Both nuclear and mitochondrial cytochrome *c* oxidase mRNA levels increase dramatically during mouse postnatal development

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The steady-state levels of 13 of 16 cytochrome *c* oxidase (COX) mRNAs and mitochondrial DNA were measured during the postnatal development of mouse skeletal muscle, ventricle, kidney and brain as well as during the differentiation of mouse myoblasts into myofibres in cell culture. These experiments indicate that large co-ordinated increases in COX mRNA levels and isoform switching are important for the elaboration of this enzyme during postnatal development and demonstrate the importance of gene-regulatory mechanisms in controlling COX activity. On a per nucleus basis, the levels of the mitochondrial- and most nuclear-encoded COX mRNAs co-ordinately increase 3–10-fold during postnatal development, with the highest levels obtained in ventricle and skeletal muscle. However, concentrations of mito-

chondrial and nuclear COX mRNAs remain constant during the differentiation of myoblasts into fibres in cell culture. A gradual change from the liver to the heart isoform of COX subunit VIa mRNA occurs during postnatal development of skeletal muscle and ventricle and is nearly complete 3 days after the formation of myofibres in cell culture. Mitochondrial DNA increases proportionally with COX mRNAs during mouse postnatal development but not during myoblast differentiation in cell culture, in which mitochondrial DNA levels increase 5-fold and mitochondrial mRNA levels remain constant. This suggests that mitochondrial DNA replication may control mitochondrial RNA concentrations during postnatal development but not during myoblast differentiation in cell culture.

INTRODUCTION

Cytochrome *c* oxidase (COX) plays a crucial role in oxidative metabolism, acting as the terminal component of the electron-transport chain in which electrons are passed from cytochrome *c* to molecular oxygen. Mammalian COX is comprised of 13 polypeptides, three encoded by mitochondrial DNA and ten by the nuclear genome [1,2]. The mitochondrial subunits (I, II and III) are analogous to prokaryotic subunits and perform the actual catalytic function of the complex. The nuclear subunits are probably involved in the regulation of the catalytic activity, assembly or stability of the enzyme. Also, tissue-specific isoforms of three nuclear subunits (VIa, VIIa and VIII) have been identified and are involved in specifying the different oxidative capacities of the tissues [3,4].

The activity of oxidative enzymes, including COX, differs in different adult tissues [5] and is generally high in heart and skeletal muscle, although different types of skeletal muscle have different concentrations of oxidative enzymes [6,7]. It is not known how the tissue-specific levels of COX are attained during development and whether there are large changes in the expression of most COX mRNAs during development. Previous experiments indicate that there is little or no net change in COX mRNA levels between myoblasts and myofibres in cell culture, although a transient decrease occurs in some culture systems [8–10]. Furthermore, in some cells, mitochondrial DNA levels are proportional to concentrations of mitochondrial RNAs and nuclear COX mRNAs suggesting that mitochondrial DNA replication is linked to the expression, possibly at the transcription level, of nuclear COX mRNAs [11–14]. It is not known whether this proportionality is maintained during development. Furthermore, although it is known that isoforms are differentially expressed in adult tissues and that a switch from the liver to the heart isoform of subunits VIa and VIII occurs during develop-

ment, little is known about the timing or the control of the isoform switch [15 and references therein, 16–19].

To help identify control points for COX expression during development we have measured the levels of 13 of 16 COX mRNAs and mitochondrial DNA during postnatal development of mouse leg muscle, ventricle, kidney and brain and during myoblast differentiation in cell culture. These experiments show that the establishment of adult COX mRNA levels during postnatal development is complex and involves large co-ordinated changes in mRNA concentrations in all tissues examined as well as isoform switching in striated muscles. Furthermore, mitochondrial DNA generally increases proportionally with COX mRNAs during postnatal development *in vivo* but not during myoblast differentiation in cell culture, implying different mechanisms of regulation of mitochondrial DNA and mRNA in these two systems.

