The Evolution of an Osmotically Inducible \textit{dps} in the Genus \textit{Streptomyces}

Paul D. Facey*, Matthew D. Hitchings, Jason S. Williams, David O. F. Skibinski, Paul J. Dyson, Ricardo Del Sol

Institute of Life Science, College of Medicine, Swansea University, Swansea, United Kingdom

Abstract

Dps proteins are found almost ubiquitously in bacterial genomes and there is now an appreciation of their multifaceted roles in various stress responses. Previous studies have shown that this family of proteins assemble into dodecamers and their quaternary structure is entirely critical to their function. Moreover, the numbers of \textit{dps} genes per bacterial genome is variable; even amongst closely related species - however, for many genera this enigma is yet to be satisfactorily explained. We reconstruct the most probable evolutionary history of Dps in \textit{Streptomyces} genomes. Typically, these bacteria encode for more than one Dps protein. We offer the explanation that variation in the number of \textit{dps} per genome among closely related \textit{Streptomyces} can be explained by gene duplication or lateral acquisition, and the former preceded a subsequent shift in expression patterns for one of the resultant paralogs. We show that the genome of \textit{S. coelicolor} encodes for three Dps proteins including a tailless Dps. Our \textit{in vivo} observations show that the tailless protein, unlike the other two Dps in \textit{S. coelicolor}, does not readily oligomerise. Phylogenetic and bioinformatic analyses combined with expression studies indicate that in several \textit{Streptomyces} species at least one Dps is significantly over-expressed during osmotic shock, but the identity of the ortholog varies. \textit{In silico} analysis of \textit{dps} promoter regions coupled with gene expression studies of duplicated \textit{dps} genes shows that paralogous gene pairs are expressed differentially and this correlates with the presence of a \textit{sigB} promoter. Lastly, we identify a rare novel clade of Dps and show that a representative of these proteins in \textit{S. coelicolor} possesses a dodecameric quaternary structure of high stability.

Background

Almost two decades after the first Dps protein (PexB) was characterized in \textit{E. coli} [1,2], there has been a continued effort to identify and characterize homologous genes in other prokaryote genomes. Subsequently, Dps proteins have been found in almost all the bacterial groups, including archaea [3]. As a result, the literature now contains an abundance of data on Dps proteins; providing a wealth of knowledge pertaining to their structure [4], interaction with DNA [5] and their importance in the stress response [6]. Interestingly, there is an apparent evolutionary link between the iron sequestering Ferritin (and bacterioferritin) proteins and Dps proteins [7] and, moreover there also appears to be ferritin-like proteins that share functional properties of Dps [8]. Indeed, a few studies have elucidated the crystalline structure of a few Dps proteins [9,10] and have shown that like ferritins, Dps proteins assemble into oligomers (albeit dodecamers as opposed to 24mers) and their overall three-dimensional shape is entirely critical to their function [11]. Moreover, it is because of the smaller nature of Dps oligomers (when compared to ferritins) that Dps proteins are often referred to as mini-ferritins; as opposed to maxi-ferritins [12]. However, despite similarities between these two protein families, they can be distinguished based on examination of their secondary structure. For example, Dps have a small central helix (often called the BC helix) which is absent in ferritins. Similarly, ferritins possess a helix towards their C-terminus that is absent in Dps [13]. However, both ferritins and Dps both form similar tertiary structures.

The current literature suggests that Dps proteins provide macromolecule protection either by oxidizing and storing ferrous iron in a bioavailable form or by binding and physically shielding DNA. Iron detoxification, which Dps proteins contribute to through the abatement of the Fenton reaction, occurs at the ferroxidation centre. These sites lie at the interface between two anti-parallel subunits [10] and references therein) and are therefore found within the hollow, inner cavity of the self-assembled dodecamer. However, not all Dps proteins have ferroxidase activity and not all bind DNA [14]. To the exterior of the dodecamer, the variable length N- and C-terminal tails of each monomer have been implicated in DNA binding and dodecamer assembly [13,16]. For example, removal of the N and C terminal tails of \textit{Mycobacterium smegmatis} Dps-1 prevents assembly of the dodecamer. However, other Dps proteins are “tailless” and still assemble into dodecamers [17] suggesting that, although in some genera the tails are important for dodecamer assembly, this is not always the case. However, whilst differences in tail length may reflect important variation in structure (and possibly function), this has only been studied in a few bacteria; studies on
the evolution and origin of these tails appears scant. Indeed, until recently, the focus of many Dps studies has been on their role in protection during nutrient limitation and under oxidative conditions, usually through the abatement of Fenton Chemistry [18]. However, there now appears some recognition of the multifaceted roles of dps genes [see 19,20,21,22,23] and that different Dps proteins within the same genome may be capable of different functions. With increasing numbers of completely sequenced microbial genomes, multiple dps homologs within the same species are now being identified and their structures and functions elucidated [24,25,26]. Having more than one dps gene per genome is common amongst bacteria [27]. However, for many genera, variation in the number of dps per genome amongst closely related species is yet to be satisfactorily explained. This is exemplified in species of mycobacteria. Gupta et al., [28] and Roy et al., [29] characterized two dps genes in free-living Mycobacterium smegmatis. Yet, remarkably, dps homologs are absent in the genomes of closely related, but pathogenic, M. leprae, M. bovis and M. tuberculosis. Additionally, more intricate studies are now characterizing the control of dps at the transcriptional level [27,30] and, not only is there evidence for multiple sigma factor complexes contributing to the transcription of dps [30,31] but, it also appears that expression of these genes may be driven by suites of different sigma factors e.g. msds1 [31] and msds2 [27]. Together, this suggests that the evolutionary history of dps in many genera contains duplications, losses and possible lateral acquisitions.

