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INTER-TISSUE DIFFERENCES IN MITOCHONDRIAL ENZYME ACTIVITY, RNA AND DNA IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Summary

We examined whether the relationships between mitochondrial enzyme activity, mitochondrial DNA (mtDNA) and mitochondrial RNA (mtRNA) were conserved in rainbow trout (*Oncorhynchus mykiss*) tissues that differ widely in their metabolic and molecular organization. The activity of citrate synthase (CS), expressed either per gram of tissue or per milligram of total DNA, indicated that these tissues (blood, brain, kidney, liver, cardiac, red and white muscles) varied more than 100-fold in mitochondrial content. Several-fold differences in the levels of CS mRNA per milligram of DNA and CS activity per CS mRNA were also observed, suggesting that fundamental differences exist in the regulation of CS levels across tissues. Although tissues varied 14-fold in RNA g^{-1}, poly(A^+) RNA (mRNA) was approximately 2 % of total RNA in all tissues. DNA g^{-1} also varied 14-fold across tissues, but RNA:DNA ratios varied only 2.5-fold. The relationship between two mitochondrial mRNA species (COX I, ATPase VI) and one mitochondrial rRNA (16S) species was constant across tissues. The ratio of mtRNA to mtDNA was also preserved across most tissues; red and white muscle had 10- to 20-fold lower levels of mtDNA g^{-1} but 7- to 10-fold higher mtRNA:mtDNA ratios, respectively. Collectively, these data suggest that the relationship between mitochondrial parameters is highly conserved across most tissues, but that skeletal muscles differ in a number of important aspects of respiratory gene expression ('respiratory genes' include genes located on mtDNA and genes located in the nucleus that encode mitochondrial protein) and mtDNA transcriptional regulation.

Key words: mitochondria, rainbow trout, *Oncorhynchus mykiss*, muscle bioenergetics, mtDNA, citrate synthase, mitochondrial biogenesis.

Introduction

Most ATP required by the cell under resting conditions is generated aerobically within mitochondria *via* oxidative phosphorylation. Increases in ATP demand result in the stimulation of oxidative phosphorylation, which is achieved *via* acute regulation of the existing pool of mitochondria. Apart from acutely regulating oxidative phosphorylation, tissues are capable of altering their mitochondrial content in response to environmental and metabolic factors including cold exposure (Buser *et al.* 1982), cold acclimation (Eggington and Sidell, 1989; Guderley, 1990), thyroid state (Sterling and Brenner, 1995; Ballantyne *et al.* 1992; Leary *et al.* 1997), endurance training (Farrell *et al.* 1991; Tyler *et al.* 1998) and chronic electrical stimulation (Williams *et al.* 1987). Although mitochondrial proliferation has been observed in many tissues and species (see Moyes *et al.* 1998), the regulation of the process of mitochondrial biogenesis has been studied primarily in mammalian skeletal muscle (e.g. Hood *et al.* 1989, 1994; Gagnon *et al.* 1991) and, to a lesser extent, in liver (Martin *et al.* 1993; Izquierdo *et al.* 1995).

While aerobic tissues share a dependence upon oxidative phosphorylation for energy, they differ in a number of respects expected to impact upon the molecular regulation of mitochondrial properties. Tissues such as liver and kidney maintain mitotic potential, whereas others such as brain and striated muscle are post-mitotic. Biosynthetic tissues such as liver possess a high mitochondrial content primarily for producing energy and carbon intermediates for anabolic pathways and experience less of a 'rest-to-work' transition. In contrast, heart has a high mitochondrial content and constantly works at a high proportion of its $\dot{V}_{O_2max}$ (see Moyes, 1995). Slow oxidative fibers only approach $\dot{V}_{O_2max}$ during exercise, while fast glycolytic fibers rely upon mitochondrial metabolism primarily during rest and recovery from exercise (see Moyes and West, 1995).

