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Molecular identification and expression of the phosphoglucomutase (PGM) gene from the Pacific oyster Crassostrea gigas

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Abstract:

Phosphoglucomutase is a key enzyme in glycolysis and has been widely studied in vertebrates and some invertebrates but no molecular information is available in marine invertebrates despite the importance of this marker in ecological and genetical studies. In this work, we isolated a cDNA and the corresponding genomic sequence that encode PGM-2 locus in the Pacific oyster *Crassostrea gigas*. We used sequences drawn from the database to construct an evolutionary framework for examining the position of mollusc PGM sequences among prokaryotic and eukaryotic homologues and showed that oyster PGM gene organization was closer to vertebrates PGM genes than other invertebrates as previously found in other Lophotrochozoa species. We also investigated PGM mRNA expression in oyster tissues in response to xenobiotics (i.e hydrocarbons and pesticides). The results obtained showed that PGM mRNA expression is mostly up-regulated in the first steps of the response to pollutant exposure and is xenobiotic-dependant.

Keywords: Phosphoglucomutase; Mollusc; Xenobiotics; Gene expression

Abbreviations: PGM, PhosphoGlucoMutase; UTR, unTranslated Region; ADI, Atrazine–Diuron–Isoprouron

1. Introduction

Phosphoglucomutase (PGM; EC 2.7.5.1) is a ubiquitous metallo-enzyme that is expressed in all organisms from bacteria to plants and animals. PGM catalyses the inter-conversion of Glucose-1-phosphate and Glucose-6-phosphate in the presence of glucose-1,6-diphosphate and Mg^{2+} and plays a pivotal role in the synthesis and breakdown of glycogen (Ray and Roscelli, 1964). Early genetic studies of PGM revealed that the human isozymes are encoded by three gene loci, PGM1, PGM2 and PGM3 on separate human chromosomes and other studies have demonstrated multiple loci for PGM and PGM like enzymes in several eukaryotic and prokaryotic species (Hopkinson and Harris, 1968; Rattazzi et al, 1983). Recently, the primary sequence, the gene organization and the existence of polymorphism for Pgm in a invertebrate species, *Drosophila melanogaster* has been described (Verelli and Eanes, 2000). Studies of the evolution of PGM among all organisms showed that the phylogenetic history of PGM sequences is complex and marked by duplications and translocations with two cases of transkingdom horizontal gene transfer being identified (Whitehouse et al., 1998). In humans, the physical and biochemical properties of the PGM isozymes are very similar but present some differences in substrate specificity. There appears to be relatively few species where PGM is encoded by a single locus (e.g., *D. melanogaster)* (Lindsley and Zimm, 1992). These isoforms present distinct properties in terms of molecular weight, thermostability, substrate specificity and tissue distribution (Mc Alpine et al., 1970 a,b). The processes of activation and inhibition of PGM by chelating agents and metallic ions has also been widely studied (Milstein, 1970a,b). In marine species, studies dealing with PGM are essentially based on allozyme data and at present, no molecular data are available for PGM in marine invertebrates despite the importance of this enzyme in energetic metabolism and existing data on the selective effect of environmental parameters on its allozyme polymorphism. However numerous studies showed evidence of selection on metabolic enzyme polymorphisms (Watt et al., 1985; Koehn et al., 1988). In marine

molluscs, most studies dealing with the potential selective effect of environmental parameters on the genetic structure at a population level are based on allozymic data. Phosphoglucomutase is routinely scored in genetic studies on marine molluscs and selection of particular Pgm alleles in response to anthropogenic pollutants has been previously demonstrated in crustaceans (Nevo et al., 1983, Nevo et al., 1984), gastropod (De Nicola et al., 1992) and in molluscs (Tanguy et al., 1999; Moraga and Tanguy, 2000). PGM2 locus in *C. gigas* has also been shown to be one of five loci involved in a significant positive correlation between multiple-locus heterozygosity and adult body weight (Fujio, 1982). More recently, strong evidence for selection has been shown in relation with temperature in several species such as the sea anemone *Metridium senile* (Hoffmann 1985) and *D. melanogaster* (Verelli and Eanes 2000).

In this study, isolation and characterization of the full-length cDNA and genomic sequence that encode PGM-2 of the Pacific oyster, *C. gigas* (CgPGM) is described. We have therefore done a phylogenetic analysis to situate the mollusc PGM sequence among other species and studied the expression of the PGM according to various experimental conditions.

2. Material and Methods

2.1. PGM cDNA sequencing

Total RNA was extracted from adductor muscle of *C. gigas* according to the guanidium isothiocyanate method (Strohman et al., 1977). Partial cDNA was prepared using degenerate primers. Primer design was based in the alignment of PGM sequences from *Homo sapiens* (AL109925), *D. melanogaster* (Q9VUY9) and *Caenorhabditis elegans* (Q21742). First-strand synthesis was performed using an anchored oligo-dT primer (5'CGCTCTAGAACTAGTGGATCT$(T)_{10}$3') and M-MLV reverse transcriptase. Polymerase chain reactions were performed using buffer (2mM magnesium ions), Taq polymerase (Promega, 1U), primers sense (5'GRRGARAGBTTYGGDACYGG3',15 pM) and antisense (5'CKGAARATGATDCGAGAACC3', 15 pmol), reverse transcription products (200 ng), dNTPs (2.5 mM of each) and water. All reactions were performed in a 50-µl volume with an initial 5-min denaturation at 94°C, followed by a 2-min annealing at 50°C and 90 sec elongation at 72°C; then 35 amplification cycles were performed as follows: 94°C, 30 sec ; 50°C, 40 sec and 72°C, 90 sec, followed by a final 10 min at 72°C.

