that a kinase cascade effected BRP phosphorylation. At least potentially, BRP can represent a direct substrate for the cyclic AMP–dependent protein kinase, since BRP purified by acid solubilization, which destroys endogenous kinase activity, serves as direct substrate for the catalytic subunit of the cyclic AMP–dependent protein kinase. Phosphorylation by an isolated protein kinase in vitro of course does not prove that it serves as a substrate in vivo. For example, the major H1 histone isotype serves as an in vitro substrate but exhibits no cyclic AMP–dependent phosphorylation in the intact cell. Furthermore, it is not unequivocally established that the catalytic subunit of the cyclic AMP–dependent kinase can translocate to the nucleus (*11*, *17*).

The determination of the primary sequence of BRP and characterization of the phosphorylated site (or sites) requires a purification of this low-abundance protein, which is present at approximately 1 percent of the mass of the major H1 histone isotype. This protein is clearly distinct from the developmentally regulated histone isotype referred to as $H1^0$ (*18*).

These data are most compatible with a model in which the holoenzyme resides in, or the activated catalytic subunit of the cyclic AMP–dependent protein kinase translocates to, the nucleus and phosphorylates at least one minor basic chromosomal protein, which regulates the transcription of specific genes. In this model, cyclic AMP would exert a transcriptional effect, as it does in prokaryotes (*1*), but effects of the cyclic AMP–receptor complex on gene transcription would thus be indirect, mediated by phosphorylation of chromatin-associated proteins. Alternatively, a "kinase cascade" could be involved. It is possible that the content, genomic distribution, or covalent modification of BRP (or all) directly or indirectly determines the altered binding of RNA polymerase leading to increased transcription of a restricted set of loci, including the prolactin gene, in a fashion analogous to the effects of cyclic AMP–CRP binding to specific sites in the bacterial genome. Such a model can potentially be tested with current biochemical and molecular biological techniques.

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19. We thank Rodrigo Franco and Marian Waterman for discussion and suggestions regarding the manuscript and Susan Taylor for the cyclic AMP–dependent protein kinase. Supported by grants from the National Institutes of Health.

17 September 1982

Chromosomal Assignment of the Endogenous Proto-Oncogene C-*abl*

Abstract. *Abelson murine leukemia virus (A-MuLV) is a replication-defective retrovirus that transforms lymphocytes of the B-cell lineage. This virus is a recombinant between the parental Moloney murine leukemia virus and a cellular gene termed C-*abl. *By analysis of a series of mouse × Chinese hamster hybrid cell lines containing various mouse chromosomes, we have mapped the C-*abl *gene to mouse chromosome 2.*

Abelson murine leukemia virus (A-MuLV) is one of many replication-defective transforming retroviruses. Like most such viruses, A-MuLV is a hybrid of elements of a replication-competent leukemia virus and sequences derived from the normal mouse genome. These sequences, contiguous in the virus, are spaced apart by intervening sequences in the mouse genome, spanning more than 20 kilobases (kb) of DNA. The mouse gene has been termed C-*abl*, and its viral homolog V-*abl* (*1*). A-MuLV is unusual in its tissue specificity; it transforms fibroblasts and immature lymphocytes of the B-cell lineage, but not cells of the T-

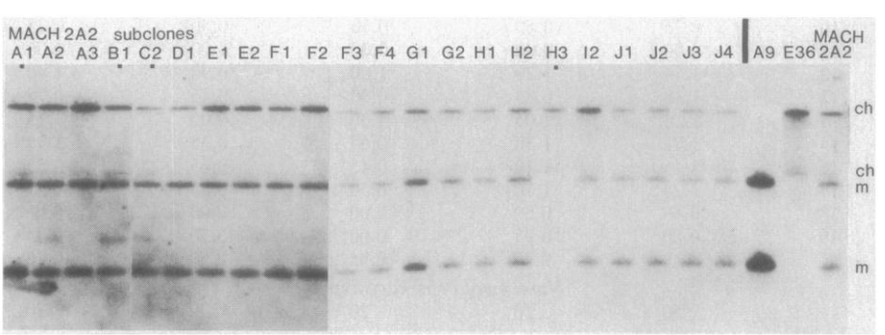

Fig. 1. Detection of C-*abl* DNA fragments in mouse (A9), Chinese hamster (E36), and hybrid cell genomic DNA. DNA digested with Hind III enzyme was fractionated by electrophoresis in agarose, transferred to nitrocellulose, and hybridized with pAB3sub3 DNA isotopically labeled by nick-translation in vitro. The four subclones of hybrid MACH 2A2 subjected to detailed karyotic and isoenzyme analysis (Table 2) are indicated (■); *ch*, Chinese hamster; *m*, mouse.