The aim of the present study is to investigate the evolutionary history of the dps genes in Streptomyces. Recent studies have identified three dps genes (herein named dpsASc, dpsBSc and dpsCSc) in the genome of the model actinomycete S. coelicolor [19], dpsASc has been shown to be part of the well characterized, osmotically-induced Sigma B regulon and is significantly overexpressed as a result of osmotic stress and heat shock [19,30]. Similarly, dpsCSc is also mildly induced during heat shock. However, despite contributing to nucleoid compaction, dpsBSc appears not to be induced during osmotic or heat shock. Phylogenetic analysis indicates that horizontal gene transfer (HGT) and gene duplication are plausible explanations for the distribution of dps genes among Streptomyces.

Results

Distribution of DpsSc orthologs in Bacteria

We used the three Streptomyces coelicolor Dps proteins as BLASTP queries for homology searches among Bacterial and Archaeal lineages. Our searches yielded 1120 unique protein sequences and included sequences from 299 completely sequenced prokaryote genomes across 8 different phyla. Retrieved sequences varied in annotation and included Dps family ferritin, starvation induced dps, ferritin, DNA-binding Dps and hypothetical proteins. All sequences were confirmed to be Dps by the possession of signature amino acids and the helical pattern characteristic of Dps protein’s secondary structure [6,19] (figure 1 provides a schematic representation showing the positions of the helices in the three Dps proteins in S. coelicolor). The results of our homology searches showed that the number of dps genes per genome was variable – even amongst closely related species. However, most (75%) genomes encoded for only one Dps protein, with 18% encoding for two Dps proteins, 5% encoding for three Dps proteins and less than one percent of all genomes scrutinized had more than 3 dps genes.

Interestingly, there was no significant (P>0.05; Mann-Whitney) distributional bias in the number of homologs per genome among the 8 phyla. Similarly in Streptomyces, the number and distribution of dps genes among closely related species was unequal. Moreover, the genomes of S. coelicolor and S. ghanaensis are within a minority of bacteria that contain 3 dps genes.

Variation in tail length of bacterial Dps and assembly and stability of S. coelicolor Dps

We uncovered significant differences in the distributions of tail lengths in Dps proteins within and among bacterial genera. Interestingly, all tail lengths appeared to occur randomly throughout the bacterial phyla - and this variation is also evident in Streptomyces. Moreover, not only does the S. coelicolor genome encode for three Dps proteins, each of these has different tail lengths (Figure 1). However, as expected from the variable distribution of Dps in Streptomyces, this pattern is not conserved. Indeed, in our dataset, the presence of three dps genes in the same genome, each encoding for a protein with a different secondary structure is unique. In other genomes that have three dps (e.g. S. ghanaensis) at least two Dps homologs have similar tail lengths. For example, in S. ghanaensis, two Dps are orthologous to DpsBSc and have both short N- and C-terminal tails and the other is orthologous to DpsASc and has a longer N-terminal tail (compared to C-terminal tail). The most frequent tail length arrangement (83% of all Dps proteins in Streptomyces) is short/negligible tails, making this secondary structure almost ubiquitous in Streptomyces. Only 30% of Dps proteins in Streptomyces have longer C-terminal tails and 20% with longer N-terminal tails.