The procedure for the generation of the 3' and 5' un-translated region (UTR) of cDNA was carried out according to the following protocol: first strand synthesis was performed using an anchored oligo-dT primer and M-MLV reverse transcriptase. The product was purified on a column (Microcon-QIAGEN). A polyA tail was then generated on the 5' end of the product by using the following protocol: 5 µl of Terminal Transferase buffer and 1 µl of 10 mM dATP were added to 18 µl of purified product. After 5' at 95°C, 1 µl (100 U) of Terminal Transferase was added to the mix and placed at 37°C for 30 min then at 70°C for 10 min. Amplification of 5' UTR region of mRNA was performed using the PCR anchor primer (5'CGCTCTAGAACTAGTGGATCT$(T)_{10}$3)' and a specific antisense primer (5'ATAGTATCGTCCATCGCCTCCGATGACTAGAGT3') designed according to the previous

sequence. Amplification of the 3'UTR region was performed using the sense and antisens primers, 5'CATTATGACTTTGGTGCTGCATT3' and 5'CGCTCTAGAACTAGTGGATCT3'. The thermal cycling program used to amplify the PGM 3' and 5' UTR fragments began with an initial 5-min denaturation at 94°C then 10 amplification cycles of 94°C, 30 sec ; 58°C, 50 sec and 72°C, 30 sec, then 25 amplification cycles of 94°C, 30 sec ; 58°C, 30 sec and 72°C, 50 sec with an increase of 15' at each cycle and a final 10 min elongation at 72°C. All PCR products were isolated, gel-purified using the QIAEX® II Gel Extraction Kit (Qiagen, Hilden, Germany), cloned in pGEM-T vector (Promega, Madison, WI, USA), and sequenced by extension from both ends using T7 and Sp6 universal primers (Thermo Sequenase Primer Cycle Sequencing Kit, Amersham Bioscience).

2.2. Cloning of the PGM gene

The genomic sequence of *C. gigas* PGM gene was obtained by PCR amplification on isolated DNA using several combinations of primers. The *C. gigas* PGM cDNA sequence was cut in 11 hypothetical exons according to the size and the position of the corresponding exons of the human PGM gene. All the primers were designed to amplify the intronic sequence between two successive exons. Polymerase chain reactions were performed using buffer (2mM magnesium ions), Taq polymerase (Eurobio, 1U), primers (15 pM each), genomic DNA (100 ng), dNTPs (2.5 mM of each) and water. All reactions were performed in a 50-µl volume. The thermal cycling program used to amplify the PGM fragments began with an initial 5-min denaturation at 94°C followed by a 2-min annealing at 56°C and 5 min elongation at 72°C. Then 35 amplification cycles of 94°C, 40 sec ; 56°C, 1 min and 72°C, 5 min, followed by a final 10 min at 72°C. All the PCR products obtained were isolated on a gel, purified using the QIAEX® II Gel Extraction Kit (Qiagen, Hilden, Germany) and inserted into the pGEM-T vector (Promega, Madison, USA). The inserts were sequenced by extension from both ends using T7

and Sp6 universal primers (Thermo Sequenase Primer Cycle Sequencing Kit (Amersham Bioscience).

2.3. Molecular phylogenetic analysis

Analyses were performed on PGM cDNA sequence from various organisms including bacteria, plants, invertebrates and vertebrates. Amino acid sequences were aligned with Clustal X Software (Thompson et al., 1997). Molecular phylogenetic trees were constructed using the Neighbor-Joining (NJ) algorithm in the PHYLIP software and the phylogenetic package MEGA2 (Kumar et al., 2001). Amino acid differences between sequences were corrected for multiple substitutions using a gamma correction. In this correction, α, the shape parameter of the gamma distribution, was set to 2. With $\alpha = 2$, the distance between any two amino sequences, dij, is approximately equal to Dayhoff's PAM distance per site. Phylogenetic trees were also generated using parsimony. For this analysis, amino acid changes were unweighted; thus a change from one amino acid to any other was equally probable. Support for the major nodes within both distance and parsimony trees were evaluated by bootstrapping the data; 1000 bootstrap replicates of the whole data set were examined.

