

Molecular Mechanism of Heme Biosynthesis

HIROYOSHI FUJITA

*Department of Molecular Biology & Applied Physiology,
Tohoku University School of Medicine, Sendai 980-77*

FUJITA, H. *Molecular Mechanism of Heme Biosynthesis*. Tohoku J. Exp. Med., 1997, **183** (2), 83-99 — Two of the major organs producing heme are bone marrow and the liver. δ -Aminolevulinate synthase (ALAS) plays the key role to regulate heme biosynthesis in hepatocytes as well as in erythroid cells. In the liver, nonspecific (or housekeeping) isozyme of ALAS (ALAS-N) is expressed to be regulated by its end product, heme, in a negative feedback manner. The way to regulate ALAS-N in the liver is suitable to supply a constant level of heme for a family of drug metabolizing enzymes, cytochrome P-450 (CYP). In erythroid tissues, not only erythroid-specific isozyme of ALAS (ALAS-E) but also ALAS-N are expressed, and regulated by distinctive manners. Although heme regulates ALAS-N in a negative feedback manner even in erythroid cells, ALAS-E is upregulated by induced heme concentration. ALAS-N in undifferentiated erythroid cells, therefore, is suggested to produce heme for CYP, whereas heme for accumulating hemoglobin (Hb) in cells undergoing differentiation is synthesized via ALAS-E. In this article, we describe the molecular mechanisms to regulate heme biosynthesis in non-erythroid as well as in erythroid tissues, and discuss the pathological significance of the mechanisms in patients with inherited disorders, porphyrias. — heme biosynthesis; δ -aminolevulinate synthase; hepatocytes; feedback regulation; erythroid differentiation © 1997 Tohoku University Medical Press

Major sites of heme synthesis are the liver and erythroid tissues where most of heme is incorporated into cytochrome P-450 (CYP) and hemoglobin (Hb), respectively, as the prosthetic group (Kappas et al. 1995). It is generally accepted that 75% of heme is synthesized in bone marrow cells, and most of the remainder is synthesized in the liver. Heme synthesis in the liver has been extensively studied and proved to be regulated by δ -aminolevulinate synthase (ALAS) in a negative feedback manner (Kikuchi and Hayashi 1982). On the contrary, little is known about the mechanism to regulate heme biosynthesis in the erythroid cells, except for observations on differentiating murine erythroleukemia (MEL) cells (Sassa 1976).

Recently, cDNAs for all enzymes concerning heme biosynthesis have been cloned to throw a light on the mechanisms to regulate these enzymes in erythroid

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tissues. One of the special features of erythrocytes is that the protein concentration of cells ($>30\%$ w/v) is much higher than those in the other cells (10 to 15% w/v). Furthermore, almost of all protein in erythrocytes is Hb, indicating that cells have to accumulate huge amount of the protein during their differentiation. It is, therefore, probable that erythrocytes have a special mechanism to produce heme for Hb. Present review describes the well known regulation of hepatic heme synthesis, followed by the way to regulate erythroid heme metabolism.

Negative feedback regulation of heme in the liver

In the liver, 2/3 of heme is utilized for the formation of CYP (Kappas et al. 1995). Biosynthesis of hepatic heme is controlled at the step of δ -aminolevulinic acid (ALA) production in a negative feedback manner. In other words, gene activation (hence the activity induction) of hepatic ALAS is regulated by free heme (or regulatory heme) pool. This regulation is suitable for production of the end-product, heme, for CYP in a steady level. However, once some step (s) of heme biosynthesis following ALAS is inhibited by either genetical and/or environmental etiology, the negative feedback regulation of ALAS becomes harmful against life; i.e., the inhibition of heme biosynthetic pathway followed by induction of ALAS results in an accumulation of huge amount of ALA, which has neurotoxicity. Hereby, patients with hepatic porphyrias develop severe symptoms of central nerve disorder (Fujita and Nagai 1993).

Fig. 1 indicates a typical study to demonstrate a negative feedback regulation of hepatic ALAS in rats treated with trichloroethylene, one of the most frequently used solvents in the modern industry (Fujita et al. 1984). The solvent is catalyzed by CYP to form trichloroethylene epoxide as a reactive intermediate. ALA dehydratase (ALAD), the second enzyme in heme biosynthetic pathway, has as much as 64 SH residues/mole, including 8 essential SH residues (Fujita et al. 1986). Highly active trichloroethylene epoxide reacted with the essential SH residues in ALAD molecule, resulting in a marked inhibition of the enzyme in a dose dependent manner. Inactivation of ALAD resulted in a marked decrease in hepatic free heme indicated by a decline in CYP concentration as well as by a reduced heme saturation of tryptophan pyrrolase. According to the decrease in free heme (or regulatory heme), dose dependent induction of ALAS was observed in trichloroethylene treated rat liver, indicating a negative feedback regulation of the enzyme. Yamamoto et al. (1988) suggested that the regulation of ALAS in the liver is mainly based on the transcription level, however, factors concerning the regulation have not yet been well elucidated.