While these generalizations are valid for most vertebrates, fish differ from mammals in a number of respects and therefore represent an interesting model for studying the fundamental properties of mitochondria in vertebrate tissues and the evolutionary processes that shape them. Fish red blood cells, unlike those of mammals, are nucleated, transcriptionally active (see Currie and Tufts, 1997) and derive most of their energy from oxidative phosphorylation (Boutilier and Ferguson, 1989;

Ferguson *et al.* 1989). Fish skeletal muscle is separated into two anatomically discrete red and white fiber types that collectively comprise 60–70 % of the body mass. Fish white muscle contains 2–4 % mitochondria per unit volume, similar to the range for most mammalian skeletal muscles (e.g. Howald *et al.* 1985; Johnston *et al.* 1997), but the mitochondrial volume density of fish red muscle is approximately ten times higher and approaches that of mammalian cardiac muscle at 25–44 % (Suarez, 1996; Battersby and Moyes, 1998). Fish mitochondria also possess many unusual properties with respect to fuel preference (see Moyes *et al.* 1990), ultrastructure (Moyes *et al.* 1992) and energy metabolism (Moyes *et al.* 1992).

Mitochondrial biogenesis and mitochondrial DNA (mtDNA) replication are thought to be under nuclear control (see Attardi and Schatz, 1988; Poyton and McEwen, 1996). Thus, it is assumed that differences in mitochondrial capacities among tissues are due to nuclear regulation. Recently, we reported that trout red and white muscles differ several-fold in levels of mtDNA, when expressed per gram tissue; however, when mtDNA levels are expressed relative to levels of nuclear DNA, there were no differences between fiber types (Battersby and Moyes, 1998). It was therefore intriguing to consider the possibility that inter-tissue differences in mtDNA per gram might arise primarily from differences in the number of nuclei per gram (an ultrastructural parameter) rather than from differences in the regulation of the nucleus. In the present study, we measured levels of DNA, mtDNA, RNA, poly(A^+) RNA and respiratory gene mRNA and the activity of citrate synthase in seven tissues from rainbow trout (*Oncorhynchus mykiss*) to investigate the nature of the relationships among mitochondrial parameters.

Materials and methods

Source and maintenance of animals

Adult male and female rainbow trout [*Oncorhynchus mykiss* (Walbaum)] ranging in mass from 0.8 to 1.2 kg were purchased from Pure Springs Trout Farm (Belleville, Ontario, Canada). Fish were held for 2 days at 15 °C in a single flow-through tank under a 12 h:12 h light:dark photoperiod and fed standard trout chow *ad libitum*. Fish were killed with a swift blow to the head, and samples of liver, kidney, heart, blood, white muscle, red muscle and brain were flash-frozen in liquid nitrogen. Tissues were then pulverized into a powder slurry using a mortar and pestle cooled under liquid nitrogen and stored at –80 °C for up to 48 h.

Enzymes

Powdered tissue was weighed and homogenized in 9 vols of homogenization buffer (20 mmol l^{-1} Hepes, 1 mmol l^{-1} EDTA, 0.1 % Triton X-100) using a Polytron tissue homogenizer (Kinematica). Citrate synthase (CS) activities were determined at 25 °C using a Molecular Devices Spectramax 250 spectrophotometer as described previously (Moyes *et al.* 1997). Equal volumes of the same homogenates were also used for determinations of tissue mtDNA content (see DNA isolation and analysis).

Construction of cDNA probes

Fish-specific probes for 16S, ATPase VI and COX I RNA were constructed as described previously (see Battersby and Moyes, 1998). The probe for CS was amplified using the polymerase chain reaction at 57.5 °C from first-strand cDNA prepared from total RNA of rat gastrocnemius muscle. Degenerate primers were designed on the basis of the pig/rabbit consensus sequence published by Annex *et al.* (1991). A 765-base-pair product was cloned into pCRII (Invitrogen), transfected into One-Shot cells (Invitrogen) and found to be 90 % identical to human CS mRNA (MOBIX, McMaster University, Canada). All radiolabelled probes for northern blots were prepared by adding 50 ng of cDNA and 1.85 MBq of [^{32}P]dCTP to Ready-to-Go labelling beads (Pharmacia).

