The high content of natural suppressor serine tRNA in dystrophic mouse muscle

Teruaki HITAKA,* Takaharu MIZUTANI,*§ Kimitsuna WATANABE† and Tsuyoshi TOTSUKA‡

*Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467, Japan,

†Department of Life Chemistry, Tokyo Institute of Technology, Nagatsuta, Yokohama 227, Japan, and

‡Department of Physiology, Institute for Developmental Research, Aichi Prefectural Colony, Aichi 480-03, Japan

In order to gain an insight into the pathogenesis of mouse muscular dystrophy, we investigated the natural suppressor serine tRNA. The natural suppressor seryl-tRNA was distinguished from the other seryl-tRNAs on the basis of its specific property of being converted into phosphoseryl-tRNA by a tRNA kinase. On a wet-weight basis, the content of total tRNA in dystrophic muscles was 47% of that in normal muscles. Although the serine-accepting activities of tRNA were similar in muscles of 3-month-old dystrophic and normal mice, the ratio of [³²P]phosphoseryl-tRNA (suppressor tRNA) to the total serine tRNA was significantly enhanced in dystrophic muscles compared with that in normal muscles. This high content of suppressor tRNA in dystrophic muscles was further confirmed by dot-blot hybridization experiments with the DNA probes CGTAGTCGGCAGGAT and CGCCCGAAAGGTGGAA for major tRNA^{Ser}_{IGA} and suppressor tRNA respectively. At the early postnatal age of 3 weeks, when only a week had elapsed since the first manifestation of the dystrophic symptom (hindleg dragging), the ratio of suppressor tRNA to major tRNAs in dystrophic hindleg muscles was abnormally increased. Thereafter it decreased with age in normal mice but remained almost unchanged in dystrophic mice. Consequently, at 3 months old, it was 1.7 times higher in dystrophic than in normal mice. The suppressor tRNA is now accepted to play a role in the synthesis of glutathione peroxidase. The present study showed that the content of this enzyme was abnormally elevated in dystrophic mice. Previously we had demonstrated that the docosahexaenoic (C_{22:6}) acid content in phospholipids was decreased, possibly resulting from the enhanced oxidative milieu caused by the dystrophic condition. Thus far, the findings suggest that an increase in the contents of suppressor tRNA and glutathione peroxidase in dystrophic muscle may have been secondarily induced by such a highly oxidative state in the dystrophic condition. However, it is difficult to exclude the possibility that the natural suppressor tRNA plays a primary role in the pathogenesis of muscular dystrophies.

INTRODUCTION

Natural suppressor tRNA, corresponding to the opal nonsense codon UGA, is present in vertebrates [1–3]. This suppressor tRNA accepts serine, and the seryl-tRNA is subsequently converted into phosphoseryl-tRNA (Ps-tRNA) by a tRNA kinase [1,4]. Meanwhile, the opal nonsense codon UGA was found in the in-frame of the mRNA of glutathione peroxidase (GSHPx) as the codon of selenocysteine (SeCys), which is present at the active site of GSHPx [5,6]. Results obtained with perfused rat liver revealed that the carbon source for the SeCys skeleton was provided by serine [7]: specific labelling of SeCys with ¹⁴C did not come from cysteine but came from serine. These results strongly suggest that natural suppressor seryl-tRNA is converted into SeCys-tRNA via Ps-tRNA and that the SeCys-tRNA is used for the co-translational incorporation of SeCys into GSHPx. We have previously presented some evidence for the enzymic conversion of bovine suppressor Ps-tRNA into SeCys-tRNA [8,9].

