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Review

Alternative respiratory pathways of *Escherichia coli*: energetics and transcriptional regulation in response to electron acceptors

G. Unden *, J. Bongaerts

Institut für Mikrobiologie und Weinforschung, Universität Mainz, Becherweg 15, 55099 Mainz, Germany

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Abstract

The electron-transport chains of *Escherichia coli* are composed of many different dehydrogenases and terminal reductases (or oxidases) which are linked by quinones (ubiquinone, menaquinone and demethylmenaquinone). Quinol:cytochrome *c* oxido-reductase ('bc_1 complex') is not present. For various electron acceptors (O_2, nitrate) and donors (formate, H_2, NADH, glycerol-3-P) isoenzymes are present. The enzymes show great variability in membrane topology and energy conservation. Energy is conserved by conformational proton pumps, or by arrangement of substrate sites on opposite sides of the membrane resulting in charge separation. Depending on the enzymes and isoenzymes used, the H^+/e^- ratios are between 0 and 4 H^+/e^- for the overall chain. The expression of the terminal reductases is regulated by electron acceptors. O_2 is the preferred electron acceptor and represses the terminal reductases of anaerobic respiration. In anaerobic respiration, nitrate represses other terminal reductases, such as fumarate or DMSO reductases. Energy conservation is maximal with O_2 and lowest with fumarate. By this regulation pathways with high ATP or growth yields are favoured. The expression of the dehydrogenases is regulated by the electron acceptors, too. In aerobic growth, non-coupling dehydrogenases are expressed and used preferentially, whereas in fumarate or DMSO respiration coupling dehydrogenases are essential. Coupling and non-coupling isoenzymes are expressed correspondingly. Thus the rationale for expression of the dehydrogenases is not maximal energy yield, but could be maximal flux or growth rates. Nitrate regulation is effected by two-component signal transfer systems with membraneous nitrate/nitrite sensors (NarX, NarQ) and cytoplasmic response regulators (NarL, NarP) which communicate by protein phosphorylation. O_2 regulates by a two-component regulatory system consisting of a membraneous sensor (ArcB) and a response regulator (ArcA). ArcA is the major regulator of aerobic metabolism and represses the genes of aerobic metabolism under anaerobic conditions. FNR is a cytoplasmic O_2 responsive regulator with a sensory and a regulatory DNA-binding domain. FNR is the regulator of genes required for anaerobic respiration and related pathways. The binding sites of NarL, NarP, ArcA and FNR are characterized for various promoters. Most of the genes are regulated by more than one of the regulators, which can act in any combination and in a positive or negative mode. By this the hierarchical expression of the genes in response to the electron acceptors is achieved. FNR is located in the cytoplasm and contains a 4Fe4S cluster in the sensory domain. The regulatory concentrations of O_2 are 1–5 mbar. Under these conditions O_2 diffuses to the cytoplasm and is able to react directly with FNR without involvement of other specific enzymes or protein mediators. By oxidation of the FeS cluster, FNR is converted to the inactive state in a reversible process.

* Corresponding author. Fax: +49 6131-392695; E-mail: unden@mzdmza.zdv.uni-mainz.de

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Reductive activation could be achieved by cellular reductants in the absence of O_2. In addition, O_2 may cause destruction and loss of the FeS cluster. It is not known whether this process is required for regulation of FNR function.

Keywords: Aerobic electron transport; Anaerobic electron transport; Regulation; FNR; ArcA; Oxygen sensor; (*Escherichia coli*)

Contents

1. Introduction

Escherichia coli is one of the preferred bacteria for studies on the energetics and regulation of respiration. The diversity and variability of the respiratory chains [1] hampered their investigation for a long time. By genetic means strains with defined composition in respiratory enzymes can be constructed. The nucleotide sequences of all respiratory enzymes are known [2,3] and most of the enzymes have been isolated and characterized. Combination of the data from physiological, biochemical and molecular genetic studies provide detailed knowledge on the organization and function of the respiratory chains and their adaptation to environmental and bioenergetic demands (for previous reviews on various aspects, see [1,4–12]). The major topic of this review is the adaptation of the respiratory system of *E. coli* to specific requirements, e.g., presence of electron acceptors. To understand principles and rationales behind regulation, here the energetics and properties of the involved enzymes will be discussed first.

2. Alternative respiratory systems of *Escherichia coli*

The respiratory chains of *E. coli* consist of (primary) dehydrogenases and of terminal reductases or oxidases which are linked by quinones. Due to the multitude of primary dehydrogenases, quinones and terminal reductases a large variability in the composition of the respiratory chains is observed. The respiratory chains are branched at the quinone level, and other potential branching points like quinol:cytochrome *c* oxidoreductase ('bc_1 complex' or 'cytochrome *c* reductase') and diffusible cytochrome *c* are missing. Since the terminal reductases as well as the dehydrogenases are expressed only under specific conditions, the number of enzymes which are actually

able to interact is restricted [11]. In addition, energetic constraints preclude the cooperation of some dehydrogenases and terminal reductases [13,14]. Since the H^+/e^- ratios differ for the enzymes, the variability has consequences for energy conservation.

2.1. Aerobic and anaerobic respiratory chains

2.1.1. Diversity of the respiratory enzymes

The respiratory chains of *E. coli* consist of 15 primary dehydrogenases and of ten terminal reductases (Table 1). These numbers include isoenzymes for some of the electron donors (H_2, formate, NADH and glycerol-3-P) and acceptors (O_2 and nitrate). The isoenzymes are completely different in their genetic and biochemical properties. Since the dehydrogenases and terminal reductases use quinones as a common substrate or redox mediator each of the dehydrogenases should be able to react with any of the terminal reductases. Only formate hydrogenlyase may be regarded as a electron transfer complex consisting of two enzymes (formate dehydrogenase and H_2-evolving hydrogenase) which are functionally linked by protein subunits [15]. The electron donors or acceptors of the enzymes have largely differing midpoint potentials, ranging from -0.43 V (formate) to $+0.03$

Table 1
Oxido-reductases of the respiratory chains of *Escherichia coli*

Enzyme	Redox couple		Genes	Map position (min)	Signal sequence subunit (Aa residues)
	Pair	E'_m(V)			
Primary dehydrogenases (DH):					
Formate DH_N	HCO_3^-/HCO_2^-	−0.43	*fdnGHI*	33.0	FdnG (1–33) (pot.)
Formate DH_O	HCO_3^-/HCO_2^-	−0.43	*fdoGHI*	88.03	FdoG (1–33) (pot.)
Formate hydrogen-lyase			*fdhF, hycA-H*	92.6; 61.35	n.s.
Hydrogenase 1	H^+/H_2	−0.42	*hyaABCDEF*	22.26	HyaA (1–45)
Hydrogenase 2	H^+/H_2	−0.42	*hybABCDEFG*	68.53	HybA (1–26/27)
NADH DH I	NAD^+/NADH	−0.32	*nuoA-N*	51.64	n.s.
NADH DH II	NAD^+/NADH	−0.32	*ndh*	25.17	n.s.
Glycerol-3-P DH_O	DHAP/Gly-3-P	−0.19	*glpD*	76.89	n.s.
Glycerol-3-P DH_N	DHAP/Gly-3-P	−0.19	*glpACB*	50.76	n.s.
Pyruvate oxidase	acetate + CO_2/Pyruvate		*poxB*	19.42	n.s.
D-Lactate DH	pyruvate/D-lactate	−0.19	*dld*	47.80	n.s.
L-Lactate DH	pyruvate/L-lactate	−0.19	*lctD*	81.55	n.s.
D-Amino acid DH	2-Oxoacid + NH_4^+/ Amino acid		*dadA*	26.64	n.s.
Glucose dehydrogenase	glucose/gluconate	−0.14	*gcd*	2.97	n.s.
Succinate DH	fumarate/succinate	+0.03	*sdhCDAB*	16.37	n.s.
Terminal reductases:					
Quinol oxidase *bo*$_3$	O_2/H_2O	+0.82	*cyoABCDE*	9.78	CyoA (1–24) (pot.)
Quinol oxidase *bd*	O_2/H_2O	+0.82	*cydAB*	16.67	n.s.
Quinol oxidase III (Cyx)	O_2/H_2O	+0.82	*appBC* (= *cyxAB)*	22.42	n.s.
Nitrate reductase A	NO_3^-/NO_2^-	+0.42	*narGHJI*	27.53	n.s.
Nitrate reductase Z	NO_3^-/NO_2^-	+0.42	*narZYWV*	33.09	n.s.
Nitrate reductase, periplasmic	NO_3^-/NO_2^-	+0.42	*napFDAGHBC*	49.5	NapB (1–34) (pot.)
Nitrite reductase	NO_2^-/NH_4^+	+0.36	*nrfABCDEFG*	92.42	NrfA (1–26) NrfB (1–31) (pot.)
DMSO reductase	DMSO/DMS	+0.16	*dmsABC*	20.32	DmsA (1–16)
TMAO reductase	TMAO/TMA	+0.13	*torCAD*	21.61	TorA (1–39)
Fumarate reductase	fumarate/succinate	+0.03	*frdABCD*	94.4	n.s.

