Relationship Between Transcription Factors and S14 Gene Expression in Response to Thyroid Hormone and Age

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Abstract. **In this report, we have examined the effect of aging on thyroid hormone regulated S14 gene expression by comparing levels of the mRNA in the liver of young (6-month) and aged (26-month) rats of various thyroid states. Although levels of mRNA-S14 were stimulated by L-triiodothyronine (T3) in both young and aged animals, net activity of the gene in response to the hormone was reduced approximately 2-fold in the aged rats. Next, we wondered whether the effect of age and T3 on levels of the mRNA correlated with changes in the DNA binding activity of two transcription factors, PS-1 and P1 that increase and decrease, respectively, S14 gene activity. The DNA binding activity of PS-1 correlated closely with both age-related and T3-induced S14 mRNA expression. Whereas the DNA binding activity of P1 was significantly reduced in aged rats, T3 failed to influence the activity of P1. Based on these observations, we postulated that age-related increases in S14 gene expression arise from combined changes in activity of the transcription factors, PS-1 and P1. However, T3 regulated S14 gene activity is more closely related to PS-1.** [P.S.E.B.M. 1994, Vol 207]

The L-triiodothyronine (T3) induction of rat S14 mRNA is a useful model for studying the mechanisms of thyroid hormone action. In adult hypothyroid animals, hepatic mRNA-S14 is induced within 10 min following the injection of a receptor saturating dose of T3. Although function of the S14 protein is not known, recent studies showed that it is a nuclear matrix protein found in lipogenic tissues such as liver, fat (brown and white), and mammary gland (1–12). In addition to T3, the S14 gene is also regulated by a variety of factors including age, circadian variation, dietary manipulation, developmental changes, and tissue specificity (1–12). Given the multitude of factors known to influence expression of the S14 gene, we were interested in examining the combined effects of two factors, age and T3, on the expression of this gene.

Previous studies have shown that expression of selected genes may vary with age of the animal (13, 14). For example, basal activity of a gene and its responsiveness to important dietary or hormonal modulation may be reduced in parallel with aging (15, 16). In other models, basal expression of a gene remains unchanged but its response to hormonal stimulation is significantly reduced in aged animals (17–19). However, a relatively uncommon pattern is a discordance between age-related increases in basal expression of a gene that appears in the setting of reduced responsiveness to hormonal stimulation. It is possible that gene S14 may exhibit this response pattern because of previous results showing activity of this gene to be increased in aged animals (5, 20–23), and in the majority of genes studied to date there is a generalized reduction in sensitivity to thyroid hormone with age (15,

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19–22). To determine the pattern of S14 gene expression in response to thyroid hormone and age, we measured steady state levels of hepatic S14 mRNA in young and aged rats of various thyroid states, hypo-, eu-, and hyperthyroid.

To gain a better understanding of the molecular mechanisms that may account for the observed alterations in S14 gene expression, we correlated changes in abundance of S14-mRNA with DNA binding activity of transcription factors known to regulate the S14 gene. We have recently identified two hepatonuclear proteins, PS-1 and P1 that enhance and repress, respectively, transcription of this gene. The DNA binding activity of PS-1 and P1 increased with the level of T3 in 2-month-old rats (24–27). Both PS-1 and P1 bind to a region of S14 DNA that is preferentially digested by DNase I. This so-called DNase I hypersensitive site, HS-1 is located immediately adjacent to the point of transcription initiation. Previous studies demonstrated that the ability of DNase I to cleave within the HS-1 site correlated with both age of the animals and S14 gene expression (28, 29). Based on these observations we asked whether the DNA binding activity of PS-1 or P1 was influenced by the combined effects of age and exposure to T3. Accordingly, we studied the relationship between the DNA binding activity of PS-1 and P1 in both young and aged animals of varying thyroid states. Results arising from these experiments provide novel insights into the role of transcription factors that regulate S14 gene activity in response to T3 and aging.

