Journal of Bioenergetics and Biomembranes, Vol. 27, No. 2, 1995

Molecular Defects of Erythroid 5-Aminolevulinate Synthase in X-Linked Sideroblastic Anemia

Sylvia S. Bottomley,[1] Brian K. May,[2] Timothy C. Cox,[2,4] Philip D. Cotter,[3] and David F. Bishop[3]

Received January 18, 1995; accepted February 1, 1995

The erythroid-specific isozyme of 5-aminolevulinate synthase (ALAS2), the first and rate-limiting enzyme of heme biosynthesis, is expressed concomitantly with the differentiation and maturation of the erythroid cell in order to accommodate generation of the large amounts of heme required for hemoglobin production. During the past few years the ALAS2 gene and its transcript have been characterized and the amino acid sequence of the enzyme deduced. The human genetic disorder X-linked sideroblastic anemia, previously postulated to be caused by defects of ALAS, has now been analyzed at the molecular and tissue-specific level. A heterogeneous group of point mutations in the catalytic domain of the ALAS2 enzyme has been found to cause the disorder. Impaired activity of recombinant mutant ALAS2 enzymes has also been demonstrated. Characterization of molecular defects in individuals with X-linked sideroblastic anemia has provided improved diagnosis for at-risk family members.

KEY WORDS: X-Linked sideroblastic anemia; heme biosynthesis; erythroid 5-aminolevulinate synthase; nucleotide substitution; pyridoxine-responsive.

INTRODUCTION

X-Linked sideroblastic anemia (XLSA) represents the most common form among the inherited types of the disorder (reviewed in Bottomley, 1993) and is not readily recognized in clinical practice. Its diagnostic hallmark, as in all sideroblastic anemias, is the presence of ring sideroblasts (erythroblasts with large, perinuclear, Prussian blue-positive granules representing amorphous iron deposits in mitochondria) in the bone marrow (Fig. 1). The erythrocytes derived from these cells are typically hypochromic and microcytic, reflecting the lack of a normal complement of hemoglobin. These morphologic features are regularly expressed in affected males and the resulting anemia varies from mild to severe. Female carriers of XLSA often show a dimorphic population of normal and aberrant erythrocytes. Less frequently they have a normal erythrocyte profile and only rarely exhibit the anemia, consistent with nearly complete lyonization of the mutant or normal alleles, respectively.

Several earlier observations implicated a defect in 5-aminolevulinate synthase (ALAS), the first enzyme of the heme synthetic pathway, as the cause of XLSA. Pyridoxine supplementation improved or corrected the hemoglobin levels in at least one-third of the cases (Horrigan and Harris, 1964; Bottomley, 1993). Incorporation of [^{14}C] glycine into heme in reticulocytes was reduced in a patient with pyridoxine-responsive sideroblastic anemia, suggesting a deficiency of ALAS (Vogler and Mingioli, 1965). Subsequently, bone marrow ALAS enzymatic activity was shown to be reduced in other cases (Aoki *et al.*, 1974) and could be enhanced or restored to normal by

[1] Department of Medicine, University of Oklahoma College of Medicine and Veterans Administration Medical Center, Oklahoma City, Oklahoma 73104.
[2] Department of Biochemistry, University of Adelaide, Adelaide, South Australia 5005.
[3] Department of Human Genetics, Mount Sinai School of Medicine, New York, New York 10029.
[4] Current address: Telethon Institute of Genetics and Medicine, San Raffaele International Biomedical Science Park, Milano, Italy, 20132.

0145-479X/95/0400-0161$07.50/0

the addition of its cofactor pyridoxal 5′-phosphate *in vitro* (Konopka and Hoffbrand, 1979) or only following the supplementation of pyridoxine *in vivo* (Aoki *et al.*, 1979).

Demonstration of erythroid and nonerythroid forms of ALAS in guinea pig tissues (Bishop *et al.*, 1981) and of immunologic differences between the enzyme isoforms in rat tissues (Yamamoto *et al.*, 1986) was followed by the isolation of an erythroid-specific ALAS gene (ALAS2) in the chicken (Riddle *et al.*, 1989) and in the mouse (Schoenhaut and Curtis, 1989). Subsequent cloning and sequencing of the human erythroid-specific gene (Bishop, 1990; Cox *et al.*, 1991) and its mapping to the X chromosome (Cox *et al.*, 1990; Bishop *et al.*, 1990) set the stage for the demonstrations that mutations in the ALAS2 gene cause XLSA (Cotter *et al.*, 1992a; Cox *et al.*, 1994; Cotter *et al.*, 1994).