GSHPx plays an important role in the detoxification of H₂O₂ and organic hydroperoxides, of which the

major components are lipid peroxides of polyunsaturated fatty acids such as arachidonic (C_{20:4}) acid and docosahexaenoic (C_{22:6}) acid. This peroxidation is increased in dystrophic muscles [10], and the concentration of malondialdehyde, a peroxidation product of polyunsaturated fatty acids, is also increased in the muscles [11]. Lipid peroxides are formed when oxygen radicals in solution react with polyunsaturated fatty acids in lipids [12]. Polyunsaturated fatty acids in the phospholipids of membranes are especially susceptible to such damage by peroxidation. We have previously reported a decrease in the docosahexaenoic acid content in phospholipids of skeletal muscles in dystrophic mice [13]. It has also been reported that the content of GSHPx relevant to the decomposition of hydroperoxide is increased in human dystrophic muscles [11,14]. It seems likely that the high content of GSHPx may be caused by the high content of the above natural suppressor tRNA. In order to understand the role of the natural suppressor tRNA in the pathogenesis of muscular dystrophy, we determined the content of natural suppressor tRNA in mouse skeletal muscles by dot-blot hybridization with DNA probes [15] and by the phosphorylation of sup-

Abbreviations used: SeCys, selenocysteine; Ps, phosphoserine; GSHPx, glutathione peroxidase.

§ To whom correspondence should be addressed.

pressor seryl-tRNA in a crude tRNA preparation, as well as the content of GSHPx in mouse skeletal muscles.

MATERIALS AND METHODS

Animals

Dystrophic (*dy/dy*) and normal (*?/+*) mice of the strain C57BL/6J-*dy* were used. This strain originated in the Jackson Laboratory (Bar Harbor, ME, U.S.A.) and has been maintained by sib mating for over 30 generations in our laboratory. Since symptoms of dystrophy (hindleg dragging) first appear 2 weeks after birth, normal mice of a mother strain (C57BL) of the dystrophic strain were used to obtain younger control values for the above dystrophic mice.

Materials

Seryl-tRNA synthetase, tRNA kinase and bovine tRNA were prepared as described in previous papers [4,16,17]. We used bovine tRNA as a control mammalian tRNA because all primary structures of tRNA are universal in mammals. Yeast tRNA and *Escherichia coli* tRNA were purchased from Sigma Chemical Co. DNA probes complementary to the region near the 3'-terminus of major tRNA^{Ser}_{IGA} [18] and suppressor tRNA^{Ser}_{NCA} [18] were prepared by means of a DNA synthesizer (Applied Biosystems 381A) and purified by h.p.l.c. These sequences are 5'-CGTAGTCGGCAGGAT-3' (probe *a*) and 5'-CGCCCGAAAGGTGGAA-3' (probe *b*) respectively. The labelled compounds were [γ -³²P]ATP (5000 Ci/mmol; Amersham) and [³H]serine (20 Ci/mmol; Amersham).

General methods

The serine-accepting activity of tRNA was measured as described previously [4]. The activity of GSHPx in the supernatant of muscle extracts was assayed as described in the literature [19]. The amount of selenium was determined fluorimetrically by the method using diaminonaphthalene [20].

Preparation of tRNA and its phosphorylation

tRNA was prepared in accordance with a previous paper [17], as follows. The hindleg muscles were removed from mice, homogenized with quartz sand and then mixed with 5 vol. of 150 mM-NaCl/10 mM-MgCl₂/0.5 mM-EDTA/20 mM-Tris/HCl buffer, pH 7.0, containing 3 mM-diethyl pyrocarbonate. The mixture was centrifuged at 8000 *g* for 10 min and the supernatant was further centrifuged at 105000 *g* for 60 min. The supernatant was mixed with an equal volume of 90% phenol including 1% (w/v) SDS. This solution was vigorously shaken and centrifuged. The extracted tRNA was precipitated by the addition of 2 vol. of ethanol. The tRNA preparation was deacylated by incubation in 0.1 M-Tris buffer, pH 9.1, for 30 min.