Difference of ALAS in the liver and erythroid tissues

If ALAS in erythroid cells is regulated by heme under a similar way observed in the liver, the cells could not accumulate enough amount of heme for Hb. Thus, the mechanism to activate ALAS gene in erythroid tissues should be different from

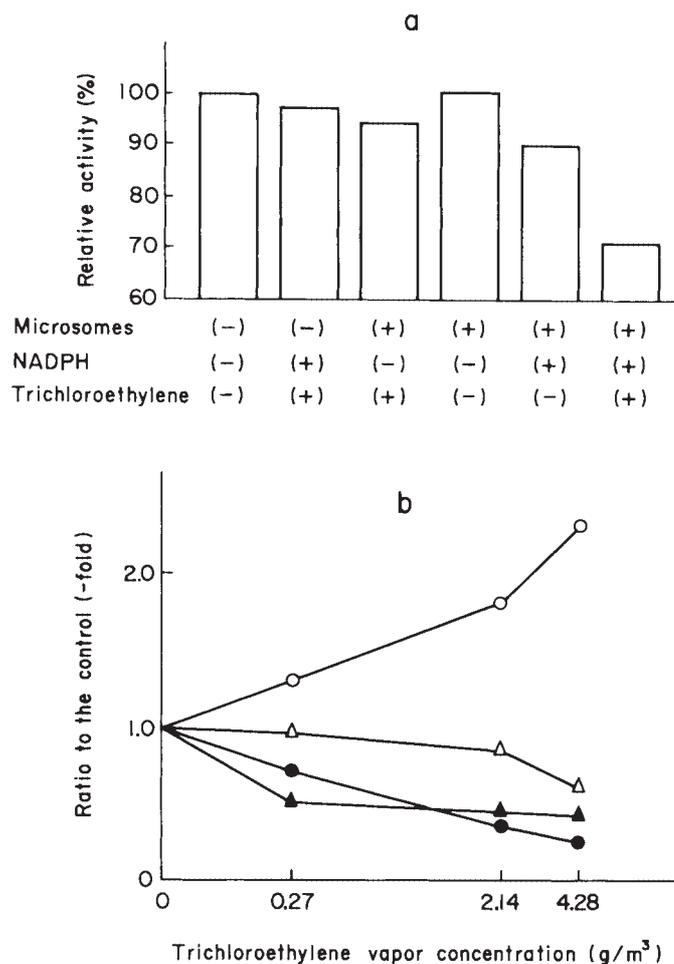


Fig. 1. Effects of trichloroethylene exposure on heme biosynthesis.

(a) Inhibition of ALAD activity by trichloroethylene *in vitro*.

Purified ALAD ($6 \mu\text{g/ml}$, i.e., $1.7 \mu\text{M}$ of subunit, which contains $13.7 \mu\text{M}$ of SH residues) in 0.5 ml of 100 mM Tris -acetate buffer, pH 7.1, was incubated with trichloroethylene ($60 \mu\text{M}$) either in the presence or absence of the mixed function oxidase system for 60 minutes, followed by ALAD activity determination in the presence of dithiothreitol and zinc. ALA dehydratase activity is inhibited after bioactivation of trichloroethylene by cytochrome P-450. The activity without trichloroethylene and mixed function oxidase system is taken as 100%.

(b) Effects of trichloroethylene exposure on heme biosynthesis in rat liver.

Male Wistar rats (8 rats for each group) were exposed to various concentrations of trichloroethylene vapor for 10 days. The liver was removed after perfusion with isotonic KCl to minimize blood contamination. Effects of trichloroethylene exposure on hepatic ALAS activity (○—○), ALAD activity (●—●), cytochrome P-450 concentration (△—△), and heme saturation of tryptophan pyrrolase (▲—▲) were determined.

the negative feedback system. One of the early studies, hemin mediated differentiation of murine erythroleukemia cells was reported, suggesting that ALAS in erythroid cells could be regulated by heme in a positive feedback manner (Sassa 1976). To elucidate the erythroid-specific heme metabolism, we have carried out immunochemical analyses of heme enzymes (Fig. 2) (Yamamoto et al.

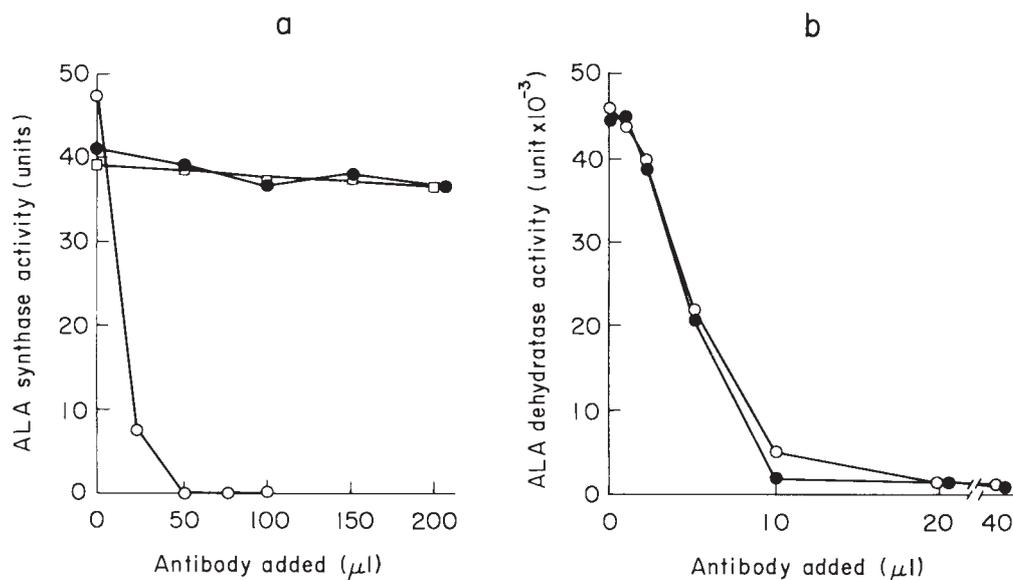


Fig. 2. Immunochemical studies of ALAS (a) and ALAD (b).

Immunoprecipitation against purified enzymes from rat liver (\circ) or from erythrocytes (\bullet) was carried out either with anti-hepatic ALAS antibody (a) or with anti-erythroid ALAD antibody (b) for 12 hours at 4°C , centrifuged at $15\,000\times g$ for 10 minutes, then enzyme activities in supernatant were determined.

