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Cloning and characterization of *DjPRPS* gene in freshwater planarian *Dugesia japonica*

[*DjPRPS* geninin tatlısu planaryası *Dugesia japonica*'da tanımlanması ve klonlanması]

Changying Shi[1],
Zimei Dong[1],
Hecai Zhang[1],
Fangfang Cheng[1],
Guangwen Chen[1],
Dezeng Liu[2]

[1] Henan Normal University, College of Life Sciences, Xinxiang 453007, China
[2] Heilongjiang Academy of Science, Institute of Natural Resources, Harbin 150031, China

Correspondence Address
[Yazışma Adresi]

Prof. Dr. Guangwen Chen

Henan Normal University, College Of Life Sciences, No.46, Jianshe East Road, Xinxiang 453007, China
Phone: +86 15836064961
E-mail: chengw0183@sina.com

Translated by [Çeviri] Dr. Aylin Sepici Dinçel

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ABSTRACT

Objective: To clone the phosphoribosyl pyrophosphate synthetase gene from *Dugesia japonica*, and to do the bioinformatics and spatial expression analysis of the gene.

Methods: The phosphoribosyl pyrophosphate synthetase gene (*DjPRPS*) was cloned from *Dugesia japonica* using the rapid amplification of cDNA ends method and its sequence was analyzed with biological softwares. Spatial expression pattern of the gene was detected by whole-mount in situ hybridization.

Results: The full length cDNA of DjPRPS was 1,086 bp, containing a 80 bp of 5'-untranslated region, a 55 bp of 3'- untranslated region and a 951 bp of open reading frame encoding a protein of 316 amino acids with a calculated molecular mass of 35.18 kDa and isoelectric point of 8.14. The deduced amino acid sequence shared the conserved eukaryotic PRPS family motif. Phylogenetic analysis supported the traditional concept of zoological taxonomy in large scale. Intense positive signals were detected as bilateral lines of patches in the ventral side where the ovaries and yolk glands located in the intact sexual planarians. In the regenerating planarians, the expression pattern was similar to that of the intact samples, and no intense transcipt signals were detected in the blastema.

Conclusion: *DjPRPS* sequence was highly homologous with that of other eukaryotic phosphoribosyl pyrophosphate synthetase gene. Intense transcript signals of *DjPRPS* were not detected in the blastema but in ovaries and yolk glands, suggesting that its function was probably reproduction-related and maybe not involved in regeneration.

Key Words: *Dugesia japonica*, *DjPRPS*, cloning, whole-mount in situ hybridization

Conflict of Interest: The authors have no conflict of interest.

ÖZET

Amaç: *Dugesia japonica*'dan fosforibozil pirofosfat sentetaz geninin klonlanması ve genin mekansal ekspresyon analizi ile bioinformatik çalışmaların yapılmasıdır.

Metod: Fosforibozil pirofosfat sentetaz (*DjPRPS*) geni *Dugesia japonica*'dan cDNA ucunun hızlı amplifikasyon metodu kullanılarak klonlandı ve dizilim biyolojik yazılımlar ile analiz edildi. Genin mekansal ekspresyon motifi whole-mount in situ hibridizasyon ile saptandı.

Bulgular: *DjPRPS* geninin tüm cDNA uzunluğu 1,086 bp'dir. Bu yapı, 80 bp 5'-çevrilmemiş bölge, 55 bp 3'-çevrilmemiş bölge ve 951 bp'lik izoelektrik noktası 8.14 ve hesaplanan moleküler kütlesi 35.18 kDa olan 316 amino asitden oluşan proteini kodlayan açık okuma ucu içerir. Ortaya çıkarılan amino asit dizilimi korunmuş ökaryotik PRPS ailesi motifini paylaşmıştır. Filogenetik analizler büyük oranda geleneksel zoolojik taksonomi kavramlarını desteklemiştir. Bozulmamış seksüel planaryalarda yerleşmiş yumurtalık ve yolk keselerinde ventral bölgede yoğunlaştırılmış pozitif sinyaller bilateral yama tarzında çizgiler olarak tespit edildi. Rejenere olan planaryalarda da ifade edilme kalıbı benzerdi ve embriyonik hücre gruplarında yoğun transkripsiyon sinyalleri tespit edilmedi.

