The ISME Journal (2017) 11, 1722–1726
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SHORT COMMUNICATION

Animal-like prostaglandins in marine microalgae

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Diatoms are among the most successful primary producers in ocean and freshwater environments. Deriving from a secondary endosymbiotic event, diatoms have a mixed genome containing bacterial, animal and plant genes encoding for metabolic pathways that may account for their evolutionary success. Studying the transcriptomes of two strains of the diatom *Skeletonema marinoi*, we report, for the first time in microalgae, an active animal-like prostaglandin pathway that is differentially expressed in the two strains. Prostaglandins are hormone-like mediators in many physiological and pathological processes in mammals, playing a pivotal role in inflammatory responses. They are also present in macroalgae and invertebrates, where they act as defense and communication mediators. The occurrence of animal-like prostaglandins in unicellular photosynthetic eukaryotes opens up new intriguing perspectives on the evolution and role of these molecules in the marine environment as possible mediators in cell-to-cell signaling, eventually influencing population dynamics in the plankton.

The ISME Journal (2017) **11,** 1722–1726; doi:10.1038/ismej.2017.27; published online 28 March 2017

Prostaglandins (PGs) are considered 'local hormones' participating in intercellular signaling, sustaining both homeostatic functions and mediating pathogenic mechanisms (Wiktorowska-Owczarek *et al.*, 2015). They are enzymatically derived from 20-carbon polyunsaturated fatty acids (PUFA) and together with oxylipins constitute a unique class of lipid derivatives known as eicosanoids (Wolfe, 1982).

PGs are present in all vertebrates, in some terrestrial (Stanley, 2006) and aquatic invertebrates (Rowley *et al.*, 2005; Varvas *et al.*, 2009), and have also been identified in some macroalgae of the genera *Gracilaria* and *Laminaria* (Sajiki and Kakimi, 1998; Ritter *et al.*, 2008).

Here we report, for the first time, the presence of PGs in marine microalgae, specifically in two strains of the diatom *Skeletonema marinoi* (FE7 and FE60), known to differ for the production of oxylipins, high in FE7 and low in FE60 (Gerecht *et al.*, 2011).

Analysing the transcriptomes of the two strains, we have identified the sequence of three main enzymes involved in PG biosynthesis (Figure 1a, Supplementary Figure S1), prostaglandin-endoperoxide G/H synthase 1 or cyclooxygenase-1 (COX-1), microsomal prostaglandin E synthase 1 (PTGES) and prostaglandin-H2 D-isomerase (PTGHI or PTGDS), and of a prostaglandin transporter (PTGT). Interestingly, we found that the FE60 transcriptome lacked the annotation for the enzyme COX-1 that initiates PGs synthesis.

Real-time-qPCR experiments confirmed the expression of these transcripts in both strains, revealing the presence of COX-1 also in the FE60 strain. Expression levels varied slightly in different phases of growth for each strain (Figures 1b–d; growth curve in Supplementary Figure S2). In particular, in strain FE7 COX-1 was down-regulated (DR) in the senescent phase with respect to the exponential phase, while in strain FE60 PTGDS and PTGT were DR in the stationary phase (Figures 1b and c). Overall, there was a lower expression level of the PG pathway in the FE60 strain compared to FE7 (Figure 1d).

The presence of prostaglandin metabolites assessed by liquid chromatography/mass spectrometry (LC/MSMS) analyses (Supplementary Figures S3 and S4) confirmed qPCR results. The identified metabolites derived not only from the main PUFA precursors eicosapentaenoic acid (EPA), the most abundant in diatoms (Stonik and Stonik, 2015), but also from eicosatrienoic (ETE) and arachidonic (AA) acids, both found in very low amounts in diatoms (d'Ippolito *et al.*, 2004). In accordance with the qPCR results, quantitative analysis revealed an overall lower production of prostaglandins in the FE60

