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Deletion in erythrocyte band 3 gene in malaria-resistant Southeast Asian ovalocytosis

PETR JAROLIM*, JIRI PALEK*†, DOMINICK AMATO‡, KHALID HASSAN§, PETER SAPAK¶, GEORGE T. NURSE‖, HILLARD L. RUBIN*, SEN ZHAI*, KENNETH E. SAHR*, AND SHIH-CHUN LIU*

*Department of Biomedical Research and Division of Hematology/Oncology, St. Elizabeth's Hospital, Tufts University School of Medicine, Boston, MA 02135; ‡Mt. Sinai Hospital, University of Toronto, Toronto, ON M5G 1X5, Canada; §Institute for Medical Research, Kuala Lumpur, Malaysia; ¶Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea; and ‖University of Papua New Guinea, Boroko, Papua New Guinea

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ABSTRACT **Southeast Asian ovalocytosis (SAO) is a hereditary condition that is widespread in parts of Southeast Asia. The ovalocytic erythrocytes are rigid and resistant to invasion by various malarial parasites. We have previously found that the underlying defect in SAO involves band 3 protein, the major transmembrane protein, which has abnormal structure and function. We now report two linked mutations in the erythrocyte band 3 gene in SAO: (*i*) a deletion of codons 400–408 and (*ii*) a substitution, A → G, in the first base of codon 56 leading to substitution of Lys-56 by Glu-56. The first defect leads to a deletion of nine amino acids in the boundary of cytoplasmic and membrane domains of band 3. This defect has been detected in all 30 ovalocytic subjects from Malaysia, the Philippines, and two unrelated coastal regions of Papua New Guinea, whereas it was absent in all 30 controls from Southeast Asia and 20 subjects of different ethnic origin from the United States. The Lys-56 → Glu substitution has likewise been found in all SAO subjects. However, it has also been detected in 5 of the 50 control subjects, suggesting that it represents a linked polymorphism. We conclude that the deletion of codons 400–408 in the band 3 gene constitutes the underlying molecular defect in SAO.**

Southeast Asian ovalocytosis (SAO) is an asymptomatic hereditary condition that is widespread in certain ethnic groups of Malaysia, Papua New Guinea, the Philippines, and Indonesia (1–3). Ovalocytic erythrocytes are rigid and exhibit reduced expression of many erythrocyte antigens (4, 5). A remarkable feature of ovalocytes is their resistance to invasion *in vitro* by several strains of malaria, including *Plasmodium falciparum* and *Plasmodium knowlesi* (6–8). Moreover, in areas of endemic malaria, the ovalocytic subjects contain reduced numbers of intracellular parasites *in vivo* (9).

In a recent communication (10), we reported that the underlying molecular defect in SAO involves the band 3 protein, the major transmembrane protein of the erythrocyte membrane. The ovalocyte band 3 had a markedly reduced lateral mobility in the membrane and it bound more tightly to ankyrin. Limited tryptic digestion of band 3 from subjects with SAO revealed an abnormal electrophoretic mobility of one of the tryptic peptides that was coinherited with the SAO phenotype.

Here we report that the underlying molecular defect in SAO is an intraexon deletion of codons 400–408 from the erythrocyte band 3 gene. This gene defect leads to a deletion of nine amino acids in the boundary of the cytoplasmic and membrane domains of the band 3 protein and is linked with the SAO phenotype in all of the 30 ovalocytic subjects examined.

MATERIALS AND METHODS

Subjects. Blood from SAO and control subjects was collected in sterile tubes containing citrate/phosphate/dextrose and shipped on ice to Boston. A total of 30 SAO subjects and 30 matched controls from Northern and Southern Papua New Guinea, Malaysia, and the Philippines was studied. These individuals included one family from Malaysia (4 SAO and 5 controls), one family from Papua New Guinea (6 SAO and 4 controls), two siblings from the Philippines (SAO and control), and 19 unrelated SAO and 20 control subjects. All 3 subjects from the Philippines are currently living in Canada. In addition, 20 randomly chosen unrelated White, Black, and Chinese individuals from the United States were examined. All SAO subjects contained 50% ovalocytes or more, some of which had a transverse ridge or a central or transverse slit.