Sonuç: *DjPRPS* gen dizilimi diğer ökaryotik fosforibozil pirofosfat sentetaz geni ile yüksek oranda homoloji göstermektedir. Yumurtalıklar ve yolk kesesinde yoğun transkripsiyon sinyalleri tespit edilmiş olmasına rağmen embriyonik hücre gruplarında *DjPRPS* için yoğun transkripsiyon sinyalleri tespit edilmemesi genin fonksiyonunun üreme ile alakalı olduğunun, rejenerasyon sürecine dahil olmayabileceğini akla getirmektedir.

Anahtar Kelimeler: *Dugesia japonica*, *DjPRPS*, klonlama, whole-mount in situ hibridizasyon

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Planarians are bilateral symmetry and triploblastic animals. They belong to phylum Platyhelminthes and are viewed as members of the Lophotrochozoa (together with annelids and molluscs), which is one of the three major phylogenetic groups of bilaterally symmetric animals [1,2]. The great powers of regeneration shown by freshwater planarians have attracted the interest of scientists throughout history [3]. In addition to their regenerative capability, planarians have the unique ability to convert from an asexual state to a sexual state and *vice versa*, known as "sexualization" and "desexualiation", respectively [4]. It is reported that some planarians, e.g. those of genus *Dugesia*, comprise at least three races with respect to the reproductive mode, namely asexual race, sexual race, and physiological race [5]. In the asexual race, worms reproduce by fission without sexual organs. In the sexual race, worms with hermaphroditic sexual organs can copulate and lay cocoons filled with several fertilized eggs. They are not likely to fission. In the physiological race, worms convert between asexual and sexual reproduction seasonally [6].

The mechanisms that lead to germ-cell formation in planarians, or the signals that instruct ovaries, testes and the complicated reproductive apparatus to be formed in the appropriate place in the flatworm have not been studied very deeply up to now [7]. At the molecular level, the first germ-line markers being identified in planarians were a receptor tyrosine kinase (*DjPTK1*) [8] and two vasa-like genes (*DjvlgA* and *DjvlgB*) from *D. japonica* [9]. Then *DeY1* in *D. etrusca* [10], *Dryg* [11] in *D. ryukyuensis*, nanos-like genes in *D. japonica* (*Djnos*) [12], *Schmidtea mediterranea* (*Smednos*) [13] and *D. ryukyuensis* (*Dr-nanos*) [14], boule-like genes in *Macrostomum lignano* [15] and *Drpiwi* in *D. ryukyuensis* [16] were also cloned and their expression and function were explored via whole-mount in situ hybridization (WISH), RNA interference (RNAi) and/or other methods.

The enzyme 5-phosphoribosyl-1-pyrophosphate synthetase (PRPS; EC 2.7.6.1), also called ribose-phosphate pyrophosphokinase, is responsible for the synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP), a precursor required for the production of purine, pyrimidine and pyridine nucleotides and the amino acids histidine and tryptophan [17]. PRPS is required for both the *de novo* and salvage pathways and therefore represents a key enzyme in intermediary metabolism [18]. As one of the more conserved genes during evolution, *PRPS* gene has been cloned and sequenced from a variety of organisms, including bacteria [19], yeast [20], protozoa [21], nematodes [22], plants [23], rat [24] and human [25] etc. In many organisms, PRPS can be encoded by more than one genes [26,18]. PRPS gene together with other six genes was cloned in planarian *D. ryukyuensis* [27], and its spatial expression in yolk gland which is an organ specific to sexual worms indicated that *PRPS* might be one of sexual reproduction related genes in planarians.

In this paper, we cloned and identified the full-length *PRPS* cDNA sequence from *Dugesia japonica* and examined its spatial expression pattern in intact and regenerating planarians. This study will lay the foundation for further studying the function of *DjPRPS*.

Materials and Methods

Animals

Planarians used in this study belong to the sexual strain of *D. japonica*, collected from Yuquan spring (Henan Province, China). They were cultured in dechlorinated tap water in dark at 20°C and fed once a week with fresh fish spleens. Animals were starved for at least 1 week before being used in the experiments.

