JOURNAL OF VIROLOGY, Sept. 1989, p. 3678–3682
0022-538X/89/093678-05$02.00/0

Vol. 63, No. 9

Identification of Two Murine Loci Homologous to the v-*cbl* Oncogene

DANIEL C. REGNIER,[1] CHRISTINE A. KOZAK,[2] DAVID M. KINGSLEY,[3] NANCY A. JENKINS,[3] NEAL G. COPELAND,[3] WALLACE Y. LANGDON,[1,4†] AND HERBERT C. MORSE III[1]*

Laboratory of Immunopathology[1] and Laboratory of Molecular Microbiology,[2] National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892; Mammalian Genetics Laboratory, Bionetics Research Inc.–Basic Research Program, National Cancer Institute–Frederick Cancer Research Facility, Frederick, Maryland 21701[3]; and Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia[4]

Received 17 April 1989/Accepted 1 June 1989

The virally transduced oncogene v-*cbl* transforms fibroblasts in vitro and induces early B-cell-lineage lymphomas in vivo. A series of probes derived from a molecular clone of v-*cbl* were used to map related sequences in the mouse genome. Analyses of Chinese hamster × mouse somatic-cell hybrids showed that two related genes, *cbl-1* and *cbl-2*, were located on chromosomes 6 and 9, respectively. Restriction enzyme studies of DNA from hybrid cells containing either chromosome 6 or 9 suggested that *cbl-1* resembles v-*cbl* and may be a processed gene, whereas *cbl-2* has a complex genomic structure. Analyses of *Mus domesticus*/*M. spretus* interspecific backcross mice showed that *Cbl-1* maps between the immunoglobulin kappa light chain and T-cell receptor beta chain loci and that *Cbl-2* is tightly linked to *Thy-1*.

Acutely transforming retroviruses contain nonviral sequences within their genomes that represent portions of normal cellular genes (c-*onc* genes) involved in regulation of cell growth and differentiation. Studies of these virally transduced sequences have resulted in the identification of numerous oncogenes and have provided evidence that deregulated expression of these genes is associated with neoplastic transformation (1).

We recently described a new, virally transduced oncogene, termed v-*cbl*, that transforms fibroblasts in vitro and induces pre-B-cell lymphomas and occasional myeloid tumors in vivo (12, 14). Nucleotide sequence analyses showed no significant homology between v-*cbl* and previously described oncogenes, but the deduced amino acid sequence was related to that of the yeast transcriptional activator GCN4 in both the DNA-binding and activation domains (14). We report here the use of somatic cell hybrids and Mendelian genetics to localize mouse cellular sequences related to v-*cbl*.

MATERIALS AND METHODS

Mice. Inbred strains of mice were obtained from the colonies of the National Institutes of Health or from Jackson Laboratory, Bar Harbor, Maine. Backcross progeny from (C57BL/6J × *Mus spretus*)F_1 × C57BL/6J crosses (3) were generated at the National Cancer Institute–Frederick Cancer Research Facility. The *M. spretus* (Spain) mice were a gift from E. Eicher (Jackson Laboratory).

Somatic-cell hybrids. Hybrids used in the linkage analysis were produced by fusion of the Chinese hamster cell line E36 with cells of three different inbred mouse strains, BALB/c, A/HeJ and NFS.AKR-*Akv-2* (11).

Southern blot analysis. High-molecular-weight DNA prepared from mouse tissue or cell lines was digested with appropriate restriction endonucleases, separated on horizontal agarose gels, and transferred to nitrocellulose or nylon by using standard techniques. Prehybridization and hybridization conditions were as described elsewhere (11; D. M. Kingsley, N. A. Jenkins, and N. G. Copeland, submitted for publication). Fragments of nonviral v-*cbl* sequences cloned in pUC18 and used as probes labeled by random primer extension are shown in Fig. 1 and included (i) a 498-base-pair *Sac*I-*Eco*RI fragment from the 5′ end of this region (probe 1), (ii) a 418-base-pair *Eco*RI-*Pst*I fragment from sequences immediately 3′ of the *Sac*I-*Eco*RI probe (probe 2), and (iii) a 100-base-pair *Pst*I-*Pst*I fragment from the 3′ end of v-*cbl* (probe 3).