A native protein Western blot (Figure 2A) revealed that DpsASc and DpsCSc readily assemble into higher oligomeric states in vivo in contrast to DpsBSc that does not. Moreover, our in vivo observations of DpsASc shows that it appears to be present in two major species on a 7% native PAGE gel (lanes 1 – 3). The mobility of the lower DpsA oligomer indicates that it is significantly smaller than a dodecamer (but larger than DpsB) whilst the upper species is believed to be a dodecamer. S. coelicolor Dps oligomers also display differences in their resistance to denaturation. Of the two Dps that assemble into dodecamers (DpsA and DpsC), we found that DpsC dodecamers are significantly more resistant to denaturation than those of DpsA. The native PAGE gels (Figure 2B) indicate that whilst the dodecamer of DpsA is denatured by 8 M urea, the DpsC oligomer is much more stable. Even after extended incubation with 8 M urea, the DpsC dodecamer exhibits very little disassembly - indicating that the molecular interactions maintaining the oligomeric structure are very strong.

The distribution of orthologous protein clusters and tail lengths

Highlighted on the Maximum-likelihood reconstructed phylogeny of Actinobacterial Dps sequences (figure 3) are three distinct clades. These correspond to the three S. coelicolor orthologous Dps protein clusters. Remarkably, proteins that are orthologous to DpsASc are rare in bacteria, and even rarer in Streptomyces – certainly, orthologs of DpsASc were found in only five Streptomycetes. Thus, this very narrowly distributed clade contains very interesting Dps proteins. Similarly, orthologs of DpsBSc are rare in Streptomyces, and, this is suggestive of non-linear inheritance. In contrast, many Streptomycetes possess an ortholog of DpsCSc (indicated in figure 3 by the large cluster of Streptomycetes) and the origin of this large cluster of orthologous proteins has a very deep node - indicative, maybe, of very early divergence in the Streptomyces. In all cases we found that the GC content of dps genes was consistent with the local GC content of neighbouring genes and also the genome average.
Duplication and expression of Dps in *Streptomyces* genomes

Our expression analysis shows that there is a trend for at least one *dps* per genome to be upregulated during osmotic stress although the identity of the upregulated ortholog differs among *Streptomyces* (Figure 4). Moreover, possession of an osmotically regulated *dps* appears to have arisen in many genomes after gene duplication. The ML phylogenetic tree identifies a duplication of *dps* genes in a few *Streptomyces* genomes. The genomes of *S. avermitilis*, *S. scabies*, *S. ghanensis*, *S. griseoflavus*, *S. viridochromogenes* and *S. sviceus* contain two highly similar copies (pairwise mean percentage similarity = 97% ± 1.5 S.D) of a *dps* that is orthologous to *dps*ASc. Interestingly, expression analysis after osmotic upshock shows that only one member of each paralogous gene pair is induced (Figure 4) and these group together within the tree (Figure 3).

Figure 5 summarizes the results of our searches for *sigB*-like promoter motifs upstream of *dps* genes in 17 completely sequenced *Streptomycetes*. Although *dps*ASc is transcribed from a *sigB*-like promoter, our approach of mapping putative *sigB* promoters using an *in silico* approach revealed that not all orthologs of *dps*ASc in other species possess a recognizable SigB-dependent promoter. Furthermore, for species that lack an ortholog of *dps*ASc, or where this ortholog is present but lacks a *sigB*-like consensus promoter sequence upstream, the genome often contains an alternative ortholog with a putative SigB-dependent promoter. In species where we identified a duplicated *dps*, in all cases one of these copies had a *sigB* promoter motif. Moreover, in four species tested, we confirmed that *dps* genes with a *sigB*-like promoter motif upstream of their ORF are significantly upregulated during osmotic stress (Figure 2).

Gene synteny and chromosomal location of *dps* in *Streptomyces*

In order to provide insights into an evolutionary history of *dps*, which may have included gene duplications and lateral acquisitions in *Streptomyces*, we performed synteny comparisons among all completely sequenced and assembled Streptomycete genomes. In addition, we also compared the chromosome locations of *dps* genes in 17 streptomycete genomes. Our comparisons of gene location (Figure 5) showed that orthologs of *dps*ASc and *dps*CSc are generally located outside of the core genome (i.e. in the chromosome arms - demarcated using a consensus of existing published coordinates [32,33,34]); the exceptions are in *S. albus* and *S. griseus*. The distribution of *dps*ASc orthologs is more complex. In species where we identified duplicated copies of *dps*ASc, at least one of these copies is always located nearer to the ends of the chromosome. In contrast, very poor gene synteny occurred around the *dps*ASc without a *sigB*-like promoter. The other copy, without the *sigB* promoter is found more centrally in the chromosome. In addition, a comparison of the genomic neighborhood around these paralogs (figure 6) revealed a high degree of gene conservation around the *dps* lacking a putative *sigB*-like promoter.