2.4. Experimental design

Adult oysters (10-11 cm), *Crassostrea gigas*, were collected from "La Pointe du Château" (Brittany, France) in winter 2001 for hydrocarbon experiment and in fall 2002 for pesticide experiments. After an acclimation period of 7 days in aerated 0.22µm-filtered seawater at constant temperature and salinity (15°C and 34‰, respectively), oysters were challenged as follows. Groups of 20 oysters were exposed to 0.1% of hydrocarbon mixture (consisting of the water-soluble fraction of fuel homogenised for three days in filtered seawater) according to the protocol described in previous study (Boutet et al., 2004), for 3 weeks. Groups of 20 oysters were exposed to a mixture of three herbicides (atrazine 2µg/l; diuron 0.5µg/l and isoproturon

1µg/l, ADI) and to 2µg/l of glyphosate for four weeks as described in previous study (Tanguy et al., 2005). Another group of 20 oysters was maintained in seawater, without contaminant, as a control. No mortality was observed either in the control or in the exposed oysters for the whole experiments.

2.5. Phosphoglucomutase expression study by semi-quantitative RT-PCR

Total RNA was extracted from the digestive gland of control and stress-exposed oysters according to the method described above and submitted to reverse transcription using oligo dT anchor primer (GAC CAC GCG TAT CGA TGT CGA $CT_{(16)}V$) and M-MLV reverse transcriptase (Promega). The PCR reaction contained 200 ng of reverse transcription product, 2 mM $MgCl_2$ and 10 pmol of each primer (5'-CACGAATACCTACCAGTGGCC-3' and 5'-TCAGCAACATATGCATTTAGATTTGCCAT-3'). A first round of PCR was conducted to determine the most appropriate number of PCR cycles that would allow avoid signal saturation for band intensity quantitation. PCR products (435 pb) were analysed after 15, 20, 25, 30, 35, 40 and 45 cycles and a standard curve was designed (data not shown). The number of 30 cycles was chosen for further analysis. The cycling parameters consisted of denaturation at 94°C for 2 min, hybridization at 58°C for 2 min and elongation at 72°C for 40 sec, then 30 cycles at 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s, with a final elongation at 72°C for 7 min. A control for the efficiency of both the reverse transcriptase and the polymerase, 28S ribosomal DNA, was used to standardize all samples. It was amplified as described above using sense primer 5'-CCATCAGATTCTTTGGCTGTTTTAGCACA-3' and antisense primer 5'-TTTAGATTTGCCATCATCTTATTGGCTGG-3'. The number of cycles for 28S was determined to avoid signal saturation for band intensity quantitation and 18 cycles were used for all samples. The resulting PCR products were electrophoresed in a 0.5X TBE/1.5% agarose gel and stained with ethidium bromide then visualized with U/V light. Quantification of band intensities was measured by using Gene Profiler 4.03 Software (Scanalytics Inc.) and ratio

between PGM OD and 28S OD were calculated. The expression of PGM mRNA was analyzed in digestive gland from control and stress-exposed oysters from all the experiments

2.6. Statisticals analysis

To compare GSII mRNA expression between treatment and groups, a statistical analysis using a Student test was performed on expression data. For each gene on each treatment, differences were tested by sampling date pair (first date versus second date, third date versus second date, etc).

3. Results

3.1. PGM-cDNA isolation

A partial cDNA fragment of about 400 bp and coding for PGM was first isolated using degenerate primers. From the corresponding sequence, specific primers were then designed to amplify the full cDNA sequence. The CgPGM cDNA encoded a deduced protein of 555 amino acid residues with a calculated molecular mass of 61 kD and a calculated pIs of 6.82. The 5' untranslated region contains 18 bp and the 3' untranslated region, 97 bp not including the poly(A) tail.

3.2. Organization of the CgPGM gene

The *C. gigas* PGM gene corresponding to the cDNA fragment was isolated using several combinations of primers designed in predicted exons. The CgPGM gene spans 7071 bp from the ATG codon to the poly adenylation signal. CgPGM gene contains 8 coding exons of 81, 53, 151, 52, 83, 61, 45 and 29 amino acids respectively, separated by seven introns of 296, 841, 377, 227, 664, 1307 and 1594 bp respectively. All the intron borders of CgPGM start and end with the consensus GT and AG splicing signals. Two (GA) microsatellites have been detected in introns 4 and 5 (Fig 1).

3.3. Molecular phylogeny

Twenty-one PGM amino acid sequences corresponding to true phosphoglucomuatse enzymes were selected. The sequences spanned several kingdoms (vertebrate, invertebrate, plants, fungi and prokaryotes). Phylogenetic trees obtained either from parsimony or the distance method had nearly identical topologies with each of the 21 proteins falling into one of the five major lineages (Fig 1). The tree shows that oyster PGM sequence constitute a distinct branch from the other homologous sequences from the vertebrate and invertebrate groups. PGM from *C.*

gigas seems closer to the vertebrate group than the invertebrate group comprised of insects and nematods but the associated bootstrap value remain low suggesting that the tree branch of C. gigas is not strongly attached to the vertebrates group. The lack of PGM sequence in other invertebrate species and more particularly in mollusc could explain this isolated branch for *C. gigas*. The highest amino acid sequence identity for CgPGM was the deduced PGM sequences from human and rabbit (57%), and the invertebrates as *Drosophila melanogaster* (55%) and *C. elegans* (50%); these identity values contributing to the tree observed with the oyster branch closer from vertebrates than other invertebrates species.

