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Cellular and Molecular Physiology of *Escherichia coli* in the Adaptation to Aerobic Environments

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Upon exposure to oxygen, *Escherichia coli* increases the expression of enzymes essential for aerobic respiration, such as components of the TCA cycle and terminal oxidase complexes. This increase requires the elimination of repression mediated by the Arc regulatory system under anaerobic conditions. Coordinately, the synthesis of enzymes that function in anaerobic processes such as fermentation decreases, partly due to the inactivation of the transcription factor Fnr. *E. coli* is thus able to adjust the levels of respiratory enzymes to fit its environmental circumstances, and in this case, reduces the production of the less energy efficient fermentation enzymes in favor of the aerobic pathways. In contrast to the advantage in energy production, aerobiosis brings a disadvantage to *E. coli*: the production of reactive oxygen species (ROS), *i.e.* superoxide anion radical ($O_2 \cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH). These byproducts of aerobic respiration damage many biological molecules, including DNA, proteins, and lipids. To alleviate the toxicity of these compounds, *E. coli* induces the synthesis of protective enzymes, such as Mn-dependent superoxide dismutase (SodA) and catalase I (HP I), and this induction is controlled by the regulatory proteins SoxRS, OxyR, and ArcAB. Thus, ArcAB, Fnr, SoxRS, and OxyR function in concert so that *E. coli* can optimize its energy production and growth rate. Fnr and SoxRS are cytoplasmic, DNA-binding proteins, and these regulatory systems utilize iron-sulfur clusters as cofactors which may directly sense the redox environment. OxyR is also a cytoplasmic, DNA-binding protein, and appears to respond to redox potential through the oxidation state of a specific cysteine residue. In the ArcAB system (which belongs to the family of two-component regulatory systems), ArcB, a membrane protein, functions as the sensor, and ArcA, a DNA-binding protein, directly controls target gene expression. Under anaerobic conditions, ArcB undergoes autophosphorylation and transphosphorylates ArcA, stimulating ArcA's DNA-binding activity. During aerobic growth, the transphosphorylation of ArcA does not occur. In this signal transduction mechanism, the ArcB C-terminal or "receiver" domain plays a critical role; that is, it stimulates or abolishes the transphosphorylation depending on the metabolic state of the cell, which in turn is influenced by the availability of oxygen. *E. coli* thus employs at least four global regulatory systems which monitor the cellular oxidative/metabolic conditions, and adjust the expression of more than 70 operons to give the organism a better aerobic life.

Key words: ArcAB, Fnr, iron-sulfur cluster, oxidative damage, respiration.

The chromosome of *Escherichia coli* is 4,700 kb long, and is estimated to contain 3,000 to 5,000 genes. Many of these genes are not expressed under particular conditions, and are induced only when a specific stimulus is present in the environment. Depending on the stimulus, the expression of groups of genes or operons may be simultaneously altered, and this coordinated control is frequently accomplished by a single regulatory pathway. This type of regulation, where a set of operons is controlled by one regulatory protein, is called global control.

Several global control systems have been discovered in *E. coli*, and the focus of this review will be the ArcAB, Fnr, OxyR, and SoxRS systems (*1-4*). These systems respond to growth conditions as determined by the availability of oxygen, and control the expression of proteins that function in aerobic respiration, anaerobic metabolism, and defense against hydrogen peroxide and superoxide. Although these systems operate independently, each shares some of its target operons with the others so that these target genes are expressed in a finely-tuned, sophisticated manner.

In this review, we will briefly describe the adaptation of *E. coli* to aerobic environments with an emphasis on the control of gene expression by the global regulatory systems.

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Physiology

(i) Anaerobic energy metabolism. *Escherichia coli* can extract energy from substrates both aerobically and anaerobically, but without O_2 or any other electron acceptors, energy production relies completely on fermentation. This

process can be divided into two parts (Fig. 1A). The first part couples the degradation and oxidation of glucose with the reduction of NAD^+, resulting in the production of pyruvate. The second part consists of the reduction of pyruvate coupled to the regeneration of NAD^+. *E. coli* has several pathways for this second step, and therefore produces various amounts of several fermentation products —lactate, succinate, formate, acetate, and ethanol. Since the overall oxidation states of the fermentation products are the same as that of glucose, the extracted energy is apparently produced by the cleavage of the C3-C4 bond of glucose, and most of the energy is still preserved in the fermentation products. Fermentation proceeds at high rates in the anaerobically grown cells, due in part to the strong expression of the fermentative enzymes under these conditions. One such example is pyruvate formate lyase, encoded by *pfl*. Transcription of *pfl* is elevated approximately 15-fold during anaerobic growth, and this expression is stimulated by the Fnr and ArcAB systems (*5*). Another example is aspartase, encoded by *aspA*, which is also transcriptionally activated by Fnr under anaerobic conditions (*2*).