Figure 1. Amino acid alignment of *S. coelicolor* Dps proteins. Amino acid alignment of the three *S. coelicolor* Dps proteins showing the position of the five characteristic Dps helices along with the position of the different length N- and C-terminal tails. doi:10.1371/journal.pone.0060772.g001

Figure 2. A: Immunoblot of a native PAGE gel. Immunoblot showing the in vivo oligomeric state of DpsASc, DpsBSc, and DpsCSc overexpressed using a thiostrepton inducible promoter. Overexpression of DpsA (lane A), DpsB (lane B) and DpsC (lane C) from a thiostrepton inducible promoter. Black arrow indicates the non-dodecameric DpsB. B: Coomassie blue stained native PAGE gel assessing the stability of assembled *S. coelicolor* Dps oligomers. Coomassie blue stained native PAGE gel showing the stability of assembled *S. coelicolor* Dps oligomers after incubation with 8 M urea. Lane A− = DpsASc without 8 M urea, lane A+ = DpsASc + 8 M urea, Lane C− = DpsCSc without 8 M Urea, lane C+ = DpsCSc + 8 M Urea. doi:10.1371/journal.pone.0060772.g002
the copy where we identified a sigB-like promoter motif upstream of corresponding ORFs. Orthologs of dpsASc and dpsCSc displayed very poor gene synteny in the genomes analysed.

Discussion

The ancestral dps in Streptomyces possessed short N- and C-terminal tails and was not involved in the osmotic stress response

Our in silico predictions, coupled with expression analysis, is suggestive that in many Streptomyces there is a requirement for an osmotically inducible dps. However, the presence of such a dps within the genomes of Streptomyces appears fairly recent in evolutionary history. Indeed, we provide evidence that an osmotically inducible dps arose either after gene duplication and functional divergence or, in other cases, through the lateral acquisition of an osmotically inducible dps from other Actinobacteria. In support of the former, the orthologous relationships in many Streptomyces are on a one-to-many basis - e.g. S. coelicolor contains a single copy of dpsBSc, yet, S. avermitilis, S. scabies, S. ghanensis, S. griseoflavus, S. virochromogenes and S. sviceus contain two homologous dpsB sequences with a high pairwise percentage similarity. The occurrence of two highly similar copies of dps in these genomes provides strong evidence for paralogy. Interestingly, a very distinct dichotomy could be observed among paralogous gene pairs based on chromosome location, gene synteny and presence of a putative sigB-like promoter motif. Principally, paralogous pairs could be divided into (i) those that were located within 2 Mb of the chromosome ends, had a poorly conserved genomic neighbourhood and possessed a sigB-like promoter motif upstream and (ii), those that were located within the chromosome core, had a highly conserved genomic neighbourhood and did not possess a sigB-like promoter motif.

In addition, the presence of a sigB promoter motif also conferred expression after osmotic upshock in all paralogs tested. This, coupled with the almost ubiquitous nature of the second class of dpsB genes in the genomes of most Streptomyces makes them ideal candidates for the ancestral dps within this genus. Certainly, in support of this, Bentley’s comparisons between S. coelicolor and C. diphtheriae chromosomes indicated that the last common ancestor (LCA) of these taxa may have shared the core region (but not the arms) of the S. coelicolor chromosome. Hence, as the core of the C. diphtheriae genome, like many Streptomycetes, possesses an ortholog of dpsBSc, this is consistent with the hypothesis that the LCA encoded a short-tailed Dps protein and is the ancestral dps in the genus Streptomyces. Therefore, osmotically-inducible dpsBSc orthologs appear to have arisen more recently; possibly as a result of an environmental pressure for osmotic protection. Furthermore, despite the large amount of data implementing Dps tails in the binding of DNA during the stress response, the fact that we show the presence or absence of tails does not correlate with osmotic inducibility suggests that tails are not required for the function of these proteins under osmotic shock.

Figure 3. Majority rule consensus phylogenetic tree of Actinobacterial Dps orthologous proteins. Maximum-likelihood reconstructed phylogenetic tree of Actinobacterial Dps proteins. Indicated are three orthologous protein clusters (shaded boxes). DpsASc, DpsBSc and DpsCSc indicates the position of S. coelicolor Dps in the tree. Paralogous gene pairs of DpsB in Streptomyces are indicated using matched Roman numerals. σB indicates those proteins where a putative sigB-like promoter was identified. Bootstrap values >60% are indicated next to major nodes.

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Figure 4. Normalized fold change in *dps* transcript abundance among five *Streptomyces* in response to osmotic stress. q RT PCR monitoring of *dps* transcript abundance after 1 hour incubation with 250 mM KCl in S. co. = *S. coelicolor*; S. gha = *S. ghanaensis*; S. alb = *S. albus*; S. av = *S. avermitilis*; S. ven. = *S. venezuelae*. Relationships to *S. coelicolor dps* orthologs are indicated in parentheses. *dps* transcript abundance is normalized to principal sigma factor *hrdB*. Paralogous gene pairs are sequentially numbered. Presence of a *sigB*-like promoter motif upstream of the ORF is indicated with an asterisk. Error bars represent standard deviation of the mean normalized transcript abundance. A broken Y-axis has been used.