EXPERIMENTAL

Mice and cell culture

Tissues were isolated from male B6/D2 mice. Adult mice were from 60 to 120 days of age. The MM14DZ myoblast cell line derived from Balb/c mice was cultured as described previously [20,21]. Medium was changed 24 h after plating the cells. Myoblast fusion begins about 60 h after plating and is complete by day 4 (96 h). Between day 4 and 6 of culture the myofibres increase in size and smaller myofibres fuse to form larger ones.

Isolation of nucleic acids

RNA was isolated from tissue culture cells as described previously [21,22]. To isolate RNA from tissues, small pieces were gently homogenized in 5 vol. of buffer (7 M guanidinium chloride,

Abbreviation used: COX, cytochrome *c* oxidase.

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Figure 1 Pattern of expression of COX mRNAs (per cellular DNA) during development

Relative levels of specific COX mRNAs per total RNA at different developmental stages were determined by quantitative RNA gel-blot techniques as described in the Experimental section. The values at each time point were multiplied by the corresponding RNA/DNA ratio derived from Figure 2 for developing mice and from previous data for differentiating myoblasts [37]. The resulting mRNA/cellular DNA ratio in adult kidney was set equal to one and the values from the other developmental time points were adjusted proportionally. For subunit VIaH, which is not expressed in kidney, the RNA/cellular DNA ratio was set equal to one in adult leg muscle. (a) Patterns of expression of COX mRNAs during development *in vivo*. ■, Ventricle; ●, leg muscle; ○, kidney; □, brain; ▼, extensor digitorum longus; ▽, soleus. (b) Patterns of expression of COX mRNAs during differentiation of myoblasts into myofibres in cell culture. Myo, day-2 cultures containing myoblasts; Fib4, day-4 cultures containing newly formed myofibres; Fib6, day-6 cultures containing more mature myofibres.

0.5% *N*-lauroylsarcosine, 30 mM sodium acetate, pH 5.5 and 1 mM EDTA) in a Brinkmann Polytron. Then 1.2 vol. of 100 mM Tris/HCl, pH 8.0, containing 10 mM EDTA and 2 mg/ml proteinase K, was added and the resulting solution was incubated for 20 min at 37 °C. The solution was extracted twice with phenol/chloroform and once with chloroform. DNA was separated from the RNAs by extraction with 2 M LiCl and further purified by digestion with RNAase A followed by phenol/chloroform extraction.

Hybridization probes

Hybridization to gel blots was performed as described previously [21,22]. The gel-separated *Pvu*II fragment (nucleotides 6717–7220 in the mouse mitochondrial genome) was isolated from a mouse mitochondrial DNA clone [23]. The *Sac*I–*Pvu*II fragment (nucleotides 9047–10084) was used to detect COXIII. COXIV, Va, Vb, VIaL, VIaH, VIIaL, VIIc and VIII were detected using mouse cDNA hybridization probes. These cDNAs were isolated from a cDNA library derived from myofibres formed in culture using the corresponding human cDNAs for subunits IV [24], Va [25], Vb [26], VIaL [27], VIIa [28] and VIIc and VIII [29] and using a PCR-generated probe for subunit VIaH. The identity of

the mouse cDNA clones was confirmed by DNA sequence analysis. The probes for subunits VIb and VIc were generated by PCR starting with myofibre cDNA. The GATGGAGCCAGT-TCAGATCTTCC and CTTTGTACAGCCGCTTCCCCAAC primers were used for VIb based on the bovine sequence [30], and CTCCAGCCTTCCTCATCTCGTC and ATGAGTTCCGGT-GCACTGTTGCC primers were used for VIc based on the rat sequence [31]. The identities of the PCR-generated probes were also determined by DNA sequence analysis.

RNA gel-blot analysis

Equal amounts of RNA isolated from tissues or tissue cultured cells were denatured with glyoxal [32], fractionated on agarose gels, transferred to nylon paper and hybridized to nick-translated radioactive hybridization probes. In order to compare directly the signals from blots derived from different developmental time points, for which the hybridization efficiency, specific radioactivity of the probe or exposure time may vary, RNA isolated from adult kidney and leg muscle was included on each gel. For each autoradiogram, the signals from the different tissues were divided by the signal from adult kidney or, for COX VIaH, leg

muscle, so that for a given COX mRNA species the signals from different time points are expressed on the same scale.