We also compared the CgPGM gene organization to the human and fruit fly PGM gene (Fig 2). The CgPGM exon 3 corresponds to the merging of exons 3, 4 and 5 and exon 5 to the merging of exons 7 and 8, in human sequence, respectively. The size of other exons is similar in the two species and the exon borders correspond to the same amino acid position. In *D. melanogaster* as in *C. elegans*, only four exons were found in the PGM gene and the exon borders are not conserved compare to the *C. gigas* and human genes. A large decrease in intronic sequence size was seen in *D. melanogaster* and *C. elegans* compared to the human PGM gene.

3.4. Expression studies

The results of RT-PCR showed regulation of *Cg*PGM mRNA according to the experimental conditions. Expression of PGM was first analyzed in several tissues (digestive gland, gill, adductor muscle and mantle) to determine a potential tissue specific expression of PGM mRNA in *C. gigas*. *Cg*PGM expression was higher in digestive gland and adductor muscle compared to gill and mantle (Fig 3A). *Cg*PGM expression was then evaluated only in digestive gland for all the samples (control and treated) in other experiments. From all the experiments, *Cg*PGM mRNA levels were determined in the control oysters, and none of them showed any significant variation in any experiments.

In the pesticide experiment, an increased level of PGM mRNA expression was detected in individuals exposed for 7 days only in ADI experiments compared to the control ($t= 4.857$, $p<0.05$) following by a regular decrease in PGM mRNA level to reach RNA levels similar to the control (Fig. 3B). Statistical analysis of the means showed no significant difference ($p<0.05$) between samples at 7, 14, 21 and 30 days compare to the control in glyphosate experiments even a slight increase was observed after 7 days of exposure ($t= 2.598$, $p<0.1$). In the hydrocarbon experiment, significant higher levels of PGM mRNA were observed after 7 days ($t= 5.568$, $p<0.05$) and a lower increase was also observed at 14 days of exposure ($t = 3.126$, $p<0.01$) (Fig. 3C).

4. Discussion

At present, very few genomic sequences encoding for PGM have been characterized either in invertebrates or vertebrates. Here, we identified for the first time both cDNA and gene of PGM in a mollusc species, the Pacific oyster *C. gigas*. The cDNA obtained from the adductor muscle produced only one of the two loci coding for PGM in *C. gigas*, the one commonly called PGM-2. Among the two loci, only PGM-2 was polymorphic when using allozyme electrophoresis, while PGM-1 was monomorphic (Pogson, 1989, 1991). The molecular characterization of the Pgm-2 genomic sequence was based on adductor muscle cDNA sequence but similar cDNA sequence has been identified in digestive gland. The PGM-cDNA we identified encodes a protein of 555 amino acids and corresponds to a gene of about 7 Kbp that comprises 8 exons. In the *Cg*PGM gene sequence, the phosphoserine motif (ILTASHNP) which contains the serine of rat PGM that is phosphorylated during catalysis and the Mg-binding motif (DGDGDR) are similar to those described in *C. elegans* and *H. sapiens* (Milstein and Sanger, 1961). But the putative sugar-binding motif has several amino acid differences (EEERFG in *C. gigas* and GEESFG *in C. elegans* and human) and it has been demonstrated that this motif is less well conserved (Whitehouse et al., 1998). As in others species, the three motifs occur in the same linear order: the Mg-binding site is central, the phosphoserine site on the amino terminal side and the sugar-binding site on the carboxy-terminal side. Moreover, the mean distances between TASHNP, DGDGDR, and EEERFG are 162 and 80 amino acids, respectively.

Phylogenetic analysis using amino acid alignments showed that PGM from *C. gigas* seems closer to vertebrates than other invertebrate species such as *C. elegans* or *D. melanogaster* that are the closest invertebrates species in which PGM gene have been fully sequenced. With 8 coding exons and 7 introns, CgPGM gene has a comparable organization in terms of exon size with human PGM gene except for exons 3 and 5 in *C. gigas* that represent the merging of three exons and two exons in the corresponding human PGM sequence respectively. Both *C. elegans*

and *D. melanogaster* Pgm genes possess only 4 exons and 3 short introns (all comprised of between 48 and 534 bp). Recent studies showed that in Lophotrochozoe species, genes organization (number of introns and exons) is quite similar to their vertebrate orthologs and much higher than in the ecdysozoans species such as *Drosophila* and *C. elegans* (Raible et al., 2005). In the same studies, authors demonstrated that worms and human proteins were found to be closely related to each other than to their ecdysozoan orthologs as we observed in *C. gigas*. Previous phylogenetic studies on the PGM super-family also revealed a complex evolutionary history marked by duplications, translocations and at least two cases of transkingdom horizontal gene transfer. Some of the duplicated genes performed novel functions such as in yeast, where one PGM is constitutively expressed whereas the other is induced by galactose (Oh and Hopper, 1990). In human and mice, a PGM1 paralog has lost phosphoglucomutase activity and constitute a structural protein that interacts with cytoskeletal proteins in the adherens type cellular junctions (Belkin et al., 1994). The lack of PGM sequences in invertebrates and more especially in mollusc species makes difficult the comparison of the PGM oyster sequence with closer related species.