The second substrate for each of the enzymes are quinones which are not given. Molecular properties of the structural genes, map positions and signal sequences (n.s., no signal sequence; pot., potential signal sequence) were obtained from the *Escherichia coli* database [3]

V (succinate) for the donors and from +0.82 V (O_2) to +0.03 V (fumarate) for the acceptors. Three different quinone types, ubiquinone (UQ), menaquinone (MK) and demethylmenaquinone (DMK) are used [16–19].

Each of the structural genes of the respiratory enzymes and their positions on the *E. coli* genetic map are known (Table 1). Knowledge of the structural genes enabled a clear-cut identification and differentiation of the individual enzymes and isoenzymes. Due to the presence of isoenzymes the enzymes are most clearly identified by their gene names (Table 1). The operons encoding the respiratory enzymes map all over the bacterial chromosome. With the exception of formate hydrogen-lyase, the structural genes of the enzymes are generally organized in one operon. Some of the operons, like *cyoABCDE*, *narGHJI*, *napFDAGHBC*, *nrfABCDEFG* and the hydrogenases contain in addition genes required for maturation or processing of the enzymes or for the biosynthesis of cofactors. The respective proteins are not found in the mature proteins [15,20–23]. By genetic methods strains can be constructed in which interferring enzymes are inactivated, enabling studies with defined enzymes and respiratory chains.

2.1.2. Structure and composition of the respiratory enzymes

The respiratory enzymes show a great variability with respect to composition and prosthetic groups. The enzymes can be composed from one or up to 14 subunits like NADH dehydrogenase I (*nuo* genes) which is one of the most complex enzymes in *E. coli*. As prosthetic groups the Mo cofactor, Ni, FAD, FMN, FeS clusters, PQQ and heme *b*, *c*, *d* or *o* are used [1]. PQQ, the cofactor of glucose dehydroge-

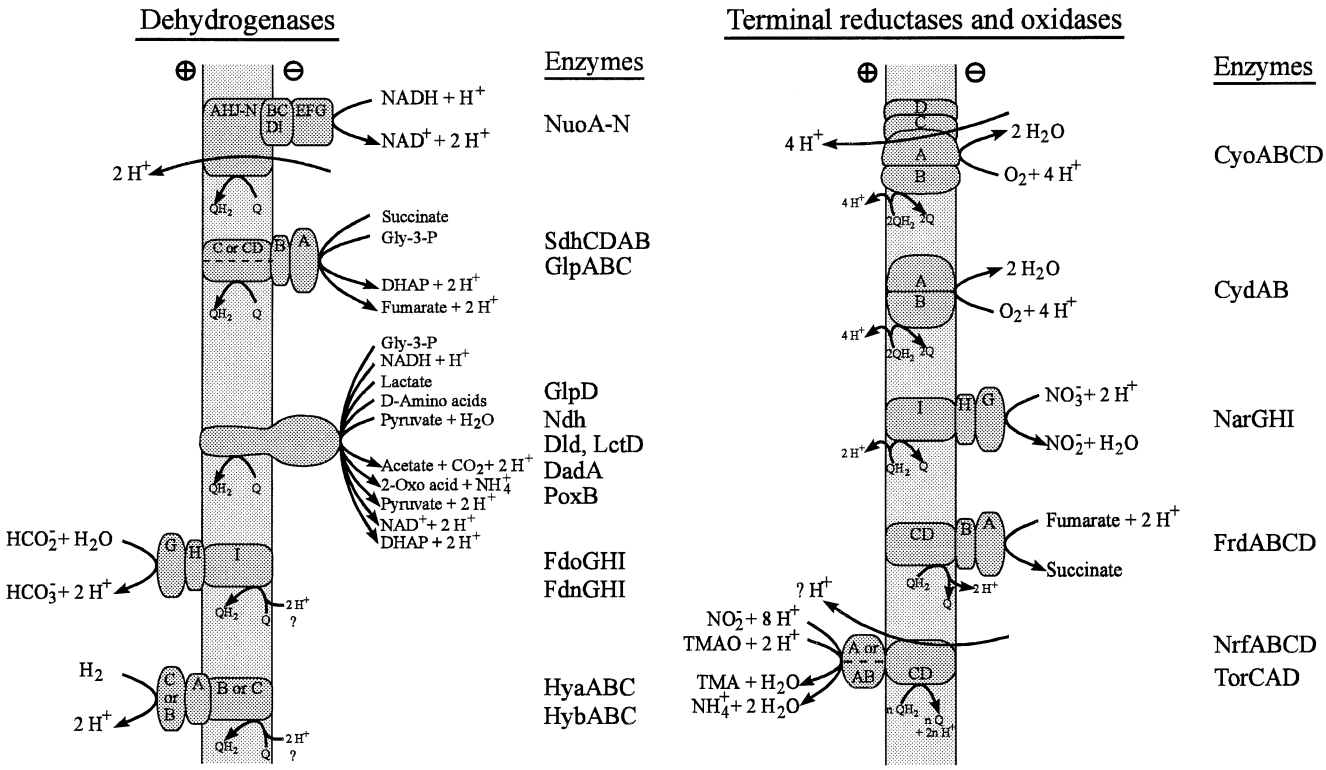


Fig. 1. Topology and arrangement of the enzymes of aerobic and anaerobic respiration in *E. coli*. Topology and membrane insertion was derived from biochemical data or from protein sequence properties such as hydrophobicity or signal sequences. The subunits are not drawn to scale. Designation of the enzymes and the subunits is according to the gene names (compare Table 1). Q and QH_2 stand for quinones and reduced quinones which might either be ubiquinone or menaquinone. The orientation of the quinone sites (i.e., site of H^+ release or uptake) is either according to experimental evidence or in accordance with the H^+/e^- ratios (see text). For enzymes with unknown orientation of the quinone site, the protons released or taken up are placed within the cytoplasmic membrane. The orientation and topology of the subunits was derived from the *E. coli* database [3] and experimental evidence (see text for references and [128]).

nase, cannot be synthesized by *E. coli* and has to be taken up from the medium.

The coding sequences of subunits from hydrogenases 1 (HyaABC) and 2 (HybABC), formate dehydrogenases *O* (FdoGHI) and *N* (FdnGHI), nitrite reductase (NrfABCD), periplasmic nitrate reductase (NapABC) and of TMAO reductase (TorCAD) contain (putative) *N*-terminal signal peptides (Table 1). The sequences direct secretion of the respective subunits to the periplasm causing periplasmic location of the subunit. The signal peptides of the two Nrf subunits (NrfA, NrfB), NapB and of TorA are characteristic for the general secretory pathway. They consist of an *N*-terminal hydrophilic 'N region' with a net positive charge, followed by the hydrophilic 'H domain' and the 'C domain' of six residues including an Ala-Thr-Ala cleavage site [24]. The 'double arginine' signal sequence (Arg-Arg-Xxx-Phe-Xxx-Lys) of the hydrogenase und formate dehydrogenase subunits, however, is typical for secreted proteins binding complex redox cofactors which do not use the general secretory pathway [15,25].

2.1.3. Topology of the respiratory enzymes

The topology of the enzymes in the cytoplasmic membrane is important for evaluating the H^+/e^- ratios of the enzymes (Fig. 1). Informations on the topology are derived from the accessibility of the active sites for substrates, inhibitors, chemicals and proteases, or from probing the orientation of transmembrane and hydrophilic loops with *phoA* or *lacZ* protein fusions. In addition the protein sequences provide informations on transmembrane helices and the presence of export signals.

