

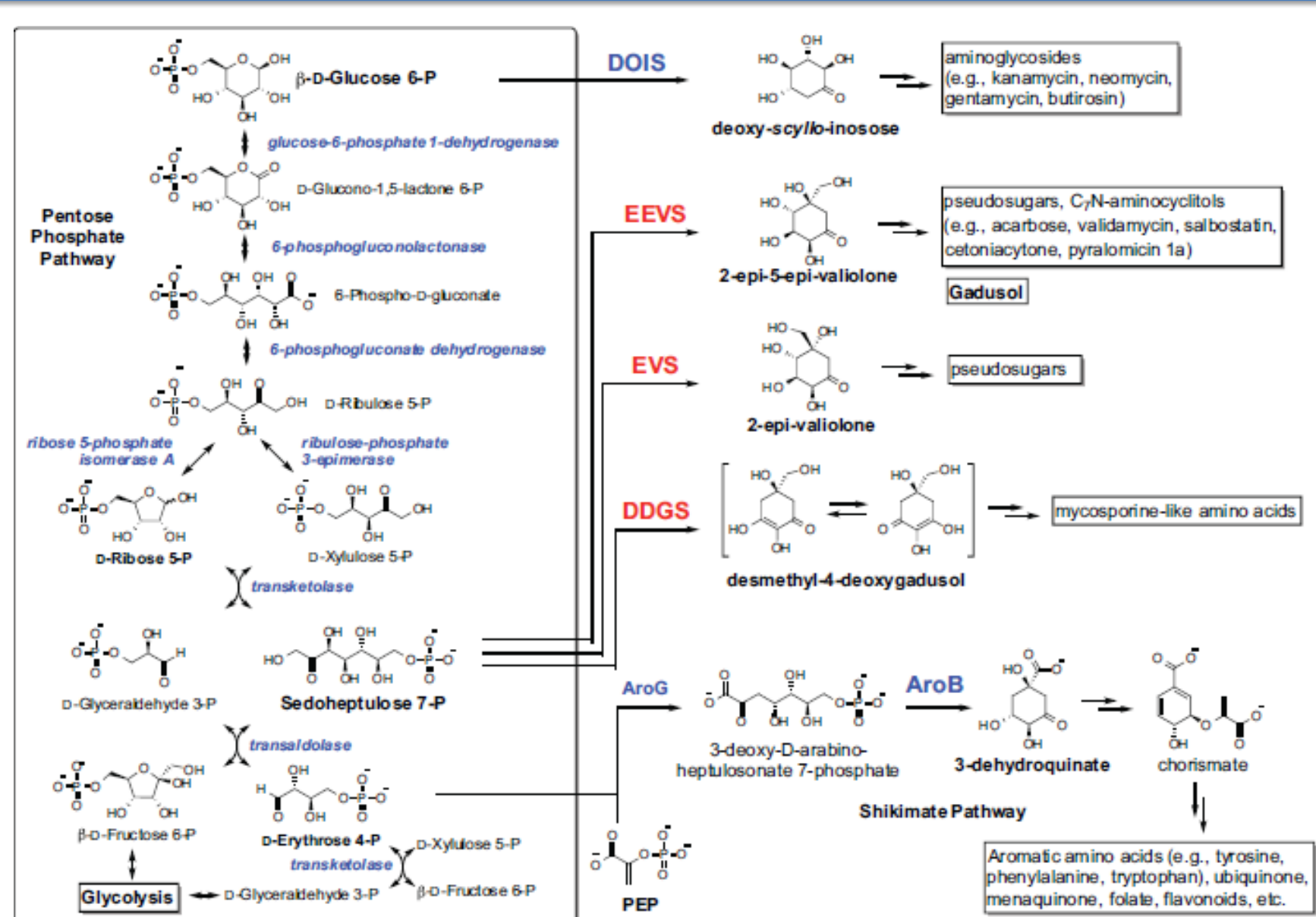
De novo synthesis of a sunscreen compound in vertebrates