Materials and Methods

Animals. Male Fischer 344 rats were obtained from the National Institute of Aging colony maintained by Harlan Laboratories (Indianapolis, IN). Six-month-old animals (young) were compared with 26-month-old animals (aged). The 6-month-old rat is considered a mature adult, whereas the 26-month-old animal would be considered in the senescent category. Approximately 10% of the cohort at birth will reach the age of 26 months (30). These animals had free access to standard laboratory rat chow (Teklad Laboratories, Madison, WI) and water. All the animals were housed in individual cages. Body weight and food intake were measured every other day for a total of 4 weeks. Within 4 to 5 days of arrival at our animal facility, the food intake of all rats had stabilized and the studies were initiated within 7 days. The data on body weight changes are published previously (31). Rats of both age groups were rendered hypothyroid with 0.025% methimazole (Sigma Chemicals, St. Louis, MO) in the drinking water over 4 weeks. The serum thyroxine level in hypothyroid rats prior to being sacrificed was less than 16.1 nmol/l (the lower limit of the assay used for these studies). Rats were made hyperthyroid with a receptor saturating dose of 15 μg T3/100 g body wt ip daily for 10 days. Euthyroid untreated rats served as controls. The number of rats per group were as follows: 6-month-old hypothyroid, $n = 7$; 6-month-old euthyroid, n = 10; 6-month-old hyperthyroid, n = 10; 26-month-old hypothyroid, n = 7; 26-month-old euthyroid, n = 10; and 26-month-old hyperthyroid, n = 10. The internal organs of all the animals were inspected for gross pathology. Animals ($n = 4$) bearing tumors were excluded from the analysis. Serum creatinine level was determined to exclude renal failure.

Northern Blot Analysis of S14 mRNA. Total hepatic RNA from animals in the various groups were extracted using methods described by Chirgwin *et al.* (32). Northern blot analysis to measure the relative abundance of the mRNA was performed according to procedures described previously (24). Hybridization of the blots were performed with radiolabeled S14-cDNA generously provided by Dr. H. C. Towle, University of Minnesota, or mouse β-actin cDNA given to us by M. Getz, Mayo Clinic, Rochester, MN. Relative levels of hepatic mRNA-S14 were measures in tumor free rats of different ages and thyroid status.

Gel-Retardation Assay. Procedures by radiolabeling the PS-1 binding motif or F1, DNA containing the P1 binding site that spans nucleotide −67 to −44 and −389 to −279, respectively of the S14 gene, incubation of labeled probe with nuclear protein extracts, separation of the protein-DNA complex using low ionic strength native polyacrylamide gel electrophoresis, and detection of the signals have been described previously (15). All reactions contained 2 μg of nuclear protein extract. Nuclear extract from four animals in each of the different groups described in the preceding section were analyzed.

Quantitation of Northern or Gel-Retardation-Signals. Quantitation of the signal intensity arising from mRNA-S14 on the blots or DNA binding activity of PS-1 or P1 was assessed by videodensitometry (33). The relative amount of protein:DNA complex in each gel-retardation reaction was calculated by dividing optical density of the shifted material by the sum of the shifted material plus unbound probe. All the results are presented as mean ± SD. The statistical analysis was done by two-way analysis of variance (ANOVA) followed by Student's t test for unpaired variables with Bonferoni correction. $P < 0.05$ was considered the limit for statistical significance.

Results

T3 Induction of mRNA-S14 is Lower in Aged Animals. Northern blot analysis of total RNA from the liver of hypothyroid, euthyroid, hyperthyroid young (6-month) and aged (26-month) rats are shown in Figure 1. Autographs of Northern blots hybridized with radiolabeled S14 cDNA are shown in Figure 1A. The same blots hybridized with β-actin cDNA (actual

