Volume 332, number 3, 203–207 FEBS 13088 October 1993

Minireview

Redox control of transcription: sensors, response regulators, activators and repressors

John F. Allen*

Plant Cell Biology, Lund University, Box 7007, S-220 07 Lund, Sweden

Received 18 August 1993

In a growing number of cases, transcription of specific genes is known to be governed by oxidation or reduction of electron carriers with which the gene products interact. The biological function of such control is to activate synthesis of appropriate redox proteins, and to repress synthesis of inappropriate ones, in response to altered availability of specific electron sources and sinks. In prokaryotic systems this control appears to operate by two general classes of mechanism: by two-component regulation involving protein phosphorylation on histidine and aspartate; and by direct oxidation–reduction of gene repressors or activators. For the first class, termed 'two-component redox regulation', the term 'redox sensor' is proposed for any electron carrier that becomes phosphorylated upon oxidation or reduction and thereby controls phosphorylation of specific response regulators, while the term 'redox response regulator' is proposed for the corresponding sequence-specific DNA-binding protein that controls transcription as a result of its phosphorylation by one or more redox sensors. For the second class of redox regulatory mechanism, the terms 'redox activator protein' and 'redox repressor protein' are proposed for single proteins containing both electron transfer and sequence-specific DNA-binding domains. The structure, function and biological distribution of these components are discussed.

Electron transport; Transcriptional control; Redox response regulator; Redox sensor; Redox activator protein; Redox repressor protein; Oxidative stress

1. INTRODUCTION

Oxidation–reduction (redox) reactions involve electron or hydrogen transfer and play a fundamental role in cell energetics. The electron transfer chains of photosynthesis and respiration in particular involve components the activity ratio of oxidised [ox] to reduced [red] forms of which is influenced by intrinsically variable environmental factors, such as availability of light, oxygen, and other electron sources and sinks. The redox potential of all electron and hydrogen carriers is concentration dependent, in accordance with the Nernst equation:

$$E = E_m - \frac{RT}{nF} \cdot \ln \frac{[red]}{[ox]}$$

where E is the redox potential, E_m is the mid-point potential characteristic of the chemical species in question, and n is the number of electrons transferred. Changes in redox potential will therefore result from changes in environmental factors that influence or participate in cellular electrochemistry. However, many redox components function safely only within relatively narrow ranges of redox potential. Cells have evolved a variety of adaptive responses that tend to maintain redox poise, thereby permitting them to exploit environmental conditions effectively while minimising the destructive effects of oxidation or reduction of inappropriate substrates.

An example of such an adaptive response is transcriptional control of gene expression. A number of individual proteins or pairs of interacting proteins have recently been implicated simultaneously in electron transfer reactions and in DNA binding. In each case there is evidence that transcription may thereby be regulated in response to changes in redox potential, yet there is currently no general term used to describe such proteins that consistently makes clear this common function. Here I describe two major classes of redox regulatory system, and suggest appropriate terms for these classes and their components. The structural and functional properties of these components are discussed, together with their biological distribution and role in maintenance of redox homeostasis in both prokaryotic and eukaryotic cells.

2. TWO-COMPONENT REDOX REGULATION: REDOX SENSORS AND REDOX RESPONSE REGULATORS

Bacterial two-component regulatory systems control gene expression by means of environmental sensors and

*Corresponding author. Fax: (46) 46 10 40 09. Email: john.allen@placebio.lu.se.

response regulators [1,2]. The sensor is usually a membrane protein and becomes phosphorylated in response to the environmental signal. Its substrate, the response regulator, is a DNA-binding protein that initiates transcription of a specific gene or genes by interacting, in its phosphorylated form, with an RNA polymerase. Sensors undergo histidine phosphorylation at a conserved site, and response regulators are phosphorylated on aspartate by the action of their corresponding sensors as phosphotransferases or kinases [2].

I therefore propose the terms 'redox sensor' for any electron carrier that initiates control of transcription upon oxidation or reduction; and 'redox response regulator' for the corresponding DNA-binding protein that modifies gene expression as a result of the action of a redox sensor. Any redox sensor together with its corresponding redox response regulator comprises a 'two-component redox regulatory system'.