The enzymes differ largely with respect to the orientation of the active sites to the periplasm or cytoplasm and the relative portion of hydrophilic and hydrophobic subunits or transmembrane helices (Fig. 1). With respect to these criteria, one class of enzymes is characterized by the composition from hydrophilic as well as hydrophobic (membrane integral) subunits. Representatives of this group are dehydrogenases for formate (FdoGHI, FdnGHI), succinate (SdhCDAB), NADH (NuoA-N), hydrogen (hydrogenases HyaABC, HybABC) and gly-3-P (GlpABC) and the terminal reductases using nitrate (NarGHI), fumarate (FrdABCD), DMSO (DmsABC), TMAO (TorCAD) and nitrite (NrfABCD). The active sites for the mentioned substrates are located on the hydrophilic subunits which can protrude considerably into the water space. The hydrophobic subunits serve as membrane anchors and carry the quinone (or quinol) reactive sites. The membrane anchor mostly consists of 1–2 hydrophobic subunits with transmembrane α-helices. In NADH dehydrogenase I (NuoA-N), seven subunits with altogether 56 (predicted) transmembrane helices make up the membrane fragment [26]. A second class of enzymes is represented by the terminal oxidases (CyoABCD, CydAB) which are integral membrane proteins without large hydrophilic regions. In both classes enzymes can be found, such as NADH dehydrogenase I or quinol oxidase bo_3, which are able to operate as proton pumps.

The third class of enzymes is represented by the simple dehydrogenases using glycerol-3-P (GlpD), NADH (NADH dehydrogenase II, Ndh), pyruvate (PoxB), lactate (DldD, LctD) or D-amino acids (DadA). The enzymes consist of one hydrophilic protein which is associated to the membrane mostly by one or few hydrophobic stretches as concluded from hydropathy profiles. Some of the dehydrogenases like PoxB lack such a hydrophobic stretch for anchoring in the membrane. Many of the enzymes can be rather easily displaced from the membrane and isolated as water-soluble species. Membrane association of theses enzymes presumably is only required for the donation of electrons to the quinones,

Table 2
Quinones in *E. coli* after aerobic and anaerobic growth on glucose and the electron acceptors O_2, nitrate, fumarate or DMSO

Quinone	E'_m(V)	Content(μmol quinone per g dry weight)		
		O_2	nitrate	fumarate (or DMSO)
Ubiquinone (UQ)	+0.11	0.36	–	0.09
Demethylmenaquinone (DMK)	+0.04	0.22	0.69	0.14
Menaquinone (MK)	−0.08	0.02	0.29	0.66

but is not sufficient for the translocation of electrons or protons across the membrane. For the hydrogenases, and nitrite (NrfABCD), periplasmic nitrate (NapABCD) and TMAO (TorCAD) reductases the topology of some subunits is not clear. The hydrophilic subunits of DMSO reductase have cytoplasmic orientation [27,28]. Therefore the significance of the periplasmic signal sequence in DmsA is not clear (Table 1).

2.1.4. Quinones in aerobic and anaerobic respiration

E. coli and related enteric bacteria synthesize three different quinones, ubiquinone (UQ) and the naphthoquinones menaquinone (MK) and demethylmenaquinone (DMK) [16,19,29,30]. In aerobic growth the major quinone is ubiquinone (UQ), which is replaced by menaquinone in anaerobic growth with fumarate or DMSO as acceptor (Table 2). In anaerobic growth with nitrate, DMK levels are highest [30]. The deydrogenases and the terminal reductases mostly show no substrate specifity for the various quinones presupposed that the midpoint potentials of the quinones are suitable for the electron donors or acceptors. The quinone reactive sites of the enzymes are not well characterized so far. The quinones which donate electrons to the terminal reductases are well known from studies with quinone biosynthesis mutants, reconstitution experiments and isolated enzymes (Fig. 2). NADH dehydrogenases I and II, and presumably most of the other dehydrogenases, transfer electrons to UQ as well as to the naphthoquinones [14,31]. Succinate dehydrogenase is restricted to UQ as an acceptor [16]. Most of the terminal reductases and oxidases on the other hand are restricted to specific quinones (Fig. 2) [17,18]. The oxidases accept electrons from ubiquinol. Ubiquinol, however, is too electro-positive to operate as an electron donor for fumarate, DMSO and TMAO and the respective reductases accept electrons only from the more electro-negative naphthoquinones MKH_2 or $DMKH_2$. Nitrate reductase (NarG) on the other hand uses MKH_2 or UQH_2 as a donor.

2.2. Energetics of the respiratory pathways and H^+/e^- ratios

In *E. coli* the determination of H^+/e^- ratios for individual enzymes or respiratory chains was largely complicated by the presence of isoenzymes and the branched electron-transport chains. Use of mutant strains with defined, non-branched respiratory chains or the use of isolated enzymes allowed clear-cut results for some enzymes. The H^+/e^- ratios for the different enzymes is in the range from 0 to 2 H^+/e^- (Table 3). For many enzymes the value is much below the theoretical upper limit. Proton potential can be generated by conformational proton pumps (e.g., NuoA-N, CyoABCD) or by redox loops. Redox loops are found in enzymes which carry the active sites for the substrate and the quinone on opposite sides of the membrane. By this arrangement chemical or scalar protons are produced and consumed on opposite sides of the membranes and only electrons are transferred across the membrane (compare Fig. 1). In Fig. 1 the topology and orientation of the active sites is given for the enzymes as far as known.

The H^+/e^- ratios of the terminal reductases have been determined in some detail. The quinol oxidase *bo_3* (CyoABCD) similar to other heme copper oxi-

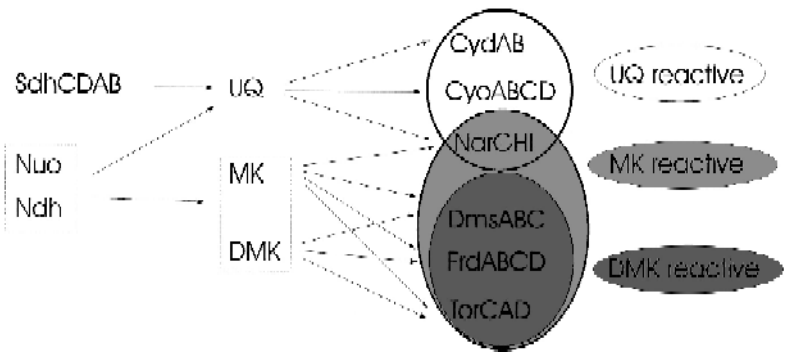


Fig. 2. Quinone specificity of the dehydrogenases and terminal reductases of aerobic and anaerobic respiration of *E. coli*. The dehydrogenases which are not shown presumably donate electrons to ubiquinone and the naphthoquinones as well.

Table 3
H^+/e^- ratios for respiratory enzymes and pathways of *E. coli*

Reaction	H^+/e^- (enzymes)		H^+/e^- total	Comment
$NADH \rightarrow O_2$	2 (NuoA-N)	+2 (CyoABCD)	4	
	2 (NuoA-N)	+1 (CydAB)	3	
	0 (Ndh)	+2 (CyoABCD)	2	major path
	0 (Ndh)	+1 (CydAB)	1	
Formate $\rightarrow NO_3^-$	1? (FdnGHI)	+1 (NarGHI)	2?	
$NADH \rightarrow NO_3^-$	2 (NuoA-N)	+1 (NarGHI)	3	
$H_2 \rightarrow$ Fumarate	1? (HybABC)	+0 (FrdABCD)	1?	
NADH → Fumarate	2 (NuoA-N)	+0 (FrdABCD)	2	major path
	0 (Ndh)	+0 (FrdABCD)	0	
NADH → DMSO	2 (NuoA-N)	+0 (DmsABC)	2	

The H^+/e^- ratios for the individual enzymes (donor + quinone → $donor_{ox}$ + quinol, or quinol + acceptor → quinone + $acceptor_{red}$) and for the overall electron transport are given. The numbers given for the enzymes include the protons translocated by conformational pumping and by arrangement of the substrate sites in redox lops. ? indicates that the H^+/e^- ratio is preliminary. The H^+/e^- ratio of the electron-transport chain gives the total of protons translocated. Compare also Fig. 1 for the H^+/e^- ratios of the enzymes and the overall reaction. See text for refences.

