Volume 313, number 3, 251–254 FEBS 11798 November 1992

Deletion of subunit 9 of the *Saccharomyces cerevisiae* cytochrome bc_1 complex specifically impairs electron transfer at the ubiquinol oxidase site (center P) in the bc_1 complex

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Received 4 August 1992; revised version received 5 October 1992

Deletion of *QCR9*, the nuclear gene encoding subunit 9 of the mitochondrial cytochrome bc_1 complex in *Saccharomyces cerevisiae*, results in inactivation of the bc_1 complex and inability of the yeast to grow on non-fermentable carbon sources. The loss of bc_1 complex activity is due to loss of electron transfer activity at the ubiquinol oxidase site (center P) in the complex. Electron transfer at the ubiquinone reductase site (center N), is unaffected by the loss of subunit 9, but the extent of cytochrome *b* reduction is diminished. This is the first instance in which a supernumerary polypeptide, lacking a redox prosthetic group, has been shown to be required for an electron transfer reaction within the cytochrome bc_1 complex.

bc_1 complex; Subunit 9; QCCR9; Ubiquinol oxidase; Protonmotive Q cycle

1. INTRODUCTION

The cytochrome bc_1 complexes of mitochondria contain 9–11 polypeptides [1], while the counterpart complexes which have been purified from prokaryotes typically contain many fewer subunits [2]. For example, the complexes from *Paracoccus denitrificans* [3] and *Rhodospirillum rubrum* [4,5] contain only three electron transfer proteins, cytochrome *b*, cytochrome c_1 and Rieske iron–sulfur protein. The additional subunits found in the mitochondrial bc_1 complexes are not known to contain any redox prosthetic groups, and their functions in assembly and/or catalytic activity of the bc_1 complex are not known. Because these polypeptides are numerically in excess of the number known to be essential for electron transfer and energy transduction in the bacterial bc_1 complexes, we have referred to these proteins as 'supernumerary' subunits [6].

We recently cloned and sequenced *QCR9*, the nuclear gene encoding the 7.2 kDa subunit 9 of the yeast cytochrome bc_1 complex [7]. Yeast strains in which the chromosomal copy of *QCR9* is deleted are respiratory deficient (*petite*), due to an almost complete loss of ubiquinol–cytochrome *c* oxidoreductase activity. The *petite* phenotype resulting from deletion of subunit 9 differs from that which results from deletion of other nuclear-encoded subunits, in that the subunit 9 deletion strain assembles an optically detectable cytochrome bc_1 complex. In contrast, the loss of bc_1 complex activity which results from deletion of core protein 1 [8], subunit 7 [9], or subunit 8 [10], can be attributed to lack of cytochrome *b* and/or incomplete assembly of the remaining subunits of the complex.

The mechanism of electron transfer in the cytochrome bc_1 complex is known [11]. To better understand the molecular basis for the lack of ubiquinol–cytochrome *c* oxidoreductase activity in the bc_1 complex lacking subunit 9, we have examined the presteady-state reduction of cytochromes *b* and c_1 in mitochondrial membranes lacking this subunit, and used inhibitors which block electron transfer at unique sites [12] to examine discrete electron transfer steps within this electron transfer complex. Here we report that subunit 9 is required for electron transfer from ubiquinol to cytochrome c_1 and cytochrome *b* through the ubiquinol oxidase site, center P, but is not required for electron transfer to cytochrome *b* through the ubiquinone reductase site, center N.

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2. MATERIALS AND METHODS

The parental yeast strain, W303-1A, the subunit 9 deletion strain, JDP1, and methods for growing the yeast were described elsewhere [7]. Mitochondrial membranes were isolated with glass beads [13,14]. Prior to breaking the cells, 0.5 mM diisopropylfluorophosphate was added to the buffer to prevent proteolysis of the iron–sulfur protein, as evaluated by Western blots of cytochrome bc_1 complex subunits after SDS-PAGE of the mitochondrial membranes.

To eliminate uncontrolled reduction of the cytochromes by endogenous substrate, mitochondrial membranes were suspended in 5 μM phenazine methosulfate, 500 μM potassium ferricyanide, and washed two times with 150 mM KCl. Residual phenazine methosulfate in the membranes caused an ascending baseline in the optical spectra, but did not mediate a bypass of electrons within the bc_1

complex, as evidenced by the effective blockage of cytochrome *b* reduction by inhibitors in the membranes from the subunit 9 deletion strain.