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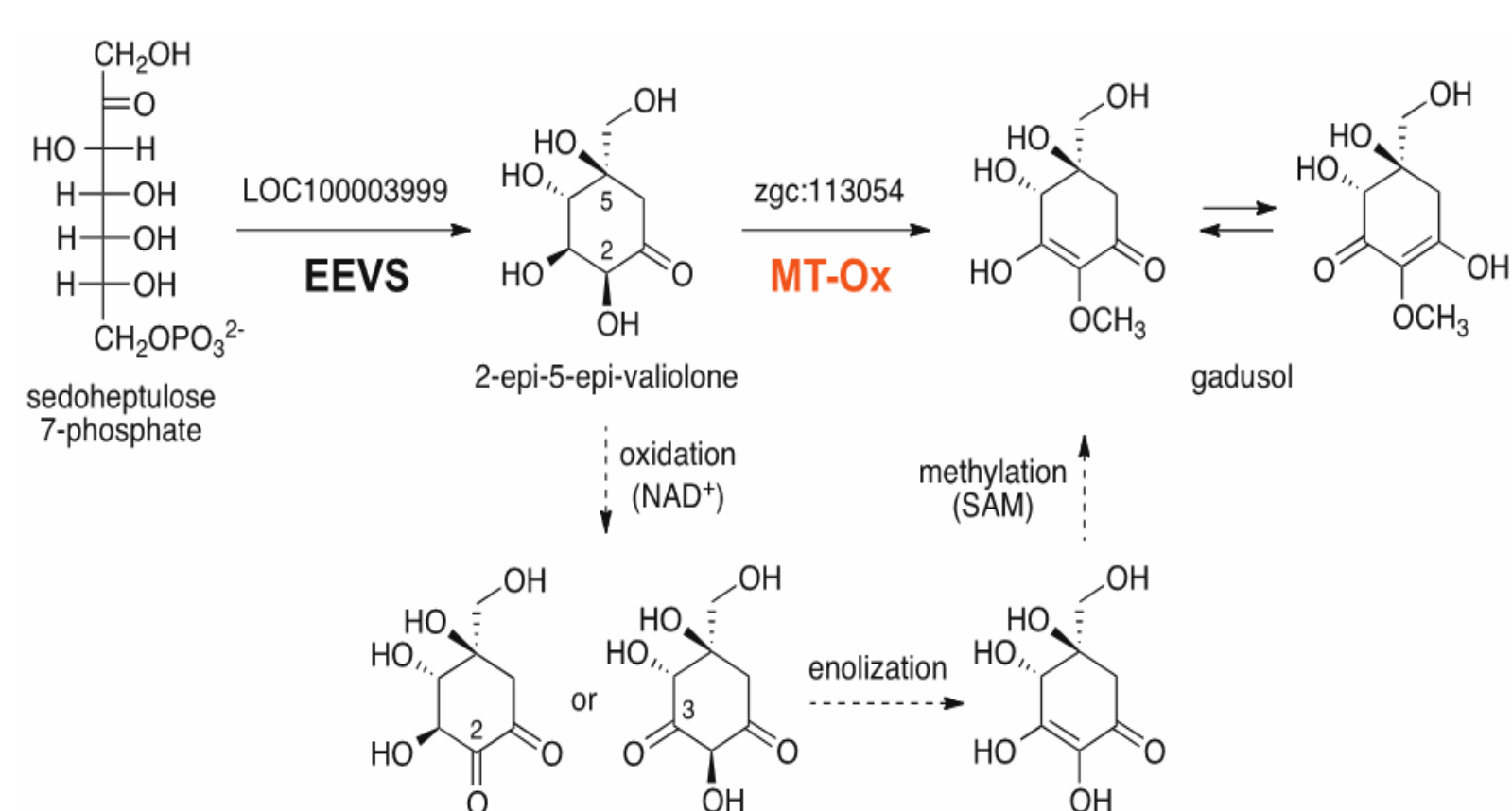
Introduction

Current paradigms suggest that gadusol, a sunscreen and antioxidant found in fish, is derived from 4-deoxygadusol, a precursor of mycosporine-like amino acids (MAA) produced by cyanobacteria, fungi, algae, and marine invertebrates. The accumulation of these compounds in marine animals has been proposed to be of dietary or symbiont origin^{1,2}. Here, we report that gadusol is synthesized *de novo* in zebrafish (*Danio rerio*) from a pentose phosphate pathway intermediate, sedoheptulose 7-phosphate, by the sugar phosphate cyclase (SPC) 2-epi-5-epi-valiolone synthase (EEVS) and a methyltransferase/oxidoreductase (MT-Ox). This is the first example of a SPC in vertebrates.