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Figure 5. *dps* location maps among 17 *Streptomyces* chromosomes. Approximate chromosome location of *dps* orthologs in 17 *Streptomyces* chromosomes. Species names are abbreviated to the left of the diagram: S. gha = *S. ghanaensis*; S. griseof = *S. griseoflavus*; S. prist = *S. pristinaespiralis*; S. co. = *S. coelicolor*; S. liv. = *S. lividans*; S. alb = *S. albus*; S. flavo = *S. flavogriseus*; S.gris = *S. griseus*; S. virido = *S. viridochromogenes*; S. scab = *S. scabies*; S. aver = *S. avermitilis*; S. clav = *S. clavuligerus*; S. gris. gris = *S. griseus* subsp. *griseus*; S. him = *S. himastatinicus*; S. svic = *S. sviceus*; S. griseoaur = *S. griseoaurantiacus*; S. venez = *S. venezuelae*. Chromosomes were orientated based on the centrally located initiator protein (black arrow). Light grey arrows represent orthologs of *dpsASc*, clear arrows represent orthologs of *dpsCSc*, striped arrows represent orthologs of *dpsBSc*. Figures above arrows represent approximate chromosome location in Mb. Figures in parentheses = chromosome size. Asterisks indicate those genes where we identified *sigB*-like promoter motifs upstream of ORFs. Scale bar = 1 Mb.

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Evidence for lateral acquisition of dps genes in Streptomyces

Phylogenetic reconstruction using all retrieved Dps orthologs indicates that the evolution of Dps proteins in some bacterial species results from HGT rather than by lineal descent. Indeed, HGT has already been demonstrated for dps genes elsewhere (e.g. the lactic acid bacterium, Lactococcus spp.; [35]) and recent evidence purports that HGT as a mechanism is prevalent in Streptomyces [36]. More specifically, it has been shown that the terminal regions at either end of many Streptomyces chromosomes are “hot spots” for laterally acquired genes - with these regions spanning up to 2 Mb in length [32]. Certainly, in S. coelicolor, our data are suggestive of HGT as both dpsA and dpsC are found within such a region [23]. Together, this would suggest that, at least in S. coelicolor, dpsA and dpsC were additions to the genome. Certainly, orthologs of DpsA and DpsB were under-represented in other Streptomyces species (being found only in S. coelicolor and S. albus) making the evolution of these orthologs inconsistent with a common ancestry. Interestingly, in agreement with other studies on actinobacterial Dps proteins in the DpsA clade (i.e. that of Mycobacterium smegmatis; [37]), DpsA orthologs also assemble into two different sized oligomers – suggesting that proteins within this clade may assemble in a similar fashion and could therefore have these similar evolutionary origins. Furthermore, as with Mycobacterium smegmatis Dps, the removal of the C-terminal tail of DpsA and N-terminal tail of DpsC in S. coelicolor confers an inability of these proteins to assemble correctly (unpublished).

In support of lateral acquisition of dpsC-like genes, structural and phylogenetic comparisons highlight a clade containing only nine actinobacterial sequences. This is despite the initial BLAST searches using very relaxed E-values. Interestingly, this clade is populated by some species that have been shown to grow in high temperature environments [26–29]. Thus, this very narrowly distributed clade contains very interesting Dps proteins. Indeed, S. coelicolor DpsC protein dodecamers are highly resistant to denaturation by urea (8 M over night incubation). Our initial modeling studies of this group of proteins (unpublished) suggest that the very long N-terminal tails of these proteins may act to stabilize the dodecamer. As only a small number of species contain orthologs of this gene, they are more likely to have been acquired by horizontal gene transfer rather than having been lost in the majority of species.

The fact that DpsB in S. coelicolor does not assemble in our in vivo observations is also very interesting. Whilst it is possible that DpsB assembles under different conditions to the other two Dps proteins in S. coelicolor, we hypothesise that this protein (and orthologs of this protein in other Streptomyces), being the ancestral Dps in this genus, may have become redundant as the number of dps genes increased in these genomes. However, further analyses are required to test this.