Subcloning and Sequencing of SAO Band 3 cDNA. Total reticulocyte RNA, isolated as described (11), was reverse-transcribed using a band 3-specific PCR primer, P134 (5′-TCCGACACTCCCATCTGGTT-3′; bases 1316–1297), and PCR-amplified with primers P122 (5′-GGAACGAGTGGGAACGTA-3′; bases −150 through −133) and P134 using the Perkin–Elmer GeneAmp PCR reagents kit (35 cycles: 1 min at 94°C, 1 min at 46°C, 2 min at 72°C). An aliquot of the PCR product was cloned into plasmid pCR1000 using the TA cloning kit (Invitrogen, San Diego), and inserts were sequenced in their entirety with the T7 sequencing kit (Pharmacia) and a set of nested sequencing primers.

Sequencing of Genomic DNA. Genomic DNA was isolated from patients with SAO and controls as described (12), PCR-amplified using primers P183 (5′-TAGTGCTGCCTCCCACCGATG-3′; bases 956–976) and P159 (5′-GGTGACAGTGCAGCAAAGTAG-3′; bases 1256–1236), cloned, and sequenced as described above. PCR amplification was 35 cycles: 1 min at 94°C, 2 min to 50°C, 1 min at 50°C, and 30 sec at 72°C.

PCR Amplification of Genomic DNA and cDNA in SAO and Control Subjects. PCR amplification of genomic DNA and/or cDNA was used for detection of the mutation in SAO subjects and controls. PCR amplification was as follows. Genomic DNA: primers P198 (5′-GGGCCCAGATGACCCTCTGC-3′; bases 1098–1117) and P199 (5′-GCCGAAGGTGATGGCGGGTG-3′; bases 1272–1253), 30 cycles 1 min at 95°C, 1 min at 70°C; the size of the normal PCR product was 175 base pairs (bp). cDNA: primers P183 (bases 956–976) and P134 (bases 1316–1297), 30 cycles 1 min at 94°C, 2 min to 54°C, 1 min at 54°C, 30 sec at 72°C; the size of the normal PCR product was 361 bp.

Lack of Salt-Induced Shape Change (LISC). One of the features of SAO erythrocytes and their ghosts is their resistance to changes in shape, as induced by echinocytogenic

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Abbreviations: SAO, Southeast Asian ovalocytosis; LISC, lack of salt-induced shape change.

†To whom reprint requests should be addressed.

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stimuli, including changes in salt concentration. The shape response of ghosts to changes in salt concentration was examined as described (10) with the following modification: 10 μl of blood was lysed in 0.1 ml of distilled water and, after 1 min, 0.9 ml of isotonic saline was added. After another minute, the ghosts were fixed with 0.25% glutaraldehyde and examined with a phase-contrast microscope. Whereas control erythrocytes shrank and became echinocytic (LISC negative), the SAO erythrocyte ghosts retained their original size and smooth contours (LISC positive).

RESULTS

SAO Band 3 cDNA Contains Two Linked Mutations: Substitution AAG → GAG (Lys-Glu) in Codon 56 and Deletion of Codons 400–408. We have isolated total reticulocyte RNA from a subject with SAO and a matched control from Papua New Guinea and cloned and completely sequenced cDNA corresponding to the entire cdb3, the first transmembrane and ectoplasmic segments, and an additional 150 bases in the 5′ untranslated region (bases −150 through 1316). All control clones and ≈50% of SAO clones had the expected size of 1466 bp and the previously published sequence (13). In contrast, the remaining SAO clones were found to be shorter by 27 bp and to contain two mutations (Fig. 1): a substitution, A → G, in nucleotide 166 (codon 56 AAG → GAG, Lys → Glu) and a deletion of 27 bases (bp 1198–1224) corresponding to amino acids 400–408 (AFSPQVLAA) that are located at the junction of cytoplasmic and membrane domains of band 3. We have verified by sequence analysis the presence of both mutations in four additional unrelated SAO subjects from two different region of Papua New Guinea, one SAO subject from the Philippines, and one SAO subject from Malaysia. PCR amplification of cDNA coding for the rest of the membrane domain of band 3 did not reveal any additional deletion or insertion (not shown).

The Nine-Codon Deletion Represents an Intraexon Deletion. To decide whether the deletion of amino acids 400–408 is due to abnormal mRNA processing or is present at the gene level, we have studied the organization of the part of band 3 gene coding for amino acids 320–436. This region consisted of one full exon and parts of two other exons with two intervening introns of 233 and 180 bp, respectively (Fig. 2*A*). Positions and sequences of the exon–intron boundaries were compared with those for band 3 exons 10, 11, and 12 in mouse (14) and, because of the close homology, human exons and introns were provisionally numbered as those in the mouse erythrocyte band 3 gene. Sequencing again revealed a deletion of 27 bases (cDNA bases 1198–1224) from exon eM11 in one allele of SAO subjects. The corresponding cDNA clone is depicted in Fig. 2*B* and the position of the two mutations in the band 3 protein is schematically shown in Fig. 2*C*.