Several procedures were followed in order to increase the accuracy of the data. To eliminate variability resulting from potential differences between animals, RNA was isolated from tissues pooled from three or more animals. The amount of RNA loaded on to the gels was analysed by staining the gel before blotting the RNA to paper. If a sample was under- or over-loaded, the corresponding density of the autoradiogram was adjusted accordingly. However, in no case was the difference in loading greater than 40%. If the RNA preparation showed signs of significant degradation as indicated by a 28S/18S rRNA ratio of less than 1, fresh RNA was isolated and the measurement was repeated.

Two trials indicate that the experimental results generated using this methodology are highly reproducible. In the first trial, the same two RNA samples isolated from adult liver and kidney were assayed five different times for ten of the COX subunits. The average standard deviation was 23% of the mean. Of the 50 different data points, none was more than 60% different from the mean, only one differed by 50–55% and four by 40–45%. In the second trial, three different pooled RNA preparations from each adult tissue and three different preparations of the RNA isolated from tissue culture cells were assayed for six different COX mRNAs. The variability was similar but slightly less than that described in the above trial.

Mitochondrial DNA/nuclear DNA ratios

The amount of mitochondrial DNA per nuclear DNA in the various tissues was determined by Southern-blot hybridization [33]. Total cellular DNA was isolated from tissues pooled from three or more animals, digested with *Bam*HI, fractionated in an agarose gel and blotted to nylon paper. The blot was hybridized to p5B ribosomal DNA probe [34] to control for the amount of DNA and to a mitochondrial DNA probe (nucleotides 3338–3565 and 4275–15742) either sequentially after stripping the p5B probe or simultaneously. The mitochondrial DNA/nuclear DNA ratio was also determined using slot-blot analysis. In this case, whole cell extracts were incubated in 0.4 M NaOH/1 M NaCl for 40 min at 50 °C to denature DNA and destroy hybridizable RNA. An equal volume of 1 M NaCl/1 M Tris/HCl, pH 7.2, was added and dilutions were applied to a nylon membrane using a slot-blot apparatus. The resulting blots were hybridized sequentially to the nuclear ribosomal DNA and mitochondrial DNA probes. In order to compare the results from different blots, a sample from adult kidney was included in each blot and the data were normalized as described for the RNA blots.

Total RNA/DNA measurements

To determine the total RNA/DNA ratio, tissues pooled from three or more animals were thoroughly homogenized in a Brinkmann Polytron in 0.01 M Tris/HCl pH 7.4, containing 2 M NaCl and 2 mM EDTA. Portions were removed to measure DNA using the Hoechst assay [35], with purified calf thymus DNA as the standard, and total RNA by the orcinol reaction [36], with purified yeast tRNA as the standard.

RESULTS

COX mRNA levels increase dramatically during development in a tissue-specific pattern

The levels of 13 COX mRNAs per total cellular RNA were measured by RNA gel-blot analysis 1, 10, 20 and 43 days after birth, and in adult mice (Figure 1a) and during the differentiation

Figure 2 Total cellular RNA/cellular DNA during development

RNA was measured by the orcinol reaction [36] and DNA with the Hoechst reagent [35]. The following differences are statistically significant at the 0.05 level ($n = 2$). By day 20 the RNA/DNA ratio has significantly increased in both leg muscle (●; ▼, extensor digitorum longus; ▽, soleus) and ventricle (■) but not brain (□) or kidney (○). By day 43 the ratio in brain has significantly increased. There are no further significant increases in leg muscle, ventricle muscle or brain after this time. The RNA/DNA ratio in kidney does not increase at all during development and is significantly less than those in leg muscle, ventricle and brain in adults. The standard error of these measurements ranged from 3 to 21% of the mean.

