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Genomics update

Microbial sunscreens

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Basking in the sun on an Australian beach, I began to feel a burning sensation from excessive UV irradiation and decided not to wait for the ozone layer to return. As first aid I scraped some algae and cyanobacteria from the blistering rocks and smeared them on my exposed skin. Why? Because these organisms contain sunscreen compounds to protect themselves from harmful doses of UV-B (280–315 nm) and UV-A (315–400 nm) radiation. Cyanobacteria are prominent in many superficial habitats exposed to high solar irradiance, including deserts, polar regions and intertidal marine flats. On rocky marine substrates, many cyanobacteria form crusts or small cushions in the high intertidal or supratidal zone. In response to intense solar radiation, cyanobacteria and some other microorganisms have evolved a variety of defence mechanisms including the biosynthesis of UV-absorbing/screening compounds such as mycosporine-like amino acids (MAAs) and scytonemin. So far, scytonemin has been found to be produced mainly by cyanobacteria (Fig. 1), while mycosporine and MAAs are widespread and are accumulated by a range of microorganisms, prokaryotic (cyanobacteria) as well as eukaryotic (microalgae, yeasts and fungi), and a variety of marine macroalgae, corals and other marine life forms. Excellent reviews on this topic can be found in Klisch and Hader (2008), Sinha and Hader (2008), Rastogi and Sinha (2009), Rastogi *et al*. (2010) and Singh *et al*. (2010a).

The very recent elucidation of biosynthetic pathways and identification of associated genes now allows data mining of microbial genomes to assess their potential for producing sunscreen compounds. A brief overview is given here to wet your appetite.

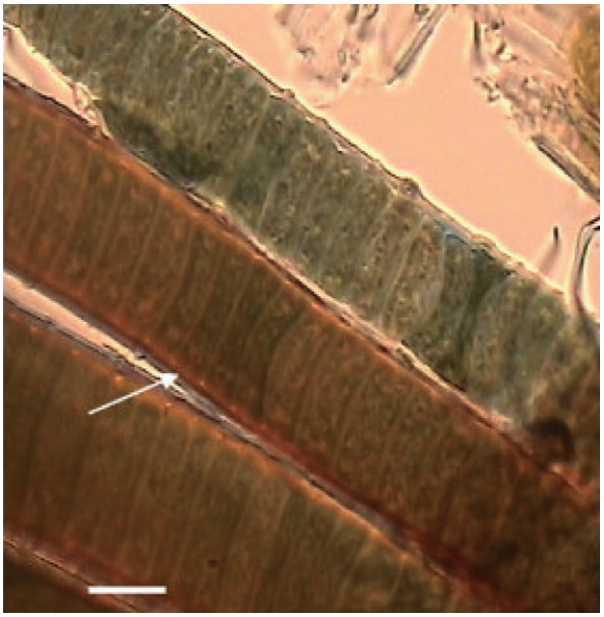

Fig. 1. Filaments of the cyanobacterium *Lyngbya* sp. with sheaths coloured by the yellow to red-brown UV protectant scytonemin (see arrow); bar = 10 μm. Reprinted from Sinha and Hader (2008) with permission from Elsevier.

Mycosporines and MAAs

Mycosporines and MAAs are colourless compounds found intracellularly in many marine and freshwater organisms (Sinha *et al*., 2007; Klisch and Hader, 2008; Llewellyn and Airs, 2010). These natural products are characterized by a cyclohexenone or cyclohexenimine chromophore core conjugated with amino acids or imino alcohol substituents (Fig. 2). These are attached to the core through imine linkages, leading to a combination of resonance tautomers which facilitates absorption of UV light. Differences in the absorption spectra of MAAs, with maxima ranging from 310 to 360 nm (Fig. 3C), are due to variations in the attached side groups and nitrogen substituent. Figure 2A shows chemical structures of

