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P_1-450 and P_3-450 gene expression and maximum life span in mice

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Summary

The effects of β-naphthoflavone on the inducibility of hepatic P_1-450 and P_3-450 mRNA were investigated in male B10.RIII/Sn, C57BL/10Sn, C3H/HeSnJ, and A/WYSn mice. Previous work has shown that the maximum level of aryl hydrocarbon hydroxylase induction in these strains correlates with maximum life span. In this study we found that the maximum inducible levels of P_1- and P_3-450 RNA were significantly different among the strains, and these levels also correlate with life span. The differences were not due to strain-specific differences in the kinetics of P_1- or P_3-450 RNA induction. The differences were specific to expression of the P-450 genes, since the levels of hepatic α-actin and albumin RNA were not significantly different among the strains, and specific RNA levels were normalized to the level of total polyadenylated RNA. β-Naphthoflavone was found to induce α-actin mRNA approximately 2-fold and to transiently repress albumin RNA about 50% in all mouse strains.

Maximum P_1- and P_3-450 gene expression correlated directly with the 10th deciles of survival of the mouse strains. Longer-lived strains expressed higher combined levels of P_1- and P_3-450 RNAs. Maximum P_1- and P_3-450 gene expression also correlated generally with the reported aryl hydrocarbon hydroxylase receptor levels of each strain. It is unlikely that the hepatic P_1- and P_3-450 genes are ever maximally induced under the sheltered laboratory conditions used to determine maximum life span, as we consistently find very low levels of P-450 expression in uninduced animals. These uninduced levels were not statistically different between the strains. Therefore, the reason for the relationship between maximum life span and maximum P_1- and P_3-450 inducibility is unclear at present.

Cytochrome P_1-450 (P_1-450) is a principal source of hepatic aryl hydrocarbon hydroxylase (AHH) activity, which metabolizes drugs, chemical carcinogens and other environmental pollutants. The P_1-450 gene is closely related structurally and functionally to the cytochrome P_3-450 (P_3-450) gene (Kimura et al., 1984). These genes apparently evolved from a common ancestor. Together these genes are classified as P450IA genes (Nebert et al., 1987). Expression of both genes is induced by polyaromatic hydrocarbons, and this induction is dependent upon occupancy of the *Ah* receptor (Okey et al., 1979; Tukey et al., 1982).

The *Ah* receptor mediates induction of the hepatic P450IA genes (reviewed in Nebert and

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Gonzalez, 1987). Nebert et al. (1984) have shown that *Ah* receptor levels are generally related to fertility, fitness and average life span in mice; however, they did not provide maximum life span data. Koizumi et al. (1986, 1987) presented evidence that maximum life span in mice correlates closely with the maximum inducible level of hepatic AHH activity. Longer-lived strains have higher inducible levels of AHH.

Significant difference have been reported in the maximum life spans of male B10.RIII/Sn (RIII), C57BL/10Sn (B10), C3H/HeSnJ (C3H) and A/WYSn (A) mice (Smith and Walford, 1977). Furthermore, when 3 sets of H-2 congenic partners, on B10, C3H and A strain backgrounds, were compared, a close relationship was found between the H-2 locus and maximum life span. However, the non-H-2 background also influenced longevity. In the present study, we examined the relationship between maximum life span and P_1- and P_3-450 gene expression. We report that longevity correlates directly with maximum P-450 RNA induction, expressed as the sum of the hepatic P_1- and P_3-450 RNA levels. Animals able to express higher levels of P-450 RNA live significantly longer.

Materials and methods

Animals

Male B10.RIII/Sn (RIII), C57BL/10Sn (B10), C3H/HeSnJ (C3H) and A/WYSn (A) mice 6 weeks of age were purchased from Jackson Memorial Laboratories (Bar Harbor, ME) and maintained in our colony at UCLA until use at 3–4 months of age. They were maintained under conditions very similar to those described in the previous life span and acryl hydrocarbon hydroxylase induction studies (Smith and Walford, 1977; Koizumi et al., 1986). The RIII and B10 strains of mice are H-2 congenic partners, whereas the C3H and A strains have separate backgrounds. H-2 congenic partners are genetically 'identical' except for the chromosome region containing the H-2 complex. The H-2 types of the 4 strains are respectively r, b, k and a. The animals were injected with 83 mg/kg body weight β-naphthoflavone (β-NF) suspended in 50 μl of corn oil or with corn oil alone every 24 h and killed at the times indicated in the figures. Survival at this dose of β-NF approached 100%. Higher doses sharply decreased survival. Animals showed no gross signs of pathology at the time of sacrifice.