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Received 28 April 2016; revised 18 January 2017; accepted 27 January 2017; published online 28 March 2017

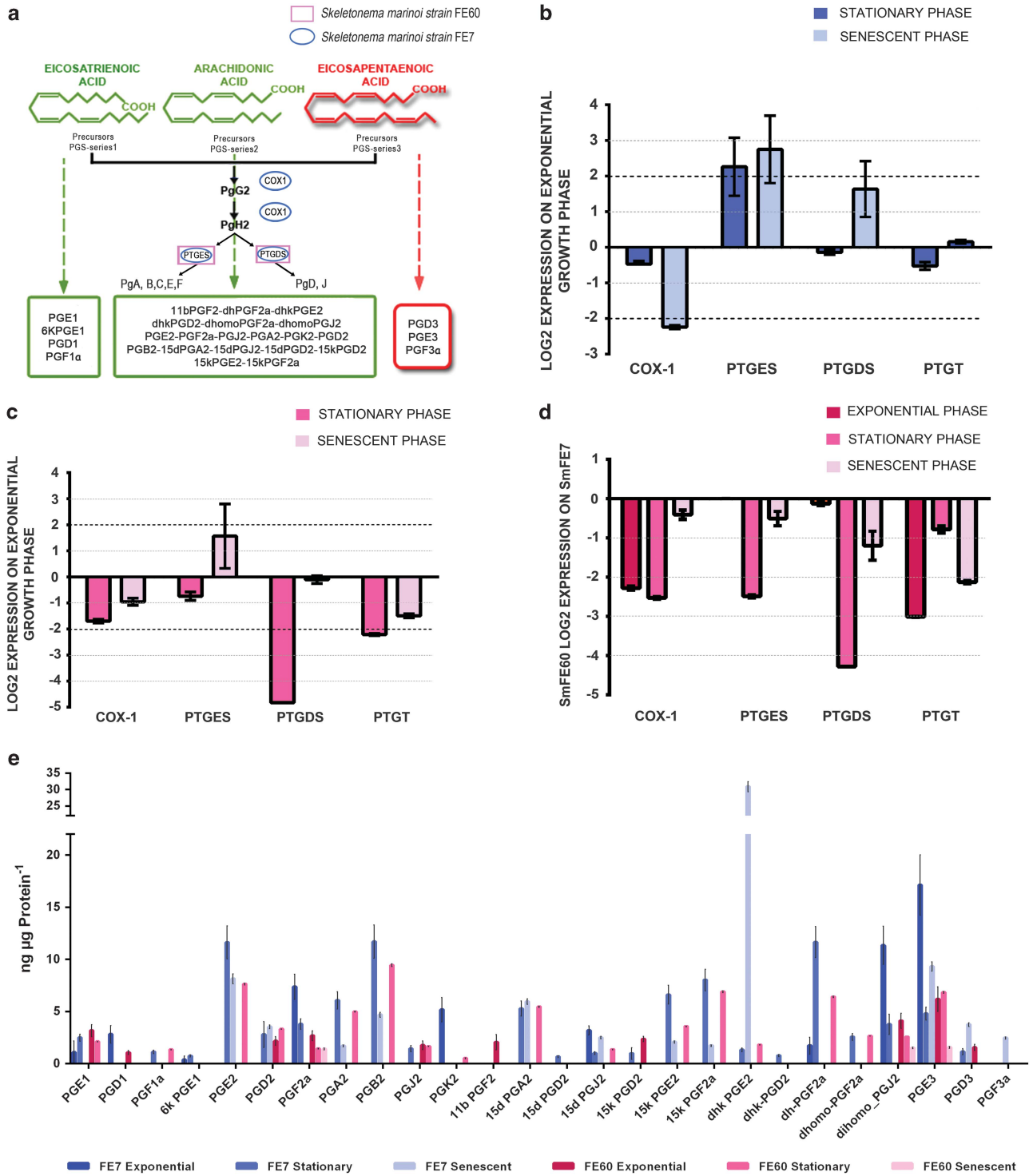

Figure 1 The Prostaglandin pathway: expression and abundance of the prostaglandin metabolites synthesized by *Skeletonema marinoi* FE7 and FE60 strains. (**a**) Schematic representation of prostaglandin synthesis pathway, starting from the three main precursors: eicosatrienoic, arachidonic and eicosapentaenoic acids. The most abundant precursor PUFAs in diatoms is shown in red. Square box contains the prostaglandins identified in our study in relation to their respective PUFA precursors. Colored rectangles or ovals indicate presence of each enzyme in the annotation table of *Skeletonema marinoi* strains FE7 or FE60 (see legend). (**b–d**) Expression levels of enzymes responsible for PGs synthesis and of the prostaglandin transporter measured by Real-time qPCR in exponential, stationary and senescent phases of growth. Results were analysed with the REST software and reported as 2 log expression ratio in the exponential phase (control) ± s.d. (**b**, FE7 strain; **c**, FE60 strain) or as 2 log expression ratio of FE60 with respect to FE7 strain (**d**). Statistical analysis ($N = 3$) was performed using the Pair Wise Fixed Reallocation Randomization test by REST. Relative expression ratios above two fold were considered significant. (**e**) Abundance of the prostaglandin metabolites identified in our *Skeletonema marinoi* strains, quantified with LC/MSMS in the three different phases of growth ($N = 3$). Results are means of three technical replicates (**b–e**) for each biological replicate ($N = 3$).