1986). Although an antiserum raised against rat erythrocyte ALAD completely crossreacted with hepatic ALAD, an antiserum against rat hepatic ALAS failed to recognize erythrocyte enzyme. These observations strongly suggested that ALAS in the liver and in erythrocytes are isozymes, while ALAD in both tissues are not (Fujita et al. 1986). It is, therefore, probable that isozymes of ALAS are under different regulation. Recently, it became obvious that human ALAS-E and ALAS-N are localized to different chromosomes, i.e., the former is assigned at Xp11-q12 and the latter is at 3p21, respectively (Sutherland et al. 1988; Bishop et al. 1990; Cox et al. 1990; Cotter et al. 1992).

Role of ALAS-N in housekeeping type heme biosynthesis

Expression of ALAS-N and ALAS-E were examined by Northern blot analysis. ALAS-E was detected only in erythroid tissues, while the other organs expressed only ALAS-N mRNA. This observation is in good agreement with previous reports which indicated heme mediated negative feedback regulation of ALAS not only in the liver but also in the brain and kidneys (Barns et al. 1971; Woods 1988). In this review, we define heme production in non-erythroid tissues as housekeeping type heme synthesis.

To elucidate the specific character(s) of housekeeping type heme synthesis, we have examined effects of 2-allyl-2-isopropylacetamide (AIA) on rat liver. In the examined animals, CYP is induced to catabolize AIA, resulting in an acceleration of heme biosynthesis. Though AIA induced ALAS-N to as high as 10-times of the control, effects on the other heme enzymes were essentially nil. Thus, it is

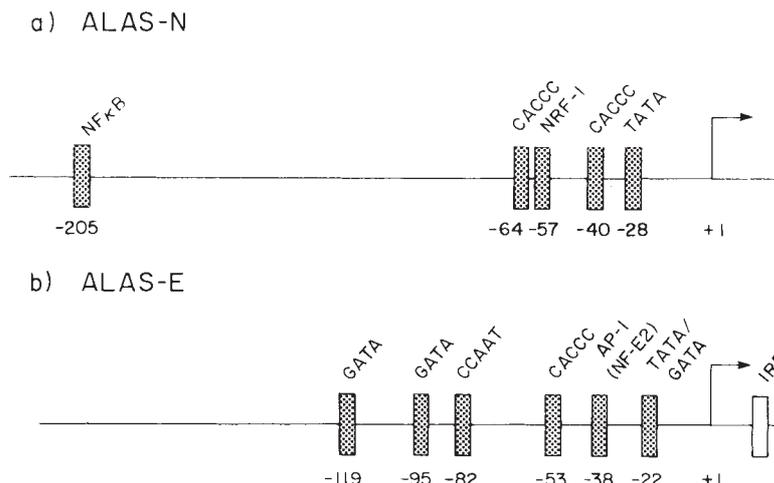


Fig. 3. Potential cis-acting elements in 5' flanking region of rat ALAS-N gene (A) and ALAS-E gene (B).

clearly demonstrated that the housekeeping type heme synthesis is regulated only by ALAS-N (Nagai et al. 1997).

For the further analyses, we examined 5' flanking region of rat ALAS-N gene (Yomogida et al. 1993). As shown in Fig. 3a, TATA box consensus was found at -28 in the promoter region. In addition, two CACCC motifs (-40 and -64), NRF-1 binding sequence (-57), NF- κ B binding sequence (-205), and GATA motif (-649) were identified. Since CACCC and GATA sequence 5' to erythropoietin gene has been reported to be positive and negative regulator of hypoxia mediated gene activation in the hepatoma cells (Imagawa et al. 1994, 1996) whose oxygen sensor is heme (Goldberg et al. 1988), these elements might be responsible for negative feedback regulation of ALAS-N gene. Recently, we found an oxygen mediated negative regulation of ALAS-N gene via NF- κ B binding site.

Unique regulation of heme metabolism in the Harderian gland

Harderian gland in rodents, a bi-lobed alveolar gland located within the orbit, is well known to contain an extremely high level of protoporphyrin (Margolis 1971; Eida et al. 1975; Payne et al. 1979; Shirama et al. 1981). Our data indicates that mRNAs for ALAS-N, ALAD, porphobilinogen deaminase (PBGD), uroporphyrinogen decarboxylase (UROD), coproporphyrinogen oxidase (CPO) were maximally induced in the gland, while concentration of mRNA encoding ferrochelatase (FeC) was only 40% of that in the liver (Nagai et al. 1997). These results explain the mechanism to accumulate protoporphyrin. Furthermore, mRNA level for heme oxygenase (HO) was higher than that in the liver by > 10-times, in good agreement with heme concentration in the gland was less than 1% of that in the liver. Our observations indicated that lack of heme in MEL cells resulted in a reduction of FeC mRNA level without any decrease in transcription (Fukuda et al. 1993a, b), therefore, the extremely low level of heme in the Harderian gland may also reduce FeC mRNA.