dases functions as a proton pump (1 H^+/e^-) (Fig. 1 and Table 3) [32]. In addition, 1 H^+/e^- is contributed by the opposite orientation of the sites for H^+ release during UQH_2 oxidation and H^+ consumption during O_2-reduction, resulting in a H^+/e^- ratio of 2 for the overall reaction [33,34]. Quinoloxidase *bd* (CydAB) does not operate as a proton pump and yields only 1 H^+/e^- by the latter mechanism. The H^+/e^- ratio obtained by nitrate reductase (NarGHI) from *E. coli* and other bacteria is less clear-cut. The enzyme supposedly is able to translocate 1 H^+/e^- during quinol oxidation, e.g., by the orientation of the substrate sites [35–39]. For fumarate reductase of *Wolinella succinogenes*, and presumably also of *E. coli*, on the other hand, the H^+/e^- ratio is zero. The menaquinol:fumarate reductase neither operates as a proton pump nor translocates protons due to arrangement of the substrate sites. Proton release during MKH_2 oxidation and proton consumption during fumarate reduction both take place at the cytoplasmic aspect of the membrane [40]. The missing energy conservation by this enzyme is in agreement with the low redox difference between the donor and the acceptor ($\Delta E \cong 110$ mV). The situation for the DMSO reductase is similar. The periplasmic nitrite and TMAO reductases, which consume (chemical) protons in the periplasm would have to translocate H^+ by some mechanism, to explain generation of a proton potential and growth by TMAO or nitrite respiration.

The H^+/e^- ratios of the dehydrogenases mostly have not been studied in detail. NADH dehydrogenase I is suggested to operate as a proton pump with $H^+/e^- = 2$ similar to the homologous enzyme from mitochondria [26,41]. The membrane associated enzymes which lack large transmembrane domains or subunits like NADH dehydrogenase II (Ndh) or glycerol-3-P dehydrogenase (GlpD) obviously do not operate as proton pumps. Due to the oxidation of the substrates in the cytoplasm only chemical protons are released which do not contribute to the generation of a proton potential. For succinate dehydrogenase the H^+/e^- ratio should be 0 due to the low redox difference between donor and acceptor (Table 1) and no proton translocation has been reported for this enzyme. The formate dehydrogenases (FdoGHI, FdnGHI), the hydrogenases (HyaABC, HybABC), and the anaerobic glycerol-3-P dehydrogenase (GlpABC), would be able to couple the redox reaction to proton translocation. The proton translocation ($H^+/e^- \approx 1$) reported for the formate dehydrogenase(s) and the hydrogenase(s) could be achieved by the orientation of the substrate sites for formate (or H_2) and the quinones (see Fig. 1) [42,43].

2.3. Alternative respiratory pathways in E. coli

2.3.1. Selective interaction of specific dehydrogenases and terminal reductases

In principle any respiratory dehydrogenase of *E. coli* is able to react with any terminal reductase or oxidase due to the common use of the quinones as redox mediators. In practice, however, the combinations of interacting dehydrogenases and terminal reductases are significantly restricted. The expression of the enzymes is transcriptionally regulated by electron acceptors like O_2 and nitrate (for reviews, see [1,11,12,44]). Therefore various enzymes are not produced under the same conditions and only restricted sets of dehydrogenases and terminal reductases are able to interact (Fig. 3). In the presence of O_2 a large number of dehydrogenases is synthesized which transfer electrons to the oxidases. In anaerobic respiration with fumarate and DMSO a different set of dehydrogenases is produced (Fig. 3). The dehydrogenases used in nitrate respiration on the other hand, are mostly the same as in aerobic or in fumarate respiration. As a consequence, most dehydrogenases donate electrons preferentially to selected terminal reductases. Thus, e.g., H_2 donates electrons mainly to fumarate, formate mainly to nitrate. Generally, if the same substrate, e.g., NADH, glycerol 3-P or formate, donates electrons to different acceptors, often the alternative isoenzymes are used for the different acceptors.

2.3.2. Use of dehydrogenase isoenzymes in different respiratory chains

For the NADH dehydrogenase isoenzymes the transcriptional regulation and their role in aerobic and anaerobic respiration has been compared (Fig. 4). In aerobic growth most of the electron flux is directed through NADH dehydrogenase II and the bo_3 type oxidase, although NADH dehydrogenase I is expressed, too [13,31,45]. Under the 'energy limited' growth conditions of fumarate and DMSO respiration, however, the use of the coupling NADH dehydrogenase I was essential [14]. The availability of isoenzymes with differing H^+/e^- ratios therefore provides the bacteria with a mechanism to vary and to optimize the efficiency of energy conservation. Other isoenzymes like glycerol-3-P dehydrogenases (GlpD/GlpABC), too, operate with different terminal acceptors and might have different H^+/e^- ratios. Inspection of Fig. 3 and Fig. 4 shows that in aerobic respiration preferentially dehydrogenases are used which are not able to conserve the free energy of the redox reaction in a proton gradient (Ndh, GlpD, PoxB, SdhCDAB). If coupling dehydrogenases are

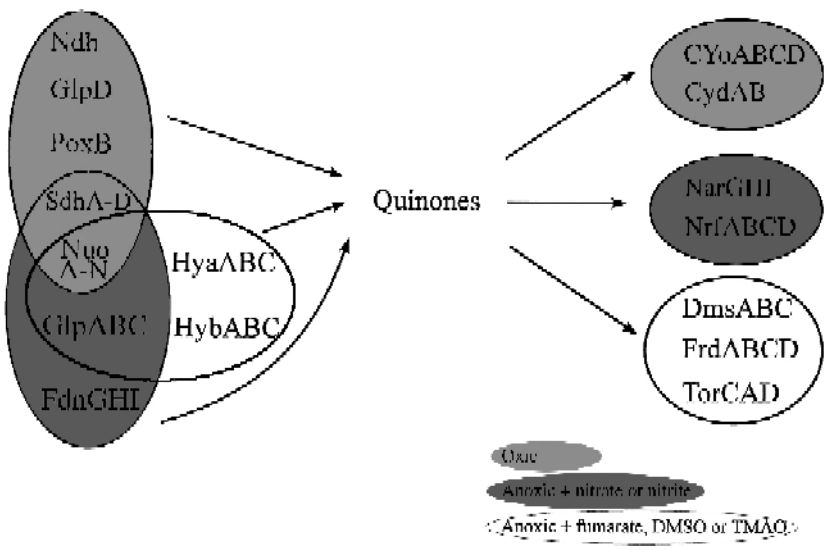


Fig. 3. Dehydrogenases and terminal reductases of aerobic and anaerobic respiration of *E. coli*. The figure gives the conditions (i.e., presence of electron acceptors) for the synthesis of the enzymes, and which dehydrogenases and reductases are able to interact in respiration. Only major conditions for synthesis are considered.

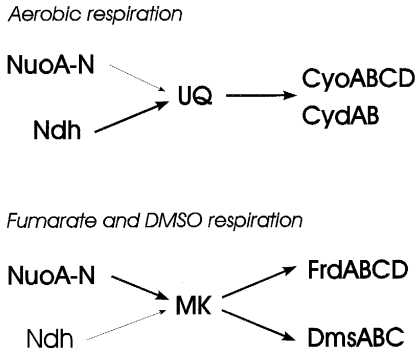


Fig. 4. Scheme showing the participation of NADH dehydrogenases I (NuoA-N) and II (Ndh) in aerobic, fumarate and DMSO respiration of *E. coli*. The relative amount of enzymes present and their contribution to electron transport under the respective conditions is schematically indicated by line thickness. UQ, ubiquinone; MK, menaquinone; FrdABCD, fumarate reductase; DmsABC, DMSO reductase; CyoABCD and CydAB, ubiquinol oxidases.

present like the proton pumping NADH dehydrogenase I (NuoA-N) their contribution to electron transport is minor. In fumarate or DMSO respiration on the other hand energy conserving dehydrogenases (NuoA-N, HyaABC, HybABC and potentially GlpABC) predominate.