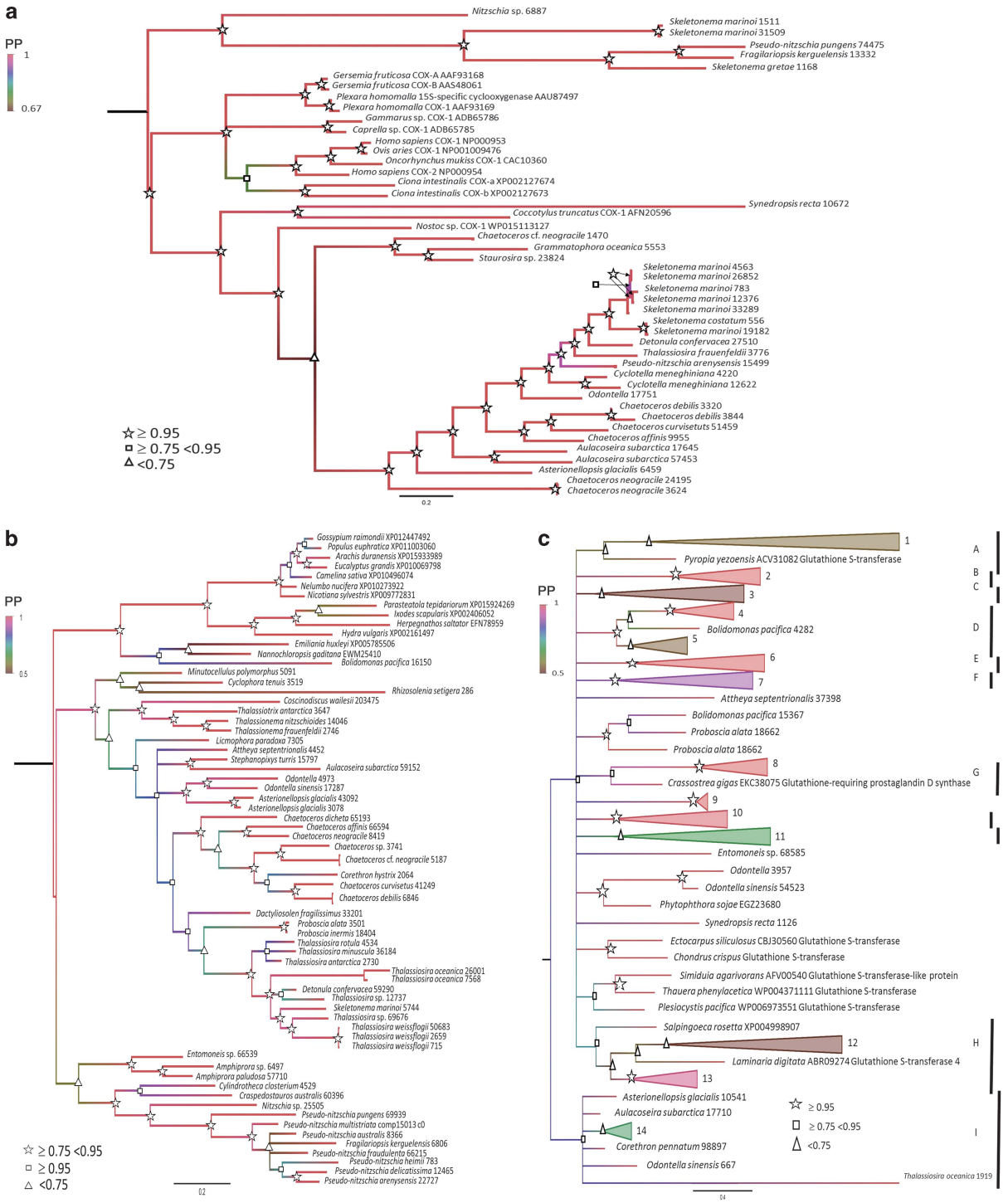

Figure 2 Phylogenetic analysis of the enzymes involved in the prostaglandin pathway. (**a**–**c**) Bayesian mid-point routed phylogenetic trees of COX-1 (**a**), PTGE Synthase (**b**), PTGD Synthase (**c**) proteins from pennate and centric diatoms. Sequences from other organisms are also included as outgroup. Sequences used for phylogenetic analyses are listed in Supplementary Table S3. The trees were built on scale in the branch length (scale bars reported). Sequences are identified by the species name and annotation (where applicable), followed by their MMETSP ID deprived of the taxon ID for simplicity or GenBank accession number. Posterior probability (PP) values are represented with symbols at the nodes: star represents PP above or equal to 0.95; square PP between 0.75 and 0.95; triangle below 0.75. Branch coloring refers to PP, color code is reported in figure. (**a**) COX and COX-like proteins (2 000 000 generations, split frequency standard deviation (s.d.) = 0.002); (**b**) PTGE synthase proteins (5 000 000 generations, split frequency s.d. = 0.01 (**c**) PTGD synthase (10 000 000 generations, split frequency s.d. = 0.04). For simplicity, large sequence clusters were condensed and identified by numbers and the nine main clades were identified by letters highlighted with vertical bars on the right side of the picture. Numbers do not correspond to a rank (see details in Supplementary Information and Supplementary Figure S7).

strain, even considering the biological variability among replicates (Figure 1f, Supplementary Table S1). Molecule quantity decreased during the stationary and senescent phases of growth, especially in the FE7 strain, coherently with the DR of COX-1 transcript expression. Interestingly, the major EPA metabolite expected, prostaglandin E3 (PGE3), is at least two and six times more abundant in the FE7 strain with respect to FE60 during both exponential and senescent phases, respectively, while in the stationary phase the amounts are comparable.

We also verified if a nutrient stress condition (Supplementary information), such as silica starvation (36 μM $Si(OH)_4$), might affect the expression levels of prostaglandin-synthesizing enzymes in the FE7 strain. Results showed a high variability of expression levels of the three enzymes with no significant differences in silica starvation versus standard growth conditions (Supplementary Figure S5).