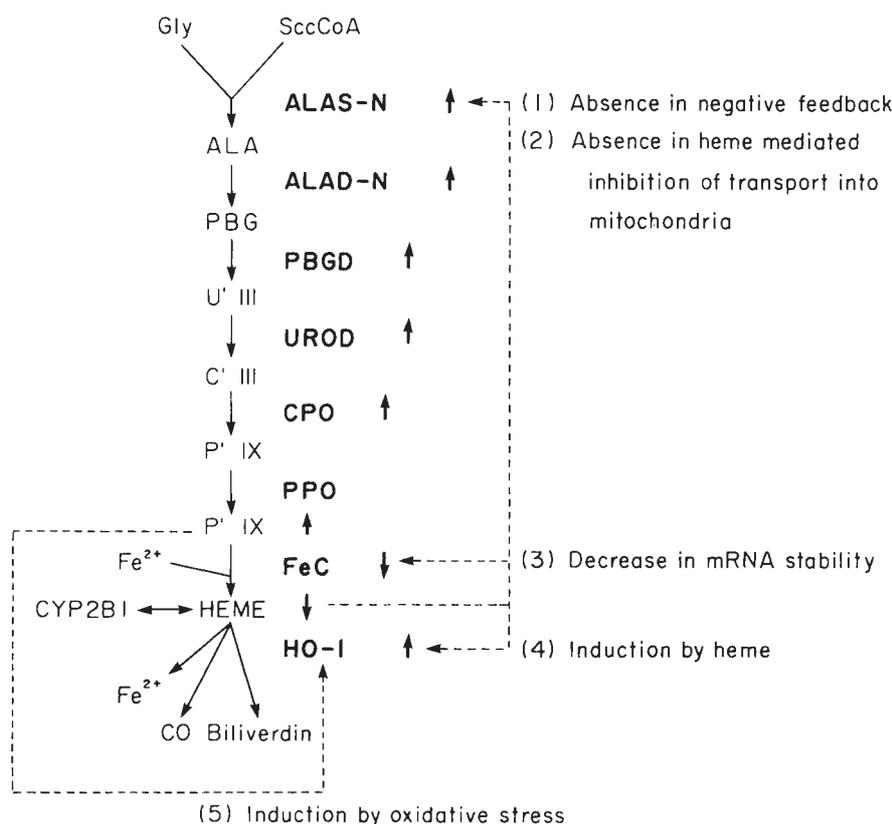


Fig. 4. Scheme for possible mechanisms of heme metabolism in the Harderian gland.

(1) to (5) Possible mechanisms to result in the Harderian gland specific heme metabolism. Abbreviations: ALA, δ -aminolevulinic acid; PBG, porphobilinogen; U' III, uroporphyrinogen III; C' III, coproporphyrinogen III; P' IX, protoporphyrinogen IX; P IX, protoporphyrin IX; ALAS, ALA synthase; ALAD, ALA dehydratase; PBGD, PBG deaminase; UROD, uroporphyrinogen decarboxylase; CPO, coproporphyrinogen oxidase; PPO, protoporphyrinogen oxidase; FeC, ferrochelatase; HO-1, heme oxygenase-1; CYP2B1, cytochrome P-450 2B1.

Since the extremely decreased heme thought to activate ALAS-N in the gland to maximum level, hemin treatment of the cultured tissues has been carried out. To our surprise, the hemin treatment failed to reduce ALAS-N level, suggesting a novel regulation of ALAS-N in the gland. This observation is in good agreement with the finding on CYP2B1, which could not be induced after AIA treatment in the Harderian gland. Hemin can induces HO in the gland, thus it is obvious that factor (s) for heme mediated regulation of HO is different from those for ALAS-N. To examine the difference in ALAS-N regulation between the liver and the Harderian gland, gel mobility shift analysis was carried out. The preliminary data indicates that nuclear extract from the liver can recognize cis-element harboring -100 bp upstream to ALAS-N gene whereas nuclear extract from Harderian gland cannot. It is therefore suggested that a transcription factor (s) for ALAS-N gene is missing in the gland. None of mRNA for erythroid type protein has been observed. Our data demonstrated that there are at least two

tissue-specific regulation of heme biosynthesis among non-erythroid organs. Scheme of heme metabolism in the Harderian gland is presented in Fig. 4.

Not only ALAS-E but also ALAS-N are expressed in erythroid cells

B. May and coworkers had concluded that erythroid ALAS mRNA was identical to hepatic ALAS, and that difference between hepatic and erythroid heme biosynthesis was based on different regulations of single ALAS gene in those organs (Elferink et al. 1987, 1988). Their reports have not been agree with our immunochemical observations, which demonstrated the difference in antigenecities between hepatic and erythroid ALAS (Yamamoto et al. 1986). We, therefore, carried out Northern hybridization analysis of RNA from MEL cells and clearly indicated that not only ALAS-E but also ALAS-N were expressed in erythroid cells (Fig. 5) (Fujita et al. 1991a). Far different from the remarkable increase in ALAS-N reported by B. May's group (Elferink et al. 1988), ALAS-N mRNA is markedly decreased during erythroleukemia differentiation. Though the exact explanation of discrepancy between B. May's report and ours has not yet been well explained until today, many possible reasons which may introduce B. May's observations were discussed in a recent review (Yamamoto et al. 1994).

Since ALAS-E as well as ALAS-N were identified in uninduced MEL cells, we quantitated both mRNA to understand isozyme(s) which is responsible for erythroid heme synthesis (Fig. 5). The concentration of mRNA for ALAS-E (20 pg/5 μ g total RNA) was 10 times higher than mRNA for ALAS-N (2 pg/5 μ g total RNA), suggesting that erythroid type heme biosynthesis is mainly regulated by ALAS-E gene even in uninduced cells. During erythroleukemia differentiation, mRNA for ALAS-E increased to as high as 50-times of that in uninduced cells, while mRNA for ALAS-N decreased to less than 10% of that in uninduced cells. The ratio ALAS-E mRNA to ALAS-N mRNA in differentiated cells is calculated to be almost 5000 : 1, therefore, it is obvious that almost of all heme in erythrocytes is synthesized through ALAS-E to saturate tremendous amount of globin protein essential for erythrocytes (Fujita et al. 1991a).