Sugar phosphate cyclases and their enzymatic reactions



Proposed gadusol biosynthesis

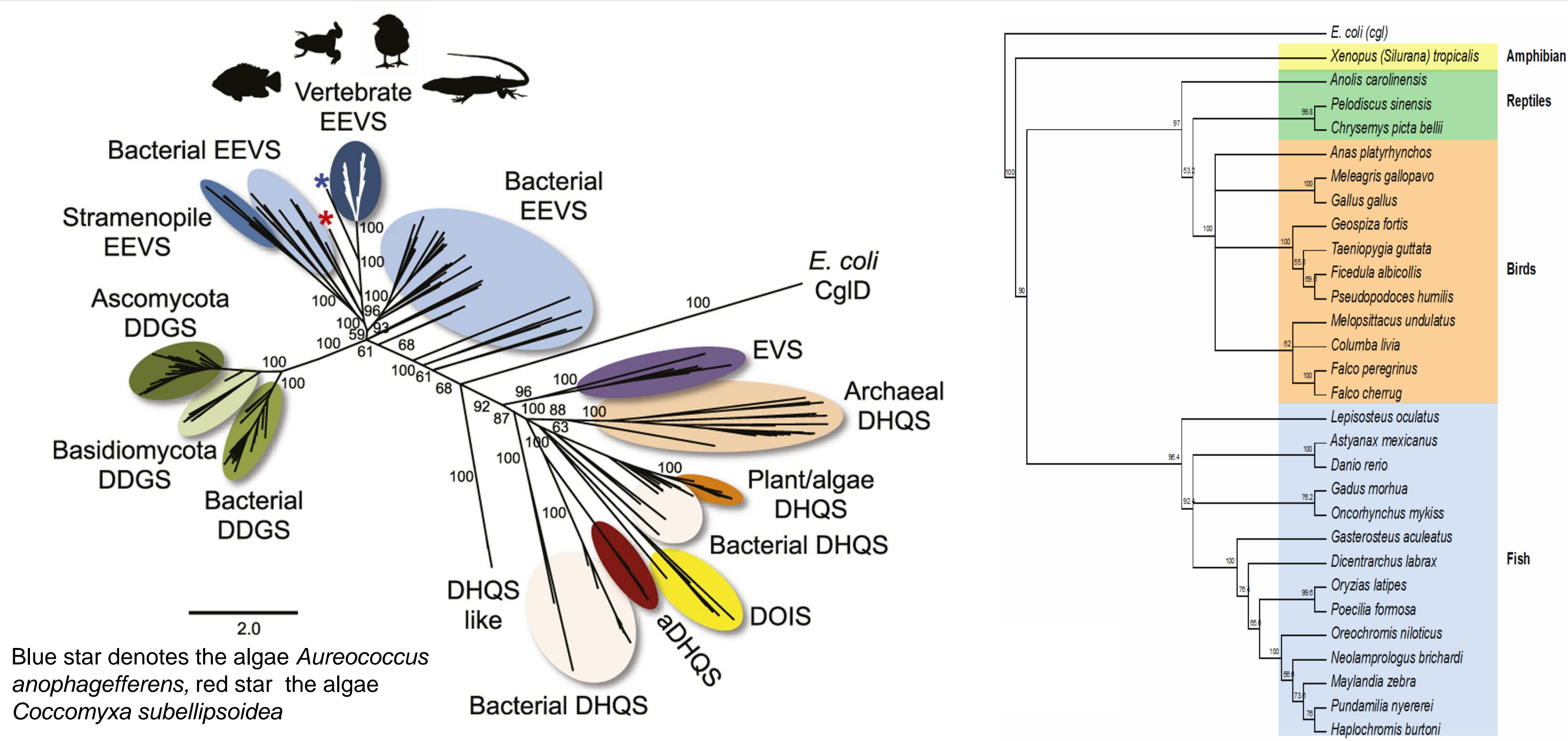


Experiments

- A phylogenetic analysis of SPCs.
- Zebrafish genes were codon optimized and expressed in *Escherichia coli* and enzyme assays were performed.
- Transcription levels of each gene were measured in zebrafish embryos.
- Gadusol was isolated from zebrafish embryos at 72 hpf and the compound was analyzed by HPLC, MS, and NMR (NMR not shown).
- Heterologous expression of EEVS and MT-Ox genes in yeast was carried out in *S. cerevisiae* BY4742 *tal1Δ::KanMX4 trp1Δ::URA3* strain and gadusol production was analyzed by UV, HPLC, MS, and NMR (NMR not shown).
- The UV exposure experiments were performed on wild-type and *rad1Δ* yeast mutant strains using a 302 nm lamp.

