Journal of Bioenergetics and Biomembranes, Vol. 29, No. 4, 1997

Mitochondrial Biogenesis in the Liver during Development and Oncogenesis

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Received April 25, 1997; accepted July 15, 1997

The analysis of the expression of oxidative phosphorylation genes in the liver during development reveals the existence of two biological programs involved in the biogenesis of mitochondria. Differentiation is a short-term program of biogenesis that is controlled at post-transcriptional levels of gene expression and is responsible for the rapid changes in the bioenergetic phenotype of mitochondria. In contrast, proliferation is a long-term program controlled both at the transcriptional and post-transcriptional levels of gene expression and is responsible for the increase in mitochondrial mass in the hepatocyte. Recently, a specific subcellular structure involved in the localization and control of the translation of the mRNA encoding the β-catalytic subunit of the H^+-ATP synthase (β-mRNA) has been identified. It is suggested that this structure plays a prominent role in the control of mitochondrial biogenesis at post-transcriptional levels. The fetal liver has many phenotypic manifestations in common with highly glycolytic tumor cells. In addition, both have a low mitochondrial content despite a paradoxical increase in the cellular representation of oxidative phosphorylation transcripts. Based on the paradigm provided by the fetal liver we hypothesize that the aberrant mitochondrial phenotype of fast-growing hepatomas represents a reversion to a fetal program of expression of oxidative phosphorylation genes by the activation, or increased expression, of an inhibitor of β-mRNA translation.

KEY WORDS: Mitochondrial biogenesis; differentiation of mitochondria; proliferation of mitochondria; liver; oxidative phosphorylation genes; regulation gene expression; development; oncogenesis; mitochondrial DNA; mRNA localization.

INTRODUCTION

Considerable differences in the structure, number, and function of mitochondria exist between the different cell types of mammals. What is even more evident is that mitochondria show profound qualitative and quantitative changes within a defined cellular type during development. Despite this, the mechanisms that control the biogenesis of mitochondria in mammalian cells have been poorly studied. Thus, it is not a surprise that we know very little about the molecular alterations that promote the expression of an aberrant mitochondrial phenotype in tumor cells.

Biogenesis of mitochondria requires the coordinated expression of the nuclear and mitochondrial genomes that code for the molecular components of the organelle (Fig. 1) (Attardi and Schatz, 1988). The control of the biogenesis of mitochondria is believed to be exerted at the nuclear level (Attardi and Schatz, 1988; Clayton, 1991; Nagley, 1991; Scarpulla, 1996), where the vast majority of mitochondrial proteins are encoded. In the last years, the promoters of several nuclear genes involved in mitochondrial functions have been characterized (Tomura *et al.*, 1990; Li *et*

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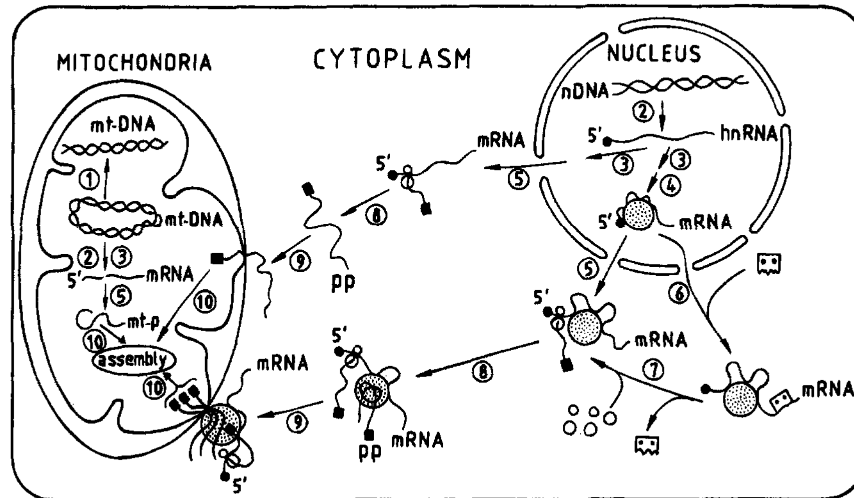

Fig. 1. Molecular processes that control the expression of the genes involved in mitochondrial biogenesis. (1) Replication of mitochondrial DNA (mtDNA). (2) Transcription of nuclear (nDNA) and mitochondrial genes. (3) Processing and maturation of the primary transcripts (hnRNA in the nucleus). (4) For some nuclear-encoded genes (β-F1-ATPase) the mRNA product is assembled in complex cluster structures in the nucleus of the hepatocyte before export to the cytoplasm. Other nuclear-encoded mRNAs (α-F1-ATPase) are exported without obvious assembly in a defined structure involved in mRNA localization. (5) This stage involves the export (nuclear-encoded mRNAs) and translation of the mRNAs. (6) For certain nuclear-encoded mRNAs (β-F1-ATPase), the exported RNP could experience a "masking" event, i.e., a translational repression, by the binding of regulatory proteins to the 3'-UTR of the mRNA (). This masking event most likely influences also the stability of the transcript. (7) As a result of certain signals the unmasking of the mRNA occurs because the binding activity of the translational repressor vanishes. (8) The synthesis of mitochondrial precursor proteins (pp), both delocalized (α-F1-ATPase) and localized (β-F1-ATPase), is completed. (9) Precursor proteins synthesized delocalized in the cytoplasm might encounter import receptors on mitochondrial membranes by random diffusion. In contrast, precursor proteins synthesized in RNP structures are presumed to migrate attached to the RNP along elements of the cytoskeleton toward mitochondria. In both localized and delocalized import pathways of the precursor proteins, molecular chaperones are expected to assist the translation and sorting of the precursor. (10) Incoming precursor proteins are processed, sorted, and often assembled with the proteins synthesized in mitochondria (mt-p).

al., 1990; Suzuki *et al.*, 1991; Chau *et al.*, 1992; Villena *et al.*, 1994) and specific transcription factors for some of these genes have been isolated (Evans and Scarpulla, 1990; Virbasius *et al.*, 1993). The finding that the promoters of some of the genes involved both in the bioenergetic and metabolic function of the organelle and in the replication and transcription of mitochondrial DNA share essential *cis*-acting elements has provided a scheme for explaining, at the transcriptional level, the concerted expression of both genomes (Virbasius and Scarpulla, 1994; Scarpulla, 1996). However, the control of gene expression is not only exerted at the DNA level. Processes that regulate the localization, stability, and translation of the mRNAs, as well as the turnover of the proteins, are all potential target sites for controlling gene expression (Fig. 1). In fact, recent findings indicate that the control of the expression of oxidative phosphorylation genes during liver development, and therefore mitochondrial biogenesis, is exerted at the post-transcriptional level (Izquierdo *et al.*, 1990; Luis *et al.*, 1993; Izquierdo *et al.*, 1995b; Ostronoff *et al.*, 1995, 1996; Izquierdo and Cuezva, 1997).

