Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 3117–3121, April 1990
Genetics

Ankyrin and the hemolytic anemia mutation, *nb*, map to mouse chromosome 8: Presence of the *nb* allele is associated with a truncated erythrocyte ankyrin

(conserved chromosomal segments/recombinant inbred lines/reticulocyte ghosts/inside out vesicles/immunoblots)

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Communicated by Elizabeth S. Russell, January 26, 1990

ABSTRACT **Mice with normoblastosis, *nb/nb*, have a severe hemolytic anemia. The extreme fragility and shortened lifespan of the mutant erythrocytes result from a defective membrane skeleton. Previous studies in our laboratory indicated a 50% deficiency of spectrin and an absence of normal ankyrin in erythrocyte membranes of *nb/nb* mice. We now report genetic mapping data that localize both the *nb* and erythroid ankyrin (*Ank-1*) loci to the centromeric end of mouse chromosome 8. Using immunological and biochemical methods, we have further characterized the nature of the ankyrin defect in mutant erythrocytes. We do not detect normal sized (210 kDa) erythroid ankyrin by immunoblot analysis in *nb/nb* reticulocytes. However, *nb/nb* reticulocytes do contain a 150-kDa ankyrin immunoreactive protein. The 150-kDa protein is present with normal-sized ankyrin in *nb/+* reticulocytes but is not found in +/+ reticulocytes. Our genetic and biochemical data indicate that the *nb* mutation results from a defect in the erythroid ankyrin gene. A human hereditary spherocytosis putatively resulting from an ankyrin defect maps to a segment of human chromosome 8 that is homologous to the *nb*–ankyrin region of mouse chromosome 8. The linkage data suggest that the mouse and human diseases result from mutations in homologous loci.**

The erythrocyte membrane skeleton is a two-dimensional net-like structure composed primarily of spectrin and proteins that bind spectrin subunits to one another and to the membrane (for review, see refs. 1 and 2). Ankyrin is the major link between spectrin and a transmembrane protein, band 3 (3, 4). Weaker interactions occur that join spectrin to other integral membrane proteins, such as the glycophorins, but ankyrin appears to play a dominant role in stabilizing the erythrocyte membrane skeleton (5, 6).

Spectrin deficiencies in mutant mice cause severe hemolytic anemia (7, 8). One such mutant, normoblastosis (gene symbol, *nb*) (9), has erythrocytes that are 50% deficient in spectrin (7, 8). Bodine *et al.* (8) showed that spectrin synthesis, spectrin assembly into the membrane skeleton, and spectrin mRNA translational activity are all essentially normal in *nb/nb* reticulocytes. Since *nb/nb* erythrocytes lack detectable ankyrin (8), it was hypothesized that the primary defect in *nb/nb* mice is in ankyrin and that the spectrin deficiency is a secondary effect. In the present report, we have established a relationship between ankyrin and *nb* by genetic mapping studies and have shown that although we do not detect normal-sized 210-kDa ankyrin in *nb/nb* erythrocytes, there is a 150-kDa ankyrin-like protein that is unique to the *nb* mutation.

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MATERIALS AND METHODS

Animals. Inbred mice of the SWR/J (SWR) strain were purchased from the Production Colony at The Jackson Laboratory. The *nb* mutation was maintained by forced heterozygosis in our research colony on both the WB/Re (WB) and C57BL/6J (B6) inbred strains (10). Viable *nb/nb* mice were obtained by crossing the WB and B6 heterozygotes.

Linkage Analysis. The *nb* locus was mapped by isozyme analysis of interstrain backcross mice. Heterozygous (*nb/+*) mice were identified by an osmotic fragility test (10). The glutathione reductase (*Gr-1*) and glutamate oxalate transaminase (*Got-2*) genotypes were determined by cellulose acetate electrophoresis of kidney lysates (11, 12). Erythroid ankyrin (*Ank-1*) and tissue plasminogen activator (*Plat*) genotypes were detected by restriction fragment length polymorphism (RFLP) analysis (13, 14). The DNA probes used were a 4.6-kilobase (kb) cDNA (mAnk-1) for *Ank-1* (R.A.W. and S.E.L., unpublished data) and a 1.7-kb cDNA (pTAM50) (16) for *Plat*. Recombinant inbred (RI) line genomic DNA was obtained from the Jackson Laboratory DNA Service. Nylon filter blots of restriction enzyme-digested DNA from C57BL/6J × *Mus spretus* backcross progeny were provided by Ceci *et al.* (17). In the backcross experiments, map distances were calculated as number of crossovers, divided by number of progeny tested, times 100 and reported as map units (mean ± SEM). For the RI line analysis, map distances and 95% confidence levels were determined according to Silver (18).

