 TCTCCCATGA	1430 CAACTITITI 1550 CAGAGGETCC AGGCTCTGA 1670 AGGCCTCTGA TCTTGGTTAA 1910 TCCAGGCCTC 2030 GCCTCTGCCA 22150 CAGGGAGGCAA	1440 BCCCTCC 1560 ATBGA99 GABTGCT 1800 GBAATGA 1920 TTTGCCC 2040 GBATCTA 2160 C000000
1330 CCCABCTGCAAAGG CCTTCTCCCCCAAG CCTTCTCAAGTCCAA ACCCTTCTCTCTGTA ACCCTTCTCTGTA ATCAACGAATGAAT AAGAAAGAAAACTTC AGCCCATGAGCACA	1340 1340 1340 1460 1460 1580 1580 1580 1580 1580 1620 1620 1620 1620 1620 1620 1620 1620 1640 1640 1580 1700 1640 1580 1700 1640 1580 1700 1640 1580 1700 1640 1580 1700 1640 1700 1640 1700 1640 1700 1640 1700 1640 1700 1640 1700 1640 1700 1640 1700 1640 1700 1640 1700 1640 1700 1640 1700 1640 1700 1640 1700 1640 1700 1640 1640 1700 1640 1700 1640 1640 1700 1640 1640 1640 1640 1700 1640	1350 CCACCCCACC 1470 CTTGACATGT 3000 3000 3000 3000 3000 3000 3000 3	1360 564TACBA99 1480 CTTCCST99T CAATTACATT T6CCC99CCC 1840 66CA9696A9A 1960 T979TCT8A 2080 CCT69CA6AT	1370 GGCTGTGCGA 1490 GGGGGCAGGGG 1490 CCCACCACCC CCACCACCACCC CCACCACCACCC CCACCA	1380 TCCC9CA9CC BAAGGCAGA9 TBT9CCCCA9 TBT9CCCCA9 TCCTCA9CCA9 CTCT69CTGA CTCT69CTGA CTCT69CTGA AATA9CACA9 AATA9CACA9 2100 CAAA9GAA9A9	1390 BCTCCCTCCB 1510 CCCABACTCT 1450 CATBCTCTTA BAGGBGAAGG CATBCTCTTA 1870 GAGGBGAAGG CATBCTCTAA 1990 CCTTCCATBC 2110 TCCTCAAATG	1400 BBBCTCCTCC 1520 TBBABCASTTI 1640 CATCABCCCA CAGBCCCTBC 1880 CTAABBCATB 2000 CCCCBACCCCC 2120 TAGABTATAT	1410 CATTTATCCC 311530 311CCCCCTG 31650 51CCCA66CG ACCCA66CG 31770 ACA6A617A6C 31890 5CTGA66ATTC ACCCCAA6CC 2010 ACCCCAA6CC 2130 TTTTTCTTT	1420 AGTGGCTCAA GGGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT 1900 AAGCCACCAT TCTCCACTAG 2020 TCTCCACTAG	1430 CAACTITITI 1550 CAGAGGECTCC 1670 AGGECTCTGA TCTTGGTTAA 1910 TCCAGGECTCC 2030 GCCTCTGCCA 2150 CAGGAAGCAA	1440 BCCCTCC 1560 ATBGABG 1680 BAGTGCT 1800 GGAATGA 1920 TTTGCCC 2040 GGATCTA 2160 GAAAGAA
1330 CCCA9CT9CA4A96 CCTTCTCCTCCATC CCTCTCA9GTCCA4 ACCCTTCTCTTGT/ ACCCTTCTCTTGT/ ATCAACGAATGAAT AAGAAAGAAAGAAACTTC AAGAAAGAAAGAAACTTC AGCCCATGAGCACC	1340 1340 1360 1460 1350 1350 1350 1350 1350 1350 1350 135	1350 CCACCCACC 1470 CTTGACATGT 1590 3AAGCCTCTC 1710 CCAACACCCC 1830 FGGACCTCTG 1950 FGCACTCTCC 2070 TATCCCAAGAA	1360 GGATACSAGG 1480 CTTCCSTGGT 1600 CAATTACATT 1720 TGCCCGGCCC 1840 GGCAGGGAGAA 1960 TGTGTTCTGA 2080 CCTGGCAGAT	1370 GGCTGTGCGA 1490 GGGGGCAGGGG 1610 CCCACCACCACCC 1730 CTGAGCTGCC 1850 CCTGGGTCTTT 1970 GTCCCTGGCC 2090 GTGGCTGCTC	1380 TCCC9CA9CC 1500 BAAGGCAGA9 1620 TGTGCCCCA6 TGTGCCCCA6 TCTCGGCTGA CTCTGGCTGA CTCTGGCTGA ATAGCACA6 2100 CAAGGAAGA	1390 9CTCCCTCC9 1510 CCCASACTCT 1630 AAABBCCCCC 1750 CATBCTCTTA 1870 9ABBGBAABB 1990 CCTTCCATBC 2110 TCCTCAAATG	1400 BBBCTCCTCC 1520 TBBABCABTH 21640 CATCABCCCA 2000 CTAAGBCCTBC 2000 CCCGACCCCC 2120 TAGAGTATAT	1410 CATTTATCCC 1530 BTTCCCCCTB 1650 BTCCCAGBCB ACAGABTABC 1770 ACAGABTABC 1870 BCTBAGATTC 2010 ACCCCAAGCC 2130 TTTTCCTTT	1420 AGTGGCTCAA 1540 GGGACTCTGT 1660 ACTCGTCAAGC ACTCGTCAAGC AAGCCACCAT 1780 AAGCCACCAT AAGCCACCAT 2020 TCTCCCACTAG 2140 TCTACCATGA	1430 CAACTITITI 1550 CAGAGGETCC AGGCCTCTGA 1670 AGGCCTCTGA TCTTGGTTAA 1910 TCCAGGCCTC BCCTCTGCCA 2030 BCCTCTGCCA 2030 BCCTCTGCCA	1440 BCCCTCC 1560 ATBGA99 GABTGA 1680 GBAATGA 1920 TTTGCCC 2040 GBATCTA 2140 GBAAGGAA