Reduction of the cytochromes was monitored and optical spectra recorded on an Aminco DW-2a spectrophotometer equipped with a Nicolet digital oscilloscope [3]. Mitochondrial membranes were suspended at approximately 0.25 μM cytochrome c_1. Spectra were recorded before and after each reaction to confirm the oxidation–reduction status of the cytochromes. An analogue of ubiquinol containing a 9-carbon sidechain was used as substrate for reduction of cytochrome c_1, which was monitored at 553 vs. 539 nm. 2-Methyl-3-undecyl-1,4-naphthoquinol was used as substrate for reduction of cytochrome *b*, which was monitored at 564 vs. 575 nm. Antimycin (12 μM) or methoxyacrylate (20 μM) were added where indicated.

3. RESULTS AND DISCUSSION

Under presteady-state conditions there are two pathways for reduction of cytochrome *b* [11]. When ubiquinol is added to fully oxidized bc_1 complex, it is preferentially oxidized by the Rieske iron–sulfur protein, which transfers one electron to cytochrome c_1 and generates a strongly reducing ubisemiquinone anion, which in turn reduces the low potential heme of cytochrome b_{566}. The iron–sulfur protein/cytochrome *b* interfase which catalyzes this oxidant-induced reduction of cytochrome *b* is referred to as center P.

If reduction of cytochrome *b* through center P is blocked, cytochrome *b* can be alternatively reduced in an iron–sulfur protein-independent reaction in which electrons are transferred to the high potential heme of cytochrome b_{562} at center N. Since b_{566} and b_{562} are electronically connected, both can be reduced either through center P or center N, provided that the quinol substrate is of sufficiently low potential.

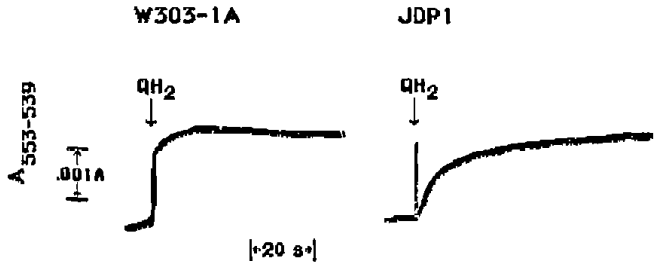

Fig. 1. Presteady-state reduction of cytochrome c_1 by ubiquinol in mitochondrial membranes from yeast in which subunit 9 of the cytochrome bc_1 complex is present or absent. The tracing on the left shows reduction of cytochrome c_1 in membranes from the wild-type parent, and the tracing on the right shows reduction of cytochrome c_1 in membranes from JDP1, the yeast strain in which the nuclear gene (*QCR9*) encoding subunit 9 of the cytochrome bc_1 complex, is deleted.

Presteady-state reduction of cytochrome c_1 and oxidant-induced reduction of cytochrome *b* via center P are blocked by methoxyacrylates, which, however, allow *b* reduction via center N [15]. Reduction of cytochrome *b* via center N is blocked by antimycin, which permits cytochrome c_1 reduction and oxidant-induced reduction of cytochrome *b* via center P [16].

The tracings in Fig. 1 show cytochrome c_1 reduction by ubiquinol through center P, in mitochondrial membranes from the parental (W303-1A) and subunit 9 deletion strains (JDP1). In the parental strain, c_1 reduction is so rapid that the rate ($t_{1/2}$ < 100 ms) is masked by the