DNA isolation and analysis

Total cellular DNA was isolated from 750 mg of powdered tissue after digestion in 100 mmol l^{-1} NaCl, 25 mmol l^{-1} EDTA, 10 mmol l^{-1} Tris (pH 8.0), 0.5 % SDS and 0.1 mg ml^{-1} proteinase K for 18–24 h at 50 °C. Tissue samples were then extracted in an equal volume of phenol/chloroform/isoamyl alcohol (24:25:1 by volume), vortexed and centrifuged at room temperature (21–25 °C) for 5 min at 3000 ***g***. The aqueous phase was removed, and the DNA was precipitated overnight at 4 °C using 2 vols of 100 % ethanol and 0.5 vols of 7.5 mol l^{-1} ammonium acetate. Samples were centrifuged, the pellet was resuspended in TE buffer (10 mmol l^{-1} Tris-Cl, 1 mmol l^{-1} EDTA, pH 8.0), and potential RNA contaminants were removed by incubating the mixture for 1 h at 37 °C with 0.1 % SDS and 1 μg μl^{-1} DNAase-free RNAase (see Ausubel *et al.* 1992). DNA was then re-extracted twice and precipitated as described above. Samples were resuspended in TE buffer (pH 8.0), and the genomic DNA concentration was quantified spectrophotometrically in triplicate. Throughout the procedure, recovery volumes were measured to ensure that extractions were quantitative.

Total DNA (5 μg) was diluted to 200 μl in NaCl (0.5 mol l^{-1}), NaOH (0.25 mol l^{-1}) and water, denatured at 95 °C for 15 min, and rapidly cooled on ice. An equal volume of 0.25× standard saline citrate (SSC; 1× SSC is 150 mmol l^{-1} NaCl, 15 mmol l^{-1} sodium citrate) was added, and the samples were dot-blotted onto Duralon nylon membrane (Stratagene) and washed with 6× SSC. The membrane was dried, and DNA was fixed by cross-linking under ultraviolet irradiation. The membrane was then prehybridized in 6× SSC, 5× Denhardt's reagent, 0.5 % SDS and 100 μg ml^{-1} denatured salmon sperm DNA at 65 °C in a Hybaid mini-hybridization oven. After 5 h, prehybridization solution was replaced with hybridization solution (6× SSC, 0.5 % SDS and 100 μg ml^{-1} denatured sheared herring sperm DNA) and COX I cDNA probe. Following hybridization for 12–18 h at 65 °C, the membrane was washed at the same temperature for 15 min, twice with 1× SSC/0.1 % SDS and twice with 0.1× SSC/0.1 % SDS. The membrane was then exposed to autoradiography film (NEN) at room temperature. Relative mtDNA levels were quantified using a Molecular Dynamics densitometer and ImageQuant software. Four exposures were used to ensure the linearity of the film. Single exposure times

did not allow for accurate quantification of all band intensities within the linear range of the film, owing to several-fold differences in mtDNA levels across tissues. As a result, all values for a given tissue were expressed relative to the value for white muscle because this represented an intermediate value between tissues expressing high and low levels of mtDNA.

RNA isolation and analysis

Powdered tissues were weighed in a cold room (4 °C), and total RNA was extracted using the RNeasy kit (Qiagen) and the acid–phenol method of Chomczynski and Sacchi (1987). For tissues with high glycogen contents (liver, white muscle), an added wash in 4 mol l^{-1} LiCl (diethyl-pyrocarbonate-treated) was incorporated into the method prior to the final resuspension. Total RNA measured using both methods was quantified spectrophotometrically in triplicate. Total RNA (5 μg) from acid–phenol extractions was glyoxylated (see Ausubel *et al.* 1990) and electrophoresed on a 1.4 % agarose gel at 4.0 V cm^{-1}. RNA was transferred overnight from the gel onto a Duralon nylon membrane (Stratagene) by capillary transfer in 20× SSC (Sambrook *et al.* 1989). The membrane was rinsed with 2× SSC, air-dried and ultraviolet-crosslinked.