A clear example is the Arc system of *Escherichia coli*, where mutations in two genes, *arcA* and *arcB*, increase the anaerobic expression of a number of genes normally repressed by anoxia [3,4]. ArcB is a protein kinase which contains two membrane-spanning helices and an extensive cytoplasmically exposed C-terminal domain, the latter containing a histidine which is the site of autophosphorylation. Autophosphorylation occurs in response to decreased redox potential, rather than in response to decreased oxygen concentration per se [5,6]. ArcA is a soluble protein which is phosphorylated by ArcB [7] and which contains the helix-turn-helix motif of DNA-binding proteins. I suggest that the terms redox sensor and redox response regulator are appropriate descriptions of ArcB and ArcA, respectively.

Another example of two-component redox regulation in *E. coli* is the NarX/NarL system [8]. NarX is a membrane-bound sensor the phosphorylation of which, on histidine, causes phosphorylation of an aspartate of NarL. This activates transcription of genes for nitrate reductase and formate dehydrogenase while repressing transcription of the gene for fumarate reductase.

In the purple non-sulphur bacterium, *Rhodobacter capsulatus*, mutations in a single regulatory DNA sequence inhibit the opposing effects of light and oxygen on synthesis and assembly of photosynthetic reaction centre and light-harvesting complexes [9]. The regulatory gene product has been termed PPBP, for *puf* promoter binding protein [10], and the gene has been termed *regA* [11]. Like ArcA, RegA shows the helix-turn-helix motif of DNA-binding proteins and sequence similarities with other response regulators. RegA is therefore likely to be a redox response regulator in the sense defined here.

The sensor controlling expression of nitrogen-fixation genes in *Rhizobium meliloti* is a membrane haemoprotein with protein kinase and phosphotransferase activity, termed FixL [12]. FixL catalyses its own phosphorylation, and is a protein kinase for FixJ, the response regulator. Thus, certain redox sensors may also be haemoproteins, haem redox sensors standing in relation to haem oxygen sensors as cytochromes do to haemoglobins and myoglobins.

Examples of known and probable redox sensors and redox response regulators are listed in Table I. Fig. 1 shows a general scheme for their functional interaction.

3. REDOX ACTIVATOR AND REDOX REPRESSOR PROTEINS

In other activator or repressor proteins, sensor and response regulator domains are present in a single protein, as exemplified by the catabolite activator or repressor protein (CAP or CRP) [2,3]. The *E. coli* FNR protein represses fumarate and nitrate reductases under aerobic conditions and other conditions of high redox potential. FNR is a helix-turn-helix DNA-binding protein with an N-terminal segment containing cysteines that ligate a redox-active iron atom [2,3]. Reduction of the iron from Fe^{III} to Fe^{II} is thought to cause a protein structural change that regulates transcription. From effects of poising the growth medium at different potentials on expression of a reporter gene for *frd* (encoding fumarate reductase), the standard mid-point potential (Em_7) of the FNR response has been estimated at +400 mV [13].

Another redox regulatory system of *E. coli* is the soxRS system, responding to a variety of specific treatments causing oxidative stress, and governing transcription of a number of genes, including that for manganese superoxide dismutase [14]. Both soxS and soxR have helix-turn-helix DNA-binding motifs, and soxR, like

Table I

Examples of redox sensors and redox response regulators

Redox sensor	Presumed prosthetic group	Redox response regulator	Regulating genes for:	Organism and reference
ArcB	Ubiquinone/ubisemiquinone/ ubiquinol	ArcA	Aerobic respiratory chain components	*Escherichia coli* [2–5]
NarX	Unknown	NarL	Nitrate reductase	*Escherichia coli* [8]
FixL	Haem	FixJ	Nitrogenase	*Rhizobium meliloti* [12,17]
Unknown	Unknown	RegA	Photosynthetic reaction centre and light-harvesting components	*Rhodobacter capsulatus* [10,11]