RNA extraction and DjPRPS cDNA cloning

Total RNA was extracted using E.Z.N.A.TM Mollusc RNA Kit (Omega, USA) and 2 µg RNA was used for reverse transcription. Based on *PRPS* gene sequences of planarian *D. ryukyuensis* (Drs6 in [27]; GenBank accession number: AB284777) and other close-related animal species, a set of primers (forward: 5'-TGCC GGAG CAAA TCAT AT-3' and reverse: 5'-GGTA TTGT GTTT GTTA CTAC-3') designed by Oligo 6.0 software was used to amplify the partial fragment of *PRPS* from *D. japonica*. The PCR program was as follows: 94°C for 5 min, followed by 35 cycles of 94°C for 40 s, 48°C for 30 s, 72°C for 1 min and a final extension step at 72°C for 10 min. The PCR products were gel-purified and sequenced. Based on the known expressed sequence tag (EST), the 5'-gene specific primer (Inner primer: 5'-TATGACATGGCTCAGA CAAAAG-3' and Outer primer: 5'-CTGCACTGCTTA-CACTTAAACTG-3') and 3'-gene specific primer (Inner primer: 5'-TGCT ACTC ATGG AATG TTAT CAG-3' and outer primer: 5'-AAGATTGATGGAAGTTGGCG-3') were designed for rapid amplification of cDNA ends (RACE). Both 5'-RACE and 3'-RACE were carried out using a RACE cDNA amplification kit (TaKaRa, China) according to the protocol. The PCR products were gel-purified, ligated into the pMD19-T Vector using the pMD19-T Vector kit (TaKaRa, China) in terms of the manufacturer's instruction, and submitted for sequencing. The amplified gene was named *DjPRPS* (*Dugesia japonica PRPS*), and the sequence was deposited into the GenBank database.

Bioinformatics analysis of deduced protein

The open reading frame (ORF) was identified using the ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The physicochemical properties were analyzed by Protparam (http://web.expasy.org/protparam/). The signal peptide and subcellular localization were predicted by SignalP-4.1 (http://www.cbs.dtu.dk/services/SignalP) and BaCelLo (http://gpcr.biocomp.unibo.it/bacello/pred.htm), respectively. SMART (http://smart.embl-heidelberg.de) was used to predict the function domain and Swiss-model

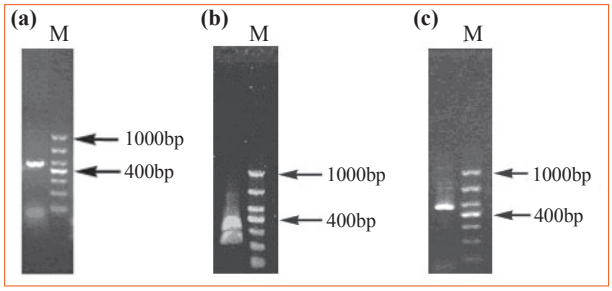

Figure 1. Electrophoresis results of EST **(a)**, 3' **(b)** and 5' **(c)** DNA segments of *DjPRPS*. M: 1000 bp Marker.

(http://swissmodel.expasy.org/) to predict the three-dimensional structure of the deduced protein.

Homology analysis and phylogentic tree reconstruction

The similarity analysis of nucleotide and amino acid sequences were carried out by using BLASTn and BLASTp at web servers of the National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast). Pairwise protein and nucleotide sequences identities were calculated using the MegAlign program in DNAStar software package, respectively. Protein multiple sequences alignment were done using Clustal_1.83 (http://www.clustal.org/). Phylogenetic relationship analysis was carried out based on NJ (neighbor-joining) and UPGMA (unweighted pair-group method with arithmetic means) methods using Mega 4.0 program (http://www.megasoftware.net/), and statistical support was provided by 1,000 bootstrap replications.