2.4. Carrier systems for substrates of anaerobic respiration

In aerobic and anaerobic respiration in parts different transmembrane carriers are required for the transport of specific substrates or products like glycerol-3-P, nitrate or fumarate. The NarK protein has been assigned a role in nitrate/nitrite antiport [46,47], but recent experiments indicate that NarK functions in electrogenic excretion of nitrite [48]. An interesting situation is found for C_4-dicarboxylates like succinate, fumarate and malate which have different roles in aerobic and anaerobic metabolism. In aerobic growth, the C_4-dicarboxylates are completely oxidized to CO_2. Thus the carriers from aerobically grown bacteria (Dct carriers) catalyse only uptake (Fig. 5) [2,49,50]. In anaerobic growth, however, C_4-dicarboxylate exchange, efflux and uptake activities are found [11,50–53]. Exchange is essential in fumarate respiration where fumarate (or malate as the metabolic precursor of fumarate) is reduced to succinate which has to be excreted. During anaerobic growth on some substrates succinate is a fermentation product which has to be exported. Uptake of C_4-dicarboxylates is required if C_4-dicarboxylates are used as the carbon source.

The anaerobic C_4-dicarboxylate transport is effected by three homologous secondary carriers, DcuA, DcuB and DcuC [52,53]. Each of the carriers is predicted to be an integral membrane protein with 12 or 14 transmembrane helices. The carriers can replace each other for the most part, but DcuB is the most important carrier. Use of mutant strains carrying only one of the Dcu carriers, show that each of the carriers is able to catalyse exchange as well as uptake (Fig. 5). The DcuA, B and C carriers also contribute to the efflux activity, but an additional specific efflux carrier appears to be present [53]. Exchange is an electro-neutral antiport of the dicarboxylates, presumably with a symport of $3H^+$ in both directions. Uptake and efflux are electrogenic, e.g., a symport of $3H^+$ with the C_4-dicarboxylates [50,51]. As a consequence, fumarate/succinate exchange does not con-

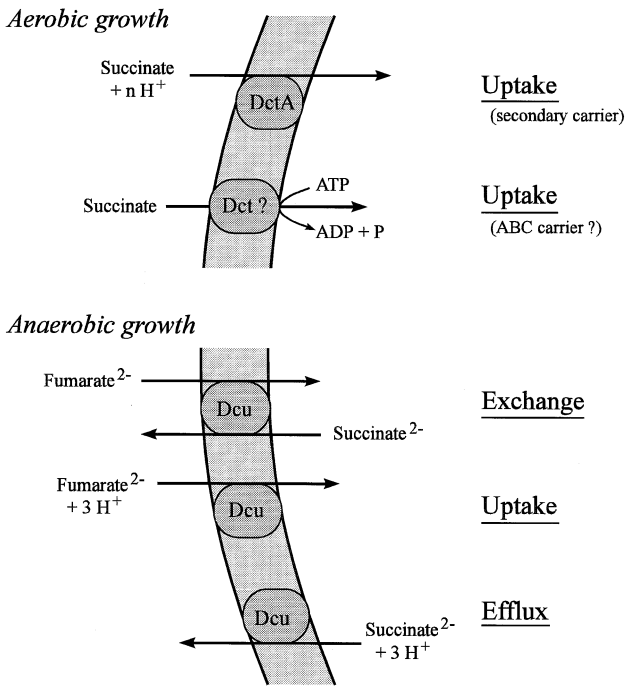


Fig. 5. C_4-dicarboxylate carriers of aerobically or anaerobically grown *E. coli* and their mode of action. DctA and the Dcu carriers (DcuA, DcuB, and DcuC) are secondary carriers. The second aerobic carrier (Dct?) is supposed to be a binding protein (*yiaO* or 0328 gene) dependent carrier (ABC carrier, ATP consuming) [129].

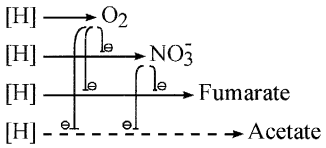
sume energy, whereas the uptake is driven by the proton gradient over the membrane. The efflux might generate a proton gradient similar to lactate efflux in *Streptococcus cremoris* [54]. The differing C_4-dicarboxylate systems in aerobically and anaerobically grown *E. coli* demonstrate that facultatively anaerobic bacteria have to catalyse many reactions by alternative mechanisms under both conditions for energetic and mechanistic reasons [11].

3. Transcriptional regulation by O_2 and other electron acceptors

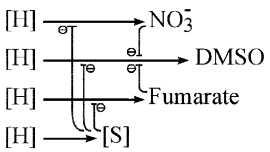
3.1. Expression of respiratory enzymes

3.1.1. Hierarchical use of electron acceptors according to maximal energy yield

In facultatively anaerobic bacteria, electron acceptors often are used in a specific order or hierarchy (Fig. 6). In *E. coli* O_2 represses all anaerobic respiratory pathways and fermentation, whereas nitrate is the preferred acceptor under anaerobic conditions and represses other anaerobic pathways [5,9,11,44]. The hierarchy is effected by transcriptional regulation of the corresponding terminal reductases by O_2 and nitrate. Preferential use of the electro-positive acceptors is explained by the higher energy yields. Since with different acceptors not only respiratory chains but also the overall catabolism including the central pathways can be changed, the use of the acceptors should be related to the $\Delta G^{0\prime}$ values or the ATP yields of the corresponding pathways (Fig. 6) [11]. The correlation of these parameters with the sequence by which the acceptors are used, supports the regulatory significance of the energy yields for the bacteria. *Bacillus macerans* is also able to use alternatively O_2, nitrate and fumarate as acceptors for respiration or to grow by fermentation. This bacterium expresses the various pathways in a hierarchical order similar to *E. coli* [55]. For other bacteria different regulatory criteria might be valid. *Wolinella succinogenes* is able of respiratory growth with nitrate, nitrite, fumarate, DMSO and polysulfide as the acceptors. In this bacterium the use of the acceptors is regulated, too [56,57]. But here polysulfide, the most electro-



E. coli	E_m' (mV)	$\Delta G^{0\prime}$ (kJ/mol Glukose)	μ (h^{-1})	Sequence of use ('Hierarchy')
[H] → O_2	+820	-2830	1.33	I
[H] → NO_3^-	+420	-858	0.58	II
[H] → Fumarate	+30	-550	0.21	III
[H] - - - → Acetate	-	-218	-	III



W. succinogenes	E_m' (mV)	$\Delta G^{0\prime}$ (kJ/mol Glukose)	μ (h^{-1})	Sequence of use ('Hierarchy')
[H] → NO_3^-	+420	-168	0.4	II
[H] → DMSO	+160	-116	0.05	III
[H] → Fumarate	+30	-90	0.46	II
[H] → [S]	-270	-37	0.58	I

Fig. 6. Regulation of respiratory pathways and of fermentation in *E. coli* and *Wolinella succinogenes* by electron acceptors ('hierarchy'). [H] → acceptor indicates the various respiratory chains or redox reactions in fermentation. − indicates transcriptional repression of the pathway by the electron acceptors shown. E_m' gives the midpoint potentials of the electron acceptors. For *E. coli* $\Delta G^{0\prime}$ refers to growth on glucose plus the respective acceptors, and the following growth reactions: glucose + 6 O_2 → 6 CO_2 + 6 H_2O; Glucose + 4 NO_3^- → 2 acetate$^-$ + 4 NO_2^- + 2 HCO_3^- + 4 H^+; Glucose + 4 fumarate^{2-} → 2 acetate$^-$ + 4 succinate^{2-} + 4 H^+; Glucose + H_2O → acetate$^-$ + ethanol + 2 formate$^-$ + 3 H^+ [10]. Growth rates of *E. coli* (μ) are given for growth on glycerol plus acceptors. For *W. succinogenes* $\Delta G^{0\prime}$ and μ are given for growth on formate plus acceptor. The hierarchical use of the acceptors is shown by the numbers I–III with I being the preferred and III the least preferred acceptor.

negative acceptor which also shows lowest ATP yields, is the preferred substrate. This could reflect an adaptation to specific ecological conditions or to maximal growth rates (Fig. 6) [58]. In *E. coli*, too, the growth rates correlate with the hierarchical use of the acceptors (Fig. 6). Therefore this parameter could be of similar significance as the energy or ATP yields.

