Assembly and Repair of Membrane-Bound Electron Transport Complexes: Impact on Plant Physiology and Medicine

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Redox signalling in chloroplasts and mitochondria: genomic and biochemical evidence for two-component regulatory systems in bioenergetic organelles

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Abstract

Redox chemistry is central to the primary functions of chloroplasts and mitochondria, that is, to energy conversion in photosynthesis and respiration. However, these bioenergetic organelles always contain very small, specialized genetic systems, relics of their bacterial origin. At huge cost, organellar genomes contain, typically, a mere 0.1 % of the genetic information in a eukaryotic cell. There is evidence that chloroplast and mitochondrial genomes encode proteins whose function and biogenesis are particularly tightly governed by electron transfer. We have identified nuclear genes for 'bacterial' histidine sensor kinases and aspartate response regulators that seem to be targeted to chloroplast and mitochondrial membranes. Sequence similarities to cyanobacterial redox signalling components indicate homology and suggest conserved sensory and signalling functions. Two-component redox signalling pathways might be ancient, conserved mechanisms that permit endogenous control over the biogenesis, *in situ*, of bioenergetic complexes of chloroplasts and mitochondria.

Key words: evolution, photosynthesis, redox, regulation, respiration.

Abbreviations used: FNR, fumarate and nitrate reductase.

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Introduction

Free-living bacteria are usually opportunistic and flexible in the way in which they use energy and nutrient sources; bacteria rarely possess a single electron transport chain with a uniform set of components. This generalization applies both to respiration [1] and to photosynthesis [2]. In fact, many purple photosynthetic bacteria are able to switch between phototrophy and chemotrophy by changing the components of modular electron transport chains. For example, the same protonmotive cytochrome *b*–*c* complex can be used for either photosynthesis or respiration, merely by replacing the complexes to which it donates electrons and from which it accepts them. The various options for bacterial electron transport are selected on the basis of environmental and metabolic circumstances. An altered oxidation–reduction state of one or more components of the chain is usually the signal that initiates the appropriate change in gene expression.

There are two chief mechanisms by which redox reactions govern gene expression in bacteria [2,3]. One is exemplified by the FNR (for 'fumarate and nitrate reductase') redox activator–repressor of *Escherichia coli*, which is essentially a DNA-binding ferredoxin [4,5]. The other mechanism that couples electron transfer with gene expression is two-component redox signal transduction [3,6,7]. The latter mechanism employs a

redox sensor, a special case of the ubiquitous bacterial histidine sensor kinase. A redox sensor detects a change in redox potential that is initiated by altered light intensity or quality, or by a change in the availability of a respiratory substrate or a terminal electron acceptor.

It is likely that the control of gene expression by electron transport is an ever-present requirement of bioenergetic systems. Accordingly, the 'bacterial' components of the control pathways might have been conserved, even through the evolutionary transitions that gave rise to chloroplasts and mitochondria. There is evidence for direct coupling between electron transfer and genes for key components of electron transfer. The genes whose expression must be placed under endogenous control, together with genes for components of the required genetic system, might now comprise the genome of any bioenergetic organelle [3,7]. If this hypothesis is correct, then what the structural proteins encoded by organellar genomes have in common is that there is a premium on the direct redox control of their biosynthesis.

Two-component signal transduction

Signalling through two-component systems is widespread in bacteria [8]. The eponymous components are a sensor and an effector. The sensor is also described as a 'histidine kinase', 'histidine sensor kinase' or 'sensor histidine kinase'. The effector is now more commonly termed a 'response regulator'. There are actually two reactions catalysed by the 'sensor kinase' enzyme. The first is transfer of the γ-phosphate of ATP to a histidine side chain of the protein itself, to form a phosphoamide linkage (autophosphorylation):

$$\text{Sensor-His} + \text{ATP} \rightleftharpoons \text{Sensor-His} \sim \text{P} + \text{ADP} \quad (1)$$

The second reaction performs the transfer of the phosphate from the histidine residue to an aspartate residue on the corresponding effector or response regulator:

$$\text{Regulator-Asp} + \text{Sensor-His} \sim \text{P} \rightleftharpoons \text{Regulator-Asp} \sim \text{P} + \text{Sensor-His} \quad (2)$$

Because the phosphohistidine residue acts as a covalent chemical intermediate in the transfer of the phosphate group between ATP and the response regulator, 'sensor kinases' that catalyse the overall reaction:

$$\text{Regulator-Asp} + \text{ATP} \rightleftharpoons \text{Regulator-Asp} \sim \text{P} + \text{ADP} \quad (3)$$