Whole-mount in situ hybridization (WISH)

A fragment of *DjPRPS* was used as templates for the sense and antisense digoxigenin-labeled RNA probes, which were synthesized using the RNA in vitro labeling kit (Roche). Whole-mount in situ hybridization was performed on intact and regenerating planarians essentially as reported [28,29] by treating planarians with 2% HCl, and then fixed in 4% paraformaldehyde for 4 h at 4°C. After being rinsed twice with NaPBSTw and bleached in 6% H_2O_2 at room temperature under UV light for more than 6 h, planarians were treated with 10 µg/ml proteinase K and fixed in 4% paraformaldehyde for 1 h at room temperature, followed by twice rinse with NaPBSTw. Prehybridization was done at 55°C for 3 h in prehybridization solution. Hybridization was performed at 55°C for 24 h with digoxigenin (DIG)-labeled riboprobes in hybridization solution. Planarians were blocked in 1% blocking reagent (Roche) for 1 h and incubated with an anti-DIG antibody at 4°C overnight. The color was developed using NBT/BCIP for 20 min in dark until signals were visible, then the reaction was stopped by rinse in NaPBSTw. Samples were observed with a Leica DMLB stereomicroscope and images were captured with a Leica DFC300FX camera.

Results

cDNA cloning and sequencing of planarian DjPRPS

The PCR product amplified by the specific primers was about 460 bp (Fig.1a), and its nucleotide sequence was significantly similar to other known *PRPS* genes. *DjPRPS* 3'-terminal specific primer and TaKaRa 3'-RACE inner primer were used to amplify a fragment about 330 bp (Fig.1b). *DjPRPS* 5'-terminal specific primer and TaKaRa 5'-RACE inner primer were used to amplify a fragment of 460 bp (Fig.1c). The complete cDNA sequence

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                                                CGCGGATCCACAGCCTACTGATGATCAGTCGATGGATTATATAATTAATGACCTTCAGCGTAAACTATGTTCTCTAAACT

81   ATGTTGAATATTAGATTATTTTCGGGCTCTTCAAATGTTTTATTGACAGAAAAAATAGGAGAAAGGATTGGAATTAAACTATCTGAAGCT
1    M  L  N  I  R  L  F  S  G  S  S  N  V  L  L  T  E  K  I  G  E  R  I  G  I  K  L  S  E  A

171  ATTTTAAACAAATTTAGCAATAATGAAACCAGTGTTCAAATAAAAGAATCAGTAAGAGGTAAAGACGTTTTTATTTTACAGTCTGGATAT
31   I  L  N  K  F  S  N  N  E  T  S  V  Q  I  K  E  S  V  R  G  K  D  V  F  I  L  Q  S  G  Y

261  ATAGGTGTTAATAATCATTTGATGGAGCTACTTATTATGATAAATGCGTGTAAAAATGCGTCATCTGCTCGCATTACAGCAGTTTTACCA
61   I  G  V  N  N  H  L  M  E  L  L  I  M  I  N  A  C  K  N  A  S  S  A  R  I  T  A  V  L  P

351  TATATGCCATATTCTCGTCAAAGCAAGAAAGATAATAACCGTGTGCCAATTACAGCAAAATTGGTTGCTGATTTATTGCACATTGCTGGA
91   Y  M  P  Y  S  R  Q  S  K  K  D  N  N  R  V  P  I  T  A  K  L  V  A  D  L  L  H  I  A  G

441  GCCAATCATATTATAACAATGGATTTACATGCTAGTCAAATTCAAGGATTTTTTAATGTTCCAGTAGATAATCTTTTGTCTGAGCCATGT
121  A  N  H  I  I  T  M  D  L  H  A  S  Q  I  Q  G  F  F  N  V  P  V  D  N  L  L  S  E  P  C

531  CATATATCATGGATCAAGAAAAATATTTCCAAATGGGAAGAGATTACCATATTCAGTTTAAGTGTAAGCAGTGCAGAACGTGTTGCCAAT
151  H  I  S  W  I  K  K  N  I  S  K  W  E  E  I  T  I  F  S  L  S  V  S  S  A  E  R  V  A  N

621  ATGGCTAATACCATTAAAGTAAGATTTGGATTAATATATGTGGAAAGAAAAGAAGCTTCAGAACAGATCATAGTAATCGGAGACGTAGTT
181  M  A  N  T  I  K  V  R  F  G  L  I  Y  V  E  R  K  E  A  S  E  Q  I  I  V  I  G  D  V  V

