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Bacterial responses to photo-oxidative stress

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Abstract

Singlet oxygen is one of several reactive oxygen species that can destroy biomolecules, microorganisms and other cells. Traditionally, the response to singlet oxygen has been termed photo-oxidative stress, as light-dependent processes in photosynthetic cells are major biological sources of singlet oxygen. Recent work identifying a core set of singlet oxygen stress response genes across various bacterial species highlights the importance of this response for survival by both photosynthetic and non-photosynthetic cells. Here, we review how bacterial cells mount a transcriptional response to photo-oxidative stress in the context of what is known about bacterial stress responses to other reactive oxygen species.

Many key biological processes are dependent on molecular oxygen (O_2). A consequence of the use of O_2 in bioenergetic or other metabolic pathways is the formation of reactive oxygen species (ROS). There are two classes of ROS, created through either electron transfer (type I) or energy transfer (type II) reactions[1,2] (FIG. 1). Electron transfer to O_2 can produce superoxide, hydrogen peroxide and hydroxyl radicals, all of which are toxic to cells. Study of the cellular responses to type I ROS has provided considerable information on the mechanisms that cells use to survive in their presence[3]. In the second type of reaction, energy transfer to O_2 results in the formation of singlet oxygen ($^1O_2$). In contrast to the responses to the first class of ROS, the cellular responses to $^1O_2$ have only recently begun to be analysed. Here, we review recent investigations that have led to the identification of genes and proteins that are involved in $^1O_2$-dependent stress responses.

Photosynthesis and evolution of oxygen

When photosynthetic organisms acquired the ability to produce O_2, they substantially altered the Earth's atmosphere and the forms of life that could be sustained[4,5]. In particular, the accumulation of atmospheric O_2 allowed the evolution of bioenergetic pathways, such as aerobic respiration, that couple the reduction of O_2 to the formation of a proton gradient[4,6]. The advent of aerobic respiration was followed by the evolution of complex organisms, including animals and plants.

ROS

Despite the energetic gains from the presence of atmospheric O_2, serious consequences also arose owing to the adventitious formation of ROS in respiratory and other reactions[1,3]. O_2, or dioxygen, is a stable diradical containing two unpaired, spin-aligned electrons in its outer *p* molecular orbitals[1,2] (FIG. 1). This spin-aligned configuration constrains the reactivity of O_2 with most non-radical molecules. However, these unpaired, spin-aligned electrons make O_2 receptive to accepting electrons[1,2]. A one-electron transfer reduction of O_2 produces a superoxide radical anion ($O_2^{\bullet-}$), commonly referred to as superoxide. An electron transfer

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reaction to superoxide produces a peroxide anion (O_2^{2-}), which exists as hydrogen peroxide (H_2O_2) in biological systems. In turn, H_2O_2 reacts with ferrous iron (Fe^{2+}) in the Fenton reaction to produce a hydroxyl radical ($OH^{\bullet}$).

The transfer of excitation energy to ground state (or triplet) O_2 can cause a rearrangement of the outermost electrons, producing one of two forms of $^1O_2$. The biologically relevant state of $^1O_2$, designated $^1\Delta g$, results when the spin-aligned, unpaired electrons of O_2 pair with each other, thereby producing an outer *p* orbital structure in which one orbital has paired electrons and the other orbital is empty[1,2,7] (FIG. 1). This electron rearrangement removes the spin restriction, converting O_2 to $^1O_2$, and makes $^1O_2$ the most reactive of oxygen species.

In vitro studies have shown that ROS cause damage to various biomolecules. Primarily, O_2^- and H_2O_2 damage proteins through oxidation[1,2], whereas $OH^{\bullet}$ damages DNA[1,2], resulting in various lesions that are potentially mutagenic and may even be lethal[1]. Cells can be killed by $^1O_2$, which has been shown to react with numerous cellular components *in vitro*, including membranes, proteins and DNA[1,7–9]. Each of these ROS (FIG. 1) can damage cells, but there is some disagreement as to which chemical reactions occur, and at what rates individual reactions occur, *in vivo*[1,10].

Sources of $^1O_2$

$^1O_2$ can be formed during solar energy capture, when photosynthetic pigments (in particular, chlorophyll) are raised to a higher energy level and reach a triplet excited state owing to the absorption of light energy[11,12] (FIG. 1). The pigments can then transfer energy to O_2 to produce $^1O_2$ (REFS [11,12]). Because of this well-known mechanism of forming $^1O_2$, the terms ‘photo-oxidative stress’ and ‘singlet oxygen stress’ are often used interchangeably. Other biological sources of $^1O_2$ production include energy transfer from excited photosensitizers, from the activity of several peroxidases, from the macrophage respiratory burst and from other enzymatic reactions[7,13] (FIG. 2).

Oxidative stress responses

The ROS molecules H_2O_2 and $O_2^{\bullet-}$ can be produced in aerobically grown bacteria by the auto-oxidation of respiratory chain components, by various endogenous activities or by exogenous agents[1–3]. Because ROS can cause damage to DNA, proteins and membranes, mounting a rapid response is crucial for survival[1].

Bacterial adaptive or stress responses to H_2O_2 and $O_2^{\bullet-}$ were identified over 20 years ago following the observation that low doses of either H_2O_2 or $O_2^{\bullet-}$ (generated by the biological activation of paraquat) could protect *Escherichia coli* and *Salmonella enterica* subsp. *enterica* serovar Typhimurium from subsequent exposure to higher doses that would otherwise have been lethal[14–16]. Using two-dimensional gel electrophoresis, it was shown that $O_2^{\bullet-}$ and H_2O_2 induced the synthesis of approximately 30–40 proteins when compared with non-challenged cells[15,17–20].