HUMAN ERYTHROID 5-AMINOLEVULINATE SYNTHASE

5-Aminolevulinate synthase is a nuclear encoded enzyme, and following its synthesis it is targeted to the mitochondrion and imported into the mitochondrial

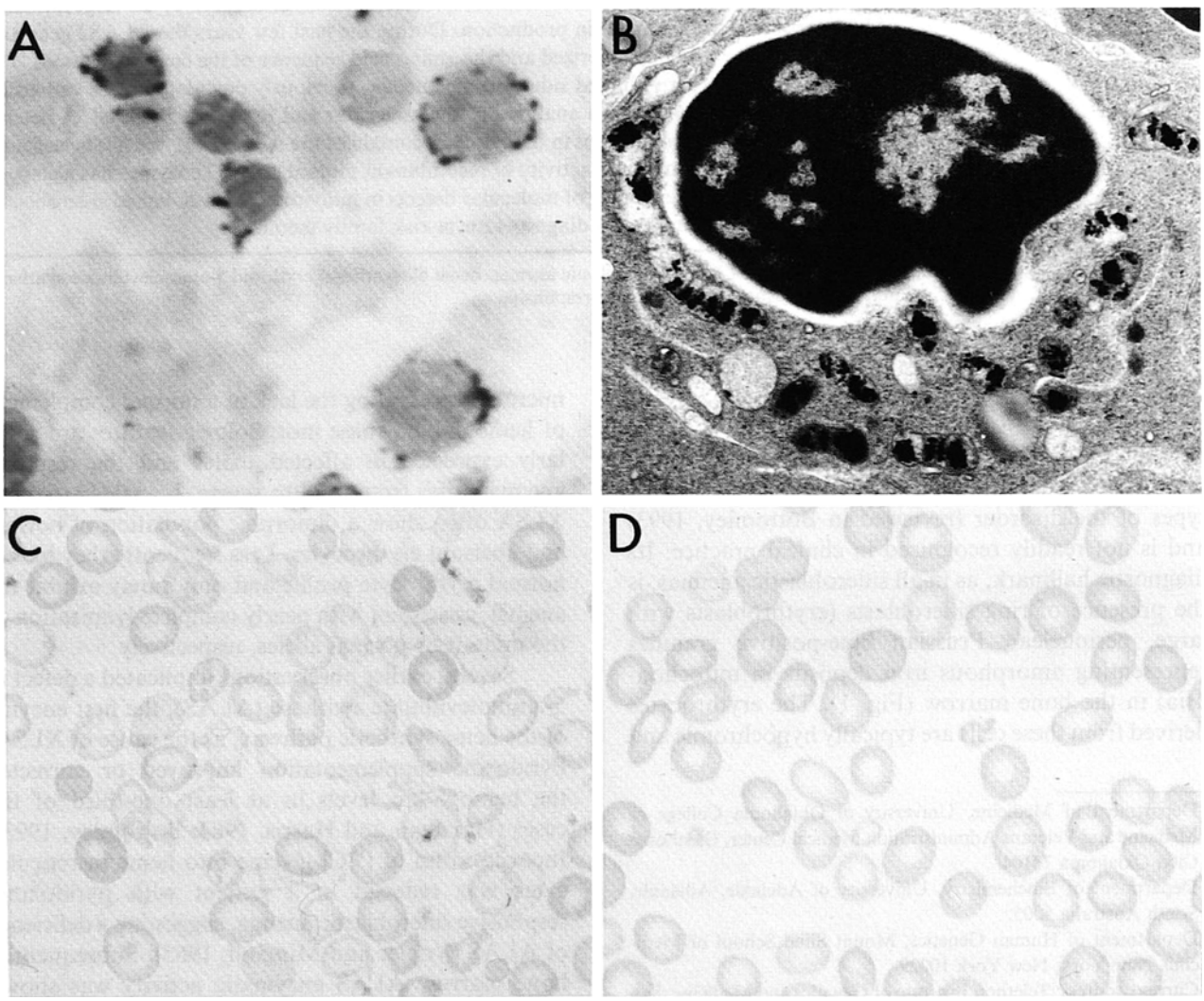

Fig. 1. The morphologic features of sideroblastic anemia. (A) Bone marrow smear (Prussian blue stain) with ring sideroblasts. (B) Electron micrograph of an erythroblast with iron-laden mitochondria. (C, D) Blood smears (Wright's stain) of severe and mild sideroblastic anemia.

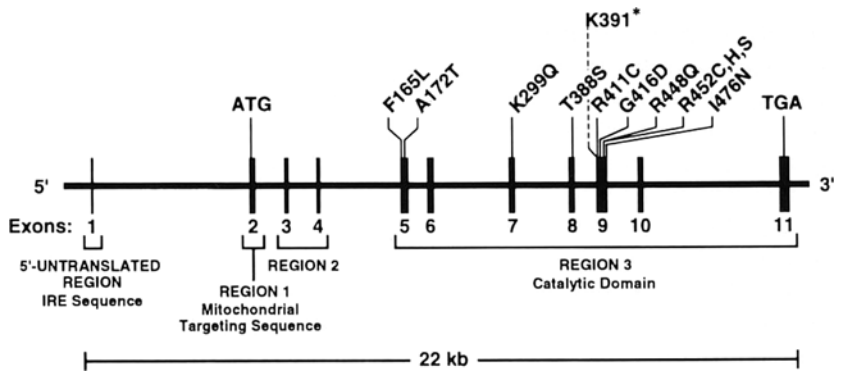

Fig. 2. The genomic organization of the human ALAS2 gene. The relative exon positions and encoding regions of the ALAS2 isozyme are indicated. IRE = iron-responsive element. The various ALAS2 mutations identified to date are shown above their corresponding exonic locations. *The pyridoxal 5′-phosphate binding lysine.