1330 CCCABCTBCAAABB CCTTCTCCCCCAABC CCTTCTCCCCCAABC CCTCCCAABGCCAA ACCCTTCTCTCTGT/ ATCAACGAATGAAT AAGAAAAGAAACTTC AGCCCATGAGCACA	1340 1340 1996ATCAGCC 1460 196CTTCCCAGC 1580 1580 1580 1580 1620 1820 1820 1820 1820 1820 1820 1940 1940 1940 1940 1940	1350 CCACCCCACC 1470 CTTBACATGT 3AABCCTCTC 1590 3AABCCTCTC 1830 rGGACCTCTG 1950 rGCACTCTCC 2070 rATCCCAAGA	1360 564TACBA99 1480 CTTCCST99T CAATTACATT T6CCC89CCC 1840 56CA969A9A 1960 T979TTCT9A 2080 CCT69CA9AT	1370 GGCTGTGCGA 1490 GGGGGCAGGGG 1490 CCCACCACCA CCACCACCACCC CCACCACCACCC CCACCA	1380 TCCC9CA9CC BAAGGCAGA9 TBT9CCCCA9 TBT9CCCCA9 TCCTCA9CCGA 1740 TCCTCCA9CCGA 1780 AATA9CACA9 AATA9CACA9 2100 CAAA9SAA6A	1390 BCTCCCTCCB 1510 CCCAGACTCT AAA3GCCCCC CATGCTCTTA GAGGGGAAGG CATGCTCTTA 1870 GAGGGGAAGG CCTTCCATGC 2110 TCCTCAAATG	1400 BBBCTCCTCC 1520 TBBABCABCTT 1640 CACAGECCCA CACEGECCCTGC 1880 CTAAGECATB 2000 CCCCGACCCCC 2120 TAGAGTATAT	1410 CATTTATCCC 31530 3TTCCCCCTG 31650 5TCCCAGGCG 1770 ACAGAGATAGC 31890 5CTGAGATAGC 31890 5CTGAGATAGC 2010 ACCCCAAGCC 2130 TTTTCCCTT	1420 AGTGGCTCAA GGGACTCTGT 1540 GGGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT 1900 AAGCCACCAT 2020 TCTCCACTAG 2140 TCTACCATGA	1430 CAACTITITI 1550 CAGAGGECTCC 1670 AGGECTCTGA TCTTGGTTAA 1910 TCCAGGGCCTC 2030 GCCTCTGCCA 2150 CAGGAAGCAA	1440 BCCCTCC 1540 ATBGABB 1680 BABTGCT 1800 BGAATGA 1920 TTTGCCC 2040 BGATCTA 2160 GAAAGAA
1330 CCCA9CT9CA4A96 CCTTCTCCTCCATC CCTCTCAA9CTCCATC ACCCTTCTCTTGT/ ACCCTTCTCTTGT/ ATCAACSAATGAAT AAGAAAGAAAGAAACTTC AGCCCATGAGCAC/ 2170	1340 1340 1340 1360 1350	1350 CCACCCCACC 1470 TTGACATGT 1590 30AGGCCTCTC 1710 CCAACACCCC 1830 TGGACCTCTG 1950 TGCACTCTCC 2070 TATCCCAAGAA 2190	1360 1480 CTTCCSTGST 1480 CTTCCSTGST 1600 CAATTACATT 1720 TGCCCSGCCC 1840 GGCAGGGAGAGA 1940 TGTGTTCTGA 2080 CCTGGCAGAT 2200	1370 GGCTGTGCGA GGGGGGCAGGGG 1490 GGGGGGCAGGGG 1610 CCCACCACCACCC 1730 CCTGAGCTGCC 1950 CCTGGGTCTTT GTCCCTGGGTCTTT 2090 GTCGCTGCCCC 2210	1380 TCCC9CA9CC 1500 BAAGGCAGA9 1620 TGTGCCCCA6 TGTGCCCCA6 TCTGGCCCCA6 1740 TCCTCGACCCA6 T740 TCCTCGACCA6 1980 AATAGCACA6 2100 CAAGGAAGA	1390 BCTCCCTCCB 1510 CCCASACTCT 1630 AAABBCCCCCC 1750 CATBCTCTTA 1870 BABBBBAABB CCTTCCATBC 1990 CCTTCCATBC 22110 TCCCCASATB 2230	1400 BBBCTCCTCC 1520 TBBABCABTTI 1640 CATCABCCCA 2140 CABBCCCTBC 1980 CTABBCCTBC 2000 CCCGBCCCCC 2120 TABBBCATBT 2240	1410 CATTTATCCC 1530 BTTCCCCCTB 1650 BTCCCAGBCB ACAGABTAGC 1870 BCTGAGATTC 2010 ACCCCAAGCC 2130 TTTTTCCTTT 2250	1420 AGTGGCTCAA 1540 GGGACTCTGT 1660 CCCGTCAAGC ACTCATTAAT 1900 AAGCCACCAT 2020 TCTCCACTAG 2140 TCTACCATGA	1430 CAACTITITI 1550 CAGABGETCC 1670 AGGECTCTGA 1670 TCTTGGTTAA 1910 TCCAGGGCTC 2030 GCCTCTGCCA 2150 CAGGGAGGAA	1440 BCCCTCC 1560 ATBGA89 BABT9CT 1800 BGAATGA 1920 TTTGCCC 2040 BGATCTA 2160 GGAAGGAA

