Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 4671–4674, June 1991
Genetics

Steel-Dickie mutation encodes a c-Kit ligand lacking transmembrane and cytoplasmic domains

CAMILYNN I. BRANNAN*, STEWART D. LYMAN†, DOUGLAS E. WILLIAMS‡, JUNE EISENMAN‡, DIRK M. ANDERSON†, DAVID COSMAN†, MARY A. BEDELL*, NANCY A. JENKINS*, AND NEAL G. COPELAND*

*Mammalian Genetics Laboratory, ABL-Basic Research Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD 21702-1201; and Departments of †Molecular Biology and ‡Experimental Hematology, Immunex Corporation, 51 University Street, Seattle, WA 98101

Communicated by M. F. Lyon, March 11, 1991 (received for review February 11, 1991)

ABSTRACT **Mice homozygous for the viable *Sl* allele steel-Dickie (Sl^d) are sterile, severely anemic, and black-eyed white. The nature of the Sl^d mutation was investigated at the molecular level and was found to be due to a 4.0-kilobase intragenic deletion in mast cell growth factor (MGF) genomic sequences, providing conclusive evidence that *Sl* encodes MGF. As a consequence of this deletion, Sl^d is only capable of encoding a soluble truncated growth factor that lacks both transmembrane and cytoplasmic domains. Northern analysis indicates that Sl^d mRNA is expressed at approximately wild-type levels in adult tissues, and yeast expression studies suggest that the Sl^d protein is as biologically active as wild-type soluble MGF. These studies provide a molecular basis for explaining the Sl^d phenotype, a description of a germ-line mutation in the transmembrane and cytoplasmic domains of a membrane-bound growth factor, and *in vivo* evidence for the importance of membrane-bound forms of growth factors in mammalian development.**

Mice carrying mutations at the dominant white spotting (*W*) and steel (*Sl*) loci have deficiencies in pigment cells, germ cells, and blood cells (reviewed in ref. 1). While phenotypically similar, *W* mutations act cell-autonomously whereas *Sl* mutations exert their effects in the extracellular environment. The *W* locus encodes the c-*kit* protooncogene product (c-Kit), a tyrosine kinase receptor (2, 3). A ligand for c-Kit has recently been identified which is variously known as mast cell growth factor (4–6) (subsequently referred to here as MGF), stem cell factor (7–9), and c-Kit ligand (10, 11). This growth factor is a good candidate for the *Sl* gene product (5, 9, 11). Like several other growth factors characterized to date (12–18), MGF exists in both membrane-bound and soluble forms (6, 8, 11). The primary translation products of these growth factors are membrane-bound, whereas the soluble form(s) is derived by proteolytic cleavage.

We and others have shown (5, 9, 11) that MGF coding sequences are totally deleted in mice homozygous for lethal *Sl* alleles, whereas no gross structural alterations are associated with most viable *Sl* alleles. These results suggest, but do not prove, that MGF is encoded by *Sl*. A simple interpretation of these data is that the lethal *Sl* alleles result from the complete absence of MGF protein and that homozygous viable alleles either encode a mutant form of MGF or produce lower levels of the wild-type gene product. This interpretation is consistent with a previous study showing that an intragenic deletion in c-*kit* produces a homozygous lethal phenotype (19). It remains possible, however, that MGF deletion mutants are viable but other genes mapping near MGF are deleted in mice carrying lethal *Sl* alleles and that these genes are responsible for the lethality.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

In this report we show that one of the viable mutant *Sl* alleles, steel-Dickie (Sl^d), has a 4.0-kilobase (kb) intragenic deletion that truncates the *Sl* coding sequence. These results provide direct evidence that *Sl* encodes MGF and a molecular basis for explaining the Sl^d phenotype.

MATERIALS AND METHODS

Southern Blot Analysis. Preparation of genomic DNAs, restriction endonuclease digestions, agarose gel electrophoresis, Southern blot transfers, and hybridizations were performed as described (20), except that Zetabind membrane (Cuno) was used. MGF probes were derived from the MGF.4 cDNA clone (6).

Genomic Clone Analysis. Murine genomic MGF clones were isolated from a C57BL/6J library by screening with the 1-kb *Sal* I fragment of MGF cDNA. The restriction map was generated by digesting the phage DNA with *Eco*RI, *Hin*dIII, or *Eco*RI plus *Hin*dIII and probing a Southern blot of these digests with subfragments of the MGF cDNA clone.