Phosphorylation of [³H]seryl-tRNA with [³²P]ATP by tRNA kinase was carried out, as described previously with seryl-tRNA isolated on Sephacryl S-200 and unlabelled ATP [21]. [³²P]Phosphoseryl-tRNA was separated from free [³²P]ATP on Sephacryl S-200. The peak of Ps-tRNA was precipitated by addition of 3 vol. of ethanol and collected. Ps-tRNA was chromatographed on a BD-cellulose (benzoylated DEAE-cellulose) column (0.6 cm × 5 cm) by elution with a linear gradient of 0.4 M-NaCl to 2 M-NaCl/15% (v/v) ethanol in 10 mM-MgCl₂/10 mM-acetate buffer, pH 4.6 (total volume 6 ml).

Radioactivity in the eluate, blotted on filter paper, was measured after washing with cold 10% (w/v) trichloroacetic acid.

Dot-blot hybridization

The dot-blot hybridization was carried out according to the method of Kumazawa *et al.* [15], as follows. The DNA probes were labelled with [γ -³²P]ATP by T₄ polynucleotide kinase (Boehringer). The specific labelling is with a 10⁶ c.p.m./pmol DNA probe. The tRNA solution was dotted on nylon membranes (Pall Biodyne A) and then cross-linked by u.v. irradiation at 302 nm for 30 min. After the membranes had been incubated for 1 h at 43 °C for probe *a* and at 47 °C for probe *b* (probe concentration 0.1 nM) in a standard hybridization buffer (0.9 M-NaCl/5 mM-EDTA/0.1% SDS/50 mM-NaH₂PO₄/5 × Denhardt's solution), the membranes were incubated in the presence of the ³²P-labelled probe for 16 h at the appropriate temperature. (1 × Denhardt's solution is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin.) The membranes were then washed with 3 × SSC for 1 h with several changes of 3 × SSC. (1 × SSC is 150 mM-NaCl/15 mM-sodium citrate buffer, pH 7.0.)

Analysis with a Bioimage-Analyzer

The sensitivity of the Bioimage-Analyzer (Fuji Film Co.) for ³²P and ¹⁴C is about 1000 times higher than that of autoradiography with X-ray film. The content of suppressor tRNA in a crude tRNA preparation of muscles was about 0.03% of total tRNA. The radioactivity of tRNA (0.01 A₂₆₀ unit) dotted on the membrane was marginal for determination by autoradiography and scintillation counting, and therefore we used the Bioimage-Analyzer to determine the amount of radioactivity on the membranes. The membranes were placed in the cassette with an imaging plate and left to stand for 20 h. Then the activated imaging plate was analysed by using the Bioimage-Analyzer.

RESULTS

Table 1 shows the weights of some organs in dystrophic mice as percentages of those of normal mice at 2 months old. The values for spleen and liver were in proportion to the value for the body weight, but that for skeletal muscle (24%) was only about half that for the body weight (55%). Thus the atrophy of skeletal muscle was a specific feature in dystrophic mice. On the other hand, the value for tongue muscle (77%) was higher than that for the body weight (55%), showing that the tongue striated muscle had grown normally, unlike skeletal

Table 1. Dystrophic/normal ratios of weight of the body and some organs

Dystrophic/normal (Dy/N) ratios are given as means ± S.E.M. (n = 6).					
	Body	Spleen	Liver	Tongue muscle	Skeletal muscle
Mean wt. of normal mice	17.8 g	86 mg	1.03 g	53 mg	1.12 g
Dy/N ratio (%)	55 ± 1	59 ± 6	63 ± 3	77 ± 1	24 ± 1

striated muscles. Such an apparent hypertrophy has been reported in the case of the tongue in human muscular dystrophy also [22].