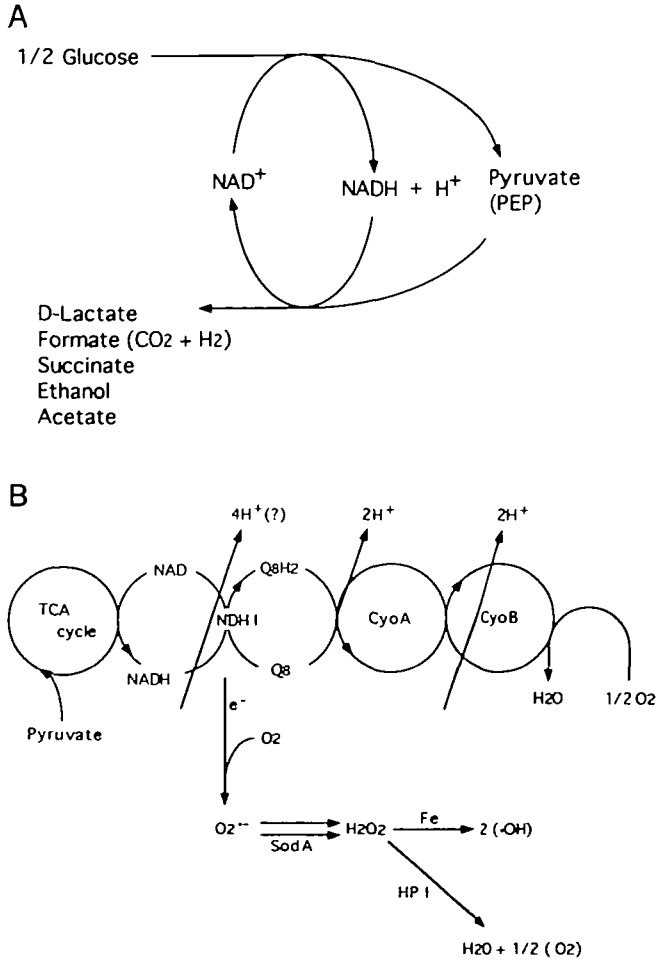

Fig. 1. **A: A simplified presentation of fermentation in *E. coli*.** Succinate is produced *via* phosphoenole pyruvate (PEP), whereas the other 4 fermentation products are generated *via* pyruvate. **B: A simplified presentation of the electron transport chain and superoxide metabolism in *E. coli*.** The translocation of protons from the cytoplasm or inner membrane to the periplasm is represented with arrows. $O_2\cdot^-$ can be converted to H_2O_2 by SodA or non-enzymatically.

(ii) Aerobic energy metabolism. Aerobic energy production is far more efficient than anaerobic metabolism. In the presence of O_2, pyruvate flows primarily to the tricarboxylic acid (TCA) cycle (Fig. 1B). The TCA cycle oxidizes substrates through 8 enzymatic reaction steps, transferring hydrogen molecules from the substrates mainly to NAD^+. (In one step, succinate dehydrogenase transfers hydrogen molecules to FAD.) The NADH is then oxidized through ubiquinone and the terminal oxidase complexes. Recent studies have revealed that the TCA cycle does not simply consist of 8 enzymes, but is rather complicated due to the existence of several isozymes. These isozymes include two aconitases, three fumarases, and two enzymes involved in the interconversion between succinate and fumarate (succinate dehydrogenase and fumarate reductase) (*6, 7*). The expression of these isozymes is differentially controlled at the transcriptional level, but the overall level of enzyme activity for each step is higher aerobically than anaerobically. A genetic analysis clearly showed that all members of the TCA cycle, except for the minor isozymes, are transcriptionally derepressed under aerobic conditions due to the inactivation of the ArcAB system (*8-10*).

When one pyruvate molecule is oxidized through the TCA cycle *via* pyruvate dehydrogenase, 4 NADH molecules are produced. These NADH molecules are in turn oxidized by NADH dehydrogenase I (NDH I), a reaction coupled to the reduction of ubiquinone. During this process, the membrane-associated dehydrogenase is thought to pump 4 protons across the cytoplasmic membrane per molecule of NADH (or per 2 e^-) (Fig. 1B) (*11*). *E. coli* contains another NADH dehydrogenase (NDH II), but this enzyme does not function as a proton pump. Interestingly, NDH I provides *E. coli* with a competitive advantage in stationary phase over a strain lacking this enzyme (*12*). This result implies that NDH I either provides *E. coli* with an energetic advantage in stationary phase, produces less superoxide than NDH II, or enables *E. coli* to store more energy supplies during log phase. At present, the regulation of NDH I gene expression is not elucidated, but the NDH II gene is known to be repressed by Fnr under anaerobic conditions, a rare example of negative regulation by Fnr (*2*).

E. coli possesses three aerobic terminal oxidase complexes, called Cyo, Cyd, and Cyx (*11*). Cyo and Cyd, encoded by the *cyoABCDE* and *cydAB* operons respectively, are the major terminal oxidases, but Cyo is the most efficient energetically. This terminal oxidase complex consists of at least four subunits in the cytoplasmic membrane, and two subunits are directly involved in the catalytic activity (Fig. 1B). CyoA is responsible for the oxidation of ubiquinol, whereas CyoB transfers electrons from CyoA to oxygen (*11, 13*). Both are transmembrane proteins, and together they pump 4 protons across the cytoplasmic membrane during the transport of 2 electrons. The resulting electrochemical gradient is then available for many kinds of energy dependent reactions. The metabolic steps of the TCA cycle and electron transport system extract the maximum possible chemical energy from the substrates. As might be expected, the expression of the Cyo complex is elevated during aerobiosis, and like the compo-

nents of the TCA cycle, this induction results from the elimination of ArcAB-mediated repression (*8, 14, 15*).