Phenylhydrazine Treatment. Reticulocytosis (≥95%) was produced in +/+ and *nb/+* adult mice by five daily injections of phenylhydrazine chloride [Sigma; P-7126; 0.04 mg/g (body weight)] (19). Blood was collected 2 days after the last injection.

Antiserum. The antiserum used in these experiments was provided by Vann Bennett (Duke University Medical Center, Durham, NC). It is a monospecific anti-human erythrocyte ankyrin IgG prepared as described by Bennett and Davis (4). We have designated the antiserum as aHEA in the present study.

Preparation, Electrophoresis, and Immunoblots of Reticulocyte Ghosts and Inside Out Vesicles (IOVs). Hemoglobin-depleted erythrocyte ghosts were prepared by the procedure of Dodge *et al.* (20) as modified by Bodine *et al.* (8). Phenylmethylsulfonyl fluoride (Sigma; P-7626) at 20 μg/ml and EDTA (Sigma; ED-255) at 1.0 mM were included in the lysis and wash buffers as protease inhibitors. Protein concentrations were determined by the method of Lowry *et al.*

Abbreviations: RFLP, restriction fragment length polymorphism(s); RI, recombinant inbred; IOV, inside out vesicles.

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(21). SDS/PAGE was performed according to either Fairbanks *et al.* (22) or Laemmli (23) using a 5% stacking gel and 10% running gel. Protein transfers and immunoblotting were performed as described by Burnette (24) and Towbin *et al.* (25). Ankyrin-enriched, spectrin-depleted IOVs were prepared according to the procedures of Bennett and Branton (26) and Tyler *et al.* (27). Spectrin was isolated from the supernatant of the IOV preparation by the method of Litman *et al.* (28).

RESULTS

Linkage of the *Ank-1* Gene and the *nb* Locus on Mouse Chromosome 8. The linkage of *nb* to markers on mouse chromosome 8 was established in a series of interstrain backcross experiments. A total of 17 biochemical and morphological markers distributed throughout the genome were analyzed. Linkage was detected between *nb* and the well-established chromosome 8 marker, *Gr-1*. This linkage was further investigated by a three-point marker analysis in the cross illustrated in Table 1. *nb*, *Gr-1*, and another well-defined marker locus, *Got-2*, were typed in 290 progeny from this backcross. The results position the *nb* locus near the centromere on chromosome 8, 8.3 ± 1.6 map units proximal to *Gr-1*.

The linkage of the erythrocyte ankyrin gene, *Ank-1*, on the centromeric end of mouse chromosome 8 was established by the use of recombinant inbred strains of mice (29, 43). *Ank-1*/*Hin*dIII and *Plat*/*Bam*HI RFLPs allowed us to type these two loci in the AKR/J × C57L/J (AKXL) and C57BL/6J × C3H/HeJ (BXH) RI lines. The results of this analysis are shown in Table 2. *Ank-1* shows linkage to three independently mapped proximal chromosome 8 markers: tissue plasminogen activator, *Plat* (17, 32); endogenous xenotropic virus 12, *Xmv-12* (33); and cryptdin, *Defcr* (34). There were no crossovers between *Ank-1* and *Plat* in the 30 RI lines tested. At the 95% confidence level this indicates that these two loci are within 3.5 map units of each other (18). The RFLPs and strain distribution patterns for *Ank-1* and *Plat* are given in Table 2.

The localization of *Ank-1* on chromosome 8 was further defined by analysis of 77 progeny from the interspecific backcross outlined in Table 3. Nylon-filter blots of restriction enzyme-digested genomic DNA from the backcross progeny were kindly provided by Ceci *et al.* (17). Hybridization and RFLP analysis using the mAnk-1 probe were done in our laboratory. RFLP typing for *Plat* was provided by Ceci *et al.* (17). No crossovers between *Ank-1* and *Plat* were detected in the 77 progeny tested. Statistically, this positions the two loci 0 ± 4 map units from each other, agreeing completely with the findings of the RI line analysis.