RNA Manipulation. Total RNA was isolated from tissues by the guanidinium thiocyanate/phenol/chloroform method (21) and size-fractionated in a 1% agarose/formaldehyde gel. The RNA was transferred to Zetabind membrane, hybridized, and washed (22). Total RNA from both +/+ and Sl^d/Sl mice was used as a template to synthesize single-stranded cDNA with the Copy Kit (Invitrogen, San Diego).

Amplification of cDNA. cDNA was used as a template in PCRs with the following oligodeoxynucleotide amplimers: A (GATTCCAGAGTCAGTGTCAC), B (CATGGCATTGCCGGCTCTC), C (CGCACAGTGGCTGGTAACAG), and D (CTGGACACATGTTCTTGTCC) (see Fig. 1). cDNA was mixed with 400 ng of each primer and amplified during 30 cycles of the following conditions: 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min.

Sequence Analysis. The Sl/Sl^d cDNA sequence was determined by direct dideoxy sequencing of PCR-derived brain cDNAs (23) using the amplimers A and D shown in Fig. 1. Specifically, the above PCR products were excised from a 0.8% low-melting-point agarose gel and diluted 4-fold. A second round of PCR was performed using 1 μl of the diluted product mixed with 400 ng of one primer and 8 ng of a second primer; the reaction proceeded for 40 cycles of the following conditions: 94° for 1 min, 55°C for 1 min, and 72°C for 2 min. The single-stranded product was purified by spin dialysis using a Centricon 100 column (Amicon) and was used as a template for dideoxy sequencing (Sequenase protocol, United States Biochemical).

RESULTS

Southern blot analysis of $Sl^d/+$ genomic DNA identified an MGF restriction fragment length polymorphism unique to Sl^d

Abbreviation: MGF, mast cell growth factor.

(5, 9). This finding suggested that Sl^d may contain an intragenic mutation of MGF. To explore this possibility further, additional Southern blot analyses comparing $Sl^d/+$ and wild-type (+/+) DNA were performed (Fig. 1 *Lower*). A 1-kb cDNA probe encompassing most of the known coding region of MGF (*Sal* I fragment in Fig. 1 *Upper*), detected an *Eco*RI fragment unique to Sl^d, as described previously (5). *Eco*RI digestion of +/+ DNA produced fragments 12.0, 11.0, 8.5, 6.4, and 3.9 kb in length (lane 2). In $Sl^d/+$ DNA, the 12.0-kb fragment was reduced in intensity by 50% and a new 8.0-kb fragment was detected (lane 1). In addition, the hybridization intensity of a 3.5-kb *Hin*dIII fragment (lane 3) was 50% that detected for wild-type DNA (lane 4).

To further localize the MGF structural defect, the probe was subdivided into several nonoverlapping restriction fragments and hybridized to $Sl^d/+$ and wild-type DNA. Hybridization of a *Sal* I–*Eco*RI fragment representing 5′ MGF coding sequences failed to detect any alterations in $Sl^d/+$ DNA compared with +/+ (Fig. 1, lanes 5–8). In contrast, an *Eco*RI–*Bgl* I fragment from the middle of the MGF coding region detected the *Eco*RI polymorphism (lane 9), while a 3′ *Bgl* I–*Sal* I fragment detected the 3.5-kb *Hin*dIII polymorphism described previously (lane 15). These results suggest that the MGF structural alteration occurs in the 3′ half of the MGF coding region.

To facilitate the characterization of the MGF structural alteration, we constructed a partial restriction map of the MGF wild-type genomic locus (Fig. 2 *Upper*). The polymorphic 3.5-kb *Hin*dIII restriction fragment was also subcloned and used as a hybridization probe for Southern blot analysis. The 3.5-kb *Hin*dIII fragment detected a number of novel bands in $Sl^d/+$ DNA. Both the wild-type 12.0-kb *Eco*RI fragment and an altered 8.0-kb *Eco*RI fragment were observed in mutant DNA (Fig. 2, lane 5). A 6.5-kb *Hin*dIII fragment that was not observed with the cDNA probe was also detected in mutant DNA in addition to the wild-type 3.5-kb *Hin*dIII fragment (lane 3). A *Hin*dIII/*Eco*RI double digest produced fragments of 3.3 and 3.5 kb in mutant DNA, but only the 3.5-kb band was found in wild-type DNA (data not shown). Finally, a rearrangement not detected with the cDNA probe was observed in *Bgl* II-digested mutant DNA (Fig. 2, lane 1). These data are consistent with a 4-kb deletion in Sl^d genomic DNA that removes a *Hin*dIII (Fig. 2 *Upper*) and a *Bgl* II site.