Studies on hepatocytes and the Harderian gland demonstrates tissue specific regulations of ALAS-N by heme, therefore, effects of heme on both ALAS isozymes were examined in MEL cells (Fig. 6). In cells treated with hemin, ALAS-E mRNA increased whereas ALAS-N mRNA decreased. Inhibition of endogenous heme biosynthesis by succinylacetone (SA), one of the potent inhibitors of ALAD, increased ALAS-N mRNA without any change in ALAS-E mRNA. These results demonstrate that ALAS-N gene in erythroid cells is controlled in the way as same as that observed in hepatocytes. This fact also indicates that "single ALAS gene with different regulations" in hepatic and erythroid cells (Elferink et al. 1987, 1988) is not reproducible. In contrast to a negative feedback regulation of ALAS-N, ALAS-E gene is regulated by heme under a positive feedback manner. Thus, it is obvious that ALAS-E and ALAS-N genes are independently regulated

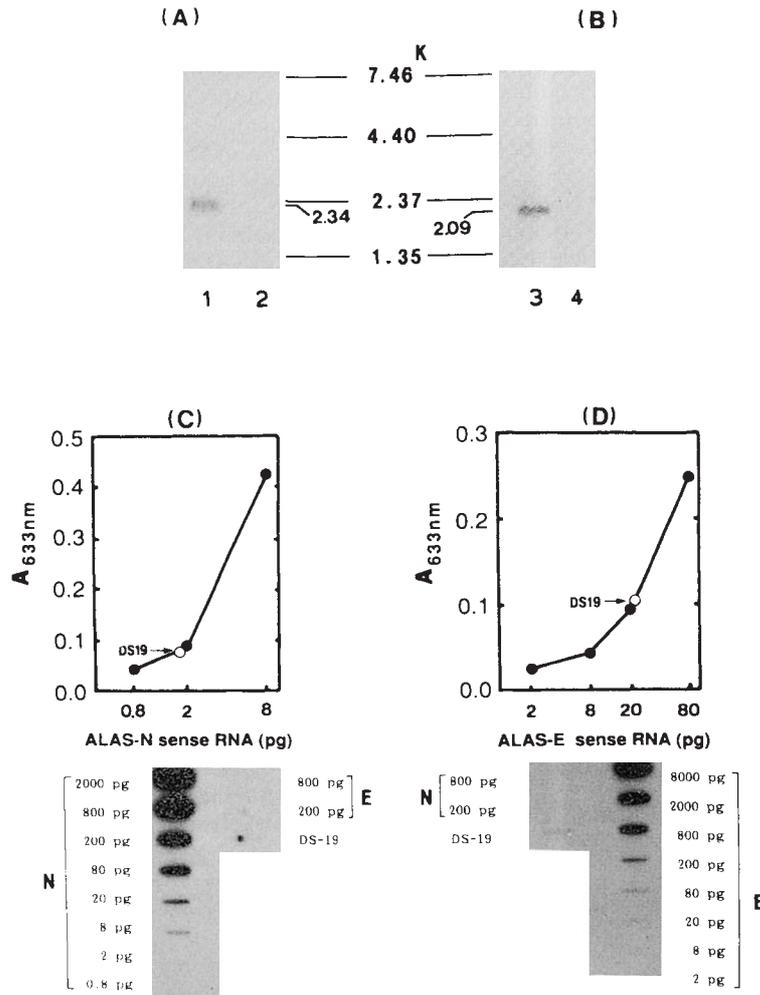


Fig. 5. Expression of ALAS isozymes in uninduced MEL cells.

(A) and (B): Twenty μg of total RNA from uninduced MEL cells (lanes 1, 3), 200 pg of ALAS-E sense RNA (lane 2), and 200 pg of ALAS-N sense RNA (lane 4) were subjected to Northern blot analysis, and hybridized either with ALAS-N cRNA probe (A) or with ALAS-E cRNA probe (B). After hybridization, filters were treated with RNase A, followed by washing under highly stringent condition. The sizes of mRNA encoding ALAS-N (lane 1) and ALAS-E (lane 3) estimated by RNA ladders (Bethesda Res. Lab.) are 2.34 and 2.09 kb, respectively.

(C) and (D): Slot blot analysis was carried out using sense RNAs for ALAS-N and ALAS-E, as well as total RNA from uninduced MEL cells. Filters were hybridized with ALAS-N cRNA probe (C) or ALAS-E cRNA probe (D), treated with RNase A, and washed under highly stringent condition. Densitometrical estimation reveals that ALAS-N and ALAS-E transcripts in uninduced MEL cells are 2 pg/ $5 \mu\text{g}$ total RNA and 20 pg/ $5 \mu\text{g}$ total RNA, respectively.

N: 0.8 to 2000 pg of sense ALAS-N RNA.

E: 2 to 8000 of sense ALAS-E RNA.

DS-19: $5 \mu\text{g}$ of total RNA from uninduced DS-19 cells.

in erythroid cells. The observation that decline in heme concentration has no effect on ALAS-E gene explains the absence of both remarkable ALA excretion in urine and neuropathy in patients with erythropoietic porphyrias (Fujita and

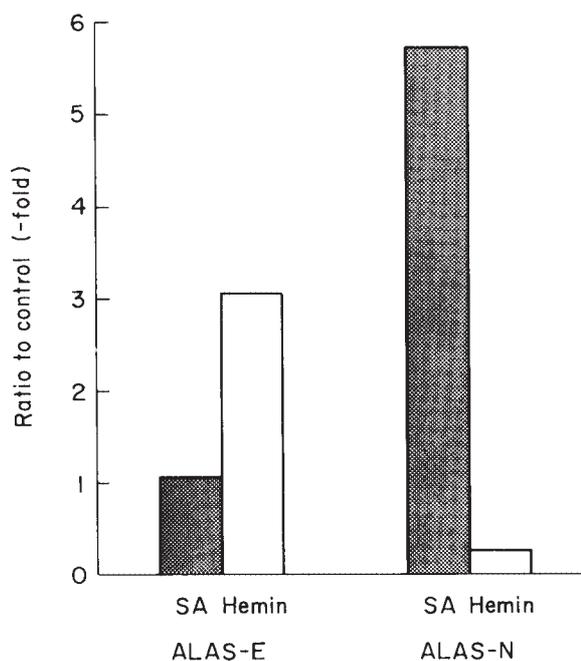


Fig. 6. Effects of heme on ALAS isozymes.