of mouse myoblasts into myofibres in cell culture (Figure 1b). If the total RNA/DNA ratio is the same in all tissues at the different time points, then the level of mRNA per total cellular RNA actually reflects the level of mRNA on a per gene or per cellular DNA basis, which is most useful when analysing gene expression. Therefore RNA/DNA ratios for each tissue at various developmental time points were determined, and statistically significant differences were detected (Figure 2). RNA/DNA ratios in ventricle and leg muscle increase significantly and then plateau by day 20. In brain a significant increase is not detected until day 43. No significant increase occurs in kidney during postnatal development. We have previously measured RNA/DNA ratios during the differentiation of myoblasts into myofibres [37]; it does not increase during myoblast differentiation and is comparable with that in adult leg muscle. To obtain a measurement of specific mRNA per cellular DNA (Figure 1), specific COX mRNA concentrations per total RNA measured in RNA gel blots were multiplied by the corresponding RNA/DNA ratio. Taking into account the variability of RNA gel-blot analysis and the RNA/DNA ratios (see the Experimental section and legend to Figure 2), only differences greater than 2-fold should be considered significant.

Three different patterns of COX mRNA expression per cellular DNA were obtained. The first is exhibited by most COX mRNAs including both mitochondrial (COX I, II, III) and nuclear (COX IV, Va, Vb, VIb, VIIaL and VIIc) subunits (Figure 1a): the level of mRNA per cell generally increases 3–10-fold from day 1 to adult, with the greatest increase and the highest level attained in ventricle followed by skeletal muscle. Brain and kidney generally have lower concentrations of these mRNAs, although they also increase 3–5-fold during development. In contrast, there is no significant increase in the steady-state levels of these mRNAs during the differentiation of myoblasts into myofibres in cell culture (Figure 1b); mRNA concentration in cultured muscle cells is slightly less but still comparable with their levels in day-43 or adult muscle. The pattern is not absolutely identical for all

Figure 3 Southern-blot analysis

Total DNA from day-2 myoblasts (lane 1), day-4 newly formed myofibres (lane 2) and day-6 more mature myofibres (lane 3) were digested with *Bam*HI and analysed using Southern-blot techniques as described in the Experimental section. The blots were hybridized to probes for nuclear ribosomal DNA and mitochondrial DNA.

these mRNAs, and some such as IV and VIIaL deviate slightly from the pattern. Interestingly, subunit VIIaL is expressed in a similar fashion to subunits not having isoforms except it is expressed at relatively higher levels in kidney. Previous experiments have also shown that the pattern of expression of VIIa isoforms is very different from the expression of the VIa and VIII isoforms [15].

The second pattern of expression is displayed by COX subunits VIaL and VIII L: mRNA concentration decreases or remains nearly constant during postnatal development in ventricle and skeletal muscle and increases about 5-fold in kidney and brain (Figure 1a). Similarly to the situation in developing heart and skeletal muscle, the levels of these mRNAs decrease by one-third to one-quarter after myoblast differentiation into myofibres (Figure 1b). Interestingly, the levels of VIaL and VIII L mRNAs, especially COX VIII L, are higher in myoblasts than in any muscle or non-muscle tissue *in vivo*.

The third pattern is displayed by subunit VIaH. This mRNA is not detected in brain and kidney during postnatal development. However, it is detected in both leg muscle and ventricle at day 1 and gradually increases 10-fold during development (Figure 1a). COX VIaH mRNA is not expressed at detectable levels in

myoblasts but begins to accumulate after myoblasts differentiate into myofibres and reaches a level approximately one-half of that of adult skeletal muscle 3 days after myoblast fusion (Figure 1b).