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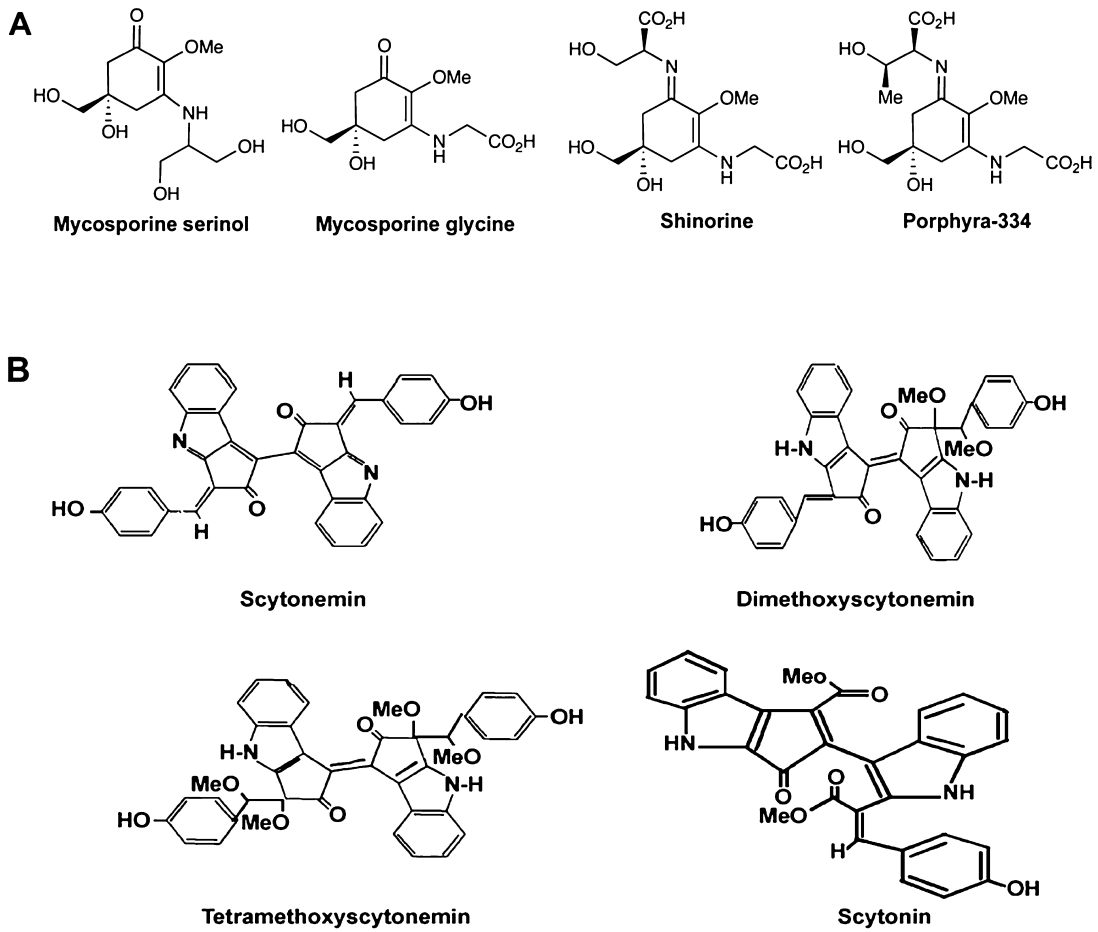

Fig. 2. Chemical structures of representative (A) mycosporines and MAAs from fungi (mycosporine serinol) and cyanobacteria (mycosporine-glycine, shinorine and porphyra-334); from Balskus and Walsh (2010), reprinted with permission from American Association for the Advancement of Sciences; and (B) scytonemin and derivatives; reprinted from Sinha and Hader (2008) with permission from Elsevier.

representative MAAs, and many more structures are described in Sinha and Hader (2008) and Rastogi and Sinha (2009) and in the database of mycosporine-like amino acids (Sinha *et al*., 2007). Cyanobacteria make primarily mycosporine-glycine, shinorine, porphyra-334 and palythinol, while fungi make mainly mycosporine-glutaminol/glutamicol-glucoside and macroalgae make various other MAAs (Sinha *et al*., 2007). MAAs found in higher animals are derived from their algal diet (Newman *et al*., 2000).