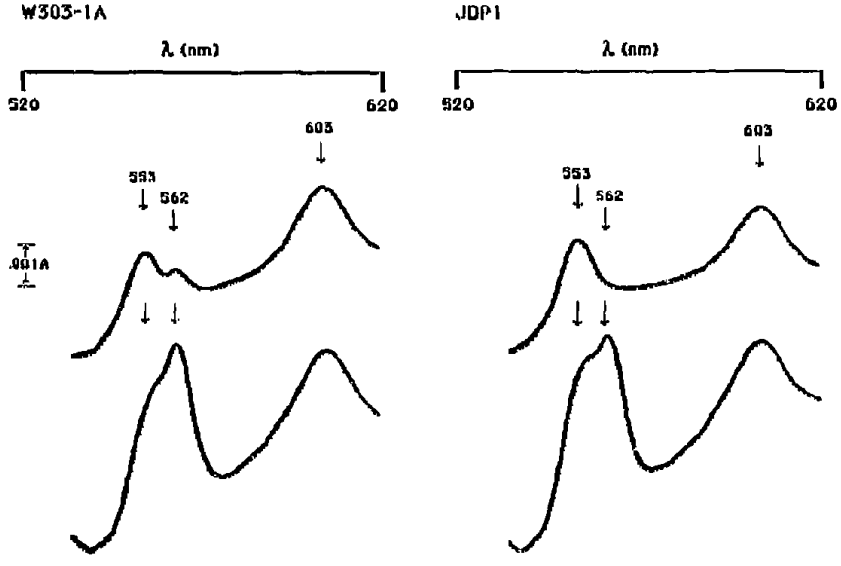

Fig. 2. Optical spectra of mitochondrial membranes from yeast in which subunit 9 of the cytochrome bc_1 complex is present or absent, after reduction of the cytochromes with ubiquinol or dithionite. The tracing on the top left shows the optical spectrum of mitochondrial membranes from the wild-type parental strain (W303-1A), and the tracing on the top right shows the analagous spectrum of mitochondrial membranes from the subunit 9 deletion strain (JDP1), after reduction with ubiquinol. The bottom tracings show spectra of the same membranes as above, after reduction with dithionite.

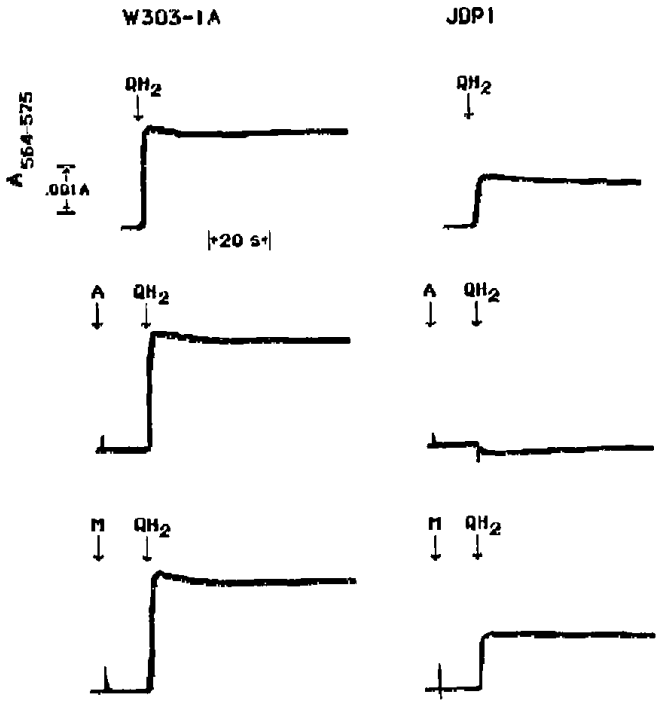

Fig. 3. Presteady-state reduction of cytochrome *b* by menaquinol in mitochondrial membranes from yeast in which subunit 9 of the cytochrome bc_1 complex is present or absent, showing the effects of bc_1 complex inhibitors on reduction of cytochrome *b*. The tracings on the left are of cytochrome *b* reduction in the wild-type parental strain (W303-1A), and those on the right are of *b* reduction in the subunit 9 deletion strain (JDP1). The arrows show additions of menaquinol (QH_2), antimycin (A), or methoxyacrylate (M).

mixing time (1 s) of the spectrophotometer. In the subunit 9 deletion strain the rate of c_1 reduction is markedly slowed, having a half-time in excess of 10 s. This result suggests that electron transfer through center P is impaired in JDP1.

Optical spectra taken after reduction of the bc_1 complex by ubiquinol (Fig. 2) show reduction of cytochrome c_1, evidenced as an absorption peak at 553 nm, in both parental and subunit 9 deletion strains. Ubiquinol also elicits oxidant-induced cytochrome *b* reduction in the parental strain, resulting in an absorption peak at 562 nm. This oxidant-induced *b* reduction is not observed after ubiquinol reduction of the bc_1 complex in the subunit 9 deletion strain (Fig. 2), confirming that electron transfer through center P is impaired in the absence of subunit 9. Under the conditions of the experiments in Figs. 1 and 2 there is virtually no reduction of cytochrome *b* via center N, owing to the relatively high potential of the ubiquinol substrate, which is further increased by oxidation of ubiquinol by cytochrome c_1.

