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Role of Mitochondria in Carcinogenesis

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INTRODUCTION

In 1926 Warburg noticed that tumour cells had a deficient respiration to which he attributed an important role in carcinogenesis. Further work involving nuclear genes, in particular oncogenes, in the successive steps of the tumour process, put mitochondria to a second frame. Recent work based on mitochondrial DNA (mtDNA) structural differences in cancer cells raises again the problem of mitochondrial involvement in both carcinogenesis and maintenance of the cancer state.

The majority of mitochondrial proteins are encoded by the nuclear genome; this supposes that mitochondria strictly depend upon it. In turn, however, mitochondrial genes may have some regulating influence on the nuclear genome [1] since mitochondrial DNA lesions are at the origin of changes in activity of several nuclear genes involved in the biogenesis of the cell surface [2]. Moreover, mouse mtDNA is involved in the expression of the surface antigen Mta [3].

Several differences in mitochondrial morphology, number and type of enzymatic constituents and transport mechanisms, have been described in several tumour cell lines. Although many are due to misprogramming of proteins coded for by the nuclear genome, several may result from changes in mtDNA arising during carcinogenesis [4].

MITOCHONDRIAL DNA PREDISPOSITION TO MUTATIONS

Mitochondrial DNA is constitutively more accessible to chemical attacks than nuclear DNA since it is not protected by histones. Its super-coiled circular structure favours the binding of alkylating agents such as *N*-nitrosomethylamine [5] that accumulate in membranes [6], to which mtDNA and its replicating enzymes bind [7].

Fukanaga and Yielding postulated that mtDNA has no base repair mechanisms [8]. However, a few systems not strictly devoted to DNA damage repair exist, but they are poorly efficient [9].

Mitochondrial DNA is particularly exposed to free radicals produced by complex III of the respiratory chain [10] leading to the formation of the cross-linking agent malonaldehyde [11]. The activities of mitochondrial superoxide dismutase (Mn^{2+}dependent) and of catalase are highly reduced, if not absent, in transformed cells. Therefore, the concentration of oxygen radicals in tumour mitochondria is elevated [12]. Mitochondria consume about 90% of cellular oxygen and their respiratory chain is a continuous source of oxygen radicals [13]. Moreover, superoxide production increases with age [14]. Hydroxyl radicals cause mtDNA breakage [15]. In conclusion, the high level of mtDNA oxidative damages is due to the combination of important fluxes of oxygen-free radicals, of a poorly efficient mtDNA repair system and of a lack of mtDNA protection.

Mitochondrial DNA is a privileged target for several mutagens and carcinogens. Polycyclic aromatic compounds covalently bind mtDNA [16], as do the dihydrodiol-epoxide derivative of benzo[a]pyrene [6], certain drugs used in cancer therapy such as dequalinium [17] and the intercalating agent diterqualinium [18] which eliminates mtDNA from cultured mouse leukaemic cells. The anti-leukaemic agent daunomycin acts through a specific inhibition of mtDNA transcription. Seemingly, ethidium bromide, a largely used compound in molecular biology research, preferably binds and mutates mtDNA [19].

MITOCHONDRIAL DNA AND CARCINOGENESIS

General considerations

Mitochondrial DNA replication is more regulated by the cell mass that mitochondria must serve than by direct coupling to nuclear DNA replication. If the amount of mtDNA is similar in both normal cells and immortalised cells [20], mtDNA of cancer cells differs by its integrity. A large number of unusual mtDNA forms containing two or more concatenated molecules have been visualised in certain cancer tissues and in cells transformed by oncoviruses [21]. Molecules two to three times larger than normal have been found in HeLa cells and in leukaemic leucocytes, resulting probably from the non-parting of molecules after replication [22]. An important increase of a rare dimeric form has been observed in certain malignant tissues such as acute myeloid leukaemia, salivary gland carcinoma and, to a lower extent, a number of solid tumours [23]. The significance of these mtDNA complex forms remains unknown.