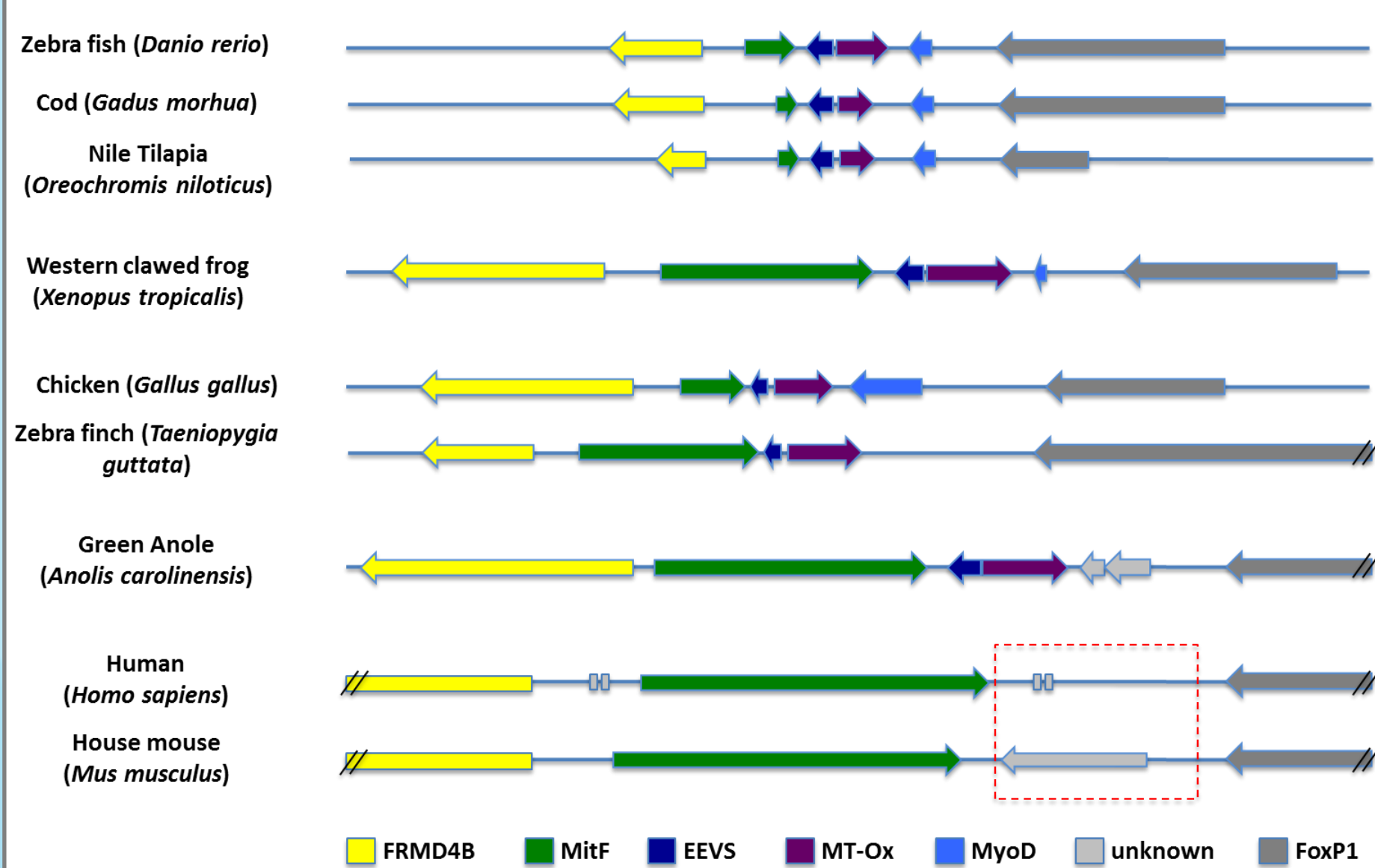
Results

Protein phylogeny of the vertebrate EEVS and sugar phosphate cyclases



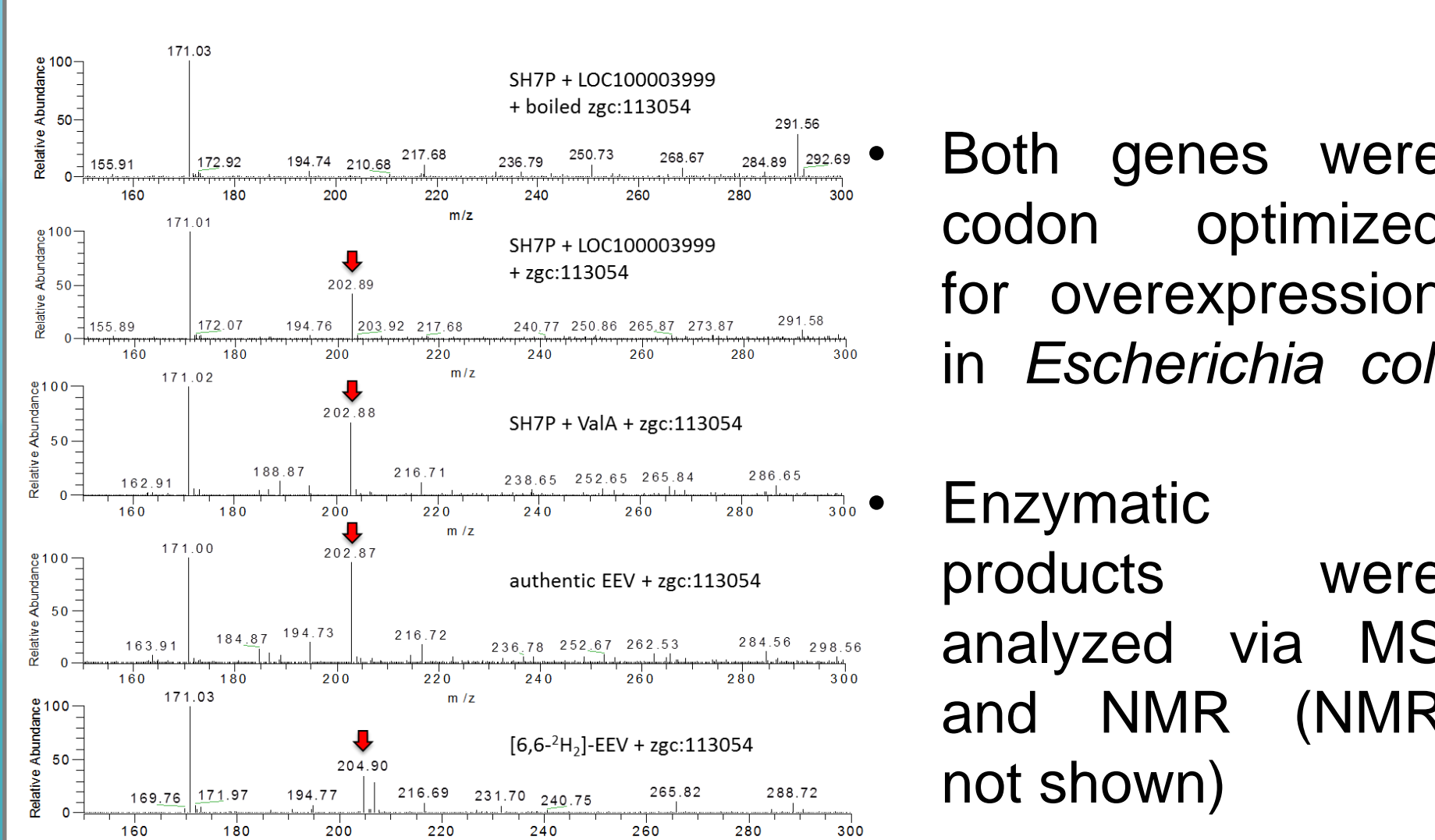
Blue star denotes the algae *Aureococcus anophagefferens*, red star the algae *Coccomyxa subellipsoidea*

Transcription factors are clustered with the vertebrate EEVS gene



The EEVS and MT-Ox genes are clustered with a suite of conserved transcription factor genes. Homologous gene clusters were also identified in the genomes of other vertebrates, *i.e.* amphibians, reptiles, and birds. Humans and other mammals, do not have the EEVS and MT-Ox genes, but maintain the transcription factor gene cluster. It is postulated that these ancient genes were lost during the evolution of mammals *ca.* 220 million years ago.