The expression of CydAB becomes maximal under nearly anaerobic conditions, and this terminal oxidase has a higher affinity for O_2 than the Cyo complex (*16*). The Cyd complex however can pump only 2 protons across the cytoplasmic membrane per 2 e^- transferred, and is less efficient than Cyo therefore in its ability to generate the proton gradient (*11*). It has been proposed that this terminal oxidase complex scavenges residual oxygen under micro-aerobic growth conditions, and thereby inhibits the inactivation and degradation of oxygen-sensitive enzymes. The expression of the *cyd* operon is reduced by deletion and insertion mutations in either the *arc* or *fnr* genes (*15, 17-19*). Although it is not clear whether these two regulatory systems are directly involved in the peak *cyd* expression under micro-aerobic conditions, the effect of Fnr is likely to be indirect (see below).

(iii) Toxicity of oxygen. Oxygen ($E_{m,7} = 0.82$ mV) is the most favorable electron acceptor for energy production, but this molecule can also be modified to form harmful, highly-reactive respiratory byproducts: namely, superoxide anion radical ($O_2\cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH) (Fig. 1B) (*3, 4*). The first of these reactive oxygen species (ROS) to be generated during electron transport, mainly by NDH II, is $O_2\cdot^-$ (Fig. 1B) (*4, 11, 20*). The $O_2\cdot^-$ is then converted to H_2O_2 either enzymatically or non-enzymatically, and ·OH can be produced non-enzymatically from H_2O_2 in the presence of Fe ion (*3, 4*). The hydroxyl radical is the most reactive of these species, but all three ROS are toxic to *E. coli*, and can damage virtually all biological molecules, including lipids, proteins, and DNA. In the case of superoxide, its toxicity is clearly shown in its effects on fumarase A, fumarase B, and aconitase, each of which contains active [4Fe-4S] clusters. $O_2\cdot^-$ rapidly inactivates the fumarase and aconitase activities, and converts the iron-sulfur clusters of the fumarases to an unstable oxidation state (*21, 22*). The [4Fe-4S] clusters may be among the primary targets of $O_2\cdot^-$.

There is no pathway by which to eliminate the ROS in one step, and *E. coli* produces two types of enzymes to combat these agents—superoxide dismutase and catalase. The former reduces $O_2\cdot^-$ to H_2O_2, and the latter converts H_2O_2 to H_2O, so that the combination of the two enzymes results in the detoxification of both harmful ROS (Fig. 1B) (*4*). *E. coli* has three superoxide dismutases, named SodA, SodB, and SodC (*3, 4, 23*). These enzymes are Mn-, Fe-, and CuZn-dependent respectively, and each appears to play a distinct and specific defensive role (*24* and references therein). SodA expression is derepressed under aerobic conditions, and this enzyme accounts for a large portion of the superoxide dismutase activity in aerobic cultures. SodA possesses DNA-binding activity, and thus it was proposed that SodA is responsible for protection against DNA damage. SodB expression does not respond to aerobiosis, and is stimulated only 2-fold by the Fur protein (ferric iron uptake). This enzyme appears to be constitutively expressed, and is thought to protect the cytoplasm from oxidative damage. SodC, a periplasmic enzyme, most likely protects the cell from external superoxide produced by the environment. Although *sodA* gene expression is induced by the presence of oxygen, it can be experimentally augmented by the addition of superoxide-generating reagents or nitric oxide (NO·). Furthermore, exposure of *E. coli* to superoxide-generating reagents increases the levels of about 30 proteins in addition to SodA, suggesting that the induction of SodA is part of a much larger response to prevent or repair oxidative damage. Similarly, the addition of H_2O_2 to *E. coli* cultures induces the synthesis of catalase I (HP I) and approximately 40 other proteins (*3, 4, 24*).

(iv) Aerobic capture of Fe ion. CyoB and several of the TCA cycle enzymes possess heme or iron-sulfur clusters as cofactors. In the presence of oxygen, however, free Fe is not readily available, because ferrous ion (Fe^{2+}) is easily oxidized to ferric ion (Fe^{3+}), which is essentially insoluble in an aqueous environment. To alleviate this problem, *E. coli* contains several transport systems through which ferric compounds are efficiently taken up into the cytoplasm. Each transport system consists of an inner membrane protein with a broad specificity, and an outer membrane protein specific to a substrate containing Fe^{3+}. The substrates are iron chelating agents called siderophores, which are produced by many microorganisms. Examples of the outer membrane proteins are FhuA (TonA) for the uptake of ferrichrome, FepA for ferric enterobactin, FecA for ferric citrate, and FhuE for coprogen and ferric rhodotorulate (*25*). To control the cellular concentration of Fe ion, *E. coli* employs the regulatory protein Fur, which represses transcription of the siderophore transport operons when cellular Fe^{2+} concentration is high; when the iron concentration is low, Fur is inactivated, derepressing the transport systems. The Fur regulatory activity maintains the total cellular iron concentration at a constant level irrespective of the presence or absence of O_2 (*26*). The expression of the siderophore transport systems responds most dramatically to changes in the external concentration of iron, and so in aerobic environments, the operons encoding siderophore transport systems appear to be expressed only a little more highly than in the absence of O_2. In contrast, the *sodA* operon, which is also under the control of Fur, is quite significantly repressed anaerobically and derepressed aerobically (*26, 27*). The repression of the *sodA* promoter may require a higher level of active Fur than the siderophore transport operons, indicating that Fur may have a lower affinity for the *sodA* promoter.