This minireview has a twofold purpose: in the first place, to define the two programs that control mitochondrial biogenesis in the hepatocyte, for which we will summarize findings on the development of mitochondrial function and on the expression pattern of oxidative phosphorylation genes in rat liver during development. Based on these findings, we further sug-

gest that the altered content and phenotype of mitochondria in rapidly growing hepatomas might result from a reversion to a fetal program of expression of oxidative phosphorylation genes. A more ambitious purpose is to contribute to the stimulation of basic research in this field, because it may be anticipated that not all mammalian tissues share the same basic regulatory mechanisms for controlling the biogenesis of mitochondria (Izquierdo and Cuezva, 1993a; Izquierdo and Cuezva, 1997).

DEVELOPMENT: A HELPFUL EXPERIMENTAL SYSTEM

Development presents a succession of different steady-state situations for the expression of the genes that define the phenotypes through which the organism progresses in fulfillment of its morphogenetic and differentiation programs. In this regard, development offers an attractive *in vivo* experimental system to study the mechanisms that control the biogenesis of mitochondria. This is so because the maintenance of a defined mitochondrial content at each stage of development should result from changes in the expression pattern of oxidative phosphorylation genes. As discussed recently (Izquierdo *et al.*, 1995a,b), the expression of the nuclear encoded β-catalytic subunit of the H^+-ATP synthase and of counterpart subunits encoded in the mitochondrial genome (ATPase 6–8) provide suitable markers of mitochondrial biogenesis in the liver.

PATHWAYS OF ENERGY PROVISION DURING LIVER DEVELOPMENT

As in most mammalian cellular types, metabolic energy in the adult liver is provided by the oxidation of substrates to carbon dioxide and water in a process efficiently coupled to oxidative phosphorylation. Respiration and phosphorylation are catalyzed by proteins located in the inner mitochondrial membrane. During embryonic (Morriss and New, 1979; Clough and Whittingham, 1983) and fetal (Burch *et al.*, 1963; Hommes *et al.*, 1973; Berger and Hommes, 1973; Hommes, 1975; Chico *et al.*, 1979) stages of development, the liver meets most of its energy demands by glycolysis, because both the number of mitochondria per cell (Rohr *et al.*, 1971; Lang and Herbener, 1972; David, 1979; Aprille, 1986) and the bioenergetic activity of the existing mitochondria (Pollak and Duck-Chong, 1973; Nakazawa *et al.*, 1973; Pollak, 1975; Aprille and Asimakis, 1980; Hallman, 1971; Valcarce *et al.*, 1988) are very low compared to that present in adult liver cells (Fig. 2). A remarkable characteristic of fetal mitochondria is the low specific activity of the enzymatic machinery that allows the oxidation of pyruvate (Knowles and Ballard, 1974; Chitra *et al.*, 1985) and fatty acids (Foster and Bailey, 1976; Asins *et al.*, 1995), the main substrates that support mitochondrial activity in the neonatal and adult liver. In addition, fetal rat liver mitochondria have a very low activity of key regulatory enzymes of specific metabolic pathways of the hepatocyte: Pyruvate carboxylase (Ballard and Hanson, 1967; Snell, 1974) for gluconeogenesis, ornitine transcarbamylase (Belbekouche *et al.*, 1985) for ureogenesis and HMG-CoA synthase (Serra *et al.*, 1993, 1996), and β-hydroxybutyrate dehydrogenase (Levy and Toury, 1970) for ketogenesis, provide some examples. In other words, both the phenotype of liver mitochondria and the total mitochondria capacity of the fetal hepatocyte are different from, and much lower than, that of the adult.

Since the total respiratory capacity of fetal hepatocytes is very low, the fetal liver has very high rates of lactate production by glycolysis (Burch *et al.*, 1963; Hommes *et al.*, 1973; Berger and Hommes, 1973) (Fig. 2). Concurrently, it is not a surprise that the activity of the enzymes of the glycolytic pathway is higher in fetal than in adult liver (Burch *et al.*, 1963). Furthermore, several glycolytic enzymes of the fetal liver are different isoforms of the protein expressed in normal adult liver (Ballard and Oliver, 1964; Middleton and Walker, 1972). The high rate of lactate production in the growing fetal liver is then supported by the continuous nonrestrained transfer of glucose from maternal circulation (Fig. 2). Glycolysis in the liver is rapidly turned off after birth (Burch *et al.*, 1963) when mitochondrial activity in the hepatocyte starts as a result of the immediate postnatal switch-on of mitochondrial biogenesis (Valcarce *et al.*, 1988; Izquierdo *et al.*, 1995b). Biochemically, this phenomenon is expressed by the increase in the specific activity, both in the isolated organelle and in the hepatocyte as a whole, of enzymes involved in energy transduction and of liver specific metabolic pathways, as a result of *de novo* synthesis of proteins of the mitochondria (Valcarce *et al.*, 1988; Izquierdo *et al.*, 1995b; Ostronoff *et al.*, 1996).

