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THE MOLECULAR GENETICS OF SUPEROXIDE DISMUTASE IN *E. COLI*

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The biological role and the regulation of superoxide dismutase (SOD) in *E. coli* have been investigated using genetics. Cloning of both *E. coli* SOD genes permitted construction of mutants completely lacking SOD. The conditional oxygen sensitivity of those mutants, together with their increased mutation rate, demonstrated the essential biological role of SOD. SOD-deficient mutants constitute a powerful tool to assess a possible role of O^{-2} or SOD in biological processes. Complementation of their deficiencies by the expression of SOD originating from a different organism is used for screening libraries for SOD genes of other species. Regulation of MnSOD has been studied using protein and operon fusions with the lactose operon, and isolating regulation mutants. These studies reveal multiregulation of MnSOD including response to the superoxide mediated oxidative stress and response to variations of the intracellular redox state induced by metabolic changes.

KEY WORDS: Oxidative stress, superoxide dismutase, *E. coli*, mutants, regulation, mutagenesis.

INTRODUCTION

Cells subjected to oxidative stress protect themselves from the potential damages by several means: – oxidative agents can be eliminated by enzymatic or nonenzymatic processes, – oxidative lesions can be eliminated or repaired, unfaithfully repaired DNA lesions leading to mutagenesis, – alternative pathways to bypass the damaged functions can also be developed.

In *E. coli*, in normal aerobic growth conditions, superoxide radicals (O_2^-) produced by cellular metabolism are rapidly eliminated by the dismutation reaction catalysed by superoxide dismutase (SOD), giving rise to hydrogen peroxide (H_2O_2) which is eliminated in turn by catalases and peroxidases. Since elimination of O_2^- produces another toxic oxygen species, H_2O_2, and since an excess of either O_2^- or H_2O_2 will favor formation of hydroxyl radicals ($OH^{\cdot}$), a subtle equilibrium, implying rigourous controls, should be maintained to allow the cells to survive.

Indeed when intracellular flux of O_2^- increases, MnSOD is induced,[1] resulting in an overproduction of H_2O_2. The increase of H_2O_2 concentration induces in turn catalases and peroxidases.[2] Together with the SOD or catalase induction, each one being triggered by the oxygen species it eliminates, numerous other functions are induced in response to the stress provoked by an excess of O_2^- or H_2O_2. Up to 30 proteins have been shown to be overproduced after H_2O_2 stress.[2] Several of them are controlled by a same transcriptional activator, the *oxyR* gene product.[2] Few of them are identified (catalase hydroperoxidase I, alkyl hydroperoxide reductase). Similarly, it has been shown that several proteins are overexpressed in response to O_2^- stress.[3,4] Moreover, several of these O_2^- induced proteins – including MnSOD, endonuclease IV repair

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enzyme,[5] glucose-6-phosphate deshydrogenase[4] – are under the control of a same regulatory protein (B. Demple, this issue). Other functions as the catalase hydroperoxide II and the repair enzyme exonuclease III, both positively regulated by the *katF* gene product, are involved in the response to oxidative stress;[6] it is not known which signal triggers it on.

Beside of the direct response to the oxidative stress created by an increase of O_2^- intracellular flux, it seems to be a response to the redox state of the cells, mediated by iron, as suggested by the anaerobic inductions of MnSOD by iron chelator[7,8] or by growth conditions positively changing the redox potential.[9-11] The above incomplete scheme outlines the need, studying or acting on a particular function to not forget all these interactions. The balance has to be maintained for cells to survive.

RESULTS AND DISCUSSION

SOD in E. coli: Biological Role

We have used genetics as an approach to understand the biological role and mechanisms which control SOD expression in *E. coli*.[12]

The two *E. coli* SOD genes have been cloned, *sodA* gene for manganese SOD[13] and *sodB* for iron SOD,[14] and they have been further sequenced.[15,16] The cloning permitted construction of mutants devoid of one or both SOD.[17] They were obtained by insertional mutagenesis in the cloned gene, followed by an allele exchange with the corresponding wild-type chromosomal allele. The use of transposons conferring different antibiotic resistances for the insertional mutagenesis, allowed each of the mutation to be readily transferred to a wide variety of genetic backgrounds. This also permit to construct a double mutant completely lacking SOD.