FIG. 2. Nucleotide sequence of p1715. The DNA-sequencing strategy of p1715 is outlined in Fig. 1. The fusion junction of the hybrid message is located between nucleotides 503 and 504, as indicated by the arrow. MYL and RARA designate the cDNA regions derived from chromosome 15 and chromosome 17, respectively. The two arrowheads indicate sites of alternative splicing.

abnormality was performed to determine whether there was rearrangement of the unknown gene sequence. A PCRamplified 1.2-kb DNA fragment (nucleotides 504 to 1704 [Fig. 2]) was used as the probe for hybridization. As shown in Fig. 6A and B, 12 of the 17 APL DNA samples show rearranged bands. DNAs from HL-60 cells and patients without the t(15;17) translocation breakpoint did not show rearranged bands. This result indicates that translocation breakpoints in chromosome 15 cluster within the same gene. In addition, samples from patients 9 and 10 (Fig. 6B) represent DNAs isolated from the same patient in acute phase and in remission. A rearranged band was found in the acute-phase DNA sample but not in the remission sample. This result suggests that the rearrangement is not a result of restriction fragment length polymorphism.

**Expression of the myl gene sequence in human leukemia** samples. The myl cDNA was used to hybridize to the Northern blots of RNA samples of different leukemia patients. As shown in Fig. 7A, mRNA species of 5.5, 4.2, and 3.2 kb were detected. In one APL patient, an additional mRNA of 2.6 kb (lane 6) was also detected. The myl cDNA appears to be expressed at high levels in all leukemic cell types. The 3.2-kb mRNA is the predominant band and appeared in all samples tested. No additional bands were observed in the other APL RNA sample (lane 7). Since the additional band in lane 6 did not appear to be a sharp band, he61nPheLeuArgCys81n61nCys61nA1a61uA1aLysCysProLysLeuLeuProCysLeuHisThrLeuCysSer61yCysLeuG1uA1aSer61yHet61n 40 AlaGluGluGluF CysProlleCysBlnAleProTrpProLeuGlyAleAspThrProAleLeuAspAsnValPhePheGluBerLeuGlnArgArgLeuSerValTyrArgGlnIleValAspAleGlnAle 80 GTGTGCACCCGCTGCAAAGAGTCGGCCGACTTCTGGTGCTTTGAGTGCAGCAGCAGCCCCTCGCGCCAAGTGCTTCGAGGCACACCAGTGGTTCCTCAAGCACGAGGCCCGGCCCCTAGCA Val CysThrArgCysLysBl uSerAl aAspPheTrpCysPheBl uCysBl uBl nLeuLeuCysAl aLysCysPheBl uAl aHi sBl nTrpPheLeuLysHi sBl uAl aArgProLeuAl a 120 GGAGCTGGACOCCATO SerLysProLeuCysCysSerCysA1aLeuLeuAspSerSerHisSer61uLeuLysCysAspI1eSerA1a61uI1e81n61nArg61n61uL6uAspA1aMetThr61nA1aLeu 200 AlaHisValArgAlaBinBiuArgBiuLeuLeuGiuAlaValAspAlaArgTyrBinArgAspTyrBiuBiuHetAlaSerArgLeuBiyArgLeuAspAlaValLeuBinArgIleArg 280 970 980 990 1000 1010 1020 1030 1040 1050 1060 1060 1070 1080 CAABCTBCCBTBCBCACCBATBBCTTCBACBABTTCAABGTBCBCCTCBABGACCTCABCTCTTBCATCACCGABGBBAAABATBCABCCABCABBCCABCCABABBCTBCC GInAlaAlaValArgThrAspBlyPheAspBluPheLysValArgLeuGInAspLeuSerSerCysIleThrGInBlyLysAspAlaAlaValSerLysLysAlaSerProGluAlaAla 300 1130 1140 1150 1160 1170 1180 1190 1200 3ASAGAGTGAABSCCCAGGTTCABSCCCTBBGGCTGAGGCCCAGCCTATSSCTGTGGTACAGTCAGTCAGTCCCC 1090 1100 1110 1120 AGCACTCCCA666ACCCTATT6AC6TT6ACCT6<u>CCC6A66A86CA6</u> Ser ThrProArgAspProI1eAspValAspLeuProBluBluAlaBluArgValLysAlaBlnValBlnAlaLeuBlyLeuAlaBluAlaBlnProMetAlaValValBlnSerValPro400 1330 1340 1350 1360 1370 1380 1370 1400 1410 1420 1430 1440 GTCATCAABATBBAGTCTGABGAGBGAABBAGBCAABBTTGBCTCBGABCTCCCCBBABCAGCCCABBCCCCCABBCACCTCCCAABBCABTCTCACCACCCCBBATBBACCBCCTABC Val 11 eLysMet 61 u Benst Er tendendeutsandsonstander i feber 1600 freder for European tendendeutsander for tendendeutsander feber 1600 freder 1600 fr Sp ArgProAlaValHisArg8lyIlaArgTyrLeuLeuTyrArgAlaBlnArgAlaIleArgLeuArgHisAlaLeuArgLeuHisProBlnLeuHisArgAlaProIleArgThrTrpSer640 1930 1940 1950 1960 1970 1980 1970 2000 2010 2020 2030 2040 CCCCATGEGGECCAGGCACTCCEGCCATCACGAGGGCCCCCCAACCATCCEGCCAGGAACATCCEGCCGGCEGCGGCACCAGGCACCCCCCCCGGATACSAGGGG ProhisValValBlnAlaSerThrProAlalleThrGlyProLeuAsnHisProAlaAsnAlaGlnGluHisProAlaGlnLeuGlnArgGlyIleSerHisProThrGlyTyrGluGly680 2410 2420 2430 2440 2450 2460 GCTGCCTCCCTCCABCCCATGCTCTTACAGGCCCTGCACAGAGTAGCACTCATTATTCTTGGTTAC ABBAATBAATCAACBAATBAATBGCTATBCATBGACCTCTBBGCABGBAGACCTF ICTOTCACCCTTOCACTCTCCTOTOTICTGAGTCC 