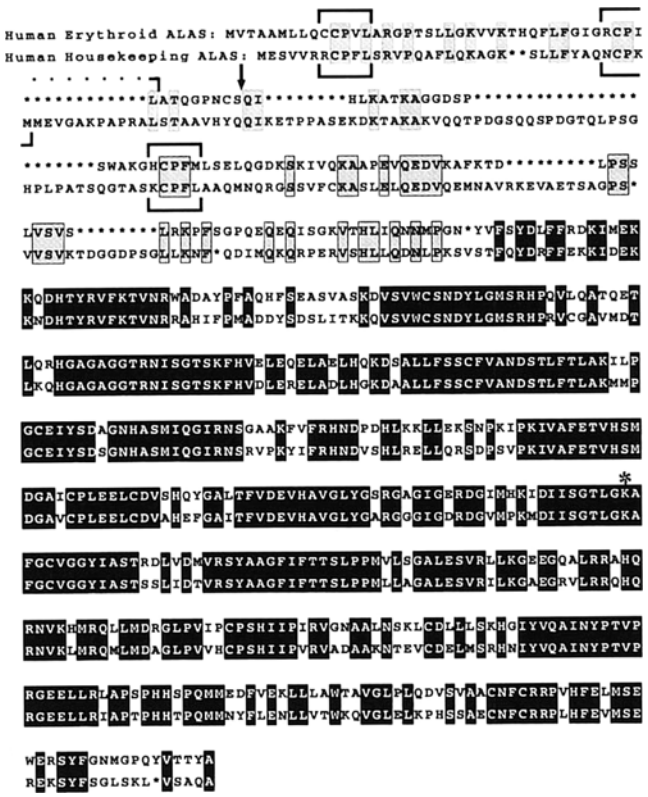

Fig. 3. The deduced amino acid sequences of human ALAS2 and ALAS1. The common amino acids are shaded. The highly conserved C-terminal domain is shown in bold. The putative proteolytic cleavage site is indicated by the arrow, and the active site lysine with an asterisk. Sequences in large brackets represent the putative heme-binding regions (Lathrop and Timko, 1993). (Modified from May *et al.*, 1995, with permission.)

matrix, its site of action (Jordan, 1991). The enzyme catalyzes the formation of 5-aminolevulinate from glycine and succinyl CoA and requires pyridoxal 5′-phosphate as a cofactor. Expression of the ALAS2 isozyme is developmentally regulated in erythroid cells, while heme negatively regulates the synthesis of the housekeeping ALAS isozyme (ALAS1), which is expressed in all tissues (May *et al.*, 1995). In man, the gene for ALAS2 is located at Xp11.21 (Cotter *et al.*, 1992c; Cox *et al.*, 1992) and the ALAS1 gene resides on chromosome 3 (3p21.1) (Sutherland *et al.*, 1988; Bishop *et al.*, 1990; Cotter *et al.*, 1995).

The human ALAS2 gene consists of 11 exons spanning 22 kb (Fig. 2) (Conboy *et al.*, 1992). Its promoter region contains several *cis*-acting elements identified in other erythroid-specific promoters, namely GATA-1, CACCC box, and NF-E2 binding sites (Cox *et al.*, 1991) that are not found in the ALAS1 gene (Braidotti *et al.*, 1993). The short 5′-untranslated region of the ALAS2 mRNA (predominantly encoded by exon 1) forms a secondary structure that confers iron-dependent regulation of translation to the mRNA (Cox *et al.*, 1991; Bhasker *et al.*, 1993). This structure is very similar to that of the iron-responsive element (IRE) first found and characterized in both ferritin and transferrin receptor mRNAs. The post-transcriptional regulation of these mRNAs is imparted by the iron-mediated binding of a cytosolic protein, the IRE-binding protein (IRE-BP), to the mRNAs (Klausner *et al.*, 1993). Exon 2 encodes the mitochondrial targeting presequence of the enzyme which, based on the known proteolytic cleavage site of the signal sequences for the chicken and rat ALAS1

isozymes (Borthwick *et al.*, 1985; Srivastava *et al.*, 1988), is predicted to be cleaved between the serine 49 and glutamine 50 residues (Fig. 3). Exons 5 to 11 encode the proposed catalytic domain of the protein. This domain is conserved in both human ALAS isozymes (Fig. 3) as well as throughout all species, including bacterial ALAS proteins (Cox *et al.*, 1991). Exons 3 and 4 encode the remaining N-terminal region of the mature ALAS protein. The role of this region is unknown; it is absent from bacterial ALAS proteins.