Table 2 shows the dystrophic/normal ratios for the amount of tRNA per wet weight and for the serine-accepting activity per A_{260} unit of tRNA in some organs. The tRNA contents in liver, spleen and tongue muscle in dystrophic mice were similar to, or slightly higher (115–124 %) than, the respective values in normal mice. However, the tRNA content in skeletal muscle of dystrophic mice was $9.0 \pm 1.3 A_{260}$ units/g (mean $\pm$ S.E.M.; $n = 6$) and this value was clearly only half (47 %) that in normal mice ($19.6 \pm 3.0 A_{260}$ units/g). This abatement is statistically significant by Student's *t* test ($P < 0.01$), compared with no alteration in the values for dystrophic liver, spleen or tongue muscle. The serine-accepting activities of the tRNA preparations of dystrophic liver, tongue muscle and skeletal muscle were almost normal, but that for spleen was greatly increased; the reason for the great increase in the tRNA activity of the spleen remains to be clarified. The serine-accepting activity of tRNA in dystrophic skeletal muscle is slightly high but is within the normal limit, i.e. the difference between dystrophic liver and skeletal muscle is not statistically significant. Thus in skeletal muscle the content of tRNA per wet weight was significantly low in the dystrophic condition but the activity of this tRNA was almost identical with that in normal mice.

The amount of suppressor tRNA in the total tRNA of skeletal muscle was assessed from the radioactivity of ^{32}P in phosphoseryl-tRNA synthesized from [^3H]seryl-tRNA by the tRNA kinase. The $^{32}\text{P}/^3\text{H}$ relative ratio in dystrophic tRNA was 350 %, where the $^{32}\text{P}/^3\text{H}$ ratio in normal tRNA was taken as 100 %. The amount of suppressor tRNA from dystrophic mice was about 3 times more than that from normal mice. This result and the decreased content of tRNA as shown in Table 2 indicated that in dystrophic mice the content of suppressor tRNA was not changed in spite of the fact that the content of major tRNA decreased. Then we determined the amount of suppressor tRNA in dystrophic and normal mice from chromatographic patterns of [^{32}P]phospho[^3H]seryl-tRNA on BD-cellulose columns. Suppressor tRNA was recovered as a peak of ^{32}P at the later-eluted position on the chromatographic pattern. The pattern of tRNA in dystrophic mice showed a clear peak of ^{32}P , but the peak height of ^{32}P on the pattern for normal mice was about one-fifth of that for dystrophic

Fig. 1. Autoradiogram of the membrane dotted with several tRNAs and hybridized with ^{32}P -labelled probe *b*

(a) shows the results with mammalian tRNA (M), yeast tRNA (Y) and *E. coli* tRNA (each $0.05 A_{260}$ unit). (b) shows the results with some mammalian tRNA fractions obtained from a BD-cellulose column (each $0.025 A_{260}$ unit). 1 is the first fraction on BD-cellulose and 13 is the last fraction containing suppressor tRNA. 5 is the fraction containing the major serine tRNA_{IGA}. 4-3 is the further purified fraction of fraction 13 on a reverse-phase-chromatography-type column.

mice. The patterns of total [^3H]seryl-tRNA of dystrophic and normal mice were similar. In order to confirm the high content of suppressor tRNA in dystrophic mice, we further measured the amount of suppressor tRNA by dot-blot hybridization, which is a very precise method suitable for the evaluation of minor tRNA.

By the dot-blot hybridization method we first confirmed the specificity of probe *a* (major tRNA_{IGA}^{ser}) and probe *b* (suppressor tRNA). Mammalian tRNA, *E. coli* tRNA and yeast tRNA (each $0.05 A_{260}$ unit) were separately dotted on a membrane and the hybridization was carried out as described in the Materials and methods section. Fig. 1(a) shows the results of hybridization experiments with probe *b*. Similar results were obtained with probe *a*. From these results one can see that these probes do not hybridize with yeast tRNA and *E. coli* tRNA preparations but definitely hybridize with mammalian tRNA. Fig. 1(b) shows the hybridization patterns with probe *b* of some fractions (each $0.025 A_{260}$ unit) obtained by chromatography of mammalian tRNA. Suppressor tRNA was rich in fraction 13 (the last fraction) by chromatography on BD-cellulose as described previously [16], and probe *b* strongly hybridized with fraction 13 among the fractions, 1, 5, 10, 11 and 13, as shown in Fig. 1(b). Fraction 4-3 obtained by further purification of fraction 13 through a reverse-phase-chromatography-type column was hybridized with probe *b* much more than fraction 13. Meanwhile, probe *a* was strongly hybridized with fraction 5, which contained major serine tRNA, and weak hybridization was found with fraction 10 (results not shown). No hybridization was found with other fractions. Thus the specificity of probes *a* and *b* was clearly confirmed.