FIG. 3. Nucleotide sequence of the *myl* cDNA pMYL211. Numbers on top of each line indicate nucleotide positions. Numbers on the right indicate positions of the last amino acid on each line. The polyadenylation site AATAAA is underlined. Sp indicates the position of alternative splicing. The underlined region from nucleotides 1114 to 1257 is not found in p1715.

## 1. GGCGCCGGGGAGGCAG<u>GTAGGGAG</u>AGGAACGCG

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### 3. GGCGCCGGGGAGGCAG<u>GTAGGGAG</u>GTGGGTAGGGCAG-------1.5KB----CCCCTTCCCCGTTTCAGAGGAACGCG

FIG. 4. The additional 8-bp DNA sequence found in the cDNA of p1715 is a result of alternative splicing. Line 1 shows the DNA sequences of p1715 between nucleotides 817 and 849 (Fig. 2). Line 2 shows the homologous DNA sequence from pMYL211, between nucleotides 1501 and 1525 (Fig. 3). Line 3 shows the genomic DNA sequence of the intron/exon junctions within this region. The 8 bp involved in the alternative splicing are underlined.

the Northern blot was repeated (Fig. 7C). An abnormal band of 2.6 kb was then clearly visible (lane 3). The Northern blot in Fig. 7A was stripped and rehybridized to the <sup>32</sup>P-labeled probe of pRA1 (Fig. 7B). The two RARA mRNA species of 2.7 and 3.7 kb were detected in all samples tested. In lane 6, containing a sample from an APL patient, two abnormal mRNA species of 4.2 and 3.2 kb were found. Since the pRA1 cDNA consists of about 520 bp of exon 2 of the RARA gene, a 2.6-kb abnormal mRNA should be detectable. However, failure to identify it may be explained by its comigration with the 2.7-kb normal RARA mRNA. In a second APL patient (lane 7), two abnormal mRNAs of 4.8 and 3.8 kb (slightly overlapped with the 3.7-kb normal band) were found. Samples from both APL patients tested in this Northern blot were analyzed for RARA gene rearrangement. The breakpoint for both patients was localized to intron 2 (data not shown). This observation indicates that the difference in the sizes of the abnormal mRNAs from the two APL patients was the result of different breakpoint sites on chromosome 15.

Since the *myl* cDNA probe isolated from p1715 did not hybridize to any of the abnormal mRNA species detected by the RARA cDNA (Fig. 7A through C), the 4.2- and 3.2-kb mRNAs detected in lane 6 and the 4.8- and 3.8-kb mRNAs detected in lane 7 may represent hybrid *myl*/RARA mRNA rather than RARA/*myl* RNA. In addition, the size difference of the two abnormal mRNAs from the two APL patients was 1 kb, similar to the size difference of the two normal RARA mRNAs (3.7 and 2.7 kb). Our sequence analysis of pRA1 (Fig. 1) at the 3' end indicates that a second polyadenylation site is used by the RARA gene, in contrast to what was previously reported for cDNA sequences (22, 37). Recently, it was reported that a single transcription start site is used by the RARA gene (5). Therefore, different polyadenylation sites may have resulted in the two different RARA mRNA species.

Detection of the two different fusion transcripts RARA/myl and myl/RARA in APL. To conclusively prove that both fusion transcripts RARA/myl and myl/RARA are expressed in the same APL patient, two sets of oligonucleotide primers were designed to amplify the fusion junction of the hybrid mRNA by PCR. RNAs from three APL patients were analyzed. As shown in Fig. 8A, primer set R5/D4 amplified an expected DNA fragment of 760 and 620 bp in three APL samples. Sequence analysis of the amplified DNA fragments confirmed that these DNA fragments were derived from the fusion junction of the RARA/myl mRNA (Fig. 8B). The two amplified DNA fragments differed by approximately 140 bp; this is the result of alternative splicing (Fig. 3, nucleotides 1114 to 1257). Sequence analysis of RARA/myl mRNA as



FIG. 5. Chromosome assignment of the *myl* gene sequence of p1715. A 1.2-kb cDNA fragment obtained from PCR amplification (nucleotides 504 to 1704 of p1715) was oligolabeled to  $10^9$  cpm/µg DNA and hybridized to the chromosome-specific DNA dot blot as described in Materials and Methods. (A) Positions of chromosome spots of each filter disc. (B) X-ray autoradiograph of hybridized filters. Arrow indicates the specific hybridization of the unknown gene sequence to the chromosome 15 spot. An additional signal fell outside the chromosome 3 or 4 spot and is thus not a hybridization signal.

shown in Fig. 8B indicates a clear sequencing tract in all four lanes. However, the sequencing tracts beyond nucleotide 1113 (Fig. 3) become unreadable, presumably due to the overlapping of two different sequences (data not shown). Primer set R2/15U1 amplified a DNA fragment of 1.2 kb in all three APL RNA samples. DNA sequence analysis confirmed that the PCR amplified a DNA fragment derived from the hybrid mRNA of *myl*/RARA (Fig. 8B). Our results conclusively prove that both RARA/*myl* and *myl*/RARA mRNAs were coexpressed in all three APL patients.