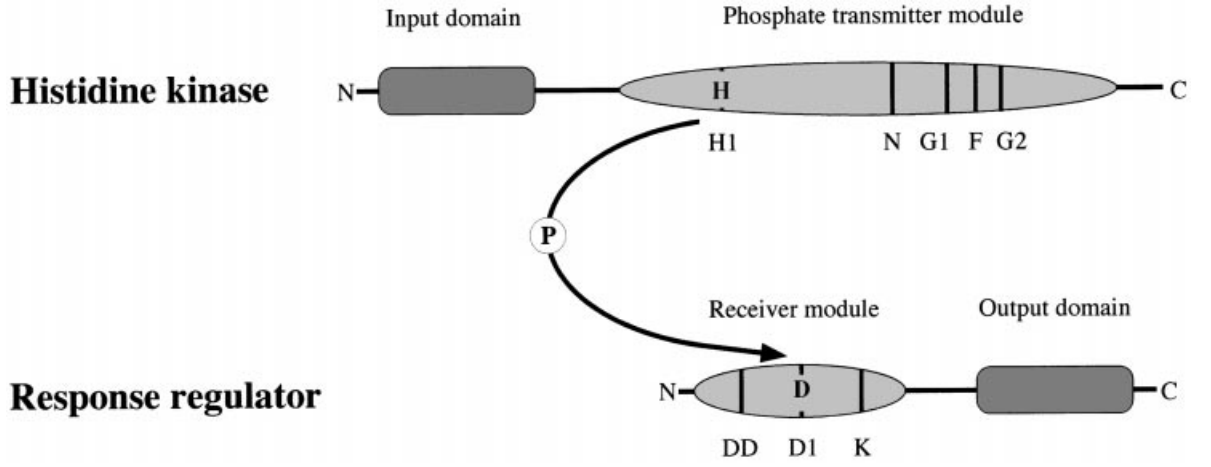
are really 'response regulator kinases' but the latter term does not correspond to current usage.

The histidine sensor kinase performs autophosphorylation if, and only if, an environmental precondition is met, and on an invariant histidine residue. The phosphate group is then transferred to the aspartate residue of one or more response regulators. Phosphorylation of the response regulator activates a response to the environmental change that produced the original histidine phosphorylation. A 'model histidine kinase' consists of an input domain and a phosphate transmitter module. The 'model response regulator' consists of a phosphate receiver domain and an output module (Figure 1).

Figure 1

Model for basic signalling through a two-component system and presentation of conserved motifs of histidine kinase phosphate transmitter modules and response regulator receiver modules

This figure is adapted from [9,10].



There are variations on and additions to these elements in two-component systems. For example, response regulators can occur without the output module, and histidine kinases can also contain, in addition to the input domain and the phosphate transmitter module, the sequence for a response regulator [8]. Histidine kinases of the latter type are called hybrid histidine kinases. There are many unrelated classes of input domains of histidine kinases, whereas the phosphate transmitter modules are relatively uniform and contain several stretches of conserved amino acids [8–10] (Figure 1). Several conserved motifs can also be found in the sequences of response regulators [8,10] (Figure 1). Two-component system elements of both prokaryotes and eukaryotes share the same conserved sequence signatures for histidine kinases and response regulators.

Organellar sensors and response regulators in plants

The chloroplastic genomes of rhodophytes such as *Porphyra purpurea* [11], the cryptophyte *Guillardia theta* [12] and the raphidophyte *Heterostigma akashiwo* [13] encode elements of two-component systems. In chlorophytes and land plants, the chloroplastic genomes themselves are apparently devoid of sensor and response regulator genes. However, the nuclear genomes of *Arabidopsis thaliana* and *Zea mays* contain genes coding for histidine kinases and response regulators that seem to be targeted to the chloroplast.