The Nine-Codon Deletion Is Linked to the SAO Phenotype. We have used PCR amplification of genomic DNA and/or total reticulocyte RNA for the testing of additional subjects. PCR with primers flanking the deletion produced a single band in controls and two bands differing in size by 27 bp in SAO heterozygotes (Fig. 3). We have detected the 27-bp deletion in all of the 30 ovalocytic subjects from different areas of Southeast Asia. PCR amplification of cDNA yields similar intensities of bands corresponding to individual band 3 alleles, indicating synthesis of similar amounts of mRNA for both alleles. In contrast, PCR amplification of genomic DNA gives higher intensity of the normal product. This could be due to simultaneous amplification of genes for the band 3-related proteins (AE2 and AE3) that are, by comparison with those in mouse (15) and rat (16), highly homologous with the erythroid band 3 (AE1) gene within the amplified region of exon eM11.

In an attempt to correlate the band 3 gene deletion with a functional manifestation, we have examined the previously described phenomenon of resistance of ovalocytes to crenation, as revealed by LISC (6, 10, 17). In all 40 subjects studied (19 SAO and 21 control subjects), the positive result of the LISC test correlated with the SAO genotype (Fig. 3).

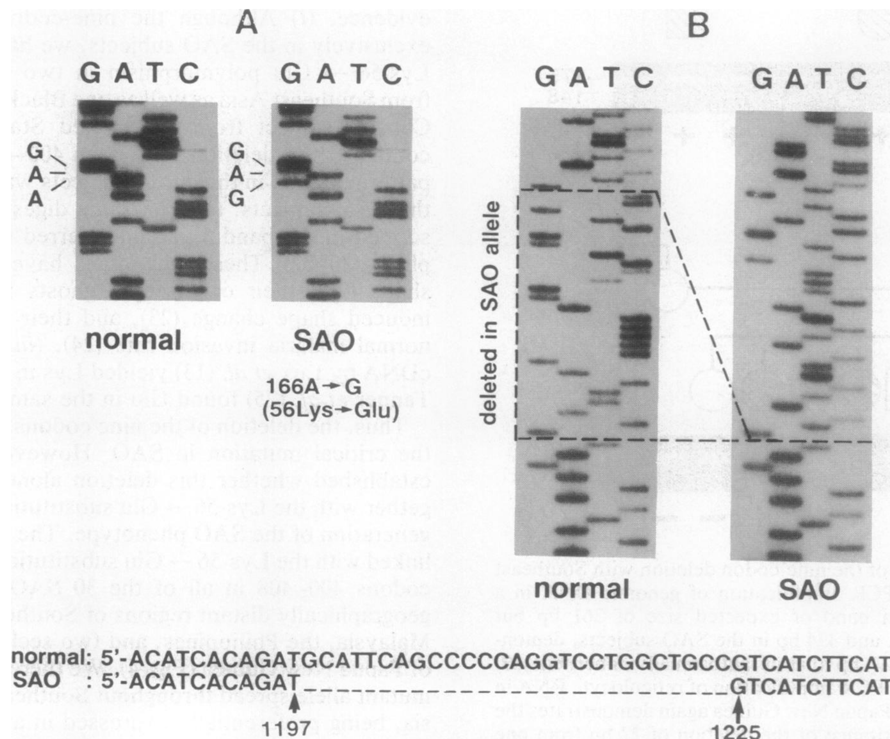

FIG. 1. Sequencing autoradiograms of cDNA from both band 3 alleles in SAO. (*A*) Substitution A → G in nucleotide 166, changing the codon for lysine 56 (AAG) to glutamic acid (GAG). (*B*) Deletion of codons 400–408.