SOD lacking mutant survive aerobically, but shows a conditional sensitivity to oxygen. (i) poor growth in rich medium, (ii) hypersensitivity to oxygen and redox cycling compounds, (iii) no growth on minimal medium unless the 20 amino-acids (aa) are provided. Although all aa are necessary to restore complete growth, some are more effective than others suggesting several targets in the chains of aa biosynthesis with differential sensitivity to superoxide, (iv) spontaneous mutagenesis is increased in strains lacking SOD. It is oxygen dependent, RecA independent, and largely depends on an active exonuclease III, a repair enzyme which recognizes oxidative lesions on DNA.[18] In anaerobiosis, the *E. coli* SOD negative behave as wild-type cell. All these deficiencies are complemented by the introduction of a gene expressing SOD. This complementation is obtained not only with *E. coli* SOD, but also with evolutionary unrelated SOD from other organisms, as human Cu–Zn-SOD.[19]

Mutants lacking SOD recovered the property to grow on minimal medium at a pretty high frequency (unpublished observations). We have not yet identified the secondary mutation(s) which is responsible for the loss of aa autotrophy. Those new mutants, still SOD negative, present a leaky phenotype. Several observations suggest that there is a diminution of the intracellular superoxide flux in these mutants, such that O_2^- concentration do not reach the critical threshold above which the growth in minimal medium is impaired.

Is superoxide or a derivative responsible for the premutagenic lesions on DNA in SOD lacking cells? We suspect that it is not superoxide, but more likely hydroxyl radical. Indeed challenge with 150 mM H_2O_2 increases mutagenesis above the level

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which occurs normally in SOD negative mutant and do not change the mutagenesis level in wild-type cells.[21] This synergistic action of O_2^- (in absence of SOD) and H_2O_2 to enhance mutagenesis likely results from the formation of hydroxyl radicals. Mutation frequency in SOD lacking mutants both with or without H_2O_2 challenge returns to wild-type level in strains which lack exonuclease III, suggesting that the premutagenic lesions on DNA are the same.

SOD lacking mutants can be, have been, used to test presupposed effects of O_2^- or SOD in biological processes as long as those can be tested in *E. coli*.[22,23]

Also, their complementation by SOD originating from other organisms constitute a tool to screen libraries for SOD genes of other species (A. Hass *et al.*; W. Van Camp *et al.*; this issue).

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MnSOD regulation

Global SOD level in *E. coli* is modulated by MnSOD expression. MnSOD is not usually expressed anaerobically, but is expressed in presence of iron chelators or in growth conditions which positively change the redox potential, as growth on nitrate.[9-11] Aerobically, MnSOD is overproduced in presence of product able to generate endogenous superoxide, as paraquat.[1] To determine at which level, transcriptional, translational or post-translational, the expression of MnSOD was regulated, studies using protein or operon fusions with the lactose operon genes were undertaken.[24,25] We conclude to a multiregulation of MnSOD, including (i) a positive transcriptional control via O_2^-, (ii) a negative transcriptional control via Fe^{++}, (iii) an autogenous regulation and (iv) a post-translational activity modulation via metal ion concentration.

Our present approach is to identify the effectors of MnSOD expression and their targets on DNA by isolating regulation mutants. Mutants *cis*-acting (targets) or *trans*-acting (effectors) in which MnSOD is expressed anaerobically have been isolated (with H. Hassan). Preliminary characterization of effector mutants permitted to identify at least two regulators, among which the Fur protein. Both are involved in a negative transcriptional control which need iron to be active. They act synergistically and do not affect significantly the aerobic induction of MnSOD by redox cycling compounds.

Therefore MnSOD appears to be submitted to two types of controls acting at the transcriptional level. One is the response to the oxidative aggression via O_2^-, and is likely mediated by the *soxR* gene product (B. Demple, this issue). The other seems to have the function of maintaining a steady state of intracellular O_2^-, and responds to cell metabolism variations which modify the redox potential. In this later control iron might play a role of sensor of the redox state of the cells.

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References

1. H.M. Hassan and I. Fridovich (1979) Intracellular production of superoxide radicals and hydrogen peroxide by redox active compounds. *Archives of Biochemistry and Biophysics*, **136**,385–395.
2. M.F. Christman, R.W. Morgan, F.S. Jacobson and B.N. Ames (1985) Positive control of a regulon

