The cytochrome \( bc \) complexes found in mitochondria, chloroplasts and many bacteria play critical roles in their respective electron transport chains. The quinol oxidase (Qo) site in this complex oxidizes a hydroquinone (quinol), reducing two one-electron carriers, a low potential cytochrome \( b \) heme and the “Rieske” iron-sulfur cluster. The overall electron transfer reactions are coupled to transmembrane translocation of protons via a “Q-cycle” mechanism, which generates proton motive force for ATP synthesis. Since semiquinone intermediates of quinol oxidation are generally highly reactive, one of the key questions in this field is: how does the Qo site oxidize quinol without the production of deleterious side reactions including superoxide production? We attempt to test three possible general models to account for this behavior: 1) The Qo site semiquinone (or quinol-imidazolate complex) is unstable and thus occurs at a very low steady-state concentration, limiting \( \mathrm{O}_2 \) reduction; 2) the Qo site semiquinone is highly stabilized making it unreactive toward oxygen; and 3) the Qo site catalyzes a quantum mechanically coupled two-electron/two-proton transfer without a semiquinone intermediate. Enthalpies of activation were found to be almost identical between the uninhibited Q-cycle and superoxide production in the presence of antimycin A in wild type. This behavior was also preserved in a series of mutants with altered driving forces for quinol oxidation. Overall, the data support models where the rate-limiting step for both Q-cycle and superoxide production is essentially identical, consistent with model 1 but requiring modifications to models 2 and 3.

The cytochrome (cyt)\(^2 \) \( bc_1 \) complex (EC 1.10.2.2) (cyt \( bc_1 \) complex) is found on the inner membrane of mitochondria and energy transducing membranes of many bacteria (1–3). It is structurally and functionally homologous to a taxonomically wide spread group, collectively referred to as “\( bc \) complexes,” additionally consisting of the cyt \( bc_1 \) complexes and the menaquinol oxidizing complexes in many bacteria (4–6). In all well studied cases, cyt \( bc \) complexes couple the oxidation of a substrate quinol (\( \mathrm{QH}_2 \)) to the formation of a proton motive force across the energy transducing membrane in which the given complex resides, the energy of which is ultimately stored as ATP.

Cytochrome \( bc_1 \) complexes contain four redox-active metal centers, arranged in two separate chains (2, 5, 7–9). The “high potential chain” consists of the Rieske iron-sulfur [2Fe2S] cluster (in the Rieske, or “iron-sulfur subunit,” hereafter “the [2Fe2S] cluster”), and a \( c \)-type cyt, known in mitochondria and Gram-negative bacteria as cyt \( c_1 \). The “low potential chain,” which is liganded to the cyt \( b \) subunit of ubiquinol oxidizing complexes, consists of two \( b \)-type hemes, cyt \( b_1 \) and \( b_4 \) labeled for their relatively lower and higher electrochemical potentials. Crystal structures from several taxonomic sources (2, 8, 10, 11) suggest a well conserved placement of the cofactors throughout the various cyt \( bc \) complexes.

Three enzymatic binding sites participate in catalysis on the cyt \( bc_1 \) complex, the quinol oxidase (Qo) site, quinol reductase (Q) site, and a docking site for soluble cyt \( c \) on cyt \( c_1 \). The Qo site is located toward the positively charged side of the membrane, where protons are released during turnover of the enzyme. The Q site is located toward the negatively charged surface of the membrane, where protons are taken up during catalysis. The water-soluble cyt \( c \) docking site is located on the \( c \)-type cyt representing the terminal electron carrier within the cyt \( bc_1 \) complex on the positive side of the membrane.