RESULTS

Assignment of v-*cbl*-related sequences to chromosomes 6 and 9. Mouse sequences homologous to v-*cbl* were initially mapped by the analysis of somatic-cell hybrids. Experiments with the *Eco*RI-*Pst*I fragment of v-*cbl* (probe 2) as a hybridization probe showed that *Eco*RI-digested hamster DNA contained a single cross-reactive fragment of 9.4 kilobases (kb), whereas mouse DNA restricted with the same enzyme contained three fragments of 6.5, 3.6, and 1.4 kb (Fig. 2, lane a). DNA from 22 hybrid lines was examined for the presence of the mouse-specific fragments. Unexpectedly, some lines (7 of 22) contained only the 3.6-kb fragment, while 4 contained the 6.3- and 1.4-kb but not the 3.6-kb fragment; 9 of the remaining 11 lines contained all three fragments, whereas the other 2 had none (Fig. 1, lanes b through f). These results indicated that the mouse genome contained two sequences related to v-*cbl*. Analyses of the chromosome contents of these hybrids showed that these sequences were located on chromosomes 6 and 9; the 3.6-kb hybridizing fragment was contained on chromosome 6 (Table 1), and the 6.5- and 1.4-kb fragments mapped to chromosome 9 (Table 2). We have designated the chromosome 6 sequence *Cbl-1* and the chromosome 9 sequence *Cbl-2*.

The demonstration that two chromosomes contained v-*cbl*-related sequences could mean either that the transduced material contained sequences from two unrelated genes, as described for a number of other acutely transforming retro-

* Corresponding author.
† Present address: Division of Human Immunology, Institute of Medicine and Veterinary Science, Adelaide, South Australia, Australia.

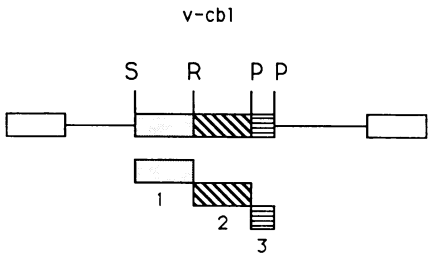

FIG. 1. Probes derived from v-*cbl* for hybridization studies. Three regions of v-*cbl* were subcloned in pUC18 and designated probes 1, 2, and 3 (see Materials and Methods). S, *Sac*I; R, *Eco*RI; P, *Pst*I.

viruses (4, 10, 16), or that two related copies of a single sequence were present at different locations in the genome, a possibility with multiple precedents (for example, see references 2 and 19). To discriminate between these possibilities, DNA from hybrids containing either chromosome 6 or chromosome 9 was digested with *Eco*RI and hybridized with the three probes from v-*cbl* described in Fig. 1 (data not shown). These analyses showed that chromosome 6 as well as chromosome 9 contained sequences homologous to both the 5′ and 3′ ends of the transduced v-*cbl* gene, indicating that these chromosomes contained related genes.

Structural analyses of c-*cbl* genes. To evaluate the structural relationships between v- and c-*cbl* sequences, the organization of the two cellular genes was examined by using enzymes that generate characteristic fragments from v-*cbl*. DNAs from somatic-cell hybrids digested with *Sac*I and *Pst*I were hybridized with probe 1 or probe 2. Hybrids that contained chromosome 6 but not chromosome 9 exhibited a single fragment of 0.8 kb that was reactive with both probes and corresponded to the size of the internal *Sac*I-*Pst*I fragment of v-*cbl*. Digestion of the same hybrid cell DNA with *Eco*RI and *Pst*I generated a 0.4-kb band hybridizing with probe 2 from v-*cbl* that, again, was identical in size to the *Eco*RI-*Pst*I fragment of virally transduced sequences.