PGM resides at the glycolytic pathway branch leading to glycogen synthesis and variations in its activity could contribute to the regulation of carbohydrate storage through the breakdown or synthesis of glycogen. Studies focusing on the functional properties of PGM variants in many organisms from insect to human showed correlations between PGM polymorphism, glycogen content and PGM activity (Leigh Brown, 1977; Sugie et al., 1988). In previous works, it was demonstrated that phosphoglucomutase activity in the mantle and adductor muscle tissues of *C. gigas* was significantly correlated to the season of collection, intertidal location and PGM2 genotype (Pogson, 1991). Similar results were found in other marine mollusc species, where metabolic enzyme activity levels have exhibited strong seasonal fluctuations (Gabbot and Head, 1980; Livingstone and Clarke, 1983), in particular, according to their reproductive cycle stage (Gabbot, 1983). In our experimental studies, we observed that PGM mRNA expression was higher in adductor muscle and digestive gland compared to gill or

mantle. Our results also showed that PGM mRNA expression could be modulated by some xenobiotics such as hydrocarbons or pesticides. But because of a lack of similar works in literature, we can not compare these results. However, we noticed a comparable response of mRNA regulation in both hydrocarbon and pesticide experiments. Following the pesticide exposure, a significant increase of PGM is observed in the first week after exposure followed by a regular decrease of mRNA level to reach the control level in the next weeks. These results were only obtained in ADI experiment but not in response to glyphosate exposure, showing a differential effect of both pesticides treatment. A similar pattern is noticed in response to hydrocarbons exposure but with an increase of mRNA PGM level observed essentially in the first week of exposure. Nevertheless, in both case, the observed increases remains low (less than a two-fold increase) suggesting a moderate regulation of PGM in response to the xenobiotic used. The elevation of PGM level in the beginning of the pollutant exposure could reflect a higher production of energy to overcome of the pollutant stress and can be considered as an "early stress response". One explanation of PGM MRNA increase could be a higher need in energy at the beginning of the pollutant exposure. The activation of PGM mRNA expression helps to produce more sugars that enter in the energy cycle to produce ATP. Also because we have no information about the possible correlation between the amount of PGM mRNA and the corresponding protein concentration and/or its activity, it does not appear clearly that the activation of PGM mRNA by xenobiotics can be linked to the energy requirement. In the mussel *Perna viridis*, a drastic drop of condition index (significant decrease in a fuel reserve indicator) has been observed in mussels exposed to metals for three days when compared to control mussels (Yap et al., 2004) suggesting the same pattern of energy consumption at the beginning of a stress process. This phenomenon could be due to higher energy requirement as a result of more enzyme synthesis in order to overcome of the metal stress. In previous studies, we showed that other genes related to energetic metabolism such as phosphoenol pyruvate kinase, aspartate amino transferase and ATP syntase, were up-regulated by hydrocarbon exposure (Boutet et al.,

2004; Tanguy et al., 2005). All theses results suggest that metabolic genes are regulated by stress factors but they also show that their expression is not stress specific and their regulation mostly at the beginning of the xenobiotic exposure may reflect a classical increase in energy need and consumption associated to the synthesis of many “stress-regulated” specific genes and related metabolisms.

Other studies showed a strong positive correlation between PGM activity and glycogen biosynthesis that is dependant on diet, and that PGM deficiencies resulted in glycogen-storage disease in humans (Leigh Brown, 1977). Line-specific PGM activity variations have also been correlated with glycogen content in *D. melanogaster* (Clark and Keith, 1988; Clark, 1989). It is also possible that PGM amino acid mutation decreases enzyme activity and are deviations from optimal protein function (Knowles, 1991). Temperature can also play a major role in catalyzing enzymatic reactions and an increase in PGM activity can reflect compensation for temperature to maintain constant glycogen content. Thermal compensation can be accomplished by altering transcription levels to maintain enzyme activity (Crawford and Powers, 1992). In *D. melanogaster*, it was clearly showed that PGM amino acid polymorphism was significantly associated with enzyme activity and glycogen content and could alter flux in the pathway to glycogen synthesis (Verelli and Eanes, 2000). It is possible that glycogen storage may be causally connected to starvation resistance by being an energy reserve (Chippendale et al., 1996). Differences among genotypes for such fitness-related characters as fecundity, hatchability, developmental rate, and mating capacity were also found in males in *D. pseudoobscura* (Marincovic and Ayala, 1975). In previous studies, we demonstrated the existence of selective effect of pesticides and tributyltin on particular allele at the PGM locus in *C. gigas* (Tanguy et al., 1999; Moraga and Tanguy, 2000) and many other studies showed a selective effect of heavy metals, essentially copper and cadmium on PGM allozyme in several marine invertebrate species (Nevo et al., 1983; Nevo et al., 1984; De Nicola et al., 1992; Yap et al., 2004). Nevertheless, in none of these studies and because of a lack of molecular data, no direct correlation between

PGM genotype and corresponding protein activity or sugar concentration variations was demonstrated.