Further studies identified the transcription factors involved (OxyR for H_2O_2 and SoxRS for $O_2^{\bullet-}$), their target genes and how their activity is regulated[3]. OxyR activity is increased by H_2O_2. The OxyR regulon includes genes that are involved in oxidant elimination (*katG*, *ahpC* and *ahpF*), in maintenance of the balance between thiol groups and disulphide bonds (*gorA*, *grxA* and *trxC*) and in limiting Fe^{2+} availability (*dps* and *fur*) to minimize the occurrence of the Fenton reaction[3]. Once SoxR is activated by $O_2^{\bullet-}$, it directly stimulates transcription of *soxS*. SoxS acts in a second cascade of the transcriptional response to $O_2^{\bullet-}$ to directly activate gene transcription. The SoxRS regulon includes genes that are involved in O_2^- elimination (manganese superoxide dismutase (*sodA*)), in DNA repair (endonuclease IV (*nfo*)) and in

increasing cellular pools of reduced pyridine nucleotides for glutathione-dependent repair reactions (glucose-6-phosphate dehydrogenase (*zwf*)). Additional SoxRS regulon members include *fur*, superoxide-resistant isozymes of fumarase (*fumC*) and aconitase (*acnA*), which allow continued Krebs cycle function, and several flavodoxin genes (*fpr*, *fldA* and *fldB*), the products of which are presumably important for reducing Fe–S clusters[3]. Both the OxyR and SoxRS regulons also protect cells from other noxious chemicals, including reactive nitrogen species and organic solvents[3].

Photo-oxidative stress in bacteria

Most studies on the microbial or cellular response to ROS have centred on protection from $O_2^{\bullet-}$ and H_2O_2. However, in the past decade our understanding of the response to $^1O_2$ (or to photo-oxidative stress) has greatly improved.

Evidence for photo-oxidative stress in bacteria

Photo-oxidative stress and its consequences have been observed in numerous biological systems. The presence of photo-oxidative stress in bacteria was reported over 50 years ago, when the analysis of wild-type and carotenoid-deficient strains of the purple photosynthetic bacterium *Rhodobacter sphaeroides* showed that the presence of carotenoids protected the cells (BOX 1) from so-called photodynamic damage that was caused by the introduction of air to photosynthetically growing cultures[21]. Decades later, the identity of the toxic byproduct of photodynamic damage was discovered when triplet excited bacteriochlorophyll *a*(^{3}Bchl*a**) was identified as the light-excited sensitizer that reacts with O_2 to produce $^1O_2$ (REFS [11,12,22]). Carotenoids, along with Bchl*a*, are components of the photosynthetic apparatus, both in photosynthetic reaction centres (the bacterial ancestors of photosystems in O_2-evolving phototrophs) and light-harvesting complexes[12]. Carotenoids have been shown to directly quench $^1O_2$, but their primary protective effect *in vivo* is likely to be the rapid quenching of ^{3}Bchl*a** to reduce $^1O_2$ formation[12,22].

Box 1

Rhodobacter sphaeroides as a model system

Rhodobacter sphaeroides is a Gram-negative, purple non-sulphur bacterium that has a long history of serving as a model system for studies of photosynthesis and other metabolic processes[63,64]. This alphaproteo-bacterium is normally found in either freshwater or marine environments. *R. sphaeroides* is a facultative bacterium with the ability to grow using respiratory and photosynthetic lifestyles. This diversity of lifestyles makes *R. sphaeroides* the model system of choice in many studies, particularly regarding the process and control of photosynthesis. Results from bioenergetic, biochemical, genomic and other studies have provided unparalleled insight into mechanistic, structural and chemical details of the photosynthetic apparatus of *R. sphaeroides*[11,12,21]. Furthermore, the ease with which substantial amounts of singlet oxygen ($^1O_2$) are formed during light energy capture by the photosynthetic apparatus, the availability of systems to monitor $^1O_2$ effects in vivo and the ability to use genomic approaches make this bacterium the best system for defining the response to this reactive oxygen species.

Detection of $^1O_2$

Several methods have been used to detect $^1O_2$. In *R. sphaeroides* cultures, the change in Dane-Py fluorescence[23] was used to measure the amounts of $^1O_2$ *in vivo*24. As expected, $^1O_2$ was detected in both wild-type and carotenoid-deficient cells that had been exposed to high light levels, but more $^1O_2$ was detected in the carotenoid-deficient strain[24]. Both electron

paramagnetic resonance spin trapping with 2,2,6,6-tetramethylpiperidine[25] and direct near-infrared (NIR) emission at 1,270 nm[26,27] have been used to detect $^1O_2$ in *R. sphaeroides* reaction centres that were isolated from carotenoid-deficient strains. By contrast, $^1O_2$ was not detectable in wild-type reaction centres. Recently, a sensitive NIR emission method was used to provide the first *in vitro* detection of $^1O_2$ that was generated in wild-type *R. sphaeroides* reaction centres[28]. Given the high reactivity of $^1O_2$ and the sensitivity of the assays, it is possible that each of these methods underestimates the actual amount of ROS generated *in vivo* or *in vitro*, as each method only reports on the amount of ROS that interact with the probe. Conversely, when using exogenous photosensitizers or other chemical agents as sources of $^1O_2$ with whole cells, the effective concentration or primary site of action (for example, whether it is extracellular or in a subcellular compartment) of ROS is often unknown. Therefore, it is frequently difficult to determine the cellular concentration of $^1O_2$.

Responding to photo-oxidative stress

The ease with which $^1O_2$ is generated through photochemistry has made *R. sphaeroides* the bacterial model system to study the response to this ROS (BOX 1). To begin to investigate the genes that might be regulated by $^1O_2$, real-time PCR was used to examine the transcriptional activity of genes that were reasoned to have possible roles in the response[24]. Two candidate genes in this study were activated under photo-oxidative conditions (light plus methylene blue): *RSP_2389*, which encodes a putative glutathione peroxidase, and *RSP_0799*, which encodes a putative Zn-dependent hydrolase of the glyoxalase II family[24,29]. Oxidative damage to proteins can result in the production of protein peroxides, and glutathione peroxidase activity has been shown to detoxify protein peroxides *in vitro* through degradation[30]. Glyoxalase enzymes can cleave thioesters that are accumulated during oxidative stress; therefore, RSP_0799 may have a general role in removing several classes of glutathione adducts that are caused by $^1O_2$ stress[29]. Activation of a glutathione peroxidase-like gene is consistent with studies in the unicellular alga *Chlamydomonas reinhardtii*, in which transcription of a glutathione peroxidase homologue (*GPX5*; also known as *GPXH*) is increased following $^1O_2$ stress[31] (see below). Interestingly, expression of the *R. sphaeroides* carotenoid biosynthesis genes *crtA* and *crtI* did not increase under photo-oxidative stress conditions[24].