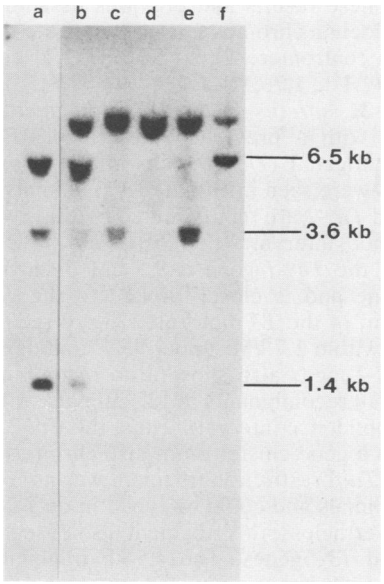

FIG. 2. Southern blot hybridization of DNAs from hamster × mouse somatic-cell hybrids. DNAs were digested with *Eco*RI and hybridized with probe 2 from v-*cbl*. Lanes: a, BALB/c liver; b, hybrid containing mouse chromosomes 6 and 9; c and e, hybrids containing mouse chromosome 6 but not chromosome 9; d, hybrid containing neither mouse chromosome 6 nor chromosome 9; f, hybrid containing mouse chromosome 9 but not chromosome 6. The weakly hybridizing 1.4-kb band in lane f is much more apparent on longer exposures.

TABLE 1. Analysis of concordance between specific mouse chromosomes and the presence of *Cbl-1* in hamster × mouse somatic cell hybrids

Mouse chromosome	No. of hybrids scored for *Cbl-1*/chromosome				% Discordance
	+/+[a]	−/−	+/−	−/+	
1	12	2	4	3	33.3
2	13	1	3	5	36.4
3	6	0	5	4	60.0
4	7	4	9	2	50.0
5	2	2	14	3	81.0
6	16	6	0	0	0.0
7	14	1	2	5	31.0
8	8	5	6	1	35.0
9	9	2	7	4	50.0
10	2	6	14	0	63.6
11	0	4	10	0	71.4
12	7	3	2	2	28.6
13	12	1	2	5	35.0
14	3	5	12	1	61.9
15	10	0	0	4	28.6
16	6	3	7	3	52.6
17	13	0	3	6	40.9
18	10	3	5	3	38.1
19	8	5	7	1	38.1
20	12	3	4	3	31.8

[a] Presence (+) or absence (−) of a 3.6-kb *Eco*RI fragment hybridization with probe 2 from v-*cbl* with respect to the presence or absence of a particular mouse chromosome among 22 hybrids examined.

TABLE 2. Analysis of concordance between specific mouse chromosomes and the presence of *Cbl-2* in hamster × mouse somatic cell hybrids

Mouse chromosome	No. of hybrids scored for *Cbl-2*/chromosome				% Discordance
	+/+[a]	−/−	+/−	−/+	
1	9	3	3	6	42.9
2	12	3	1	6	31.8
3	6	3	2	4	40.0
4	8	8	5	1	27.3
5	5	9	7	0	33.3
6	9	2	4	7	50.0
7	13	3	0	6	27.3
8	7	6	5	2	35.0
9	13	9	0	0	0.0
10	2	9	11	0	50.0
11	0	7	7	0	50.0
12	4	2	3	5	57.1
13	11	3	0	6	30.0
14	3	8	9	1	47.6
15	7	0	0	7	50.0
16	6	6	4	3	26.8
17	12	2	1	7	36.4
18	9	5	3	4	33.3
19	6	6	6	3	42.9
20	8	2	5	7	54.5

[a] Presence (+) or absence (−) of 6.5- and 1.4-kb fragments hybridizing with probe 2 from v-*cbl* with respect to the presence or absence of a particular mouse chromosome among 22 hybrids examined.

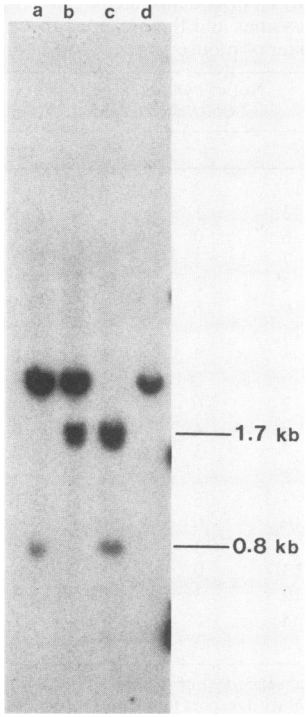

FIG. 3. Southern blot hybridization of DNAs from hamster × mouse somatic cell hybrids. DNAs were digested with *Sac*I and *Pst*I and hybridized with probe 2 from v-*cbl*. Lanes: a, hybrid containing mouse chromosome 6 but not chromosome 9; b, hybrid containing mouse chromosome 9 but not chromosome 6; c, BALB/c liver; d, E36 hamster cells.