Gadusol was identified by MS and NMR

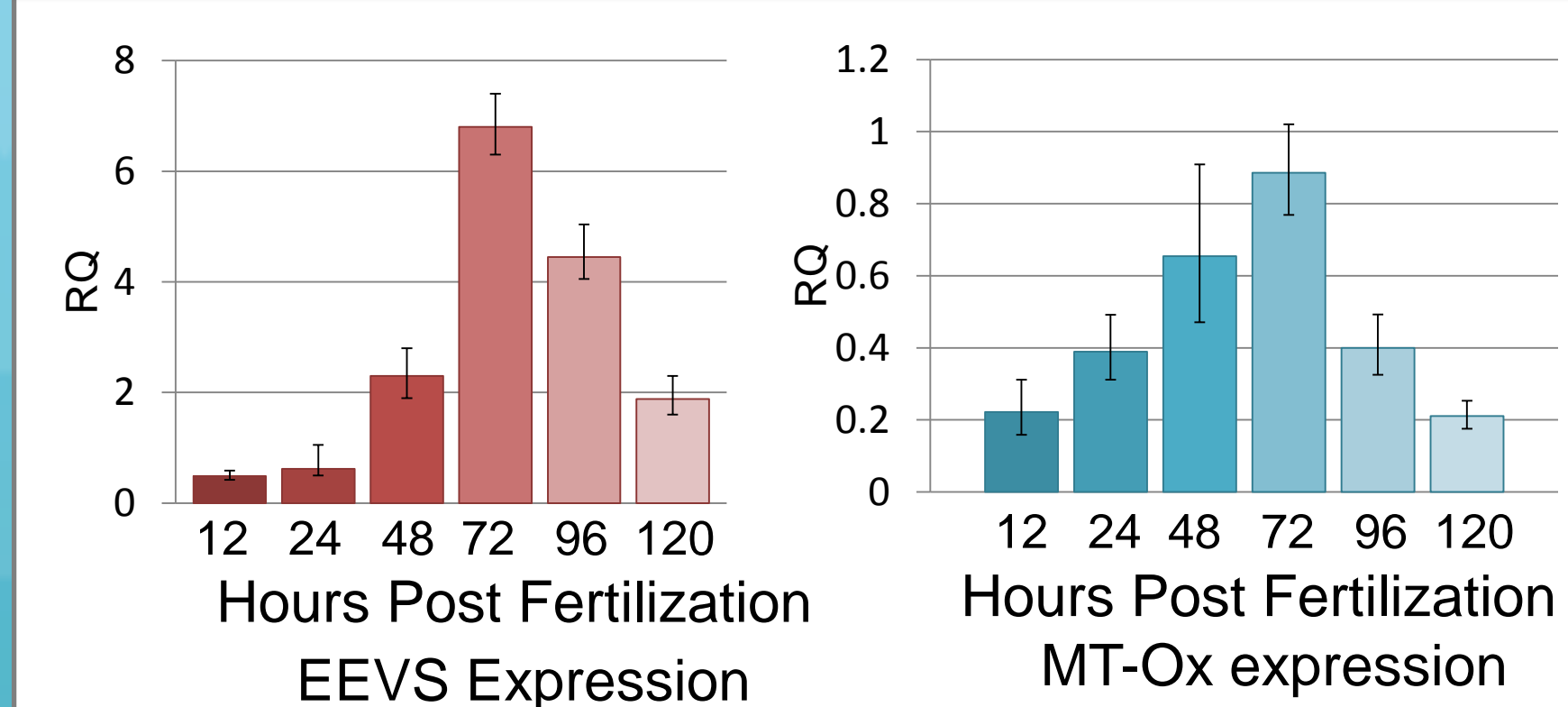


- Both genes were codon optimized for overexpression in *Escherichia coli*
- Enzymatic products were analyzed via MS and NMR (NMR not shown)