Conclusions

In summary, the presence of an osmotically inducible dps in Streptomyces has resulted from duplications and lateral acquisitions. That dpsC is very poorly represented in the bacterial lineage is interesting, and this, coupled with the stability of DpsCs, dodecamers, is suggestive of either very selective acquisition of a gene encoding for a protein with a highly specific function or, significant gene loss. Certainly, the proteins within this clade warrant further study. Moreover, through environmental selection pressure, the genomes of many Streptomyces now contain an osmotically induced dps. This may have arisen in two ways. Either by duplication, whereby one of the paralogs later becomes part of the osmotically inducible SigB regulon, or, in the absence of such an event, Streptomyces have acquired an osmotically induced dps that is subsequently transcribed as part of the SigB regulon. The order with which these events have occurred is difficult to ascertain. However, the absence of dpsB but the presence of dpsA with an upstream sigB-like promoter motif in S. flavogriseus and S. albus is suggestive that acquisition of dpsA preceded or paralleled duplication of dpsB in other species. In those species where an osmotically induced dps is absent, or where our in-silico analysis has not identified a sigB promoter motif upstream of a dps ORF (i.e. S. venezuelae), this may indicate that there is not a requirement for this trait in the host environment - indeed, S. venezuelae is significantly less salt tolerant than S. coelicolor (unpublished). Lastly, the variable distribution of dps genes in other bacterial genomes is consistent with the patterns we have shown for Streptomyces. In summary, we propose that the evolutionary pathway that we describe here, which involves both HGT and gene duplication, may be applicable to the evolution of Dps proteins in many other species.

Methods

Bacterial strains, media, RNA isolation and Q RT PCR

Streptomyces strains were grown at 30°C on the surface of MS (mannitol soya flour) agar or on cellophane discs [38]. Liquid cultures of nutrient broth were set up in 10 mL volumes. Liquid cultures were incubated at 30°C with shaking (250 r. p. m.). To
induce osmotic stress, cellophane disc cultures were grown on MS agar for 16 hours and then transferred to MS agar containing 250 mM KCl. For each species tested, total RNA was isolated from three independent cultures (5 biological replicates), reverse transcription and q RT PCR procedures were performed as previously described [30]. The quantification of dps ortholog transcript abundance was performed on five Streptomyces species. These were specifically chosen to provide Streptomyces that, (i) possessed three different Dps proteins (S. coelicolor), (ii) duplicated dps with and without putative sigB promoters (S. avermitilis and S. ghanensis and S. albus), and (iii) a single dps, orthologous to dpsC_{S. venezuelae}.

Secondary structure predictions, we manually counted the number of alpha helix for each protein. Structures (i.e. those with confidence levels lower than 40%) were grouped together with bacterioferritin/ not Dps). In addition, to characterize Dps proteins, secondary structure annotations were scrutinized and poorly predicted structures were discarded. In all species, specificity of the reaction was assessed using melt analysis. Fold change in transcript abundance was calculated using the efficiency corrected Pfaffl method [39].

Results from these initial searches were then re-blasted back to correct for erroneous annotations and to exclude bacterioferritins from the orthologous data list, firstly, a distance based limiting results to S. coelicolor. Secondly, the secondary structure of all Streptomyces putative Dps orthologs and S. coelicolor, were predicted using JPRED3 [43]. All secondary structure annotations were scrutinized and poorly predicted gene in all samples. Specificity of the reaction was assessed using melt analysis. Fold change in transcript abundance was calculated using the efficiency corrected Pfaffl method [39].

Obtaining an oothologous data set of Dps proteins

An actinobacterial orthologous data set of Dps proteins was obtained using a Reciprocal Best Hit (RBH; [40]) approach. Briefly, the three S. coelicolor Dps protein sequences were used as queries for retrieving homologous sequences using the Blastp algorithm [41] and a relaxed E value threshold of 1E−05. Default parameters were used throughout - however, to limit data redundancy, we retrieved sequences from the refseq database. Results from these initial searches were then re-blasted back limiting results to Streptomyces coelicolor. Only proteins that came out as best hits bi-directionally were retained. Sequences of retrieved proteins were aligned using ClustalW implemented in Molecular Evolutionary Genetics Analysis software (MEGA; [42]). Moreover, to correct for erroneous annotations and to exclude bacterioferritins from the orthologous data list, firstly, a distance based (Neighbor-joining) tree was reconstructed that included all putative Dps orthologs and Streptomyces coelicolor bacterioferritin (Genbank: NP626370) and secondly, the secondary structure of all proteins were predicted using JPRED3 [43]. All secondary structure annotations were scrutinized and poorly predicted structures (i.e. those with confidence levels lower than 40%) were manually removed. Sequences were excluded from the data set if they grouped together with S. coelicolor bacterioferritin and also lacked the characteristic BC helix of Dps (i.e. those sequences that were bacterioferritin/ not Dps). In addition, to characterize Dps homologs in terms of N- and C- terminal tail length, using the secondary structure predictions, we manually counted the number of amino acid upstream of the first and downstream of the last alpha helix for each protein.