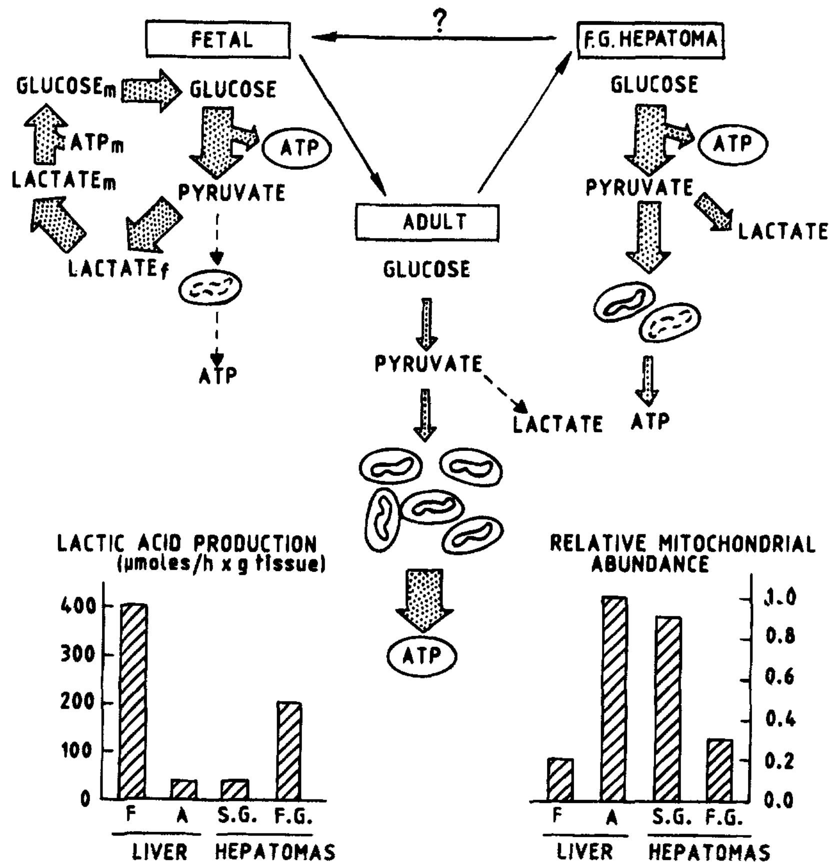

Fig. 2. Glycolytic and mitochondrial phenotypes in the liver during development and oncogenesis. The size of the arrows indicates qualitative flux of carbon skeletons through the pathway and the yield of ATP produced either by glycolysis or mitochondrial oxidative phosphorylation. Mitochondria are depicted with "kidney shape" either differentiated (continuous inside line) or undifferentiated (discontinuous inside line). Under the fetal phenotype the "lactate cycle" established between fetal producing tissues and the mother is indicated. The lactate transferred from the fetus is converted into glucose by maternal gluconeogenic tissues (liver and kidney) (Valcarce *et al.*, 1984, 1985). The histograms illustrate the glycolytic flux of the liver and the relative mitochondrial abundance of the hepatocyte, in the fetal (F) and adult (A) stage, as well as in slow- (S.G.) and fast-growing (F.G.) hepatomas (Pedersen, 1978). This figure has been designed using data from references (Landau *et al.*, 1958; Burch *et al.*, 1963; Pedersen, 1978; Izquierdo *et al.*, 1995b).

TWO BIOLOGICAL PROGRAMS CONTROL THE BIOGENESIS OF LIVER MITOCHONDRIA

Differentiation of Mitochondria

When considering the changes in the content and/or activity of mitochondria in the hepatocyte during development, it is necessary to distinguish between the short-term and long-term regulated events, because they illustrate two different biological programs responsible for the biogenesis of mitochondria. Short-term regulation of mitochondrial biogenesis occurs in the liver immediately after birth. Within the first hour of extrauterine life the pre-existing liver organelles are transformed into energy conserving mitochondria (Valcarce *et al.*, 1988; Cuezva *et al.*, 1990). In contrast to previous hypothesis (Pollak and Sutton, 1980), it has been shown that this program is responsible for the increase in inner membrane proteins relative to other mitochondrial proteins (Valcarce *et al.*, 1988; Cuezva *et al.*, 1990) in the absence of changes in the relative mtDNA content (Ostronoff *et al.*, 1996). Thus, it certainly requires a concerted expression of the

nuclear and mitochondrial genomes (Valcarce *et al.*, 1988; Cuezva *et al.*, 1990; Izquierdo *et al.*, 1995b; Ostronoff *et al.*, 1996). At variance with many other cellular activities that are induced in the liver at this stage of development, this program is controlled at two post-transcriptional levels that involve the regulation of the stability (Izquierdo *et al.*, 1995b; Ostronoff *et al.*, 1995) and translational efficiency (Luis *et al.*, 1993; Izquierdo *et al.*, 1995b; Ostronoff *et al.*, 1996) of the mRNAs encoding mitochondrial proteins. The program is thus responsible for transforming the "incomplete" fetal liver organelles into mature, or adult-type, mitochondria. As a program responsible for the rapid and profound changes in the ultrastructural, molecular and functional phenotype of the organelle (Pollak and Sutton, 1980; Valcarce *et al.*, 1988; Cuezva *et al.*, 1990), it has been defined as *mitochondrial differentiation.* It should be noted that differentiation of mitochondria also occurs in other experimental systems, such as the regenerating liver (Guerrieri *et al.*, 1995), other mammalian tissues (Luis and Cuezva, 1989; Prieur *et al.*, 1995; Schönfeld *et al.*, 1997), and in other organisms (Rouslin and Schatz, 1969; Pollak and Sutton, 1980; Vallejo *et al.*, 1996).

The postnatal enrichment in adenine nucleotides undergone by liver mitochondria after birth (Pollak, 1975; Valcarce *et al.*, 1988) also contributes to the maturation of an efficient energy-conserving membrane (Aprille, 1986; Valcarce *et al.*, 1988, 1990). In fact, the H^+-leak of the inner membrane of fetal mitochondria experiences a rapid reduction immediately after birth (Valcarce *et al.*, 1990), which may be triggered by the interaction of the nucleotides with the adenine nucleotide translocase (Valcarce and Cuezva, 1991). However, this "maturation" event of the membrane does not require the synthesis of mitochondrial proteins because it can be mimicked *in vitro* by addition of the nucleotides (Valcarce and Cuezva, 1991) and *in vivo* it is resistant to protein synthesis inhibitors (Valcarce *et al.*, 1988; Cuezva *et al.*, 1990).

Proliferation of Mitochondria

At birth, mechanisms for long-term regulation of mitochondrial biogenesis in the liver are also started off. These mechanisms belong to the program of biogenesis responsible for the increase in mitochondrial mass relative to other cellular components of the hepatocyte, once the bioenergetic phenotype of adult-type mitochondria has been acquired (Valcarce *et al.*, 1988). It also requires that during the proliferation of the hepatocytes the synthesis of mitochondrial components exceeds their breakdown. At the end, i.e., when the adult steady-state condition is reached, the number of mitochondria per cell (Rohr *et al.*, 1971; Lang and Herbener, 1972; David, 1979), or any other parameter that could represent the mitochondria, such as the ratio mtDNA/nuclear genome (Ostronoff *et al.*, 1996; Cantatore *et al.*, 1986) or mt-protein/total cellular protein (Izquierdo *et al.*, 1995a,b), is higher than at initial stages of development. In contrast to the program of differentiation, the expression of nuclear and mitochondrial genomes during long-term regulation of mitochondrial biogenesis appears to be controlled both at the transcriptional (Izquierdo *et al.*, 1995b) and post-transcriptional levels (Izquierdo *et al.*, 1995a; Ostronoff *et al.*, 1996). Therefore, long-term regulation of mitochondrial biogenesis is not mechanistically responsible for the changes in the phenotype of mitochondria, but rather for the increase in the total mitochondrial capacity of the cell; thus, it is defined as *mitochondrial proliferation.*