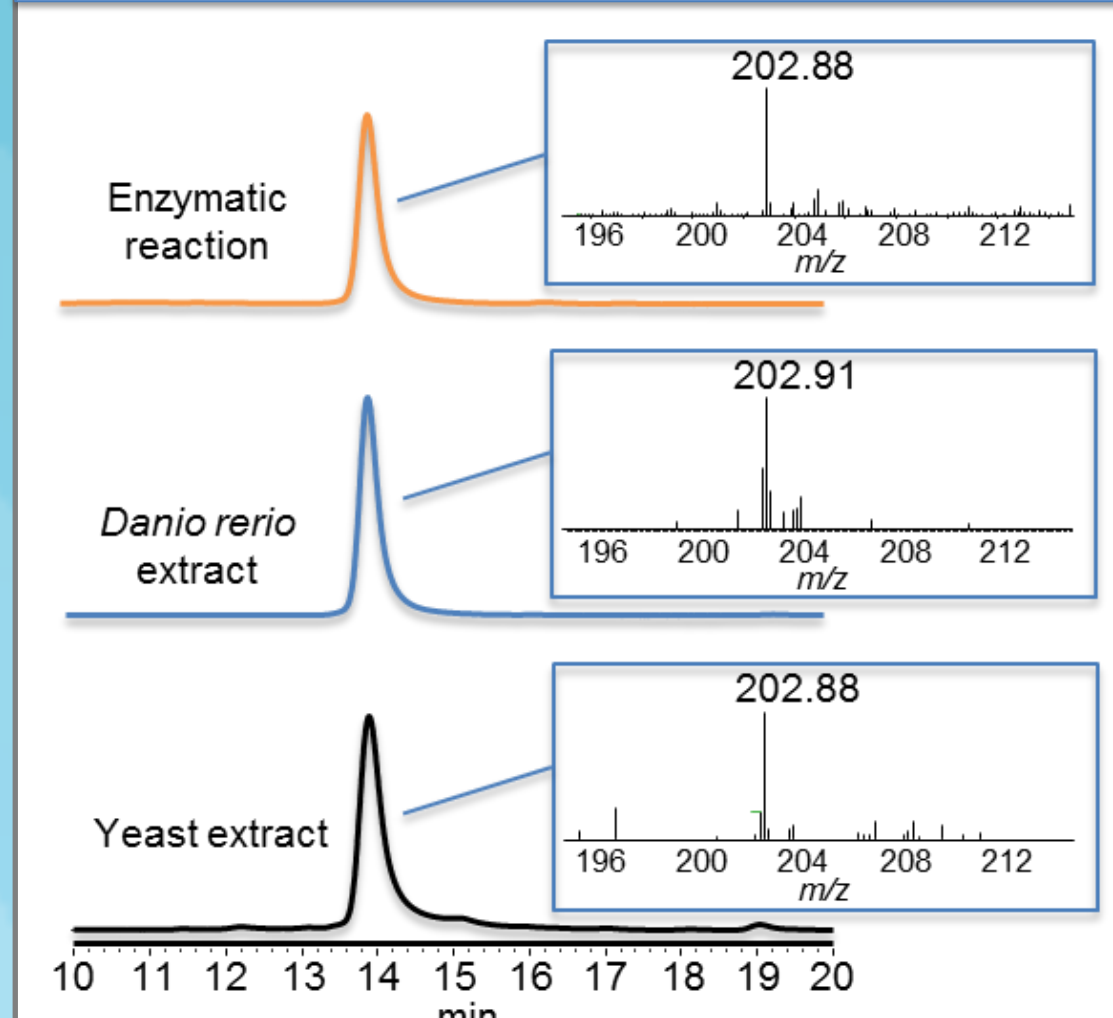
Conserved animal genome organization



EEVS and MT-Ox are expressed during zebrafish development

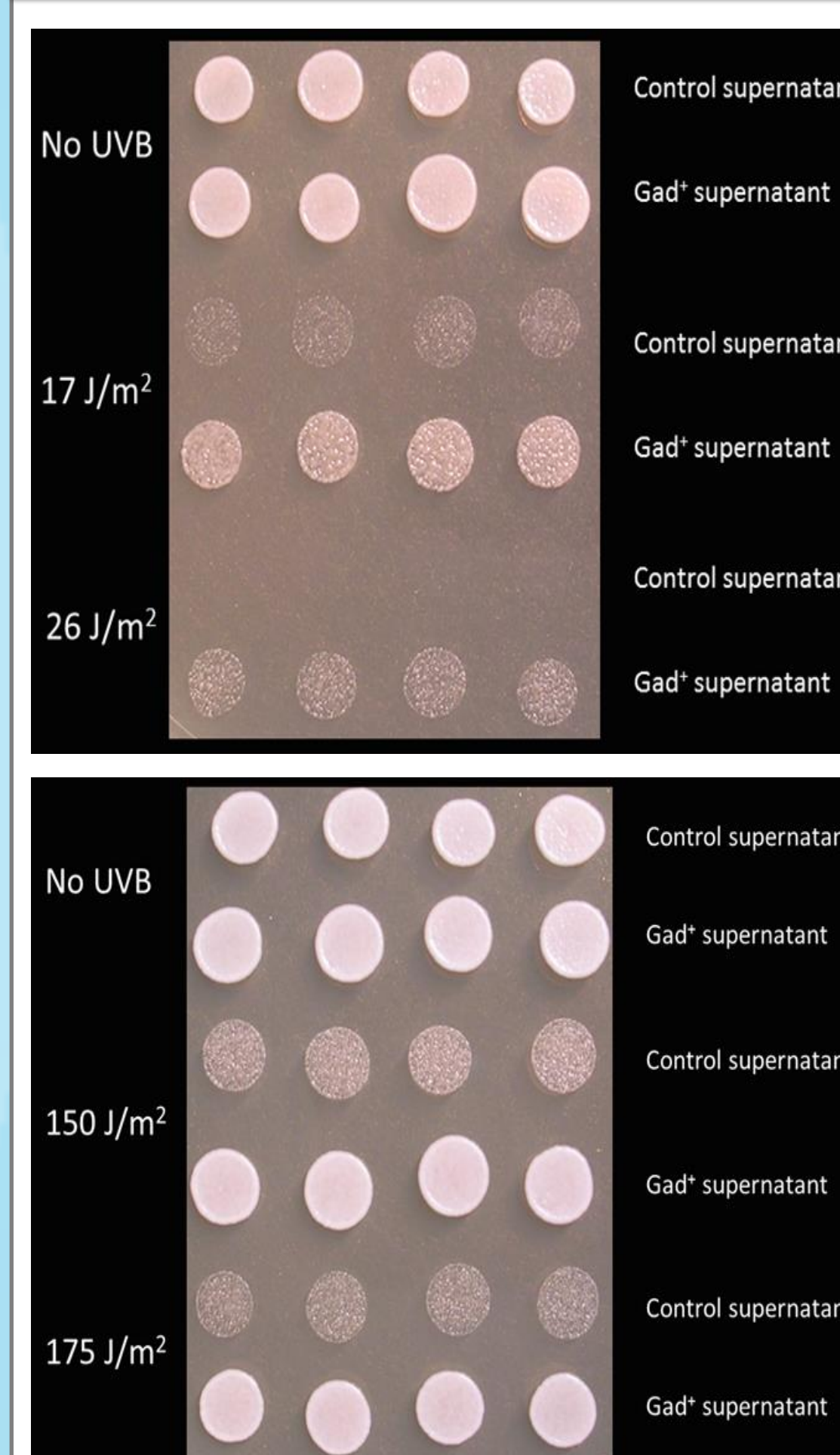


Gadusol was identified by HPLC and MS from Zebrafish embryos and Yeast



- Gadusol was identified in :
1. 72 hpf zebrafish embryos
- Gadusol-producing *S. cerevisiae*

Gadusol can act as a sunscreen in Yeast



- The *S. cerevisiae rad1Δ* mutant is UV sensitive. This yeast strain was treated with gadusol through suspension in a supernatant from a gadusol-producing *S. cerevisiae*. After the gadusol treatment is applied, the mutant can survive exposure to UV at 302 nm.
- The same effect is seen when gadusol is applied to *S. cerevisiae* that has a functional *rad1* gene.

Conclusions

Gadusol, previously thought to be accumulated through the diet of marine fish, is actually produced *de novo* in zebrafish. A sugar phosphate cyclase, 2-epi-5-epi-valiolone synthase, and a methyltransferase-oxidoreductase are responsible for producing gadusol. Genetic data suggests that non-mammalian vertebrates (birds, amphibians, reptiles) also produce gadusol. The function of gadusol in vertebrates remains unknown. The genes responsible for gadusol production were inserted into *S. cerevisiae* and gadusol was produced. Gadusol acted as a sunscreen for the UV-sensitive *S. cerevisiae rad1Δ*.

Acknowledgements

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