Gene synteny and chromosome location of dps genes in completely sequenced Streptomyces genomes

In the absence of horizontal gene transfer and duplication, the chromosome location of orthologous genes and gene synteny surrounding them is often conserved amongst closely related species. Thus, we chose to compare location of dps genes in 17 completely sequenced and assembled Streptomyces genomes. In addition, we manually compared gene synteny of genes surrounding dps. Chromosome coordinates of orthologous dps genes used to construct gene location maps were retrieved from NCBI. To calibrate chromosome orientation, we chose to use the direction of the initiator protein, dnaA, located in the central oriC region of streptomycete chromosomes [44,45].

Phylogenetic reconstruction

A Maximum-likelihood based phylogeny using Actinobacterial Dps ortholog sequences was reconstructed using MEGA using the amino acid substitution model JTT + F + I. The consensus tree was drawn using the Majority Rule criteria. To assess the robustness of inferred phylogenies, we used 500 bootstrap pseudoreplicates.

Identification of SigB dependent promoters upstream of dps genes

The conservation of a sigB-like regulon among dps orthologs in Streptomyces was investigated using an in-silico approach. An in-silico search using the degenerative motif GNNTN(N) 14–16 GGGYAY was performed on the complete genomic sequences of 17 Streptomyces using COMplex PAttern of sequence search software (COMPAsss; [46]). Organisms were only included in the analysis if they were classified down to the species level. The occurrence of this motif, which represents the −10 and −35 regions of the dpsA/sigB recognizable promoter [30] in the 17 Streptomyces genomes, was retrieved using the COMPAsss built-in database mining capability linked to the NCBI nucleotide database. Generated hit tables were scrutinized for putative functional promoters by limiting hits to those found in intergenic regions and to motifs located within 200 nucleotides of dps ORFs.