It should be noted that the distinction between differentiation and proliferation is operational, because they are both likely to occur in the liver simultaneously at all stages of development, perhaps representing two different phases of the process of mitochondrial biogenesis (Pollak, 1975, 1976). In this regard, two mitochondrial populations, differing in the ultrastructural morphology (Valcarce *et al.*, 1988; Cuezva *et al.*, 1990), sedimentation (Pollak and Munn, 1970) and osmotic (Almeida *et al.*, 1997) behaviors, DNA (Davis and Clayton, 1996) and protein synthesis (Duck-Chong and Pollak, 1973) capacity, and fluorescence intensity (Lopez-Mediavilla *et al.*, 1989; Hernandez Berciano *et al.*, 1993) have been shown to co-exist in mammalian cells. Further, it has been shown that the relative proportion of each population changes abruptly in the liver at birth (Cuezva *et al.*, 1990; Almeida *et al.*, 1995, 1997), suggesting that fetal liver provides a unique developmental condition where the differentiation of the organelle is arrested.

GENE EXPRESSION AND MITOCHONDRIAL DIFFERENTIATION

Evidence for the Control of mRNA Stability

Paradoxically, during the last days of fetal development and first hours of life, a time when mitochon-

dria are less abundant in the developing liver (Fig. 2), the steady-state content of the transcripts of oxidative phosphorylation encoded in nuclear (β-F1-ATPase mRNA) and mitochondrial (ATP 6-8 mRNA) genomes are more abundantly represented than in the adult liver (Luis *et al.,* 1993; Izquierdo *et al.,* 1995b) (Fig. 3). This situation has also been noted for other nuclear-encoded transcripts of mitochondrial proteins (Kelly *et al.,* 1989; Bailly *et al.,* 1991). How are these mRNA levels attained in the fetal liver? Recent findings have

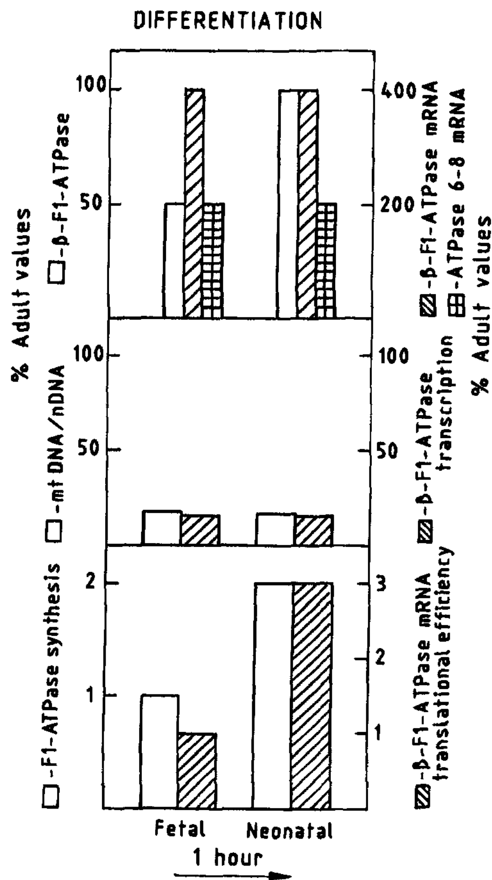

Fig. 3. Rapid postnatal differentiation of liver mitochondria is controlled at the level of translation. The values of β-F1-ATPase protein/mg mitochondrial protein determined in isolated liver mitochondria from fetal and 1-h-old neonates is presented as percentage of that in adult mitochondria. The steady-state levels of the mRNAs of oxidative phosphorylation (β-F1-ATPase and ATPase 6-8) and the relative representation of the mitochondrial genome (mtDNA/nDNA) in the liver, as well as the rates of transcription of the β-F1-ATPase gene determined in isolated liver nuclei, are expressed as percentage of adult values. The *in vivo* synthesis rate of the F1-ATPase complex and of the *in vitro* translational efficiency of the β-F1-ATPase mRNA in 1-h-old neonatal samples are relatively compared to fetal values. For other details see references (Luis *et al.,* 1993; Izquierdo *et al.,* 1995b; Ostronoff *et al.,* 1996).

provided evidence that the accumulation of transcripts of oxidative phosphorylation is not the result of an increased transcriptional activity in the fetal liver (Izquierdo *et al.,* 1995b). In fact, the transcription rate of the nuclear β-F1-ATPase gene is at its lowest level at this stage of development (Izquierdo *et al.,* 1995b) (Fig. 3). A similar situation is observed for the expression of the mitochondrial genome. Transcription rates of mtDNA do not change during development (Ostronoff *et al.,* 1996) and the ratio of mtDNA/nuclear genome is also at its minimum level in the fetal liver (Ostronoff *et al.,* 1996) (Fig. 3). Interestingly, when compared to the adult liver, the *in vivo* turnover of the mRNAs encoded in both nuclear (Izquierdo *et al.,* 1995b) (Fig. 4) and mitochondrial (Ostronoff *et al.,* 1995) genes revealed a sharp increase in the half-life

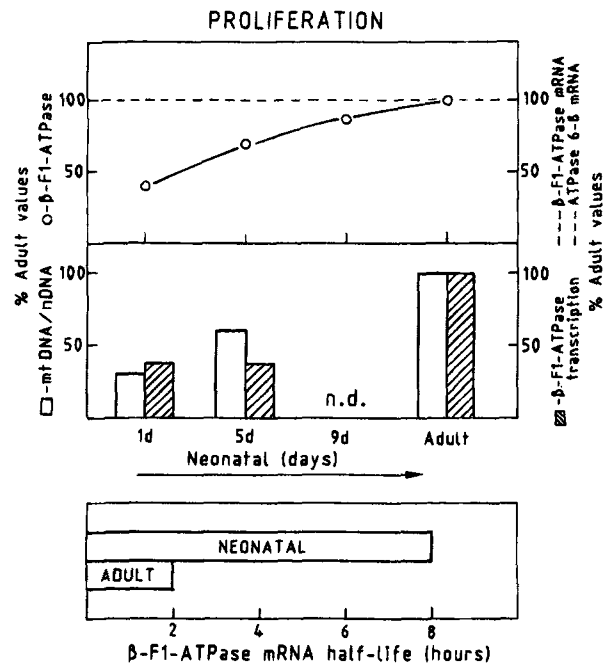

Fig. 4. Proliferation of liver mitochondria during neonatal development. The content of β-F1-ATPase protein/g liver (open circles), the steady-state levels of the mRNAs of oxidative phosphorylation (β-F1-ATPase and ATPase 6-8) (discontinuous line), and the relative representation of the mitochondrial genome (mtDNA/nDNA ratio) in the liver, as well as the rates of transcription of the β-F1-ATPase gene determined in isolated liver nuclei are expressed as percentage of adult values at different stages of neonatal development. The representation of mtDNA at day 5 has been obtained from reference (Cantatore *et al.,* 1986), and normalized to the changes of the mtDNA/nDNA ratio found in liver during development (Ostronoff *et al.,* 1996). The half-life of β-F1-ATPase mRNA in the liver was determined *in vivo* after administration of actinomycin D to adult and newborn rats. For other details see references (Izquierdo *et al.,* 1995a,b; Ostronoff *et al.,* 1996).