Membranes were prehybridized in 25 mmol l^{-1} K_2HPO_4 (pH 7.4), 5× SSC, 5× Denhardt's reagent, 50 μg ml^{-1} denatured salmon sperm DNA and 50 % formamide for 3–6 h at 42 °C in a Hybaid mini-hybridization oven. Following prehybridization, hybridization solution (25 mmol l^{-1} K_2HPO_4, pH 7.4, 5× SSC, 5× Denhardt's reagent, 50 μg ml^{-1} denatured salmon sperm DNA, 50 % formamide and 25 g l^{-1} dextran sulphate) was added along with the radiolabelled probe of interest. After 12–18 h, membranes were washed twice (15 min) with 1× SSC/0.1 % SDS at 42 °C and twice (15 min) with 0.1× SSC/0.1 % SDS at 65 °C. Bands were quantified by autoradiography and densitometry as described previously.

Upon confirmation that RNA extractions yielded undegraded RNA from all tissues (see Fig. 1), two methods were used to determine the ratio of poly(A^+) RNA to total RNA. First, 1 μg of total RNA was quantified in duplicate spectrophotometrically, denatured in the plate wells with 0.1 mmol l^{-1} NaOH, and dot-blotted as described above in the section on DNA isolation and analysis. The membrane was subsequently prehybridized, hybridized and washed at room temperature (21–25 °C) using protocols identical to those described above except that the formamide was removed from both the prehybridization and the hybridization solutions. The poly(A^+) probe was prepared by end-labelling oligo(dT) (15-mer; Promega) with [γ^{32}P]ATP for 30 min at 37 °C using T4 polynucleotide kinase (Pharmacia). Second, poly(A^+) RNA was extracted from 140 μg of total RNA from the sample that represented the median value for total RNA g^{-1} within each tissue type using MPG streptavidin-coated beads and biotinylated oligo(dT) (CPG, New Jersey, USA). Following quantification at 260 nm, 2 μg of poly(A^+) RNA was glyoxylated, electrophoresed and transferred overnight using protocols identical to those described above. The poly(A^+) northern blot was prehybridized and hybridized at 42 °C in 6× SSC, 5× Denhardt's solution and 0.5 % SDS.

Statistical analysis

Significant differences (P<0.05) between tissues for RNA:DNA and mtRNA species were detected using one-way analysis of variance on ranks and identified using Dunn's test (see Zar, 1996).

Results

Total RNA and poly(A^+) RNA

The two methods used to determine levels of RNA g^{-1} tissue yielded slightly different values, but were highly consistent within tissues (Table 1). Differences in RNA yield between

Table 1. *Citrate synthase activity and RNA and DNA levels in rainbow trout (*Oncorhynchus mykiss*) tissues*

Tissue	DNA content (mg g^{-1})	Total RNA content (mg g^{-1})			RNA:DNA§	Citrate synthase activity (μmol min^{-1} g^{-1} tissue)
		RNeasy*	Acid–phenol*	Ratio‡		
Heart	3.17±0.14	0.64±0.10	1.09±0.052	1.7	0.346±0.016[a]	29.9±1.2
Red muscle	0.82±0.14	0.40±0.0054	0.61±0.026	1.5	0.888±0.237[b]	20.3±0.5
Brain	2.83±0.12	0.74±0.089	1.06±0.030	1.4	0.377±0.021[a]	8.4±0.4
White muscle	0.41±0.039	0.10±0.021	0.28±0.028	2.8	0.730±0.164[b]	4.1±0.3
Kidney	4.31±0.48	2.68±0.16	3.64±0.12	1.4	0.894±0.112[b]	10.8±1.1
Liver	5.91±0.27	3.80±0.17	2.54±0.37	1.5	0.636±0.069[b]	4.0±0.5
Blood	1.05±0.20	0.39±0.04	0.34±0.041	1.1	0.527±0.223[a,b]	0.27±0.04
N	5	3	5			5

Values are means ± S.E.M.

*Trade name of the RNA extraction kit (see Materials and methods).

†Ratio of highest to lowest yield of mg total RNA g^{-1} tissue expressed as either acid–phenol/RNeasy or RNeasy/acid–phenol.

§Total RNA values used to calculate RNA:DNA represent the method of extraction that resulted in the highest RNA yield per gram tissue.

Values with different superscripts are significantly different (P<0.05).