Given the importance of carotenoids during photo-oxidative stress in *R. sphaeroides*[21,25–27], when it was found that inactivation of an early enzyme in carotenoid biosynthesis, phytoene synthase (CrtB), slightly increased the activity of the *R. sphaeroides* alternative σ-factor, σ^E (also known as RpoE) (BOX 2), researchers proposed a connection between $^1O_2$ stress and this transcriptional regulator[32]. Using the promoter from *rpoE* (which is dependent on its own gene product, σ^E) fused to the β-galactosidase gene (*lacZ*), increased rates of β-galactosidase activity were measured after exposing anaerobic, photosynthetic cells to O_2 and light — conditions known to cause the production of $^1O_2$. A sustained increase in β-galactosidase activity from this reporter gene was observed only when both O_2 and light (at wavelengths known to excite pigments of the photosynthetic apparatus) were introduced to the photosynthetic cultures, consistent with the hypothesis that $^1O_2$ is an inducer of σ^E activity[32]. To provide independent support for this hypothesis, cultures grown aerobically (which therefore lack photosynthetic pigments) were exposed to light and methylene blue to induce $^1O_2$ generation. This confirmed that $^1O_2$ causes a marked increase in σ^E activity, as a high rate of β-galactosidase activity from the *rpoE–lacZ* reporter was only observed in the presence of oxygen, light and methylene blue. Control experiments in which one of the components needed to generate $^1O_2$ was omitted resulted in no significant increase in σ^E activity, further indicating that this ROS was responsible for the increase in σ^E-dependent transcription[32]. In addition, activation of this σ^E-dependent response did not occur when cells were exposed to other ROS[32], suggesting that $^1O_2$ is a specific activator of this transcriptional pathway. This σ^E-dependent transcriptional response was crucial to survival in the presence of $^1O_2$, as generation

of $^1O_2$ rapidly killed σ^E-deficient cells that were grown under conditions that restrict carotenogenesis[32]. Therefore, in *R. sphaeroides* loss of the quenching ability of carotenoids combined with the lack of the σ^E-dependent transcriptional pathway creates a synthetic lethal pair in the response to $^1O_2$.

Box 2

σ-factors

Bacterial transcription occurs through the function of RNA polymerase. Core RNA polymerase is a multiprotein complex composed of a β-, a β′- and two α-subunits. Promoter specificity is achieved when core RNA polymerase associates with one of many σ-subunits to form a holoenzyme[65]. The primary σ-factor is a member of the so-called σ^{70} superfamily and is referred to as the housekeeping σ-factor[66]. Alternative σ-factors provide a mechanism by which bacteria can control gene expression in response to certain environmental or developmental cues, including stress conditions. One class of σ-factor is the group IV σ-factors[50,51]. *Rhodobacter sphaeroides* σ^E (also known as RpoE) is a group IV σ-factor and, like many group IV σ-factors, it is typically maintained in an inactive state by forming a complex with its cognate anti-σ factor, ChrR[41–44]. σ^E–ChrR forms a heterodimeric complex from which σ^E is released on reception of the singlet oxygen signal[44]. Once released, σ^E positively autoregulates its own expression (by activating transcription of the *rpoEchrR* operon) and also activates transcription of additional target genes[41–44,47].

Other bacteria have been reported to mount a transcriptional response to $^1O_2$. Carotenogenesis is increased during photo-oxidative stress in *Myxococcus xanthus*[33–35]. In this non-photosynthetic bacterium, the tetrapyrrole protoporphyrin IX acts as an endogenous photosensitizer as it accumulates to high levels during stationary phase[33]. It has been shown that the activity of *M. xanthus* CarQ, an alternative σ-factor (BOX 2), is increased by photo-oxidative stress. In turn, CarQ increases the expression of the *carQRS* operon to initiate a gene expression cascade that promotes carotenogenesis and potentially other currently undiscovered functions that protect against $^1O_2$ (REFS [34,35]). Photo-oxidative stress conditions in *M. xanthus* carotenoid-deficient mutants result in cell lysis[33], presumably owing to $^1O_2$ toxicity. In the eukaryotic microorganism *Phaffia rhodozyma*, $^1O_2$ also controls carotenoid biosynthesis. Generation of $^1O_2$ in *P. rhodozyma* (using exogenous photosensitizers) caused a modest but sustained increase in carotenoid content when compared with that of non-challenged cultures[36]. Although the mechanism that is used to increase carotenoid content in *P. rhodozyma* has not been described, a possible explanation is that $^1O_2$ stress activates the expression of carotenoid genes.

In *E. coli*, forming $^1O_2$ through thermodissociation of exogenous disodium 3,3′-(1,4-naphthylidene) diproprionate ($NDPO_2$) endoperoxide activated expression of the SoxRS regulon in a *soxR*-dependent manner[37]. The effect of exogenous $NDPO_2$ was not altered by antioxidants, such as mannitol, glutathione, catalase or superoxide dismutase (SOD), suggesting that the response might be specific to $^1O_2$. In another study, overexpression of *E. coli* OxyR decreased the bactericidal effect of $^1O_2$ (which was generated by methylene blue plus light), diminished protein oxidation as measured by carbonyl content, and increased catalase and SOD-specific activities[38]. Consistent with this observation, an *oxyR* deletion mutant is hypersensitive to $^1O_2$ and exhibits increased protein damage in the presence of this ROS[38], suggesting that OxyR protects cells from photo-oxidative damage by increasing the expression of antioxidant enzymes.

In *Agrobacterium tumefaciens*, three iron-dependent SODs are reported to protect against $^1O_2$ that is generated by the photosensitizer rose bengal[39,40]. Each of these SODs is

reported to have a different expression pattern and cellular localization[39], but studies with strains containing single or multiple mutations indicate that SodBI has the largest protective role[40].

In considering some of the above work, the chemical differences between $^1O_2$ and $O_2^{\bullet-}$ or H_2O_2 (FIG. 1) make it difficult to explain how SOD activity or members of the OxyR or SoxRS regulons protect cells against photo-oxidative stress. For example, it is unclear whether there is specificity in the response to each type of ROS, whether there is a cross-protective ability of proteins in each stress response or whether cellular damage caused by $^1O_2$ can increase the production of other ROS.