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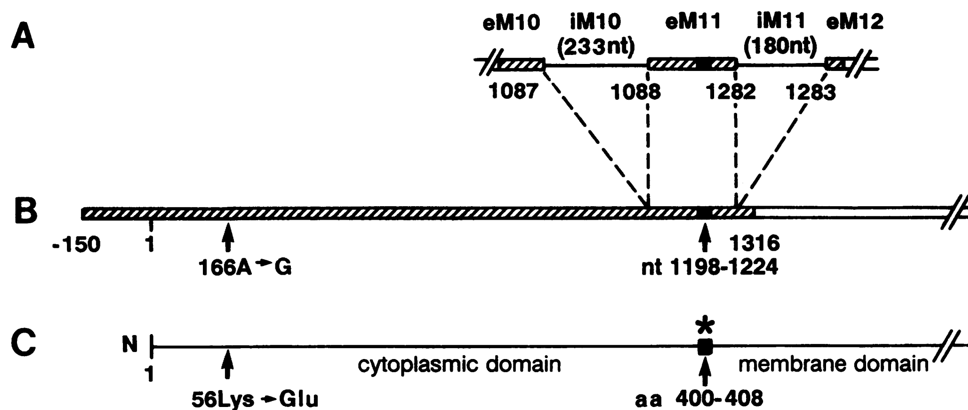

FIG. 2. Schematic diagram of the underlying defect of the SAO band 3 protein at the level of genomic DNA, cDNA, and the band 3 protein. The deletion of nine codons is detected in genomic DNA and in reticulocyte RNA of SAO subjects. (*A*) Intraexon deletion (black box) in a 195-nucleotide (nt) exon, eM11. The PCR-amplified and sequenced part of the gene is hatched. (*B*) Schematic representation of the cDNA clone containing the nt 166 A → G polymorphism and the nt 1198–1224 deletion. The region of cDNA that was PCR-amplified and sequenced is hatched. (*C*) Schematic depiction of a part of the band 3 protein with positions of the b3M polymorphism (Lys → Glu) in position 56 and the SAO-specific deletion of amino acids (aa) 400–408. This deletion is localized at the boundary (marked by asterisk) of cytoplasmic and membrane domains of band 3 that was deduced from the hydropathy plot (13).

The Substitution Lys-56 → Glu Represents a Linked Polymorphism, Whereas the Deletion of Codons 400–408 Is Unique to SAO. Although the nine-codon deletion was found to be unique for the SAO subjects, we have detected the Lys-56 → Glu substitution not only in all SAO subjects but also in two unrelated normocytic controls from Southeast Asia and in three controls from the United States. None of these five individuals contained the deletion of codons 400–408 and their erythrocyte ghosts exhibited normal salt-induced crenation.

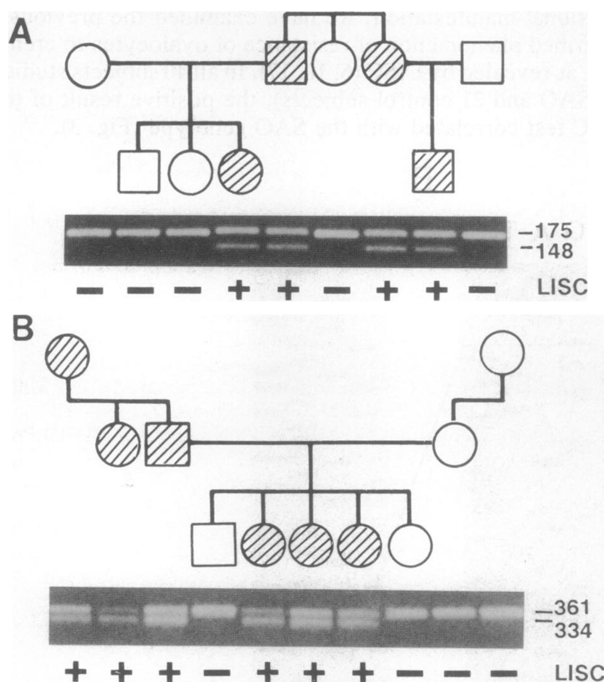

FIG. 3. Coinheritance of the nine-codon deletion with Southeast Asian ovalocytosis. (*A*) PCR amplification of genomic DNA in a Malay family produces a band of expected size of 361 bp but produces a doublet of 361 and 334 bp in the SAO subjects, demonstrating the deletion of 27 bp that is autosomally inherited. (*B*) Reverse transcription and PCR amplification of reticulocyte RNA in a family from the north of Papua New Guinea again demonstrates the autosomal dominant inheritance of the deletion of 27 bp from one allele in SAO (doublet of 175 and 148 bp vs. single band of 175 bp in controls). The band 3 gene deletion is linked with the LISC in SAO erythrocytes.