Expression of COX subunits was also examined in an adult fast (extensor digitorum longus) and slow (soleus) skeletal muscle to determine whether there are differences in COX mRNA expression between these tissues. Slow twitch muscle is reported to have a higher concentration of mRNA for certain oxidative proteins than fast twitch [38]. Hybridization of the RNA gel blots to myoglobin showed that soleus contains about 10-fold more myoglobin mRNA than extensor digitorum longus, confirming the difference in energy metabolism between these muscles (results not shown). Figure 1(a) shows that nuclear COX mRNA concentrations are about 2-fold higher in soleus than extensor digitorum longus except for VIaL and VIII L subunits which are expressed in low levels in these cells. Interestingly, mitochondrial COX mRNAs (I, II, III) are expressed at the same level in extensor digitorum longus and soleus. This relative difference is reproducible and was verified by rehybridizing blots originally hybridized to mitochondrial COX probes to nuclear COX mRNA probes and vice versa. This result suggests differential regulation of the nuclear and mitochondrial COX subunits in slow and fast twitch muscle. However, this difference is small when compared with the overall increase in COX mRNAs in leg muscle during development.

These experiments do not accurately measure differences in the absolute levels of different COX mRNA species, because of the difficulty of controlling for differences in specific radioactivity of different probes and hybridization efficiencies for different COX mRNA species. In contrast, these factors are controlled when relative increases or decreases in individual COX mRNA species are measured (Figure 1). However, one large difference in the absolute concentrations of different mRNA species was noted. We reproducibly observed that the exposure times for blots hybridized to probes complementary to mitochondrial COX mRNAs were one-third to one-twentieth that of blots hybridized to probes to nuclear COX mRNAs. It is unlikely that such large reproducible differences in exposure times are due to differences in hybridization efficiencies or specific radioactivities of the probes for mitochondrial and nuclear COX mRNAs which both

Figure 4 Mitochondrial DNA per nuclear ribosomal DNA during myoblast differentiation (a) and during *in vivo* development of mice (b)

The following differences are significant at the 0.05 level ($n = 2$), and the average standard error was 28% of the mean. The increase during differentiation of myoblasts into myofibres is significant (see Figure 1 for definition of abbreviations). The increases in ventricle (■) from day 10 to day 20 and from day 20 to adult are significant. The increase in leg muscle (●; ▽, soleus; ▼, extensor digitorum longus) and brain (□) from day 20 to adult is significant. The amount of mitochondrial DNA in ventricle at day 1 is significantly higher than in the other tissues. At day 20 and day 43, ventricle, leg muscle and brain/kidney (○) are all significantly different from each other, and in adults all four tissues are significantly different from each other.

varied about 3-fold. Rather, these large differences in exposure time suggest that the absolute levels of mitochondrial COX mRNAs are 3–20-fold higher than those of nuclear COX mRNAs.

Mitochondrial DNA increases in parallel with mitochondrial mRNA during *in vivo* development but not during myoblast differentiation in cell culture

The steady-state concentration of mitochondrial DNA per cellular DNA was also measured during postnatal development of mice and during myoblast differentiation in cell culture using Southern and slot-blot techniques (see the Experimental section). Figure 3 shows a typical Southern blot derived from myoblasts and myofibres that was hybridized to probes to mitochondrial DNA and nuclear ribosomal DNA to control for potential differences in DNA loaded into the wells. These data as well as those obtained using developing mice are plotted in Figure 4. Mitochondrial DNA per ribosomal DNA increases 3–5-fold after myoblasts differentiate into myofibres and attains a level that is about one-half that in adult leg muscle. Mitochondrial DNA per cellular DNA also increases significantly during the postnatal development of ventricle, leg muscle and brain but not kidney, and there are significant differences among these tissues in the final concentration of mitochondrial DNA attained (Figure 4).

To determine whether mitochondrial COX mRNAs and mitochondrial DNA increase in parallel during development, the ratio of the former to the latter was calculated. No differences greater than about 2-fold, which is not significant, were detected during postnatal development (compare Figures 1a and 4b). In contrast, the ratio significantly decreases during myoblast differentiation (compare Figures 1b and 4a) because mitochondrial DNA increases whereas mitochondrial COX mRNA concentration remains constant after myoblasts differentiate into myofibres. This indicates that mitochondrial DNA and mitochondrial COX mRNAs are differentially regulated during myoblast differentiation.