$^1O_2$ stress response regulons

The finding that σ^E is a required part of the transcriptional response to $^1O_2$ in *R. sphaeroides* led to the investigation of target genes that are directly and indirectly activated by this ROS[32]. Wild-type cells have low σ^E activity owing to the sequestering function of ChrR, the anti-σ factor of σ^E (REFS [41–44]) (BOX 2). On the basis of this, expression profiles for wild-type and ΔChrR cells (which have constitutively increased σ^E activity) were compared to identify genes that are involved in this response[32]. As expected, *rpoE* showed increased expression in ΔChrR cells. In addition, ~180 genes, corresponding to ~60 operons, had a three-fold or more increase in expression in ΔChrR cells. When upstream promoter DNA from nearly half of these operons was tested, only four additional σ^E-dependent operons were identified (*RSP_1091–1087*, *RSP_2143–2144*, *RSP_0601* and *RSP_1409*; TABLE 1)[32], suggesting that the presence of $^1O_2$ had many indirect effects on gene expression. One of the direct targets of σ^E, *RSP_0601*, encodes an alternative σ-factor of the heat shock family (RpoH2)[45], showing that $^1O_2$ activates a transcriptional cascade[32] (FIG. 3). In an independent microarray study, these genes were also found to have increased expression in wild-type *R. sphaeroides* that was grown under semi-aerobic conditions in the presence of light[46]. On the basis of these observations, the challenge became to develop methods that could distinguish $^1O_2$-induced genes that were directly σ^E-dependent from those that were part of the response but dependent on RpoH2 or other unknown cellular adaptations to this ROS. The practical nature of this challenge is illustrated by the fact that genes (namely *RSP_2389* and *RSP_0799*) that were previously identified as being $^1O_2$ responsive[24,29] are not direct σ^E targets[32,47] (see below).

Direct σ^E target genes

To distinguish between direct and indirect σ^E targets, publicly available *R. sphaeroides* gene expression microarray data sets were analysed using hierarchical clustering to identify patterns of co-regulated genes. Of the genes that showed differential expression between wild-type and ΔChrR cells, one cluster contained the previously identified members of this regulon (including the *rpoE* operon) and *RSP_1852* (REFS [32,46,47]). As expected, when the upstream DNA sequences for each operon were aligned, a sequence motif with features that are typical of bacterial σ-factor binding sites was discovered. When this motif was converted into a position-specific weighted matrix (PSWM) and used to query a putative promoter library, 15 potential σ^E promoters were identified (TABLE 1) when at least 75% correspondence to the PSWM was required[47]. *In vivo* and *in vitro* testing of the new σ^E candidates indicated that *RSP_1852*, *RSP_3336* and *RSP_6222* are direct σ^E targets[47]. Weak activities of the *RSP_3336* and *RSP_6222* promoters may explain why they were not identified as candidates in the clustering of gene expression data[47].

To test whether this approach was successful in finding most or all of the σ^E target genes, chromatin immuno-precipitation microarray (ChIP-chip) assays were carried out to monitor the occupancy of σ^E or the β′ subunit of RNA polymerase (as a reporter of active transcription) across the *R. sphaeroides* genome[47]. As expected, the known strong σ^E-dependent promoters

showed enrichment for σ^E and β′ in ΔChrR cells[47]. Although a small number of additional potential σ^E binding sites were found in this analysis, none of these was located in upstream promoter DNA, suggesting that all of the major direct σ^E targets (15 genes in 9 potential operons; TABLE 1) have been discovered[47].

Indirect targets of σ^E

Interestingly, none of the other *R. sphaeroides* genes found to be differentially expressed when comparing wild-type and ΔChrR cells contained promoters matching the σ^E motif[47]. As RpoH2 is essential for viability during the $^1O_2$ response[48], some of these differentially expressed genes are likely to be targets of this alternative σ-factor. Results that are consistent with this hypothesis were reported recently for some of these genes[48]. In addition, the same high-throughput approaches have identified dozens of RpoH2 target genes in *R. sphaeroides*, some of which have been confirmed as direct targets by *in vitro* transcription assays (T.J.D., Y.S. Dufour and H.A. Green, unpublished observations). Some of these genes contain promoters that are only recognized by RpoH2 whereas others contain promoters that are also recognized either by RpoH1 (FIG. 3), a second heat shock σ-factor in *R. sphaeroides*[49], or by RNA polymerase containing the housekeeping σ-factor (T.J.D., Y.S. Dufour and H.A. Green, unpublished observations). RpoH1 and RpoH2 have previously been shown to transcribe promoters upstream of the same genes, but there are also gene promoters that are specific to one or the other of these heat shock family σ-factors[45] (FIG. 3).

σ^E–ChrR orthologues

Linking the knowledge discussed above about the *R. sphaeroides* $^1O_2$ stress response to the large microbial genome database provides an opportunity to test the evolutionary conservation of this system among bacteria. In *R. sphaeroides*, *rpoE* is co-transcribed with *chrR*[41] (BOX 2). Seventy-three bacteria containing a group IV σ-factor gene[50,51] (such as *rpoE*) adjacent to a *chrR* homologue were analysed, most of which were alphaproteobacteria and gammaproteobacteria, with only one betaproteobacterium and one deltaproteobacterium being found to contain σ^E and ChrR homologues[47]. A phylogenetic analysis of the σ^E and ChrR proteins across bacterial divisions also suggested that σ^E–ChrR evolved before the divergence of the alphaproteobacteria and gammaproteobacteria[47]. Although Cyanobacteria, which are predicted to be close relatives of the original oxygenic phototrophs[4,5], contain multiple group II alternative σ-factors[51], isolates with sequenced genomes do not contain group IV σ-factors[51] or homologues of σ^E and ChrR[47]. This has been taken as evidence that the σ^E-dependent bacterial response to $^1O_2$ did not precede the accumulation of atmospheric O_2 (FIG. 4) from the water-splitting activity of photosystem II from ancestors of modern-day cyanobacteria[52]. Therefore, the natural history of σ^E and ChrR suggests that they, and also this photo-oxidative stress response (see below), originated later than the Great Oxidation Event that gave rise to molecular oxygen[52].