We next tested the relation between the amount of tRNA and radioactivity of ^{32}P -labelled probe *b* on membranes by analyses with the Bioimage-Analyzer. At a constant concentration (0.1 nM) of probe *b* in hybridization buffer, the radioactivity of ^{32}P -labelled probe hybridized on tRNA increased in proportion to the increase in tRNA in the range 0–0.05 A_{260} unit of tRNA. A similar pattern was obtained with probe *a*. Therefore we blotted 1 μl of tRNA solution at a con-

Table 2. Dystrophic/normal ratios of the content and serine-accepting activity of tRNA prepared from some organs

Dystrophic/normal (Dy/N) ratios of tRNA content (determined as A_{260} units/g) and serine-accepting activity (determined as [^{14}C]serine d.p.m./ A_{260} unit) are given as means $\pm$ S.E.M.

Organ (<i>n</i>)	Dy/N ratio of tRNA content (%)	Dy/N ratio serine-accepting activity (%)
Liver (4)	115 $\pm$ 6	91 $\pm$ 9
Spleen (4)	119 $\pm$ 27	431 $\pm$ 154
Tongue muscle (4)	124 $\pm$ 11	89 $\pm$ 15
Skeletal muscle (6)	47 $\pm$ 3	139 $\pm$ 43

Fig. 2. Autoradiogram of the membranes dotted with tRNA from six normal (left) and six dystrophic (right) mice and hybridized with probe *a* (upper two lanes, major serine tRNA) and probe *b* (lower two lanes, suppressor tRNA)

Each tRNA sample was dotted in duplicate (vertical two points in the lanes).

centration of 10 A_{260} units/ml (total 0.01 A_{260} unit) in the subsequent experiments. This value is about 100 times lower than the amount of tRNA necessary for the assay of serine-accepting activity. Each tRNA (0.01 A_{260} unit) was dotted on two membranes. One membrane was used for hybridization with probe *a* (major tRNA) and the other with probe *b* (suppressor tRNA). Fig. 2 shows the similar density of each dot for tRNAs from six 3-month-old dystrophic and six 3-month-old normal mice with probe *a*. In this experiment we used six mice as one group because this is the standard number for a bioassay. The differences in density among the six dots in Fig. 2 show deviation in one group. By contrast, with probe *b* the enhanced hybridization of the tRNA from dystrophic mice is obvious from the stronger density of each dot compared with that with the tRNA from normal mice. Thus the ratio of suppressor tRNA to major tRNA in dystrophic mice was about twice that in normal mice. This difference between dystrophic and normal mice is significant ($P < 0.05$).

Next, in order to study the relation between growth and the content of suppressor tRNA, we prepared tRNA from skeletal muscle of dystrophic and normal mice that were 3 weeks, 6 weeks, 3 months and 4 months old. Since the dystrophic symptom first appears at 2 weeks of age, it is difficult to discriminate between dystrophic and normal littermate mice before 2 weeks. Therefore, in order to obtain information about a younger control value, we examined tRNA samples from 8-day-old normal mice of the mother strain C57BL.