3.1.2. Expression of dehydrogenases is not adapted to maximal energy conservation

The expression of the dehydrogenases is regulated by electron acceptors, too, and most of the dehydrogenases are expressed preferentially in the presence of specific acceptors (Fig. 3). The expression of NADH dehydrogenase I (NuoA-N), for example, is stimulated by nitrate and fumarate, but also by O_2, compared to fermentative conditions [14,44]. The alternative NADH dehydrogenase II (Ndh), is expressed mainly under aerobic conditions and is then the major enzyme due to high turn-over numbers [59,60].

Most of the dehydrogenases operating in aerobic respiration (Ndh, GlpD, PoxB, SDH) do not contribute to the generation of the proton potential by proton pumping or oriented redox loops, although each of the dehydrogenases would be able for thermodynamic reasons to translocate at least one H^+/e^- by reaction with ubiquinone. The proton pumping NADH dehydrogenase I is expressed under these conditions, too, but has only a minor role in NADH $\rightarrow O_2$ respiration due to the high activity of NADH dehydrogenase II [13]. It is obvious that in aerobic respiration dehydrogenases are prevailing which do not contribute to Δp formation. Therefore, the use of the dehydrogenases is not selected for high H^+/e^- ratios or maximal energy conservation. Other needs such as high metabolic fluxes or high growth rates due to low coupling coefficients, or accelerated NAD regeneration could be more important than maximal energy conservation in aerobic metabolism [13]. Preference of high metabolic fluxes or growth rates is in accordance with the assumption that bacteria are optimized for high growth rates rather than for maximal energy yield [58]. Energy conservation by the dehydrogenases of fumarate or DMSO respiration on the other hand is essential since this provides the only coupling site in these respiratory chains.

3.2. Transcriptional regulators

3.2.1. Regulators responding to electron acceptors: two-component regulatory systems

The transcriptional regulation is mainly effected by regulators responding to O_2 and nitrate, but other acceptors like fumarate might have regulatory affects, too. Regulation by O_2 is effected by the O_2 sensor-regulators FNR and ArcA/B [4,9,61–63], regulation by nitrate by the sensor-regulators NarX/L and NarP/Q [6,8]. The latter are two-component regulatory systems, which consist of a membraneous sensory kinase (ArcB, NarX, NarQ) and a cytoplasmic response regulator (ArcA, NarL and NarP) (Fig. 7). The sensors typically comprise a *N*-terminal histidine kinase sensory domain and a *C*-terminal cytoplasmic transmitter domain. In the transmitter domain a conserved histidine residue is autophosphorylated by a histidine kinase upon stimulation. The phosphoryl residue is then transferred by the kinase to an aspartyl residue in a conserved receiver domain of the response regulator. As a consequence, the output domain of the regulator with a DNA-binding helix-turn-helix motif is in the active state and binds to DNA.

3.2.2. O_2 responsive regulators

In variation of the general construction of two-component sensors, ArcB contains an *C*-terminal extension with a receiver domain and a second histidine protein kinase domain the role of which is not clear so far [64,65]. Since the cytosolic portion of ArcB is very large, it has been suggested that this domain might be important for sensing of oxic or anoxic conditions [64].

The second O_2-sensor, FNR, is of completely different type and function. The protein is a typical procaryotic gene regulator with a helix-turn-helix DNA-binding motif [61,66]. The protein shows significant similarity to the CRP protein (*c*yclo AMP *r*eceptor *p*rotein) of *E. coli* which effects catabolite repression [66]. However, FNR and FNR-like sensor proteins contain an *N*-terminal extension with three or four cysteine residues, three of which are part of a cluster of four conserved and essential cycteine residues in the protein [67–69,71]. The cysteine residues serve as ligands for the O_2-responsive [4Fe 4S] cluster of FNR [70,72–77].

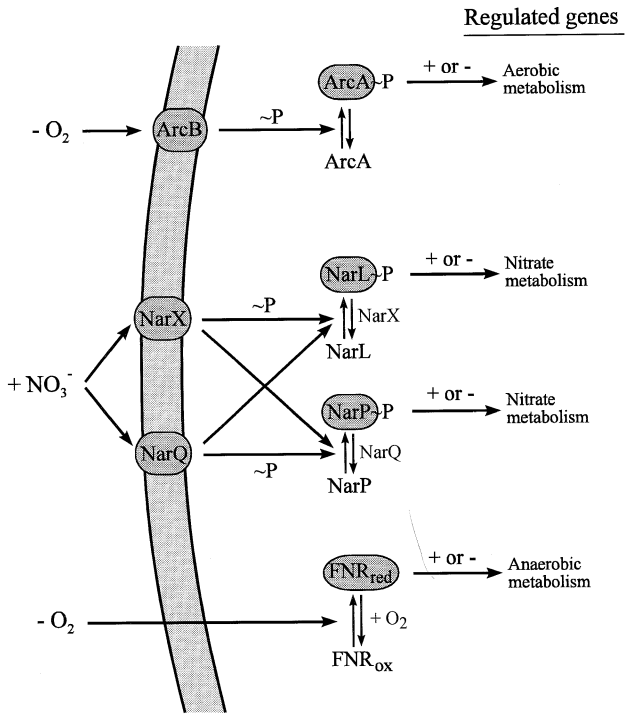


Fig. 7. Model for the sensors and regulators involved in the O_2 and nitrate regulated gene expression and the response to the signals. The model shows the stimuli (O_2, nitrate), the sensors (ArcB, NarX, NarQ), the response regulators (ArcA, NarL, NarP) and the cytoplasmic DNA sensor-regulator FNR. Activation and repression of the target gene expression is given by + or −. For the response regulators the active phosphorylated states (ArcA-P, NarL-P, NarP-P) and the inactive dephosphorylated forms are given. It is shown which (sensory) protein acts as the kinase, which as the phosphatase of the response regulators. With nitrite the same sensors (NarX, Q) and regulators (NarL, P) as with nitrate respond. But phosphorylation of NarL by NarX is low, resulting in low NarL-P concentrations under these conditions. Thus with nitrite, NarP-P is the major regulator; with nitrate NarL-P and NarP-P are functional.

3.2.3. Nitrate and nitrite sensing and regulation

Nitrate and nitrite regulation recently has been reviewed comprehensively by Stewart and Rabin [8]. Two sensor-regulator systems interact to achieve the nitrate and nitrite dependent gene expression. Sensing of nitrate and nitrite is mediated by the homologous membrane bound sensor proteins NarX and NarQ which control the activity of the homologous response regulators NarL and NarP via phosphoryl transfer [78–82]. Each of the sensors responds to nitrate and nitrite as well, and each is able to phosphorylate both regulators NarL and NarP. Nitrate generally is the more efficient signal for both sensors compared to nitrite. But NarL mainly serves as a nitrate regulator and becomes only weakly phosphorylated with nitrite. The phosphorylation state of NarP on the other hand, is high with nitrate and nitrite as well. The phosphatase activity required for a set-back of the regulators to the inactive state, is provided or controlled by the sensors. Dephosphorylation of NarP is restricted to NarQ, dephosphorylation of NarL to NarX.

Both NarL and NarP activate the expression of nitrate and nitrite catabolic genes and repress other anaerobic respiratory systems like fumarate reductase (Frd) or genes involved in fermentation. NarL has a broader spectrum of target genes compared to NarP and is the more general nitrate regulator (reviewed in [8]). Target genes may be regulated by NarL and NarP in a different mode. Thus the respiratory nitrite reductase (*nrf* genes) is repressed by NarL and activated by NarP. Other genes respond only to NarL like nitrate reductase A (NarGHI).