It should be noted that the genome sequence data set used for this analysis could be biased towards alphaproteobacteria and gammaproteobacteria, as most of the bacterial genome sequences that are currently available are from these groups. However, the failure to identify σ^E and ChrR homologues in the large number of enteric bacteria that were included in this analysis is evidence that this system is not present in all members of the gammaproteobacteria[47]. Until genome sequences from a more representative set of other bacterial divisions become available, the full extent of the conservation of the σ^E–ChrR pair or the transcriptional response to $^1O_2$ in the bacterial kingdom will not be known.

The core σ^E–ChrR regulon

To explore the extent and content of the photo-oxidative stress networks across bacterial species, the same 73 genomes were analysed for genes that are orthologous to those in the *R.*

sphaeroides σ^E–ChrR regulon and for genes with promoters that contain the σ^E motif. When a phylogenetically determined PSWM was used to score the promoter regions of annotated genes in the various genomes to identify possible σ^E homologue binding sites, many of the known *R. sphaeroides* σ^E–ChrR regulon members were identified in members of the alphaproteobacteria and gammaproteobacteria. The most conserved σ^E targets, which constitute a so-called core σ^E–ChrR regulon across species (TABLE 1), include the *rpoE–chrR* operon (loci *RSP_1092–RSP_1093* in *R. sphaeroides*), *phrA* (*RSP_2143* in *R. sphaeroides*), cyclopropane fatty acyl-phospholipid synthase (*cfaS; RSP_2144* in *R. sphaeroides*) and *RSP_1091–RSP_1087* (REF. [47]). Therefore, it is not unreasonable to propose that these photosynthetic and non-photosynthetic bacteria encounter $^1O_2$ in their environment[47]. Alternatively, some bacterial species might use the σ^E–ChrR system to respond to other stresses. Recent studies of the σ^E–ChrR system in *Caulobacter crescentus* have shown a rapid response to $^1O_2$ and organic hydroperoxides and a slower response to both ultraviolet A and cadmium exposure[53], suggesting that there are either additional signals in some bacteria or methods by which one stress can activate other systems.

The predicted core σ^E–ChrR regulon includes the positive master regulator (*rpoE*) and its inhibitor (*chrR*) and encodes proteins that are proposed to be involved in the cellular response to $^1O_2$ (REF. [47]). In the presence of $^1O_2$, the integrity of the membrane fatty acid bilayer may be maintained by *cfaS*, which encodes a putative cyclopropane fatty acid synthase. Using its substrate, *S*-adenosylmethionine, as a methyl donor, CfaS creates a methylene bridge across unsaturated fatty acid double bonds[54]. In the case of $^1O_2$ assault, this modification would make the membrane less accessible to chemical modification, thereby minimizing susceptibility to any further damage that could result in increased membrane permeability in the presence of $^1O_2$. RSP_1091 and RSP_1090 have unknown functions but do have limited amino acid similarity to cyclopropane fatty acid synthetases. Repair of light-induced damage to DNA may be accomplished by PhrA, a predicted DNA photolyase[55] that could repair pyrimidine dimers. Additional important functions are likely to be carried out by proteins that are encoded by the core σ^E–ChrR regulon, as the functions of some of these genes are unknown, and there are few clues regarding their putative roles[47].

The extended σ^E–ChrR regulon

A group of genes that are not as prevalent among the 73 bacterial species that were analysed seem to constitute an extended σ^E–ChrR regulon. As with the core regulon, the extended regulon contains several genes of unknown function[47]. There are also gene sets that are found mostly in specific groups or genera of bacteria, suggesting that there are functions in this response that may be associated with the environment in which these organisms are found[47].

The so-called extended σ^E–ChrR regulon often contains *RSP_0601* (REF. [47]), which encodes RpoH2[45]. RpoH2 is found in only a few alphaproteobacteria and in none of the gammaproteobacteria for which genome sequences are currently available[47]. Most alphaproteobacteria that have RpoH2 homologues also have σ^E binding motifs in the RpoH2 promoter region[47]. This suggests that the σ^E–RpoH2 cascade is a conserved transcriptional network in this group of alphaproteobacteria for the response to $^1O_2$. One of the genes that is directly transcribed by RpoH2 is *RSP_2389*, which encodes a putative glutathione peroxidase (T.J.D., Y.S. Dufour and H.A. Green, unpublished observations), a class of enzymes that is known to be a key part of the defence against ROS in animals and plants[56,57].

Interestingly, *RSP_2389* homologues in several gammaproteobacteria that lack RpoH2 but contain σ^E–ChrR homologues have a conserved σ^E motif in their promoter regions[47]. This suggests that *RSP_2389* is directly activated by σ^E in some organisms that lack the second, RpoH2-dependent part of the photo-oxidative stress cascade. These two transcriptional network schemes for the activation of bacterial *RSP_2389* homologues, together with studies

examining the role of the homologous *GPX5* in algae[31] (see below), indicate that glutathione peroxidase is a part of the photo-oxidative stress response that is conserved across kingdoms.

$^1O_2$ and oxygenic phototrophs

Photo-oxidative stress responses have also been studied in eukaryotic phototrophs. Unlike purple photosynthetic bacteria (which are anaerobic phototrophs that do not form O_2 during photochemistry), eukaryotic systems are continually generating $^1O_2$, as photosystem II activity both generates triplet state chlorophyll pigments and releases O_2 (REFS [5,58]).

The first demonstration of gene induction by $^1O_2$ in a photosynthetic organism was that of *GPX5* in *C. reinhardtii*[31]. This induction was rapid, robust and showed specificity for $^1O_2$, with $O_2^{\bullet-}$ and H_2O_2 having only small effects on GPX5 expression[31,59,60]. Further studies provided evidence that *C. reinhardtii* can acclimatize to $^1O_2$ stress with the help of GPX5 and glutathione *S*-transferase[61], highlighting the importance of these detoxifying functions in the $^1O_2$ response. In recent *C. reinhardtii* studies, $^1O_2$ was detected in the cytoplasm[62], providing evidence for either a signalling pathway between the thylakoid membrane (which is the source of this ROS) and the nucleus or a second pathway that generates $^1O_2$ outside the chloroplast.