Global regulatory proteins

One parameter, the presence of O_2, can activate or inactivate 4 global regulatory systems, but the regulators do not necessarily recognize the oxygen molecule. One regulator, Fnr, appears to sense oxygen directly (Fig. 2), but the other regulators apparently recognize cellular conditions or metabolites as determined by the availability of O_2. We briefly describe here how Fnr, ArcAB, SoxRS, and OxyR become functional depending on whether the bacteria are exposed to aerobic or anaerobic conditions.

Fnr

Three independent groups originally isolated mutants which lack the activities of nitrate, nitrite, and fumarate reductases, all of which can catalyze terminal electron transfer to acceptors under anaerobic conditions. The responsible gene, found at 29 min and named *fnr* (fumarate and nitrate reduction), is now known to activate 22 and inactivate 8 operons (*2, 28*). The finding of sequence similarity between Fnr and Cap, a transcription factor that

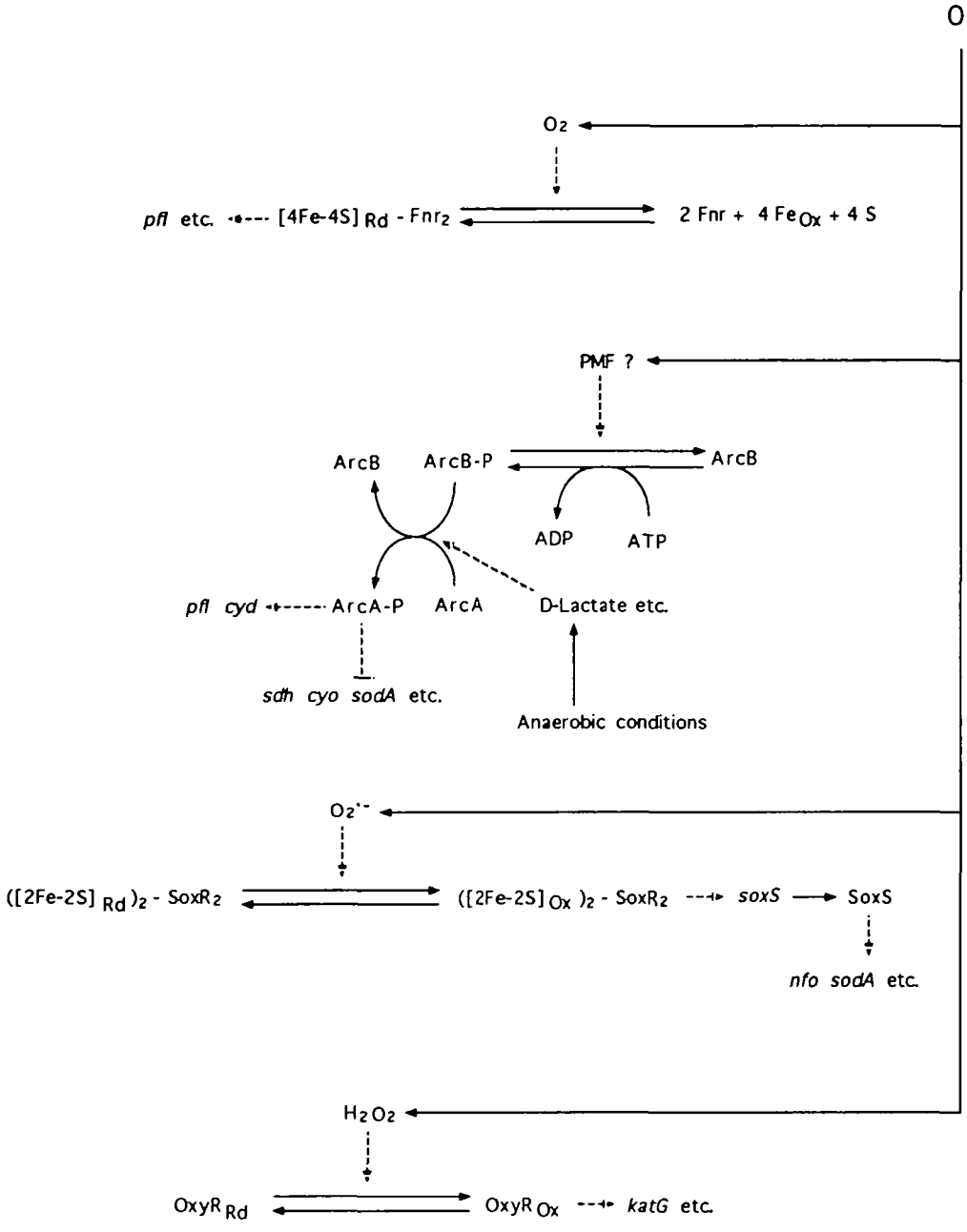

Fig. 2. **A schematic diagram of regulatory pathways involved in the adaptation of *E. coli* to aerobic environments.** Four global systems are represented—Fnr, ArcAB, SoxRS, and OxyR. For each system, signal transduction mechanisms and representative target genes are shown. Solid arrows indicate metabolic reactions, dotted arrows represent stimulation, and dotted bars indicate inhibition. Gene names are italicized. Rd and Ox designate the reduced and oxidized forms of a molecule, respectively. PMF indicates proton motive force.