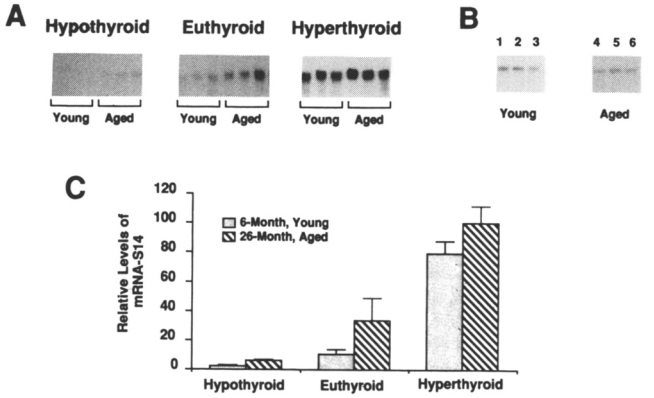

Figure 1. Effect of age on hepatic S14 mRNA levels in various thyroid states. Panel A shows the autoradiographs of northern blots hybridized with S14 cDNA. Each lane contains 10 μg per lane of total hepatic RNA from young and aged rats. Samples from hypo-, eu-, and hyperthyroid rats appear in the left, middle, and right panels, respectively. Lanes 1–3 and 4–6 in each panel contains RNA from young and aged animals, respectively. Panel B contains autoradiographs of Northern blots hybridized with mouse β-actin cDNA. Each lane contains 10 μg per lane of total hepatic RNA from young and aged rats. Lanes 1–3 contain total hepatic RNA from young hypo-, eu-, and hyperthyroid rats, respectively. Lanes 4–6 contain total hepatic RNA from aged hypo-, eu-, and hyperthyroid rats, respectively. Panel C, contains a graph showing levels of mRNA-S14 relative to β-actin mRNA in animals from the various groups. Each column and error bar show the mean + SD, respectively. The number of animals per group were the following: young hypo-, 7; young eu-, 10; young hyper-, 9; old hypo-, 6; old eu-, 9; and old hyperthyroid, 9. Both euthyroid and hyperthyroid levels of S14 mRNA in either the 6- or 26-month-old groups of animals were significantly ($P < 0.05$) different from age-matched hypothyroid controls.

blot not shown) and the ratio of S14 mRNA relative to that of β-actin for each sample yielded results appearing in the graph of Figure 1C. A representative blot showing the content of β-actin in a single animal from each group appears in Figure 1B. As expected, S14 mRNA levels correlated with thyroid state in both young and aged rats. In young rats, the level of S14 mRNA increased 5- and 35-fold in euthyroid and hyperthyroid rats, respectively when compared with the hypothyroid animal. Aged rats subjected to the identical protocol yielded a 5- and 15-fold rise in euthyroid and hyperthyroid rats, respectively when compared with the hypothyroid animal. In the aged rats, S14 mRNA levels were consistently higher than those found in young rats regardless of the thyroid status. This observation indicates that age-related increases in S14 mRNA are separate from the effects of thyroid hormone.

When we compared the T3-induction of S14 mRNA from the euthyroid with hyperthyroid state, a 7-fold increase was observed in young rats. The same comparison in aged animals revealed only a 3-fold rise in the level of S14 mRNA between the two thyroid states. This observation indicated that the net induction of S14 mRNA by T3 in euthyroid animals was reduced with aging by roughly 2-fold. A similar analysis from hypo- to euthyroid state was attempted in young and old animals, but, due to the extremely low levels of S14 mRNA in the hypothyroid states, a reliable estimate could not be obtained. Together these observations showed that the aging process blunted the induction of S14 mRNA in euthyroid animals.

Changes in Transcription Factor Activity with T3 and Aging. Since previous studies have demonstrated that age and T3 influenced transcription of the S14 gene, we wondered whether there were changes in activity of hepatonuclear factors known to regulate the S14 gene (24–27). Therefore, we measured the DNA binding activity of two recently described rat hepatonuclear factors, PS-1 and P1 that are known to enhance and repress, respectively transcription of the S14 gene. DNA binding activity of PS-1 is represented by multiple complexes detected by gel-retardation (Fig. 2A). Etiology of the multiple bands observed for the binding of PS-1 to its recognition site has not been investigated. But in previous studies (24), we showed that the formation of all complexes was inhibited by the addition of oligonucleotide homologous to the binding motif for PS-1. Results of current studies detailing the DNA binding activity of PS-1 in young and aged rats of varying thyroidal states appear in Fig. 2. PS-1 activity was always higher in aged rats compared with young animals, irrespective of thyroidal status. In both age groups, the DNA binding activity of PS-1 rose with T3 treatment revealing a close correlation between this parameter and thyroid status of the animal.