Conclusions and future directions

Determining how cells sense and respond to $^1O_2$ stress is necessary for a complete understanding of cellular survival to this toxic ROS. The recent use of traditional, high-throughput and genomic approaches has greatly advanced our understanding of photo-oxidative stress responses in both photosynthetic and non-photosynthetic bacteria. The prevalence of the σ^E–ChrR proteins that control this response and of the conserved core members of the σ^E regulon across diverse bacterial species suggest that $^1O_2$, generated by either light-dependent or light-independent processes, is encountered more widely in nature than has previously been appreciated. In the future, it will be interesting to identify the sources of $^1O_2$, particularly in the many non-photosynthetic bacterial species that are predicted to contain the regulators and target genes of a response that was initially identified in the photosynthetic bacterium *R. sphaeroides*. Much work remains to be carried out to determine the mechanistic details of how cells sense, respond to and protect themselves from $^1O_2$, both in bacteria and eukaryotes. Currently, it is also unclear whether bacteria and eukaryotes use similar gene products or processes to combat $^1O_2$ stress. Some of the genes that are induced by $^1O_2$ stress have proposed protective or repair functions, providing some insight into the nature of this response. However, many genes encode proteins of unknown function, which is currently preventing us from comprehending the full extent of the cellular response to $^1O_2$.

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Glossary

Diradical	A molecular species with two electrons occupying two degenerate molecular orbitals, typified by high reactivity and a short lifespan
Fenton reaction, The reaction in which free	$H_2O_2 + Fe^{2+} \rightarrow OH^- + FeO^{2+} + H^+ \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$

ferrous iron (Fe^{2+}) transfers an electron to hydrogen peroxide (H_2O_2) to produce a hydroxyl radical ($OH^{\bullet}$)	
Triplet excited state	The higher energy state of a molecule that is characterized by an electron paramagnetic resonance spectrum with three peaks
Photosensitizer	A chemical compound that readily undergoes photoexcitation and then transfers its energy to other molecules, resulting in a reaction mixture that is sensitive to light
Respiratory burst	The rapid release of reactive oxygen species from neutrophils and macrophages of the immune system
Paraquat	A redox cycling agent that can be oxidized by dioxygen, resulting in the production of superoxide; also called methyl viologen
Isozymes	Any of the electrophoretically distinct forms of an enzyme, which represent different polymeric states but have the same function
Carotenoids	A class of pigments that are widely distributed in nature
pigments can be yellow	orange, red or purple
Photosynthetic reaction centre	A site where molecular excitations originating from light are transformed into a series of electron transfer reactions. It is composed of a multiprotein complex containing pigmented cofactors (such as chlorophyll)
Phototroph	An organism that uses light as a source of metabolic energy
Dane-Py	(3-(*N*-diethylaminoethyl)-*N*-dansyl-aminomethyl-2,5-dihydro-2,2,5,5-tetramethyl-1-*H*-pyrrole.) A fluorescent molecular trap that is specific for singlet oxygen
excitation occurs at 337 nm	with emission at 545 nm
Electron paramagnetic resonance spin trapping	Experiments in which a particular electron spin state of a molecule is isolated to measure its spectrum
σ-factor	A specificity subunit of prokaryotic RNA polymerase that directs the RNA polymerase holoenzyme to the promoter DNA of specific genes. The primary σ-factor in cells is responsible for the recognition of so-called housekeeping genes, and alternative σ-factors are devoted to specialized functions
Methylene blue	A photosensitizer that is used to generate singlet oxygen when it is exposed to both oxygen and light
Rose Bengal	A widely used stain that also functions as a photosensitizer, specifically to generate singlet oxygen from ground state triplet oxygen
Position-specific weighted matrix	A statistical representation of patterns in biological sequences, composed of a matrix of score values that gives a weighted match to any given substring of fixed length

Photolyase	An enzyme that repairs DNA that has been damaged by ultraviolet light exposure by breaking pyrimidine dimers
Thylakoid	An internal membrane system occupying the main body of a plastid; particularly well-developed in chloroplasts.

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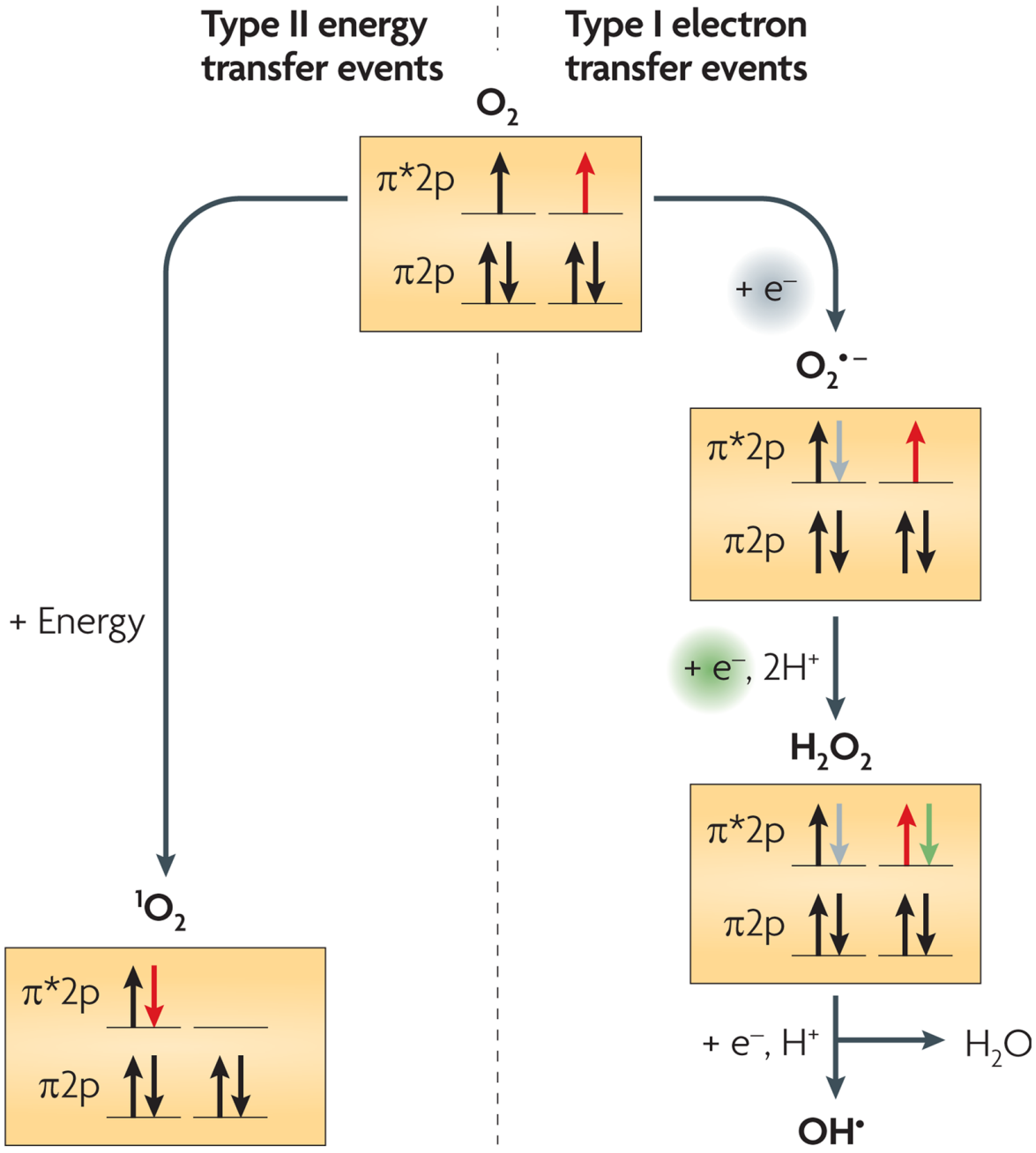