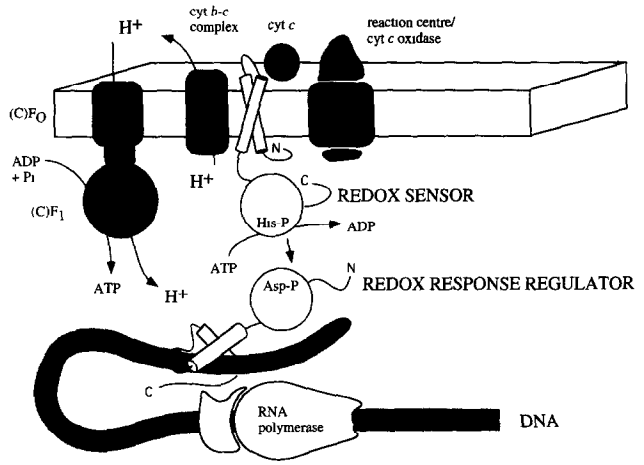

Fig. 1. Two-component redox regulation: schematic outline. A redox sensor is a membrane phosphoprotein that becomes phosphorylated on a histidine side chain when oxidised or reduced by components of an electron transport chain. Its substrate, the redox response regulator, is a sequence-specific DNA-binding protein that becomes phosphorylated on aspartate, regulating transcription.

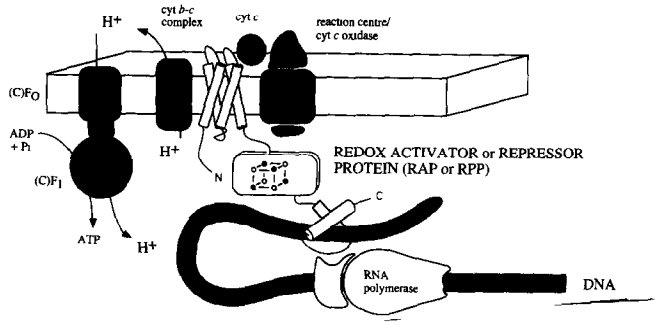

Fig. 2. Redox activator or repressor proteins: schematic outline. A single protein contains a redox-active prosthetic group (depicted as a cubane structure as found in, for example, Fe_4S_4 ferredoxins), and a sequence-specific DNA-binding domain. The redox-active group may be associated with a membrane domain or with a polar domain, the latter as in the soluble redox repressor, FNR. Membrane-bound redox activators and repressors may provide points of attachment between DNA and bioenergetic membranes.

FNR, has a cluster of cysteines that suggests [14] a metal-binding site responsible for redox control. Superoxide dismutase synthesis is also regulated at a transcriptional level by the two-component redox regulatory Arc system [14].

FNR is clearly a redox counterpart of CAP. I therefore propose the general terms 'redox activator protein' (RAP) and 'redox repressor protein' (RRP) for any protein containing both electron transfer and DNA-binding domains. Only a loose distinction can be drawn between 'redox activator' and 'redox repressor', since activation of transcription by a decrease in redox potential is equivalent to repression of transcription by an increase in redox potential, and vice versa. Whether a given gene is activated or repressed under optimal growth conditions depends upon the physiology of the species in question. Examples of redox activator or repressor proteins are listed in the Table II. Fig. 2 schematically depicts their function.

4. STRUCTURAL AND FUNCTIONAL PROPERTIES OF REDOX REGULATORY COMPONENTS

The *E. coli* redox sensor, ArcB [6], is a membrane protein of 778 amino acids. Its N-terminal domain contains two putative membrane-spanning α-helices, from positions 23–50 and 58–77. The phosphorylated His-292 lies in the extensive C-terminal, cytoplasmic domain. In view of its function in sensing redox potential of the respiratory chain, it seems likely that one or both of the two membrane helices contains a redox-active centre. However, no sequence elements are present that obviously suggest any particular prosthetic group. Because of its presumed operation at approximately the level of the cytochrome *b/c* complex, it is possible that the N-terminal domain of ArcB responds to the redox state of the ubiquinone pool, or to $UQ/UQ^{\bullet-}$ or $UQ^{\bullet-}/UQH_2$. Potentiometric redox titration of its action in phosphorylating ArcA should distinguish between such possibilities. Redox titration of protein phosphorylation has demonstrated redox control of the chloroplast thylakoid light-harvesting complex at the level of the quinone pool or the cytochrome *b/f* complex [15]. Medium-potential redox sensors operating at approximately +50 mV would be strategically positioned to respond to changes in redox potential in a variety of photosynthetic and branched respiratory electron transport chains. It remains to be seen whether similar membrane histidine phosphoproteins are found with sensor domains and redox-active prosthetic groups in N-terminal membrane domains. An 85 kDa cyanobacterial thylakoid membrane protein has been shown to be phosphorylated when the plastoquinone pool is reduced [16], and this is therefore a candidate for a cyanobacterial counterpart of ArcB.