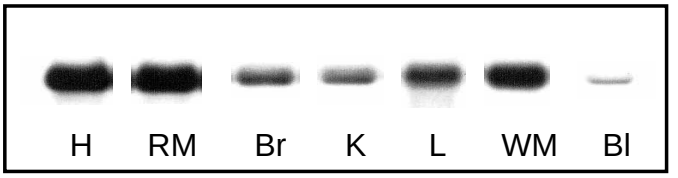

Fig. 1. Representative northern blot probed for COX I (5 μg of total RNA loaded per lane). H, heart; RM, red muscle; Br, brain; K, kidney; L, liver; WM, white muscle; Bl, blood.

methods probably reflect the limitations of each extraction procedure; low molecular mass species such as tRNAs and small rRNAs (e.g. 5.8S) are frequently lost during RNA extractions using the Qiagen RNeasy kit, while extractions with the acid–phenol method, particularly in conjunction with LiCl washes, can lead to non-specific RNA losses. It was therefore assumed that inter-method variability reflected differences in RNA yield. As a result, the values used for subsequent calculations involving total RNA were taken from the method of extraction that resulted in the higher yield. A 14-fold difference in total RNA g^{-1} was observed across tissues.

To measure reliably the proportion of poly(A^+) to total RNA, it is important to ensure that RNA extractions yield undegraded RNA. This may be achieved by visual inspection of a northern blot that has been probed for either a nuclear or a mitochondrial signal. Once probing of a northern blot (5 μg of each RNA) had established that total RNA was not degraded (Fig. 1), two independent methods were used to determine the proportion of RNA that was poly(A^+) RNA. A dot-blot probed with radiolabelled oligo(dT) showed that poly(A^+) RNA was a relatively constant proportion of total RNA, varying less than twofold across all tissues (Fig. 2). Only the oligo(dT)/streptavidin method provided an absolute estimate of poly(A^+) RNA: 1.7–2.9 % of total RNA (N=1; heart, 2.6 %; red muscle, 2.9 %; brain, 2.6 %; white muscle, 2.5 %; kidney, 1.7 %; liver, 2.2 %; blood, 2.5 %).

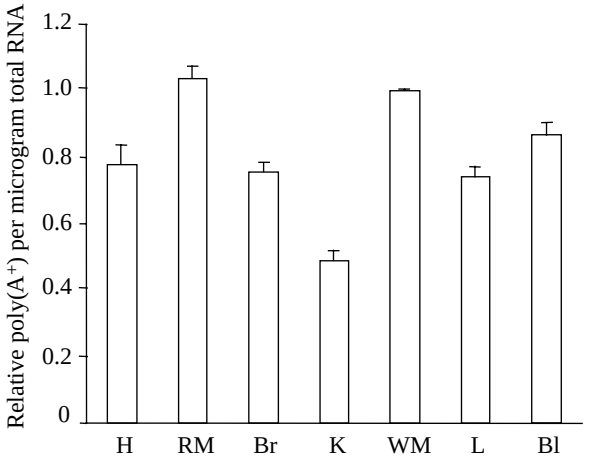

Fig. 2. Poly(A^+) mRNA per total RNA (values are expressed as means + S.E.M. relative to the median value for white muscle; N=5 except for liver and blood, where N=4). H, heart; RM, red muscle; Br, brain; K, kidney; L, liver; WM, white muscle; Bl, blood.

Total DNA and mtDNA

As with total RNA g^{-1}, total DNA g^{-1} varied 14-fold across tissues (Table 1). It was highest in liver and kidney, followed by heart, brain, blood, red muscle and white muscle. Tissues with a high DNA content also had a high RNA content, such that RNA:DNA ratios only varied 2.5-fold, being significantly lower in heart and brain than in all other tissues except blood (P<0.05) (Table 1).

The proportion of mtDNA mg^{-1} tissue varied 44-fold across tissues, and the proportion of mtDNA mg^{-1} DNA varied 10-fold across tissues (Figs 3A, 4). In each tissue, the relationship between relative levels of two mitochondrial mRNA species (COX I and ATPase VI) and one mitochondrial rRNA (16S) was invariant (P>0.05) (Fig. 3B). The ratio mtRNA:mtDNA was also preserved across most tissues, except for red muscle and white muscle which had 10- to 20-fold lower mtDNA g^{-1} tissue but 7- to 10-fold higher mtRNA:mtDNA ratio, respectively.