DISCUSSION

Our laboratory and others have previously reported abnormalities of the band 3 protein in SAO as revealed by abnormal limited trypsin and papain digestion, tighter ankyrin-band 3 binding (10), decreased lateral and rotational mobility (10, 18), increased phosphorylation, and an N-terminal extension (19). The structural abnormality of the band 3 protein, as revealed by trypsin digestion, was tightly linked with SAO (10). We now report the presence of two linked mutations of the SAO band 3 gene: (*i*) substitution A → G in nucleotide 166, changing the codon for lysine 56 (AAG) to glutamic acid (GAG), and (*ii*) deletion of codons 400–408.

We suggest that the nine-codon deletion constitutes the underlying molecular defect in SAO, whereas the Lys-56 → Glu represents a linked polymorphism based on the following evidence. (*i*) Although the nine-codon deletion is found exclusively in the SAO subjects, we have also detected the Lys-56 → Glu polymorphism in two normocytic controls from Southeast Asia as well as one Black, one White, and one Chinese subject from the United States, none of whom contained the deletion of codons 400–408. The proteolytic pattern of cdb3 in the above subjects was identical to that of the SAO subjects. (*ii*) The same digestion pattern was described in the band 3 variant referred to as "band 3 Memphis" (20–22). These individuals have normal erythrocyte shape (20), their erythrocyte ghosts have a normal salt-induced shape change (23), and their erythrocytes have a normal malaria invasion rate (24). (*iii*) Cloning of band 3 cDNA by Lux *et al.* (13) yielded Lys in position 56, whereas Tanner *et al.* (25) found Glu in the same position.

Thus, the deletion of the nine codons appears to represent the critical mutation in SAO. However, it remains to be established whether this deletion alone or its presence together with the Lys-56 → Glu substitution is required for the generation of the SAO phenotype. The SAO phenotype was linked with the Lys-56 → Glu substitution and the deletion of codons 400–408 in all of the 30 SAO subjects from four geographically distant regions of Southeast Asian, including Malaysia, the Philippines, and two secluded coastal regions of Papua New Guinea (Fig. 4). We therefore believe that this mutant allele spread throughout Southeast Asia and Melanesia, being preferentially expressed in areas of endemic malaria because of the survival advantage resulting from the resistance of SAO erythrocytes to invasion by malarial parasites (6–9).

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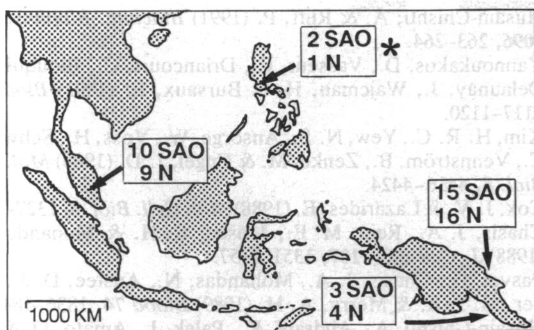

FIG. 4. Deletion of codons 400–408 is detected in SAO subjects from geographically unrelated areas of Southeast Asia. The number of examined SAO subjects vs. the number of matched controls from each of the four geographic locations under study is shown. In all 30 SAO subjects studied, the normal and deletion-containing alleles were detected by PCR amplification of genomic DNA and/or reticulocyte RNA. Moreover, the presence of the deletion of codons 400–408 was verified by sequencing of either cDNA or genomic DNA in seven unrelated SAO subjects (at least one SAO subject from each location). The deletion has not been detected in any of the 30 matched controls from Southeast Asia and, likewise, in any of 20 unrelated randomly chosen White, Black, and Chinese subjects from the United States.