activates promoters sensitive to catabolite repression, contributed greatly to the understanding of Fnr. The amino acid sequence of Fnr is 22% identical to Cap, and all of the structural elements of Cap are conserved in Fnr, including the helix-turn-helix motif of the DNA-binding domain (*2*). In addition, both proteins recognize similar core nucleotide sequences: TTGAT for Fnr, and GTGA for Cap. The difference in the first nucleotides of these sequences, T in the case of Fnr and G in the case of Cap, is critical for specific recognition by the two regulators. In fact, the Fnr binding sequence can be converted into a Cap binding sequence by changing the first T to G, and *vice versa*. However, Fnr has a major difference with Cap in the N-terminus where a cysteine-rich sequence exists. This cysteine-rich sequence was thought to be an iron binding site, and to function in perceiving the redox environment. Although iron was detected in purified Fnr, the roles of the cysteine-rich domain and iron have been difficult to determine because attempts to purify active Fnr were unsuccessful (*2*). Recently, Fnr mutant proteins were isolated, which are partially resistant to oxidation. One mutant protein was found to contain a [3Fe-4S] cluster, and higher DNA-binding activity than the others. Exposure of the [3Fe-4S]-Fnr to oxygen or a chelator decreased the binding activity to one-tenth or one-sixth respectively, and thus the iron-sulfur cluster appears to be required for high-affinity binding. Furthermore, a high proportion of this purified mutant protein exists as a dimer, in contrast to wild-type Fnr which forms an inactive monomer during purification. These results led the authors to suggest that in anaerobic cultures, Fnr exists as an active dimer containing one iron-sulfur cluster. This cluster is likely to be [4Fe-4S],

since this cluster is often oxidized and detected as [3Fe-4S]. Upon exposure to aerobic environments, the iron-sulfur cluster is easily destroyed, and Fnr monomer, which does not bind to DNA, is released (Fig. 2). Hence according to this model, Fnr dimerization occurs prior to DNA binding and only in anaerobic cultures (*29*). As an alternative model, it was proposed that Fnr monomers bind to DNA irrespective of the presence or absence of oxygen, and that under anaerobic conditions, neighboring monomers assemble into stable dimers (*30*). This model was derived from results with a mutant Fnr protein which exists as a monomer in solution, but has DNA-binding activity. In order to clarify which model is correct for Fnr, the properties of the two mutant proteins, and the primary consequences of the mutations, must be studied in greater depth. When wild type Fnr was isolated by an improved purification procedure, the protein was found to contain iron and sulfur, and displayed a spectroscopic pattern characteristic for [3Fe-4S] or [4Fe-4S]. Furthermore, gel filtration of the protein in the presence of iron showed that it is larger than the monomer (*31*). Although it has been demonstrated using hexacyanoferrate (III) that redox potential, and not the oxygen molecule, is the direct stimulus for conversion between the active and inactive forms *in vivo* (*2*), oxygen is no doubt the natural substance which provides high redox potential.

Fnr can either stimulate or repress transcription, and these different activities appear to correlate with the location of its binding site in target promoters. In the cases where Fnr functions as a transcriptional activator, the Fnr binding site is centered at −39 to −49 relative to the start of transcription. In the examples where Fnr inhibits transcription (including the *fnr* gene itself), Fnr binds to a site which either overlaps or is adjacent to the sigma 70 recognition sequence, and presumably blocks the binding of RNA polymerase holoenzyme (*2*).

ArcAB

It has long been known that the levels of respiratory enzymes are coordinately elevated in the presence of oxygen, but this regulatory mechanism was never genetically explored. To address this problem, we constructed a strain that contains β-galactosidase controlled by the promoter of a respiratory enzyme, succinate dehydrogenase (*sdh′-′lac*). We then used this strain to isolate mutants that display an elevated transcription of many aerobic respiratory genes under anaerobic conditions. The elevated enzymes include several dehydrogenases of the flavoprotein class, the Cyo terminal oxidase complex, and members of the TCA cycle, glyoxylate shunt, and the pathways for fatty acid degradation. The mutant alleles were found at two loci, min. 0 and 69.5, and the genes were named *arcA* and *arcB* (aerobic respiration control) respectively (*8, 14*). Subsequently, the *sodA* gene was also found to be derepressed by mutations in either *arcA* or *arcB*. In addition, the mutants exhibited a decreased anaerobic expression of two enzymes associated with oxygen-limited conditions—pyruvate-formate lyase and Cyd. At present, the ArcAB system is known to repress 17 and activate 9 operons under anaerobic conditions (*28*). The *arcB* gene is constitutively expressed irrespective of the availability of oxygen, and the *arcA* gene is expressed anaerobically at a 4-fold higher level than aerobically (*9, 32*). There are several transcription start sites in the *arcA* promoter, and one of them is dependent on the Fnr protein (*32*).

ArcAB belongs to the family of two-component regulatory systems (*33-35*). In these systems, a sensor protein detects an environmental signal and modifies the activity of a response-regulator protein, often a DNA-binding transcription factor. In the Arc system, ArcB, an 82 K protein, functions as the sensor, and ArcA, a 29 K protein, is the cognate regulator. Upon recognition of its specific stimulus, ArcB signals the information to ArcA by transphosphorylation, similar to other two-component systems. ArcA-P then binds to its target DNA sequences, and represses or activates transcription (*1, 36, 37*). Since the genetic disruption of the *arcB* gene completely disturbed the response of this global system to changes in the availability of oxygen, the recognition of the environment by the Arc system is totally dependent on the ArcB sensor protein (*1, 9*).