A supplementary 40 base pair *Hinf* I fragment was found in the gene coding for subunit I of cytochrome oxidase of six different renal oncocytomas [24]. Other anomalies of mtDNA were reported: restriction fragments of abnormal length in leukemic lymphoblasts [25], partial deletion in genes coding for $tRNA^{Tyr}$ and $tRNA^{Trp}$ and an insertion of two nucleotides in the gene coding for $tRNA^{Cys}$ in a dimethylaminobenzene-induced rat hepatoma [26]. However, since proteosynthesis is not affected, the authors think that these anomalies do not accompany heteroplasmy. Damaged and deleted mtDNA were found in cirrhotic liver surrounding hepatic tumours, a discovery supporting the idea that mutations in mtDNA are one of the numerous endogenous factors favouring carcinogenesis in a precancerous state [27].

A mechanism has been proposed to explain mutations occurring during mtDNA replication [28]. At the moment of replication, and because of the high number of repeated sequences in the mitochondrial genome, an abnormal curvature affects one of the two strands that brings two repeated sequences into contact. A recombination event may take place after breakage of the mtDNA sequence at the level of particular break-points. Such a mechanism produces mtDNA molecules of different sizes at the origin of a heteroplasmy.

Transcription of mitochondrial genes in tumour cells

Increased amounts of mitochondrial primary transcripts are often related to abnormal mtDNA molecules. The levels of mtDNA coding for subunit II of cytochrome oxidase are 10-fold increased in either embryonic fibroblasts transformed by

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the polyoma virus or in cells immortalised with the E1A adenovirus or the *myc* oncogene. Glaichenhaus *et al.* [29] suggest that a direct *trans*-regulating effect may act on the synthesis of mitochondrial primary transcripts, without neglecting the possible involvement of regulating signals issued by the nucleus.

In a similar way, transcription of mitochondrial genes in hepatoma cells is 10-fold increased after growth stimulation [30]. Mitochondrial gene expression is increased before phase S of cell cycle is reached, at the moment when cells need energy to proliferate. Since initiation of L strand transcription produces a primer starting at the O_H origin, the increase of transcription due to growth stimulation may also be important for mitochondrial proliferation.

Corral *et al.* [31] noticed a high increase of mRNA corresponding to a portion of the mitochondrial gene coding for subunit V of NADH dehydrogenase (ND5). This selective increase is not simply explained by a stimulation of transcription since the latter produces a polycystronic mRNA, but rather involves some differences in the stability of mitochondrial mRNAs. An increase of mitochondrial transcripts has also been observed in neoplastic cells of familial polyposis coli [32]; a change of synthesis and/or degradation rate of mitochondrial mRNAs may be at the origin of such phenomena.

The mechanism that governs global or selective mitochondrial messengers' increase in neoplastic cells has not yet been elucidated. Among the possible causes, two hypotheses may be proposed. First, the expression of genes coding for mitochondrial RNA polymerase and/or for mitochondrial transcription factor (mtTF1) may be regulated at the nuclear level; the amount of proteins produced may determine the transcriptional activity after their translocation into mitochondria has taken place. Second, mitochondrial RNA polymerase and/or mtTF1 may both have an active and an inactive form; their interconversion may be regulated either through cell proliferation or by the amount of proteins translocated into mitochondria.