Next, we examined the DNA binding activity of a transcription factor, P1 that repressed S14 gene activ-

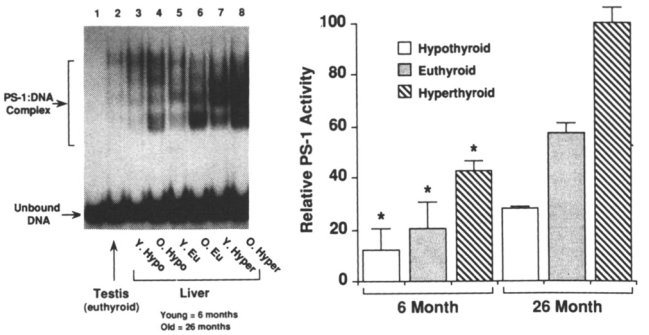

Figure 2. DNA binding activity of PS-1. Panel A contains the autoradiographs of gel-retardation studies showing the ability of hepatonuclear extracts from young and aged animals of various thyroid states to bind radiolabeled DNA homologous to the PS-1 binding motif. Each reaction was performed with the identical amount of hepatonuclear extract (2 μg). All lanes contained radiolabeled probe and hepatonuclear protein extract from the following: 1, none; 3, young hypothyroid; 4, aged hypothyroid; 5, young euthyroid; 6, aged euthyroid; 7, young hyperthyroid; and 8, aged hyperthyroid. Lane 2, shows the activity in nuclear extract from euthyroid testis. Panel B contains a graph showing the relative PS-1 binding activity in animals ($n = 4$) from the various groups. Each column and error bar show the mean ± SD, respectively. *A statistically significant ($P < 0.05$) difference between 26- and 6-month animals of the same thyroid status.

ity. The binding activity of P1 was higher in young versus aged animals (Fig. 3). Thus, reduced DNA binding activity of P1 in aged rats correlated inversely with increased expression of S14 mRNA. In young or aged animals, the DNA binding activity of P1 did not change with thyroidal status.

Since PS-1 and P1 are known to have opposing effects on S14 gene transcription, results showing changes in the activity of these factors with age and T3 imply that they play important roles in modulating S14 gene expression. The close correlation between activity of PS-1 and S14 mRNA suggests that this factor has a direct influence in modulating the S14 gene response to age and T3. In contrast, P1 correlated inversely with S14 gene activity in response to only age and therefore, may point to a permissive effect of this nuclear factor on S14 gene activity. For example, lower levels of the repressor would facilitate the positive effectors of S14 gene expression.

Discussion

In this report, we have presented data that will give us a better understanding of how age and T3 modulate hepatic expression of the S14 gene. Inspection of the results clearly indicate that steady state levels of the mRNA are increased in aged rats irrespective of thyroidal status. This confirms and extends previously published observations on the maturational changes of S14 mRNA levels in rats (5, 8, 23). Of particular interest arising from an analysis of the results suggested that net responsiveness of gene S14 in the hyperthyroid state was reduced by roughly 2-fold in aged rats