ArcB has the common primary structure for members of the two-component sensor family; it possesses a transmitter domain, which includes a kinase domain and a canonic His residue at position 292, and two putative transmembrane segments at the N-terminus. However, this sensor protein also has special features. In addition to the N-terminal transmitter domain, this hybrid sensor possesses a C-terminal domain which is homologous to the phosphate-acceptor or receiver domains of the response-regulator proteins (*34, 35*). The ArcB receiver domain is essential for the proper response to aerobic or anaerobic environments, and for optimal communication with ArcA. The analysis of the ArcB receiver domain was carried out both genetically and biochemically. Truncation of the ArcB C-terminus, including the receiver domain, resulted in a 12-fold decrease in the range of *sdh′-′lac* expression levels when expression was compared under aerobic and anaerobic conditions. Despite this reduction in expression range, the *sdh′-′lac* fusion was still induced 2-3-fold aerobically. Interestingly, the narrowed response range was caused by both incomplete anaerobic repression and incomplete aerobic derepression, suggesting that the ArcB receiver domain anaerobically stimulates, but aerobically inhibits, the phosphorylation of ArcA. Thus, while the ArcB C-terminal domain may not be absolutely essential for Arc signal transduction, this domain greatly amplifies the signal from ArcB to ArcA (*9, 38*).

Receiver domains contain three well-conserved acidic amino acids which form "a pocket" and act as the phosphate-acceptor site; the phosphorylated residue at this site is always aspartic acid (*33-35*). An analysis of wild-type and mutant ArcB proteins showed that the putative acidic pocket of the ArcB receiver domain is phosphorylated by His292 of the transmitter domain. Furthermore, the substitution of alanine for each of the aspartic acid residues of this pocket (positions 533 and 576) completely eliminated anaerobic repression of the *sdh′-′lac* fusion. These results suggest that when the acidic pocket is not phosphorylated, the receiver domain abolishes signalling to ArcA (*9, 10, 38*).

In all two-component regulatory systems, the sensor protein transduces the signal by first autophosphorylating a histidine residue in its transmitter domain, and then transferring this phosphoryl group to the cognate response-regulator protein. This mechanism is conserved in the Arc

system, as His292-P of the ArcB transmitter domain can directly phosphorylate the receiver domain of ArcA (*10, 38*). Signal transduction in the Arc system possesses additional complexity, however, as His292-P can also phosphorylate the His717 residue of the same protein, and His717-P, like His292-P, can transfer its phosphoryl group to ArcA (*39, 40*). ArcB thus contains two possible phosphoryl donor sites, and it appears likely that both sites transphosphorylate ArcA under anaerobic conditions. His717-P was further shown to phosphorylate another response-regulator protein, OmpR, which controls porin gene expression in response to osmotic conditions (*39*). *In vivo*, the overexpression of ArcB was able to compensate for the absence of EnvZ, the sensor protein that normally signals information to OmpR. Although these results indicate the possibility of "cross-talk" between Arc and other two-component systems (*39*), His717-P was shown to predominantly phosphorylate ArcA over OmpR (*40*). Under physiological conditions, OmpR does not appear to be a substrate of His717-P, and thus this phosphate donor is most likely specific for ArcA (*9*). These results, together with those obtained by truncation of the ArcB C-terminus (*9, 38-40*), suggest that His717 is necessary for the maximal transphosphorylation of ArcA, and hence for the severe anaerobic repression of aerobic respiratory genes. Since all phosphoryl groups in ArcB are derived from His292, the question remains as to why two donor sites are preferrable to His292 alone.

One important problem currently under investigation is the nature of the signal that activates the Arc system and is directly sensed by ArcB. Fermentation products, such as D-lactate, pyruvate, and NADH, were shown to inhibit the intrinsic dephosphorylation activity of ArcB-P *in vitro*. The resulting higher levels of ArcB-P in turn stimulated the transphosphorylation of ArcA. The effects of these metabolites are mediated by the ArcB receiver domain, because ArcB mutants lacking this domain, or substituting Ala for Asp533 or Asp576, did not exhibit this enhancement of ArcB signalling activity (*38*). While these metabolites may play an important role in Arc regulation *in vivo*, it seems likely that there is another signal which initiates the autophosphorylation of ArcB. Our experiments have excluded the possibility that this signal consists of either the O_2 molecule, or a redox reaction involving the cysteine residues of ArcB (*8, 9, 15*). It has been suggested that ArcB may detect changes in the proton motive force (*28*) or cellular proton concentration. Our current hypothesis for the regulation of Arc signal transduction is described in Fig. 3.