It appears that, in APL, translocation breakpoints result in two different hybrid gene structures. A scheme which illustrates the expression of normal and fusion transcripts of the RARA and myl genes in APL is outlined in Fig. 9. In one, the RARA gene promoter is upstream of the myl gene. Transcriptional control by the RARA promoter element results in a hybrid mRNA consisting of exons 1 and 2 of the RARA gene, which is truncated downstream by the myl gene (RARA/myl or p1715 in this report). In the other, the myl gene promoter is upstream of the RARA gene. Transcriptional control of the myl gene results in a hybrid mRNA consisting of the 5' portion of the myl gene truncated downstream by exons 3 to 9 of the RARA gene. In this instance, the use of the two polyadenylation sites should not be affected by the translocation (the 4.2- and 3.2-kb or the 4.8- and 3.8-kb abnormal mRNAs found in Fig. 7, lanes 6 and 7, in this report or the fusion gene as reported by de The et al. [15]). Both the RARA and myl genes are expressed at high levels in hematopoietic cells, and both promoter elements apparently remain active after translocation.

### DISCUSSION

The translocation breakpoint t(15;17) of APL transcribes two different types of hybrid mRNA. We have identified and



FIG. 6. Southern blot analysis of 17 APL DNA samples with *myl* gene probe. Ten micrograms of each DNA sample was restriction digested with *Bgl*II (B2), *Bam*HI (Bm), *Hin*dIII (H3), or *Eco*RI (R1), electrophoresed on a 0.75% agarose gel, transferred to a Nytran membrane, and hybridized to an oligolabeled 1.2-kb probe as described for Fig. 5. (A) Samples 1 to 7 and 9 are from APL with the t(15;17) breakpoint, and sample 8 is from APL with a  $17q^-$  abnormality; H represents DNA isolated from human leukemia cell line HL-60. (B) DNA samples 1 through 9 were obtained from t(15;17)-positive APL. Sample 10 is the remission sample of patient 9. Rearranged bands are indicated by arrows.

characterized a cDNA (RARA/myl) encoded from the nonrandom translocation breakpoint t(15;17)(q22;q21) of APL. This cDNA represents the hybrid mRNA regulated by the promoter element of the RARA gene, which is different from the myl/RARA fusion gene reported by de The et al. (15). We have demonstrated that both RARA/myl and myl/RARA fusion transcripts are coexpressed in three different APL patients. This suggests that two different types of hybrid messages are transcribed following the t(15;17) translocation, one from each of the reciprocal translocations. This



FIG. 7. Northern blot analysis of poly(A) RNA isolated from different leukemia patients. In panels A and B, lanes 2 through 10 contain RNAs isolated from acute myelogenous leukemia (AML) samples. Lanes: 1, HL-60 RNA; 2, chronic lymphocytic leukemia; 3, acute lymphoblastic leukemia; 4, acute monoblastic leukemia (AML-M5); 5, acute myelomonocytic leukemia (AML-M4); 6 and 7, APL (AML-M3); 8, AML with maturation (M2); 9, AML without maturation (M1); 10, acute undifferentiated leukemia (AML-M0). Designations of different AML are according to the FAB (French-American-British) classification (2). Panel A shows hybridization to the <sup>32</sup>P-labeled PCR-amplified 1.2-kb probe of *myl* cDNA, and panel B shows hybridization to the pRA1 cDNA probe. Panel C is a repeated Northern blot of panel A. Samples 1, 2, 3, and 4 are the same samples as 10, 9, 6, and 5, respectively. The upper parts of each panel show the same filter hybridized to a  $\beta$ -2 microglobulin cDNA probe (47).

finding differs from those of previous studies on other translocation breakpoints related to hematologic neoplasia, where only one of the translocated genes was transcriptionally active and only one type of hybrid mRNA was found encoded from the breakpoint region (18, 21, 25, 40, 45).

Molecular pathogenesis of t(15;17) translocation breakpoint in APL. RA is a morphogen, as demonstrated in chick limb experiments (7). Since RA can interact directly with RARA (42), RARA may be the key element in regulating RA concentration in the nucleus to achieve differentiation and development. In APL, the translocation breakpoint splits exons I and II from exons III to IX of the RARA gene.

The fusion cDNA RARA/myl consists of the first and second exons of the RARA gene. Recently, alternative splicing of the mouse RARA gene has been found, and several different cDNA molecules with different 5' end (exons I and II) sequences have been isolated (31). This finding indicates that exons I and II of RARA are important for a specific function. The first 59 amino acids of the predicted fusion protein were derived from the RARA exon II. This portion of the protein is believed to be involved in transactivation and target gene specificity (43). The finding of an additional 8-bp sequence in the p1715 cDNA as a result of alternative splicing is particularly interesting. We have shown that RARA/myl fusion transcripts with and without the 8-bp sequence exist in the APL RNA sample. Thus, RARA/myl with the eight additional nucleotides will be translated into a short fusion protein with a molecular weight of 18,500 due to a switch of the reading frame. RARA/myl without the 8-bp sequence would be able to encode a polypeptide of 382 amino acids with a molecular weight of about 40,000. Recently, we have identified two different myl cDNAs with additional 29- and 640-bp sequences (unpublished data) at nucleotide 1568 (Fig. 3). These additional sequences also result in an early translation stop of the myl mRNA. Alternative splicing of the myl gene appears to be a complicated process. We have now identified 20 cDNA clones of myl. Characterization of these clones and the myl genomic DNA will help us understand the mechanisms of alternative splicing of the myl gene. We found several cysteine-rich regions between amino acids 10 and 44, 82 and 105, and 142 and 180 (Fig. 3) which resemble a zinc finger motif found in a new family of DNA-binding proteins (20). In addition, a putative leucine zipper sequence was found between amino acids 164 and 200 (Fig. 3). On the basis of these findings, it was postulated that myl may be a transcription factor (16, 27). The biological function of myl is currently unknown; however, the extensive alternative splicing mechanisms suggest an important functional gene.