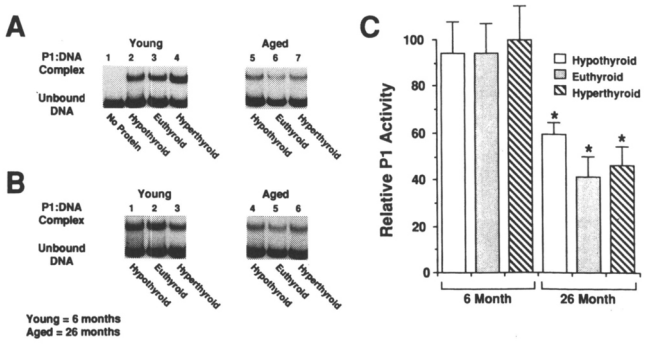

Figure 3. DNA binding activity of P1. Panel A contains the autoradiographs of gel-retardation studies showing the ability of hepatonuclear extracts from young and aged animals of various thyroid states to bind radiolabeled DNA homologous to the P1 binding motif. Each reaction was performed with the identical amount of hepatonuclear extract (2 μg). All lanes contained radiolabeled probe and hepatonuclear protein extract from the following: 1, none; 2, young hypothyroid; 3, young euthyroid; 4, young hyperthyroid; 5, aged hypothyroid; 6, aged euthyroid; and 7, aged hyperthyroid. Panel B shows another series of studies using extracts from a different set of animals. Panel C contains a graph showing the relative P1 binding activity in animals (n = 4) from the various groups. Each column and error bar show the mean ± SD, respectively. *A statistically significant ($P < 0.05$) difference between 6- and 26-month animals of the same thyroid status.

despite elevated basal levels of the mRNA in animals of this chronologic group. In previous studies, we reported a similar observation in the age-related reduction of malic enzyme (ME) mRNA response to T3 (15). The only difference in the ME studies was that basal level of the mRNA, unlike that of S14 mRNA, is reduced in aged animals. This interpretation of the results is dependent on previous results showing that expression of S14 and ME genes is maximal in the presence of receptor saturating doses of T3 (5). These observations taken together support the notion that aging is associated with reduced T3 responsiveness of specific genes irrespective of the changes in their basal level of expression.

The changes in mRNA S14 levels with age and in response to T3 correlate closely with the DNA binding activity of PS-1, a hepatonuclear factor that enhances S14 gene expression (24, 25). The age-related reduction in the DNA binding activity of P1, a repressor (26, 27) of S14 gene activity would also facilitate expression of S14 mRNA. Although P-1 binding activity did not correlate with S14 gene in response to thyroid hormone treatment in 6- (young) or 26- (aged) month-old animals, it is clearly associated with T3 action in the 2-month-old rats (27). One explanation is that P-1 responsiveness to T3 is lost at an early stage during developmental maturation of the animal.

Based on the findings contained in this report it is tempting to postulate the following model as an explanation for the combined actions of PS-1 and P1 on S14 response to age and T3. The basic premise is that the gene is activated and repressed by PS-1 and P1, respectively. Age and T3 related increases in S14 gene expression are mediated by mechanisms that augment the function of PS-1, a factor (or factors) that increases transcription of the gene. In addition to a rise in the DNA binding activity of PS-1 there is a reduction in P1, the repressor (27). Therefore, the combined effects of elevations in PS-1 and reductions of P1 have cumulative effect to augment S14 gene expression. It is difficult to ignore the possibility that cellular mechanisms which control PS-1 and P1 function have been coordinated by a yet unknown process in which discordant behavior of the DNA binding activity of PS-1 and P1 leads to increased S14 gene expression. Overall, it is evident that the additive effect of the age-related rise and fall in PS-1 and P-1, respectively, enhance the net activity of the S14 gene in aged rats. This novel observation complements the previously published finding of age-related reduction in methylcytosine content of S14 DNA as the molecular basis for increased S14 gene expression with age (22).

Despite the unknown biological function of S14 gene product, it has been generally accepted as a valuable marker of thyroid hormone action. Within this context, the data presented in this study supports the

notion that aging is a state of relative resistance to the effects of thyroid hormone. This idea is underscored by the clinical disorder of apathetic hyperthyroidism in the elderly. The age-related reduction in the response of gene S14 to thyroid hormone is due in part to alterations in the activity of two hepatonuclear transcription factors, PS-1 and P1. Although we have interpreted our results to be consistent with a relative resistance to thyroid hormone, the underlying observation is a simple rise in S14 gene expression with age. Future studies are needed to clarify the mechanisms by which responsiveness to T3 appears in early development but disappears with aging.

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