0036-8075/82/1224-1317$01.00/0

cell lineage (*2*). Its transforming ability appears to reside in the V-*abl* gene inasmuch as mutations of that gene alter or abolish the transforming activity of A-MuLV (*3–5*). A polypeptide with a molecular weight of about 150,000 that is antigenically cross-reactive with the V-*abl* gene product has been found in normal murine lymphoid cells (*6*). RNA transcripts from C-*abl* are found in many cell types and at especially high levels in established fibroblastic cell lines (*7*). To characterize the C-*abl* gene further, we have now determined its chromosomal location, using a V-*abl* DNA probe to screen recombinants (Southern blots) (*8*) of genomic DNA from mouse × Chinese hamster somatic cell hybrids.

Table 1. Hybrid cell panel tested for murine C-*abl* gene.

Hybrid cell line	Mouse chromosomes retained*	C-*abl*†
BEM 1-6	1,*2*,3,4,6,8,9,10,12,13,14,15,16,17,18,*19*,X	+
BEM 1-4	1,*2*,3,5,6,8,10,12,13,14,15,16,17,18,*19*,X	+
MACH 7A13-3B3	*2*,5,7,9,12,13,14,15,16,17,18,*19*	+
MACH 4A63	*2*,7,12,13,15,16,17,18,*19*	+
MACH 4A64-A1	1,*2*,7,12,15,17,*19*	+
MACH 4B31-AZ3	*2*,7,8,12,15,16,17,*19*	+
MACH 2A2	1,*2*,3,4,6,7,8,9,10,12,13,14,15,16,17,18,*19*,X	+
MAE 28	12,X	−
MAE 32	16,X	−
ECm4e	14,15	−
R44-1	17	−

*The detailed characterization of this panel of cell lines, by a combination of karyotypic and isoenzyme analyses, has been described (*9*). Chromosomes shown as retained were found at frequencies greater than 0.1 per cell. †Genomic DNA was scored by Southern blotting for the presence (+) or absence (−) of mouse-specific DNA fragments reactive with the isotopically labeled pAB3Sub3 probe.

Table 2. Detailed analysis of MACH 2A2 and subclones.* Subclones of hybrid line MACH 2A2 were generated by plating cells at limiting dilution in tissue culture flasks (25 cm^2), in antibiotic-free Dulbecco's modified essential medium, high glucose formulation, plus 10 percent fetal bovine serum (Gibco, Grand Island, New York). Individual colonies were picked using cloning rings. Samples of the same cell population from which genomic DNA was isolated for Southern blotting were subjected to karyotypic analysis by the combined trypsin–Giemsa and Hoechst 33258 staining techniques (*15*) and were assayed for the presence of isoenzyme markers previously assigned to mouse chromosomes 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 17, and 19 (*16, 17*). The number shown is the mean number of copies of the chromosome found per cell by karyotypic analysis.

Mouse chromosome	MACH hybrid cell line†				
	2A2	2A2-A1	2A2-B1	2A2-C2	2A2-H3
1	0.26	0.30	0.80	0.50	0.65
2	1.08	0.85	1.10	0.60	0.00
3	0.68	0.70	0.75	0.45	0.75
4	0.15	0.15	0.35	0.10	0.15
5	0.00	0.00	0.00	0.00	0.00
6	0.34	0.35	0.60	0.00	0.80
7	0.82	0.70	0.45	0.55	0.85
8	0.26	0.80	0.60	0.40	0.90
9	0.34	0.55	0.80	0.85	0.90
10	0.26	0.80	0.70	0.50	0.75
11	0.00	0.00	0.00	0.00	0.00
12	1.54	1.20	1.00	0.90	1.40
13	1.18	0.85	0.00	0.45	0.75
14	1.36	1.05	1.30	0.00	1.15
15	1.72	1.30	0.65	0.35	1.30
16	1.16	1.10	0.45	0.65	0.55
17	1.24	1.10	0.50	0.85	1.05
18	0.66	0.60	0.00	0.00	0.60
19	0.80	0.95	0.00†	0.50	0.95
X	0.40	0.70	0.35	0.60	0.70
	Number of cells karyotyped				
	50	20	20	20	20
	*Reaction with C-*abl *probe*				
	+	+	+	+	−

*This cell line expressed the mouse form of the isoenzyme marker glutamate oxalate transaminase-1 (E.C. 2.6.1.1), previously mapped to chromosome 19 (*16*), but contained no recognizable copies of the chromosome. In all other cases, results of isoenzyme and karyotypic analyses were concordant. †Scored as shown in Fig. 1.

