

CLASSIC PAPER

Mitochondria as a source of reactive oxygen species

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INTRODUCTION

Nowadays, it is generally accepted that the mitochondrial respiratory chain is an important source of ROS (reactive oxygen species) in biological systems. This view arose from work by a number of laboratories from the 1960s onwards that provided evidence that the mitochondrial respiratory chain could produce ROS. Three papers published in the *Biochemical Journal* between 1972 and 1980 by Britton Chance (Figure 1) and colleagues had a fundamental impact on our understanding of mitochondria as a source of ROS. The implications of these papers are still stimulating research today, as we continue to unravel the sources and consequences of ROS production *in vivo*. In this *Biochemical Journal* Classics article, we discuss the important role that these papers played in developing our current understanding of mitochondrial ROS metabolism.

From investigating the interactions of ionizing radiation with biological systems, it was known that ROS, such as the superoxide ($O_2^{\bullet-}$) and hydroxyl (HO^{\bullet}) radicals, as well as non-radicals such as hydrogen peroxide (H_2O_2), could damage biological molecules [1]. The similarities between the pathological damage seen on exposure to ionizing radiation and that found to accumulate during aging led to the proposal that endogenously produced ROS may cause some of the damage seen in normal aging and in other pathologies [2]. The discovery of an enzyme, Cu,Zn-SOD (copper/zinc superoxide dismutase), that detoxified superoxide and was present at high concentrations in the cytoplasm of a range of tissues strongly supported a role for ROS in biological systems [3], but the sources of these endogenous ROS were uncertain.

MITOCHONDRIA AS A SOURCE OF HYDROGEN PEROXIDE

By the 1960s, the role of mitochondria in respiration and ATP synthesis was well established, and the mechanism of oxidative phosphorylation was keenly sought. The first indication that the mitochondrial respiratory chain might be a source of ROS came in 1966 from studies with SMPs (submitochondrial particles), which are inverted vesicles of the mitochondrial inner membrane that can sustain respiration and proton pumping across the membrane to establish a protonmotive force (Δp). In SMPs, there was a significant flux of hydrogen peroxide produced as a by-product of respiration under some conditions [4]. However, it was unclear whether this ROS production was a consequence of disrupting mitochondria to make SMPs, therefore it was important to see whether intact mitochondria also produced hydrogen peroxide. To this end, Britton Chance introduced a sensitive spectrophotometric method for measuring hydrogen peroxide production using the absorption changes that occur during the catalytic cycle of cytochrome *c* peroxidase on reaction with hydrogen peroxide (reviewed in [5]). This innovation enabled the flux of hydrogen peroxide to be measured in cytosolic extracts, including isolated mitochondria [6–9],



Figure 1 Britton Chance

Britton Chance made seminal contributions to the development of several fields, including enzymology, bioenergetics and the biology of ROS, as well as winning an Olympic Gold medal for the United States in sailing at the 1952 Olympic Games in Helsinki. In mitochondrial research, his achievements include the development of the double-wavelength spectrophotometer, helping to determine the pathway of electron movement down the respiratory chain and introducing the concepts of 'state 3' and 'state 4' respiration.

making it clear that mitochondria were a significant source of hydrogen peroxide within the cell (Figure 2). These studies included two seminal *Biochemical Journal* papers [7,8], which have been cited 1145 and 794 times respectively up to the end of 2008. Most interestingly, the citation rate for both papers peaked around 2003–2004, more than 20 years after their publication; the vast majority of papers reach their peak citation rate within a few years of publication. The discovery that mitochondria are a significant source of hydrogen peroxide is one of the many fundamental contributions made by Britton Chance and his collaborators to understanding mitochondrial function.

HOW ISOLATED MITOCHONDRIA PRODUCE ROS

After the Chance laboratory demonstrated that mitochondria produce hydrogen peroxide, a number of other studies refined our understanding of this process. It was shown that hydrogen peroxide arose from the dismutation of the proximal ROS superoxide, itself produced directly by the interaction of molecular oxygen with an electron leaking from the respiratory chain [10,11]. This superoxide was then dismutated to hydrogen peroxide by a mitochondrial Mn-SOD (manganese superoxide dismutase) [12], and the hydrogen peroxide could subsequently diffuse from the mitochondria. Complex III in the respiratory chain could be induced to produce large amounts of superoxide