Cyt \( bc_1 \) complex catalysis is thought to occur by a “Q-cycle” mechanism (1, 12–14). Perhaps the key reaction of the Q-cycle is the “bifurcated” electron transfer at the Qo site, the mechanism of which is still controversial (1, 4, 7, 15–17). In the bifurcated reaction, \( \mathrm{QH}_2 \) is oxidized at the Qo site, with one electron being transferred through the high potential chain to reduce cyt \( c \) and the other electron is transferred through the low potential chain, to reduce a quinoid species (Q or SQ, depending on the state of the two-electron gate) at the Q site. Two turnovers of the Qo site are required to reduce a Q site Q to a \( \mathrm{QH}_2 \).
Superoxide Production and the Q-cycle

Under some conditions, the Q-cycle can be short-circuited by various "bypass" reactions, some of which yield the physiologically deleterious superoxide (18–22). The bypass reactions are typically observed in vitro under "partially inhibited" conditions, e.g. in the presence of antimycin A (AA) or under high proton motive force, where it is thought that the [2Fe2S] cluster can oxidize QH₂ to a semiquinone (SQ), but processing of electrons by the low potential chain is hindered, resulting in the accumulation of a SQ intermediate, which in turn can reduce O₂ to superoxide (18, 20, 21). Under such conditions, superoxide production can occur at 2–10% of the uninhibited rates. Superoxide production rates approaching 100% of normal turnover were observed when the natural substrate, ubiquinol, was replaced with rhodoquinol (23).

We consider here the three classes of models, illustrated in Scheme 1, proposed to account for the high efficiency of the bifurcated reaction under uninhibited conditions. The models differ in the nature of the reactive intermediates of quinol oxidation (reviewed in Ref. 24). In the first class (Scheme 1, panel A), SQ is destabilized so that its concentration is kept very low, preventing rapid rates of O₂ reduction (25). In the second category (Scheme 1, panel B), the SQ intermediate is stabilized by the Q₅ site, making it less reactive to O₂ (26, 27). In the third category (Scheme 1, panel C), the SQ is avoided completely by forcing both quinol electrons to be transferred simultaneously (28, 29). In addition to the above models, a number of "gating" mechanisms have been proposed where the Q₅ site is modified during key stages of catalysis, preventing the escape of reactive intermediates (30–32).

In this work, we attempt to discriminate among these models by comparing the activation energetics for the uninhibited Q-cycle and superoxide production in the presence of AA in Saccharomyces cerevisiae complexes with a range of [2Fe2S] cluster redox potentials (33, 34).

EXPERIMENTAL PROCEDURES

Yeast Strains—Yeast strains with site-directed mutations in the Rieske subunit of the cyt bc₁ complex (22, 33, 34) were grown in a synthetic mixture of amino acids excluding tryptophan (Sigma) and with dextrose (Fisher) as the carbon source. Ammonium sulfate (Fisher) and yeast nitrogen base (Sigma) without amino acids were added as a source of nitrogen.

Submitochondrial Particles Preparation—Submitochondrial particles (SMPs) were prepared as described Muller et al. (20) and used as prepared or after dispersal in dodecyl maltoside (5 mg/mg protein/dodecyl maltoside) (Anatrace) on ice for 1 h, followed by centrifugation for 5 min at 16,000 × g to remove insoluble material. The concentration of cyt bc₁ complex was estimated by measuring the sodium dithionite/ascorbate absorbance difference spectrum of the cyt b hemes at 562–578 nm using an extinction coefficient of 25.6 mm⁻¹cm⁻¹ (35).

Enzyme Assays—All assays were carried out in buffer containing 50 mM MOPS, 100 mM KCl, 20 mM NaN₃, 50 μM horse heart cyt c₃ (Fisher), 50 μM decylubiquinol at pH = 8.0. Sample temperature was set using a flowing temperature controller and water jacketed cell holder and measured in situ using a thermocouple thermometer. Rates are reported as mol of cyt c⁺₃ reduced·s⁻¹·mol⁻¹·cyt bc₁ complex. In all experiments, the kinetic traces were initiated before the addition of enzyme (0.5–20 nm cyt b) to allow for the subtraction of background cyt c reduction (or background superoxide production) by free decylubiquinol. Decylubiquinol was freshly generated as needed using a modification of the technique reported in Kramer et al. (36) by reducing decylubiquinone (Sigma) dissolved in 1:1 ethanol:ethylene glycol with ~2 mg of sodium borohydride, followed by the addition of 0.1 N HCl to quench unreacted sodium borohydride. For the superoxide production experiments, the cyt bc₁ complex was treated with a saturating (20 μM) concentration of AA (Sigma).