Digestion of mouse genomic DNA with the restriction endonucleases Hind III or Xba I produced fragments reactive with the isotopically labeled V-*abl* DNA probe pAB3sub3 (*1*). Chinese hamster DNA likewise yielded fragments reactive with this probe, but these could be resolved from their mouse counterparts on the basis of size (*1*) (Fig. 1). This species-specific restriction fragment polymorphism could thus be used to detect the mouse C-*abl* gene in Southern hybridizations (blots) of DNA from mouse × Chinese hamster hybrid cell lines, and to resolve it from the Chinese hamster homolog present in the lines.

We previously developed a panel of such hybrid cell lines, carrying various limited sets of mouse chromosomes on a constant Chinese hamster background (*9*). Analysis of this panel by the Southern blot method suggested that the murine C-*abl* gene sequence was located on either chromosome 2 or chromosome 19 (Table 1). To localize C-*abl* to a single chromosome, subclones of hybrid MACH 2A2 were analyzed similarly (*8*) (Fig. 1). The one subclone that had lost the mouse C-*abl* gene and three of the 21 subclones that had retained it were analyzed in detail (Table 2). The three positive subclones had retained chromosome 2. The negative subclone, MACH 2A2 H3, had lost chromosome 2 and had retained chromosome 19. Therefore, C-*abl* could be assigned to chromosome 2.

This assignment raises the question of possible relationships among C-*abl*, the lymphocyte surface antigens Ly-6, Ly-8, and Ly-11, β_2-microglobulin, and susceptibility to radiation-induced leukemia (*Ril*-1), all of which genes have previously been mapped to chromosome 2 (*10, 11*). The polypeptides corresponding to Ly-6, Ly-8, Ly-11, and *Ril*-1 are unknown. Our finding thus emphasizes the importance of the biochemical analysis of these markers, and of the definition, at the DNA or protein level, of C-*abl*–associated polymorphisms that will allow its precise localization with respect to these other lymphocyte-specific genes on chromosome 2.

More generally, this mapping procedure should be applicable to other endogenous *onc* genes, both those defined by their presence in transforming viruses [for example, see (*12*)] and those defined by their direct transforming ability in DNA-mediated gene transfer systems (*13*). Such mapping data would provide a useful starting point for functional studies of the *onc* gene family.

Recent studies by Bodmer and Todaro (*14*) have shown that the human version

of C-*abl* maps to human chromosome 9. It is also known that the genes for the murine and human forms of adenylate kinase (AK-1) map to mouse chromosome 2 and to human chromosome 9, respectively. This may prove significant since the human AK-1 gene has been mapped regionally to the end of the long arm of human chromosome 9, and therefore the C-*abl* gene may be located close by. If this proves to be so, then one must be prepared to inquire whether the human chromosome 9/22 translocation (Philadelphia chromosome) which frequently involves the end 9q and which correlates positively with chronic myeloid leukemia (CML) may alter the expression of the human C-*abl* gene and in turn influence tumor progression in CML.

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18. This work was supported by grants GM-09966 (F.H.R.) and CA-14051 (D.B.) from the National Institutes of Health and by American Cancer Society grant VC-41 (D.B.).
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14 June 1982; revised 26 August 1982

Mouse c-myc Oncogene Is Located on Chromosome 15 and Translocated to Chromosome 12 in Plasmacytomas

Abstract. *Hybridization studies with viral oncogene probes indicate that c-myc, the cellular gene homologous to the transforming gene of avian myelocytomatosis virus, resides on mouse chromosome 15 and in many plasmacytomas is translocated to the antibody heavy chain gene locus on chromosome 12. The transcriptional orientation of the translocated c-myc sequence is opposite the orientation of the adjacent C_α gene that codes for the heavy chain of immunoglobulin A. The translocated c-myc sequence is not the same oncogene detected in murine plasmacytomas by the NIH-3T3 cell transformation assay.*

Characteristic chromosomal translocations are often associated with lymphoid tumors in both mice and humans (*1*). It has been postulated that these abnormalities may contribute to the generation of tumors by altering the expression of certain cellular genes, referred to as oncogenes (*1*). To further understand the role of translocations in tumorigenesis, it is important to clone the region of DNA containing the translocation. This has been accomplished with the use of murine plasmacytomas, which characteristically have a translocation of the distal part of chromosome 15 to chromosome 12 [t(12;15)] or 6 [t(6;15)] (*1*).