at early stages of development. Thus, it seems that the accumulation of these transcripts in the fetal hepatocyte results from the concerted regulation of the mechanisms controlling the decay of the mRNAs of both genetic units. Unfortunately, the mechanisms that might exert this type of control in mammals remain unexplored.

Molecular, Cellular, and Functional Differentiation of Mitochondria

The rapid postnatal development of the bioenergetic function of mitochondria provides a functional explanation for the paradoxical behavior of the mRNAs encoding proteins of oxidative phosphorylation in the fetal liver. Within the first hour of life the maximum rates of respiration, assessed in isolated liver organelles, increase twofold when compared to those in mitochondria from fetal liver (Valcarce *et al.*, 1988; Pollak, 1975; Sutton and Pollak, 1980; Aprille and Asimakis, 1980). This surge in the mitochondrial respiratory capacity is paralleled by a similar increase in most of the enzymatic activities of the respiratory complexes (Valcarce *et al.*, 1988; Cuezva *et al.*, 1990). Concurrently, the coupling between respiration and oxidative phosphorylation also develops within this short time interval, and is also paralleled by an increase in the activity and amount of proteins of the H^+-ATP synthase (Valcarce *et al.*, 1988; Cuezva *et al.*, 1990) (Fig. 3). The lack of changes in the steady-state content of the nuclear encoded β-mRNA (Izquierdo *et al.*, 1990, 1995b; Luis *et al.*, 1993) (Fig. 3), as well as of several transcripts of the mitochondrial genome (Ostronoff *et al.*, 1996) (Fig. 3), suggested that regulation of mitochondrial biogenesis at this stage of development is exerted at post-transcriptional levels (Izquierdo *et al.*, 1990). In fact, the administration of cytosolic and mitochondrial protein synthesis inhibitors to the newborns at the time of birth arrested the postnatal development of the bioenergetic function of mitochondria by impairing the synthesis of nuclear- and mitochondrial-encoded proteins (Valcarce *et al.*, 1988; Cuezva *et al.*, 1990; Ostronoff *et al.*, 1996). Therefore, it appears that differentiation of liver mitochondria is controlled at the level of translation by the preferential synthesis of proteins encoded by the mRNAs accumulated during fetal stages of development. Interestingly, the rates of mitochondrial protein synthesis determined in the isolated organelle during development were also higher at the time of differentiation (Ostronoff *et al.*, 1996). Since the response of mitochondrial translation in the isolated organelle is transient, occurs only at 1 h postnatal, and is dependent on the synthesis of proteins in the cytoplasm (Ostronoff *et al.*, 1996), we suggested that mitochondrial translation is a secondary response after the previous activation of the translation of nuclear-encoded transcripts. This might provide a cross-talk mechanism for exerting a concerted regulation of the expression of both genomes at the level of translation (Ostronoff *et al.*, 1996; Attardi *et al.*, 1990; McConnell *et al.*, 1996).

The rapid postnatal enrichment in bioenergetic proteins assessed in isolated liver organelles has also been demonstrated in the liver *in situ* by immunoelectron microscopy approaches (Izquierdo *et al.*, 1995b). Using antibodies against the whole F1-ATPase complex (Valcarce *et al.*, 1988) or antibodies against the β-catalytic subunit of the complex (Egea *et al.*, 1997), fetal mitochondria have been shown to contain half of the relative content of these proteins/μm^2 of mitochondrial section than 1-h-old or adult rat liver mitochondria. It also illustrates at the cellular level the rapid transformation of the "incomplete" fetal mitochondrion into a mature adult-type organelle. The accumulation of oxidative phosphorylation transcripts in the fetal liver thus seems to be a developmentally regulated response of a very specific cellular program of organelle biogenesis, the differentiation of pre-existing mitochondria.

It should be stressed that the onset of mitochondrial bioenergetic function is a prerequisite for adaptation of mammals to extrauterine life. Impairments in the onset of mitochondrial function after birth, such as those in rats when birth occurs before term gestation (Valcarce *et al.*, 1994), have been associated with the high mortality rates of the newborns in the immediate postnatal period (Cuezva *et al.*, 1990; Valcarce *et al.*, 1994). The operation of a program of organelle biogenesis controlled at post-transcriptional levels of gene expression certainly provides a mechanism of regulation of mitochondrial biogenesis allowing a faster response to environmental changes than a program of biogenesis controlled at the level of transcription.

The β-F1-ATPase mRNA: a Target for Controlling the Biogenesis of Mitochondria?

The reduced content of mitochondria in the fetal hepatocyte, despite the higher availability of oxidative phosphorylation transcripts, indicated that mRNAs of

oxidative phosphorylation must be translationally repressed in the fetal liver. Their translational activation must be triggered therefore by changes brought about by the birth process (Mayor and Cuezva, 1985). A general activation of the global rate of translation occurs in rat liver immediately after birth (Luis *et al.*, 1993). The abrupt change in the rate of translation has been associated with the covalent modification and subsequent changes in the activity of certain initiation factors of the translational machinery (Luis *et al.*, 1993). However, concurrent with this activation of translation, there is a selective increase in the *in vivo* synthesis rate of the F1-ATPase complex between the fetal and 1-h-neonatal stage of development (Izquierdo *et al.*, 1995b) (Fig. 3) (see Section 5.3.2.).

A Prominent Role in Biogenesis for Localized mRNAs?

