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Rapid report

Exposing the Complex III Qo semiquinone radical

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Abstract

Complex III Qo site semiquinone has been assigned pivotal roles in productive energy-conversion and destructive superoxide generation. After a 30-year search, a genetic heme b_H knockout arrests this transient semiquinone EPR radical, revealing the natural engineering balance pitting energy-conserving, short-circuit minimizing, split electron transfer and catalytic speed against damaging oxygen reduction.

Keywords: Complex III; Cytochrome bc_1; Semiquinone; Qo; Superoxide; Electron transfer

Around 1970, semiquinone radical (SQ) was proposed as a key element in mitochondrial respiration with a remarkable role straddling high and low potential redox chains in respiratory Complex III [1–3]. The importance of Complex III in generating reactive oxygen species (ROS) emerged at the same time [4]. A connection was made between a highly reducing SQ of Complex III and the reduction of molecular oxygen to superoxide radical, the first in a series reactions thought to signal hypoxia and initiate a cascade of events that leads either to cellular protection against low levels of O_2 or cellular destruction in apoptosis [5–7].

Since then, kinetics and crystal structures have supported the split electron transfer by reduced ubiquinone proposed by Mitchell in 1976 in his Q-cycle mechanism of Complex III [2]. One electron from hydroquinone at the Qo site passes initially to Rieske FeS cluster, before proceeding down the high potential cofactor chain of heme c_1 and cytochrome c. Essentially simultaneously or, in Mitchell's view after a brief delay in which SQ is an intermediate, the other electron passes first to a low potential heme b_L before going on to a higher potential heme b_H and then on to the Qi site near the other side of the membrane, completing the "Q-cycle" [2,8,9] (Fig. 1a). Extensive studies have made it clear that the Qo site rather than the Qi site dominates ROS generation in hypoxia [5].

The search for the pivotal SQo began soon after Mitchell's proposal. In 1979 Takamiya failed to detect the SQo signal by EPR in the presence of antimycin [10] an inhibitor that eliminates the relatively stable semiquinone at Qi. In 1981, de Vries seemed to detect SQo free radical [11], but subsequent work [12] associated these semiquinones with other respiratory complexes. Recent stopped flow work failed to see any radical at Qo [13]. SQo was never seen presumably because it is a transient and at most lightly populated intermediate between the first uphill electron transfer to FeS and the second more favourable electron transfer to heme b_L [12], although others suggested that magnetic coupling between reduced FeS and SQ makes the SQ spin in principle undetectable [14].

Recent work with cofactor knock-outs trims back the b-chain and provides a unique opportunity to arrest any transient SQ at Qo by hindering escape of the SQ electron. Mutating the heme b_H iron ligating His to Gln in photosynthetic bacteria [15] creates a Complex III with an intact and fully operational Qo-site [16] but no heme b_H. A simple flash of light activates the photosynthetic reaction centre (RC) in these membranes, creating in microseconds the two substrates of Complex III: oxidized cytochromes c_2 and hydroquinone. Electron transfer proceeds through Qo along the b- and c-chains until a quasi-equilibrium state is reached, in which the redox potential of the

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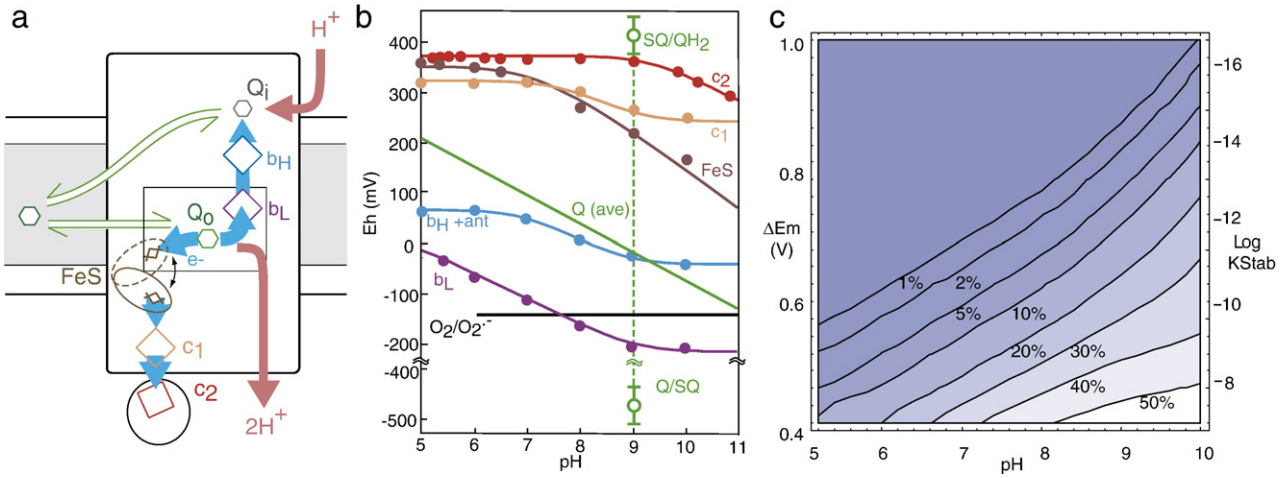