The human erythroid ALAS gene has four mRNA transcripts that are generated by alternative splicing and include the full-length transcript as well as three shorter ones lacking the sequences of exon 2, exon 4, and both of these exons, respectively (Conboy *et al.*, 1992; Bottomley *et al.*, 1994). These alternative transcripts are not differentiation-specific as they are present at all stages of erythroid development; the functional implication of these splicing events is as yet unclear. While the region encoded by exon 4 may be dispensable in that the recombinantly expressed protein lacking exon 4 retains full activity (Cox, unpublished data), this region may be important in modulating the function of the other domains of the protein by contributing to essential structure *in situ*. The mitochondrial signal sequence of the ALAS2 precursor protein, which is encoded by exon 2, contains the two heme-binding motifs (Fig. 3) implicated in regulating translocation of ALAS isozymes into mitochondria (Lathrop and Timko, 1993). ALAS2 transcripts lacking exon 2 may thus play a role in the modulation of ALAS2 import into mitochondria and thereby regulate heme synthesis.

With the distinctive features of erythroid ALAS so far observed, a model can be proposed for its pivotal role in regulating heme biosynthesis in developing erythroid cells (Fig. 4). In response to erythropoietin, transcription of the ALAS2 gene is activated together with genes for other heme pathway enzymes, the appropriate globin genes, and also the transferrin receptor gene (May *et al.*, 1995). Translation of ALAS2 mRNA, and thus protoporphyrin and heme formation, are linked to iron availability through the IRE-BP control of translation. Translation of globin chains is in turn coordinated with the supply of heme via inactivation of the eIF-2α protein kinase (or HRI) by heme to permit initiation of globin translation (Crosby *et al.*, 1994). On the other hand, heme in excess of that needed for assembly of globin chains into hemoglobin tetramers would inhibit translocation of ALAS2 into mitochondria and thus regulate its own synthesis. These post-transcriptional control mechanisms governing production of ALAS2, heme and globin would also remain important at the reticulocyte stage of erythroid cell maturation where up to one-third of the cell's hemoglobin continues to be synthesized from preexisting mRNAs.

MUTATIONS OF ERYTHROID 5-AMINOLEVULINATE SYNTHASE IN X-LINKED SIDEROBLASTIC ANEMIA

With the localization of the gene for erythroid ALAS to the X chromosome, the prediction was made that mutations of this gene could be responsible for at least some cases of XLSA (Cox *et al.*, 1990;

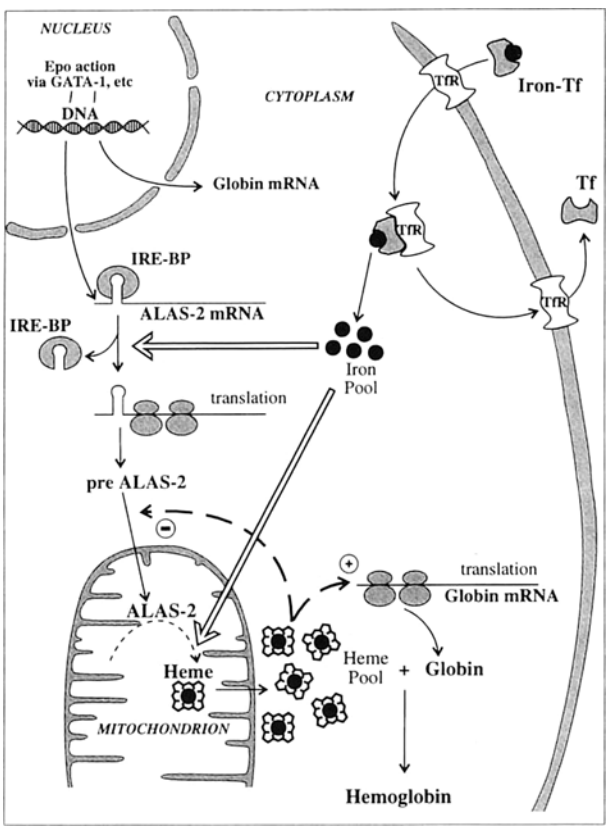

Fig. 4. A proposed model for the translational regulation of ALAS2. Erythropoietin (Epo) activates transcription of ALAS2 and other erythroid-specific genes. Tf = transferrin, TfR = transferrin receptor, IRE-BP = iron-responsive element binding protein. The iron pool regulates ALAS2 mRNA translation and is incorporated into protoporphyrin to give heme. The cytosolic heme pool may act in a negative fashion to regulate its own synthesis or regulate globin mRNA translation. (Modified from May *et al.*, 1995, with permission.)