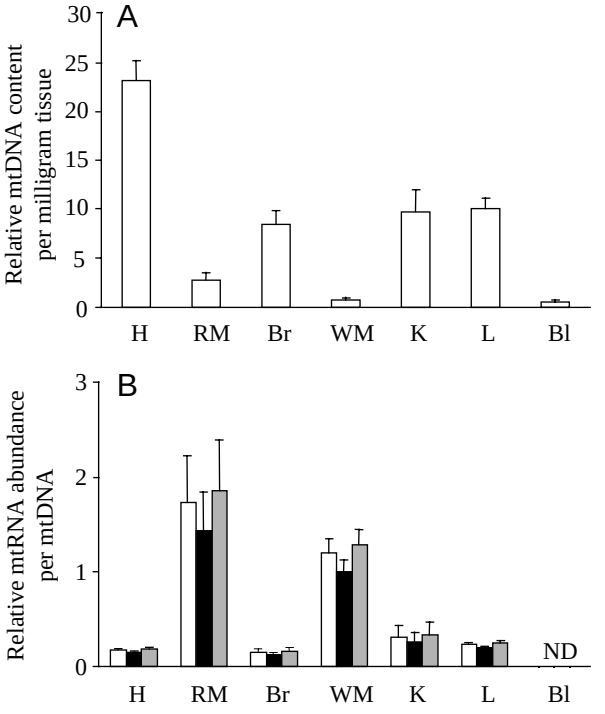

Fig. 3. Intertissue differences in mitochondrial DNA (mtDNA) parameters in rainbow trout (*Oncorhynchus mykiss*) (values are expressed as means + S.E.M. relative to the median value for white muscle). (A) mtDNA mg^{-1} tissue (N=5, except for white muscle where N=4). (B) mtRNA:mtDNA (total RNA isolated using the acid–phenol method; COX I, open columns; ATPase VI, filled columns; 16S, grey columns; N=5, except for white muscle and liver, where N=4; ND, not determined). H, heart; RM, red muscle; Br, brain; K, kidney; L, liver; WM, white muscle; Bl, blood.

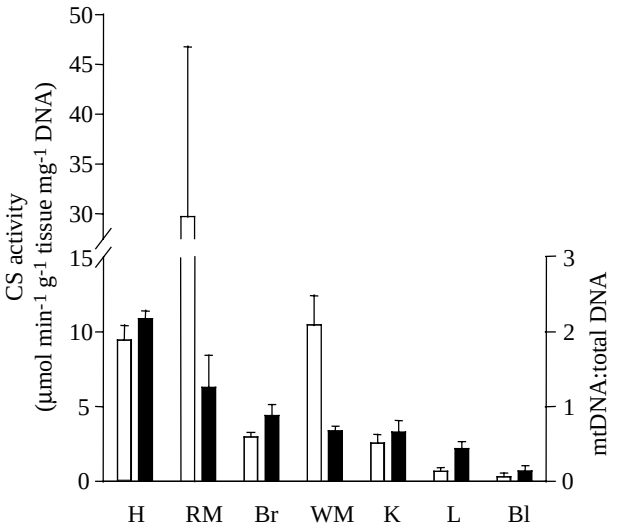

Fig. 4. Citrate synthase (CS) activity mg^{-1} DNA (open columns) and mtDNA:DNA (filled column) for seven tissues from rainbow trout (*Oncorhynchus mykiss*) (*N*=5 for CS activity; *N*=5 for mtDNA for all tissues except white muscle, where *N*=4). Values are as means + S.E.M. H, heart; RM, red muscle; Br, brain; K, kidney; L, liver; WM, white muscle; Bl, blood.