Recently, the initial steps in the biosynthesis of mycosporines and MAAs in *Anabaena variabilis* were elegantly elucidated (Balskus and Walsh, 2010). A cluster of four genes (Fig. 3A) was found to be responsible for conversion of the common pentose phosphate pathway intermediate sedoheptulose 7-phosphate into shinorine (Fig. 3B). In the first steps, a dehydroquinate synthase (DHQS) homologue 2-*epi*-5-*epi*-valiolone synthase and an *O*-methyltransferase convert the precursor into 4-deoxygadusol, after which an ATP-grasp homologue and an (NR)peptide synthetase homologue attach glycine and serine to generate mycosporine-glycine and shinorine.

Genome data mining subsequently identified this gene cluster in several cyanobacteria, fungi, dinoflagellates and even in an actinobacterium (Table 1) (Balskus and Walsh, 2010; Singh *et al*., 2010b). In cyanobacteria, all gene clusters contain the first three genes to generate the main intermediate mycosporine-glycine, while additional genes vary. Most clusters encode a conserved D-Ala D-Ala ligase homologue, presumably also to couple amino acids to the mycosporine core, while others encode a (NR)peptide synthetase and/or conserved transporter, or combinations of these.

Scytonemins

Cyanobacteria produce the indole alkaloid scytonemin as part of their response strategy for survival in environmentally stressed conditions, particularly in pulsed-irradiation