Bishop *et al.*, 1990; May *et al.*, 1990) and was confirmed (Cotter *et al.*, 1992a; Cox *et al.*, 1994; Cotter *et al.*, 1994). To date, our laboratories have identified single base changes in the ALAS2 gene in 17 unrelated kindreds or individuals with the disorder (Table I). All the mutations thus far detected are located within exonic regions encoding the catalytic domain of the enzyme (Fig. 2). While two mutations reside in exon 5 and one in exon 7, the majority (82%) are clustered near the pyridoxal 5′-phosphate-binding lysine (*K391*, Fig. 2) which is located in exon 9 (Ferreira *et al.*, 1993). Four mutations in exon 9 were found in more than one kindred. In seven kindreds (41%), arginine 452 is replaced by cysteine, serine, or histidine due to base changes at nucleotide 1406 or 1407, representing the most common altered codon in the ALAS2 mRNA. Interestingly, mutations clustered between codons 416 and 452 appear to produce a milder phenotype than the other mutations. Eleven of the 17 kindreds (65%) had mutations in CpG dinucleotides of the sense or antisense strand. These sites are known to be mutational hotspots and are thought to be caused by methylation of the cytosine and subsequent spontaneous deamination to thymine (Coulondre *et al.*, 1978). A preponderance of mutations at these sites has been noted for other genes, e.g., the Factor VIII and α-galactosidase A genes in hemophilia A and Fabry disease, respectively (Youssoufian *et al.*, 1988; Eng and Desnick, 1994). Some of the ALAS2 mutations have also introduced restriction enzyme sites in the DNA sequence, e.g., an *Mse* I site (Cotter *et al.*, 1994), or deleted others. By using these restriction site changes or by engineering ones by mismatch PCR (Haliassos *et al.*, 1989) it has been possible to rapidly analyze family members of affected individuals for carrier status.

The overall heterogeneity of the mutations may be predicted to cause diverse effects on enzyme function and in turn the clinical phenotypes of XLSA, such as variable severity of anemia and disparate responses to pyridoxine supplements. For three mutants which have been expressed in prokaryotic systems (*Escherichia coli*), residual enzyme activity in the absence of pyridoxal 5′-phosphate ranged from 0% to 50% of the wild type: I476N = 0% (Cotter *et al.*, 1992a); T388S = 50% (Cox *et al.*, 1994); F165L = 13% (Cotter *et al.*, 1994). The activity of the mutant enzymes was significantly enhanced by pyridoxal 5′-phosphate but was never restored to the level of the wild type enzyme. In two cases these observations correlated with the enzyme activities obtained in crude marrow cell lysates of the probands (Cox *et al.*, 1994; Bottomley, unpublished data). However, the severity of the patients' anemia was only loosely correlated with residual recombinant enzyme activity, with or without added pyridoxal 5′-phosphate, probably due to other *in situ* factors that would be absent in the prokaryotic expression systems as well as differences in the expression systems used. Both the I476N and T388S mutations were expressed in the pET expression system (Novagen,

Table I. Single-Base Substitutions Found in the ALAS2 Gene of Unrelated Kindreds with X-Linked Sideroblastic Anemia

Exon	Base change	Amino acid change	Number of kindreds	Severity	Response to pyridoxine	Reference
5	C547A	F165L	1	Severe	Partial	(Cotter *et al.*, 1994)
5	G566A	A172T	1	Severe	Complete	(May *et al.*, 1994)
7	A947C	K299Q	1	Severe	Complete	(Cotter *et al.*, 1992b)
8	C1215G	T388S	1	Severe	Complete	(Cox *et al.*, 1994)
9	C1283T	R411C	1	Severe	Partial	(Cotter *et al.*, 1992b)
9	G1299A	G416D	1	Moderate	Partial	(Bottomley *et al.*, 1993)
9	G1395A	R448Q	3	Mild to severe	None/partial	(Bottomley *et al.*, 1993) (Bishop, unpublished)
9	C1406A	R452S	2	Moderate	Partial	(Bottomley, unpublished)
9	C1406T	R452C	3	Mild	Partial/?	(Bishop, unpublished) (Bottomley, unpublished) (May, B. K., unpublished)
9	G1407A	R452H	2	Mild	None/partial	(Bottomley *et al.*, 1993) (Bishop, unpublished)
9	T1479A	I476N	1	Severe	Complete	(Cotter *et al.*, 1992a)

Madison, Wisconsin) using Gln 50 as the amino terminus. In this system the majority of the recombinant enzyme was in the form of inclusion bodies with only a small fraction remaining soluble. Wild type ALAS2 activity from crude lysates ranged from 70 nmol/hr/mg (Cox *et al.*, 1994) to 195 nmol/hr/mg (Cotter *et al.*, 1992a). The F165L mutation was expressed as a fusion protein allowing for affinity purification of soluble protein to a specific activity of about 16,000 nmol/hr/mg for characterization studies (Cotter *et al.*, 1994). While these mutations predicted altered secondary structure, precisely how any one mutation affects the function of the enzyme and ultimately the clinical phenotype will require stability studies and kinetic analysis of the purified mutant proteins and correlations with alterations in their three-dimensional structures.