In Fig. 1*A*, we have summarized the genetic data contained in this manuscript. The relative positions of the genes studied and their most likely order on the proximal end of mouse chromosome 8 are given. Even though the *nb* and *Ank-1* loci were mapped in separate backcross experiments, the data position the *nb* and *Ank-1* loci very near each other and are consistent with the two loci being identical.

The human genes that are homologous to the mouse genes studied in our experiments all map to the short arm of human chromosome 8 (8p). They are defensin, *DEF1* (35); glutathione reductase, *GSR* (36); tissue plasminogen activator, *PLAT* (37); hereditary spherocytosis type II, *SPH1* (30, 31); and erythroid ankyrin, *ANK1* (38, 39). Their relative positions are shown in Fig. 1*B*. The mouse homologs are *Defcr*, *Gr-1*, *Plat*, *nb*, and *Ank-1*, respectively. Note that relative to the position of the centromere, gene order is conserved between mouse and human, thus defining a conserved chromosomal segment (40) in the centromeric end of mouse chromosome 8 and the short arm of human chromosome 8.

Detection of the *nb* Mutation by PAGE on SDS/Polyacrylamide Gels. Coomassie blue-stained SDS/polyacrylamide gels prepared by the method of Fairbanks *et al.* (22) were used in our initial studies of the *nb* mutation (8). It is clear that α and β spectrin are reduced and ankyrin is missing in *nb*/*nb* ghosts when this method of detection is used (Fig. 2*A*). Because ankyrin is not well resolved from β spectrin in the

Table 1. Backcross analysis of *nb* linkage on mouse chromosome 8

Chromosome inherited from F_1 parent			
nb	*Gr-1*	*Got-2*	*n*
B	B	B	96
S	S	S	102
B × S		S	10
S × B		B	8
B		B × S	41
S		S × B	27
B × S × B			3
S × B × S			3

Cross: (C57BL/6J, *nb*/+ × SWR/J)F_1, *nb*/+ × C57BL/6J. Progeny tested: total *n* = 290. Progeny types are designated as B or S when the RFLP was inherited from the C57BL/6J or (C57BL/6J × SWR/J)F_1 mice, respectively. Regions of inferred crossover are indicated by ×. Male and female data are combined for the chromosomes inherited from F_1 parent. Gene order is chosen so as to minimize the number of double crossovers. Map distance between *nb* and *Gr-1* is 8.3 ± 1.6 map units and between *Gr-1* and *Got-2* is 25.5 ± 2.5 map units. Gene order: centromere—*nb*–(8.3 map units)–*Gr-1*–(25.5 map units)–*Got-2*—.

Table 2. RI strain analysis of *Ank-1* linkage on mouse chromosome 8

Locus	RI line	Linked markers	Discordance	Map distance	95% confidence limits
Ank-1	AKXL	*Plat*	0/18	0.00	0.00–3.50
	BXH	*Plat*	0/12	0.00	0.00–3.50
Ank-1, *Plat*	AKXL	*Xmv-12*	2/18	3.33	0.35–18.10
Ank-1, *Plat*	BXH	*Defcr*	1/12	2.38	0.05–22.75

For the *Ank-1*/*Hin*dIII RFLPs, the L,B type is 3.8 kb and the AK,H type is 3.2 kb. For the *Plat*/*Bam*HI RFLPs, the L,B type is 9.4 kb and the Ak,H type is 5.7 kb. The *Ank-1* and *Plat* strain distribution patterns were as follows. AKXL, AK type (strains 5, 8, 9, 13, 17, 19, 28, 29, 38) and L type (strains 6, 7, 12, 14, 16, 21, 24, 25, 37); BXH, B type (strains 3, 4, 7, 8, 9, 10, 12, 14) and H type (strains 2, 6, 11, 19).

Table 3. Backcross analysis of *Ank-1* linkage on mouse chromosome 8

Chromosome inherited from F_1 parent		
Ank-1	*Plat*	*n*
B	B	40
S	S	37
B × S		0
S × B		0

Cross: (C57BL/6J × *Mus spretus* [Spain])F_1 × C57BL/6J. Progeny tested: total *n* = 77. Progeny types are designated as B or S when the RFLP was inherited from the C57BL/6J or (C57BL/6J × *M. spretus*)F_1 mice, respectively. Regions of inferred cross-overs are indicated by ×. All recombination data are derived from F_1 females. *Plat* typing is from Ceci *et al.* (17). Map distance between *Ank-1* and *Plat* is 0 ± 4.0 map units.