To determine the effect of this deletion on MGF mRNA levels, we analyzed total RNA isolated from adult tissues of Sl^d/Sl and +/+ mice by Northern analysis using an MGF cDNA probe. The compound heterozygote was used to follow Sl^d MGF expression, since the *Sl* allele lacks MGF coding sequences (5, 9). MGF mRNA derived from brain and lung of Sl^d/Sl mice was slightly smaller than wild-type mRNA (Fig. 3 *Left*). Similar results have been reported for Sl^d/Sl spleen mRNA (9). These findings are consistent with the Southern blot results and indicate that the Sl^d deletion removes MGF exon sequences. MGF mRNA levels in Sl^d/Sl mice appeared similar to those observed in +/+ mice, indicating that the deletion does not affect MGF transcription.

To further localize the extent of the deletion, we used the amplimers shown in Fig. 1 *Upper* for PCR amplification of cDNA produced from first-strand synthesis of RNA isolated from brain of wild-type and Sl^d/Sl mice. The combination of amplimers A and D produced a Sl^d MGF cDNA product that was ≈150 bp smaller than that observed in wild-type control RNA (Fig. 3 *Right*, lanes b and c), whereas the combination of amplimers C and D showed no alterations (lanes f and g). Finally, the combination of amplimers B and D was not capable of generating a product from Sl^d cDNA (lane e), even though amplification of wild-type cDNA with these same amplimers resulted in a product of the expected size (lane d). These data suggest that Sl^d MGF mRNA has a deletion of ≈150 bp and that the sequences homologous to amplimer B are contained within this deletion.

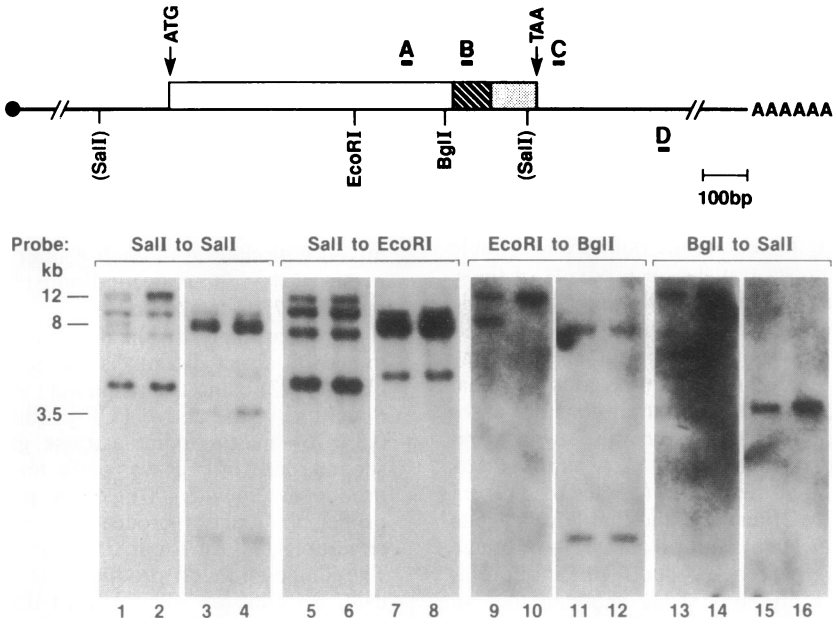

FIG. 1. MGF genomic DNA associated with the Sl^d allele has *Eco*RI and *Hin*dIII polymorphisms that map to the 3′ region of the open reading frame. (*Upper*) Schematic of wild-type MGF cDNA showing the entire open reading frame. Fragments of cDNA used as hybridization probes were generated using the restriction sites shown. The *Sal* I sites represent the endpoints of a partial cDNA clone (6) used to generate probes. The location of the amplimers (underlined A–D) used for PCR amplification (refer to Fig. 3) are also shown. The open box represents the extracellular domain, the hatched box represents the transmembrane domain, and the stippled box represents the cytoplasmic domain. bp, Base pairs. (*Lower*) Southern blot of $Sl^d/+$ DNA (odd-numbered lanes) and +/+ DNA (even-numbered lanes) probed with fragments from the MGF cDNA. DNA was digested with *Eco*RI (lanes 1, 2, 5, 6, 9, 10, 13, and 14) or *Hin*dIII (lanes 3, 4, 7, 8, 11, 12, 15, and 16). The probes used for hybridization were the 1-kb *Sal* I–*Sal* I fragment (lanes 1–4), the 565-bp 5′ *Sal* I–*Eco*RI fragment (lanes 5–8), the 200-bp *Eco*RI–*Bgl* I fragment (lanes 9–12), and the 190-bp *Bgl* I–*Sal* I fragment (lanes 13–16).