Using the program P-sort [14,15] we obtained estimates of the probabilities for subcellular locations of histidine kinases and response regulators from their predicted protein sequences in *A. thaliana* and *Z. mays* (Table 1). Only sequences with clear similarity to known two-component elements were considered. Histidine kinases should contain the H1, N, G1, F and G2 motifs; response regulators should contain stretches of amino acids corresponding to the DD, D1 and K motifs (Figure 1). We found a number of *A. thaliana* and *Z. mays* histidine kinase and response regulator protein sequences that seemed to be targeted to the chloroplast as well as a number of sequences that seemed to be targeted to the mitochondrion. The proposed subcellular locations of these sequences are shown in Table 1, each with an estimate, from P-sort, of the probability that the location is correct. Figure 2(a) shows part of the predicted amino acid sequence of the putative chloroplast thylakoid histidine kinase BAB09274 of *A. thaliana*. The sequence is aligned with those of a homologous protein from the cyanobacterium *Synechocystis* 6803 and of a plastid-encoded protein of the eukaryotic red alga *P. purpurea*. Figure 2(b) shows corresponding alignments for the putative chloroplast stromal response regulator AAD55287 of *A. thaliana* and its red algal and cyanobacterial homologues.

Phylogenetic analysis of predicted protein sequences of *A. thaliana* response regulators reveals two major groups: group A and group B. When subcellular localization is assigned with P-

Table 1

Predicted sensors and response regulators of photosynthetic organisms that are likely, in their mature forms, to be located in chloroplasts or mitochondria

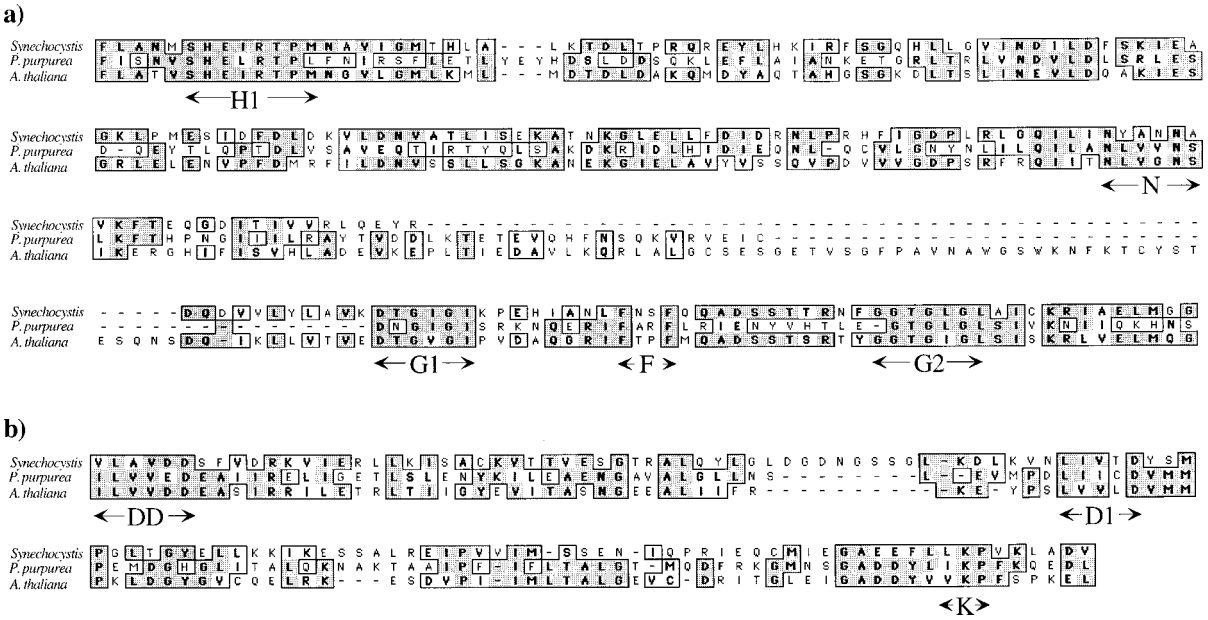
Predicted locations with $P < 1$ are derived from the program P-sort [14,15]. The probability value of 1 (*) is based on the consideration that the genes encoding these proteins are located in the chloroplast genome.

Protein ID	Organism	Type of two-component signalling element	Predicted subcellular location	Probability of location
ycf26	*Porphyra purpurea*	Sensor histidine kinase	Chloroplast	1*
BAB09274	*Arabidopsis thaliana*	Hybrid histidine kinase	Chloroplast thylakoid membrane	0.922
AAD03576	*Arabidopsis thaliana*	Hybrid histidine kinase	Chloroplast thylakoid membrane	0.632
ycf27	*Porphyra purpurea*	Response regulator	Chloroplast	1*
ycf29	*Porphyra purpurea*	Response regulator	Chloroplast	1*
AAD15535	*Arabidopsis thaliana*	Response regulator	Mitochondrial matrix space	0.628
AAD55287	*Arabidopsis thaliana*	Response regulator	Chloroplast stroma	0.864
BAB20581	*Zea mays*	Response regulator	Chloroplast stroma	0.938
BAA85112	*Zea mays*	Response regulator	Chloroplast thylakoid membrane	0.656
BAA85113	*Zea mays*	Response regulator	Chloroplast stroma	0.600