For measuring superoxide production in the presence of 20 μM AA, we used the Amplex Red assay (product number 12212; Molecular Probes, Eugene, OR). This assay was found to be much more sensitive than the cyt c reduction experiments.
Superoxide Production and the Q-cycle

TABLE 1

| Mutant   | $E_{mc}$ | $V_{QC}$ | $V_{SOP}$ | $K_m$ | $E_a$ |
|----------|----------|----------|-----------|-------|-------|
| Wild type| +285     | 185      | 2.7       | 4.5 ± 0.5 | 57.0 ± 3.1 |
| S183T    | +269     | 124      | 2.5       | 12.4 ± 1.1 | 60.4 ± 2.0 |
| Y185F    | +217     | 59       | 0.9       | 9.2 ± 3.6  | 62.3 ± 0.8 |
| S183A    | +165     | 23       | 0.15      | 4.3 ± 1.2  | 68.6 ± 1.3 |

* Data from Ref. 25.

A.)

\[
\ln(v/s) = \frac{3.30}{T/k} - 3.45
\]

B.)

\[
\ln(v/s) = \frac{3.30}{T/k} - 3.45
\]

FIGURE 1. Arrhenius plots for the uninhibited turnover (A) and superoxide production steady-state turnover (B). ■, wild-type; ○, S183T; ▲, Y185F; ◦, S183A.

RESULTS

General Enzymatic Data—The apparent Michaelis constant ($K_m$) for decylubiquinol at the cyt $bc_1$ complex Q$_o$ site in detergent-solubilized SMPs was 4–12 $\mu M$ for wild type and each of the mutants (see Table 1), consistent with previous determinations (19, 23, 37–41). In the presence of saturating concentrations of myxothiazol, we found a raised $K_m$ of ~40 $\mu M$ decylubiquinol, consistent with previous observations (19), implying that the measured $K_m$ values reflect saturation of the Q$_o$ site with substrate (and not other processes such as micelle breaking). We concluded that 50 $\mu M$ decylubiquinol essentially saturated the Q$_o$ site under steady-state uninhibited conditions.

Table 1 shows the uninhibited $V_{max}$ ($V_{QC}$) and turnover number for superoxide production ($V_{SOP}$) measured for wild-type and three mutant complexes with altered [2Fe2S] redox potentials. Our results on uninhibited rates were comparable with those made previously by Denke et al. (33). With saturating concentrations of AA, superoxide production was measured at about 1.4–2.7% the respective uninhibited rate of cyt $c_1$ reduction, with essentially the same dependence on 2Fe2S redox potential as reported previously (33, 42).

Activation Energy Measurements—The Arrhenius activation energy ($E_a$) for the uninhibited turnover of the wild type yeast enzyme was found to be $57.0 ± 3.0$ kJ mol$^{-1}$ at pH 8.0 (see Fig. 1), consistent with previous reports from the cyt $bc_1$ complex in Rhodobacter, Bos, and Saccharomyces (17, 25, 43, 44).