POLYMORPHISMS IN ERYTHROID 5-AMINOLEVULINATE SYNTHASE

Two polymorphic sites have been detected in the ALAS2 gene and have been useful for analysis of inherited sideroblastic anemia at the molecular level and for establishing or excluding linkage of the gene to XLSA. One site within intron 7 of the ALAS2 gene is a highly polymorphic compound dinucleotide repeat sequence, or microsatellite, with an expected heterozygosity of 78% and nine alleles identified to date (Cox *et al.*, 1992). With this marker, linkage to the disorder could be established in several large three-generation XLSA kindreds, and female carriers as well as affected males with a mild or questionable clinical phenotype could be detected rapidly (Cox *et al.*, 1994; Bottomley and Cox, unpublished data). Use of this polymorphism also led to exclusion of linkage of ALAS2 in one large four-generation kindred and thus strengthened the evidence for a different X-linked locus underlying the rare disorder, XLSA with ataxia (XLSA/A) (Cox *et al.*, 1992).

A second polymorphism in the 3′ region of the ALAS2 gene, a G-to-A transition that is 220 nucleotides 3′ of the AATAAA polyadenylation signal, has a gene frequency of 25% and was useful in excluding linkage of ALAS2 to the sideroblastic anemia in one family (Jardine *et al.*, 1994). In addition, two rare alleles, both silent mutations, have been identified; one is in exon 10 (C1612A, P520P) and the other in exon 11 (T1705A, S551S) (Bishop, unpublished data).

PATHOPHYSIOLOGY OF HEMOGLOBIN SYNTHESIS AND ERYTHROPOIESIS DUE TO DEFECTIVE ERYTHROID 5-AMINOLEVULINATE SYNTHASE

Since the ALAS-catalyzed reaction is the rate-limiting one of the heme biosynthetic pathway (Bottomley and Muller-Eberhard, 1988) a mutant ALAS2 with lowered activity would be expected to reduce the production of protoporphyrin and heme in the erythroid cell and, in turn, result in diminished production of globin chains. Evidence indicates that in sideroblastic anemia iron continues to enter the erythroid cell and is transferred to mitochondria at a normal rate in the face of its reduced utilization (Bottomley, 1988). In this setting, a likely surfeit of cytosolic iron may increase the rate of translation of the ALAS2 mRNA, and such a response may account for the elevated levels of ALAS activity observed in erythroid cells of several cases with XLSA, before or after pyridoxine supplementation (Bottomley *et al.*, 1992). The observation of reduced erythroblast ALAS activity in iron-deficient erythropoiesis would be consistent with such a control mechanism (Houston *et al.*, 1991). With reduced heme levels it may also be predicted that there would be an increase in the import of the mutant ALAS2 protein precursor from its site of synthesis in the cytosol into the mitochondria (Lathrop and Timko, 1993). On the other hand, high levels of iron in the mitochondria may inhibit the import of ALAS2 protein or its activity *in situ* so that the activity of mitochondrial ALAS2 remains inadequate. In most of the cases with identified ALAS2 mutations at least some response to pyridoxine has been observed. In these, greater than physiologic levels of pyridoxal 5′-phosphate may compensate for a reduced affinity of the mutant enzyme for the cofactor, enhance its stability, or facilitate its correct folding during synthesis, thus maintaining sufficient enzyme levels to lessen the heme deficit in the developing erythroid cell. Nonetheless, residual phenotypic features of the disorder uniformly remain, as reflected in the persistence of some erythrocytic microcytosis, with or without residual ring sideroblasts in the bone marrow even when an optimal hemoglobin response to pyridoxine supplementation occurs (Bottomley, 1993).

The formation of ALAS1 mRNA in normal erythroid cells is probably less than 1% of the erythroid transcript and may be even further repressed by heme during erythropoiesis. In the ring sideroblast, however,

it is possible that in the low-heme environment synthesis of this isozyme is derepressed, analogous to the effects of inhibited heme synthesis during chemically induced erythroid differentiation in the murine erythroleukemia cell culture model (Fujita *et al.*, 1991). This was suggested by findings of increased levels of mRNA for ALAS1 along with raised levels of erythroblast ALAS activity in two cases with XLSA (Bottomley *et al.*, 1992). Yet induction of this isozyme is also unable to fully compensate for defective ALAS2.

As in other erythropoietic disorders with defective cytoplasmic maturation (e.g., thalassemias), the consequence of the poor hemoglobinization of developing erythroblasts is their destruction in the marrow environment to the extent that the marrow's capacity to compensate is exceeded and anemia results (Horrigan and Harris, 1964). By a completely unknown mechanism this ineffective erythropoiesis is regularly associated with increased intestinal iron absorption and a consequent inexorable parenchymal iron overload which is phenotypically indistinguishable from hereditary hemochromatosis (Hathaway and Harris, 1967). This latter process does not always correlate with severity of the sideroblastic anemia (Peto *et al.*, 1983) and is the major factor limiting survival unless managed early (Bottomley, 1991).