DISCUSSION

Our results show that the levels of most mitochondrial and nuclear COX mRNAs increase 3–10-fold during postnatal development of all tissues studied, whereas the VIaL, VIIIL and VIaH mRNAs display tissue-specific patterns of expression. COX mRNAs reach higher levels in leg muscle and ventricle than in brain and kidney. These large increases in mRNA levels demonstrate the importance of gene-regulatory mechanisms in controlling COX activity. Total RNA/DNA also increases during development, suggesting that some of the developmental increase in COX mRNA may be due to general processes that increase expression of all genes. However, the increase in COX mRNA levels exceeds the increase in total RNA/DNA, especially in ventricle. This suggests that specific mechanisms also play a role in the control of the increased COX mRNA levels. It is not known whether this control is exerted at the level of transcription, processing or mRNA stability.

Expression of the mitochondrial and most nuclear COX mRNAs increases in unison. This implies significant co-ordination of the expression of genes even though they are derived from different genomes, transcribed by different polymerases and are products of different processing systems. However, this co-ordination is not absolute. For example, our results show that soleus and extensor digitorum longus have similar levels of mitochondrial-encoded COX mRNAs but soleus has about twice

the amount of most nuclear-encoded COX mRNAs. However, such differences were not detected in an analysis of different types of rat skeletal muscle [6], suggesting that this particular difference is not universal among mammals. Furthermore, our results show that the levels of mitochondrial COX mRNAs are considerably higher than those of nuclear COX mRNAs in mouse tissues, and previous experiments have shown that the situation is similar in human cells [7]. If the nuclear and mitochondrial COX subunits are synthesized in equimolar ratios, then nuclear COX mRNAs must be translated more efficiently than mitochondrial COX mRNAs.

On the basis of the general proportionality between mitochondrial DNA and RNA in different types of skeletal muscle, it was previously suggested that the accumulation of mitochondrial DNA is rate-limiting for the production of mitochondrial RNAs [11,12]. In this model, expression of nuclear COX mRNAs is linked to the replication or turnover of mitochondrial DNA. Consistent with this model, our results show that the level of mitochondrial DNA generally increases in parallel with nuclear and mitochondrial COX mRNAs during *in vivo* development of the mouse tissues studied. A transcription factor, nuclear respiratory factor 1, has been identified that binds to some nuclear mitochondrial respiratory genes and to the RNA subunit of mitochondrial RNA-processing endonuclease which may be involved in mitochondrial DNA replication [39]. This factor could play a role in co-ordinating the expression of mitochondrial and nuclear COX mRNAs. However, the disproportional increase in mitochondrial DNA compared with mitochondrial COX mRNAs during myoblast differentiation in cell culture and numerous other examples of the unco-ordinated regulation of mitochondrial DNA and RNAs indicate that expression of mitochondrial RNA is also determined by factors other than the level of mitochondrial DNA [40–45].

Our experiments also demonstrate that isoform switching occurs during development. All tissues express relatively high levels of COX VIaL and VIIIL mRNAs shortly after birth, which increase in kidney and brain but decrease in skeletal muscle and ventricle during postnatal development. In contrast, COX VIaH mRNA is not detected in kidney and brain, and only low concentrations are detected in skeletal muscle and ventricle at day 1; the concentration then gradually increases in skeletal muscle and ventricle during further development. Although we were unable to generate a probe for mouse COX VIIIL, it is probably regulated in a similar way to COX VIaH, because COX VIIIL is regulated in a similar way to the VIaL isoform. The ratio of COX VIaL to VIaH is similar in leg muscle and ventricle, unlike in humans where heart tissue has relatively more subunit VIaL [46].

The myoblast cell culture accurately mimics *in vivo* isoform switching. Interestingly, the switch is nearly complete 3 days after the fusion of mouse myoblasts, making this an excellent system for studying this process. Isoform switching involving VIa was also detected after fusion of human myoblasts in culture [10]. The regulation of mRNAs involved in isoform switching is more complicated than that of other mRNAs because they not only increase during development like other COX mRNAs but are also expressed in a tissue-specific manner.