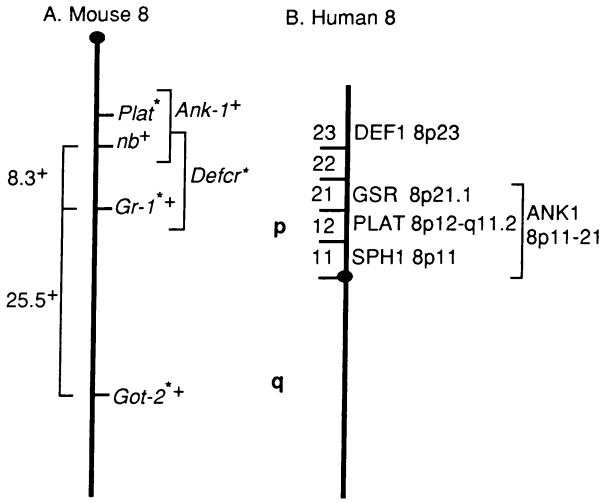

FIG. 1. (*A*) Proximal end of mouse chromosome 8. An asterisk indicates previously mapped loci. Loci typed and map distances determined in the present study are indicated by a plus. (*B*) Short (p) and long (q) arms of human chromosome 8 showing the map positions of the homologous human genes. The solid circles represent the centromeres.

Fairbanks system, we used the SDS/PAGE method of Laemmli (23) in this study. In the Laemmli system, the relative positions of ankyrin and β spectrin were reversed, but ankyrin was very clearly resolved (Fig. 2*B*).

Detection of Ankyrin Immunoreactive Proteins in Normal and *nb/nb* Mice. A monospecific anti-human erythrocyte ankyrin IgG preparation (aHEA) (4) was used to probe immunoblots of erythrocyte ghost proteins from normal and *nb/nb* mice. As predicted by the SDS/PAGE data above, aHEA detected a normal-sized ankyrin in +/+ but not in *nb/nb* (Fig. 3). However, an aHEA-immunoreactive polypeptide with an approximate molecular mass of 150 kDa was detected in *nb/nb* ghosts. Although several protease inhibitors (phenylmethylsulfonyl fluoride and EDTA) were included in our ghost preparations, it is clear that we did not block all protease activity. However, in this instance we can use this to our advantage. A comparison of the apparent degradation patterns of normal-sized ankyrin and the 150-kDa protein (Figs. 3 and 4*A*) allows several important observations. (*i*) No aHEA-reactive proteins larger than 150 kDa are detected in *nb/nb* ghosts. (*ii*) A 150-kDa aHEA-reactive protein is not apparent in +/+ reticulocyte ghosts. (*iii*) If the 150-kDa protein is a closely related or truncated

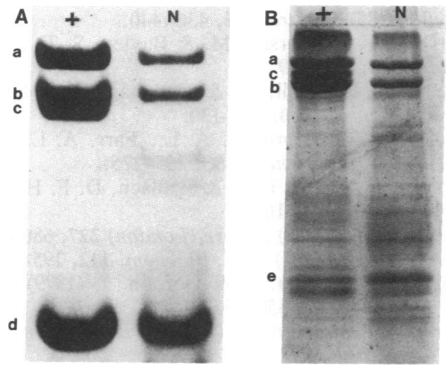

FIG. 2. Coomassie blue-stained SDS/polyacrylamide gels of equal amounts (70 μg) of ghost proteins from normal (lanes +) and normoblastic (lanes N) reticulocytes. Electrophoresis was according to Fairbanks *et al.* (22) in *A* [adapted from Bodine *et al.* (8)] and to Laemmli (23) in *B*. Bands: a, α Spectrin; b, β spectrin; c, ankyrin; d, band 3; e, band 4.1.

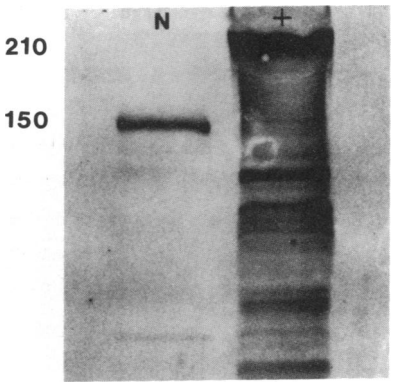

FIG. 3. Detection of a unique ankyrin immunoreactive protein among *nb/nb* reticulocyte ghost proteins. Immunoblot of 70 μg of *nb/nb* (lane N) and +/+ (lane +) reticulocyte ghost proteins probed with antiserum aHEA. Bands: 210, 210-kDa normal ankyrin; 150, 150-kDa *nb*-related ankyrin.

version of the normal ankyrin, it would be expected to share some but not all of the bands in the degradation pattern of normal ankyrin. The degradation patterns seen are consistent with this expectation.