The hybrid myl/RARA mRNA detected in this report and by others (13, 15, 34) consists of exons III to IX of the RARA gene and contains the complete DNA- and RA-binding domains (22, 37). The fusion transcript myl/RARA can be translated into a polypeptide with a molecular weight of about 83,000. Approximately 200 amino acids on the NH<sub>2</sub>terminal end are derived from the myl protein. Since the DNA- and RA-binding domains remained intact, replacing the NH<sub>2</sub> terminal of RARA with a much larger peptide may have altered the properties of RAR function and may result in its becoming oncogenic. The tumorigenic potential of the myl/RARA cDNA will be investigated in the future to elucidate this possibility.

The most important issue is the pathogenic events that lead to the leukemogenesis of APL. APL is a clonal proliferation of promyelocytes, and the t(15;17) translocation is a consistent feature of this disease. Recently, all-*trans* RA has proved capable of producing complete remission in a significant number of APL patients (9, 12, 26). The interpretation of this clinical observation could be explained if the t(15;17)translocation breakpoint in APL partially impairs the normal function of RARA, because translocation results in the production of a nonfunctional RARA. When APL patients



FIG. 8. Detection of fusion transcripts RARA/myl and myl/ RARA in APL. Primers R5 and D4 (nucleotides 258 to 278 and 869 to 887 [Fig. 2]) are designed to preferentially amplify the RARA/myl mRNA. Primers 15U1 (nucleotides 894 to 912 [Fig. 3]) and R2 (nucleotides 1288 to 1308 of RARA [22]) are designed to preferentially amplify the myl/RARA mRNA. The fusion transcripts were amplified by reverse transcriptase-PCR as described in Materials and Methods. (A) Two DNA fragments of 770 and 630 bp (0.77 and 0.63 bands) were amplified in all three APL samples by using primers set R5/D4. A single band of 1.2 kb (1.20) was amplified in the three APL samples by using primer set 15U1/R2. M, HindIIIdigested lambda DNA size marker. (B) Sequence analysis of the PCR-amplified DNA fragments. Internal primers R1 (nucleotides 443 to 467 [Fig. 2]) and R4 (nucleotides 446 to 465 of RARA [22]) were used to sequence the fusion junctions of RARA/myl and myl/RARA, respectively. The exact sites of the fusion junctions are indicated by arrows. As indicated, RARA/myl was sequenced from  $5' \rightarrow 3'$  and myl/RARA was sequenced from  $3' \rightarrow 5'$ .

are treated with all-trans RA, high concentrations of RA may overcome the RARA deficiency, possibly by saturating the RARA-binding sites and enabling the delivery of a higher concentration of RA into the nucleus. Recently it was demonstrated that cellular retinoic acid binding protein (CRABP) plays a role in mediating the RA concentration in cells (4). All-trans RA treatment of APL may have abolished the normal function of cellular RA-binding protein allowing an uncontrolled level of RA to be available in the nucleus. Differentiation of the proliferative promyelocytes in APL can then be achieved. Recently it was reported that the myl/RARA fusion protein can act as an RA-inducible transcription factor with transactivating properties different from those of RARA (16, 27, 36). From these studies, it was postulated that myl/RARA is a dominant negative oncogene product which inhibits expression of RA-responsive genes, thus interfering with promyelocyte differentiation in APL



FIG. 9. Schematic representation of the expression of genes involved in the translocation breakpoint t(15;17) in APL. Solid boxes indicate the DNA- and RA-binding domains of the RARA gene. Solid inverted arrowheads indicate the locations of the breakpoint sites of the RARA and *myl* cDNAs. Locations of the DNA- and RA-binding domains were determined as reported by Giguere et al. (22).

(16, 27). However, experimental data supporting this hypothesis is currently unavailable.

Our results demonstrate that two different types of hybrid mRNA are transcribed from the t(15;17) translocation breakpoint. Although the hybrid mRNAs transcribed from translocation breakpoints have been shown to have oncogenic potential (8, 25), we do not yet have any information on the oncogenic potential of the hybrid messages in APL. We have shown that the *myl* gene is expressed in all leukemia cells tested, and it has been proposed to encode a transcription factor (16, 17). It can be hypothesized that partial inactivation of this gene as a result of the translocation could also have deleterious effects on cell differentiation. Future studies on the functional role and the genomic organization of the *myl* gene in the t(15;17) translocation breakpoint will contribute to our understanding of APL pathogenesis.

#### ADDENDUM

After submission of this manuscript, Pandolfi et al. (36), Kakizuka et al. (27), and de The et al. (16) reported the characterization of the myl/RARA (or PML/RARA) fusion transcript and of the normal myl (or PML) cDNA.

### ACKNOWLEDGMENTS

This project was supported in part by American Cancer Society Grant MG-14, a Pennzoil Company grant, and a grant from The David Bruton, Jr. Charitable Trust Fund to K.S.C., by a Rockwell Foundation grant to S.A.S., and by NCI grant CA 39809-07 to E.J.F.