Citrate synthase (CS)

There was an approximately 100-fold range in CS activity across tissues, expressed either per gram tissue (Table 1) or per milligram DNA (Fig. 4). Interesting trends emerged when comparing activity indices across tissues. For instance, heart had an 8.7-fold higher CS activity than white muscle when expressed per gram tissue, but their activities were similar when expressed per milligram DNA because of differences in DNA content among tissues. Complex trends were also evident when comparing CS activity and mRNA levels (Table 2). While CS activity g^{-1} tissue was comparatively low in white muscle, the CS mRNA mg^{-1} DNA of white muscle was approximately 4–5 times higher than those of red muscle and heart, respectively, and 50–100 times higher than those of blood, liver, kidney and brain (Table 2). However, CS activity per CS mRNA was approximately 20-fold lower in white muscle than in kidney and brain, and 10-fold lower than in heart, liver and red muscle (Table 2).

Discussion

Mitochondrial enzyme levels are an important determinant of aerobic capacity for ATP production. Mitochondrial biogenesis requires contributions from both nuclear DNA and mtDNA in a process controlled ultimately by the nucleus. Developmental and adaptive changes in mitochondrial content require appropriate coordination of nuclear and mitochondrial events including transcription, translation, import and mtDNA replication (Williams, 1986; Hood *et al.* 1989; Moyes *et al.* 1998; Takahashi *et al.* 1998). Investigations of the factors that determine the mitochondrial content of a tissue frequently use a physiological challenge (e.g. electrical stimulation; Williams *et al.* 1987) or a developmental stimulus (e.g. myogenesis; Moyes *et al.* 1998) to induce mitochondrial proliferation. The inherent differences in mitochondrial properties among tissues are a less commonly employed experimental system for examining stoichiometric relationships among parameters such as gene copy number, transcript levels and enzyme activities. In trying to understand the molecular basis of differences in mitochondrial enzyme levels between tissues, physiological states and even species, there are important technical and mechanistic reasons to consider the potential confounding influence of parameters such as DNA g^{-1}, RNA g^{-1} and poly(A^+) RNA. Although RNA content and DNA content varied 14-fold across tissues, RNA:DNA showed much less inter-tissue variation. The level of mRNA as a proportion of total RNA was also conserved across tissues, constituting approximately 2 % of total RNA. This is consistent with steady-state values reported for a range of mammalian tissues (see Gagnon *et al.* 1991).

The relationship between nuclear DNA and mtDNA

Differentiation of NIH-3T3 fibroblasts leads to a marked increase in mitochondrial cross-sectional area and mitochondrial enzyme levels (Moyes *et al.* 1997), but the proportion of mtDNA to nuclear DNA does not change (Shay

Table 2. *Citrate synthase mRNA concentration and activity per gram tissue and per milligram DNA in rainbow trout (*Oncorhynchus mykiss*) tissues*

Tissue	CS activity (μmol min^{-1} g^{-1})	CS mRNA (g^{-1})	CS activity per mRNA (μmol min^{-1} g^{-1})	CS mRNA mg^{-1} DNA
Heart	31.79	10.64	2.99	3.36
Red muscle	21.17	4.03	5.25	4.91
Brain	8.85	1.12	7.91	0.39
White muscle	3.67	8.79	0.42	21.25
Kidney	13.35	1.59	8.31	0.37
Liver	3.14	1.52	2.06	0.26
Blood	0.15	0.59	0.25	0.56

N=1; replicates chosen for analysis represent the median value of total RA per gram for each tissue type.

et al. 1990). In most cases, however, adaptive increases in mitochondrial content are accompanied by increases in mtDNA copy number *via* replication. We examined the extent to which the relationship between mtDNA and nuclear DNA varies in tissues of widely different mitochondrial content. As in our earlier study, red and white muscles have similar ratios of mtDNA to nuclear DNA, despite several-fold differences in oxidative capacity (Battersby and Moyes, 1998). When trout tissues with a wider range of oxidative capacities are considered, the relationship between mtDNA and total DNA is not constant.