CONCLUSION

Molecular studies have uncovered heterogeneous mutations in the ALAS2 gene as the underlying defects in XLSA and permitted identification of the defects in relatives at risk. Polymorphisms in the gene have proven useful for establishing or excluding genetic linkage of ALAS2 to the clinical disorder and for detection of individuals with a mild phenotype and female carriers. Detailed characterization of effects of the mutations on the function of the enzyme and elucidation of the regulation of ALAS2 at the transcriptional, translational, and mitochondrial levels are the focus of current research. In addition, further insights into the regulation of iron metabolism in normal and sideroblastic erythroid cells, as well as for any role of the ALAS1 isozyme when ALAS2 is defective, will be needed to understand the full pathophysiology of the disrupted heme biosynthesis in XLSA. Identification of molecular defects in cases with severe disease and unresponsiveness to pyridoxine should warrant initiation of gene therapy approaches to correct the disorder.

ACKNOWLEDGMENTS

This work was supported by the Department of Veterans Affairs to S.S.B., a grant from the National Health and Medical Research Council of Australia to B.K.M., a Biomedical Postgraduate Scholarship from the National Health and Medical Research Council of Australia to T.C.C., a Predoctoral Graduate Training Fellowship from the March of Dimes Birth Defects Foundation to P.D.C., and by a grant (R01 DK408950) from the National Institutes of Health to D.F.B.

REFERENCES

Aoki, Y., Urata, G., Wada, O., and Takaku, F. (1974). *J. Clin. Invest.* **53,** 1326–1334.

Aoki, Y., Maranaka, S., Nakabayashi, K., and Ueda, Y. (1979). *J. Clin. Invest.* **64,** 1196–1203.

Bhasker, C. R., Burgiel, G., Neupert, B., Emery-Goodman, A., Kuhn, L. C., and May, B. K. (1993). *J. Biol. Chem.* **268,** 12699–12705.

Bishop, D. F. (1990). *Nucleic Acids Res.* **18,** 7187–7188.

Bishop, D. F., Kitchen, H., and Wood, W. A. (1981). *Arch. Biochem. Biophys.* **206,** 380–391.

Bishop. D. F., Henderson, A. S., and Astrin, K. H. (1990). *Genomics* **7,** 207–214.

Borthwick, I. A., Srivastava, G., Day, A. R., Pirola, B. A., Snoswell, M. A., May, B. K., and Elliott, W. H. (1985). *Eur. J. Biochem.* **150,** 481–484.

Bottomley, S. S. (1988). *Ann. N.Y. Acad. Sci.* **526,** 331–332.

Bottomley, S. S. (1991). *Hosp. Pract.* **26,** (Suppl. 3), 55–56.

Bottomley, S. S. (1993). In *Wintrobe's Clinical Hematology*, 9th edn. (Lee, G. R., Bithell, T. C., Foerster, J., Athens, J. W., and Lukens, J. N., eds.), Lea & Febiger, Philadephia, pp. 852–871.

Bottomley, S. S., and Muller-Eberhard, U. (1988). *Sem. Hematol.* **25,** 282–302.

Bottomley, S. S., Healy H. M., Brandenburg, M. A., and May, B. K. (1992). *Am. J. Hematol* **41,** 76–83.

Bottomley, S. S., Wise, P. D., Whetsell, L. H., and Schaefer, F. V. (1993). *Blood* **82** (Suppl. 1), 433a.

Bottomley, S. S., Wise, P. D., Whetsell, L. H., and Schaefer, F. V. (1994). *Blood* **84** (Suppl. 1), 361a.

Braidotti, G., Borthwick, I. A., and May, B. K. (1993). *J. Biol. Chem.* **268,** 1109–1117.

Conboy, J. G., Cox, T. C., Bottomley, S. S., Bawden, M. J., and May, B. K. (1992). *J. Biol. Chem.* **267,** 18753–18758.

Cotter, P. D., Baumann, M., and Bishop, D. F. (1992a). *Proc. Natl. Acad. Sci. USA* **89,** 4028–4032.

Cotter, P. D., Baumann, M., Rucknagel, D. L., Fitzsimons, E. J., May, A., and Bishop, D. F. (1992b). *Am. J. Hum. Genet.* **51** (Suppl. 1), A45.

Cotter, P. D., Willard, H. F., Gorski, J. L., and Bishop, D. F. (1992c). *Genomics* **13,** 211–212.

Cotter, P. D., Rucknagel, D. L., and Bishop, D. F. (1994). *Blood* **84,** 3915–3924.

Cotter, P. D., Drabkin, H. A., Varkony, T., Smith, D. I., and Bishop, D. F. (1995). *Cytogenet. Cell Genet.* **69,** 207–208.