We are grateful for the technical assistance of Jingfang Lu and Cihui Zhu and to Garland Yee for oligonucleotide synthesis. We are also grateful to Cheryl Willis for her assistance in the preparation of this manuscript.

#### REFERENCES

- 1. Aviv, H., and P. Leder. 1972. Purification of biologically active globin mRNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA 69:1408–1412.
- Bennett, J. M., D. Catovsky, M. T. Daniel, G. Flandrin, D. A. G. Galton, H. R. Gralnick, and C. Sultan. 1976. Proposals for the classification of the acute leukemia (FAB co-operative group). Br. J. Haematol. 33:451–458.
- Borrow, J., A. D. Goddard, D. Sheer, and E. Soloman. 1990. Molecular analysis of acute promyelocytic leukemia breakpoint cluster region on chromosome 17. Science 249:1577-1580.

- Boylan, J. F., and L. J. Gudas. 1991. Overexpression of the cellular retinoic acid binding protein-1 (CRABP-1) results in a reduction in differentiation-specific gene expression in F9 teratocarcinoma cells. J. Cell Biol. 112:965–979.
- Brand, N. J., M. Petkovich, and P. Chambon. 1990. Characterization of a functional promoter for the human retinoic acid receptor-alpha (hRAR-α). Nucleic Acids Res. 18:6799–6806.
- Brand, N., M. Petkovich, A. Krust, P. Chambon, H. de The, A. Marchio, P. Tiollais, and A. Dejean. 1988. Identification of a second human retinoic acid receptor. Nature (London) 332:850– 853.
- Brickell, P. M., and C. Tickle. 1989. Morphogens in chick limb development. Bioessays 11:145-149.
- 8. Butturini, A., and R. P. Gale. 1990. Oncogenes and leukemia. Leukemia 4:138–160.
- Castaigne, S., C. Chomienne, M. T. Daniel, P. Ballerini, R. Berger, P. Fenaux, and L. Degos. 1990. All-trans retinoic acid as a differentiation therapy for acute promyelocytic leukemia and clinical results. Blood 76:1704–1709.
- Chang, K. S., J. M. Trujillo, T. Ogura, C. M. Castiplione, K. K. Kidd, S. Zhao, E. J. Freireich, and S. A. Stass. 1991. Rearrangement of the retinoic acid receptor gene in acute promyelocytic leukemia. Leukemia 5:200–204.
- Chang, K. S., J. M. Trujillo, W. C. Pugh, E. Freireich, and S. A. Stass. 1990. Developmental and differential regulations of human myeloperoxidase gene in leukemia cells. Leukemia 4:497– 501.
- Chomienne, C., P. Ballerini, N. Balitrand, M. T. Daniel, P. Fenaux, S. Castaigne, and L. Degos. 1990. All-trans retinoic acid in acute promyelocytic leukemias. II. In vitro studies: structurefunction relationship. Blood 76:1710–1717.
- 13. Chomienne, C., P. Ballerini, N. Balitrand, M. E. Huang, I. Krawice, S. Castaigne, P. Fenaux, P. Tiollais, A. Dejean, L. Degos, and H. de The. 1990. The retinoic acid receptor α gene is rearranged in retinoic acid-sensitive promyelocytic leukemias. Leukemia 4:802-807.
- Deaven, L. L., M. A. van Dilla, M. F. Bartholdi, A. V. Carrono, L. S. Cram, J. C. Fuscoe, J. W. Gray, C. E. Hildebrand, R. K. Moyzis, and J. Perlman. 1986. Construction of human chromosome-specific DNA libraries from flow-sorted chromosome. Cold Spring Harbor Symp. Quant. Biol. 51:159–168.
- de The, H., C. Chomienne, M. Lanotte, L. Degos, and A. Dejean. 1990. The t(15;17) translocation of acute promyelocytic leukemia fuses the retinoic acid receptor α gene to a novel transcribed locus. Nature (London) 347:558-561.
- 16. de The, H., C. Lavau, A. Marchio, C. Chomienne, L. Degos, and A. Dejean. 1991. The PML-RARA fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. Cell 66:675–684.
- 17. Dobrovic, A., K. J. Trainor, and A. A. Morley. 1988. Detection of the molecular abnormality in chronic myeloid leukemia by use of the polymerase chain reaction. Blood 72:3063-3065.
- Fainstein, E., C. Marcelle, A. Rosner, E. Cenaci, R. P. Gale, O. Dreazen, S. D. Smith, and C. M. Croce. 1987. A new fused transcript in Philadelphia chromosome positive acute lymphocytic leukemia. Nature (London) 330:386–388.
- 19. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6–13.
- Freemont, P. S., I. M. Hanson, and J. Trowsdale. 1991. A novel cysteine-rich sequence motif. Cell 64:483–484.
- Gale, R. P., and E. Canaani. 1984. An 8-kilobase abl RNA transcript in chronic myelogeneous leukemia. Proc. Natl. Acad. Sci. USA 81:5648-5652.
- 22. Giguere, V., E. S. Ong, P. Sequi, and R. N. Evan. 1987. Identification of a receptor for the morphogen retinoic acid. Nature (London) 330:624–629.
- 23. Gubler, U., and B. J. Hoffman. 1983. A simple and very efficient method for generating cDNA libraries. Gene 25:263-268.
- 24. Gylensten, U. B., and H. A. Erlich. 1988. Generation of singlestranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA-DQA locus. Proc. Natl. Acad. Sci. USA 85:7652-7656.