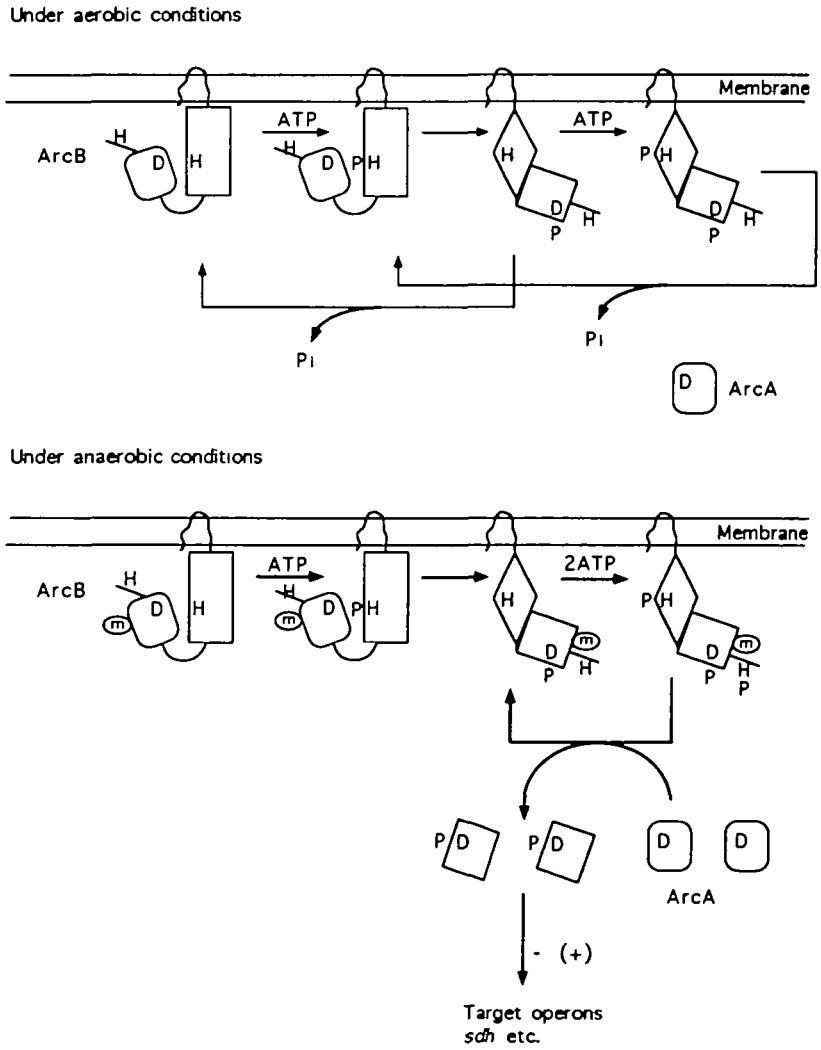

Fig. 3. **A model of ArcAB signal transduction.** H indicates His292 in the transmitter domain (adjacent to the transmembrane portion), or His717 C-terminal to the receiver domain (distal to the transmembrane portion). D represents Asp576 in the ArcB receiver domain, or Asp54 in the receiver domain of ArcA. P indicates a phosphoryl group. m represents cellular metabolites such as D-lactate and NADH. The signs + and − indicate stimulation and inhibition respectively. Under aerobic conditions, the ArcB receiver remains nonphosphorylated, due in part to an intrinsic dephosphorylation activity; this domain then blocks the transphosphorylation of ArcA. Under anaerobic conditions, H292-P phosphorylates D576 and H717, and cellular metabolites inhibit the intrinsic dephosphorylation activity of ArcB; both phosphoryl-histidines rapidly donate their phosphoryl groups to ArcA at D54.

The DNA-binding properties of ArcA were investigated in two target operons—*sodA* and *pfl*. In the *sodA* promoter, a crude extract containing overproduced ArcA protected a 65 bp segment encompassing the −35 and −10 promoter elements. This region partially overlaps with the binding site for the Fur protein, which independently represses *sodA* expression (*36*). In the *pfl* operon, purified ArcA displayed the highest affinity for a 141 bp region downstream from the promoter it activates (*37*). The large region protected in both promoters suggests that ArcA can bind to its target DNA either as an oligomer, or as a monomer that recognizes multiple sites (*36, 37*). Based on these results, a possible consensus binding sequence was proposed of TATTTaa (*37*). The incubation of ArcA with carbamoyl phosphate, which can phosphorylate other response-regulator proteins, increased the binding activity about 10-fold. Thus, phosphorylation appears to greatly stimulate, and may be required for the binding of ArcA to its target promoters, consistent with the critical role of ArcB in modulating ArcA function (*37*).

SoxRS

The *soxR* and *soxS* genes were originally identified by genetic mutations which conferred resistance to superoxide-generating agents (*41*), or exhibited high expression of the endonuclease IV promoter (*nfo′-′lac* fusion) (*42*). These genes were subsequently shown to encode DNA-binding transcription factors, and to activate the production of at least 10 proteins that protect the cell from various types of damage, such as SodA, endonuclease IV, glucose-6-phosphate dehydrogenase, fumarase C, and NADH:ferredoxin oxidoreductase (*3, 4, 24*). The *soxR* and *soxS* genes both map to 92 min on the *E. coli* chromosome, and are transcribed from divergent promoters. The activation of this system requires two steps, and while these proteins are not homologous to the family of two-component systems (either in sequence or biochemistry), SoxR functions as this system's sensor, and SoxS directly controls target gene expression. The SoxR protein is constitutively synthesized, and becomes active upon exposure to $O_2^{\bullet -}$. The active SoxR then strongly stimulates transcription of *soxS*, and SoxS activates transcription of the target genes (Fig. 2). SoxR has been purified as an active homo-dimer (holo-SoxR) with each dimer molecule containing two molecules of the [2Fe-2S] cluster (*43-45*). SoxR lacking the iron-sulfur clusters (apo-SoxR) was able to bind to the *soxS* promoter with an affinity similar to that of holo-SoxR, but did not activate *soxS* transcription (*43*). Furthermore, holo-SoxR purified from aerobic cultures stimulated *soxS* transcription, whereas the reduction of the purified protein eliminated this activity. These results suggest two possible models for SoxR function: either SoxR initially exists as the apo-protein, and becomes the active holo-protein upon exposure to the stimulus, or SoxR normally exists as an inactive holo-protein containing reduced iron-sulfur clusters, and is activated upon oxidation of the clusters by the stimulus (*24, 42*). Although the formation of $O_2^{\bullet -}$ strongly correlates with the activation of SoxR, the direct stimulus for this protein is not clear at present, since this system can also be activated by NO• (*46*). The SoxS protein binds to the consensus sequence, AN_2GCAYN_7CWA (where N is any base, Y is a pyrimidine, and W is A or T) (*47*), and can decrease expression from its own promoter by 2-fold, which is perhaps needed to prevent the overproduction of this protein (*48*).