3.2.4. Other regulators (AppY, Fis, H-NS, StpA, and IHF)

The AppY protein (*a*cid *p*oly*p*hosphatase) is involved in the anaerobic induction of hydrogenase 1 (*hyaA-F* genes), quinoloxidase AppBC, acid phosphatase (*appBC appA* genes) and some other genes [83–85]. It is not known whether AppY responds directly or indirectly to the O_2 supply. In the regulation of many genes by O_2 or nitrate a regulation due to growth rate or growth phase is superimposed. This type of regulation is effected by small DNA-binding proteins like IHF (*i*ntegration *h*ost *f*actor) [86], H-NS (*h*istone-like *n*ucleoid *s*tructuring protein) [87], Fis (*f*actor of *i*nversion *s*timulation) [88] and StpA (*s*uppressor of *t*d *p*henotype) [89]. The proteins are required for a general adaptation to changed (environmental) conditions reflected in changed growth rates or phases. The target genes responding to the regulators are found in diverse and unrelated metabolic pathways. The regulation is effected by changed amounts of the regulators rather than by changing the functional state. Thus synthesis of AppY is transcriptionally regulated by σ^s in response to the growth phase [84]. The content of H-NS is regulated by growth rate and phase and is high during slow

Table 4
Important groups of FNR-regulated genes of *E. coli*

Function of gene products	Example	Number of known genes
Respiratory enzymes (anoxic, oxic)	Fumarate reductase (*frdABCD*)	> 55
Transmembrane carriers	Nitrite efflux (*narK*)	> 9
Anaerobic catabolism, fermentation	Pyruvat formate-lyase (*pflA*)	> 6
Biosynthetic pathways	Glutamyl-tRNA dehydrogenase (*hemA*)	> 4
Gene regulators	ArcA, FNR, NarX	> 3
Toxins	Colicin E1 production (*cea kil*)	> 3

Each group contains genes which are positively or negatively regulated by FNR under anoxic conditions. For a detailed list of regulated genes, see [1,12,44]

growth and in the stationary phase [87]. The concentration of Fis on the other hand is highest in the early exponential growth phase and after shift from minimal to rich medium [90].

Fis and H-NS have been shown to modulate the expression of O_2 and nitrate regulated genes. Fis positively or negatively regulates some genes of citric acid cycle enzymes, like citrate synthase, succinate dehydrogenase, malat dehydrogenase and NADH dehydrogenase II [91–95]. By binding to regulatory sites, Fis and H-NS change DNA topology and bending [96,97]. The changed topology is suggested to promote accession of RNA polymerase and expression. The bending could also be required for interaction between RNA-polymerase and transcriptional regulators. At negatively regulated promoters H-NS was shown to occlude the promoters by binding. StpA is structurally similar to H-NS and is able to replace H-NS partially in mutants [98,99].

3.3. Genes and regulators responding to O_2 and other acceptors

FNR and ArcA, as shown earlier for NarL and NarP, control different sets of target genes (reviewed in [4,5,9,11,12,44]. FNR acts as the major regulator of anaerobic metabolism and ArcA as the regulator of aerobic metabolism. However, this classification is not strict, and some of the FNR regulated genes code for enzymes of aerobically required enzymes and vice versa. A large variety of FNR regulated genes are known which are transcriptionally activated or repressed under anaerobic conditions (Table 4). From a functional aspect, the target genes encode enzymes of anaerobic or aerobic respiration, transmembrane substrate carriers, enzymes of central metabolic pathways, enzymes for coenzyme or cofactor biosynthesis or toxins [12]. ArcA on the other hand is the major regulator of aerobic catabolism. Its major purpose is to repress genes of aerobic metabolism (aerobic respiration, enzymes of tricarboxylate cycle and of the glyoxylate shunt) in the absence of O_2.

Most of the genes regulated by O_2 and nitrate are under dual or multiple control by the regulators [4,12,44]. The regulators are able to act in any combination and in a positive or negative mode. Thus, expression of the genes by any combination of electron acceptors can be achieved, i.e., in the presence of O_2 (*cyoABCDE*), under anoxic conditions with (*narGHJI*) or without nitrate (*frdABCD*), or in the presence of any acceptor (O_2, nitrate, fumarate, *nuoA-N* genes). By this regulatory network the characteristic hierarchical expression of aerobic, nitrate, and fumarate respiratory systems described in Fig. 6 is achieved. In some examples O_2 or nitrate act via both regulators (i.e., ArcA and FNR, or NarL and NarP) which can have opposite regulatory effects at the same promoter. Thus the quinol oxidase (CydAB) is repressed by FNR and stimulated by ArcA under anaerobic conditions. This adverse regulation results in a maximal expression of the oxidase under microaerobic conditions [100–102].

3.4. Structures of O_2 and nitrate regulated promoters

From the FNR binding sites of several FNR regulated promoters a consensus of dyad symmetry (TTGAT-N_4-ATCAA) was derived [103,104]. The sequence is composed of two half sites with a strict conserved spacing of four nucleotides. Each half site

is assumed to bind one FNR monomer. The consensus sequence shows sequence similarity to the CRP binding site (TGTGA-N_6-TCACA). By substitutions at the cognate helix-turn-helix DNA binding motifs both proteins could be converted to the respective other specifity for DNA sites [105]. Thus FNR could be converted such as to bind specifically to CRP sites, CRP to bind to FNR sites. Therefore, DNA binding and recognition is very similar to well characterized procaryotic DNA binding proteins of the helix-turn-helix type. In most cases the FNR-box is centered around −41.5 with respect to the transcriptional start site of positively regulated promoters. The FNR site of *ndh* which is negatively regulated by FNR is located at position −50.5 [60]. Some promoters possess a second FNR-binding site further upstream (*ndh*: −94.5; *fdn*: −97.5) [60,106]. Obviously these sites play a role in fine-tuning of regulation.

The NarL binding site consensus TACYYMT (Y = C or Y = T, M = A or M = C) is termed NarL-heptamer [107,108]. NarL heptamers exhibit great diversity with respect to number, location, orientation, and spacing. The heptamers frequently are arranged in tandem orientation with variable spacing rather than as palindromic pairs with fixed spacing which is characteristic for dimeric helix-turn-helix DNA binding proteins such as FNR or CRP. Groups of these heptamers are found around −100 (*fdn*), −74 (*nir*), as well as +1 (*frd*) with respect to the transcription initiation site. In the *narG* operon control region, eight NarL heptamers are organized into two distinct groups (Fig. 8). One group is centered around position −200, the other around −80. There are three tandem heptamers in the first group and five heptamers in tandem and inverted orientation in the −80 region (reviewed by Stewart and Rabin [8]). At the *frd* and *pfl* operons which are repressed by NarL, the heptamers are located around the transcriptional start site (+1) preventing the RNA polymerase from binding (Fig. 8). Again both promoters contain several NarL heptamers (*frd*, 7; *pfl*, 10) as identified by footprinting [109,110]. Drapal and Sawers [111] published a putative ArcA consensus sequence, TATTTaa (lower case letters designate less well conserved nucleotides), derived from footprint studies with *pfl*. With respect to number, location, orientation and spacing of the ArcA sites the situation is similar to the NarL sites. The size of DNA fragments around the consensus sites which is protected upon binding of ArcA or NarL from DNase I digestion suggests that both proteins are bound as oligomers. However, recently a different ArcA consensus has been suggested [112].

Some complex promoters (*narG, focApflA*) with largely distant binding sites for regulators, require the support of IHF for optimal expression [113–115]. The IHF protein binds at the *nar* promoter around −125. Binding of IHF is supposed to mediate the formation of a looped DNA structure bringing the

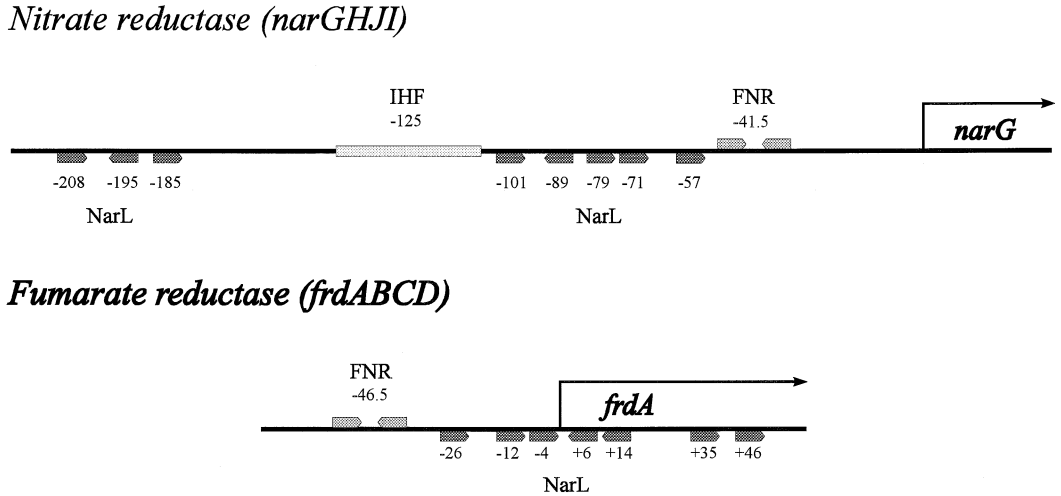


Fig. 8. DNA binding sites for NarL and FNR at the promoters of nitrate reductase (*narGHJI* genes) and of fumarate reductase (*frdABCD* genes).

distal regulatory regions into close proximity to the transcription initiation site. The situation appears to be similar for the *pfl* promoter. Thus IHF is required for optimal DNA-topology at the promoter, but not for specific sensing of the regulatory signal.