In conclusion, the molecular characterization of the oyster PGM gene will offer an opportunity to relate functional analysis at the genetic level to environmental stresses and allelic composition at the population level in various ecosystems. Situated at the glucose-6-phosphate branch point, the potential differences in PGM-2 allele enzyme activities could directly affect the partitioning of flux between glycolysis, the pentose shunt, and the synthesis of glycogen. In the aim to identify potential correlation between activities of the different PGM genotypes and the ability of oysters to survive, studies are underway to first characterize amino acid polymorphism and then try to correlate the physiological effects of this polymorphism on the metabolism of glycogen in both experimental and field oyster populations.

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Figures legend

Fig. 1

An unrooted phylogeny showing the most likely relationship between representative PGM amino acid sequences. Branch lengths are proportional to estimates of evolutionary change. The number associated with each internal branch is the local bootstrap probability that is an indicator of confidence. The sequences are *Oryctolagus cuniculus* (AAA31454), *Homo sapiens* (AAH19920), *Mus musculus* (BAB27648), *Xenopus laevis* (AAH43876), *Crassostrea gigas* (AJ512213), *Drosophila melanogaster* (AF290323), *Anopheles ga*mbia (EAA11635), *Caenorhabditis elegans* (AAA83163), *Arabidopsis thaliana* (CAB64725), *Paramecium tetraurelia* (CAA71089), *Saccharomyces cerevisiae* (P33401), *Brassica napus* (Q9SMM0), Pisum *sativum* (Q9SM59), *Acetobacter xylinus* (P38569), *Aspergillus oryzae* (P57749), *Agrobacterium* t*umefaciens* (P39671), *Escherichia coli* (P36938), *Neisseria gonorrhoeae* (P40390), *Schizosaccharomyces pombe* (O74374), *Solanum tuberosum* (Q9M4G4), *maize* (P93804).

Fig. 2

Diagram of the organization of three PGM genes (*H. sapiens*, *D melanogaster* and *C. gigas*). Exons are represented by rectangles which include the length (in amino acids) of coding sequence. Introns are represented by lines and the length is expressed in Kbp. The organization of the third and the fifth exon of CgPGM from human PGM sequence is indicated by dotted lines.

Fig. 3

Expression of the *Cg*PGM in different tissues of oyster (A), in digestive gland of individuals exposed to pesticides (B) and hydrocarbons (C) using semi-quantitative RT-PCR : results are

presented as the ratios ODCgPGM/OD28S and *n*=5 to 8 for each sampling date according to the experiment). T= time. Mean value and standard deviations are presented on the graphs.