The 150-kDa Ankyrin Immunoreactive Protein Is Unique to Mice Carrying the *nb* Gene. Ghost proteins from reticulocytes of a phenylhydrazine-treated +/+ mouse and from an animal homozygous for a different spectrin-deficient hemolytic anemia, *sph*^{ha} (8), were probed with aHEA (Fig. 4*A*). In both animals, the reticulocyte counts were comparable to those of *nb/nb* animals. It is clear that the 150-kDa protein is not common to all reticulocytes. In Fig. 4*B*, we show that although normal erythrocytes and phenylhydrazine-generated +/+ reticulocytes did not contain the aHEA-reactive 150-kDa protein, *nb/nb* reticulocytes, *nb/+* phenylhydrazine-generated reticulocytes, and *nb/+* erythrocytes did. It is interesting to note that, when 130 μg of protein is loaded in each lane, the amount of 150-kDa protein in *nb/+* reticulocytes is about half that seen in *nb/nb* reticulocytes.

The 150-kDa Ankyrin Immunoreactive Protein Fractionates with Spectrin-Depleted IOVs. Spectrin-depleted ankyrin-enriched IOVs were prepared from *nb/nb* reticulocyte ghosts

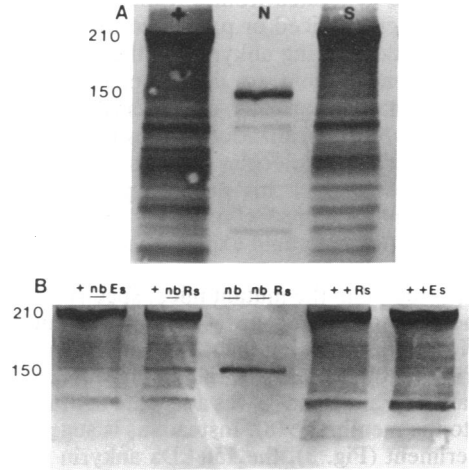

FIG. 4. Detection of ankyrin immunoreactive proteins among ghost proteins from several genotypes of mice. Each lane was loaded with 130 μg of ghost proteins and the immunoblots were probed with antiserum aHEA. Bands: 210, 210-kDa normal ankyrin; 150, 150-kDa *nb*-related ankyrin. (*A*) +, N, and S represent the +/+, *nb/nb*, and *sph*^{ha}/*sph*^{ha} genotypes, respectively. (*B*) Genotypes are given over the lanes; Rs, reticulocytes (from anemic peripheral blood); Es, erythrocytes (from nonanemic peripheral blood).

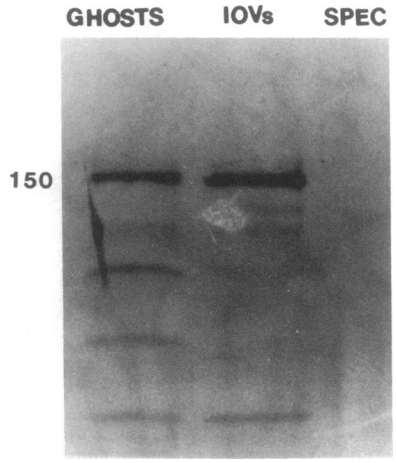

FIG. 5. Detection of the 150-kDa ankyrin immunoreactive protein in fractionated *nb/nb* reticulocyte ghosts. Immunoblots of equal amounts of *nb/nb* reticulocyte ghost proteins from whole ghosts, from IOVs, and from the spectrin (SPEC) fraction probed with antiserum aHEA.

(26–28). Both the IOV fraction and the extracted spectrin fraction were assayed for the 150-kDa protein. Immunoblots of equal amounts of protein from *nb/nb* ghosts, spectrin-depleted IOVs, and the extracted spectrin fraction were probed with aHEA. The 150-kDa protein was detected in the whole ghosts and in the IOV fraction but not in the spectrin fraction (Fig. 5).