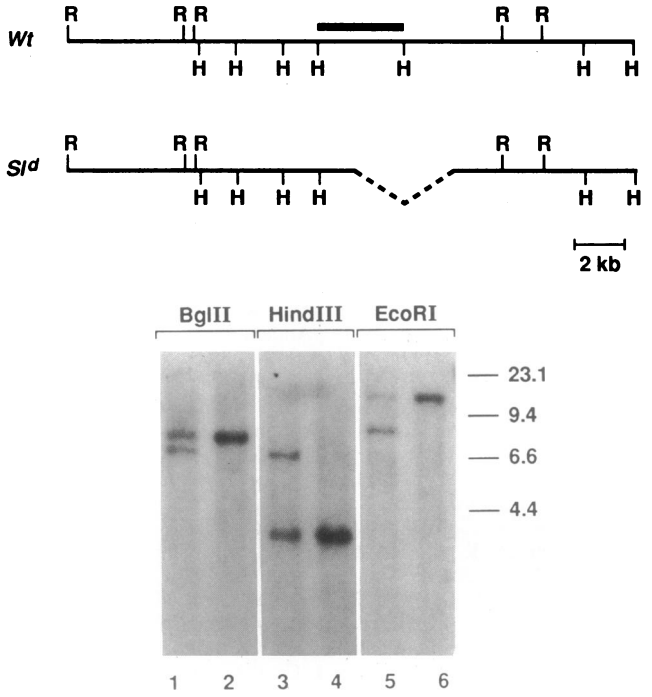

FIG. 2. Comparison of wild-type and Sl^d MGF genomic DNA. (*Upper*) Partial restriction maps for wild-type (Wt) and Sl^d MGF genomic DNA. The wild-type map was generated from four overlapping phage clones obtained from a C57BL/6J library by using *Eco*RI (R) and *Hin*dIII (H). The Sl^d map shows a 4-kb deletion (broken line). The exact endpoints of the deletion have not been accurately determined; the deletion has been arbitrarily centered around the 3′ *Hin*dIII site encompassing the 3.5-kb *Hin*dIII fragment. The 3.5-kb *Hin*dIII fragment (black bar in wild-type map) was used as a probe for Southern blot analysis. (*Lower*) Southern blot analysis of DNA isolated from $Sl^d/+$ mice (lanes 1, 3, and 5) or +/+ mice (lanes 2, 4, and 6). DNA was digested with *Bgl* II (lanes 1 and 2), *Hin*dIII (lanes 3 and 4), or *Eco*RI (lanes 5 and 6) and probed with the 3.5-kb genomic *Hin*dIII fragment. Positions and sizes (kb) of *Hin*dIII fragments of λ phage DNA are shown at right.