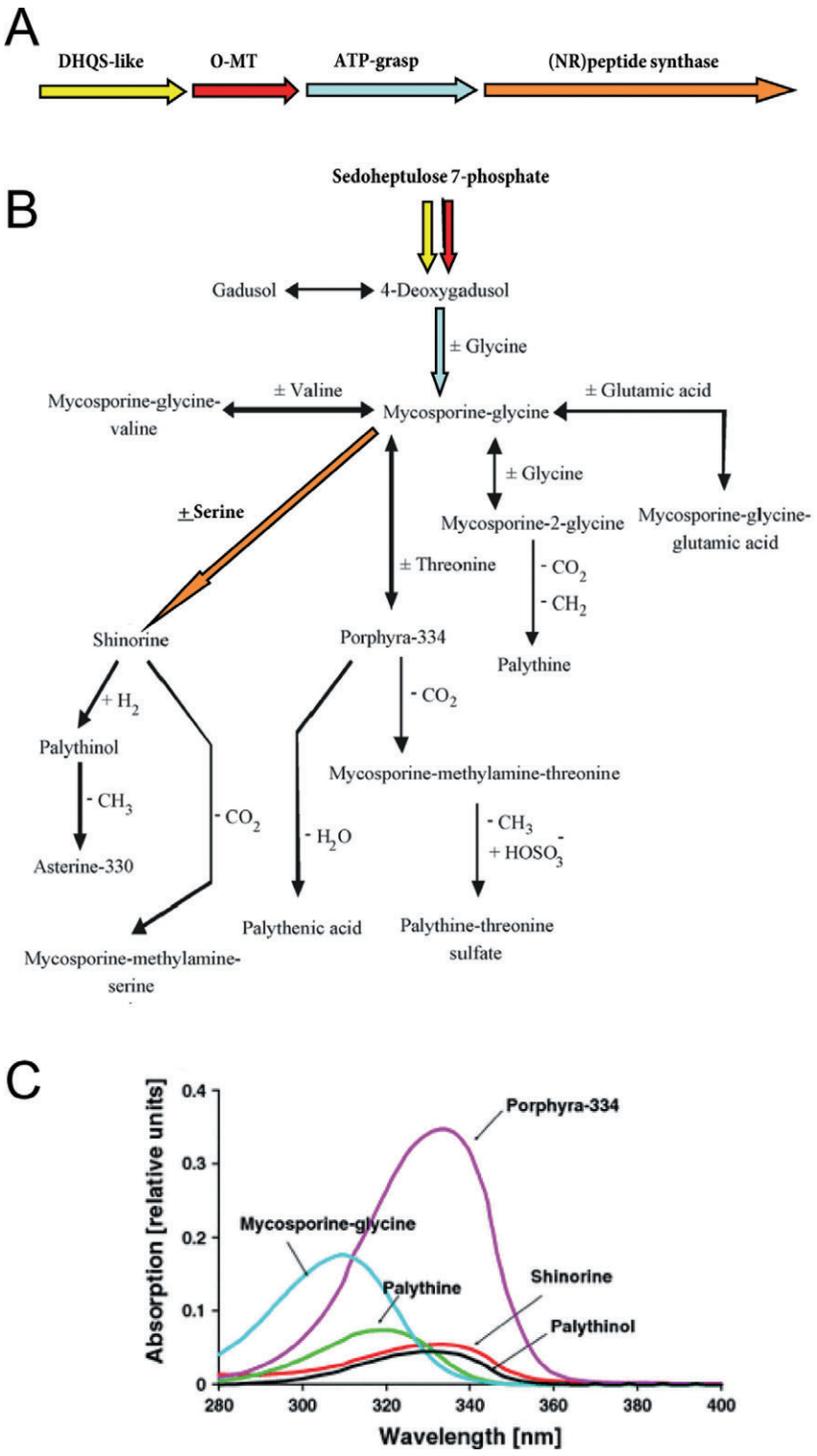

Fig. 3. A. The shinorine biosynthesis gene cluster from *Anabaena variabilis*.
B. Biosynthetic pathways for the assembly of mycosporine and MAAs from sedoheptulose 7-phosphate.
C. Absorption spectra of some MAAs.
Adapted from Sinha *et al*. (2007), Balskus and Walsh (2010) and Rastogi *et al*. (2010), with kind permission from Springer Science+Business Media.

conditions such as in hot and cold deserts. It is found as a yellow (oxidized) to red-brown (reduced), lipid-soluble pigment in the extracellular sheaths or other polysaccharide structures (Fig. 1). Its structure consists of a dimeric carbon skeleton composed of fused symmetric heterocyclic units with a conjugated double-bond distribution that allows strong absorption of UV-A radiation with a maximum absorption at 384 nm (Fig. 2).

The scytonemin biosynthesis pathway and associated genes have been partially elucidated in the cyanobacte-

Table 1. Putative mycosporine/MAA gene clusters in cyanobacteria (adapted from Balskus and Walsh, 2010).

	DHQS homologue	*O*-methyl-transferase	ATP-grasp homologue	D-Ala D-Ala ligase homologue	(NR)peptide synthetase	Transporter	Genome accession code
Cyanobacteria							
Anabaena variabilis ATCC29413	+	+	+		+		NC_007413
Nostoc punctiforme ATCC29133/PCC73102	+	+	+	+			NC_010628
Nodularia spumigena CCY9414	+	+	+	+			NZ_AAVW00000000
Cyanothece sp. PCC7424	+	+	+		+	+	NC_011729
Cyanothece sp. PCC51142	+	+	+	+		+	NC_010546
Cyanothece sp. CCY0110	+	+	+	+		+	NZ_AAXW00000000
Lyngbya sp. PCC8106	+	+	+	+		+	NZ_AAVU00000000
Microcystis aeruginosa PCC7806	+	+	+	+			
Microcoleus chthonoplastes PCC7420	+	+	+	+			NZ_ABRS00000000
Crocosphaera watsonii WH 8501	+	+	+	+		+	NZ_AADV00000000
Trichodesmium erythraeum IMS101	+	+	+	++	++	+	NC_008312
Actinobacteria							
Actinosynnema mirum DSM43827	+	+	+	+			NC_013093

+: one gene; ++: two genes; gene accession codes can be found in Balskus and Walsh (2010) supplemental material.