These data suggested that *Cbl-1* was structurally similar to v-*cbl* and, apparently lacking introns, represented a processed gene. This suggestion is strongly supported by studies of genomic clones containing v-*cbl*-related sequences that have restriction endonuclease maps with regions identical to those in v-*cbl* (M. Shapiro, D. Regnier, W. Langdon, and H. Morse, unpublished observations).

Identical analyses of a hybrid that retained *Cbl-2* on chromosome 9 but lacked chromosome 6 produced a different result. DNA digested with *Sac*I and *Pst*I and hybridized to the v-*cbl* probes revealed two fragments of 1.65 and 1.75 kb with a total size of 3.4 kb, rather than the single 0.8-kb fragment characteristic of v-*cbl* and *Cbl-1* (Fig. 3, lane b). In addition, DNA digested with *Eco*RI and *Pst*I and hybridized with the probes exhibited two fragments of 1.7 and 1.2 kb, totalling 2.9 kb, rather than the 0.4-kb fragment described above for v-*cbl* and *Cbl-1*. Thus, unlike *Cbl-1*, *Cbl-2* does not resemble v-*cbl*, and the larger size and multiplicity of fragments detected with various probes suggest a more complex genomic organization. Restriction endonuclease maps of genomic clones that hybridize with probes derived from v-*cbl* support this suggestion (M. Shapiro, unpublished observations).

Fine structure mapping of *Cbl-1* and *Cbl-2*. To localize c-*cbl* sequences more precisely on chromosomes 6 and 9, DNA samples from C57BL/6 and *M. spretus* (Spain) mice were examined by Southern blot analysis for polymorphisms in restriction fragments that hybridized with probe 2. In samples digested with *Taq*I, this probe detected bands of 7.7, 3.1, and 2.1 kb in C57BL/6J DNA and bands of 7.7, 6.0, 4.5, and 3.3 kb in *M. spretus* DNA (Fig. 4). The 4.5-kb band was not seen in all *M. spretus* animals (see below).

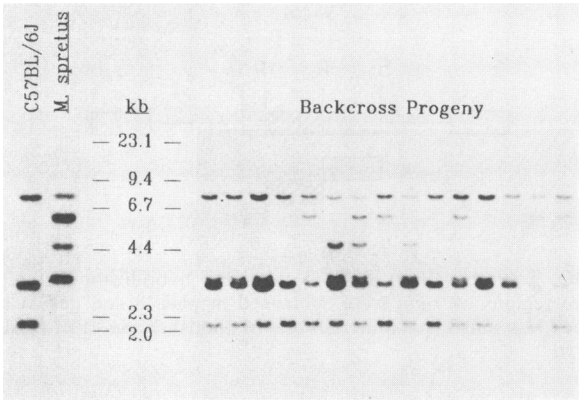

FIG. 4. Inheritance of *Taq*I RFLPs in interspecific backcross animals. DNA from the indicated animals was digested with *Taq*I restriction endonuclease, separated by agarose gel electrophoresis, transferred to nylon membrane, and hybridized to probe 2 from v-*cbl*. The backcross progeny represent individual animals from matings between (C57BL/6J × *M. spretus*)F_1 females and C57BL/6J males.

The distribution of *M. spretus*-specific restriction fragments was analyzed in progeny from a cross (3) between (C57BL/6J × *M. spretus*)F_1 females and C57BL/6J males. The presence or absence of the 6.0-kb restriction fragment was highly correlated with the segregation of *Msp*I restriction fragment length polymorphisms (RFLPs) for the T-cell receptor beta chain (*Tcrb*) and immunoglobulin kappa chain (*Igk*) genes (Fig. 5A), two chromosome 6 genes previously typed on the interspecific backcross panel (Kingsley et al., submitted). These results and previous assignments of the *Tcrb* and *Igk* loci on chromosome 6 (7) indicate the following gene order: centromere–*Tcrb*–(5.2 ± 2.1 centimorgans [cM])–*Cbl-1*–(2.1 ± 1.7 cM)–*Igk*.