To elucidate effects of heme concentration on ALAS mRNAs, DS-19 cells were treated with 0.5 mM succinylacetone (SA) or 0.1 mM hemin, respectively. Cells were harvested after 48 hours of treatment, and total RNA from cells were subjected to Northern blot analysis.

Nagai 1993).

In addition to activation of ALAS-E gene, remarkable suppression of HO, the key enzyme for heme catabolism, was observed during erythroid differentiation (Fujita and Sassa 1989). Thus, sufficient amount of heme for Hb is able to be supplied by an increase in heme synthesis coupled with a decrease in heme degradation.

Regulation of ALAS-E

Fig. 3b shows potential cis-acting element in 5' flanking region of rat ALAS-E gene (Yamamoto et al. 1994). A cluster of many potential cis-acting element for erythroid genes, such as GATA motif, NF-E2/Ap-1 binding sequence, CACCC and CCAAT motifs, were identified. Preliminary data indicates that a chimeric gene of ALAS-E 5' flanking region with CAT as a reporter had a marked CAT activity when the gene is transfected into MEL cells (Fujita and Nagai 1993; Yamamoto et al. 1994). Thus, some (or all) of these elements are shown to play a significant role (s) on activation of ALAS-E gene. Relationship between each element and erythroid differentiation is, however, unclear until today.

GATA-1 protein is already expressed in uninduced MEL cells, and the protein level is not induced by differentiation. This observation suggests that GATA-1 may not contribute the 50-times induction of ALAS-E during erythroleukemia differentiation. This factor may produce the predominant expression of ALAS-E

in uninduced MEL cells (Fig. 5). These hypothesis are supported by the finding in *c-myc* overexpressed MEL cells, which can suppress the induction of ALAS-E but had little effects on GATA-1 level during differentiation (Obinata et al. 1994). MEL cells with overexpressed MafK (small subunit of NF-E2) were able to undergo erythroleukemia differentiation (Igarashi et al. 1995), suggesting that the transcription factor might be, at least partly, responsible for ALAS-E gene activation during the period.

An iron responsive element (IRE) was identified in the 5'-untranslated region (UTR) of ALAS-E mRNA (Cox et al. 1991; Dandeker et al. 1991). IRE is also identified in the 3'-UTR and 5'-UTR of transferrin receptor (TfR) mRNA and ferritin mRNA, respectively, both of which are regulate by cytosol iron level via IRE binding protein (IRE-BP; cytoplasmic aconitase)(Altmann and Trachesel 1993). There is a significant negative correlation between the binding activity of IRE-BP and cytosol iron concentration, i.e., a decrease in iron increases binding of IRE-BP to IRE. IRE-BP binding to IRE either in 3'-UTR or in 5'-UTR of the gene inhibits the translation of mRNAs for TfR and ALAS-E or stabilizes ferritin mRNA, respectively. Accordingly, a decrease in cytosolic iron decreases TfR and ALAS-E, while increases ferritin. It is interesting that iron itself regulates its uptake by TfR, its deposit in ferritin, and its incorporation into heme.

Induction of heme enzymes during erythroid differentiation

In differentiating MEL cells, induction of ALAS-E mRNA is followed by the sequential induction of other enzymes for heme biosynthesis in the order as they appeared in their biosynthetic sequence (Fig. 7) (Fujita and Sassa 1990; Fujita et al. 1991b, 1994). Namely, mRNAs encoding ALAS-E, ALAD, PBGD, and UROD started to increase at 12, 18, 18-24 and 24 hours after the dimethyl sulfoxide (DMSO) treatment, respectively. Run off transcription assay demonstrated that their induction are based on gene activations.

Genes encoding ALAD (Bishop et al. 1991, 1996) and PBGD (Grandchamp et al. 1987) have two promoters and two first exons. In the non-erythroid cells, mRNA is transcribed using the distal promoter, while erythroid type transcription utilizes the proximal promoter (Fig. 8). Upstream of the proximal promoter, a cluster of potential cis-element, such as GATA motif, AP-1/NF-E2 binding sequence, CACCC motif, were reported. Therefore, common factors might activate genes for ALAS-E, ALAD, and PBGD in erythroid cells. Our study on UROD from a patient with hepatoerythropoetic porphyria also suggest tissue-specific expression of the gene (Fujita et al. 1987). Furthermore, gene activation of UROD in differentiating MEL cells is similar to those of ALAD and PBGD, and is far different from those of CPO, protoporphyrinogen oxidase (PPO), and FeC, therefore, the gene might have a promoter for erythroid tissues. No promoter with erythroid type cis-element has been, however, identified in the upstream region of UROD gene (Romana et al. 1987).