Radiolabeling of hybridization probes

Plasmids were ^{32}P-labeled to a specific activity of 1×10^8 cpm/μg by nick-translation (Maniatis et al., 1982). Oligo-dT (Pharmacia Inc., Piscataway, NJ) was 5′ end-labeled with ^{32}P-γ-ATP and T_4 polynucleotide kinase (Promega, Madison, WI) to a specific activity of 2×10^6 cpm/pmole (Maniatis et al., 1982).

Isolation of RNA and Northern and dot blotting

Overnight-fasted mice were killed by cervical dislocation and hepatic RNA was purified using the guanidine thiocyanate method (Chirgwin et al., 1979). Northern and dot blots were prepared as described (Crew and Spindler, 1986; Crew et al., 1987). Blots were successively probed with radioactively labeled oligo-dT, the 3′ non-coding regions of mouse P_1-450 and P_3-450 cDNA (pP_1450-3′ and pP_3450-3′, respectively; Kimura et al., 1984), and mouse α-actin (Minty et al., 1981) and mouse albumin (Minghetti et al., 1985) cDNA. The 3′ regions of the P_1-450 and P_3-450 probes used do not cross-hybridize. Autoradiographs were densitometrically scanned and absorbance quantified as described (Crew et al., 1987). All data are presented as the mean ± SD.

Results

The mouse strains used in these studies, their abbreviated strain designations and 10th deciles of survival (longevity) are presented in Table 1. Tenth decile survival, the average age reached by the longest-lived 10% of a population, is an estimate of maximum life span for a strain or species (Smith and Walford, 1977).

Strain-specific differences in P_1-450 and P_3-450 RNA inducibility

The hepatic levels of P_1-450 and P_3-450 RNA were determined in each mouse strain 48 h after initial treatment with vehicle or with the maximum technically possible dose of β-NF. Hepatic P_1-450 and P_3-450 mRNA were present at very low

TABLE 1

RELATIONSHIP BETWEEN MAXIMUM P_1-450 AND P_3-450 INDUCIBILITY, Ah RECEPTOR LEVEL AND 10TH DECILE SURVIVAL

The abbreviated names for each strain are shown in parentheses. See Fig. 1 and the indicated references for more detail.

Strain	P_1-450 [a]	P_3-450 [b]	P450IA [c]	*Ah* receptor [d]	Maximum life span (weeks) [e]
B10.RIII/Sn (RIII)	80%	100%	100%	n.d. [f]	170
C57BL/10Sn (B10)	100%	73%	96%	100%	155
C3H/HeSnJ (C3H)	50%	85%	75%	35%	138
A/WYSn (a)	18%	42%	33%	47%	134

[a] The percent of the mean maximum induced P_1-450 RNA levels is shown, calculated from the arbitrary absorbance units of Fig. 1. The relative levels of P_1-450 RNA (in arbitrary units) was 3.2 ± 0.9, 4.0 ± 0.4, 2.0 ± 0.6, and 0.7 ± 0.8 for the RIII, B10, C3H and A strains, respectively.

[b] The percent of the mean maximum induced P_3-450 RNA levels is shown, calculated from the arbitrary absorbance units of Fig. 1. The relative levels of P_3-450 RNA (in arbitrary units) was 2.6 ± 0.9, 1.9 ± 1.1, 2.2 ± 0.7, and 1.1 ± 0.6 for the RIII, B10, C3H and A strains, respectively.

[c] The values are the summation of the first 2 columns, normalized to the highest level of expression.