Figure 2

Sequence alignment of representatives of *Synechocystis* 6803, *P. purpurea* and *A. thaliana* histidine kinase phosphate transmitter motifs and response regulator receiver motifs

The representatives from *P. purpurea* are encoded by the plastid genome; the representatives from *A. thaliana* are putative chloroplast proteins. (**a**) Histidine kinases: slr2098 (*Synechocystis*), ycf26 (*P. purpurea*) and BAB09274 (*A. thaliana*). (**b**) Response regulators: slr1588 (*Synechocystis*), ycf27 (*P. purpurea*) and AAD55287 (*A. thaliana*). The positions of the different conserved motifs are indicated under the alignments. Identical or similar residues are shown in bold typeface; the former are set on a grey-shaded background.



sort, most members of group B seem to be targeted to the nucleus (results not shown). This localization has also been confirmed for two of the response regulator sequences, by transient transformation of protoplasts with fusions to green fluorescent protein [16]. We can assign the members of the group A response regulators to various subcellular locations, among them chloroplasts and mitochondria.

Two-component systems in mitochondria

Histidine phosphorylation is also present in mitochondria and sometimes has a primary role in catalysis rather than in signalling. For example, the α subunit of succinyl-CoA synthase, an enzyme of the tricarboxylic acid cycle, is phosphorylated on a histidine residue. In addition, a 37 kDa histidine phosphoprotein of plant mitochondria is proposed to be the α subunit of succinyl-CoA synthase [17].

Branched-chain α-ketoacid-dehydrogenase kinase is located in the mitochondrial matrix space [18]. This kinase from rat [19], mouse and human contains stretches of sequences, in the right order, similar to the H, N, G1 and G2 motifs characteristic of histidine kinases. Branched-chain α-ketoacid-dehydrogenase kinase does not seem to operate in the same way as the two-component system histidine kinase because it phosphorylates its substrate on serine rather than aspartate residues [20]. Nevertheless, its sequence does not resemble the sequences of serine/threonine kinases [19]. It has been proposed that branched-chain α-ketoacid-dehydrogenase kinase is an evolutionary intermediate of two-component histidine kinases and serine/threonine kinases [21]. Another enzyme of the mitochondrial matrix, pyruvate dehydrogenase kinase, is reported to contain the bacterial histidine kinase domain [22].

Evidence for redox control of gene expression in chloroplasts and mitochondria

The data in Figure 2 and Table 1 are consistent with the evolutionary hypothesis [3] that elements of bacterial signalling pathways remain, as nuclear-encoded components, in chloroplasts and

mitochondria. However, we currently have no direct indication of the function of these proteins. Nevertheless, there is a growing body of evidence that redox control of gene expression actually takes place in bioenergetic organelles. For chloroplasts, photosynthetic control of the transcription of reaction centre genes involves a signal of the redox state of the plastoquinone pool [23,24]. Furthermore, the transcriptional response is very rapid, taking place within a few minutes of perturbation of the redox state of plastoquinone, whether by selective illumination of photosystem I or photosystem II, or through the use of site-specific chemical inhibitors of electron transport [25]. Redox signalling in the control of expression of the genes involved in photosynthesis in plants is now well documented [26]. For mitochondria, the redox control of gene expression has been demonstrated at the level of protein synthesis *de novo* in isolated mitochondria, largely through use of site-specific electron transport inhibitors [27,28]. The site of control seems to be complex II (the succinate dehydrogenase complex) [28].

Whether the organellar two-component systems identified here are actually part of the mechanisms of redox signalling is the subject of current research. Certainly, cognate cyanobacterial sensors and response regulators [29] are involved in both state transitions and the regulation of photosystem stoichiometry [26], two manifestations of redox control in chloroplasts [25]. Preliminary biochemical indications that redox-responsive phosphoproteins occur in chloroplasts [25] and mitochondria [17] support the general idea that such pathways operate in bioenergetic organelles. Probing the function of these redox signalling pathways is an important direction for future research. This work is expected to provide a crucial test of the hypothesis that chloroplast and mitochondrial genomes contain the sum of those genes whose direct redox regulation *in situ* cannot be supplanted by a remote and incompetent nuclear–cytosolic level of control.

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