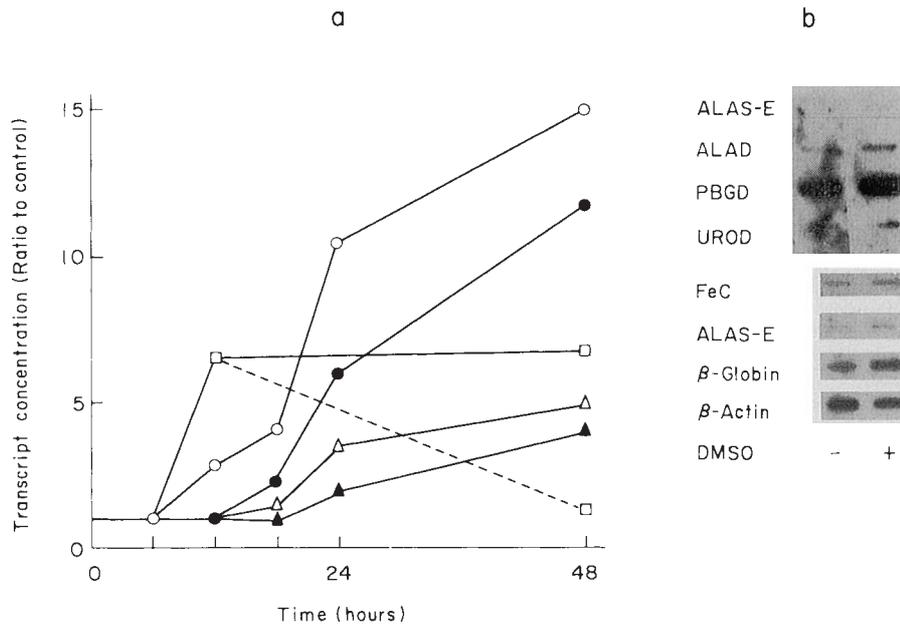


Fig. 7. Induction of enzymes for heme synthesis during erythroleukemia differentiation.

(a) DS-19 cells were treated with 1.5% (v/v) dimethylsulfoxide (DMSO) and changes in mRNAs for ALAS-E (○—○), ALAD (●—●), PBGD (△—△), UROD (▲—▲), and FeC (□—□) were estimated. Dotted line represents the changes of FeC mRNA in DMSO treated DR cells.

(b) Effect of 24 hours treatment of DMSO on transcriptional rate of each mRNA was examined by run-off transcription assay.

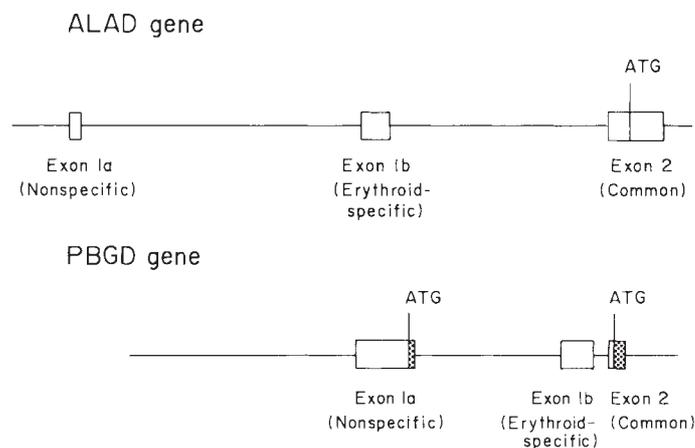


Fig. 8. Exons 1 and 2 of genes encoding ALAD and PBGD.

Both ALAD and PBGD genes have two exon 1. Exon 1a encodes housekeeping mRNA and exon 1b encodes erythroid-specific mRNA. Since there is no coding region in ALAD exon 1a and 1b, proteins for housekeeping enzyme and erythroid enzyme are identical. This observation is in good agreement with our finding on ALAD protein, i.e. the amino acid composition of erythroid ALAD is identical to that of hepatic ALAD (Fujita et al. 1986).

Different from first 4 enzymes discussed above, 3 enzymes for terminal steps for heme biosynthesis, namely, CPO, PPO, and FeC, are transcribed prior to the induction of ALAS-E mRNA (Taketani et al. 1990, 1995b; Taketani 1994). Each

gene have only one promoter with potential cis-elements for house-keeping type gene as well as for erythroid type genes (Taketani et al. 1992, 1995a; Taketani and Fujita 1995)

Expression of ALAS-E is essential for erythroid differentiation

We have established DMSO resistant (DR) clone of MEL cells, which cannot undergo erythroid differentiation after the treatment with DMSO. In DR cells, we found that (i) no ALAS-E mRNA has been identified (Fig. 9), (ii) mRNAs for ALAD, PBGD, UROD were expressed and increased after DMSO treatment, however, their maximal induction levels were approx. 1/3 of those in normal induced cells, (iii) FeC mRNA was induced to normal level in the early stage of differentiation, but cannot maintain the level (Fig. 7, broken line), (iv) ALAS-N gene was down regulated after the treatment even in cells without ALAS-E, (v) β -globin is expressed, but the level is decreased after DMSO treatment (Fujita et