The 'oxygen' sensor of *Rhizobium melioti*, FixL, is smaller than ArcB, having 505 amino acids. FixL is a membrane protein of modular structure with four predominantly hydrophobic helices between amino acids 21 and 118, and a haem-binding domain on the cytoplasmic side of the membrane. The N-terminal hydrophobic domain is not necessary for oxygen-activation in vitro [17], but can be taken as evidence that the protein is a component of a membrane complex and responds to redox potential rather than to oxygen itself. As with ArcB, potentiometric redox titration has not yet been carried out. The haem-binding domain exists as a module that is found in other sensor proteins [18]

The *E. coli* redox response regulator, ArcA, has 238 amino acids and is phosphorylated by ArcB on Asp-54 [19]. The N-terminal domain is likely to have a structure similar to that of CheY, a bacterial chemotaxis response

Table II

Examples of redox activator or repressor proteins

Redox activator/repressor protein	Presumed prosthetic group	Regulating genes for:	Organism and reference
FNR	Fe^{II}/Fe^{III}	Anaerobic respiratory chain components	*Escherichia coli* [2,3,13,27]
FixK	Fe_4S_4 iron-sulphur centre	Root nodule nitrogen fixation	*Rhizobium meliloti* [38]
PatB	Fe_4S_4 iron-sulphur centre	Cyanobacterial heterocyst formation	*Anabaena* PCC 7120 [39]
RdxA; ORFT2	Fe_4S_4 iron-sulphur centre	'Redox processes'	*Rhodobacter sphaeroides* [25]
SoxS	Metal atom	Mn-superoxide dismutase	*Escherichia coli* [17,40]
Lin-11	Fe_4S_4 iron-sulphur centre; Zn	Control of cell division	*Caenorhabditis elegans* [30]

regulator the structure of which has been determined by NMR spectroscopy [20,21]. CheY is phosphorylated on Asp-57, which is located on a surface-exposed loop between the first two anti-parallel β-strands. Although no structure has yet been obtained for the phosphorylated form of CheY, there is clear evidence from mutants that phosphorylation causes a protein structural change [21]. It is therefore likely that the effect of phosphorylation on redox response regulators involves a structural change that is communicated to RNA polymerase during control of transcriptional initiation. The FixJ response regulator of *Rhizobium meliloti* has 204 amino acids and a similar domain organisation to that of ArcA, with a phosphorylated Asp-54 and a C-terminal helix-turn-helix motif [22].

The *E. coli* redox activator–repressor protein, FNR [23], has 250 amino acids, the segment from amino acids 13–31 (SGGCAIHCQDCSISQLCIP) containing 4 cysteines that are thought to indicate an Fe-binding site, although an Fe_4S_4 cluster could also be bound at such a site. There is no obvious helical, hydrophobic segment. Amino acids 197–216 contain the helix-turn-helix motif of several classes of sequence-specific DNA-binding proteins. This and the C-terminal domain generally show similarities to the CRP catabolite repressor protein for which a structure has been obtained by X-ray crystallography [24]. The N-terminal domains of two DNA-binding proteins the sequences of which have recently been described, show clear evidence of both Fe_4S_4 clusters and membrane helices. These are RdxA and ORFT2 of *Rhodobacter sphaeroides* [25]. FNR may thus be a soluble member of the redox activator–repressor family that also includes membrane proteins. Redox activators or repressors so far described seem to be essentially DNA-binding ferredoxins. FNR may again be exceptional, since its high mid-point potential may not be that of an FeS protein. Redox titration of redox activators and repressors would be informative.

In general, bioenergetic systems have a repertoire of post-translational control mechanisms that serve to maintain optimal redox poise. In vivo, transcriptional control of synthesis of electron transport components may serve a similar function, providing a long-term solution to the problems of living in otherwise inhospitable redox environments. In particular, constitutive synthesis of components operating at extremes of redox potential may be detrimental to cell function. Such components could be compared with a useful machine operating outside its design specification, and decoupled from its intended application. In photosynthesis, photoinhibition [26] is an example of photo-electrochemistry becoming indiscriminately reactive to cellular components. In respiration, oxygen reduction is carried out by specific terminal oxidases that must be expensive to maintain in the absence of oxygen but essential if oxygen is not to be reduced at many non-specific points in a respiratory chain adapted to alternative terminal oxidants. Univalent reduction of oxygen occurs at a number of key points in photosynthesis and respiration, notably by semiquinone radicals, which perform an essential bioenergetic role in proton motive Q-cycles. Loss of control of gene expression by turnover of components of cytochrome *b/c* complexes may thus be a primary cause of oxidative stress and attendant pathological conditions.