Plasmacytomas are tumors of antibody-producing plasma cells. In diploid antibody-producing cells, there are two chromosomal copies of each antibody gene family. Plasmacytomas, like normal antibody-producing cells, have one chromosome that is rearranged and expresses an antibody polypeptide (productive re-

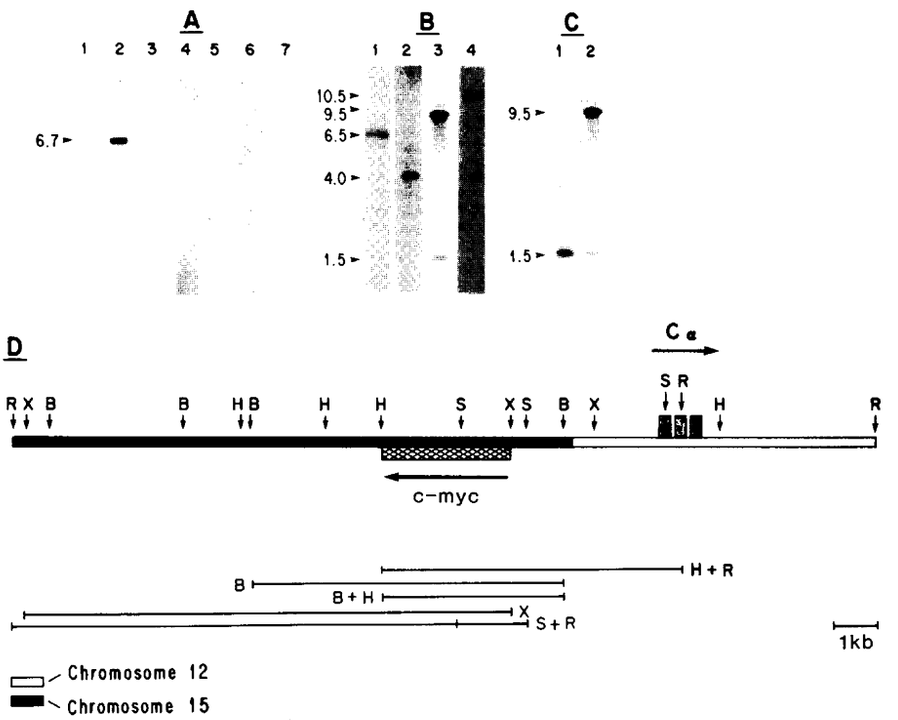

Fig. 1. Hybridization of the v-myc probe to Ch603α30 DNA. (A) Ch603α30 DNA cleaved with Bam HI (lanes 1 to 3) or Hind III (lanes 4 to 7) was subjected to electrophoresis, hybridized on nitrocellulose with the following viral oncogene probes: lane 1, v-fes; lane 2, v-myc; lane 3, v-yes; lane 4, v-src; lane 5, v-erb; lane 6, v-myb; and lane 7, v-abl. (B) Hybridization of Ch603α30 DNA digested with Hind III (lane 1), Bam HI plus Hind III (lane 2), Sst I plus Eco RI (lane 3), and Xba I (lane 4) with the v-myc probe. (C) Ch603α30 DNA digested with Sst I plus Eco RI in both lanes; lane 1 was hybridized with the 3.6-kb Sal I fragment from the v-myc clone that contains sequences from the 5′ part of the gene; lane 2 was hybridized with the 2.2-kb Sal I fragment from the v-myc gene that contains the 3′ part of the gene. [The v-myc probe contains a 1.5-kb Pst I fragment from MCV38 inserted in the Pst I site of pBR322 (*8*).] (D) Map of Ch603α30 DNA. Arrows indicate the 5′ to 3′ direction of transcription for the C_α gene segment and the c-myc sequences. Hatched area denoted "c-myc" shows the region which hybridizes with the v-myc probe, as indicated by the lines below the map showing the fragments from each digest which hybridize to the probe. R indicates Eco RI; B indicates Bam HI; H indicates Hind III, S indicates Sst I, and X indicates Xba I.

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