- Holt, J. T., C. C. Morton, A. W. Nienhuis, and P. Leder. 1987. Molecular mechanism of hematological neoplasms, p. 347–376. *In* G. Stamatoyannopoulos, A. W. Nienhuis, P. Leder, and P. W. Majerus (ed.), The molecular basis of blood diseases. W. B. Saunders Co., Philadelphia.
- Huang, M., Y. D. Ye, B. R. Chen, J. R. Chai, J. X. Lu, L. Zhoa, L. J. Eu, and Z. Y. Wang. 1988. Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. Blood 72:567– 572.
- Kakizuka, A., W. H. Miller, K. Umesono, R. P. Warrell, S. R. Frankel, V. V. V. S. Murty, E. Dmitrovsky, and R. M. Evans. 1991. Chromosomal translocation t(15;17) in human acute leukemia fuses RARA with a novel putative transcription factor, PML. Cell 66:663-674.
- Kawasaki, E. S., S. S. Clark, M. Y. Coyne, S. D. Smith, R. Champlin, O. N. Witte, and F. P. McCormick. 1988. Diagnosis of chronic myeloid and acute lymphocytic leukemias by detection of leukemia-specific mRNA sequences amplified in vitro. Proc. Natl. Acad. Sci. USA 85:5698-5702.
- 29. Koeffler, H. P. 1983. Induction of differentiation of human acute myelogenous leukemia cells. Blood 62:709–721.
- Krust, A., P. Kastner, M. Petkovich, A. Zelant, and P. Chambon. 1989. A third human retinoic acid receptor, HRAR-gamma. Proc. Natl. Acad. Sci. USA 86:5310-5314.
- Leroy, P., A. Krust, A. Zelent, C. Mendelsohn, J. M. Garnier, P. Kastner, A. Dierich, and P. Chambon. 1991. Multiple isoforms of the mouse retinoic acid receptor α are generated by alternative splicing and differential induction by retinoic acid. EMBO J. 10:59–69.
- 32. Mangelsdorf, D. J., E. S. Ong, J. A. Dyck, and R. M. Evans. 1990. Nuclear receptor that identifies a novel retinoic acid response pathway. Nature (London) 345:224–229.
- Mattei, M. G., M. Petkovich, J. F. Mattei, N. Brand, and P. Chambon. 1988. Mapping of the human retinoic acid receptor to the Q21 band of chromosome 17. Hum. Genet. 80:186–188.
- 34. Miller, W. H., Jr., R. P. Warrell, Jr., S. R. Frankel, A. Jakubowski, J. L. Gabrilove, J. Muindi, and E. Dmitrovsky. 1990. Novel retinoic acid receptor-α transcripts in acute promyelocytic leukemia responsive to all-trans retinoic acid. J. Natl. Cancer Inst. 82:1932–1933.
- Murray, S. S., L. L. Deaven, D. W. Burton, D. T. O'Connor, P. L. Mellon, and L. J. Deftos. 1987. The gene for human chromogranin A (CgA) is located on chromosome 14. Biochem. Biophys. Res. Commun. 142:141-146.
- 36. Pandolfi, P. P., F. Grignani, M. Alcalay, A. Mencarelli, A. Biondi, F. LoCoco, F. Grignani, and P. G. Pelicci. 1991. Structure and origin of the acute promyelocytic leukemias myl/RARA cDNA and characterization of its retinoid-binding and transactivation properties. Oncogene 6:1285-1292.
- Petkovich, M., N. J. Brand, A. Krust, and P. Chambon. 1987. A human retinoic acid receptor which belongs to the family of nuclear receptors. Nature (London) 330:444–450.
- Rodaway, A. R. F., C. G. Teahan, C. M. Casimir, A. W. Segal, and D. L. Bentley. 1990. Characterization of the 47-kilodalton autosomal chronic granulomatous disease protein: tissue-specific expression and transcriptional control by retinoic acid. Mol. Cell. Biol. 10:5388–5396.
- Rowley, J. D. 1984. Biological implications of consistent chromosome rearrangements in leukemia and lymphoma. Cancer Res. 44:3159–3168.
- Saito, H., A. C. Hayday, K. Wiman, K. S. Wyward, and S. Tonegawa. 1983. Activation of the cMYC gene by translocation: a model for translational control. Proc. Natl. Acad. Sci. USA 80:7476-7480.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, p. 846–851. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- 42. Sani, B. P., R. K. Singh, L. Reddy, and M. P. Gaub. 1990. Isolation, partial purification and characterization of nuclear retinoid acid receptors from chick skin. Arch. Biochem. Biophys. 283:107-113.
- Sawyers, C. L., C. T. Denny, and O. N. Witte. 1991. Leukemia and the disruption of normal hematopoiesis. Cell 64:337–350.

810 CHANG ET AL.

- 44. Sherman, M. L. (ed.) 1986. Retinoids and cell differentiation, p. 161–186. CRC Press, Inc., Boca Raton, Fla.
- 45. Shtivelman, E., B. Lifshitz, R. P. Gale, B. A. Roe, and E. Canaani. 1986. Alternative splicing of RNAs transcribed from the human abl gene and from the bcr/abl fused gene. Cell 47:277-284.
- Strickland, S., and V. Mahdavi. 1978. Induction of differentiation in teratocarcinoma stem cells by retinoic acid. Cell 15:393– 401.
- 47. Suggs, S. V., R. B. Wallace, T. Hirose, E. H. Kawashima, and K. Itakura. 1982. Use of synthetic oligonucleotides as hybridization probes: isolation of cloned cDNA sequence for human β2-microglobulin. Proc. Natl. Acad. Sci. USA 78:6613– 6617.
- 48. Zagursky, R., N. Baumeister, N. Lomax, and M. Berman. 1985. Rapid and easy sequencing of large linear double-stranded DNA and supercoiled plasmid DNA. Gene Anal. Tech. 2:89–94.