The 3.3-kb *M. spretus*-specific *Taq*I fragment segregated concordantly with a previously described RFLP for the thymus cell antigen 1 (*Thy-1*) locus on chromosome 9. No recombinants were seen in a total of 123 animals, suggesting that *Cbl-2* and *Thy-1* are tightly linked (within 2.4 cM, upper 95% confidence interval) (Fig. 5B). We have previously reported that the *Thy-1* gene is 9.8 cM distal to the *Ets-1* proto-oncogene and is closely linked to the gene for the gamma subunit of the T3 molecule (*T3g*; 0 recombinants in 173 animals; within 1.7 cM, upper 95% confidence interval) (13). The *Cbl-2* locus also showed no recombinations with the *T3g* locus (0 recombinants in 125 animals; within 2.4 cM, upper 95% confidence interval). Thus, the *Cbl-2*, *Thy-1*, and *T3g* loci form a gene cluster on mouse chromosome 9.

The 4.5-kb *Taq*I restriction fragment was not present in all *M. spretus* animals and could be typed in only 26 backcross progeny. Nevertheless, this fragment also cosegregated with the *Thy-1* and *T3g* genes. The 4.5-kb fragment may thus represent an additional polymorphism at or near the *Cbl-2* locus that is still segregating in the *M. spretus* population.

DISCUSSION

Previous studies showed that the transforming virus, Cas NS-1, acquired mouse cellular sequences directly responsible for its oncogenicity and that these sequences, designated v-*cbl*, differed from those of previously defined oncogenes

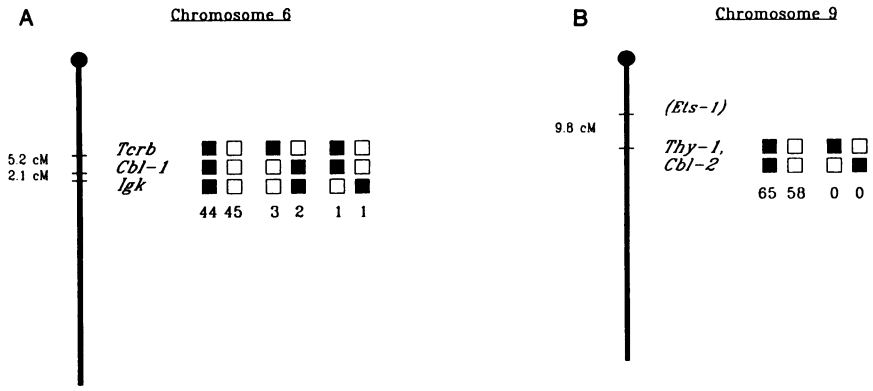

FIG. 5. Genetic localization of the *Cbl-1* and *Cbl-2* genes on mouse chromosomes 6 and 9. (A) Map of mouse chromosome 6 with the *Igk* locus positioned as shown in the work of Davisson et al. (Mouse Newsl., 1988). Previous studies have shown that the *Tcrb* locus is 7.95 ± 2.88 cM proximal to the *Igk* locus (7). On the current map, the positions of the *Cbl-1* and *Tcrb* loci relative to the *Igk* locus were determined from the interspecific backcross data summarized by the columns of boxes. Each column represents a particular pattern of species-specific RFLPs transmitted from the (C57BL/6J × *M. spretus*)F_1 parents to individual backcross progeny. The black boxes represent the presence of a C57BL/6J allele, and the white boxes represent the presence of a *M. spretus* allele. For the *Cbl-1* locus, this corresponds to the absence or presence of the 6.2-kb *Taq*I fragment. The number of offspring inheriting each gene distribution is listed at the bottom of each column. (B) Map of mouse chromosome 9 with the *Thy-1* locus positioned as shown in the work of Davisson et al. (Mouse Newsl., 1988). The *Cbl-2* locus (3.3-kb *Taq*I fragment) shows no recombinations with the *Thy-1* locus in 123 animals (block diagram as described above). The *Ets-1* locus has previously been mapped on mouse chromosome 9 and is shown in parentheses (Kingsley et al., submitted).