We also measured the rates of pre-steady-state reduction of cyt $b$ and cyt $c_1$ at a range of temperatures and decylubiquinol concentrations in cyt $bc_1$ complex isolated from wild-type yeast described in (18, 20). Resorufin fluorescence was measured using an in-house constructed fluorimeter. Fluorescence was excited at 525 nm using 2.3 KHz modulated green light emitting diodes and detected at 90° from the direction of excitation via a photodiode (Hamamatsu 1223) covered by a 590 nm band pass filter. Signals were amplified using a laboratory built amplifier and processed with a Thor Labs Inc. model LIA 100 lock-in amplifier. The assay was calibrated by titrating known amounts of hydrogen peroxide into the assay buffer with the full complement of enzymes and buffers. The calibration parameters were independent of temperature within the experimental range. All superoxide production rates are quoted as mol of superoxide generated s$^{-1}$ mol$^{-1}$ cyt $bc_1$ complex. This system yielded results that allowed us to detect as little as 100 pM superoxide produced s$^{-1}$ in our 500-μl volume. The wild-type SMPs were tested using the cyt $c$ reduction method (18, 20) and found to yield rates within 5% of that determined by the Ampyx Red system.

Pre-steady-state Kinetics—Pre-steady-state reduction of the cyt $bc_1$ complex was followed by stopped flow rapid scanning spectroscopy using an OLIS Rapid Scanning Monochromator. The temperature of the mixing chamber was varied from 10–35 °C with a thermostatically controlled Julabo F12 circulating water bath, and the temperature of the enzyme sample was equilibrated to that of the mixing chamber before each reaction. Reactions were started by mixing 3 $\mu M$ cyt $bc_1$ complex in assay buffer containing 50 mM potassium phosphate, pH 7.0, plus 1 mM sodium azide, 1 mM EDTA, and 0.05% Tween 20 against an equal volume of the same buffer containing decylubiquinol. A fresh solution of decylubiquinol substrate was prepared before each experiment, and the concentration was varied from 20 to 180 $\mu M$ as indicated in the legend to Fig. 2. Two equivalents of AA per cyt $bc_1$ were mixed with the enzyme prior to the reaction to block any reduction of cyt $b$ through the Q$_i$ site. A spectrum of oxidized $bc_1$ complex was obtained by mixing the oxidized $bc_1$ complex against assay buffer and averaging the data sets to a single scan. For each experiment, seven or eight data sets were averaged, and the oxidized spectrum was subtracted from each scan. From the three-dimensional data set comprised of wavelength, absorbance, and time, the time course and amplitude change for cyt $b$ reduction at 563 nm minus 578 nm or cyt $c_1$ reduction at 553 nm minus 539 nm was extracted using the OLIS software.
as shown in Fig. 2. These measurements have the advantage that they are not complicated by considerations of ionic strength or detergent concentration (which did alter the apparent $K_{\text{m}}$ for ubiquinol at the Qo site) as in the catalytic assay. As shown in Fig. 3, when these measurements were used to calculate activation energies, they gave values of 53 kJ mol$^{-1}$ for cyt $b$ reduction and 50 kJ mol$^{-1}$ for cyt $c_1$ reduction. When corrected for pH, these values are in good agreement with the activation energy for the catalytic turnover of the enzyme. The fact that cyt $b$ and cyt $c_1$ reduction have the same activation energy is noteworthy since these rates likely reflect the first and second oxidation steps of the ubiquinol at the Qo site. This has implications as regards the rate-limiting step in ubiquinol oxidation as discussed below.

The $E_a$ increased linearly with decreasing driving force for QH$_2$ oxidation, i.e. with decreasing $E_m$ (equilibrium redox potential (versus standard hydrogen electrode)) for the [2Fe2S] cluster (Fig. 4). The slope of $E_a$ versus $E_m$ was similar for the uninhibited Q-cycle and superoxide production in the presence of AA (see Fig. 4), and, importantly, the value of this slope was near one. Over the entire range of complexes, the $E_a$ measured for the AA bypass reaction appeared to be about 3.5 kJ mol$^{-1}$ higher than that for the uninhibited Q-cycle. This difference was significant to the 95% confidence limit.