Fig. 1

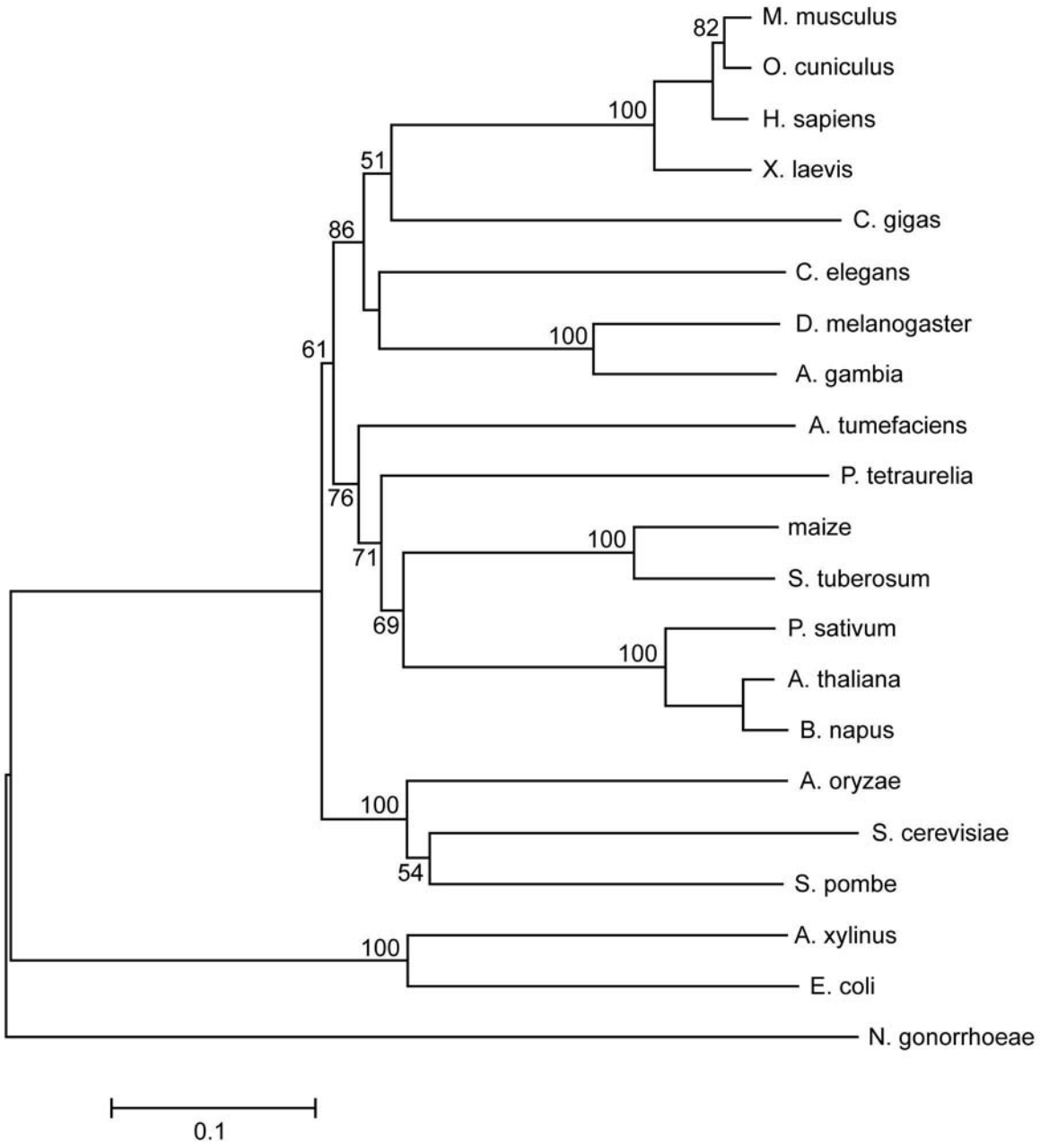

M. musculus
O. cuniculus
H. sapiens
X. laevis
C. gigas
C. elegans
D. melanogaster
A. gambia
A. tumefaciens
P. tetraurelia
maize
S. tuberosum
P. sativum
A. thaliana
B. napus
A. oryzae
S. cerevisiae
S. pombe
A. xylinus
E. coli
N. gonorrhoeae
82
100
51
86
61
100
76
71
100
69
100
100
54
100
0.1