From the results of the analysis of the ^{32}P radioactivity with the Bioimage-Analyzer, an arbitrary unit for each spot was calculated and plotted in Figs. 3(a) and 3(b). Fig. 3(a) shows the change in the content of suppressor tRNA in skeletal muscle during growth. The point at 8 days old was obtained from the mother strain C57BL. The content of suppressor tRNA in total tRNA in normal mice was decreased as growth progressed, depending upon standardization relative to the 8-day-old point, in contrast with almost no change in dystrophic mice. The high content of suppressor tRNA in dystrophic mice is coincident with the result in Fig. 2. Fig. 3(b) shows the change in the extent of major tRNA hybridization with probe *a* during the growth. The content of major tRNA was decreased as growth progressed in both normal and dystrophic mice, depending upon

Fig. 3. Change in tRNA content during growth

(a) Change in amount of skeletal-muscle suppressor tRNA hybridizing with ^{32}P -labelled probe *b*. (b) Change in amount of skeletal-muscle major tRNA_{IGA} hybridizing with ^{32}P -labelled probe *a*. (c) Change in the ratio of the suppressor tRNA to major tRNA. ○, Values with tRNA from normal mice (N); ●, values with tRNA from dystrophic mice (Dy). Each point shows the mean $\pm$ S.E.M. ($n = 6$). Statistical significance: * $P < 0.05$; ** $P < 0.01$.

standardization relative to the 8-day-old point. It is likely that a high content of serine tRNA would be necessary during muscle growth. Consistent with the result in Table 2 (dystrophic/normal ratio 139%), the tRNA content is slightly higher in dystrophic than in normal mice at all stages.

Fig. 3(c) shows age-related changes in the ratio of suppressor tRNA to major serine tRNA in normal and dystrophic muscles during growth. As the animals grew, the ratio of suppressor tRNA to major tRNA in normal muscles decreased but remained almost unchanged in dystrophic muscles. Significant differences between dystrophic and normal muscles were observed at the stages of 6 weeks and 3 months old. Notably, an alteration in the dystrophic condition was found as early as 3 weeks of age (the symptom of dystrophy appears at 2 weeks of age). Therefore it seems highly possible that the high content of suppressor tRNA may be related to the pathogenesis of dystrophy.

Thus the content of suppressor tRNA is abnormally high in skeletal muscles of dystrophic mice. Interestingly, this natural suppressor tRNA has been postulated to play a role in synthesizing GSHPx, the mRNA for which

Fig. 4. Change in the GSHPx content in skeletal muscles from normal (N, ○) and dystrophic mice (Dy, ●) during growth

Statistical significance: * $P < 0.05$; ** $P < 0.01$.

contains in-frame UGA codon. Therefore we next compared the contents of GSHPx in skeletal muscle of dystrophic and normal mice. The result is presented in Fig. 4. The content of GSHPx is significantly higher in dystrophic than in normal mice. This result agrees with the situation in human dystrophy [10]. The fact that GSHPx and suppressor tRNA are concurrently enhanced in dystrophic muscles strongly supports the assumption that natural suppressor tRNA plays a role in synthesizing GSHPx. As noted above, GSHPx contains selenium at the active site as selenocysteine, which is derived from serine. Thereupon we further determined the content of selenium in muscles of 3-month-old mice. The contents in normal and dystrophic mice were 0.097 ± 0.013 and $0.131 \pm 0.013 \mu\text{g/g}$ wet wt. of skeletal tissues (means $\pm$ S.E.M., $n = 6$) respectively. It seems likely that the alteration in selenium content in dystrophic muscles is a result of the increase in GSHPx, judged from the facts that the bound selenium in the body is mostly found in GSHPx and that this increase in selenium content is less than those of suppressor tRNA and GSHPx.

DISCUSSION

This is the first report to show an elevation of the content of natural suppressor tRNA in mouse muscular dystrophy. This tRNA, the anticodon of which is now known to correspond to the UGA stop codon [5,6], is considered to function in incorporating SeCys into GSHPx [9]. But this suppressor tRNA in dystrophic muscle may play an unusual role in synthesizing abnormal types of dystrophin [23] and myogenin [24]. SeCys-tRNA is derived from seryl-tRNA via Ps-tRNA, which is a product of phosphorylation of seryl-tRNA by a tRNA kinase [9]. However, as yet, many problems remain to be resolved. The first is how the natural suppressor tRNA discriminates GSHPx mRNA from many other mRNAs having the UGA termination codon. The second is how to discriminate the SeCys-tRNA on ribosomes from suppressor seryl-tRNA and Ps-tRNA. The concentrations of these aminoacyl-tRNAs are similar *in vivo* [25]. The third is that the suppressor tRNA is a minor constituent. Nevertheless, GSHPx is important for the detoxification of peroxides and must hence be synthesized effectively in mammals. There may be un-

known essential mechanisms in the GSHPx-synthesizing machinery. In dystrophic muscles, which contain high concentrations of peroxides, GSHPx is synthesized effectively, possibly as a result of the increase of suppressor tRNA.