OxyR

E. coli and *Salmonella typhimurium* (a related bacterial species used in studies of oxidative damage) are able to survive a sudden exposure to 60 μM hydrogen peroxide, but are killed with higher concentrations. Mutants more resistant to this reagent were isolated by exposing the bacteria to 300 μM hydrogen peroxide. These mutants were also more resistant to organic hydroperoxides, and displayed an elevated synthesis of protective enzymes, including catalase I (*katG*), alkylhydroperoxide reductase, and glutathione reductase (*3, 4, 24*). The gene responsible for this phenotype was designated *oxyR*, and encodes a DNA-binding transcription factor that activates the expression of at least 9 proteins. OxyR is constitutively synthesized irrespective of the presence or absence of H_2O_2, and appears to become activated by exposure to an oxidizing stimulus. The regulatory activity of OxyR is sensitive to redox conditions *in vitro*, as purified OxyR was able to bind to and stimulate the *katG* promoter under oxidizing conditions, but did not exhibit these activities under reducing conditions (in the presence of 100 mM dithiothreitol) (*24, 49*, and references therein). The portion of the protein that recognizes the stimulus is not clear yet, but the best candidate is a specific cysteine residue at position 199. The binding of this protein to a target promoter protects a site that is unusually long and covers a region of 45 bp. Oxidized OxyR was found to bind DNA as a homo-tetramer, and to recognize a motif consisting of four ATAG elements in four adjacent major grooves on one face of the DNA helix (*49*).

Interactions between the global regulatory systems

The global regulators in principle function independently by receiving specific stimuli and controlling particular sets of target genes. However, a complex network of interactions exists among these systems, and as a result, most of the target genes are either directly or indirectly controlled by more than one global system. With multiple systems providing information to and control over a particular target operon, the expression of that operon can be modulated in a sophisticated way. Thus target gene expression can be adapted to best fit a specific set of environmental conditions, such as the combined availability of oxygen, carbon sources, and iron.

The influence of one regulatory system on another can occur at three levels: (i) transcription of the regulatory proteins, (ii) formation of the stimuli, and (iii) transcription of the target operons. An example of the first case is found in the interaction of the Arc and Fnr systems, as transcription of the *arcA* gene is weakly activated by Fnr under anaerobic conditions (*32*). The Fnr system also influences the stimuli sensed by Arc, since Arc recognizes the metabolic conditions generated by the fermentative enzymes under Fnr's control. Through both of these mechanisms, Fnr should indirectly control all the Arc target operons, and indeed, several of these operons, such as *sdh*, *sodA*, *cyo*, and *cyd*, are moderately affected by a loss-of-function mutation in *fnr* (*11, 28*).

In contrast to these indirect mechanisms, the third case involves the direct control of a target gene by more than one

global regulator. For example, the expression of the *pfl* gene is stimulated by both Fnr and ArcA under anaerobic conditions. These regulators bind independently to the *pfl* promoters, and their effects are additive, as both transcription factors are required for full anaerobic expression (*37*). An example of opposing influences on gene expression is found in the aconitase A operon, which is activated by SoxRS, and repressed by ArcAB (*6*). The *sodA* gene provides an extreme example of multiple regulatory influences, as its expression is either activated or repressed by a total of six global regulators (*27*). Lastly, the *cyo* operon is repressed by ArcAB under anaerobic conditions (*15, 18*), but remains sensitive to the availability of oxygen in Arc deletion mutants (*19*). These results suggest the existence of another regulatory mechanism, and one interesting candidate would be the SoxRS system.

Conclusion

Several global regulatory systems are now known to control the adaptation of *E. coli* to aerobic environments, and these systems can be divided into two general categories. The first category contains regulators whose activities are directly controlled by oxidation-reduction chemistry—*i.e.* Fnr, SoxRS, and OxyR. These proteins react with O_2 or its byproducts, and in two cases (Fnr and SoxR) appear to utilize iron-sulfur clusters as sensory devices. These findings extend our understanding of the biological roles of iron-sulfur clusters, since these molecules were previously known to catalyze electron transfer in respiratory proteins.

At present, the second category consists of only one regulatory system, ArcAB, which uses a signalling mechanism based on the transfer of phosphoryl groups. Although ArcAB is unique among the systems involved in adaptation to aerobic environments, it is homologous to the family of two-component systems, which control a large and diverse group of metabolic pathways in *E. coli* and other prokaryotes. The ArcB protein has special features (such as the receiver domain) that distinguish it from the majority of sensor proteins in the two-component family, but several examples of such hybrid sensor proteins have now been found in *E. coli* and other prokaryotes. Furthermore, hybrid sensors with homology to ArcB have recently been discovered in eukaryotes as well (*Saccharomyces* and *Arabidopsis*), along with homologs of the prokaryotic response-regulator proteins (*34, 35, 39, 50*). Thus, the ArcB protein may be representative of a distinct class of hybrid sensors regulating many types of biological activities.

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