Fig. 2

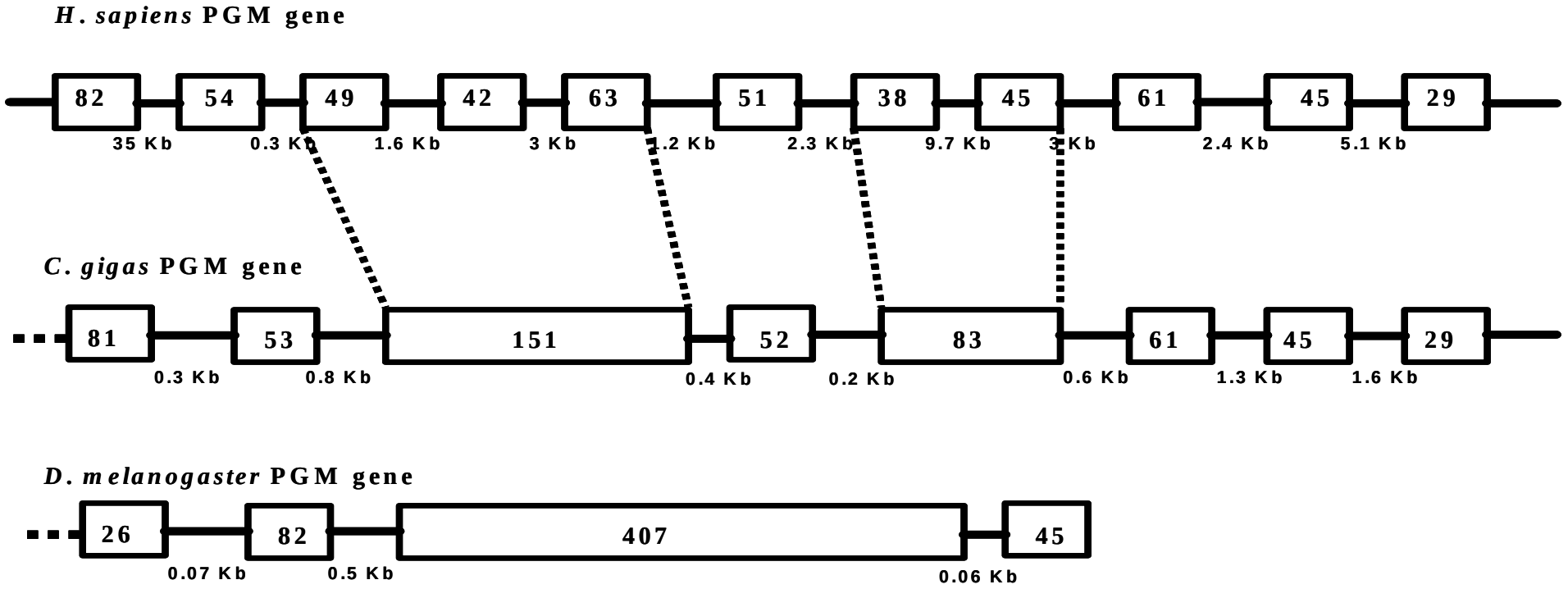
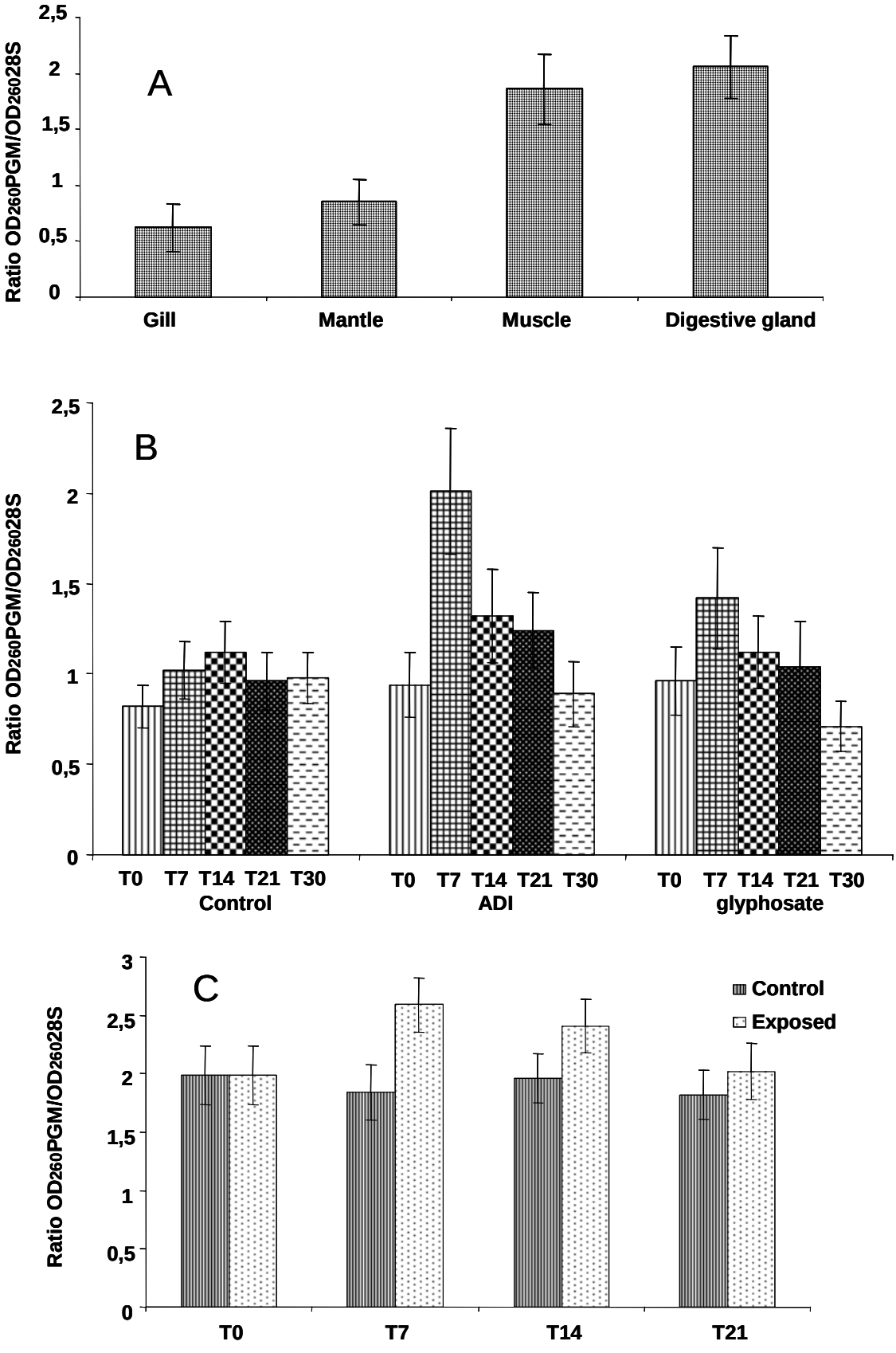

H. sapiens PGM gene
82
54
49
42
63
51
38
45
61
45
29
35 Kb
0.3 Kb
1.6 Kb
3 Kb
1.2 Kb
2.3 Kb
9.7 Kb
3 Kb
2.4 Kb
5.1 Kb
C. gigas PGM gene
81
53
151
52
83
61
45
29
0.3 Kb
0.8 Kb
0.4 Kb
0.2 Kb
0.6 Kb
1.3 Kb
1.6 Kb
D. melanogaster PGM gene
26
82
407
45
0.07 Kb
0.5 Kb
0.06 Kb

Fig. 3


A
Ratio OD260PGM/OD26028S
2,5
2
1,5
1
0,5
0
Gill
Mantle
Muscle
Digestive gland
B
Ratio OD260PGM/OD26028S
T0 T7 T14 T21 T30
Control
ADI
glyphosate
C
Ratio OD260PGM/OD26028S
3
2,5
2
1,5
1
0,5
0
Control
Exposed
T0
T7
T14
T21