Unlike the situation *in vivo*, COX mRNA/cellular DNA ratio does not increase appreciably during differentiation of myoblasts in cell culture. Levels of COX mRNAs per nucleus in both myoblasts and myofibres are comparable with those in adult skeletal muscle. In addition during myoblast differentiation, mitochondrial DNA increases 5-fold without a corresponding increase in mRNA level, again unlike the situation *in vivo*. Similar phenomena have been detected in both mouse and

human myoblast cell culture systems, suggesting that they are general properties of these cells in culture [8–10,47]. The apparent difference in the regulation of COX mRNAs and mitochondrial DNA *in vivo* and in cell culture is probably due to differences in the hormonal, nutritional and/or cellular environment of these cells. In particular, these cells lack nervous and mechanical stimulation which is known to induce metabolic activity (reviewed in ref. [48]).

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REFERENCES

- Kadenbach, B., Stroth, A., Huther, F.-J., Reimann, A. and Steverding, D. (1991) *J. Bioenerg. Biomembr.* **23**, 321–334
- Lomax, M. I. and Grossman, L. I. (1989) *Trends Biochem. Sci.* **14**, 501–514
- Anthony, G., Reimann, A. and Kadenbach, B. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1652–1656
- Rohdich, F. and Kadenbach, B. (1993) *Biochemistry* **32**, 8499–8503
- Saltin, B. and Gollnick, P. D. (1983) in *Handbook of Physiology: The Heart* (Peachez, L. P., ed.), pp. 555–631, American Physiological Society, Bethesda, MD
- Gagnon, J., Kurowski, T. T., Wiesner, R. J. and Zak, R. (1991) *Mol. Cell Biochem.* **107**, 21–29
- Van den Bogert, C., De Vries, H., Holtrop, M., Muus, P., Dekker, H. L., Van Galen, M. J. M., Bolhuis, P. A. and Taanman, J. (1993) *Biochim. Biophys. Acta* **1144**, 177–183
- Lomax, M. I., Coucouvanis, E., Schon, E. A. and Barald, K. F. (1990) *Nerve Muscle* **13**, 330–337
- Webster, K. A., Gunning, P., Hardeman, E., Wallace, D. C. and Kedes, L. (1990) *J. Cell. Physiol.* **142**, 566–573
- Taanman, J., Herzberg, N. H., De Vries, H., Bolhuis, P. A. and Van den Bogert, C. (1992) *Biochim. Biophys. Acta* **1139**, 155–162
- Williams, R. S. (1986) *J. Biol. Chem.* **261**, 12390–12394
- Williams, R. S., Salmons, S., Newsholme, E. A., Kaufman, R. E. and Mellor, J. (1986) *J. Biol. Chem.* **261**, 376–380
- Hood, D. A., Zak, R. and Pette, D. (1989) *Eur. J. Biochem.* **179**, 275–280
- Hood, D. A. (1990) *Biochem. J.* **269**, 504–506
- Anthony, G., Stroth, A., Lottspeich, F. and Kadenbach, B. (1990) *FEBS Lett.* **277**, 97–100
- Lightowlers, R., Ewart, G., Aggeler, R., Zhang, Y.-Z., Calavetta, L. and Capaldi, R. A. (1990) *J. Biol. Chem.* **265**, 2677–2681
- Ewart, G. D., Zhang, Y.-Z. and Capaldi, R. A. (1991) *FEBS Lett.* **292**, 79–84
- Fabrizi, G. M., Sadlock, J., Hirano, M., Mita, S., Koga, Y., Rizzuto, R., Zeviani, M. and Schon, E. A. (1992) *Gene* **119**, 307–312