5. BIOLOGICAL DISTRIBUTION

The two-component redox regulatory Arc and Nar systems and the redox repressor-activator FNR have all been most extensively studied in *E. coli*. [27–29]. However, there is no obvious reason why redox control of gene expression should be restricted to prokaryotic cells. The *lin-11* gene product of *Caenorhabditis elegans* [30] is probably a redox activator protein as defined here. The product of the *nifS* gene of yeast chromosome III [31] may also belong to this class. NifS in yeast was unexpected because of its evident homology with a protein regulating nitrogen fixation, a uniquely prokaryotic accomplishment. However, *nifS* is essential in yeast for mitochondrial function [31]. Further eukaryotic two-component redox regulatory proteins and redox activator and repressor proteins may be uncovered by genome sequencing projects.

The function of redox regulatory systems would be consistent with specific regulatory roles in mitochondria and chloroplasts [15,32,33]. Protein phosphorylation has been shown to be involved in regulation of tran-

scription of the *psbA* gene of the reaction centre of chloroplast photosystem II [34], a site particularly vulnerable to oxidative damage at high light intensity [26]. A chloroplast protein that may be involved simultaneously in electron transport and regulation of transcription is a nuclear-encoded DNA-binding membrane protein of 8 kDa with a pattern of cysteines similar to that of FNR [35]. The requirement for regulation of gene expression by redox potential may in principle explain the evolutionary maintenance, in eukaryotic cells, of the cytoplasmic genomes of chloroplasts and mitochondria [32,33].

Redox regulatory systems may fail, for example, under environmental conditions that lead to extreme redox potentials or as a consequence of mutation. In the event of such failure, redox damage is unlikely to be confined to chloroplasts and mitochondria. Nuclear genes encoding redox-shock proteins may then also have a requirement for redox regulatory control. It has been shown that the action of tumour necrosis factor in inducing tumour-specific gene expression is stimulated by inhibitors of mitochondrial electron transport [36]. The nuclear factor, NFκB, is an oxidative stress-responsive transcription factor involved in regulation of the pattern of gene expression determined by tumour necrosis factor and a variety of other agents [37].

An application of the idea that transcription may be controlled by redox potential per se (rather than always by specific chemical species such as oxygen or 'reactive oxygen intermediates') may lie in the interplay between nuclear and cytoplasmic responses. Redox imbalance is likely to be initiated in bioenergetic organelles and to be regulated within limits by their prokaryotic redox regulatory systems, while nuclear redox regulatory responses may arise as a more general defence against oxidative stress when the limits of organellar control are exceeded.

Acknowledgements: Related research in the author's laboratory is supported by the Swedish Natural Science Research Council and the Per-Eric and Ulla Schyberg Foundation.