[d] Shown are the mean *Ah* receptor levels from Okey et al. (1979) and Koizumi et al. (1987), expressed as percent of the levels found in the RIII and B10 strains. The values are 30 (Koizumi et al. (1987), 12 (Okey et al. (1979), and 16 (this value has been determined for the A/J strain; Okey et al. (1979)) fmole 2,3,7,8-tetrachlorodibenzo-*p*-dioxin bound/mg cytosol protein for the B10, C3H and A strains, respectively. Standard deviations are not given in the studies cited.

[e] Given are the 10th decile survivals in weeks from Smith and Walford (1977). The values are 170 ± 0.8, 155 ± 0.4, 138 ± 0.3 and 134 ± 1.7 for the RIII, B10, C3H and A strains, respectively.

[f] Not done.

levels in uninduced mice (Fig. 1A,B). These RNA were dramatically induced by 48 h of β-NF treatment (Fig. 1A,B; Table 1). The induced level of P_1-450 RNA was highest in B10 mice, and it was 80%, 50% and 18% of this level in the RIII, C3H and A strains, respectively. In contrast, the induced P_3-450 RNA was highest in RIII mice, and it was 73, 85 and 42% of this level in the B10, C3H and A strains, respectively. The summation of P_1- and P_3-450 RNA was highest for RIII mice.

These strain-specific differences in the induced levels of P_1- and P_3-450 RNA were not reflected in their uninduced levels (Fig. 1C,D). When these levels were measured after vehicle treatment, they were not significantly different. These data indicate that the strains vary in the inducibility but not the basal expression of these genes.

The strain-related differences in these P_1- and P_3-450 RNA levels were not due to differences in the kinetics of RNA induction. Induction in the shortest- and longest-lived strains (the A and RIII strains, respectively) is shown in Fig. 2A,B. Induction of both RNA was variable during the first 24 h of β-NF treatment. However, P-450 RNA reached maximum levels 48 h after the first administration of the drug to individuals of both the RIII and A strains (Fig. 2). These results indicate that the strain-related differences which were observed after 48 h of induction are not kinetic artifacts. In addition, inductions were performed in both the A and RIII species with 50% and 25% the amount of β-NF used in the inductions shown here. The levels of P_1- and P_3-450 RNA induced in each case were not significantly different (data not shown). These data indicate that the amount of β-NF used is saturating in each case. Thus, the studies in both the long- and short-lived strains are conducted at β-NF saturation.

Relationship between P_1- and P_3-450 RNA inducibility and Ah receptor levels

A general correlation between P-450 gene expression and *Ah* receptor level and/or the affinity of the receptor for inducer has been reported (Nebert et al., 1984). We also find a general correlation between *Ah* receptor level and the maximum level of P-450 induction (Table 1). The highest P-450-expressing strains of mice had the

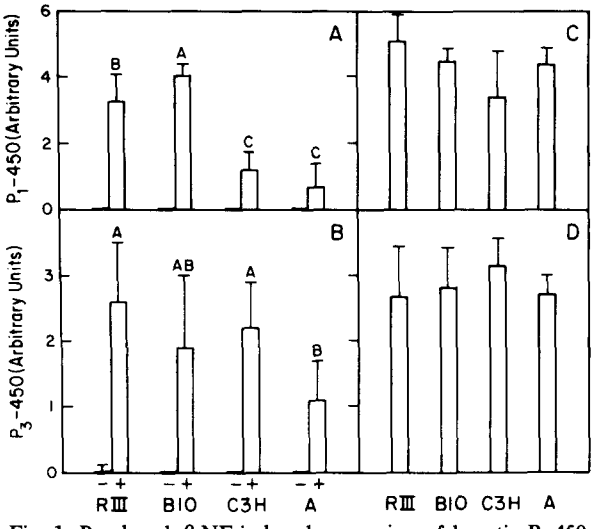

Fig. 1. Basal and β-NF-induced expression of hepatic P_1-450 and P_3-450 RNA. (A and B) Induction of P_1- and P_3-450 RNA in 4 strains of mice. Mice were treated with β-NF (+) or vehicle (−) for 48 h, and hepatic P_1-450 (A) and P_3-450 (B) RNA levels quantified by dot-blotting. (C and D) Basal levels of P_1- and P_3-450 RNA expression. Dot blots were prepared with total RNA from vehicle-treated mice and RNA expression quantified by dot-blotting after an extended period of autoradiography. In all cases, P_1-450 and P_3-450 RNA levels were normalized to the level of total polyadenylated RNA in each sample. Levels are expressed in arbitrary absorbance units. Northern blots were run on all samples to insure that the RNA was intact. Seven animals were used for each point. The data were analyzed by a 1-way ANOVA followed by Duncan's multiple range test. In panels A and B, different letters above the induced samples indicate that they have significantly different values ($P < 0.05$). The sample marked AB is not significantly different from A or B. In panel A, the least significant different (LSD) is 0.7510. In panel B, the LSD is 0.9207. The values in panels C and D are not significantly different ($P < 0.05$).