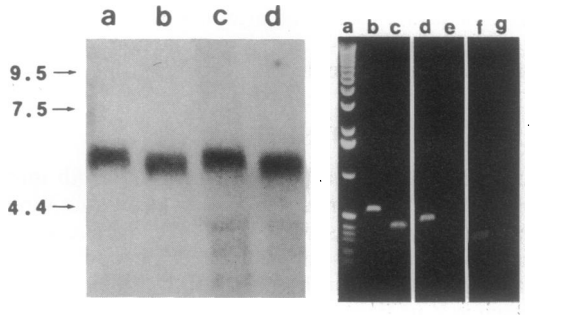

FIG. 3. MGF mRNA encoded by the Sl^d allele is smaller than wild type. (*Left*) Total RNA was examined by Northern blotting using the 1-kb *Sal* I MGF cDNA fragment as a hybridization probe. RNA (5 μg per lane) was isolated from +/+ mice (lanes a and c) and from Sl^d/Sl mice (lanes b and d). Tissues used were brain (lanes a and b) and lung (lanes c and d). Positions and sizes (kb) of RNA markers are shown at left. Ethidium bromide staining of rRNA showed that equal amounts of RNA were loaded in the various lanes (data not shown). (*Right*) PCR products were generated by using the amplimers shown in Fig. 1. Lane a shows DNA size markers (1-kb ladder; BRL). For lanes b, d, and f, +/+ cDNA was used as a template, and for lanes c, e, and g, Sl^d/Sl cDNA. For lanes b and c, DNA was amplified with amplimers A and D; for lanes d and e, with amplimers B and D; and for lanes f and g, with amplimers C and D. The expected sizes of the wild-type amplified products are 585 bp (lane b), 454 bp (lane d), and 253 bp (lane f).

The exact nature of the deletion was determined by asymmetric PCR amplification and subsequent DNA sequencing of Sl^d/Sl MGF cDNA, using amplimers A and D. It was found that the Sl^d cDNA was missing 241 bp of wild-type cDNA sequence with the addition of 67 bp of novel sequence, for a net loss of 174 bp. The novel sequence present in Sl^d MGF mRNA hybridizes to the wild-type 7-kb *Hin*dIII fragment located just 3′ of the 3.5-kb *Hin*dIII fragment, suggesting that the novel sequence represents an intron sequence that is incorporated into the Sl^d MGF mRNA as a result of the deletion. The finding of an intragenic deletion in Sl^d MGF mRNA allows us to conclude that the *Sl* locus encodes MGF.

Fig. 4 shows the region of Sl^d MGF cDNA that is different from wild-type control cDNA. The region of divergence begins 5 amino acids N-terminal to the transmembrane domain and results in termination of the Sl^d MGF open reading frame after an additional 3 amino acids. The remainder of the Sl^d MGF open reading frame was identical to wild type (data not shown). The MGF protein encoded by Sl^d would be predicted to contain a nearly full-length wild-type extracellular domain along with the N-terminal signal sequence but would lack entirely the transmembrane and intracellular domains.

To determine whether Sl^d-encoded MGF is biologically active, we constructed a recombinant cDNA form of Sl^d-encoded MGF and expressed it in yeast as previously described (6). Purified recombinant Sl^d-encoded MGF was mitogenic on MC-6 cells (a murine mast cell line) and had a specific activity of 820 units/μg compared with a specific activity of 720 units/μg for recombinant yeast MGF that

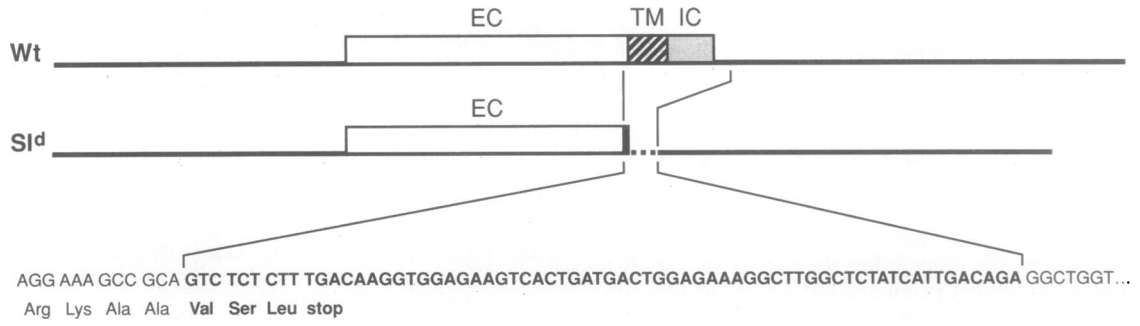

FIG. 4. Schematic of MGF deletion in Sl^d. Wild-type (Wt) MGF cDNA is shown with the open reading frame in boxes. The extracellular (EC) domain is 210 amino acids long, the transmembrane (TM) domain 27 amino acids, and the intracellular (IC) domain 36 amino acids, as previously described (6). Direct sequencing of PCR-amplified brain cDNAs (23) showed that the Sl^d cDNA encodes 205 amino acids of EC domain identical to wild-type before novel sequence is encountered. The novel sequence results in the termination of the open reading frame within 3 amino acids (black box and dotted line). After 67 bp of novel sequence (shown in bold type), the Sl^d cDNA and wild-type sequences are once again identical. A total of 241 bp of wild-type sequence is missing and 67 bp of novel sequence is present, for a net loss of 174 bp in the Sl^d cDNA sequence.

encodes the entire extracellular domain of MGF (6). Collectively, these data show that Sl^d is capable of encoding a biologically active, soluble MGF protein.