DISCUSSION

The results of this study provide additional support for our original hypothesis that an abnormal ankyrin is the primary defect in *nb/nb* mice (8). The evidence includes: colocalization of the *nb* and the *Ank-1* loci to the centromeric region of chromosome 8 (Fig. 1*A*); no apparent immunoreactive 210-kDa ankyrin in *nb/nb* reticulocytes (Fig. 2); and detection of an *nb*-specific 150-kDa ankyrin immunoreactive protein (Figs. 3 and 4).

There are several mechanisms that would account for the ankyrin defect in mice with normoblastosis. The 150-kDa protein could be generated from a truncated ankyrin gene, from an incorrectly spliced or processed mRNA, from premature termination during ankyrin synthesis, or from utilization of a nonerythroid or developmentally controlled ankyrin gene that is normally off in erythroid cells. The availability of DNA hybridization probes for ankyrin makes it possible to study the molecular genetics of the *nb* mutation.

The data presented in this manuscript suggest that the 150-kDa ankyrin is the product of the *nb* gene. The presence of the smaller fragment could explain the various membrane defects seen in *nb/nb* erythrocytes. Although no 210-kDa ankyrin is detected in the mutant mice, spectrin levels in the membrane skeleton are higher (50%) than one might expect. Our previous tenet was that the 4.1–glycophorin attachment of spectrin to the membrane, although weaker than that between ankyrin and band 3, was adequate to bind 50% of the spectrin to the membrane (8). Instead, as is suggested by the IOV experiment (Fig. 5), the 150-kDa ankyrin may be partially active. This partial activity, in addition to that provided by the band 4.1–glycophorin A complex, may be sufficient to fix 50% of the normal level of spectrin in the *nb/nb* erythrocyte.

A number of hereditary hemolytic anemias occur in man (for review, see ref. 41). One of these, dominant hereditary spherocytosis (*HS*) (30) or atypical *HS* (42), maps to the short arm of human chromosome 8 (gene symbol *SPH1*) (31). *SPH1* is associated with a deficiency of erythroid ankyrin (42, 43). The available genetic data as summarized in Fig. 1 indicate that the human *SPH1* and the mouse *nb* loci are homologous. In addition, the biochemical and genetic data suggest that both the *SPH1* mutation in man and the *nb* mutation in mouse are caused by lesions in the erythroid ankyrin gene. Although the mutations are probably not identical, analyses of disease development, pathogenesis, and therapeutic measures in the mouse will be valuable in devising potential treatment for the human disease.

We thank Dr. Vann Bennett for providing the aHEA antisera. We also thank Dr. Sidney Strickland for providing Plat cDNA. Thanks also to Drs. Nancy Jenkins and Neal Copeland for analysis of data from the B6 × *Mus spretus* backcross and to Dr. Benjamin Taylor for analysis of the recombinant inbred line strain distribution patterns. Drs. Jeffrey Saffer and John Gardner made helpful comments on the manuscript and Mrs. Nancy Hamblen maintained the *nb/nb* colony. This work was supported by Grant HL 29305 (J.E.B.) from the National Institutes of Health. The Jackson Laboratory is fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