highest receptor levels, while the lower expressers had lower levels of receptor (Table 1). However, this correspondence was not absolute. While C3H mice had less receptor than A strain mice, they expressed substantially more P_1-450 and P_3-450 mRNA. Thus, although *Ah* receptor plays a key role in determining the inducibility of the P_1- and P_3-450 genes, other strain-related factor(s) also appear to play a significant role in determining inducibility. There appears to be a small, but statistically significant MHC-dependent difference in the P_1-450 inducibility of the B10 background H-2 congenic mice (RIII and B10).

Specificity of the strain-related differences in P_1-450 and P_3-450 RNA expression

The strain-related differences in expression of the P450IA genes appear to be specific to these genes. In the various strains, no significant difference was found in the basal levels of α-actin (Fig. 3A) or albumin gene expression (Fig. 3B). Further, the strain-related differences in P-450 expression were not due to differences in the level of total hepatic mRNA. Specific RNA levels were normalized to the level of total polyadenylated RNA (mRNA) in each sample.

β-NF transiently represses albumin and induces α-actin RNA

β-NF induced α-actin RNA approximately 2-fold in these mice (Fig. 3A). While the basal levels of actin RNA were approximately the same in the 4 strains, in the RIII and B10 strains actin was induced to significantly higher levels than in the C3H and A strains. Thus, β-NF induction of α-actin RNA correlated with the hepatic *Ah* receptor levels in the strains. These results suggest that α-actin RNA induction by β-NF may be *Ah* receptor-mediated.

β-NF transiently inhibited albumin RNA by approximately 50% in all the strains examined (Fig. 3B). Inhibition appeared to be transient,

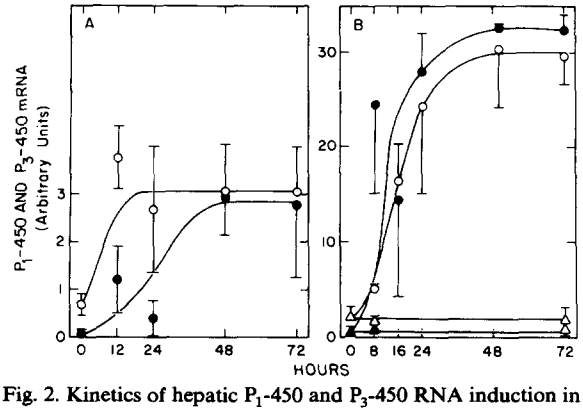

Fig. 2. Kinetics of hepatic P_1-450 and P_3-450 RNA induction in long- and short-lived mouse strains. Hepatic RNA, purified at the indicated times from β-NF-induced or vehicle-treated A strain (A) and RIII strain (B) mice were subjected to analysis by dot-blotting. Shown is the level of P_1-450 (filled symbols) and P_3-450 (open symbols) RNA in β-NF-treated (circles) and vehicle-treated (triangles) mice. 3–7 animals were utilized for each point.