DISCUSSION

These studies show that Sl^d mice have a 4.0-kb intragenic deletion in MGF genomic sequences, providing direct evidence that *Sl* encodes MGF. As a consequence of this deletion, Sl^d mice are only capable of encoding a soluble truncated growth factor that lacks both transmembrane and cytoplasmic domains.

Given the nature of the Sl^d lesion, how can we explain the Sl^d phenotype, which includes melanocyte defects, severe anemia, and sterility in the homozygous condition (24). One possibility is that deletion of MGF coding sequences affects the ability of MGF mRNA to be translated. While we consider this unlikely in view of the small size of the deletion in Sl^d mRNA (174 bp), this possibility can be tested once appropriate *Sl* antibodies become available.

A second possibility is that the Sl^d deletion somehow interferes with the ability of the Sl^d protein to be secreted into the extracellular environment. Only very small amounts of soluble MGF may be produced by Sl^d mice; while low, these levels may be sufficient to rescue the lethality associated with most *Sl* alleles. From the yeast expression studies, we know that a biologically active, soluble MGF protein can be encoded from the Sl^d locus. In support of the possibility that the Sl^d deletion interferes with secretion of the Sl^d protein, previous studies have shown that supernatants from wild-type but not Sl^d stromal cell lines contain biologically active MGF (4) and injection of pharmacologic doses of recombinant soluble rat MGF into adult Sl^d mice can partially rescue the hematopoietic defects of these mice (9).

A third, and not mutually exclusive, possibility is that membrane-bound MGF may be required in addition to soluble MGF for normal growth and differentiation. This possibility is not precluded by studies showing that the Sl^d phenotype can be partially rescued by injection of adult Sl^d mice with soluble MGF (9) or a number of other studies showing that soluble MGF is biologically active *in vitro* (4, 6–9, 11). Expression of membrane-bound MGF may be required during normal development and may provide a means to accurately specify the three-dimensional patterns of growth and differentiation in ways not easily achievable by a freely diffusible growth factor. It is of interest that the three cell types affected by the Sl^d mutation all migrate during development. The membrane-bound form of MGF may be necessary to guide these cells along the correct route.

An important role for the membrane-bound form of MGF in development is consistent with a number of previous reports. Fujita *et al.* (25) have shown that supernatants from wild-type 3T3 fibroblasts cannot support mast cell growth, whereas mast cells are able to proliferate when grown in contact with 3T3 feeder layers that express MGF. Transplantation, aggregation chimeras, and parabiosis experiments also suggest that the *Sl* gene product is not freely diffusible (26–28).

In summary, the data described here confirm that the *Sl* locus encodes MGF. The Sl^d mouse will no doubt provide a useful model system for elucidating the function(s) of the various *Sl*-encoded protein products and for evaluating the biological requirements for soluble and membrane-bound forms of growth factors in development.

We thank Debbie Gilbert, Brian Cho, Laura James, Diane Foxworthe, Julie King, and Virginia Price for technical assistance; P. Donovan, M. Justice, D. Kingsley, and L. Siracusa for critically reviewing the manuscript; and L. Brubaker and R. Handley for typing the manuscript. This research was supported in part by the National Cancer Institute under Contract NO1-CO-74101 with ABL.