The traces in Fig. 3 show reduction of cytochrome *b* by menaquinol in mitochondrial membranes from the parental and subunit 9 deletion strains. Menaquinol reduces a greater extent of the cytochrome *b* than does ubiquinol, owing to its more negative oxidation–reduction potential, and can reduce the cytochrome through either center N or center P. Thus, in the absence of any inhibitors, menaquinol reduces cytochrome *b* in both the wild-type and the subunit 9 deletion strains, as shown by the top two tracings in Fig. 3.

The two middle traces in Fig. 3 show reduction of cytochrome *b* when electron transfer through center N is blocked by antimycin. Under these conditions cytochrome *b* reduction proceeds normally through center P in the wild-type bc_1 complex. However, in the presence of antimycin there is no reduction of cytochrome *b* through center P in the bc_1 complex lacking subunit 9. This result confirms the absence of oxidant-induced reduction of cytochrome *b* inferred from the optical spectrum in Fig. 2.

If electron transfer through center P is blocked by methoxyacrylate, cytochrome *b* is reduced through center N. This pathway of *b* reduction remains operative when subunit 9 is deleted, as can be seen by comparing the bottom right and top right tracings in Fig. 3. The amount of cytochrome *b* reduced by menaquinol in the subunit 9 deletion strain, either in the absence of inhibitors or in the presence of methoxyacrylate, is about 60% of that in the wild-type cytochrome bc_1 complex. This decrease in *b* reduction is partly due to re-oxidation of the menaquinol reduced *b* which occurs in the absence of subunit 9, which was confirmed by optical spectra taken at intervals after reduction of the *b* by menaquinol (results not shown). In addition, it is possible that the lack of subunit 9 either specifically damages the heme of b_{566} or breaks electronic communication between the two hemes, such that in the absence of electron transfer through center P there is no route for reduction of the low potential b_{566}.

Acknowledgements: This research was supported by NIH Research Grant GM-20379 and a National Research Service Award GM 12775 (to L.A.G.).

REFERENCES

[1] Schägger, H., Borchart, U., Machleidt, W., Link, T.A. and Von Jagow, J. (1987) FEBS Lett. 219, 161–168.
[2] Hauska, G., Hurt, E., Gabellini, N. and Lockau, W. (1983) Biochim. Biophys. Acta 726, 97–133.
[3] Yang, X. and Trumpower, B.L. (1986) J. Biol. Chem. 261, 12282–12289.
[4] Kriauciunas, A., Yu, L., Yu, C.A., Wynn, R.M. and Knaff, D.B. (1989) Biochim. Biophys. Acta 976, 70–76.
[5] Majewski, C. and Trebst, A. (1990) Mol. Gen. Genet. 224, 373–382.
[6] Trumpower, B.L. (1990) Microbiol. Rev. 54, 101–129.
[7] Phillips, J.D., Schmitt, M.E., Brown, T.A., Beckmann, J.D. and Trumpower, B.L. (1990) J. Biol. Chem. 265, 20813–20821.
[8] Tzagoloff, A., Wu, M. and Crivellone, M. (1986) J. Biol. Chem. 261, 17163–17169.
[9] Schoppink, P.J., Berden, J.A. and Grivell, L.A. (1989) Eur. J. Biochem. 181, 475–483.

[10] Maarse, A.C., de Haan, M., Schoppink, P.J., Berden, J.A. and Grivell, L.A. (1988) Eur. J. Biochem. 172, 179–184.
[11] Trumpower, B.L. (1990) J. Biol. Chem. 265, 11409–11412.
[12] Von Jagow, G. and Link, T.A. (1986) Methods Enzym. 126, 253–271.
[13] Needleman, R.B. and Tzagoloff, A. (1975) Anal. Biochem. 64, 545–549.
[14] Graham, L.A. and Trumpower, B.L. (1991) J. Biol. Chem. 266, 22485–22492.
[15] Brandt, U., Schägger, H. and Von Jagow, G. (1988) Eur. J. Biochem. 173, 499–506.
[16] Bowyer, J.R. and Trumpower, B.L. (1980) FEBS Lett. 126, 93–97.