In recent years, it has become obvious that the localization of mRNAs within the cell is an important mechanism controlling different morphogenetic and developmental programs of the organism. In this regard, the study of the subcellular localization of α- and β-F1-ATPase mRNAs in the liver has strengthened the idea of the prominent role played by the cytoplasmic expression of the β-F1-ATPase gene in the assembly/biogenesis of mitochondria (Egea *et al.*, 1997). The subcellular presentation of these mRNA species, which encode two subunits of the same inner membrane complex (F1-ATPase), is different. Whereas the β-mRNA appears localized in rounded electron-dense clusters, often associated with mitochondria, the α-mRNA is dispersed and evenly distributed in the cytoplasm (Egea *et al.*, 1997). The study of the localization of β-mRNA in the liver during development has further allowed us to infer that differences in the translational efficiency of the transcript (Luis *et al.*, 1993) (Fig. 3) cannot be accounted for by the sequestration of β-mRNA in cluster structures, but are instead mediated by regulatory molecules that interact with the cluster (Egea *et al.*, 1997) (see Fig. 1). In fact, high-resolution immunocytochemical and hybridization approaches have revealed that β-mRNA clusters contain the β-F1-ATPase protein, 60S ribosomal subunits, and the molecular chaperone hsc 70 (Ricart *et al.*, 1997). Furthermore, a higher relative content of the β-precursor was detected in β-mRNA clusters of the neonatal than of the fetal liver (Ricart *et al.*, 1997). These findings suggest that the structure responsible for the localization of β-mRNA (Egea *et al.*, 1997) is involved in the control of its translation (Ricart *et al.*, 1997), as well as in mediating post-translational sorting of the encoded precursor to the mitochondria (Egea *et al.*, 1997; Lithgow *et al.*, 1997) (Fig. 1), and therefore, may play a prominent role in the cytoplasmic control of mitochondrial biogenesis.

Mechanism that Controls the Cytoplasmic Expression of β-mRNA

What mechanisms could promote the dormancy of β-mRNA in the fetal liver and/or the translational activation of the stored β-mRNA in the neonatal liver? Activation of "masked" mRNAs is usually mediated by covalent modification and/or trans-activation of the stored mRNAs in response to a given stimuli (Jackson and Standart, 1990; Wickens, 1990). When equal amounts of total RNA or of the poly A^+RNA fraction from fetal and 1-h-old neonatal liver were translated *in vitro*, it was found that the amount of the β-subunit precursor of the F1-ATPase immunoprecipitated from neonatal samples was three times higher than that synthesized from the fetal RNA samples (Luis *et al.*, 1993) (Fig. 3). These findings suggested that the developmental changes in the translational efficiency of β-mRNA should result from either (or a combination) of the following alternatives: (i) specific covalent changes in the mRNA itself, and (ii) the presence of a regulatory molecule that specifically inhibits (in the fetus) or activates (in the neonate) the translation of β-mRNA (Luis *et al.*, 1993).

Thus far, most of the described covalent modifications of mRNAs accompanying changes in mRNA stability and translational efficiency are those that involve modifications in the 3′-end of the transcript, either by lengthening or shortening the poly A^+ tail of the mRNA (Jackson and Standart, 1990; Wickens, 1990). However, development does not promote any significant modification of the poly A^+ tail of β-mRNA (Izquierdo and Cuezva, 1997), suggesting that the change in the translational efficiency of the β-transcript is exerted independently of the length of the poly A^+ tail and that regulation of its translation should be exerted by developmentally regulated molecules of the liver (Izquierdo and Cuezva, 1997).

The number of mechanisms involved in translational regulation of specific mRNAs during development is increasing at a fast rate (Thach, 1992; Sarnow, 1995; Hentze, 1995). In some situations, regulation of translation is exerted by differential affinity of the mRNAs for the available initiation factors and/or pro-

teins of the translational machinery. In others, regulatory proteins binding either to initiation factors or to *cis*-acting sequences located either in the 5′-, open reading frame or 3′-untranslated region of the mRNA can exert a positive or negative regulation on its expression. To add further complexity, regulatory antisense RNAs have already been described in some systems (Lee *et al.,* 1993; Rastinejad *et al.,* 1993; Hentze, 1995).

We have recently excluded most of these possibilities for the developmental regulation of the translation of β-mRNA (Izquierdo and Cuezva, 1997) and found that its translation is regulated in the liver by *trans*-acting proteins that bind at the 3′-untranslated region (3′UTR) of the mRNA (Izquierdo and Cuezva, 1997) (see Fig. 1). Remarkably, the 3′UTR of the mRNA is an essential *cis*-acting element that is necessary for *in vitro* translation of the transcript (Izquierdo and Cuezva, 1997). These findings are not that surprising if we take into consideration that mRNA regulatory sequences, involved either in localization, stability, or translation of the transcripts, are found in the 3′UTR of the mRNAs (Chen and Shyu, 1995; Decker and Parker, 1995; Hentze, 1995) and, as described above, the β-mRNA is a transcript fulfilling the three criteria, i.e., it is localized (Egea *et al.,* 1997) and subjected to translational (Luis *et al.,* 1993) and stability controls (Izquierdo *et al.,* 1995b).

These findings provide the first evidence for the cytoplasmic regulation of mitochondrial biogenesis by *trans*-acting regulatory proteins that bind the 3′-UTR of a transcript of oxidative phosphorylation (Izquierdo and Cuezva, 1997). Although the nature and functional significance of the 3′UTR-β-mRNA binding protein remain to be established in future studies, the developmental profile of its activity (Izquierdo and Cuezva, 1997) suggests that it might be a good candidate to exert the stabilization and further translational repression of β-mRNA in the fetal liver. At birth, the repressing activity vanishes (Izquierdo and Cuezva, 1997) concurrently with the relief of translation of the mRNA (Luis *et al.,* 1993; Izquierdo *et al.,* 1995b) and with further consequences on mitochondrial differentiation and onset of mitochondrial function in the neonatal liver.