REFERENCES

[1] Ronson, C.W., Nixon, T.B. and Ausubel, F.M. (1987) Cell 49, 579–581.
[2] Stock, J.B., Ninfa, A.J. and Stock, A.M. (1989) Microbiol. Rev. 53, 450–490.
[3] Guest, J.R., Green, J., Spiro, S., Prodromou, C. and Sharrocks, A. (1990) in: The Molecular Basis of Bacterial Metabolism (Hauska, G. and Thauer, R., eds.) pp. 134–145, Springer-Verlag, Berlin.
[4] Iuchi, S., Cameron, D.C. and Lin, E.C.C. (1989) J. Bacteriol. 171, 868–873.
[5] Iuchi, S., Chepuri, V., Fu, H.-A., Gennis, R.B. and Lin, E.C.C. (1990) J. Bacteriol. 172, 6020–6025.
[6] Iuchi, S., Matsuda, Z., Fujiwara, T. and Lin, E.C.C. (1990) Mol. Microbiol. 4, 715–727.
[7] Iuchi, S. and Lin, E.C.C. (1988) Proc. Natl. Acad. Sci. USA 85, 1888–1892.
[8] Stewart, V., Parales, J. and Merkel, S.M. (1989) J. Bacteriol. 171, 2229–2234.
[9] Klug, G., Gad'on, N., Jock, S. and Narro, M.L. (1991) Mol. Microbiol. 5, 1235–1239.
[10] Taremi, S.S. and Marrs, B.L. (1990) in: The Molecular Basis of Bacterial Metabolism (Hauska, G. and Thauer, R., eds.) pp. 146–151, Springer-Verlag, Berlin.
[11] Sganga, M.W. and Bauer, C.E. (1992) Cell 68, 945–954.
[12] Gilles-Gonzalez, M.A., Ditta, G.S. and Helinski, D.R. (1991) Nature 350, 170–172.
[13] Unden, G., Trageser, M. and Duchêne, A. (1990) Mol. Microbiol. 4, 315–319.
[14] Nunoshiba, T., Hidaloo, E., Amábile Cuevas, C.F. and Demple, B. (1992) J. Bacteriol. 174, 6054–6060.
[15] Allen, J.F. (1992) Biochim. Biophys. Acta 1098, 275–335.
[16] Harrison, M.A., Tsinoremas, N.F. and Allen, J.F. (1991) FEBS Lett. 282, 295–299.
[17] de Philip, P., Soupène, E., Batut, J. and Boistard, P (1992) Mol. Gen. Genet. 235, 49–54.
[18] Kahn, D. (1993) Mol. Microbiol. 8, 786–787.
[19] Drury, L.S. and Buxton, R.S. (1985) J. Biol. Chem. 260, 4236–4242.
[20] Stock, A.M., Stock, J.B. and Mottonen, J.M. (1990) Nature 337, 745–749.
[21] Johnson, L.N. and Barford, D. (1993) Annu. Rev. Biophys. Biomol. Struct. 22, 199–232.
[22] Kahn, D. and Ditta, G. (1991) Mol. Microbiol. 5, 987–997.
[23] Trageser, M., Spiro, S., Duchêne, A., Kojro, E., Fahrenholz, F., Guest, J.R. and Unden, G. (1990) Mol. Microbiol. 4, 21–27.
[24] Schultz, S., Shields, G. and Steitz, T.A. (1991) Science 253, 1001–1007.
[25] Neidle, E.L. and Kaplan, S (1992) J. Bacteriol. 174, 6444–6454.
[26] Aro, E.-M., Virgin, I. and Andersson, B. (1993) Biochim. Biophys. Acta 1143, 113–134.
[27] Guest, J.R. (1992) J. Gen. Microbiol. 138, 2253–2263.
[28] Gunsalus, R.P. (1992) J. Bacteriol. 174, 7069–7074.
[29] Iuchi, S. and Lin, E.C.C. (1993) Mol. Microbiol. 9, 9–15.
[30] Li, P.M., Reichert, J., Freyd, G., Horvitz, H.R. and Walsh, C.T. (1991) Proc. Natl. Acad. Sci. USA 88, 9201–9213.
[31] Oliver, S.G. et al. (1992) Nature 357, 38–46.
[32] Allen, J.F. (1993) Photosynth. Res. 36, 95–102.
[33] Allen, J.F. (1993) J. Theor. Biol. (in press).
[34] Tiller, K. and Link, G. (1993) EMBO J. 12, 1745–1753.
[35] Herrmann, R.G., Westhoff, P. and Link, G. (1992) in: Cell Organelles (Herrmann, R.G. ed.) pp. 275–349, Springer-Verlag, Wien.
[36] Schulze-Osthoff, K., Beyaert, R., Vandevoorde, V., Haegeman, G. and Fiers, W. (1993) EMBO J. 12, 3095–3104.
[37] Schreck, R., Albermann, K. and Baeuerle, P.A. (1992) Free Rad. Res. Commun. 17, 221–237.
[38] Anthamatten, D., Scherb, B. and Hennecke, H. (1992) J. Bacteriol. 174, 2111 2120.
[39] Liang, J.H., Scappino, L. and Haselkorn, R. (1993) J. Bacteriol. 175, 1697–1704.
[40] Fee, J.A. (1991) Mol. Microbiol. 5, 2599–2610.