When experiments were repeated in absence of added detergents, Arrhenius plots deviated from linearity at higher temperatures (data not shown), likely reflecting a shift in rate-limiting step from QH$_2$ oxidation at the Qo site to low temperature to other processes, as suggested for similar trends seen in chromatophores from Rhodobacter sphaeroides (17). However, the $E_a$ values at lower temperatures were similar to those shown in Fig. 1, suggesting QH$_2$ oxidation was not strongly modified by addition of detergent.

**DISCUSSION**

We found that the activation energies for the uninhibited Q-cycle and superoxide production in the presence of AA were nearly identical within the noise (Fig. 4). Changing the driving force for QH$_2$ oxidation by changing the redox properties of the [2Fe2S] cluster resulted in linear changes in $E_a$ for both the Q-cycle and superoxide production, with a slope near unity (Fig. 4).

The similarities in $E_a$ and its dependence on the $E_m$ of the [2Fe2S] cluster indicates that both the normal Q-cycle and superoxide production are rate-limited by a process which involves electron transfer from QH$_2$ to the [2Fe2S] cluster, most likely involving very similar chemistry (see Fig. 5). The fact that the activation energies for reduction of cyt $b$ and cyt $c_1$ under pre-steady-state conditions are identical allows us to exclude the possibility that movement of the Rieske protein prerequisite to reduction of cyt $c_1$ is the rate-limiting step at the Qo site, and it is consistent with our conclusion that electron transfer to the 2Fe2S cluster is rate-limiting. In these experiments, conducted at pH 7.0, oxidation of the first QH$_2$ at the Qo site reduces cyt $b$ and the [2Fe2S] cluster, with only partial cyt $c_1$ reduction, owing to its relatively low midpoint potential in
Superoxide Production and the Q-cycle

Taken together, these data indicate that a Qo site intermediate, likely a SQ, is the reductant for superoxide production.

Our data thus imply that production of the low potential reductant that reduces cyt b is in the Q-cycle is also involved in superoxide production and is formed via the same (or very similar) routes (i.e., these two processes probably share the same reactive intermediates, see Scheme 1, panel A). The low potential reductant may be SQ, as discussed above. An alternative suggested by Trumpower (48) is that the cyt bL reductant is a quinol-imidazolate complex formed when an electron from the quinol oxygen is delocalized into the orbitals of the imidazole ring and [2Fe2S] cluster, although the energetics for delocalization would still follow the relative redox potential of the [2Fe2S] cluster, and thus such a species would be energetically indistinguishable from a discrete SQ. In either case, we conclude that the formation of the low potential reductant occurs during both the normal Q-cycle and during superoxide production and that the reductant is formed through the same transition barrier (see Scheme 1, panel A).

This conclusion is in contradiction with Qo site models involving truly simultaneous electron transfer from QH2 to directly form Q, because they require radically different Qo site chemistries for Q-cycle superoxide production (18, 20, 24, 28). Theoretical treatments by Zusman and Beratan (49) predict distinct transition barriers and reorganization energies for sequential versus concerted (DCET) electron transfers reactions, which should be reflected in different Ea values and reorganization energies, i.e., their dependencies on driving force (compare the solid lines, representing the DCET reaction with the dashed lines representing the sequential reaction in Scheme 1, panel A). In contrast, our data show that normal Q-cycle turnover and superoxide production have very similar activation barriers and their response to the redox properties of the [2Fe2S] cluster, which clearly alters the driving force for the reactions. Overall, the simplest interpretation of our data is that DCET does not operate within the Qo site. Alternatively, one could argue that AA-inhibited enzyme acts in a completely different way, with fortuitously similar energetics, but this seems very improbable. We also cannot at present rule out another possibility that the reductant for superoxide production occurs after the Qo site-activated intermediate, e.g., a special form of cyt b with a very low redox potential able to reduce O2, thus giving similar activation behavior for both processes. In this case, though, we would have to invoke a separate mechanism for superoxide production in the presence of myxothiazol or other Qo site proximal niche inhibitors (20, 24, 28), making this option more complex.

