Both Terminal Oxidases Contribute to Fitness and Virulence during Organ-Specific *Staphylococcus aureus* Colonization

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ABSTRACT In their recent article, Hammer et al. (N. D. Hammer, M. L. Reniere, J. E. Cassat, Y. Zhang, A. O. Hirsch, M. Indriati Hood, and E. P. Skaar, mBio 4:e00241-13, 2013) described the dual functions of the two terminal oxidases encoded by *cydBA* and *qoxABCD* in *Staphylococcus aureus*. The aerobic growth of *cydB* or *qoxB* single mutant bacteria was barely affected. However, a *cydB qoxB* double mutant was completely unable to respire and exhibited the small-colony variant phenotype that is typical of menaquinone and heme biosynthesis mutants. The authors found that the two terminal oxidases play a role in pathogenesis. In a systemic mouse infection model, it turned out that in the *cydB* mutant the bacterial burden was significantly decreased in the heart, kidneys, and liver, while in the *qoxB* mutant it was decreased only in the liver. These results illustrate that both terminal oxidases contribute to fitness and virulence, representing promising candidates for the development of antimicrobials.

Unlike the respiratory system of *Escherichia coli* or *Bacillus subtilis*, that of staphylococci is not very well characterized but some details have come to light. Staphylococci possess menaquinones (MK, vitamin K_2) as their sole isoprenoid quinones, and there is a species-specific variation in the length of their isoprenoid side chains. The two principal menaquinones have seven or eight isoprene units and play important roles in electron transport and oxidative phosphorylation (1). Electrons funneled through the menaquinone cycle are transferred to the terminal oxidases with the concomitant reduction of O_2 to H_2O (Fig. 1). Studies of the staphylococcal cytochromes suggest that these bacteria possess a branched respiratory system consisting of two or three alternative and menaquinol-dependent terminal oxidases: a cytochrome *bo*, a cytochrome aa_3, and a cytochrome *bd* oxidase (2). In earlier studies, *a*- and *b*-type cytochromes were found in staphylococci; however, controversial absorption maxima were postulated for the *a*-type cytochromes: 605 nm (3, 4), 602 nm (5, 6), and 608 nm (7). On the basis of the absorption maxima, there were also several *b*-type cytochromes described in staphylococci: cytochrome *b*-552, which is present in all staphylococcal species (3), and cytochromes *b*-555 (3, 6), *b*-556 (5), *b*-557 (4, 6), *b*-559 (3), and *b*-560 (3, 4, 7), which most likely corresponds to *b*-561 (5). Cytochrome *b*-552 is present in all of the staphylococcal species tested (3), and cytochromes *b*-557 and *b*-559 are widely distributed among staphylococci. It has been assumed that electrons first pass the intermediate electron carrier cytochrome *b*-557 and finally cytochrome *b*-555, the proposed major terminal oxidase (6).

Therefore, cytochrome *b*-555 has been referred to as cytochrome *o* or cytochrome *bo* oxidase. Indeed, cytochromes *b*-557 and *b*-555 have different reactivities with regard to sensitivity to carbon monoxide and 2-heptyl-4-hydroxyquinoline-*N*-oxide (3, 8–10). Staphylococci do not possess *c*-type cytochromes such as *c*-549 and *c*-554 (3, 9).

Whether *Staphylococcus aureus* has two or three respiratory branches is not quite clear. Clements et al. postulated the presence of three branches in *S. aureus* (7): the major terminal oxidase cytochrome aa_3, cytochrome *o*, and cytochrome *bd* oxidase. Cytochrome aa_3 oxidases are usually proton pumping, as reviewed by Thöny-Meyer (11); however, it has been proposed that in *S. aureus* only the cytochrome *bo* oxidase, and not the cytochrome aa_3 (ba_3) oxidase, is proton pumping (12).