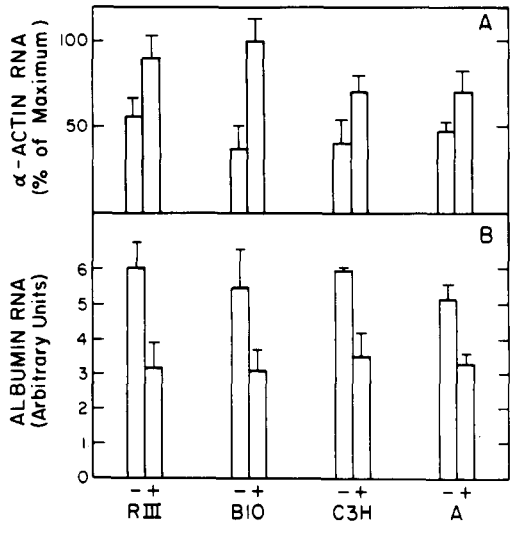

Fig. 3. Effect of β-NF on α-action and albumin RNA. Each mouse strain was treated with β-NF or vehicle. Hepatic RNA was purified after 48 h of β-NF treatment and analyzed by dot-blotting. Blots were successively probed with radiolabeled oligo-dT and α-actin cDNA (A), or with radiolabeled oligo-dT and albumin cDNA (B). Seven animals were used for each point. Using Student's *t*-test, albumin and actin mRNA levels in all species were found to be significantly different from control levels after β-NF induction ($P < 0.05$).

reaching a maximum by 24 h and returning to control levels by 72 h (data not shown), despite the sustained presence of β-NF). The transience was probably not due to a decline in the level of β-NF, since the animals were injected every 24 h, and P_1-450 and P_3-450 RNA remained maximally induced during this time (Fig. 2). Also, repression was probably not due to non-specific toxic effects of the inducer, since albumin RNA levels were normalized to the level of mRNA in each sample.

Relationship between P_1-450 and P_3-450 gene expression and longevity

The level of expression of the P_1- and P_3-450 genes appears to be directly related to longevity (Table 1). However, this correspondence is not absolute. The relationship is most pronounced when the maximum inducible P450IA RNA level is compared to longevity.

Discussion

We report here that the maximum levels of hepatic P_1-450 and P_3-450 RNA expressed in inbred strains of mice are correlated with their maximum life span. These results are consistent with those of Koimuzi et al. (1986, 1987), who found that the longer-lived strains of mice produced higher maximum levels of hepatic AHH activity.

While there is a strong correlation between maximum P_1- and P_3-450 gene expression and life span, it is not strictly quantitative. However, multiple genes are likely to influence life span. Further, the products of these genes are likely to interact in complex ways with one another and with other gene products. Thus, the existence of a strict quantitative relationship between expression of a particular gene and life span may be unlikely. Nevertheless, a significant correlation was found between life span and P_1- and P_3-450 gene expression, and this correlation is readily rationalized with the toxic and mutagenic potential of the substrates of these P-450 isozymes. In particular, aryl hydrocarbon hydroxylase has a well-characterized role in metabolizing drugs, chemical carcinogens and environmental pollutants.

It is unlikely that the hepatic P_1- and P_3-450 genes are ever maximally induced under the sheltered conditions used to determine the maximum life span of these mice (Smith and Walford, 1977). We do consistently find very low levels of hepatic P_1- and P_3-450 expression in uninduced animals, and there is no significant difference in these levels between the strains. For these reasons, the relationship between maximum inducibility and life span may reflect strain-specific differences in the sensitivity of P_1- and/or P_3-450 gene induction to very low concentrations of inducers. For example, the enhanced inducibility we have measured in the B10 and RIII strains may be reflected in a shift in the dose of polyaromatic hydrocarbons required to elicit a protective level of P_1-450. Thus, the long-lived mouse strains may be better protected from the cumulative effects of a lifetime of exposure to both very low and higher levels of environmental carcinogens and pollutants.

In addition to the detoxifying effects of the hepatic P-450 system, mixed-function oxygenases also produce activated oxygen species, which have been shown to oxidize both proteins and nucleic acids in vitro and in vivo (Rothstein, 1982; John-

son et al., 1986; Oliver et al., 1987). Thus, the interaction between the P-450 system and other enzymes such as catalase and superoxide dismutase must be considered. Further, some compounds are activated to carcinogenic, mutagenic and/or toxic forms by the hepatic P-450 system. Thus, it is unclear whether enhanced P450IA gene expression directly extends life span. Other genes linked to the *Ah* locus, or genes which are regulated by the *Ah* receptor, could influence life span instead of the genes studied here. It should be possible to directly determine the influence of enhanced P_1- and P_3-450 gene expression on life span using mice rendered transgenic using P450IA genes.

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