Protein methods

Assembly of S. coelicolor Dps into higher state oligomers was investigated in vivo using C-terminally His-tagged dpsA_{Sco}, dpsB_{Sco} and dpsC_{Sco} under the control of a thiostrepton inducible promoter to ensure sufficient protein concentration levels, encoded respectively by plasmids pDpsA9, pDpsB9 and pDpsC9 [19]. For overexpression, 24-hour liquid cultures were spiked with the antibiotic thiostrepton (25 μg ml⁻¹) for protein induction and incubated for a further hour. Cells were pelleted by centrifugation (5 000 x g for 5 min) and the media replaced with sonication buffer (50 mM Tris-HCl, pH 8, 200 mM NaCl, 15 mM EDTA, Complete protease inhibitor cocktail (Roche Diagnostics). Cells were disrupted by sonication on ice (20 seconds at 20% amplitude). Cell free extracts were obtained by centrifugation (13 000 x g for 2 min at 4°C) and removal of the supernatant. Equal volumes of supernatant were mixed with NativePAGE sample buffer (Invitrogen) and proteins were separated in a 7% polyacrylamide gel using a tris-glycine running buffer excluding sodium dodecyl sulphate (SDS) at 4°C. After electrophoresis, gels were soaked in 1 X SDS running buffer for 30 min and then equilibrated for 5 min in cold Bjerum and Schafer-Nielsen transfer buffer. Proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Hybond-P, Amersham) using a semi-dry electrophoretic transfer cell (Trans-Blot SD, BioRad). His-tagged proteins were detected with a Penta- His peroxidase conjugate (Qiagen). Immunological detection was performed using an ECL Advance Western bloting detection kit (Amersham Pharmacia Biotech). The stability of assembled S. coelicolor Dps dodecamers was investigated using recombinantly expressed Dps. Briefly, C-terminal translocation fusions to 6xhistidine tag were created as follows: the coding region of dpsA_{Sco} and dpsC_{Sco} were excised from plasmids pDpsA9 and pDpsC9 [19] as NdeI/BglII fragments and subcloned into pET26b(+)- digested NdeI/BglII to create pDpsA14 and pDpsC14 respectively. Plasmids were transformed into E. coli BL21 (DE3) and grown in 1 L cultures of 2 X YT until mid-log phase. Expression of recombinant Dps proteins was performed at 30°C for 3 h after the addition of isopropyl-1-thio-D-galactoside (IPTG) to a final concentration of 0.1 mM. Cells were harvested by centrifugation and resuspended in a sonication buffer (20 mM
| Description                      | Source                                      |
|----------------------------------|---------------------------------------------|
| E. coli BL21 (DE2)               | fhuA2 [lon] ompT gal (DE3) [dcm] ΔhsdS5 ΔEcoRI-8 int::lacI::PlacUV5::T7 gene1 i21 Δnis5 |
| E. coli ET12567 (pUZ8002)        | Dam13::Tn9 dcm6 hsdM hsdR recF143 16 zj201::Tn10 galK2 galT22 ara14 lacY1 xylS leuB6 thi1 tonA31 rpsL136 hisG4 tsx78 mtll glnV44, containing the non-transmissible oriT mobilizing plasmid, pUZ8002 |
| S. coelicolor A3(2)              |                                             |
| S. ghanaensis                    |                                             |
| S. albus                         |                                             |
| S. venezuelae                    |                                             |
| S. avermitilis                   |                                             |
| pGEMT-Easy                       | Ampicillin<sup>N</sup>                      | Promega corp. |
| pET26b+                          | Kanamycin<sup>R</sup>                      | Novagen      |
| pDpsA4                           | dpsA in pGEM-T Easy                        | Facey et al., 2009 |
| pDpsC1                           | dpsC in pGEM-T Easy                        | Facey et al., 2009 |
| pDpsA7 H                         | dpsA::His6, HygromycinR                    | Facey et al., 2009 |
| pDpsA9                           | plJ8600, tipA::dpsA::His6                  | Facey et al., 2009 |
| pDpsB9                           | plJ8600, tipA::dpsB::His6                  | Facey et al., 2009 |
| pDpsC9                           | plJ8600, tipA::dpsC::His6                  | Facey et al., 2009 |
| pDpsA14                          | dpsA coding sequence in pET26b+            | This study    |
| pDpsC14                          | dpsC coding sequence in pET26b+            | This study    |
| All species                      | hrdBFor - CCTCCCGCTGTGGTCTCT               | Facey et al., 2009 |
|                                  | hrdBRev - CTGGTAGCCCGCTTGGTGTAGTC          | Facey et al., 2009 |
| S. coelicolor                    |                                             |
|                                  | dpsAF-AGCGGAAGTGGGGACGACTAC                | Facey et al., 2009 |
|                                  | dpsAR-TCGAAAAGGTCTCGGGTGCG                 | Facey et al., 2009 |
|                                  | dpsBF-GTCTGGAAAGGCCCCGTTGTC               | Facey et al., 2009 |
|                                  | dpsBR-AGGTTGACCCGAGCGGAAGG                | Facey et al., 2009 |
|                                  | dpsCF-GGCACCAGCTAGCAGTTCC                 | Facey et al., 2009 |
|                                  | dpsCR-CGGCCAGAGCCCTTTGTTGAG               | Facey et al., 2009 |
| S. avermitilis                   |                                             |
|                                  | dpsBF-CTCCTGCTCATCGGGGAAG                 | This study    |
|                                  | dpsBR-TCGATTGACGCTGAGATG                  | This study    |
|                                  | dpsBR-TCGGATCTGCTACTCTGTC                 | This study    |
|                                  | dpsCR-AGTTGCAAGGGTAATGGAG                 | This study    |
| S. ghanaensis                    |                                             |
|                                  | dpsAF-CATGAAAGGAGCCGAGAC                 | This study    |
|                                  | dpsAR-GACGAACACTGGAAGAG                  | This study    |
|                                  | dpsBF-ATCGAGCGCACCGAGAAG                 | This study    |
|                                  | dpsBR-GAACATCCACCGCTGCTT                 | This study    |
|                                  | dpsBF-TCGGTAAAGGTCTATTGTC                | This study    |
|                                  | dpsBR-CGAGGGCGAGATCCACCAG                | This study    |
| S. venezuelae                    |                                             |
|                                  | dpsCF-CTCAACCTGCGAACAAG                  | This study    |
|                                  | dpsCR-AAGTACACCTGTTGTGGT                 | This study    |
| S. albus                         |                                             |
|                                  | dpsAF-AAGCTCATCGAACCCTCT                 | This study    |
|                                  | dpsAR-CCAGTGGATGGCTCTCCAG                | This study    |
|                                  | dpsBF-AGCCAGACATCTCTCA                   | This study    |
|                                  | dpsBR-CTAGCTGTTCCTGGCGCTC               | This study    |

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Tris/HCl, 500 mM NaCl, 50 mM Imidazole, Complete protease inhibitor cocktail [Roche Diagnostics], pH 7.5) and disrupted by sonication. A cell-free supernatant was applied to a Ni Sepharose column (HisTrap HP; GE Healthcare). Fractions containing Dps proteins were pooled and buffer exchanged using HiTrap (GE Healthcare) into 20 mM, 200 mM NaCl and 5% Glycerol. To assess stability, purified proteins were mixed with Urea (0 M) and incubated overnight; followed by native PAGE. Proteins were visualised using Coomassie Blue staining.

**Author Contributions**

Read and approved the final manuscript: PDF MDH JSW DOFS PJD RDS. Conceived and designed the experiments: PDF RDS PJD. Performed the experiments: PDF MDH JSW DOFS. Analyzed the data: PDF RDS JSW DOFS. Wrote the paper: PDF.

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