- Bonne, G., Seibel, P., Possekkel, S., Marsac, C. and Kadenbach, B. (1993) *Eur. J. Biochem.* **217**, 1099–1107
- Haushka, S. D., Linkhart, T. A., Clegg, C. H. and Merrill, G. M. (1979) in *Muscle Regeneration* (Mauro, A., ed.) pp. 211–222, Raven Press, New York
- Bowman, L. H. (1987) *Mol. Cell. Biol.* **7**, 4464–4471
- Agrawal, M. G. and Bowman, L. H. (1987) *J. Biol. Chem.* **262**, 4868–4875
- Kearsey, S. E., Flanagan, J. G. and Craig, I. W. (1980) *Gene* **12**, 249–255
- Zeviani, M., Nakagawa, M., Herbert, J., Lomax, M. I., Grossman, L. I., Sherbany, A. A., Miranda, A. F., Dimauro, S. and Schon, E. A. (1987) *Gene* **55**, 205–217
- Rizzuto, R., Nakase, H., Zeviani, M., Dimauro, S. and Schon, E. A. (1988) *Gene* **69**, 245–256
- Zeviani, M., Sakods, S., Sherbany, A. A., Nakase, H., Rizzuto, R., Samitt, C. E., Dimauro, S. and Schon, E. A. (1988) *Gene* **65**, 1–11
- Fabrizi, G. M., Rizzuto, R., Nakase, H., Mita, S., Kadenbach, B. and Schon, E. A. (1989) *Nucleic Acids Res.* **17**, 6409
- Fabrizi, G. M., Rizzuto, R., Nakase, H., Mita, S., Lomax, M. I., Grossman, L. I. and Schon, E. A. (1989) *Nucleic Acids Res.* **17**, 7107
- Rizzuto, R., Nakase, H., Darras, B., Francke, U., Farizi, G. M., Mengel, T., Walsh, F., Kadenbach, B., DiMauro, S. and Schon, E. A. (1989) *J. Biol. Chem.* **264**, 10595–10600
- Lightowlers, R. N. and Capaldi, R. A. (1989) *Nucleic Acids Res.* **17**, 5845
- Suske, G., Enders, C., Schlerf, A. and Kadenbach, B. (1988) *DNA* **7**, 163–171
- McMaster, G. K. and Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4835–4838
- Southern, E. (1975) *J. Mol. Biol.* **98**, 503–511
- Bowman, L. H., Rabin, B. and Schlessinger, D. (1981) *Nucleic Acids Res.* **9**, 4951–4966
- Labarca, C. and Paigen, K. (1980) *Anal. Biochem.* **102**, 344–352
- Schneider, W. C. (1957) *Methods Enzymol.* **3**, 680–684
- Bowman, L. H. (1987) *Dev. Biol.* **119**, 152–163
- Underwood, L. E. and Williams, R. S. (1987) *Am. J. Physiol.* **252**, C450–C453
- Evans, M. J. and Scarpulla, R. C. (1990) *Genes Dev.* **4**, 1023–1034
- Glaichenhaus, N., Leopold, P. and Cuzin, F. (1986) *EMBO J.* **5**, 1261–1265
- Meziane, A. E., Callen, J. C. and Mouniolou, J. C. (1989) *EMBO J.* **8**, 1649–1658
- Gadaleta, M. N., Petruzzella, V., Renis, M., Francasso, F. and Cantatore, P. (1990) *Eur. J. Biochem.* **187**, 501–506
- Kennedy, J. M., Lobacz, S. R. and Kelly, A. W. (1991) *Am. J. Physiol.* **261**, C1091–C1098
- Van Itallie, C. M. (1992) *Endocrinology* **130**, 567–576
- Martin, I., Vinas, O., Mampel, T., Iglesias, R. and Villarroja, F. (1993) *Biochem. J.* **296**, 231–234
- Taanman, J., Hall, R. E., Tang, C., Marusich, M. F., Kennaway, N. G. and Capaldi, R. A. (1993) *Biochim. Biophys. Acta* **1225**, 95–100
- Brunk, C. F. (1981) *Exp. Cell Res.* **136**, 305–309
- Vandenburgh, H. H. (1992) *Am. J. Physiol.* **262**, R350–R355