Annotation of the *S. aureus* genome revealed two gene clusters that encode terminal menaquinol oxidases. The *qoxABCD* operon encodes four quinol oxidase-like subunits, which are structurally related to the large family of mitochondrial-type aa_3 terminal oxidases, to the *E. coli bo* quinol oxidase, and to *B. subtilis* caa_3-605 cytochrome *c* oxidase. In *B. subtilis*, Qox oxidase (aa_3-600) is predominant during vegetative growth and its deletion caused a severe reduction of tricarboxylic acid cycle fluxes and increased overflow metabolism (13). The other terminal oxidase consists of two putative cytochrome *d* menaquinol oxidases: a subunit I homolog (*cydA*) and a subunit II homolog (*cydB*). The CydAB complex represents the cytochrome *bd* quinol oxidase (14). Recently, it has been shown that the nonpathogenic staphylococcal species encode a pyocyanin- and cyanide-insensitive cytochrome *bd* quinol oxidase, while the pathogenic species, such as *S. aureus*, encode a sensitive variant; cyanide resistance is determined by the CydB subunit (15). In *E. coli*, cytochrome *bd* oxidase (CydAB) is expressed under microaerobic conditions via the regulators Fnr and ArcA (14). As no gene cluster encoding the proposed cytochrome *bo* oxidase has been identified so far, the existence of one is questionable.

The recent study by Hammer et al. (16) has made it obvious that under aerobic growth conditions there are probably only two terminal oxidases present in *S. aureus*, and they can complement each other. A *cydB qoxB* double mutant exhibited a severe small-colony variant (SCV) phenotype similar to that seen in *menB* and *hemB* mutants, in which the menaquinone or heme biosynthesis pathway is blocked, causing a complete inhibition of aerobic respiration. Mutation of all terminal oxidases should produce a similar effect. Furthermore, similar to the *hem* mutants, the mem-

Published 3 December 2013

Citation Götz F, Mayer S. 2013. Both terminal oxidases contribute to fitness and virulence during organ-specific *Staphylococcus aureus* colonization. mBio 4(6):e00976-13. doi:10.1128/mBio.00976-13.

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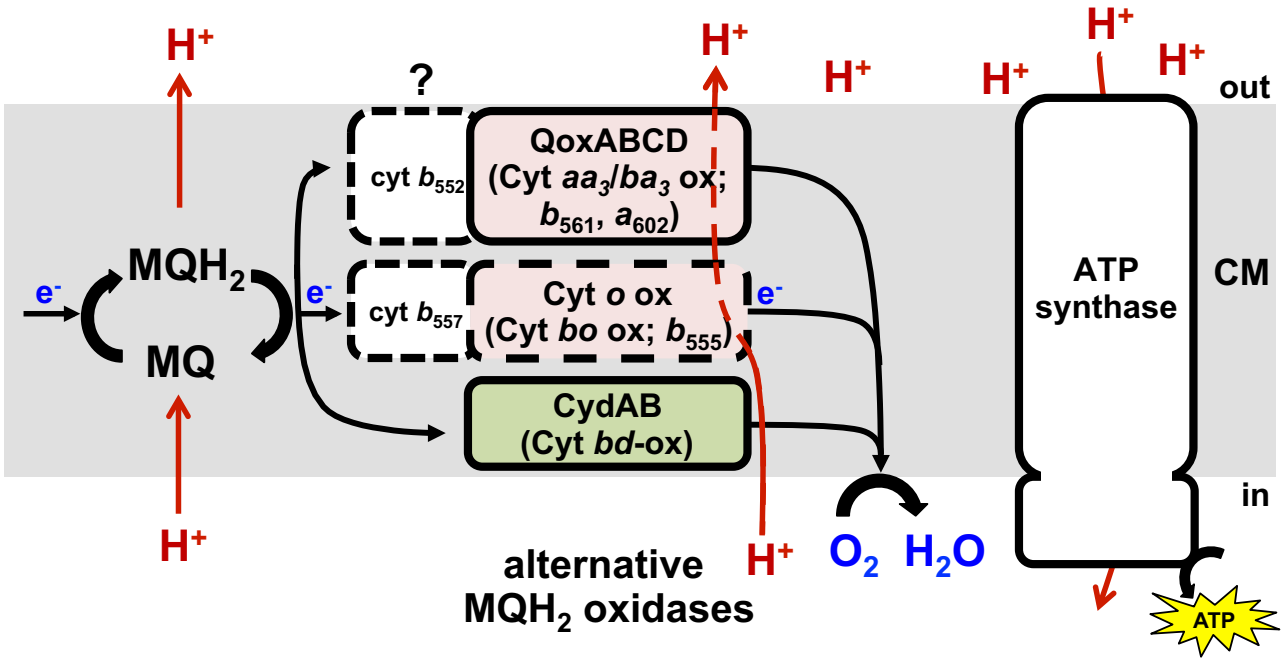