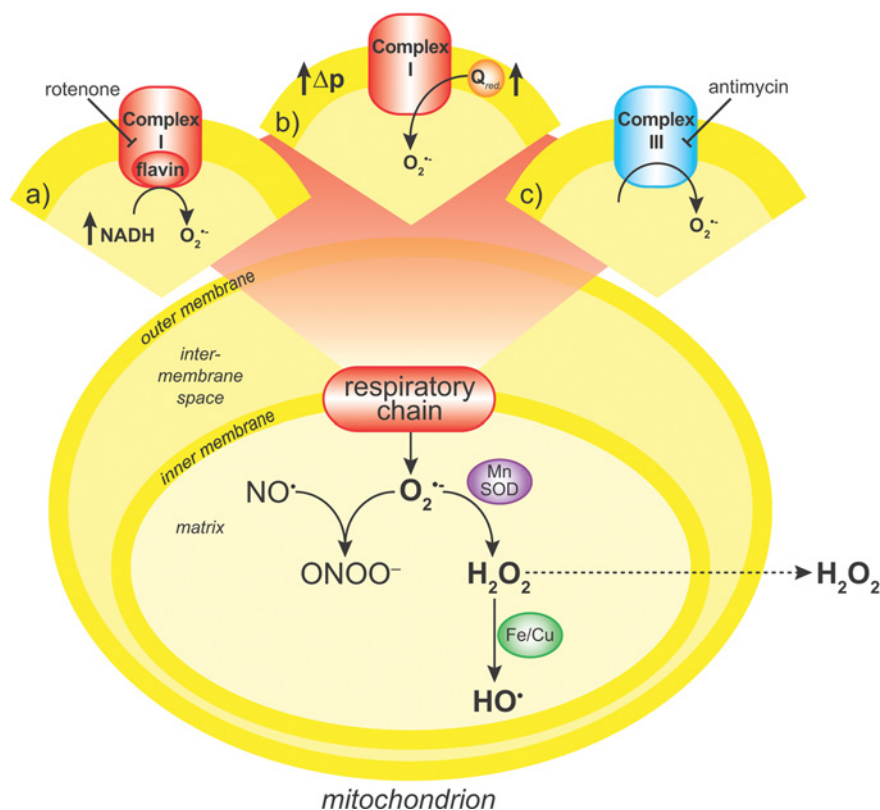


Figure 2 Production of damaging ROS by mitochondria

The proximal ROS produced by mitochondria is superoxide ($O_2^{\bullet-}$), and its production from the respiratory chain can be dramatically induced from Complex I by addition of the inhibitor rotenone in the presence of NADH (inset a), or under conditions of a high Δp and reduced coenzyme Q pool (Q_{red}) that together favour reverse electron transport (inset b). Alternatively, superoxide production can be induced from Complex III by the inhibitor antimycin (inset c). Superoxide is not particularly damaging itself, although it can disrupt certain iron–sulfur-centre proteins. Superoxide is rapidly converted into hydrogen peroxide (H_2O_2) by SOD-catalysed dismutation. Furthermore, superoxide can react with nitric oxide (NO^{\bullet}) to form the reactive and damaging peroxynitrite ($ONOO^-$). Hydrogen peroxide is also not very reactive, but it can oxidize some amino acid residues such as cysteine and methionine, and can also diffuse out of mitochondria. In the presence of metals, such as ferrous or cuprous ions, hydrogen peroxide forms the very reactive hydroxyl radical (HO^{\bullet}) that indiscriminately damages all biological molecules.

when blocked by the inhibitor antimycin [11]. The second major source of induced superoxide production in the respiratory chain is Complex I. It had been shown that inhibiting Complex I in the presence of its substrate NADH led to superoxide formation [13,14]. This was extended in another *Biochemical Journal* paper by Turrens and Boveris [15], the latter of whom was first author on the two papers from the Chance laboratory that were discussed earlier. In this work, it was shown that Complex I could produce superoxide under two conditions: when there was a high concentration of NADH in the presence of the inhibitor rotenone (now known to arise from the reduced mononucleotide within Complex I [16]), and also during reverse electron transfer when the combination of a high Δp and a reduced coenzyme Q pool drives electrons back through Complex I. Thus, from these papers and the work of many others [17], we now have a fairly complete understanding of how isolated mitochondria can be induced to produce large amounts of superoxide at Complexes I and III (Figure 2).

IMPLICATIONS FOR MITOCHONDRIAL ROS PRODUCTION *IN VIVO*

The findings outlined above provided strong evidence that mitochondria are a significant source of ROS. That mitochondria also produce ROS *in vivo* is supported by a considerable array of evidence, such as the existence of Mn-SOD and

other antioxidant enzymes within the mitochondrial matrix [12], the pathological consequences of blocking expression of these enzymes [18,19], and oxidative damage markers within mitochondria [20]. Together, these findings provide a firm basis for current investigations of mitochondrial ROS metabolism that focus on how oxidative damage arising from mitochondrial ROS leads to various pathologies, and on how mitochondrial ROS production may contribute to redox signalling, e.g. by hydrogen peroxide modifying the activity of redox-sensitive proteins in the mitochondria and cytosol (Figure 3).