1. Russell, E. S. (1979) *Adv. Genet.* **20,** 357–459.
2. Geissler, E. N., Ryan, M. A. & Housman, D. E. (1988) *Cell* **55,** 185–192.
3. Chabot, B., Stephenson, D. A., Chapman, V. M., Besmer, P. & Bernstein, A. (1988) *Nature (London)* **355,** 88–89.
4. Williams, D. E., Eisenman, J., Baird, A., Rauch, C., Van Ness, K., March, C. J., Park, L. S., Martin, U., Mochizuki, D. Y., Boswell, H. S., Burgess, G. S., Cosman, D. & Lyman, S. D. (1990) *Cell* **63,** 167–174.
5. Copeland, N. G., Gilbert, D. J., Cho, B. C., Donovan, P. J., Jenkins, N. A., Cosman, D., Anderson, D., Lyman, S. D. & Williams, D. E. (1990) *Cell* **63,** 175–183.
6. Anderson, D. M., Lyman, S. D., Baird, A., Wignall, J. M., Eisenman, J., Rauch, C., March, C. J., Boswell, H. S., Gimpel, S. D., Cosman, D. & Williams, D. E. (1990) *Cell* **63,** 235–243.
7. Zsebo, K. M., Wypych, J., McNiece, I. K., Lu, H. S., Smith, K. A., Karkare, S. B., Sachdev, R. K., Yuschenkoff, V. N., Birkett, N. C., Williams, L. R., Satyagal, V. S., Tung, W., Bosselman, R. A., Mendiaz, E. A. & Langley, K. E. (1990) *Cell* **63,** 195–201.
8. Martin, F. H., Suggs, S. V., Langley, K. E., Lu, H. S., Ting, J., Okino, K. H., Morris, C. F., McNiece, I. K., Jacobsen, F. W., Mendiaz, E. A., Birkett, N. C., Smith, K. A., Johnson, M. J., Parker, V. P., Flores, J. C., Patel, A. C., Fisher, E. F., Erjavec, H. O., Herrera, C. J., Wypych, J., Sachdev, R. K., Pope, J. A., Leslie, I., Wen, D., Lin, C.-H., Cupples, R. L. & Zsebo, K. M. (1990) *Cell* **63,** 203–211.
9. Zsebo, K. M., Williams, D. A., Geissler, E. N., Broudy, V. C., Martin, F. H., Atkins, H. L., Hsu, R.-Y., Birkett, N. C., Okino, K. H., Murdock, D. C., Jacobsen, F. W., Langley, K. E., Smith, K. A., Takeishi, T., Cattanach, B. M., Galli, S. J. & Suggs, S. V. (1990) *Cell* **63,** 213–224.
10. Flanagan, J. G. & Leder, P. (1990) *Cell* **63,** 185–194.
11. Huang, E., Nocka, K., Beier, D. R., Chu, T.-Y., Buck, J., Lahm, H.-W., Wellner, D., Leder, P. & Besmer, P. (1990) *Cell* **63,** 225–233.
12. Derynck, R., Roberts, A. B., Winkler, M. E., Chen, E. Y. & Goeddel, D. V. (1984) *Cell* **38,** 287–297.
13. Lee, D. C., Rose, T. M., Webb, N. R. & Todaro, G. J. (1985) *Nature (London)* **313,** 489–491.
14. Gray, A., Dull, T. J. & Ullrich, A. (1983) *Nature (London)* **303,** 722–725.
15. Scott, J., Urdea, M., Quiroga, M., Sanchez-Pescador, R., Fong, N., Selby, M., Rutter, W. J. & Bell, G. I. (1983) *Science* **221,** 236–240.
16. Stroobant, P., Rice, A. P., Gullick, W. J., Cheng, D. J., Kerr, I. M. & Waterfield, M. D. (1985) *Cell* **42,** 383–393.
17. Rettenmier, C. W. (1989) *Curr. Top. Microbiol. Immunol.* **149,** 129–141.
18. Kriegler, M., Perez, C., DeFay, K., Albert, I. & Lu, S. D. (1988) *Cell* **53,** 45–53.
19. Nocka, K., Tan, J. C., Chiu, E., Chu, T. Y., Ray, P., Traktman, P. & Besmer, P. (1990) *EMBO J.* **9,** 1805–1813.
20. Jenkins, N. A., Copeland, N. G., Taylor, B. A. & Lee, B. K. (1982) *J. Virol.* **43,** 26–36.
21. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
22. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1991–1995.
23. Gyllensten, U. B. & Erlich, H. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7652–7656.
24. Bernstein, S. (1960) *Mouse News Lett.* **23,** 33–34.
25. Fujita, J., Nakayama, H., Onoue, H., Kanakura, Y., Nakano, T., Hidekazu, A., Shun-ichi, T., Honjo, T. & Kitamura, Y. (1988) *Blood* **72,** 463–468.
26. Nakayama, H., Kuroda, H., Fujita, J. & Kitamura, Y. (1988) *Development* **102,** 107–116.
27. Nakayama, H., Kuroda, H., Onoue, H., Fujita, J., Nishimune, Y., Matsumoto, K., Nagano, T., Suzuki, F. & Kitamura, Y. (1988) *Development* **102,** 117–126.
28. McCulloch, E. A., Siminovitch, L., Till, J. E., Russell, E. S. & Bernstein, S. E. (1965) *Blood* **26,** 399–410.