FIG 1 Proposed terminal oxidases of the branched respiratory chain in *S. aureus*. Menaquinone (MQ) is reduced to menaquinol (MQH_2), which then transfers its electrons (e^-) to various terminal oxidases, finally reducing oxygen to H_2O. A cytochrome aa_3 oxidase (QoxABCD), which is composed of cytochromes *a*-602 and *b*-561, very likely represents the main terminal oxidase. Cytochrome *b*-552 is also involved in this respiratory branch. A second terminal oxidase, cytochrome *bd* oxidase (CydAB), is proposed to work under microaerobic conditions and is not proton pumping (shaded green). A third terminal oxidase, cytochrome bo oxidase, consisting of cytochrome *b*-555 and a preconnected cytochrome *b*-557, has been proposed. The cytochrome aa_3 oxidase and the cytochrome *bo* oxidase are thought to be proton pumping and work under aerophilic conditions (shaded pink). It is still unclear if the identified *b*-type cytochromes form the cytochrome *bo* oxidase or whether they are part of Qox and/or the cytochrome *bd* oxidase (CydAB). The precise cytochrome composition of the terminal oxidases has not been identified. The electrochemical gradient is used by the ATP synthase for ATP production. CM, cytoplasmic membrane.

brane potential was severely decreased in the *cydB qoxB* double mutant. These results support the conclusion that cytochromes encoded by the *qox* and *cyd* operons are the sole terminal oxidases used by *S. aureus* to generate a membrane potential and facilitate aerobic respiration.

Once it was confirmed that the terminal oxidases could complement each other, the next question was whether they have different roles in infection. The authors investigated the ability of the *cydB* and *qoxB* mutants to colonize the heart, kidneys, and liver by using a systemic mouse infection model. Challenging the mice with the *cydB* mutant resulted in decreased colonization of the heart, and challenging the mice with the *qoxB* mutant resulted in decreased colonization of the liver (which has already been observed earlier [17]). These data support the idea that the cytochromes have distinct roles during pathogenesis and are not functionally redundant. Challenging the mice with the *hemA* mutant resulted in decreased colonization of both the heart and the liver, which is understandable, as respiration is blocked in this mutant, and the phenotypic outcome should be similar to that obtained with the *cydB qoxB* double mutant.

The authors also verified their results by testing noniron metalloporphyrins that inhibit the growth of many species of bacteria under iron-limited conditions (18). These compounds are taken up by the iron-regulated heme transport system of *S. aureus* (Isd) and are incorporated into the cytochromes, eliminating their electron transfer activity (19, 20). Treatment of *S. aureus* with gallium protoporphyrin or zinc protoporphyrin significantly reduced growth, induced the SCV phenotype, increased fermentation products, and decreased the membrane potential. The similarities of this phenotype to that of the *hem* mutant or the *cydB qoxB* double mutant suggest that the noniron metalloporphyrins inhibit aerobic respiration. Hence, the terminal oxidases present interesting potential targets for an antimicrobial strategy, with the caveat that inhibition of *S. aureus* respiration may favor the selection of persister cells, leading to chronic infection.

In summary, the studies by Hammer et al. provide a molecular understanding of the role of the two-branched terminal oxidases involved in systemic infection and the colonization of various organs. It would be most interesting to know why the colonization of the *cydB* mutant was decreased more in the heart and that of the *qoxB* mutant was decreased more in the liver. To answer this question, a thorough biochemical analysis of these terminal oxidases is necessary. It will be particularly interesting to learn about their affinity for oxygen and their regulation patterns under various environmental conditions.

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