Even so, it is important to note that we still know relatively little about mitochondrial ROS production within living organisms. Although the papers of Chance and colleagues [6–9] indicate that mitochondria can be easily induced to produce ROS from Complexes I or III, there are still significant uncertainties about the magnitude and source of ROS production by mitochondria *in vivo* [17]. For example, it is not known whether the conditions that lead to the high fluxes of ROS from Complex I can occur *in vivo*; it may turn out that the mitochondrial ROS that contribute to mitochondrial oxidative damage come from other sources, such as matrix dehydrogenases [17]. A further issue is the magnitude of the mitochondrial ROS flux that occurs within organisms. In their work, Chance and colleagues showed that, for isolated mitochondria producing ROS by reverse electron transport at Complex I, approx. 2% of oxygen consumption was diverted from respiration to ROS production [6]. Despite the authors

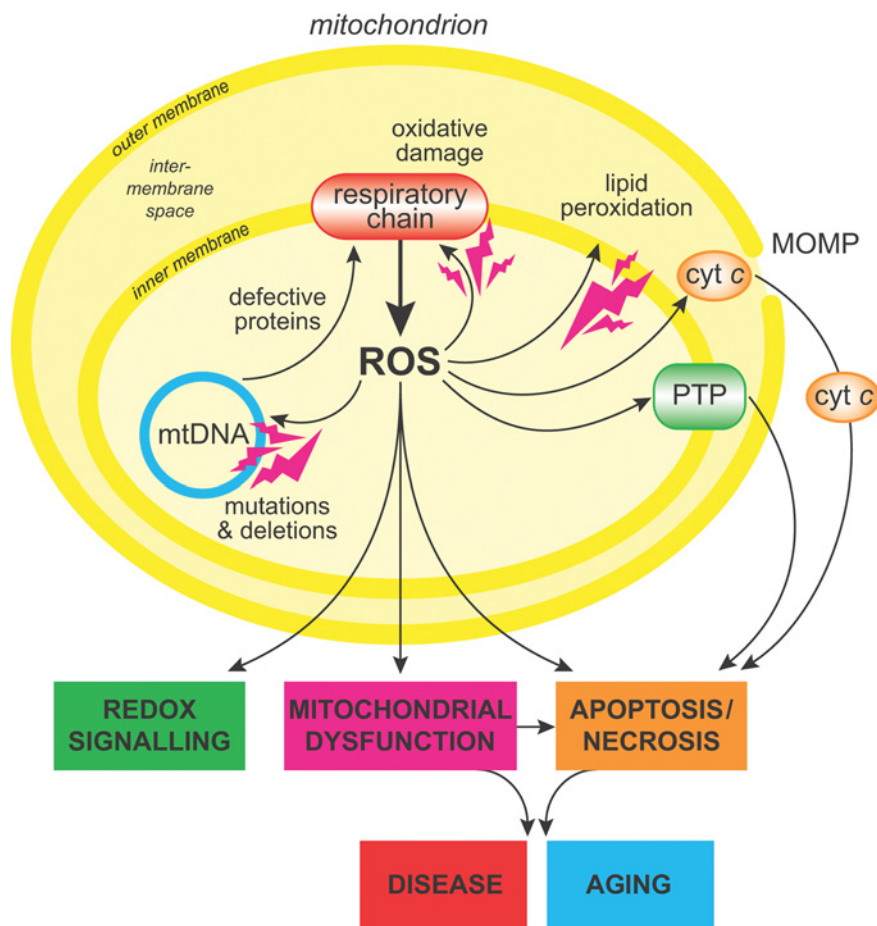


Figure 3 Consequences of mitochondrial ROS production

ROS production by mitochondria can lead to oxidative damage to mitochondrial proteins, membranes and DNA (mtDNA), impairing the ability of mitochondria to synthesize ATP and to carry out their wide range of metabolic functions, including the tricarboxylic acid cycle, fatty acid oxidation, the urea cycle, amino acid metabolism, haem synthesis and iron–sulfur-centre assembly, that are central to the normal operation of most cells. Mitochondrial oxidative damage can also increase the tendency of mitochondria to release intermembrane-space proteins such as cytochrome *c* (cyt *c*) to the cytosol by mitochondrial outer membrane permeabilization (MOMP) and thereby activate the cell's apoptotic machinery. In addition, mitochondrial ROS production leads to induction of the mitochondrial permeability transition pore (PTP), rendering the inner membrane permeable to small molecules in situations such as ischaemia/reperfusion injury. Consequently, it is unsurprising that mitochondrial oxidative damage contributes to a wide range of pathologies. In addition, mitochondrial ROS may act as modulatable redox signals, reversibly affecting the activity of a range of functions in the mitochondria, cytosol and nucleus. Reproduced from [17].

clearly indicating that this measurement was made under non-physiological conditions and that any extrapolation to the living organism required a large number of assumptions, this value of 2% of respiration going to ROS has been widely quoted as an estimate of mitochondrial ROS production *in vivo*. In fact, without considerably more knowledge of the mitochondrial Δp , the reduction potentials of the NADH and coenzyme Q pools, and the local oxygen concentration, it is difficult to estimate mitochondrial ROS production *in vivo*. So, although there is considerable evidence that mitochondria do produce ROS under physiological circumstances, the magnitude of the flux is uncertain, and the sites responsible for these ROS are unclear.

SUMMARY

The three *Biochemical Journal* papers that we have discussed have made significant contributions to confirming that mitochondria generate ROS, and opened up currently important fields of trying to understand the role of mitochondrial ROS in both normal physiology and pathology (Figure 3). However, there are still

fundamental gaps in our knowledge of how mitochondria produce ROS *in vivo*. Just as Chance and colleagues made progress by developing techniques to measure ROS production in suspensions of isolated mitochondria, future progress depends on developing techniques to assess and quantify mitochondrial function and ROS production in living organisms.

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