Our previous paper demonstrated that the content of docosahexaenoic acid in phospholipid was low in skeletal muscle of dystrophic mice whereas there was no difference in its content between tongue muscles of dystrophic and normal mice [13]. This result supports the bone-muscle imbalance hypothesis proposed previously by Totsuka [26]. Meanwhile, it is well known that docosahexaenoic acid in phospholipid is one of the biological substances most susceptible to peroxidation. The membranes are damaged by oxidation of docosahexaenoic acid and then the oxidized fatty acid is removed by phospholipase A_2 . Lysophospholipid is re-acylated to phospholipids by acyltransferase [27]. The free oxidized fatty acid is further oxidized and hydrolysed to malondialdehyde. It has been reported that the concentrations of lipid peroxides and malondialdehyde are high in dystrophic muscle [10,28–30]. These results are in agreement with our previous findings of a low docosahexaenoic acid content in phospholipids [13]. Thus membranes in dystrophic muscles are regarded as being exposed to such a toxic oxidative environment. In turn, the highly oxidative milieu will induce the synthesis of GSHPx, which decomposes peroxides. Thus it is first necessary that the suppressor tRNA must increase because it will be essential for the GSHPx synthesis. However, it is difficult to explain the mechanisms of the specific increase in suppressor tRNA compared with many other species of tRNAs.

There have been many investigations of tRNA and protein syntheses in mouse dystrophic muscle [31]. It was reported that the total RNA content did not change at any stage of mouse muscular dystrophy [32]. The turnover rate of hereditary skeletal-muscle RNA was not increased in dystrophic mice [33]. But these RNAs are mostly rRNAs, which are major constituents of control RNAs, and it is not surprising that these results are different from the present results on tRNA. Hayashi *et al.*, on a DNA basis, noted that RNA was decreased in dystrophic mice [34]. In dystrophic muscles the amino acid-incorporating activity of the poly(U)/polyribosome system was found to be abnormally enhanced [35,36]. This result agrees with our results in Table 2, in which dystrophic tRNA is low in content but high in serine-accepting activity. Although many results concerning RNA and protein syntheses [31] have been obtained, few results on tRNA, especially natural suppressor tRNA, were available before this present study.

We thank Professor Y. Takagi and Dr. N. Harada of Fujita Gakuen Health University for giving us full facilities for the use of the Bioimage-Analyzer. We thank Dr. Y. Kumazawa for preparation of DNA probes and Mr. N. Maruyama for assay of the activity of GSHPx in muscles. This work was supported in part by Research Grant no. 61571086 from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Mäenpää, P. H. & Bernfield, M. R. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **67**, 688–695