Class 2 models invoke high stabilization of the Qo site SQ, preventing it from being an effective O2 reductant (Scheme 1, panel B). We argue that our data places certain limits on the comparison to that of the [2Fe2S] cluster. During the second QH2 oxidation, cyt c1 becomes reduced. The observed rate of cyt c1 reduction is slower than cyt b reduction due to the fast equilibration with the [2Fe2S] cluster, which favors the electron residing in the [2Fe2S] cluster. The fact that cyt b and cyt c1 reduction have the same activation energy, reflecting sequential turnovers of the Qo site, indicates that they share the same rate-limiting step, QH2 oxidation, as suggested previously by Hong et al. (17) probing flash-induced kinetics in the R. sphaeroides chromatophore system.

Besides the hypothetical Qo site SQ, the most reducing species is cyt bL with an Eo between −30 and −50 mV, probably insufficiently reducing to support rapid rates of O2 reduction (20, 21), as also suggested by slow rates of cyt b oxidation in the presence of Qo energized inhibitors (e.g., myxothiazol, methoxyacrylate-stilbene, mucidin) that completely inhibit cyt b reduction (19, 20, 46, 47). These inhibitors probably allow QH2 to bind to the “distal” niche of the Qo pocket, allowing electron transfer to the [2Fe2S] cluster, forming SQ, which in turn reduces O2 (18–20, 24).
Superoxide Production and the Q-cycle

stability of the Qo site SQ. If, for instance, the Qo site SQ is highly stabilized its formation would be energetically favored (Gibbs free energy ($\Delta G < 0$), while its oxidation would require the input of energy (26). In this case, we would expect the rate-limiting and energy-requiring step to be shifted from QH$_2$ oxidation by the [2Fe2S] cluster to SQ oxidation by cyt $b_1$ and consequently little dependence on the redox properties of the [2Fe2S] cluster. Instead, we observed linear dependence of $E_a$ on changing the $E_m$ of the [2Fe2S] cluster, consistent with previous work (33, 34, 42) indicating that the rate-limiting step for the Qo site reaction involves electron transfer to the [2Fe2S] cluster. This conclusion is consistent with work by Crofts and co-workers (17) suggesting that the fractional occupancy of the intermediate states of Qo site SQ catalyzed QH$_2$ oxidation could not exceed 0.1. We add, though, that this conclusion does not exclude stabilization of a SQ species after it is formed as long as the processes involved are significantly more rapid that the initial formation of SQ from QH$_2$.

We next consider a simple “unstable” SQ model (Scheme 1, panel A), where QH$_2$ bound at the Qo site comes into equilibrium with a Q$_0$ site SQ. If the SQ is highly unstable, its formation at the Qo site would be energetically unfavorable ($\Delta G > 0$), and its concentration should be roughly proportional to the logarithm of driving force for the one-electron transfer to the 2Fe2S cluster, as indeed we observed (Fig. 4). Furthermore, if superoxide production at the Qo site is pseudo-first order (with respect to [SQ]), then the rate of superoxide production also should depend linearly on the logarithm of changes in driving force for SQ formation. Again, as shown in Fig. 5, this is precisely what we have observed, leading us to suggest that an unstable Qo site SQ comes into equilibrium with the ground state of the enzyme and that this SQ can be oxidized either by cyt $b_1$, resulting in normal Q-cycle activity, or by other oxidants including Q$_O$ resulting in superoxide production or other bypass reactions. In principle, the SQ could appear either near the transition state for QH$_2$ oxidation, as suggested by Hong and co-workers (17), or as a species that has a concentration that is tightly coupled to the concentration of the transition state.