Normal band 3 protein has a blocked N terminus (26). In contrast, SAO band 3 was reported to be accessible to Edman degradation and to have an extended N terminus. This N-terminal extension was proposed to account for the abnormal proteolytic digestion of the band 3 protein in SAO erythrocytes (19). Such digestion gives rise to two fragments: the proteolytic fragment of a normal size (the product of the normal allele) and a fragment elongated by about 3 kilodaltons (the product of the mutant allele). However, we believe that the N-terminal extension reported above (19) corresponds to a parasite protein contaminant based on the following results: (*i*) we have found a completely normal sequence in the vicinity of the translation site in 14 cDNA clones of both alleles of seven unrelated SAO subjects, (*ii*) the reported subjects (19) lived in a malaria-infested area of Papua New Guinea and the amino acid sequence of the elongated fragment was almost identical to that of a part of ring-infected erythrocyte surface antigen of *P. falciparum* (27, 28), (*iii*) PCR with a degenerate upstream primer corresponding to the reported amino acid sequence and a set of downstream band 3 primers did not produce any abnormal clone (not shown), (*iv*) the "elongated" tryptic fragment is found in the SAO subjects and in the asymptomatic carriers of band 3 Memphis (29), suggesting that it is related to the Gly-56 → Lys mutation. Indeed, in the SAO erythrocytes and in the erythrocytes of the asymptomatic band 3 Memphis carriers, electrophoresis of membrane proteins in the presence of urea abolishes differences in electrophoretic mobility between the normal and the abnormal slowly migrating cdb3 fragment, whereas it does not considerably affect the electrophoretic mobility of other erythrocyte proteins (5, 23). In agreement with the above findings, we have found that in the SAO subjects living in Canada, who have not come in contact with malaria, the N terminus of the band 3 is blocked, as is the case of the normal band 3 protein (26).

The mechanism by which the deletion of nine codons either alone or in conjunction with the Lys-56 → Gly substitution confers rigidity of the ovalocyte membranes as well as the malaria resistance is presently unclear. The deletion of codons 400–408 involves a region of band 3 cDNA that is highly conserved throughout species (13–16, 25, 30, 31) and is thus likely essential for the normal band 3 function. One of the attractive possibilities that needs experimental verification is that the membrane rigidification is a consequence of a tight binding of the SAO band 3 to ankyrin; the ensuing skeletal immobilization may preclude cell deformation. Such a possibility is supported by recent studies revealing that binding of antibodies to the exoplasmic part of another transmembrane protein, glycophorin A, markedly decreases erythrocyte deformability (32) and inhibits malarial parasite invasion (33). The former effect was abrogated in the cells containing mutant glycophorin A that was devoid of the cytoplasmic portion (Miltenberger V erythrocytes), suggesting that the increase in rigidity is mediated by interaction of the cytoplasmic domain of glycophorin A with the membrane skeleton. It is likewise of considerable interest that one or more tyrosine residues in the SAO band 3 protein is excessively phosphorylated (19, 34). However, it is presently not clear whether such band 3 hyperphosphorylation alters band 3 function, such as its binding to ankyrin, and the hyperphosphorylated residues remain to be defined.

The mechanism of malaria resistance remains likewise speculative. During the process of invasion, a profound redistribution of erythrocyte skeletal proteins takes place. Labeling with anti-band 3 antibodies revealed that the band 3 protein had been eliminated from the region of the host cell membrane in contact with the parasite (35). Similarly, the parasitophorous vacuole membrane was found to be practically devoid of spectrin, ankyrin, band 3, and glycophorin A (36). It is therefore possible that the observed marked decrease in lateral and rotational mobility of SAO band 3 protein (10, 18) precludes the redistribution of band 3, thereby interfering with the entry of malaria parasites into the cells.

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1. Lie-Injo, L. E. (1965) *Nature (London)* **208,** 1329.
2. Amato, D. & Booth, P. B. (1977) *Papua New Guinea Med. J.* **20,** 26–32.
3. Serjeantson, S., Bryson, K., Amato, D. & Babona, D. (1977) *Hum. Genet.* **37,** 161–167.
4. Mohandas, N., Lie-Injo, L. E., Friedman, M. & Mak, J. W. (1984) *Blood* **63,** 1385–1392.
5. Booth, P. B., Serjeantson, S., Woodfield, D. G. & Amato, D. (1977) *Vox Sang.* **32,** 99.
6. Castelino, D., Saul, A., Myler, P., Kidson, C., Thomas, H. & Cooke, R. (1981) *Southeast Asian J. Trop. Med. Public Health* **12,** 549–555.
7. Hadley, T., Saul, A., Lamont, G., Hudson, D. E., Miller, L. H. & Kidson, C. (1983) *J. Clin. Invest.* **71,** 780–782.
8. Kidson, C., Lamont, G., Saul, A. & Nurse, G. T. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5829–5832.
9. Cattani, J. A., Gibson, F. D., Alpers, M. P. & Crane, G. G. (1987) *Trans. R. Soc. Trop. Med. Hyg.* **81,** 705–709.
10. Liu, S. C., Zhai, S., Palek, J., Golan, D. E., Amato, D., Hassan, K., Nurse, G. T., Babona, D., Coetzer, T., Jarolim, P., Zaik, M. & Borwein, S. (1990) *N. Engl. J. Med.* **323,** 1530–1538.
11. Goosens, M. & Kan, Y. W. (1981) *Methods Enzymol.* **76,** 805–817.
12. Sykes, B. G. (1983) *Lancet* **ii,** 787–788.
13. Lux, S. E., John, K. M., Kopito, R. R. & Lodish, H. F. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9089–9093.
14. Kopito, R. R., Anderson, M. & Lodish, H. F. (1987) *J. Biol. Chem.* **262,** 8035–8040.
15. Kopito, R. R., Lee, B. S., Simmons, D. M., Lindsey, A. E., Morgans, C. W. & Schneider, K. (1989) *Cell* **59,** 927–937.
16. Kudrycki, K. E., Newman, P. R. & Shull, G. E. (1990) *J. Biol. Chem.* **265,** 462–471.
17. Saul, A., Lamont, G., Sawyer, W. H. & Kidson, C. (1984) *J. Cell Biol.* **98,** 1348–1354.
18. Tilley, L., Nash, G. B., Jones, G. L. & Sawyer, W. H. (1991) *J. Membr. Biol.* **121,** 59–66.