rium *Nostoc punctiforme* ATCC29133 (Soule *et al*., 2007; Balskus and Walsh, 2008; 2009; Soule *et al*., 2009a). An 18-gene cluster responsible for biosynthesis was identified and shown to be specifically induced by UV-A radiation (Fig. 4) (Soule *et al*., 2007; 2009a). Six consecutive genes coined *scyA–scyF* with no previously known function are likely involved in assembly of scytonemin. In this gene cluster there is a redundant set of genes coding for shikimic acid and aromatic acid biosynthesis enzymes, leading to the production of tryptophan and p-hydroxyphenylpyruvate, which are the likely precursors of scytonemin (Fig. 4). A tentative scheme of biosynthesis steps and a working model for the cellular localization of gene products have been proposed (Soule *et al*., 2009b; Rastogi *et al*., 2010). Genome data mining subsequently identified highly similar gene clusters in the cyanobacteria *Anabaena, Lyngbya, Nodularia, Cyanothece* and *Chlorogleopsis*, and two linked smaller gene clusters probably also involved in biosynthesis and regulation, based on synteny and sequence conservation (Fig. 5) (Soule *et al*., 2009b). Scytonemin biosynthesis could not be induced by UV-A in all these strains, suggesting that some gene clusters have become inactive, possibly due to long laboratory cultivation (Soule *et al*., 2009b).

Scytonemin production has not been observed in other organisms, but aquatic animals presumably accumulate scytonemins via the food chain or from symbiotic bacterial partners, as they lack the shikimate pathway for synthesizing precursors. Some derivatives of scytonemin have been discovered (Fig. 2B) suggesting that scytonemin may be the parent for a whole family of related molecules with subtle changes in radiation absorption.

Combinations of sunscreen compounds

In *Nosctoc flagelliforme*, a terrestrial cyanobacterium from arid environments exposed to intense solar radiation and known by Chinese for centuries for its edible and medicinal values, a combination was found of compounds having complementary absorption of UV-B by MAAs and UV-A by scytonemin, thereby providing protection over the whole UV radiation range from 280 to 400 nm (Ferroni *et al*., 2010). The fact that gene clusters for both scytonemin and MAAs biosynthesis are present in the sequenced genomes of *Nostoc, Anabaena, Cyanothece, Nodularia* and *Lyngbya* strains (Soule *et al*., 2009b; Balskus and Walsh, 2010) (Table 1) suggests that it is common for cyanobacteria to produce both sunscreen compounds, giving full protection over a wide UV radiation range.

Future

The ability of MAAs to prevent UV-induced damage *in vivo* in mice (de la Coba *et al*., 2009) and in human fibroblast cells (Oyamada *et al*., 2008) has been demonstrated. MAAs like shinorine and porphyra-334 from macroalgae are already used in commercial sunscreen products Helioguard 365 and Helionori to protect against UV-A