Relationship between gene copy number and respiratory gene mRNA levels

Adaptive changes in tissue proteins can be achieved through altered rates of synthesis of specific mRNA species (transcriptional control) or protection/inactivation of specific mRNA species (RNA-binding proteins). Mammalian studies have shown shifts in both the profile of individual mRNA species (Seedorf *et al.* 1986; Morrison *et al.* 1987) and the total mRNA pool (Wiesner *et al.* 1992) during physiological adaptation. Comparisons of CS mRNA levels in trout striated muscles (cardiac, red and white muscle) suggest differences in the nature of the pathways regulating CS expression. In heart and red muscle, the relationship between CS activity g^{-1}, CS mRNA g^{-1} and CS activity per milligram DNA were similar. In contrast, white muscle had lower activities of CS than would be expected on the basis of its mRNA levels. At present, it is not known whether these differences in CS expression in white muscle represent differences in transcriptional regulation or a post-transcriptional event.

During adaptation to physiological stressors, increases in mitochondrial transcription are thought to be dependent upon gene amplification *via* mtDNA replication (see Williams, 1986). Transcription of mtDNA occurs as a polycistron, with individual messages released from the primary transcript by endonucleases. The transcript can either be full length or terminated following the transcription of the 16S ribosomal subunit (for a review, see Clayton, 1992). Evidence for mitochondrial mRNA species-specific changes in stability has been found in mammalian liver (Ostronoff *et al.* 1996) and cultured myotubes (Leary *et al.* 1998). If transcript stability is equal, this results in stoichiometric relationships between mtRNA species. Within tissues, individual mitochondrial mRNA and rRNA levels were invariant, but differences in mtRNA:mtDNA occurred between tissues (Fig. 3). While these findings contrast with those of Annex and Williams (1990), who showed that the ratio of mtRNA to mtDNA was relatively constant, trends in red and white muscle suggest that mtDNA in these tissues is more transcriptionally active, as in thyroid-stimulated skeletal muscle (see Wiesner *et al.* 1992). Alternatively, the rates of mitochondrial RNA degradation may be slower in skeletal muscle (see Connor *et al.* 1996).

Unusual mitochondrial parameters in blood

Although much of our focus has been on the unique properties of muscles, blood also differed from other tissues in a number of respects. Unlike mammals, fish blood cells possess nuclei and functional mitochondria, and derive most of their energy from oxidative phosphorylation (Boutilier and Ferguson, 1989). As erythrocytes age, a decrease in mitochondrial capacity occurs (see Keen *et al.* 1989). Although genomic DNA content was within the range for other tissues, contents of mtDNA and CS were much lower. Low and variable mtDNA: total DNA ratios precluded analysis of mitochondrial transcripts per mtDNA, but levels of rRNA and mRNA species shared the stoichiometric relationship demonstrated for other tissues. Blood also maintained high poly(A^+) RNA to total RNA and CS mRNA to DNA ratios, suggesting that the differences are not due to a loss of transcriptional activity in general.

To summarize, the mitochondrial content of tissues can be described biochemically (as enzyme content), ultrastructurally (as cross-sectional area) or, less frequently, genetically (as mtDNA copy number). Direct comparisons of these biochemical, ultrastructural and genetic estimators are difficult because each is expressed in relation to a different denominator (e.g. g^{-1}, cm^{-3}, mg^{-1} DNA, respectively). Comparative studies commonly use CS activity as an indicator of mitochondrial content because it is easy to measure and the enzyme is stable when frozen. In muscles of widely different aerobic capacities, CS activity per gram is a good indicator of the amount of mitochondrial protein per gram tissue and of respiratory capacity (see Moyes *et al.* 1992). Thus, CS activity per gram reflects the capacity for aerobic ATP production in a functionally meaningful way. Nonetheless, CS and mtDNA do not appear to be equivalent indicators of mitochondrial content among fish tissues. This lack of a relationship may relate to inter-tissue differences in the transcriptional activity of mtDNA (see above).

In trout, the ratios poly(A^+) RNA to RNA, RNA to DNA and RNA to mtRNA are conserved among tissues. With the exception of red and white muscle, other inter-tissue ratios, such as mtRNA to mtDNA, are also invariant. However, the relationships between indices of CS expression, such as CS mRNA per milligram DNA and CS activity per CS mRNA, are variable among tissues. Collectively, these data indicate that the relationship between mitochondrial parameters is highly conserved among most tissues, but suggest that skeletal muscles differ from other tissues in a number of important aspects of respiratory gene expression and mtDNA transcriptional regulation.

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