2. Hatfield, D. & Portugal, F. H. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **67**, 1200–1206
3. Hatfield, D. (1985) *Trends Biochem. Sci.* **10**, 201–204
4. Mizutani, T. & Hashimoto, A. (1984) *FEBS Lett.* **169**, 319–322
5. Chambers, I., Frampton, J., Goldfarb, P., Affara, N., McBain, W. & Harrison, P. R. (1986) *EMBO J.* **5**, 1221–1227
6. Sukenaga, Y., Ishida, K., Takeda, T. & Takagi, K. (1987) *Nucleic Acids Res.* **15**, 7178
7. Sunde, R. A. & Evenson, J. K. (1987) *J. Biol. Chem.* **262**, 933–937
8. Mizutani, T. & Hitaka, T. (1988) *FEBS Lett.* **232**, 243–248
9. Mizutani, T. (1989) *FEBS Lett.* **250**, 142–146
10. Mechler, F., Imre, S. & Dioszeghy, P. (1984) *J. Neurol. Sci.* **63**, 279–283
11. Burr, I. M., Asayama, K. & Fenichel, G. M. (1987) *Muscle Nerve* **10**, 150–154
12. Del Maestro, A. F. (1980) *Acta Physiol. Scand.* **492**, 153–168
13. Futo, T., Hitaka, T., Mizutani, T., Okuyama, H., Watanabe, K. & Totsuka, T. (1989) *J. Neurol. Sci.* **91**, 337–344
14. Myllylä, V., Kihlström, M., Takala, T. E. S., Tolonen, U., Salminen, A. & Vinko, V. (1986) *Acta Neurol. Scand.* **74**, 17–24
15. Kumazawa, Y., Yokogawa, T., Miura, K. & Watanabe, K. (1988) *Nucleic Acids Res. Symp. Ser.* **19**, 97–100
16. Mizutani, T., Narihara, T. & Hashimoto, A. (1984) *Eur. J. Biochem.* **143**, 9–13
17. Narihara, T., Fujita, Y. & Mizutani, T. (1982) *J. Chromatogr.* **236**, 513–518
18. Sprinzl, M., Moll, J., Messner, F. & Hartmann, T. (1985) *Nucleic Acids Res.* **13**, r1–r49
19. Tappel, A. L. (1978) *Methods Enzymol.* **52**, 506–513
20. Hall, R. J. & Gupta, P. L. (1969) *Analyst (London)* **94**, 292–299
21. Mizutani, T. & Tachibana, Y. (1986) *FEBS Lett.* **207**, 162–166
22. Ardran, G. M., Hamilton, A. & Kemp, F. H. (1973) *Clin. Radiol.* **24**, 359–364
23. Arahata, K., Ishiura, S., Ishiguro, T., Tsukahara, T., Suhara, Y., Eguchi, C., Ishihara, T., Nonaka, I., Ozawa, E. & Sugita, H. (1988) *Nature (London)* **333**, 863–866
24. Wright, W., Sassoon, D. A. & Lin, V. L. (1989) *Cell* **56**, 607–617
25. Lee, B. J., Worland, P. J., Davis, J. N., Stadtman, T. C. & Hatfield, D. L. (1989) *J. Biol. Chem.* **264**, 9724–9727
26. Totsuka, T. (1987) *Proc. Jpn. Acad. Ser. B* **63**, 131–134
27. van Kujik, F. J. G. M., Sevanian, A., Handelman, G. J. & Dratz, E. A. (1987) *Trends Biochem. Sci.* **12**, 31–34
28. Kar, N. C. & Pearson, C. M. (1979) *Clin. Chim. Acta* **94**, 277–280
29. Jackson, M. J., Jones, D. A. & Edwards, R. H. T. (1984) *Med. Biol.* **62**, 135–138
30. Murphy, M. E. & Kehrer, J. P. (1989) *Chem.–Biol. Interact.* **69**, 101–173
31. Strickland, K. P., Hudson, A. J. & Thacker, J. H. (1979) *Ann. N.Y. Acad. Sci.* **317**, 187–205
32. Srivastava, U., Devi, A. & Sarkar, N. (1963) *Exp. Cell Res.* **29**, 289–297
33. Girkin, G., Fitch, C. D. & Dinning, J. S. (1960) *Arch. Biochem. Biophys.* **93**, 224–228
34. Hayashi, Y., Suzuki, H. & Totsuka, T. (1975) *J. Biochem. (Tokyo)* **77**, 761–768
35. Srivastava, U. (1969) *Arch. Biochem. Biophys.* **135**, 236–243
36. Nwagwu, M. (1975) *Eur. J. Biochem.* **56**, 123–127

Received 7 June 1989/6 September 1989; accepted 12 September 1989