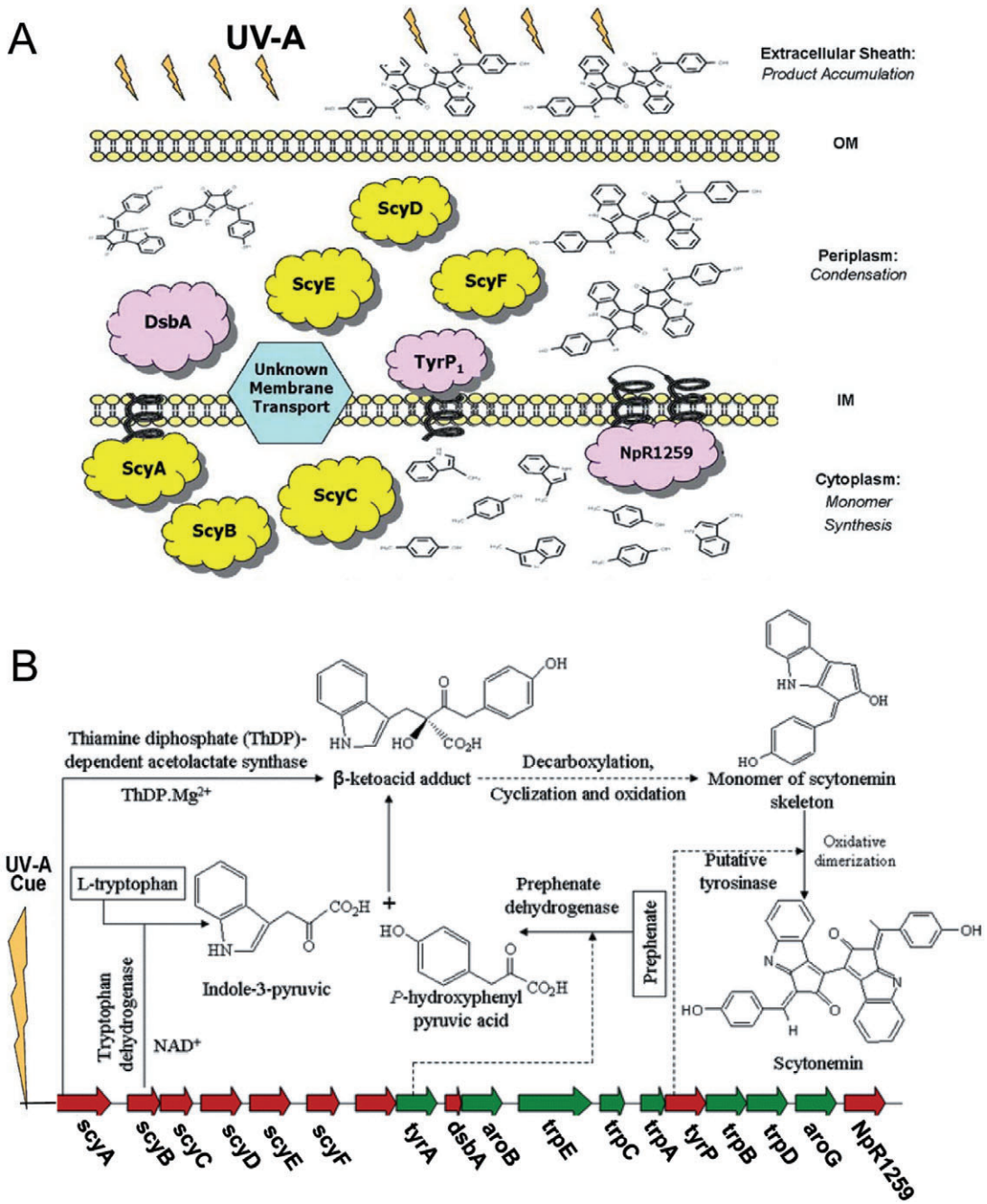

Fig. 4. A. Working model of scytonemin biosynthesis based on genomic analyses. UV-A is absorbed and activates the proposed gene cluster to produce the corresponding protein products localized according to putative protein domains. UV-A is blocked by scytonemin accumulated in the cyanobacterial sheath, which ultimately deactivates the transcription of the gene cluster and eliminates the need for the putative protein products.

B. Proposed biosynthetic pathway for scytonemin and associated gene cluster in *Nostoc punctiforme* ATCC29133. Green genes are predicted to be involved in the biosynthesis of (precursors of) aromatic amino acids, while most of the red genes (e.g. *scyA*–*scyF*) are predicted to be involved in scytonemin biosynthesis. Continuous arrows signify gene products that are functionally characterized, whereas broken arrows indicate the gene products that are still to be functionally characterized.

Adapted from Soule *et al.* (2009b) and Rastogi *et al.* (2010), with kind permission from Springer Science+Business Media.