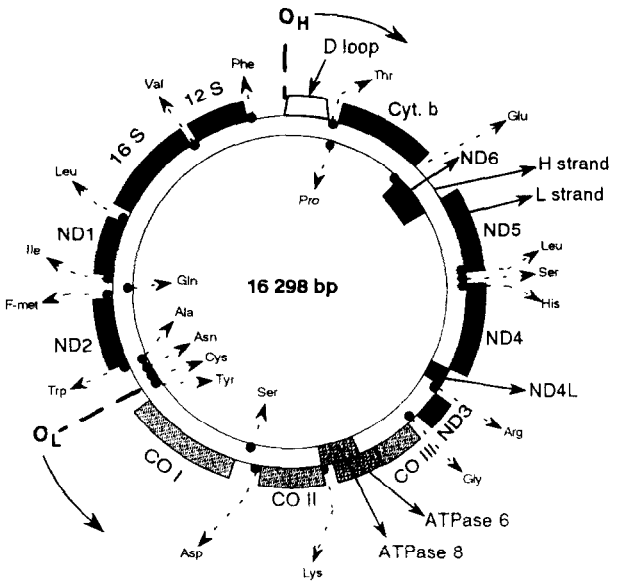

Fig. 1. Genetic map of the rat liver mitochondrial genome. The complete nucleotide sequence of the circular DNA contains 16 298 base pairs with no introns. Transcription is clockwise for the outer circle [heavy (H) strand] and counter clockwise for the inner circle [light (L) strand]. The H strand contains genes for six subunits of NADH dehydrogenase (ND1 to ND5 and ND4L), three subunits of cytochrome oxidase (COI to COIII), for cytochrome b, two subunits of mitochondrial ATPase (ATPase 8 or A6L and ATPase 6), two ribosomal RNAs (12S and 16S) and 14 tRNAs. The L strand contains genes for one subunit of NADH dehydrogenase (ND6) and 8 tRNAs. Several genes are overlapping or share several bases in common: this is the case for the genes coding for $tRNA^{Ile}$ and $tRNA^{Gln}$; COI and $tRNA^{Ser}$; ATPase 6 and ATPase 8; ND4L and ND4; and $tRNA^{Leu}$ and ND5. The D-loop contains promoter sequences and the origin of replication of the H strand (O_H), whereas the origin of replication of the L strand (O_L) is located farther on the genome. Human mitochondrial genome is similar except for its length (16 569 bp).

ROLE OF MITOCHONDRIA IN CARCINOGENESIS

Oxidative phosphorylation

It is difficult to think that rapidly proliferating cells, thus consuming a high amount of energy, may rely upon glycolysis as the sole source of ATP, without using oxidative phosphorylation. The same reasoning stands for mutations on mtDNA having no effects on the synthesis of enzymes involved in respiration. The important anomalies mtDNA of tumour cells contains are obviously at the origin of a decreased, or aborted, proteosynthesis involved in the mitochondrial respiratory activity. Because of both its mode of replication and its matrogenic lineage, only part of mtDNA may be injured, allowing the remaining intact part to balance the oxidative needs of tumour cells.

The oxidative phosphorylation system (OPS) assembles itself from 13 mitochondrial genes and about 100 nuclear genes. The adenine nucleotide translocator has a major role in the regulation of oxidative phosphorylations [33]. It is represented by at least three isoforms: the ANT1 form that is found in heart and skeletal muscle [34] and the ANT2 and ANT3 forms. The β subunit of ATP synthase represents the major component of the catalytic site that condenses ADP and inorganic phosphate to form ATP on the ATPsynthase complex.

Neoplastic transformation has a marked impact on the expression of nuclear and mitochondrial genes coding for the OPS [35]. Induction of nuclear genes coding for the ANT1 and ANT2 isoforms, for the gene coding for the β subunit of ATPsynthase and for the genes coding for 12S RNA, ND2, ATPase 8 and ATPase 6, CO III, ND5, ND6 and cytochrome b is highly increased, whereas the copy number of mtDNA decreases.

The messenger increase of the ANT1 and ANT2 isoforms in neoplastic cells explains the alteration of the ATP/ADP exchange kinetics through inner mitochondrial membranes. This has an important effect on oxidative phosphorylation in cancer cells since the translocator regulates intramitochondrial ADP concentrations and since ADP is the limiting substrate for oxidative phosphorylations.

ANT1 is especially expressed in the most oxidative tissues of the mammalian body. Its induction in cancer cells indicates that their mitochondria are more oxidative than normal ones, particularly since mRNA levels for the β subunit of ATPsynthase are increased. The concomitant increase in expression of mitochondrial genes may be related to the elevated glycolysis in tumour cells through the expression of a hexokinase form linked to the inner mitochondrial membrane [36]. As soon as ATP has been produced and transported by the adenine nucleotide translocator, it is immediately utilised by the linked hexokinase to partially phosphorylate glucose [37].

Causes for alterations of the expression of the OPS genes in tumour cells are not yet elucidated. Several hypotheses can be proposed. Changes in the expression of the OPS genes may correspond to mtDNA mutations [38]. Moreover, transformation may result from a reversion toward an embryonal energetic