Because the $E_a$ values for superoxide production and Q-cycle turnover are quite similar, the reactive intermediate of the Q-cycle could reduce either cyt $b_1$ or Q$_O$ without a large difference in enthalpic terms. This likely places the midpoint potential of the $O_2$/cyt $b_1$ reductant similar to or more negative than that of the $O_2$/Q$_O$ couple. If the solution potential for this couple operates in our system, this would imply a potential of around −150 mV or lower for the cyt $b_1$ (or O$_2$) reductant, again suggesting a very unstable intermediate.

Although this simple model accounts for the data in this paper, an important caveat needs to be considered. It has been pointed out by several authors (20, 24, 28) that superoxide production by the cyt $b_1$ complex occurs at measurable rates only when the complex is partially inhibited, e.g. by AA or high proton motive force. (It should be noted, however, there are reports of significant superoxide production by uninhibited cyt $b_1$ complexes (22, 51)). Our own experiments on the Saccharomyces cyt $b_1$ complex did not show rates of superoxide production above the background (20), and there appears to be general consensus that superoxide production is slow in the uninhibited complex. In conflict with these observations, the simple, unstable SQ model described above predicts similar transition states under conditions favoring uninhibited Q-cycle and SO production.

In one possible model to reconcile these observations, the Qo site could act primarily as a sealed “reaction chamber” (24). Under uninhibited conditions, where the lifetime of the SQ intermediate is short, the chamber can effectively shield the SQ from oxygen. However, when the Q-cycle is blocked, SQ can escape from the reaction chamber or oxygen can diffuse into the chamber, perhaps following slow (on the time scale of the overall reaction), large scale conformational fluctuations of the complex.

Several groups have suggested reasonable models that entail conformational or electrostatic gating of the Q$_o$ site reactions (52–57). In these models, the activity of the Q$_o$ site is gated by the properties of other components of the complex. For example, the Q$_o$ site might not be able to bind substrate or becomes redox-inactive when cyt $b_1$ is reduced (28), the Q$_o$ site is altered (54), substrate occupancy in one-half of the operational dimer (50) may inhibit activity in the other half of the dimer (53). Under normal conditions, such gating could effectively prevent side reactions but might be susceptible to slippage over large time scales, leading to the observed differences in SO production rates in the uninhibited and inhibited cases.

In both the “leaky reaction chamber” and the “leaky gating” models, superoxide production is at least partly controlled by conformational changes.

Acknowledgments—We thank Drs. Jonathan Cape, James Hurst, Mike Kahn, and Atsuko Kanazawa for stimulating discussion.

REFERENCES

1. Crofts, A. R. (2004) Annu. Rev. Physiol. 66, 689–733
2. Lange, C. H. C. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 2800–2805
3. Zhang, Z., Berry, E. A., Huang, L. S., and Kim, S. H. (2000) Subcell. Biochem. 35, 541–580
4. Darrouzet, E., Cooley, J. W., and Daldal, F. (2004) Photosynth. Res. 79, 25–44
5. Berry, E. A., Guergova-Kuras, M., Huang, L. S., and Crofts, A. R. (2000) Annu. Rev. Biochem. 69, 1005–1075
6. Schütz, M., Brugna, M., Lebrun, E., Baymann, F., Huber, R., Stetter, K., Hauska, G., Toci, R., Lemesle-Meunier, D., Tron, P., Schmidt, C., and Nitschke, W. (2000) J. Mol. Biol. 300, 663–675
7. Crofts, A. R., and Berry, E. A. (1998) Curr. Opin. Struct. Biol. 8, 501–509
8. Iwata, S., Lee, J. W., Okada, K., Lee, J. K., Iwata, M., Rasmussen, S., and Link, T. A., Ramaswamy, S., and Jap, B. K. (1998) Science 281, 64–71
9. Zhang, H., and Cranmer, W. A. (2004) Methods Mol. Biol. 274, 67–78
10. Berry, E. A., Huang, L. S., Zhang, Z., and Kim, S. H. (1999) J. Bioenerg. Biomembr. 31, 177–190
11. Berry, E. A., Huang, L.-S., Saechao, L. K., Pon, N. G., Valkova-Valchanova, M., and Daldal, F. (2004) Photosynth. Res. 81, 251–275
12. Mitchell, P. (1975) FEBS Lett. 56, 1–6
13. Trumpower, B. L. (1990) J. Biol. Chem. 265, 11409–11412
14. Mitchell, P. (1976) J. Theor. Biol. 62, 327–367
15. Rich, P. R. (1984) Biochim. Biophys. Acta 768, 53–79
16. Crofts, A. R., Barquera, B., Gennis, R. B., Kuras, R., Guergova-Kuras, M., and Berry, E. A. (1999) Biochemistry 38, 15807–15826
17. Hong, S., Ugulava, N., Guergova-Kuras, M., and Crofts, A. R. (1999) J. Biol. Chem. 274, 33931–33944
18. Kramer, D. M., Roberts, A. G., Muller, F., Cape, J., and Bowman, M. K. (2004) *Methods Enzymol.* **382**, 21–45