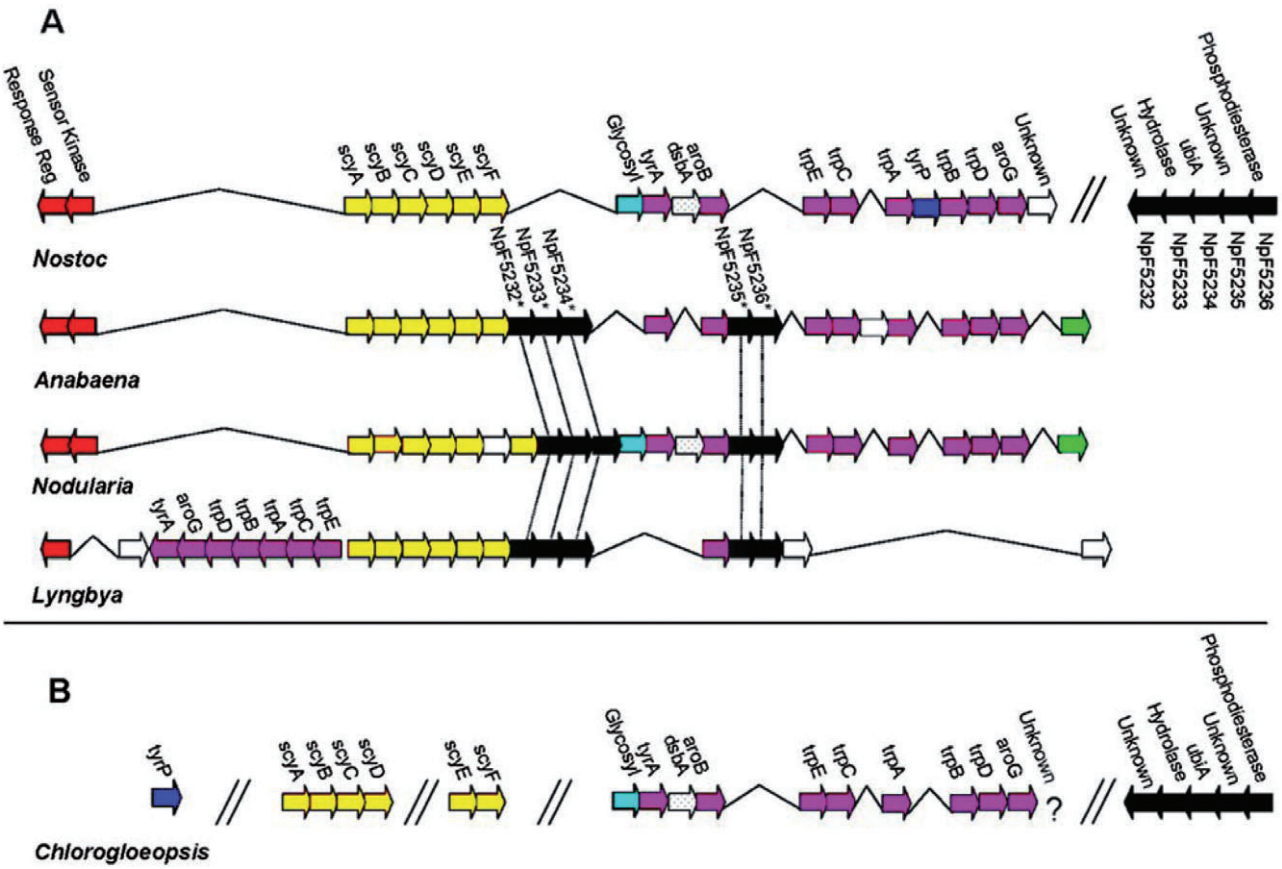

Fig. 5. Genomic region associated with scytonemin biosynthesis in several strains of cyanobacteria.
A. Genomic region in *N. punctiforme* ATCC29133, *Anabaena* PCC7120, *Nodularia* CCY9414 and *Lyngbya* PCC8106 (not drawn to scale). Red, regulatory proteins; yellow, scytonemin core genes; pink, aromatic amino acid biosynthetic genes; black, orthologues to the five-gene satellite cluster in *N. punctiforme*; white, genes without homologues among the strains studied; all other colours represent orthologues. Hash marks delimit the two gene clusters in *N. punctiforme* and carats connect adjacent genes. Orthologues to the five-gene satellite cluster from *N. punctiforme* are specified with a star.
B. Genomic region associated with scytonemin biosynthesis of *Chlorogloeopsis* CCMEE5094, vertically aligned to match *N. punctiforme* in (A). An orthologue to the last gene in the *N. punctiforme* cluster has not been identified in *Chlorogloeopsis* and *tyrP* does not appear to be integrated within the gene cluster, although it is present in the genome. Genes that are not continuously linked are shown by the insertion of hash marks.
Reproduced from Soule *et al.* (2009b).

radiation. In addition to its UV-A absorbing properties, scytonemin also has strong anti-proliferative and anti-inflammatory activities (Stevenson *et al.*, 2002a,b). Given the ever-increasing UV radiation on earth due to depletion of the ozone layer, there is clearly a great need to discover more of these natural sunscreen compounds and to identify the producing microbes. The biotechnological and commercial application of microbial sunscreen compounds appears to have a huge potential and a sunny future.

Acknowledgements

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