3.5. O_2 sensing in E. coli

The reactions and components involved in O_2 sensing by the sensors are not clear. The process of O_2 sensing often takes place at the cytoplasmic membrane by heme containing sensors. Binding of O_2 to the heme (with or without oxidation) is suggested to transfer a signal to the cytoplasmic regulator. These sensors belong to known types of signal transfer chains, e.g., to two-component signal transfer chains, such as FixL of *Rhizobium meliloti* [116] or to methyl accepting chemotaxis proteins such as DcrA of *Desulfovibrio vulgaris* [117]. Sensing of aerobic conditions by ArcB has to occur by a different mechanism. Neither O_2 reactive prosthetic groups nor interaction with O_2 directly or via the electron transfer chain could be demonstrated [4,64]. Aerobic or anaerobic conditions presumably are sensed indirectly. A product of aerobic or anaerobic metabolism, such as D-lactate, acetylphosphate or the NADH/NAD ratio could provide the signal to the cytoplasmic domain of ArcB [64,118]. Alternatively, the proton potential was suggested as a signal for the membranous sensor [119].

3.5.1. Activation and inactivation of FNR in response to the O_2 availability

O_2 sensing by FNR occurs by a different mechanism. FNR is a cytoplasmic sensor-regulator with roughly constant amounts in aerobically and anaerobically grown bacteria [120]. The sensory and regulatory (or DNA-binding) functions reside in two separate domains. Signal transfer from the sensory to the DNA-binding domain has to occur by a intramolecular conformational change which is apparently coupled to dimerization of the protein [70,73–76,121].

In vivo and in vitro studies on the function of FNR as an O_2 sensor-regulator suggest two potential steps of FNR inactivation by O_2, oxidation (step I) and destruction (step II) of the FeS cluster. Both processes are reversible and indications for both steps have been obtained in vivo and in vitro [70,74,75,122,123]. The FNR protein, previously suggested to contain an Fe ion, is now known to carry a FeS cluster (presumably 4Fe4S) which is essential for function [59,72,74,75,77,124,125]. The FeS cluster supposedly is bound by the 4 essential cysteine residues of the protein (Cys-20, Cys-23, Cys-29, Cys-122) [71,76,77]. The binding is very labile and most of the clusters are lost upon isolation of the protein, even under anaerobic conditions and other precautions. In a more stable mutant form of FNR (FNR^*) low amounts of the cluster could be identified and characterized as an 4Fe4S or 3Fe4S cluster [72,74]. A 4Fe4S cluster was also reconstituted in FNR in vitro with ferrous ions, cysteine and the NifS protein of *Azotobacter vinelandii* [75].

FNR can be inactivated in vivo by oxidants like hexacyanoferrate without involvement of O_2 (step I) [126]. Non-redox active O_2 analogs like CO and azide on the other hand are not able to inactivate FNR [127]. These results indicate that for inactivation of FNR oxidation is essential, whereas mere binding of O_2 (or O_2 analogs) is not sufficient. Inactive FNR is monomeric and becomes dimeric after reductive activation. For specific binding of FNR to DNA sites in gel retardation or in vitro footprinting dimeric FNR is required [60,70,73,74,121].

FNR is also inactivated in vivo by chelating agents or after loss of the FeS cluster upon isolation of FNR (step II) [76,77,122]. Loss of the FeS cluster (in vivo or in vitro), renders 3 to 4 of the cysteine residues accessible to modifying agents like iodoacetate. The essential cysteine residues from the N-terminus and presumably also Cys122 become accessible randomly and to a similar extent [76]. This indicates that the FeS cluster is bound by the essential cysteine residues. The FeS cluster can be reconstituted in vivo and in vitro [75,122,123]

In FNR from aerobically grown bacteria, a similar number of cysteine residues (about 3) becomes accessible [77]. This indicates that the FeS cluster becomes labile upon oxidation resulting in (partial) loss of the cluster. This process has been observed in vivo, but also in vitro after prolonged exposure of FNR to O_2 [74,75,77]. Mere oxidation of FNR might be the normal reaction to inactivate FNR in the aerobic/anaerobic switch, but under some conditions loss of the FeS cluster could occur. Therefore it is not clear so far whether in vivo the functional state of FNR is controlled only by oxidation and reduction

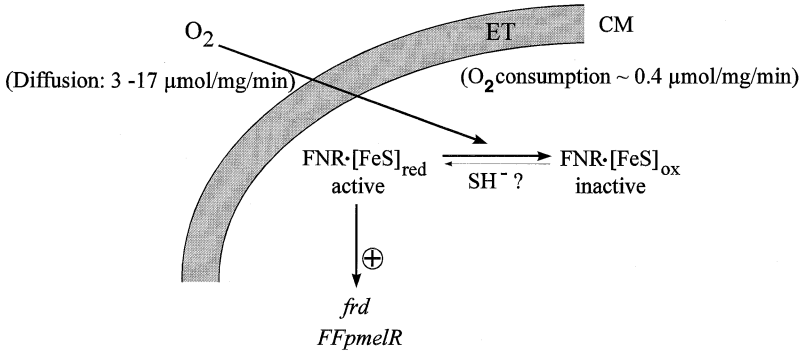


Fig. 9. Supposed interaction of O_2 and reducing agents with FNR in the control of FNR function. The diffusion of O_2 into the cytoplasm amounts to 3.4–17 μmol $O_2 \cdot min^{-1} \cdot (mg\ protein)^{-1}$ at pO_2 values (1–5 mbar) corresponding to the aerobic/anaerobic switch. O_2 consumption (mainly due to respiration) is 0.4 μmol $O_2 \cdot min^{-1} \cdot (mg\ protein)^{-1}$ at the respective p_{O2} values. In the model only oxidation, but not destruction or loss of the FeS cluster by O_2 is assumed. The oxidation (thick arrow) is supposed to be more rapid than reduction of FNR by cellular reductants like glutathion. Reduction would be efficient only at p_{O2} values below the switch point (≤ 1 mbar O_2).

(step I), or by loss and reconstitution (step II) of the FeS cluster additionally.

3.5.2. Cytoplasmic O_2 as the signal for FNR

The $pO_{0.5}$ values ('switch point') correspond to the O_2 tensions (pO_2) in the medium which cause half-maximal induction or repression of FNR regulated genes [12,120]. The $pO_{0.5}$ values are in the range of 1–5 mbar for most FNR regulated genes. The values have important consequences for the properties of O_2 as the regulatory signal for FNR (Fig. 9). The diffusion of O_2 to the cytoplasm at the regulatory O_2 concentration can be calculated to be in the range of 3.4–17 μmoles of $O_2 \cdot min^{-1} \cdot (mg\ protein)^{-1}$ [12]. This compares to an O_2 consumption rate by the bacteria of maximally 0.4 $\mu mol \cdot min^{-1} \cdot (mg\ protein)^{-1}$. The high excess of O_2 supply compared to consumption means that O_2 has to be present in the cytoplasm at the switch point and the concentrations are similar to those in the external medium. Thus, O_2 is present under these conditions in the cytoplasm to react directly with FNR. The finding that no signal transfer chain from external O_2 to FNR can be demonstrated and that O_2 is present within the cells, suggests that cytoplasmic O_2 directly reacts with FNR (Fig. 9) [127]. The electron donor for the reverse reaction, i.e., for the reductive activation of FNR, is not known. Cellular reductants like ferrous ions, glutathion or other thiol groups could be candidates for this function [127].

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