19. Muller, F., Roberts, A. G., Bowman, M. K., and Kramer, D. M. (2003) *Biochemistry* **41**, 7866–7874

20. Turrens, J. F., Alexandre, A., and Lehninger, A. L. (1985) *Arch. Biochem. Biophys.* **237**, 408–414

21. Sun, J., and Trumpower, B. L. (2003) *Arch Biochem. Biophys.* **419**, 198–206

22. Berry, E. A., and Huang, L. S. (2003) *FEBS Lett.* **555**, 13–20

23. Link, T. A. (1997) *FEBS Lett.* **412**, 257–264

24. Zhang, L., Li, Z., Quinn, B., Yu, L., and Yu, C. A. (2002) *Biochim. Biophys. Acta* **1556**, 226–232

25. Cooley, J. W., Ohnishi, T., and Daldal, F. (2005) *Biochemistry* **44**, 10520–10532

26. Cooley, J. W., Roberts, A. G., Bowman, M. K., Kramer, D. M., and Daldal, F. (2004) *Biochemistry* **43**, 2217–2227

27. Zhang, L., Li, Z., Quinn, B., Yu, L., and Yu, C. A. (2002) *Biochim. Biophys. Acta* **1556**, 226–232

28. Cooley, J. W., Ohnishi, T., and Daldal, F. (2005) *Biochemistry* **44**, 10520–10532

29. Cooley, J. W., Roberts, A. G., Bowman, M. K., Kramer, D. M., and Daldal, F. (2004) *Biochemistry* **43**, 2217–2227

30. Zhang, L., Li, Z., Quinn, B., Yu, L., and Yu, C. A. (2002) *Biochim. Biophys. Acta* **1556**, 226–232

31. Crofts, A. R. (2004) *Biochim. Biophys. Acta* **1655**, 77–92

32. Berry, E. A., and Huang, L. S. (2003) *FEBS Lett.* **555**, 13–20

33. Link, T. A. (1997) *FEBS Lett.* **412**, 257–264

34. Zhang, L., Li, Z., Quinn, B., Yu, L., and Yu, C. A. (2002) *Biochim. Biophys. Acta* **1556**, 226–232

35. Cooley, J. W., Ohnishi, T., and Daldal, F. (2005) *Biochemistry* **44**, 10520–10532

36. Cooley, J. W., Roberts, A. G., Bowman, M. K., Kramer, D. M., and Daldal, F. (2004) *Biochemistry* **43**, 2217–2227

37. Zhang, L., Li, Z., Quinn, B., Yu, L., and Yu, C. A. (2002) *Biochim. Biophys. Acta* **1556**, 226–232

38. Crofts, A. R., Lhee, S., Crofts, S. B., Cheng, J., and Rose, S. (2006) *Biochim. Biophys. Acta* **1757**, 1019–1034