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19. Jones, G. L., McLemore-Edmundson, H., Wesche, D. & Saul, A. (1991) *Biochim. Biophys. Acta* **1096,** 33–40.
20. Mueller, T. J. & Morrison, M. (1977) *J. Biol. Chem.* **252,** 6573–6576.
21. Ranney, H. M., Rosenberg, G. H., Morrison, M. & Mueller, T. J. (1990) *Br. J. Haematol.* **76,** 262–267.
22. Palatnik, M., Simoes, M. L. M. S., Alves, Z. M. S. & Laranjeira, N. S. M. (1990) *Hum. Genet.* **86,** 126–130.
23. Jarolim, P., Rubin, H. L., Mueller, T. J. & Palek, J. (1991) *Blood* **78,** Suppl. 1, abstr., in press.
24. Schulman, S., Roth, E. F., Cheng, B., Rybicki, A. C., Sussman, I. I., Wong, M., Wang, W., Ranney, H. M., Nagel, R. L. & Schwartz, R. S. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7339–7343.
25. Tanner, M. J. A., Martin, P. G. & High, S. (1988) *Biochem. J.* **256,** 703–712.
26. Drickamer, L. K. (1978) *J. Biol. Chem.* **253,** 7242–7248.
27. Favaloro, J. M., Coppel, R. L., Corcoran, L. M., Foote, S. J., Brown, G. V., Anders, R. F. & Kemp, D. J. (1986) *Nucleic Acids Res.* **14,** 8265–8277.
28. Husain-Chishti, A. & Ruff, P. (1991) *Biochim. Biophys. Acta* **1096,** 263–264.
29. Yannoukakos, D., Vasseur, C., Driancourt, C., Blouquit, Y., Delaunay, J., Wajcman, H. & Bursaux, E. (1991) *Blood* **78,** 1117–1120.
30. Kim, H. R. C., Yew, N. S., Ansorge, W., Voss, H., Schwager, C., Vennström, B., Zenke, M. & Engel, J. D. (1988) *Mol. Cell. Biol.* **8,** 4416–4424.
31. Cox, J. V. & Lazarides, E. (1988) *Mol. Cell. Biol.* **8,** 1327–1335.
32. Chasis, J. A., Reid, M. E., Jensen, R. H. & Mohandas, N. (1988) *J. Cell Biol.* **107,** 1351–1357.
33. Pasvol, G., Chasis, J. A., Mohandas, N., Anstee, D. J., Tanner, M. J. A. & Merry, A. H. (1989) *Blood* **74,** 1836–1843.
34. Husain-Chishti, A., Audrabi, K., Palek, J., Amato, D. & Liu, S. C. (1991) *Blood* **78,** Suppl. 1, abstr., in press.
35. Dluzewski, A. R., Fryer, P. R., Griffiths, S., Wilson, R. J. M. & Gratzer, W. B. (1989) *J. Cell Sci.* **92,** 691–699.
36. Atkinson, C. T., Aikawa, M., Perry, G., Fujino, T., Bennett, V., Davidson, E. A. & Howard, R. J. (1987) *Eur. J. Cell Biol.* **45,** 192–199.

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