(14). The chromosomal mapping studies described here demonstrate that the mouse genome contains two distinct loci with strong homology to v-*cbl*. One locus, termed *Cbl-1*, is located on chromosome 6 between *Tcrb* and *Igk*. This chromosome is known to carry sequences related to the *Kras* and *Raf-1* oncogenes, but they map to other portions of the chromosome (M. T. Davisson, T. H. Roderick, A. L. Hillyard, and D. P. Doolittle, Mouse Newsl. **81**:12–19, 1988). Analyses of *Cbl-1* structure showed that it resembles v-*cbl*, indicating that is a processed gene. Further analyses of genomic clones from this region and development of *Cbl-1* specific clones, if possible, will be needed to determine whether this gene is transcriptionally active. Human mapping studies that revealed a single highly homologous v-*cbl*-related sequence (see below) suggest that acquisition of *Cbl-1* occurred after the evolutionary divergence of mice and humans.

The second locus with homology to v-*cbl*, *Cbl-2*, appears to represent the authentic c-*cbl* locus. *Cbl-2* is closely linked to *Thy-1* on chromosome 9. The only other oncogene previously mapped to the proximal half of chromosome 9 is *Ets-1* (Kingsley et al., submitted). With the mapping panel used in the current studies, it was shown that *Ets-1* is located approximately 10 cM proximal to *Thy-1* (16 recombinants in 163 animals). Thus, although they are both linked to *Thy-1*, *Cbl-2* and *Ets-1* are clearly distinct loci.

The current mapping data place *Cbl-2* in the middle of a large cluster of mouse genes whose human analogs have been mapped to the long arm of human chromosome 11. This cluster extends from the *Ets-1* gene (human analog mapped to 11q23-q24 [6]), through the *Thy-1* and the *T3g*, *T3d*, and *T3e* genes (human analogs mapped to 11q22.3-q24 and 11q23, respectively [13, 18]), to 4 cM distal of *Thy-1* to include the gene for the neural cell adhesion molecule *Ncam* (human analog mapped to 11q23 [15]).

The q23 region of human chromosome 11 is disrupted by translocations involving a number of other chromosomes in a variety of leukemias and lymphomas (8, 9, 17). Studies in progress indicate that in humans there is only one locus with strong homology to v-*cbl* and that this gene localizes to 11q23 (P. Savage, H. Seuanez, S. O'Brien, M. Shapiro, W. Langdon, J. Kersey, and H. C. Morse III, unpublished observations). It will thus be of great interest to see whether the structure and/or expression of *CBL* is altered in these malignancies.

ACKNOWLEDGMENTS

We thank S. Grove for excellent secretarial assistance.

This research was supported in part by the National Cancer Institute, Department of Health and Human Services, under contract NO1-CO-74101 with Bionetics Research Inc. D.M.K. is the recipient of an American Cancer Society postdoctoral fellowship (PF-3269). The National Cancer Institute–Frederick Cancer Research Facility is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

LITERATURE CITED

1. **Bishop, J. M.** 1985. Viral oncogenes. Cell **42**:23–38.
2. **Bonner, T., S. J. O'Brien, W. G. Nash, U. R. Rapp, C. C. Morton, and P. Leder.** 1984. The human homologs of the raf(mil) oncogene are located on human chromosomes 3 and 4. Science **223**:71–74.
3. **Buchberg, A. M., H. G. Bedigian, B. A. Taylor, E. Brownell, J. N. Ihle, S. Nagata, N. A. Jenkins, and N. G. Copeland.** 1988. Localization of Evi-2 to chromosome 11: linkage to other proto-oncogene and growth factor loci using interspecific backcross mice. Oncogene Res. **2**:149–165.
4. **Coll, J., M. Righi, C. de Taisne, C. Diasous, A. Gegonne, and D. Stehelin.** 1983. Molecular cloning of the avian acute transforming virus MH2 reveals a novel cell-derived sequence (v-mil) in addition to the myc oncogene. EMBO J. **2**:2189–2194.
5. **Dean, M., C. Kozak, J. Robbins, R. Callahan, S. O'Brien, and G. F. Vande Woude.** 1987. Chromosomal location of the *met* proto-oncogene in the mouse and cat genome. Genomics **1**: 167–173.
6. **De Tainse, C., A. Gegonne, D. Stehelin, A. Bernheim, and R. Berger.** 1984. Chromosomal location of the human protooncogene c-*ets*. Nature (London) **310**:581–583.
7. **D'Hoostelaere, L. A., E. Jouvin-Marche, and K. Huppi.** 1985. Localization of CT_B and C_K on mouse chromosome 6. Immunogenetics **22**:277–283.
8. **Diaz, M. O., M. M. LeBeau, P. M. Pitha, and J. D. Rowley.** 1986. Interferon and c-*ets*-1 genes in the translocation (9;11)