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for defenses against oxidative stress and some heat shock protein in *Salmonella typhimurium*. *Cell*, **41**, 753–762.
3. L.K. Walkup and T. Kogoma (1989) *Escherichia coli* proteins inducible by oxidative stress mediated by superoxide radical. *Journal of Bacteriology*, **171**, 1476–1484.
4. J.T. Greenberg and B. Demple (1989) A global response induced in *E. coli* by redox-cycling agents overlaps with that induced by peroxide stress. *Journal of Bacteriology*, **171**, 3933–3939.
5. E. Chan and B. Weiss (1987) Endonuclease IV of *E. coli* is induced by paraquat. *Proceedings of the National Academy of Sciences, USA*, **84**, 3189–3193.
6. B.D. Sak, A. Eisenstark and D. Touati (1989) Exonuclease III and the catalase hydroperoxidase II in *Escherichia coli* are both regulated by the *katF* gene product. *Proceedings of the National Academy of Sciences, USA*, **86**, 3271–3275.
7. C.S. Moody and H.M. Hassan (1984) Anaerobic biosynthesis of the manganese containing superoxide dismutase in *E. coli*.*The Journal of Biological Chemistry*, **259**, 12821–12825.
8. S.Y.R. Pugh and I. Fridovich (1985) Induction of superoxide dismutases in *E. coli* by metal chelators. *Journal of Bacteriology*, **162**, 196–202.
9. H.M. Hassan and C.S. Moody (1987) Regulation of manganese containing superoxide dismutase in *E. coli*: anaerobic induction by nitrate. *Journal of Biological Chemistry*, **262**, 17173–17177.
10. K. Miyake (1986) Effect of nitrate on the level of SOD in anaerobically grown in *E. coli*. *The Journal of General Applied Microbiology*, **32**, 527–533.
11. C.T. Privalle, W.G. Beyer and I. Fridovich (1989) Anaerobic induction of Pro-Mn-superoxide dismutase in *E. coli*. *The Journal of Biological chemistry*, **264**, 2758–2763.
12. D. Touati (1989) The molecular genetics of superoxdie dismutase in *E. coli*. An approach to understanding the biological role and regulation of SODs in relation to other elements of the defence system against oxygen toxicity. *Free Radical Research Communications*, **8**, 1–9.
13. D. Touati (1983) Cloning and mapping of the manganese superoxide dismutase gene (sodA) of *Escherichia coli* K-12. *Journal of Bacteriology*, **155**, 1078–1087.
14. H. Sakamoto and D. Touati (1984) Cloning of the iron superoxide dismutase gene (sodB) in *Escherichia coli* K-12. *Journal of Bacteriology*, **159**, 418–420.
15. Y. Takeda and H. Avila (1986) Structure and gene expression of *E. coli* MnSOD gene. *Nucleic Acids Research*, **11**, 4577–4589.
16. A. Carlioz, M.L. Ludwig, W.C. Stallings, J.A. Fee, H.M. Steinman and D. Touati (1988) Iron superoxide dismutase. Nucleotide sequence of the gene from *Escherichia coli* K-12 and correlations with cristal structures. *The Journal of Biological Chemistry*, **263**, 1555–1562.
17. A. Carlioz and D. Touati (1986) Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide dismutase necessary for aerobic life. *The EMBO Journal*, **5**, 623–630.
18. S.B. Farr, R. D'Ari and D. Touati (1986) Oxygen-dependent mutagenesis in *Escherichia coli* lacking superoxide dismutase. *Proceedings of National Academy of Sciences, USA*, **83**, 8268–8272.
19. D.O. Natvig, K. Imlay, D. Touati and R.A. Hallewell (1987) Human copper-zinc superoxide dismutase complements superoxide dismutase-deficient *Escherichia coli* mutants. *The Journal of Biological Chemistry*, **262**, 14697–14701.
20. J.A. Fee, E.C. Nickerhoffer and C. Naranjo. First description of a variant of *E. coli* lacking superoxide dismutase activity yet able to grow efficiently on minimal, oxygenated medium. In Trace metals in man and animals (TEMA-G).
21. J. Hoerter, A. Eisenstárk and D. Touati (1989) Mutations by near-ultraviolet radiation in *Escherichia coli* strains lacking superoxide dismutases. *Mutation Research Letters*, **215**, 161–165.
22. D. Touati (1988) Molecular genetics of superoxide dismutases. *Free Radical in Biology and Medicine*, **5**, 393–402.
23. D. Touati and S. Farr (in press) Elevated mutagenesis in bacterial mutants lacking superoxide dismutase. In Oxygen radicals in biological systems, part B: oxygen radicals and antioxidants. *Methods in Enzymology*.
24. D. Touati (1988) Transcriptional and post-transcriptional regulation of manganese superoxide dismutase biosynthesis in *Escherichia coli*, studied with operon and protein fusions. *Journal of Bacteriology*, **170**, 2511–2520.
25. D. Touati (1988) Regulation of MnSOD in *E. coli*. In *Oxy Radicals in Medicine, Biology and Pathology* (P.A. Cerutti, I. Fridovich, J.M. McCord, eds), UCLA Symposia, vol. 82, A.R. Liss Inc., New York.

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