Coulondre, C., Miller, J. H., Farabaugh, P. J., and Gilbert, W. (1978). *Nature (London)* **274,** 775–780.

Cox, T. C., Bawden, M. J., Abraham, N. G., Bottomley, S. S., May, B. K., Baker, E., Chen, L. Z., and Sutherland, G. R. (1990). *Am. J. Hum. Genet.* **46,** 107–111.

Cox, T. C., Bawden, M. J., Martin, A., and May, B. K. (1991). *EMBO J.* **10,** 1891-1902.

Cox, T. C., Kozman, H. M., Raskind, W. H., May, B. K., and Mulley J. C. (1992). *Hum. Mol. Genet.* **1,** 639–641.

Cox, T. C., Bottomley, S. S., Wiley, J. S., Bawden, M. J., Matthews, C. S., and May, B. K. (1994). *N. Engl. J. Med.* **330,** 675–679.

Crosby, J. S., Lee, K., London, I. M., and Chen , J. (1994). *Mol. Cell. Biol.* **14,** 3906–3914.

Eng, C. M., and Desnick, R. J. (1994). *Hum. Mutat.* **3,** 103–111.

Ferreira, G. C., Neame, P. J., and Dailey, H. A. (1993). *Protein Sci.* **2,** 1959–1965.

Fujita, H., Yamamoto, M., Yamagami, T., Hayashi, N., and Sassa, S. (1991). *J. Biol. Chem.* **266,** 17494–17502.

Haliassos, A., Chomel, J. C., Tesson, L., Baudis, M., Kruth, J., Kaplan, J. C., and Kitzis, A. (1989). *Nucleic Acids Res.* **17,** 3606.

Hathaway, D., Harris, J. W., and Stenger, R. J. (1967). *Arch. Pathol. Lab. Med.* **83,** 175–179.

Horrigan, D. L., and Harris, J. W. (1964). *Adv. Int. Med.* **12,** 103–174.

Houston, T., Moore, M. R., McColl, K. E. L., and Fitzsimons, E. (1991). *Br. J. Haematol.* **78,** 561–564.

Jardine, P. E., Cotter, P. D., Johnson, S. A., Fitzsimons, E. J., Tyfield, L., Lunt, P. W., and Bishop, D. F. (1994). *J. Med. Genet.* **31,** 213–218.

Jordan, P. M. (1991). In *Biosynthesis of Tetrapyrroles*, Vol. 19 of New Comprehensive Biochemistry (Jordan, P. M., ed.), Elsevier, Amsterdam, pp. 1–66.

Klausner, R. D., Roualt, T. A., and Harford, J. B. (1993). *Cell* **72,** 19–28.

Konopka, L., and Hoffbrand, A. V. (1979). *Br. J. Haematol.* **42,** 73–83.

Lathrop, J. T., and Timko, M. P. (1993). *Science* **259,** 522–525.

May, A, Al-Sabah, A., Fitzsimons, E. J., Houston, T., Woodcock, B., Cotter, P. D., Wong, L., and Bishop, D. F. (1994). *Br. J. Haematol.* **87** (Suppl. 1), 148.

May, B. K., Bhasker, C. R., Bawden, M. J., and Cox, T. C. (1990). *Mol. Biol. Med.* **7,** 405–421.

May, B. K., Dogra, S. C., Sadlon, T. J., Bhasker, C. R., Cox, T. C., and Bottomley, S. S. (1995). *Prog. Nucleic Acid Res. Mol. Biol.* **51,** 1–51.

Peto, T. E. A., Pippard, M. J., and Weatherall, D. J. (1983). *Lancet* **I,** 375–378.

Riddle, R. D., Yamamoto, M., and Engel, J. D. (1989). *Proc. Natl. Acad. Sci. USA* **86,** 792–796.

Schoenhaut, D. S., and Curtis, P. J. (1989) *Nucleic Acids Res.* **17,** 7013–7028.

Srivastava, G., Borthwick, I. A., Maguire, D. J., Elferink, C. J., Bawden, M. J., Mercer, J. F. B., and May, B. K. (1988). *J. Biol. Chem.* **263,** 5202–5209.

Sutherland, G. R., Baker, E., Callen, D. F., Hyland, V. J., May, B. K., Bawden, M. J., Healy, H. M., and Borthwick, I. A. (1988). *Am. J. Hum. Genet.* **43,** 331–335.

Vogler, W. R., and Mingioli, E. S. (1965). *N. Engl. J. Med.* **273,** 347–353.

Yamamoto, M., Fujita, H., Watanabe, N., Hayashi, N., and Kikuchi, G. (1986). *Arch. Biochem. Biophys.* **245,** 76–83.

Youssoufian, H., Antonarakis, S. E., Bell, W., Griffin, A. M., and Kazazian, H. H. (1988). *Am. J. Hum. Genet.* **42,** 718–725.