(p22;q23) in human acute monocytic leukemia. Science **231:** 265–267.

9. **Fan, Y.-S., J. M. Rowe, P. Dal Cin, and A. A. Sandberg.** 1988. A translocation t(9;11) (p11;q23) in T-cell acute lymphoblastic leukemia (FAB-L2). Cancer Genet. Cytogenet. **31:**263–269.
10. **Frykberg, L., S. Palmeiri, H. Beug, T. Graf, M. J. Hayman, and B. Vennstrom.** 1983. Transforming capacities of avian erythroblastosis virus mutants deleted in the erb A or B oncogenes. Cell **32:**227–238.
11. **Hoggan, M. D., N. F. Halden, C. E. Buckler, and C. A. Kozak.** 1988. Genetic mapping of the mouse c-*fms* proto-oncogene to chromosome 18. J. Virol. **62:**1055–1056.
12. **Holmes, K. L., W. Y. Langdon, T. N. Fredrickson, R. L. Coffman, P. M. Hoffman, J. W. Hartley, and H. C. Morse III.** 1986. Analysis of neoplasms induced by Cas-Br-M tumor extracts. J. Immunol. **137:**679–688.
13. **Krissansen, G. W., P. A. Gorman, C. A. Kozak, N. K. D. Sheer, P. N. Goodfellow, and M. J. Crumpton.** 1987. Chromosomal locations of the gene coding for the CD3 (T3) gamma submit of the human and mouse CD3/T-cell receptor complexes. Immunogenetics **26:**258–266.
14. **Langdon, W. Y., J. W. Hartley, S. P. Klinken, S. K. Ruscetti, and H. C. Morse III.** 1989. v-*cbl*, an oncogene from a dual-recombinant murine retrovirus that induces early B-lineage lymphomas. Proc. Natl. Acad. Sci. USA **86:**1168–1172.
15. **Nguyen, C., M. G. Matter, J. F. Mattei, M. J. Santoni, C. Goridis, and B. R. Jordan.** 1986. Localization of the human NCAM gene to band q23 of chromosome 11: the third gene for a cell interaction molecule mapped to the distal portion of the long arm of chromosome 11. J. Cell Biol. **102:**711–715.
16. **Nunn, M. F., P. H. Seeburg, C. Moscovici, and P. H. Duesberg.** 1983. Tripartite structure of the avian erythroblastosis virus E26 transforming gene. Nature (London) **306:**391–395.
17. **Strong, R. C., S. J. Korsmeyer, J. L. Parkin, D. C. Arthur, and J. H. Kersey.** 1985. Human acute leukemia cell line with the t(4;11) chromosomal rearrangement exhibits B lineage and monocytic characteristics. Blood **65:**21–31.
18. **van Rijis, J., V. Giguere, J. Hurst, T. van Agthoven, A. G. van Kessel, S. Goyert, and F. Grosveld.** 1985. Chromosomal localization of the human Thy-1 gene. Proc. Natl. Acad. Sci. USA **82:**5832–5835.
19. **Zimmerman, K. A., J. D. Yancopoulos, R. G. Collum, R. K. Smith, N. E. Kohl, K. A. Denis, M. M. Nau, O. N. Witte, D. Toran-Allerand, C. E. Gee, J. D. Minna, and F. W. Alt.** 1986. Differential expression of *myc* family genes during murine development. Nature (London) **319:**780–783.