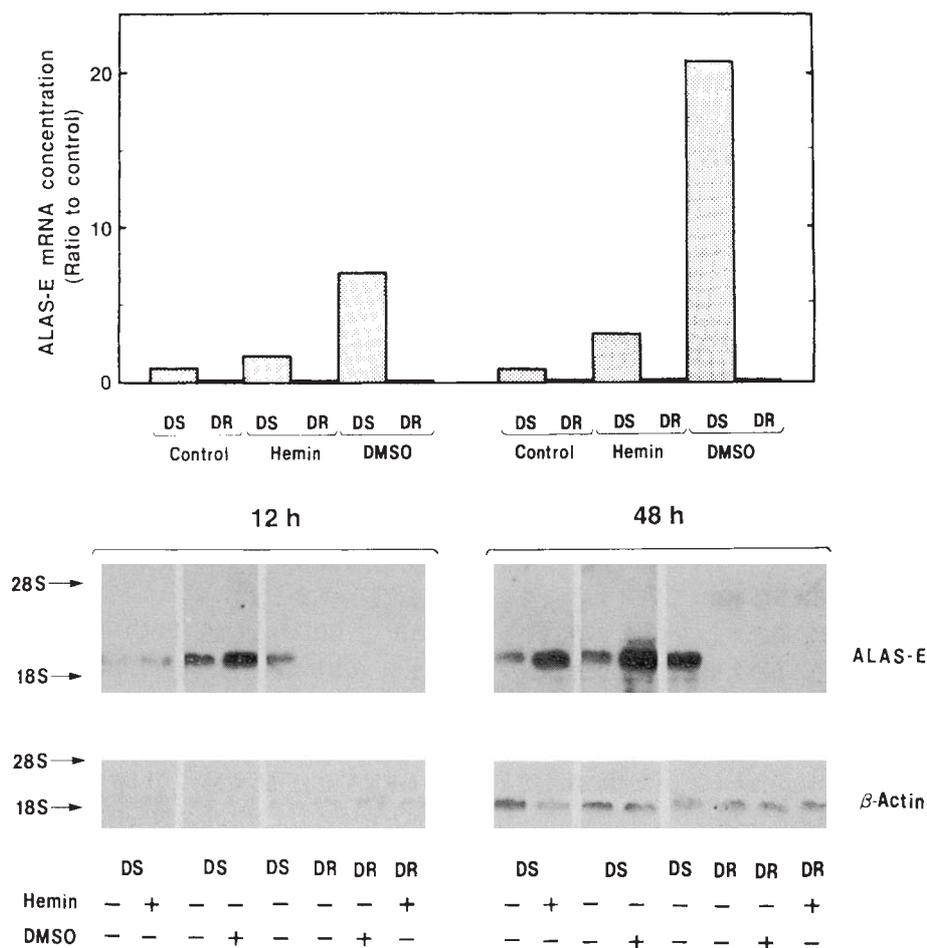


Fig. 9. ALAS-E expression in DS-19 cells and DR-1 cells.

Cells were treated with hemin or dimethylsulfoxide (DMSO) to undergo erythroid differentiation. Expression of ALAS-E was estimated by Northern blot analysis. No transcript for ALAS-E has been observed in DR cells, indicating that the absence of ALAS-E is responsible for the resistance against DMSO treatment.

al. 1991a; Mitani et al. 1992; Fukuda et al. 1993b, 1994).

Gel mobility shift assay indicated that normal level of GATA-1 was expressed in DR cells, suggesting that the cells cannot undergo differentiation primary due to the absence of ALAS-E transcript. In spite of the absence of ALAS-E, down regulation of ALAS-N gene has occurred in DR cells. It is, therefore, probable that the suppression of ALAS-N during erythroid differentiation is not a result of heme mediated negative feedback regulation. Decrease in ALAS-N observed in the latent period of erythroleukemia differentiation might be regulated by a common mechanism (s) to housekeeping genes, such as 70 kDa heat shock protein and 32 kDa heat shock protein (HO-1) (Fujita et al. 1991b).

Decline in FeC mRNA after its induction during early period after DMSO treatment is possibly due to the decrease in heme content in the DR cells. To elucidate the possibility, we inhibited endogenous heme synthesis in normal MEL cells by SA, resulting a marked decline in FeC mRNA. Any change in the FeC gene activation could not be detected after SA treatment, suggesting that heme stabilizes FeC mRNA (Fukuda et al. 1993a, b; Fujita et al. 1994). This observation is in good agreement with the accumulation of protoporphyrin in erythroid cells from patients with sideroblastic anemia, whose ALAS-E activity are known to decrease. A $[2\text{Fe}-2\text{S}]^{2+}$ cluster in the COOH terminal of FeC also regulates the half-life of the enzyme (Dailey et al. 1994). The posttranscriptional regulations of the first step, i.e., ALAS-E, as well as the terminal step, i.e., FeC, of heme biosynthesis suggest a significant role of iron in erythroid tissues.

Effects of ALAS-E suppression on heme biosynthesis

A decrease in β -globin mRNA in the DR cells was correlated with the decrease of ALAS-N mRNA, suggesting that endogenous heme may control β -globin gene (Fujita et al. 1991a). This hypothesis is supported by an observation that the hemin treatment upregulates β -globin gene in DR cells (Fukuda et al. 1994). In addition, the magnitude of induction of mRNAs for ALAD, PBGD, and UROD in DR cells were lower than those in wild type cells. We, therefore, disturbed heme biosynthesis via ALAS-E by antisense RNA expression (Meguro et al. 1995). As was expected, a decrease in ALAS-E mRNA by antisense RNA was followed by proportional decreases in the other heme enzymes and β -globin. It is probable that a transcription factor (s) regulated by heme is responsible for activations in erythroid type heme synthesis.

Igarashi et al. (1994) discovered that active form of NF-E2 is consisted with two proteins, p 45 and small MafK (p 18). In DR cells, p 45 was lower than that in wild type MEL cells. In addition, expression of ALAS-E antisense RNA seems to decrease p 45 in MEL cells (Meguro et al. 1995). Other group also reported that heme regulates NF-E2 activity (Solomon et al. 1993). Therefore, upregulation of hemoglobin synthesis in erythroid cells is controlled at least in part by NF-E2.

CONCLUSION

Two major regulations of heme synthesis, namely housekeeping type and erythroid type, are described, and possible transcription factors concerning heme pathway enzymes, especially in erythroid cells, are discussed. Among them, NF-E2 may play a significant role to accumulate large amount of heme for Hb during erythroid differentiation. In addition to these well known heme regulation, we found novel regulation of heme metabolism in the Harderian gland. Although enzymes for heme synthesis in the gland is housekeeping type, all of the enzymes, except for FeC, are maximally induced, which is similar to those in induced MEL cells. Thus, further studies on tissue-specific regulation of heme synthesis are necessary, especially from a view of evolution in heme metabolism.

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