Figure 1. Types of reactive oxygen species

Electron or energy transfer events generate the two main types of reactive oxygen species. The schematic shows the changes in occupancy of the outer *p* orbitals of molecular oxygen (O_2) during the formation of these reactive oxygen species[1,2].

H_2O_2, hydrogen peroxide; $^1O_2$, singlet oxygen; $O_2^{\bullet}$, superoxide; $OH^{\bullet}$, hydroxyl radical.

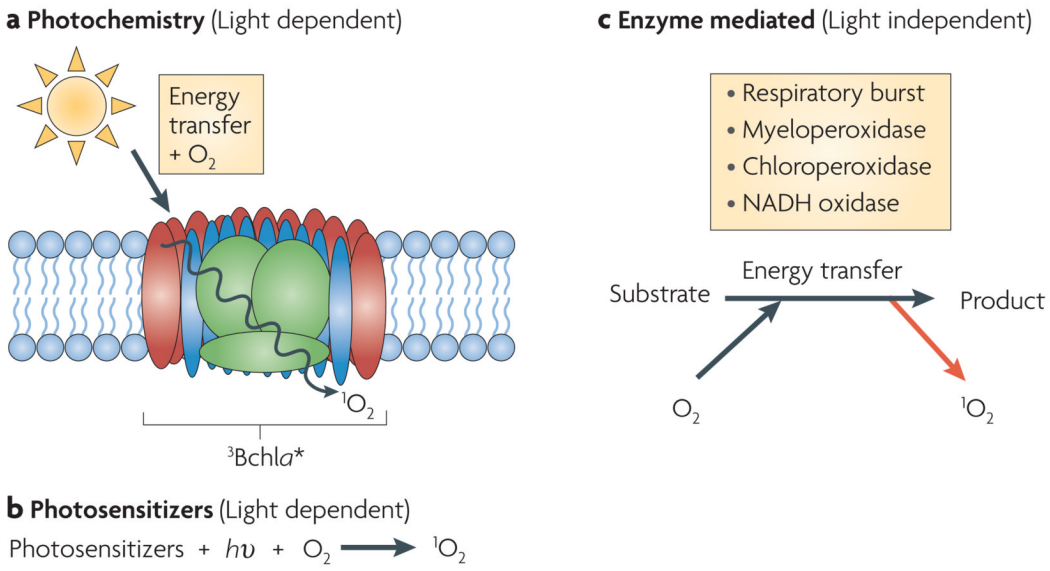

Figure 2. Sources of singlet oxygen

a,**b**. Major light-dependent sources of singlet oxygen ($^1O_2$) include energy transfer to molecular oxygen (O_2) from excited triplet state pigments such as bacteriochlorophyll *a* (^{3}Bchl*a**) in photosynthetic membranes (part **a**) and energy transfer from excited natural or exogenous photosensitizers, such as tetrapyrroles and methylene blue or rose bengal, respectively (part **b**). **c**. Light-independent pathways that generate $^1O_2$ in biological systems include enzymes (such as myeloperoxidase, chloroperoxidase and NADH oxidase) that generate this type of reactive oxygen species as a consequence of catalysis[7,13]. *hυ*, light energy.

