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Reply to Commentary

Reply to Commentary by Helmut Beinert and Patricia Kiley

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The van Niel equation provides a general description of photosynthetic assimilation of carbon dioxide:

$$2H_2A + CO_2 \xrightarrow{\text{light}} CH_2O + H_2O + 2A \qquad (1)$$

and its reverse (omitting light) is a general description of respiration. 'A' can be sulphur, nothing, organic acids such as fumarate, or oxygen, among others. Thus, photosynthesis and respiration are redox chemistry, and there is nothing special about oxygen, which is just one possible oxidation product and electron sink:

$$H_2O + CO_2 \xrightarrow{\text{light}} CH_2O + O_2 \qquad (2)$$

Admittedly, photosynthetic oxidation of water and respiratory reduction of oxygen are close to home, and the abundance of water (at 55 M), together with the large free energy change when 'A' equals 'O', has led to the dominance of oxygen chemistry in bioenergetics [1]. Within photosynthesis and respiration, however, common components are always involved [2], irrespective of the terminal electron donor or acceptor. Ref. [3] was written to draw attention to the accumulating evidence that the redox state of electron carriers may govern transcription, and to propose a nomenclature, based on that for two-component (sensor and response regulator) and one-component (repressor or activator) systems, that is logically consistent and that distinguishes between redox control and control by oxygen per se.

Beinert and Kiley [4] emphasize a possible role of reversible disassembly of iron–sulphur proteins in responses of gene expression to changes in oxygen concentration. Certainly, this process may be a component of specialist mechanisms whereby oxygen itself exerts an effect by means of iron–sulphur proteins, but haem proteins, too, can act as specific oxygen sensors [5]. At present we are only beginning to see the full range of prosthetic groups that can be involved in both redox control and oxygen control of gene expression.

Even if we confine our attention to iron–sulphur proteins (ferredoxins), the basic property of members of this group is oxidation-reduction. Most ferredoxins indeed have acid-labile iron–sulphur clusters, but pH remains relatively constant within the cell, and it is reasonable to assume that iron–sulphur clusters stay bound to their apoproteins during participation of the holoproteins in electron transport.

In experiments in vitro, the commonly used reducing agent, sodium dithionite, becomes rapidly oxidised by molecular oxygen, producing a number of strongly acidic species, including sulphites and sulphates. Dithionite stock solutions are often made up in anaerobic Tris buffer, to offset any effects of pH on the reaction under investigation. A more satisfactory way of distinguishing redox effects from side-effects of electron donors or acceptors is to carry out potentiometric redox titrations [6]. Here redox potential is measured continuously, and maintained, under anaerobic conditions, by addition of only small quantities of ferricyanide (to increase the potential) or dithionite (to decrease it). Apart from direct spectroscopic measurements, quite indirect processes such as protein phosphorylation can be measured at different redox potentials, and the mid-point (E_m) and n value of the controlling electron carrier can then be determined. In chloroplast thylakoids, for example, such an experiment provides direct evidence for redox control of protein phosphorylation, which is activated upon reduction of an electron carrier with $E_{m7} = 40$ mV, $n = 1$ [7]. Such a carrier could lie in close proximity to the high potential, Rieske iron–sulphur centre of the photosynthetic chain. As Beinert and Kiley point out [4], Rieske iron–sulphur proteins contain well-shielded, stable Fe_4S_4 clusters. This property, together with the apparent universality of Rieske iron–sulphur proteins in cytochrome *b-c* complexes [2], means that any regulatory role they possess is likely to be strict redox, not oxygen, control.

Oxygenic photosynthesis provides an example of strict redox control of protein phosphorylation and gene expression [7,8] where oxygen is continuously produced, and therefore most unlikely to be a hidden regulatory factor.

Since the 1993 letter [3], several developments can be singled out. One is the characterisation of RegB [9], the redox sensor cognate to the redox response regulator, RegA, of purple photosynthetic bacteria, and the Reg system controls transcription of the *puf* operon [10,11]. RegB is clearly an intrinsic membrane protein, becomes autophosphorylated on histidine, even in vitro, in response to decreasing redox potential, but has, as yet, no obvious prosthetic group or redox-active centre. Also in purple photosynthetic bacteria, CrtJ [12] is a single polypeptide whose affinity for a photosynthetic promoter is increased by increasing redox potential, suggesting that it acts as a redox repressor [3] in blocking transcription under oxidising conditions. From an evolutionary standpoint, we may predict that the established redox control of chloroplast and cyanobacterial membrane protein phosphorylation [7,13] and the emerging, parallel process in mitochondria [14] involve redox sensors, response regulators, and activator-repressor proteins homologous to those of purple photosynthetic bacteria.

Redox control of gene expression may have wide biological and evolutionary implications. One suggestion is that the function of the limited genetic systems of chloroplasts [15,16] and mitochondria [15] is to permit direct redox control of biosynthesis of key electron carriers of photosynthesis and

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respiration. As predicted, different redox conditions appear to select subsets of chloroplast and mitochondrial proteins for synthesis in vitro [17]. The majority of genes surviving from the endosymbionts ancestral to chloroplasts and mitochondria are now found in the nucleus, and a selective advantage for this location may be decreased free-radical mutagenesis [18]. The present distribution of genes between the three genomes of eukaryotic cells may thus be a consequence of an overriding requirement for redox control of expression of certain genes, but not of others [19]. If chloroplasts and mitochondria retain genes encoding proteins whose function is inherently mutagenic, replicating organelles should repress bioenergetic function, since otherwise organelle damage, which accumulates within and perhaps determines individual lifespan [19,20], would be inherited. The need for redox control of gene expression may thus entail separation of eukaryotic cell lines whose organelles are adapted to bioenergetic function from those whose organelles are adapted to replication. Such a division of labour is widespread, familiar, and characteristic of eukaryotes generally and muticellular organisms in particular [20].

Control by oxygen and control by oxidation-reduction are separate and distinct. I suggest that iron–sulphur proteins are most likely to participate in strict redox control, via electron transfer involving one or more Fe–S clusters of the intact holoprotein. Oxygen is just one oxidant, but its abundance and reduction to superoxide and cytotoxic and mutagenic by-products have exerted far-reaching effects. Redox chemistry is the cause: redox control is, in part, a cure.

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