The effect of stress conditions was extended to all other diatom transcriptomes in the Marine Microbial Eukaryote Sequencing Project database (Keeling *et al.*, 2014) through a bioinformatic analysis. Results (Supplementary Table S2) revealed a huge variability among species and among different culture conditions with only a few species expressing all the genes of the pathway. For the majority of species, COX-1 was not annotated. Moreover, different treatments (nutrient deprivation, temperature, CO_2 levels) did not have the same effect on different species (see COX-1 Fragments Per Kilobase of transcript per Million mapped reads-FPKM value, where present, in Supplementary Table S2). This new data disclose similarities with previous studies showing high genetic and metabolic variability among species and clones in diatoms (Gerecht *et al.*, 2011; Lamari *et al.*, 2013).

Alignment of SmCOX-1 with representative COX-1 sequences showed conservation of the aminoacidic residues involved in peroxidase and cyclooxygenase activity (Supplementary Figure S6).

A Bayesian phylogeny carried out with all COX-1 proteins from diatoms and other organisms (Figure 2a) presented three main clades: one clustering most of the diatom species, including our strains, together with the cyanobacterium *Nostoc* sp. and the red alga *C. truncates*; a second clade, sister to the first (posterior probability, PP = 1.00) clustering animals (crustaceans, *Ciona intestinalis* and mammals); a third basal clade grouping only a small portion of both pennate and centric diatom sequences (PP = 1.00). This topology may suggest that in diatoms at least two different proteins can exert the function of COX-1: the first in the large diatom clade, confirmed to be a COX-1 protein due to its phylogenetic proximity to red algal and cyanobacterial COX-1 (Varvas *et al.*, 2013; Brash *et al.*, 2014); the second was found only in four genera: *Skeletonema*, *Pseudo-nitzschia*, *Fragilariopsis* and *Nitzschia*, possibly representing a different protein sharing common domains with the first one. Indeed, *Skeletonema* and *Pseudo-nitzschia* genera are also present in the first clade, revealing that the two proteins may coexist.

Figure 2 also shows the Bayesian tree for the other enzymes required for PGs synthesis: PTGES (Figure 2b) and PTGDS (Figure 2c). PTGES tree showed a clear distinction between centric and pennate diatoms clustering separately in two sister clades (PP = 0.59 for pennates and 0.97 for centrics). Two exceptions were recorded, the two pennates *Cyclophora tenuis* and *Licmophora paradoxa* clustered with centric diatoms. PTGDS phylogeny showed at least nine clades with nodes that were variably supported statistically (PP from 0.53 to 1.00); five of these contained only diatom sequences. All but one clade contained both centric and pennate diatoms, suggesting that the nine clades cluster different proteins present in both diatom classes. This most likely indicates that in diatoms different proteins can have the same function or be expressed only in specific conditions (see Supplementary Material for details and Supplementary Figure S7). The relative PTGT Bayesian tree was complex since its sequence belongs to the broad family of ABC transporters. Results are described in Supplementary Information (Supplementary Figure S8).

Our findings show the existence of a canonical animal pathway synthesizing most of the known prostaglandins and their metabolites in a marine unicellular eukaryote. This discovery may contribute to unravel important aspects of the evolutionary history of eukaryotes and the conservation of this cell-signaling pathway from unicellular algae to humans.

Future studies are needed to shed light on the physiological and ecological role of PGs as chemical mediators in diatoms and their possible influence on plankton population dynamics.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This project was financially supported by the PharmaSea project funded by the EU Seventh Framework Program, Contract number 312184, www.pharma-sea.eu and by the SZN internal funding. IO was supported by the Italian flagship project 'Ritmare' (Contract number 2645). AA was funded by the European Union under FP7-People GA n. 600407. We thank Flora Palumbo (SZN) for figure design and Mariano Amoroso (PharmaSea) for cell culture cultivation. We are also grateful to Dr Genoveffa Nuzzo for chemical extractions and Dr Salvatore D'Aniello (SZN) for the discussions.

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Supplementary Information accompanies this paper on The ISME Journal website (http://www.nature.com/ismej)