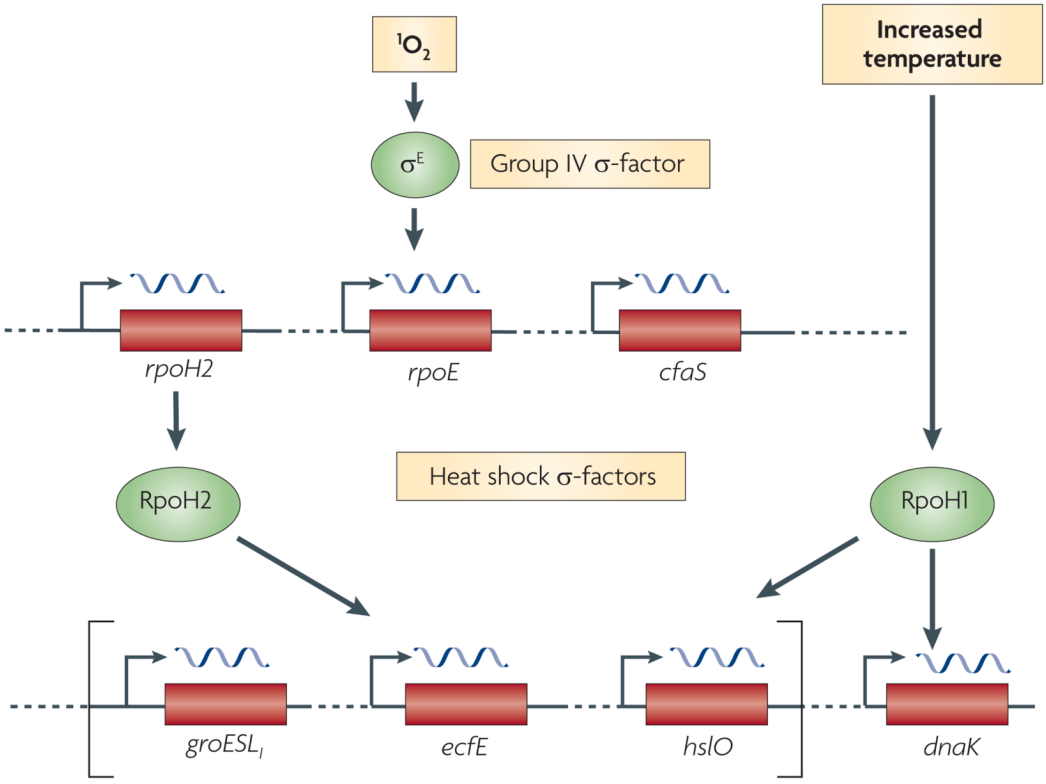

Figure 3. Singlet oxygen activates a gene expression cascade in bacteria

The cascade of transcriptional circuits that is activated by singlet oxygen ($^1O_2$) in bacteria is shown. In *Rhodobacter sphaeroides*, the formation of $^1O_2$ increases the activity of the group IV alternative σ-factor, σ^E (BOX 2). Genes that are directly transcribed by σ^E encode proteins that are predicted to protect cells from $^1O_2$ and repair damage caused by this reactive oxygen species (see text), as well as RpoH2, one of two homologues of proteins from the heat shock (σ^{32}) family of alternative σ-factors[45,47]. Although RpoH2 and RpoH1 can transcribe promoters upstream of common genes (shown in square brackets), there are other genes containing promoters that are recognized by only one of these two related σ-factors[45]. *cfaS*, cyclopropane fatty acyl-phospholipid synthase.

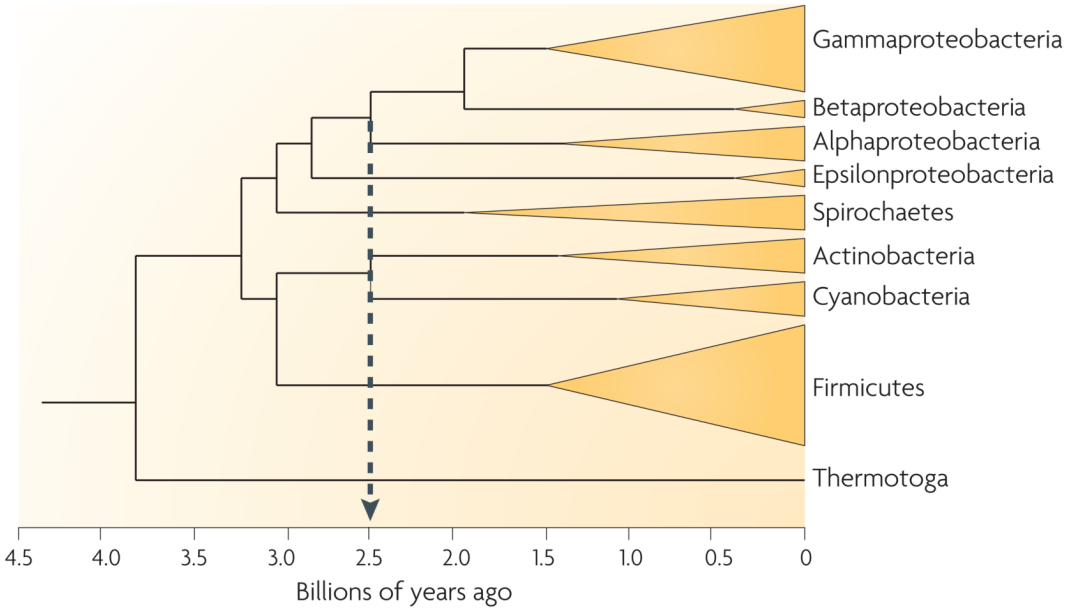

Figure 4. Natural history of the σ-^{E}-dependent bacterial response to singlet oxygen

The phylogenetic tree traces the approximate time of branching among selected important microbial groups. Most organisms that are known or predicted to contain the σ^{E}-dependent transcriptional response to singlet oxygen are members of the alphaproteobacteria or gammaproteobacteria. The dotted grey line indicates the time when the water-splitting activity of photosystem II by ancestors of modern-day cyanobacteria led to the accumulation of atmospheric O_2. Image modified from REF. [52].

Table 1

Members of the *Rhodobacter sphaeroides* σ^E–ChrR regulon*

locus designation	gene name	Annotation‡
RSP_1092§	*rpoE*	RNA polymerase σ^{70} factor||
RSP_1093§	*chrR*	Anti-σ factor (ChrR)||
RSP_2144§	*cfaS*	Cyclopropane fatty acyl-phospholipid synthase (CfaS)
RSP_2143§	*phrA*	DNA photolyase; cryptochrome 1 apoprotein (blue-light photoreceptor)
RSP_1091§	Unknown	Putative cyclopropane or cyclopropene fatty acid synthesis protein; flavin amine oxidase
RSP_1090§	Unknown	Putative cyclopropane or cyclopropene fatty acid synthesis protein
RSP_1089§	Unknown	Sugar or cation symporter; from the GPH family
RSP_1088§	Unknown	Hypothetical protein
RSP_1087§	Unknown	Short-chain dehydrogenase or reductase family member
RSP_0601	*rpoH2*	RNA polymerase σ-factor||
RSP_1409	Unknown	β-Ig-H3 or fasciclin
RSP_1852	Unknown	Hypothetical protein
RSP_0296	*cycA*	Cytochrome c_2||
RSP_3336	Unknown	ABC spermidine or putrescine transporter; inner-membrane subunit
RSP_6222	Unknown	Hypothetical protein

*Data from REFS [32,47].

‡Product name from the IMG website: http://img.jgi.doe.gov/cgi-bin/pub/main.cgi.

§Gene known or predicted to be encoded in a polycistronic operon.

||Gene product for which a biochemical function has been shown *in vitro*. ABC, ATP-binding cassette; Ig, immunoglobulin.