1. Branton, D. (1981) *Cell* **24**, 24–32.
2. Bennett, V. (1985) *Annu. Rev. Biochem.* **54**, 273–304.
3. Bennett, V. & Stenbuck, P. J. (1979) *Nature (London)* **280**, 468–473.
4. Bennett, V. & Davis, J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7550–7554.
5. Marchesi, V. T. (1985) *Annu. Rev. Cell Biol.* **1**, 531–561.
6. Bennett, V. (1989) *Biochim. Biophys. Acta* **988**, 107–121.
7. Lux, S. E., Pease, B., Tomaselli, M. B., John, K. M. & Bernstein, S. E. (1979) in *Normal and Abnormal Red Cell Membranes*, eds. Lux, S. E., Marchesi, V. T. & Fox, C. F. (Liss, New York), pp. 463–469.
8. Bodine, D. M., IV, Birkenmeier, C. S. & Barker, J. E. (1984) *Cell* **37**, 721–729.
9. Bernstein, S. E. (1969) *Genetics in Laboratory Animal Medicine* (Natl. Acad. Sci., Washington, DC), Publ. No. 1679, pp. 9–33.
10. Bernstein, S. E. (1980) *Lab. Anim. Sci.* **30**, 197–205.
11. Nichols, E. A. & Ruddle, F. H. (1975) *Biochem. Genet.* **13**, 323–329.
12. DeLorenzo, R. J. & Ruddle, F. H. (1970) *Biochem. Genet.* **4**, 259–273.
13. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
14. Botstein, D., White, R. L., Skolnick, M. & Davis, R. W. (1980) *Am. J. Hum. Genet.* **32**, 314–331.
15. Hanspal, M., Prchal, J. T., Hanspal, J. & Palek, J. (1987) *Blood* **70**, Suppl. 1, 53 (abstr.).
16. Rickles, R. J., Darrow, A. L. & Strickland, S. (1988) *J. Biol. Chem.* **263**, 1563–1569.
17. Ceci, J. D., Justice, M. J., Lock, L. F., Jenkins, N. A. & Copeland, N. G. (1990) *Genomics* **6**, 72–79.
18. Silver, J. (1985) *J. Hered.* **76**, 436–440.
19. Chui, D. H. K., Patterson, M. & Bayley, S. T. (1980) *Br. J. Haematol.* **44**, 431–439.
20. Dodge, J. T., Mitchell, C. & Hanahan, D. J. (1962) *Arch. Biochem. Biophys.* **100**, 119–130.
21. Lowry, O. H., Rosebrough, A. L., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
22. Fairbanks, G., Stech, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2616.
23. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
24. Burnette, W. N. (1981) *Anal. Biochem.* **112**, 195–203.
25. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
26. Bennett, V. & Branton, D. (1977) *J. Biol. Chem.* **252**, 2753–2763.
27. Tyler, J. M., Hargreaves, W. R. & Branton, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5192–5196.
28. Litman, D., Hsu, C. J. & Marchesi, V. T. (1980) *J. Cell Sci.* **42**, 1–22.
29. Bailey, D. W. (1971) *Transplantation* **11**, 325–327.
30. Costa, F. F., Lux, S. E., Agre, P., Watkins, P., John, K. & Forget, B. G. (1988) *Blood* **72**, Suppl. 1, 38 (abstr.).

31. Chilcotte, R. R., Le Beau, M. M., Dampier, C., Pergament, E., Verlinsky, Y., Mohandas, N., Frischer, H. & Rowley, J. D. (1987) *Blood* **69,** 156–159.
32. Rajput, B., Marshall, A., Killary, A. M., Lalley, P. A., Naylor, S. L., Belin, D., Rickles, R. J. & Strickland, S. (1987) *Somatic Cell Mol. Genet.* **13,** 581–586.
33. Frankel, W. N., Stoye, J. P., Taylor, B. A. & Coffin, J. M. (1989) *J. Virol.* **63,** 1763–1774.
34. Ouellette, A. J., Pravtcheva, D., Ruddle, F. H. & James, M. (1989) *Genomics* **5,** 223–239.
35. Sparkes, R. S., Kronenberg, M., Heinzmann, C., Daher, K. A., Klisak, I., Ganz, T. & Mohandas, T. (1989) *Genomics* **5,** 240–244.
36. George, D. L. & Francke, U. (1976) *Ann. Genet.* **19,** 253–256.
37. Yang-Feng, T. L., Opdenakker, G., Volckaert, G. & Francke, U. (1986) *Am. J. Hum. Genet.* **39,** 79–87.
38. Lambert, S., Lawler, J., Speicher, D. W., Prchal, J. T. & Palek, J. (1988) *Blood* **72,** Suppl. 1, 30 (abstr.).
39. Lux, S., John, K., Shaley, O., Forget, B., Chilcote, R., Marchesi, S., McIntosh, S., Harris, P., Watkins, P. & Bennett, V. (1988) *Blood* **72,** Suppl. 1, 46 (abstr.).
40. Nadeau, J. H. (1989) *Trends Genet.* **5,** 82–86.
41. Palek, J. (1987) *Blood Rev.* **1,** 147–168.
42. Coetzer, T. L., Lawler, J., Liu, S. C., Prchal, J. T., Gualtieri, R. J., Brain, M. C., Dacie, J. V. & Palek, J. (1988) *N. Engl. J. Med.* **318,** 230–234.
43. Taylor, B. A. (1978) in *Origin of Inbred Mice,* ed. Morse, H. C., III (Academic, New York), pp. 423–438.