GENE EXPRESSION AND MITOCHONDRIAL PROLIFERATION

In addition to the rapid postnatal differentiation of liver mitochondria, it has been shown that the number of mitochondria/hepatocyte increases during postnatal development (Rohr *et al.,* 1971; Lang and Herbener, 1972; David, 1979). In agreement with this observation, the ratio mt-DNA/nuclear genome (Ostronoff *et al.,* 1996; Cantatore *et al.,* 1986) or that of a reporter mitochondrial protein, such as the β-subunit of the ATP synthase/total cellular protein (Izquierdo *et al.,* 1995a,b), increases by a similar factor (Fig. 4). Since the relative content of the β-subunit/mg of mitochondrial protein during postnatal development is basically the same as that found in adult liver mitochondria (Izquierdo *et al.,* 1995a), it is reasonable to assume that the accumulation of this protein in liver homogenates illustrates the proliferation of mitochondria in the hepatocytes. In fact, the developmental profile for the accumulation of β-protein or mt-DNA in liver agrees with previous findings on the development of other mitochondrial enzymatic activities (Knowles and Ballard, 1974; Foster and Bailey, 1976). However, it is remarkable that during development (after 1 day postnatal) the levels of oxidative phosphorylation transcripts remain essentially unchanged (Izquierdo *et al.,* 1995a,b) (Fig. 4). In contrast, the relative accumulation of proteins that define specific functions of liver mitochondria paralleled similar changes in the relative cellular representation of the mRNAs that encode such proteins (Kelly *et al.,* 1989; Asins *et al.,* 1995; Serra *et al.,* 1993). Anyway, for both types of transcripts, and in the few cases where transcription rates of the nuclear genes have been determined, the accumulation of the proteins in the liver paralleled an increased transcriptional activity of the gene during development (Izquierdo *et al.,* 1995b; Serra *et al.,* 1996). Presumably, the dissimilar behavior on the cellular representation of the mRNAs that code for oxidative phosphorylation proteins might be an exception, just because their turnover (Izquierdo *et al.,* 1995b) (Fig. 4) and translation (Luis *et al.,* 1993; Izquierdo *et al.,* 1995b; Izquierdo and Cuezva, 1997) are subjected also to acute regulation during development.

However, hypothyroidism, which is known to influence mitochondrial number, structure, and function in mammalian cells, resulted in a significant reduction of β-mRNA levels in the liver when compared to euthyroid animals at all stages of development (Izquierdo *et al.,* 1990, 1995a). Concurrently, this condition resulted in an overall impairment in mitochondrial proliferation in the liver (Izquierdo *et al.,* 1995a). Since thyroid hormones have been shown to affect the basal transcription of the β-F1-ATPase gene (Izquierdo and Cuezva, 1993b), as well as the binding of nuclear

proteins to the promoter of the gene (Chung *et al.*, 1992), the reduction of mitochondrial proliferation in liver of hypothyroid rats most likely results from its reduced transcription (Izquierdo *et al.*, 1995a). At the present time it is not known whether the mammalian β-F1-ATPase gene is directly or indirectly regulated by thyroid hormones (Izquierdo and Cuezva, 1993b). Interestingly, the continuous increase in the transcription rates of the β-F1-ATPase gene during development (Izquierdo *et al.*, 1995b) paralleled the increase availability of thyroid hormones (Izquierdo *et al.*, 1995a), which suggests that these hormones might be controlling, at the level of transcription, the long-term accretion of mitochondrial mass in the hepatocyte.

But what about the expression of mt-genes during proliferation of mitochondria? In rabbit (Williams, 1986) and mouse (Kim *et al.*, 1995) muscle, parallel changes in mt-DNA copy number and mt-mRNA amounts have been observed, leading to the suggestion that mitochondrial genome dosage of the cell is one of the major determinants of the expression of mtDNA. In liver, the proliferation of mitochondria also matches the accumulation of mtDNA (Fig. 4). However, once again, a paradoxical situation is noted regarding the availability of the mt-encoded ATPase 6–8 mRNA, since its relative cellular content is the same throughout the proliferative phase of the organelle (Izquierdo *et al.*, 1995a,b), i.e., it does not change in parallel with changes in mitochondrial number (Fig. 4). This is not that surprising because mechanisms regulating the turnover (Ostronoff *et al.*, 1995) and translation (Ostronoff *et al.*, 1996) of the mt-transcripts are also operative in the liver during development.

What might be the putative regulatory circuit that operates during proliferation of mitochondria in the liver? In agreement with recent suggestions (Virbasius and Scarpulla, 1994; Scarpulla, 1996) it should be expected that genes involved in the bioenergetic, metabolic, and genetic function of the organelle increase their transcriptional expression during the course of development as a result of different signals, which most likely include the development of the thyroid function. The transcriptional response might be orchestrated by specific transcription factors of nuclear-encoded mitochondrial protein genes (Scarpulla, 1996), and by transcriptional activators involved in the regulation of other cellular functions, since the mitochondrial mass of the cell has to be adjusted with the general cellular activity. For some genes, the most likely response is that an increased level of transcription in the nuclei (Izquierdo *et al.*, 1995b; Serra *et al.*, 1996) will promote an increase in the relative cellular content of the mRNAs encoding mitochondrial proteins (Kelly *et al.*, 1989; Asins *et al.*, 1995; Serra *et al.*, 1996; Kim *et al.*, 1995) in parallel with the relative cellular content of mtDNA (Ostronoff *et al.*, 1996; Cantatore *et al.*, 1986). However, the mRNAs of certain nuclear (β-F1-ATPase) and mitochondrial (ATPase 6-8) genes, perhaps those playing a key role in biogenesis, are adjusted at a defined level (Izquierdo *et al.*, 1995a,b), because their expression is subjected to a more complex regulation. At the end, this scheme provides the basic scaffold promoting the accumulation of mitochondria in the hepatocyte in a time scale of days (Fig. 4). However, it is obvious that the details of the final mitochondrial building of the hepatocyte also rely on mechanisms controlling the turnover (Izquierdo *et al.*, 1995b; Ostronoff *et al.*, 1995) and translation of the mRNAs (Luis *et al.*, 1993; Izquierdo *et al.*, 1995b; Ostronoff *et al.*, 1996; Izquierdo and Cuezva, 1997) and perhaps the turnover of mitochondrial proteins.

THE ABERRANT MITOCHONDRIAL PHENOTYPE OF TUMOR CELLS

Rapidly growing cancer cells have an increased glycolytic rate while growing aerobically (Pedersen, 1978) (Fig. 2). The increased glycolysis in hepatoma cells is sustained by a high activity of the key enzymes of the glycolytic pathway, which exceeds the activities present in the adult liver (Pedersen, 1978). Hepatomas express isoforms of the glycolytic enzymes different from those present in adult liver cells (Pedersen, 1978). Strikingly, the isoforms that prevail in the glycolytic tumor are those present in the fetal liver. Concurrently, fast-growing hepatomas, unlike the adult liver, have a very low activity of the regulatory enzymes of the gluconeogenic pathway (Knox, 1967; Pedersen, 1978), which is another phenotypic manifestation in common with the fetal hepatocyte. The similarities between the fetal liver and tumor cells are also valid for the mitochondria (Fig. 2). Although there are reports in the literature illustrating alterations in the ultrastructure and function of mitochondria from tumor cells (reviewed in (Pedersen, 1978)), nowadays it is believed that the main respiratory impairment of highly glycolytic tumors is that they are markedly deficient in mitochondria (Pedersen, 1978) (Fig. 2). Anyway, it is obvious that highly glycolytic tumors show pro-

found alterations in the programs of mitochondrial biogenesis.