Fig. 1. (a) The redox centres of Complex III are grouped into high and low potential c- and b-chains on opposite sides of the Qo site. When heme b_H is knocked-out a redox quasi-equilibrium is established after a flash. (b) Our pH dependence of the redox midpoint potentials in *Rb. capsulatus*; cyt c_2 is from [30]. We estimate the two redox couples of Qo as more oxidizing than cyt c_2, and more reducing than O_2/superoxide [31]. (c) Simulated yield of SQo in heme b_H knock-out mutants following flash activated electron transfer and millisecond redox quasi-equilibrium depends upon pH and the split in the redox couples of the quinone or the commonly used apparent equilibrium stability constant. Using the midpoints of panel b and an optical kinetics determined 1:0.5:1 stoichiometry of RC:cyt c_2:Complex III, a Mathematica calculation of the redistribution of electrons to form the quasi-equilibrium state (in which the redox potential of the low potential chain, including Qo/SQo, and the high potential chain, including SQo/QoH_2, average to the potential of the Q pool) shows that much more SQ is expected after two saturating flashes progressively oxidizes the high potential c-chain (shown here) than after one flash. Similarly, much less SQo is expected if heme b_H is not knocked-out. A yield of 1% at pH 9.0 in the heme b_H knock-out corresponds to an effective K_{Stab} of $\sim 10^{-14}$ to 10^{-15}.

quinone in the Q-pool balances against the average redox potential of the high-potential c-chain and low-potential b-chain [2,16,17]. The key to promoting semiquinone at Qo is to use the knock-out to create a condition in which the b-chain can only accept one electron in heme b_L, but the c-chain can accept two and to use mildly alkaline conditions to lower the redox-midpoint values of the quinone couples [12] to favour oxidation by the high potential c-chain (Fig. 1b). Simulations of the electron transfers that lead to the quasi-equilibrium state indicate that SQo should be visible at high pH under a wide

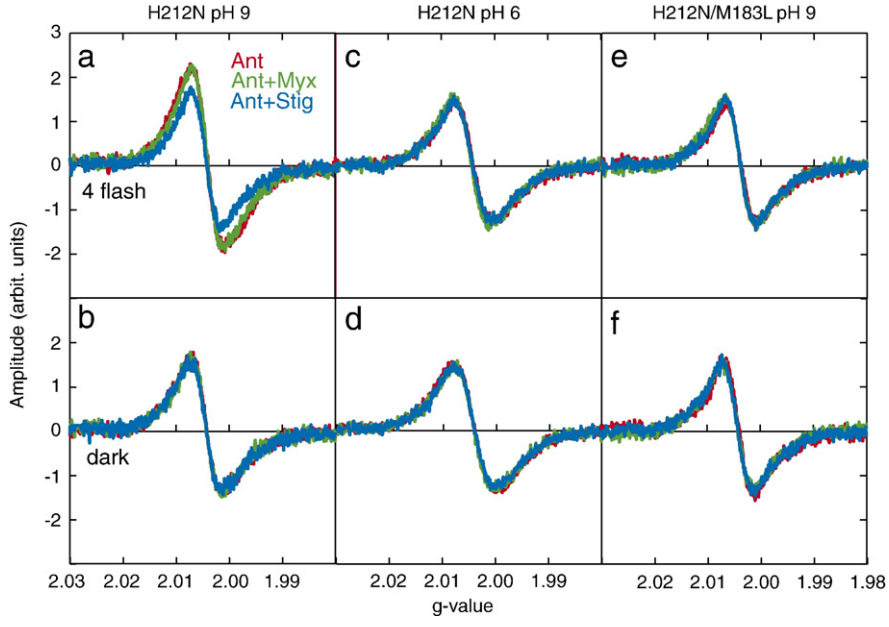

Fig. 2. EPR signals at 9.45 GHz, 0.2 mW, 130K of chromatophore membranes exposed to 4 flashes of light (a, c, e) or kept in the dark (b, d, f) for single (a–d) and double (e, f) knock-out mutants with and without Qo-site inhibitors. A stigmatellin sensitive light induced radical is present in heme b_H knockouts at pH 9 (50 mM Tris, 100 mM KCl) (a) but not at pH 6 (50 mM MES) (c) nor in the double b_H/c_1 heme knock-out (e), nor in wild-type with antimycin (not shown). All samples contain 20 μM carboxin and rotenone and 3 fold excess of antimycin and HQNO over approximately 30 μM Complex III as well as 50 μM redox mediators 2,3,5,6-tetramethyl 1,4-phenylenediamine, phenazine methosulfate, phenazine ethosulfate and 2-hydroxynaphthoquinone [19] to poise at the midpoint potential of the Q pool (−20 mV SHE at pH 9). Stigmatellin or myxothiazol when present is also in three fold excess.