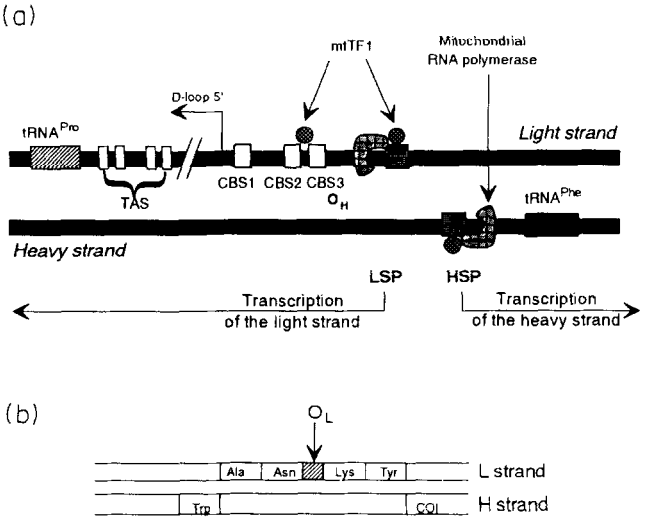

Fig. 2. Schematic organisation of the D-loop region (a) and of the L strand replication origin (b) of mammalian mitochondrial DNA. Replication of the mitochondrial DNA H strand starts at the origin O_H located in the displacement (D) loop, after the torsion of mtDNA has been relieved by DNA topoisomerases [61]. Polymerisation of both H and L template strands is due to a mtDNA polymerase (or polymerase γ) [62]. It requires the presence of RNA primers that are produced by mtRNA polymerase at the level of O_H and by a mitochondrial primase at the level of O_L. DNA synthesis is then accomplished by a mtRNase mitochondrial RNA processing at the level of the conserved sequence blocks (CSB 1 to 3) located in the D-loop, and at the level of a G-rich sequence located in O_L [63].

Transcription, due to a mtRNA polymerase, takes place in both opposite directions for each strand starting from its respective promoter (light strand promoter (LSP) for the L strand and heavy strand promoter (HSP) for the H strand, both of them located in the D-loop). It proceeds with the binding, in the promoter region, of a proteic factor activating transcription: mtTF1, which is encoded by the nuclear genome. Transcription may then start with the binding of mitochondrial RNA polymerase on the promoter complex (LSP on the light strand and HSP on the heavy strand). The H strand gives polycistronic RNAs that are subsequently matured by endonucleolytic cleavages upstream and downstream of the tRNA sequences. Messenger RNAs are then polyadenylated. The origin of replication (O_L) of the L strand is located between the genes coding for $tRNA^{Asn}$ and $tRNA^{Lys}$ on the L strand. TAS, Termination associated sequences.

metabolism [39]; this may probably be the case for the reversion to foetal forms of isozymes such as the key glycolytic enzymes. However, the adenine nucleotide translocator is not as limiting a step in the foetus as it is in the adult rat liver [40]. Other reversions to foetal forms of enzymes involved in the mitochondrial energetic metabolism may be at the origin of unusual metabolic pathways that tumour mitochondria should adopt in order for its host cell to survive in particular environmental conditions [41–43].

Mitochondrial DNA mutations and plasma membrane

Cancer cells bear structural anomalies in their plasma membrane with such consequences as the loss of control for adherence capacities and for cell divisions. When yeast cells have been treated with carcinogens, mtDNA is preferentially attacked. The resulting "petite" mutants, depleted of mtDNA, curiously present cell surface changes analogous to those observed in neoplastic cells [2]. Moreover, mtDNA regulates the expression of adherence capacity of cells that belong to established cancer lines and is necessary to maintain the transformed phenotype [44]. Therefore, mutations on mtDNA may be at the origin of these modifications [2, 45].

Mitochondria and bcl-2

The proto-oncogene *bcl-2* has been found juxtaposed to the immunoglobulin heavy chain locus in human follicular B-cell lymphoma, with a c-*myc*/*bcl-2* co-operation [46]. The product *bcl-2* is localised in the inner mitochondrial membrane. When overexpressed, it blocks the programmed cell death (apoptosis) of pre-B lymphocytes [47]. *bcl-2* is therefore involved in tumour cell transformation and has a role in the normal nucleo-mitochondrial communication circuits.

CONCLUSION

The studies mentioned above evidence the role that mitochondria and mitochondrial DNA play in carcinogenesis. The question is how they are involved in the phenomenon? Does injured mtDNA activate nuclear proto-oncogenes? Does it deregulate nucleo-mitochondrial relationships? To what extent may it be able to modify energetic and mitochondrial metabolisms in tumour cells? The next step in the study of mitochondrial carcinogenesis should be focused on the complex nucleo-mitochondrial relationships, in which a cellular oncogene with a mitochondrial localisation, *bcl-2*, is involved. The *bcl-2* gene seems to orchestrate both life and death of the cell through a genetic program that still remains to be discovered.

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