In this regard, in a rapidly growing hepatoma, it has been shown that a fivefold reduction in the content of mitochondria per cell is paralleled by a similar-fold increase in the steady-state content of nuclear and mitochondrial encoded transcripts (Luciakova and Kuzela, 1992). The up-regulation of oxidative phosphorylation transcripts has also been noted in other tumor cells (Godbout *et al.*, 1993; Faure-Vigny *et al.*, 1996; Heddi *et al.*, 1996), as well as in cellular lines transformed with viral and cellular oncogenes (Glaichenhaus *et al.*, 1986; Torroni *et al.*, 1990). In one of these studies, it is shown that transformation results in a 2–3-fold reduction in mitochondrial content (assessed by the representation of mtDNA) concurrent with a 3–10-fold increase in steady-state mRNA levels of oxidative phosphorylation transcripts (Torroni *et al.*, 1990). In other words, the fetal liver and hepatoma cells present the same paradoxical situation, a diminished content of mitochondria accompanied by an increased cellular availability of the oxidative phosphorylation transcripts.

Several hypothesis have been provided in order to explain the reduction of mitochondria in cancer cells including the mutations and deletions of mtDNA (Baggetto, 1993). This possibility seems unlikely because no alterations of mtDNA have been reported in several tumor and transformed cells (Torroni *et al.*, 1990). In this regard, mutations of mtDNA are not expected to affect the expression of nuclear-encoded genes whereas the expression of nuclear gene products has already been shown to affect the stability of mtDNA (Chen and Clark-Walker, 1995, 1997). Others have suggested that the reduction of mitochondria in tumors is the result of an increased turnover of mitochondrial proteins. In fact, a shorter half-life of the β-F1-ATPase protein has been found in hepatomas when compared to resting or regenerating liver cells (Luciakova and Kuzela, 1992). However, the molecular mechanisms that control the up-regulation of transcripts of oxidative phosphorylation still remain unexplored and it has been considered as a mere compensatory mechanism for responding to the reduced energy metabolism of tumor cells.

A Plausible Hypothesis

We think that a reasonable hypothesis to explain the up-regulation of the transcripts of oxidative phosphorylation, concurrent with a reduction in mitochondrial mass in fast-growing hepatomas, is the result of the activation of the set of post-transcriptional mechanisms that limit the differentiation and proliferation of mitochondria during embryonic and fetal stages of liver development, i.e., a reversion to a fetal phenotype of cellular energy metabolisms, the "fetalism of hepatomas" (Fig. 2). In fact, there are molecular indications that mitochondria of tumor cells are undifferentiated (Capuano *et al.*, 1996) and behave very much like fetal mitochondria (reviewed in Pedersen, 1978).

The predictions derived from this hypothesis are: *First,* transcription rates of nuclear-encoded genes involved in the bioenergetic function as well as, in the replication of mtDNA should be depressed in tumor cells when compared to the normal tissue. *Second,* the increased cellular representation of oxidative phosphorylation transcripts should result from an increased stability of the mRNAs in tumors. *Third,* the translational efficiency of nuclear-encoded mRNAs of mitochondria, at least those experiencing translational control (β-F1-ATPase mRNA), should be significantly depressed in tumor cells. *Fourth,* extracts of hepatomas should contain a 3′-UTR β-mRNA binding protein with similar activity as that present in fetal liver extracts. In this regard, preliminary observations in our laboratory indicate that in the AS-30D fast-growing hepatoma, β-mRNA fulfills the second, third, and fourth premises of the hypothesis (López-Heredia, Izquierdo and Cuezva, in preparation). Based on the fetal paradigm, it could be predicted that a repression of the translation of essential nuclear-encoded mRNAs (Izquierdo *et al.*, 1995b; Izquierdo and Cuezva, 1997) will also result in a limitation of the translation of mt-mRNAs (Ostronoff *et al.*, 1996) in tumor cells.

The translational repression of the transcripts encoded in both genetic units, added to a reduced transcription of the nuclear genes encoding proteins of the bioenergetic and replication machinery of mitochondria, will promote the progressive and concurrent decline in mitochondrial DNA and proteins observed in hepatoma cells as a result of cellular proliferation. The "dilution" of mitochondria in tumors will occur despite an increased availability of the mRNAs, because it is predicted that the transcripts will be experiencing a stabilization event that is coupled to a translational arrest, i.e., they are "masked" as in the fetal liver (Fig. 1).

The million dollar question here is who orchestrates this cellular response? Oncogenesis and cellular transformation trigger a profound modification in the

expression of the nuclear genome. Therefore, it is reasonable to assume the participation of transcription factors that, on the one hand, down-regulate specialized functions of the differentiated hepatocyte, such as oxidative phosphorylation, mtDNA replication and, perhaps, gluconeogenesis, fatty acid oxidation, etc., and, on the other hand, up-regulate the expression of glycolytic and isoform specific genes of the undifferentiated hepatocyte. Transcription factors could also play a role in the translational control and stability of the mRNAs that encode proteins of oxidative phosphorylation.

We hope that the purification and characterization of the developmentally regulated and tissue-specifically expressed β-mRNA binding protein (Izquierdo and Cuezva, 1997) will contribute to the clarification of the post-transcriptional regulation of mitochondrial biogenesis. Anyway, it is clear that much greater effort in basic research should be invested in the characterization of the mechanisms that control the expression of oxidative phosphorylation genes because we are still far from reaching the expected answer that could explain the altered energetic metabolism of tumor cells, a challenge of the scientific community set out more than 60 years ago.

ACKNOWLEDGMENTS

We thank Dr. P. L. Pedersen and P. Cantatore for the generous supply of cDNAs and mtDNAs of oxidative phosphorylation used as probes in our studies. Dr. J. Satrústegui is acknowledged for critical reading of the manuscript. M. Chamorro is acknowledged for expert technical assistance. We thank D. Jelenic and M. Sanz for secretarial assistance. L.K.O., J.R., and M.L.H. are recipients of predoctoral fellowships from Universidad Autónoma de Madrid, FPI-Ministerio de Educación y Ciencia, and Gobierno Vasco, respectively. The work described in this review has been supported by grant PB94-0159 and other previous grants from Dirección General de Investigación Científica y Técnica, Spain. We acknowledge an institutional grant from Fundación Ramon Areces.

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