711  TCTCGAACTGCTGTAATATTTTTGGACATAATTGATAATTGTGATCTAGTAATTAGTTCTGTAAAAAGATTGATGGAAGTTGGCGCGTCA
211  S  R  T  A  V  I  F  L  D  I  I  D  N  C  D  L  V  I  S  S  V  K  R  L  M  E  V  G  A  S

801  GGCGTCTATGTGATTGCTACTCATGGAATGTTATCAGAAAGATTAATGAAAGAAGTAGATGATTCCATTATCACCAAATTGGTAGTAACA
241  G  V  Y  V  I  A  T  H  G  M  L  S  E  R  L  M  K  E  V  D  D  S  I  I  T  K  L  V  V  T

891  AACACAATATGTCAGGAAAAATATGTTAAACAAAGCGATAAAATCACATGTCTCGATGTCAGTGTGATATTTGCTGAAGCTATTCGCAGA
271  N  T  I  C  Q  E  K  Y  V  K  Q  S  D  K  I  T  C  L  D  V  S  V  I  F  A  E  A  I  R  R

981  ATACATTACGGCGAATCGGTCTCATGTTTATACAATAATCTTCTAGCCTGAAATGCTTTATTATATTTTAAAGTAAACTTGTCCTTATTA
301  I  H  Y  G  E  S  V  S  C  L  Y  N  N  L  L  A  *

1071 TCAGAAAAAAAAAAAAA
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Figure 2. Nucleic acid and amino acid sequence of *DjPRPS*. The start coden, stop coden and the conserved region corresponding to the PRPS motifs characteristic of the PRPP- and divalent cation-binding sites are underlined.

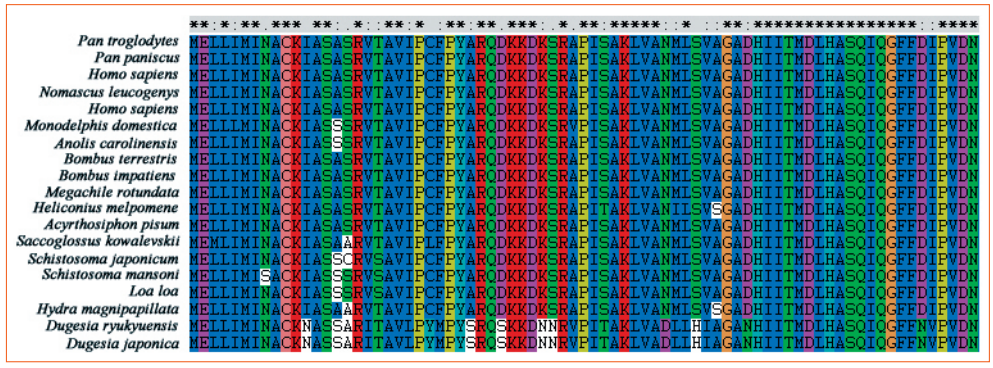

Figure 3. The alignment result of the conserved motif of DjPRPS and other eukaryotic PRPS. The asterisks indicate the identical amino acids.

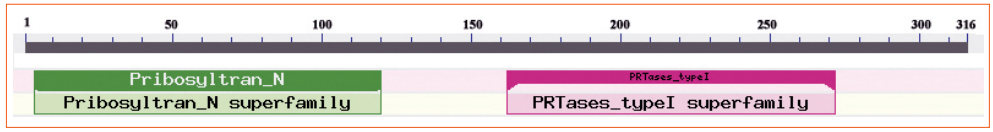

Figure 4. Distribution of two tandem conserved domains of phosphoribosyl pyrophosphate synthetase from *Dugesia japonica*.

of *DjPRPS* gene of 1,086 bp was obtained by assembling the above three fragments.

Characterization of DjPRPS

The cDNA sequence of the *DjPRPS* gene was deposited in GenBank under the accession number KC207115. The full-length cDNA of *DjPRPS* was 1,086 bp, including a 5'-untranslated region (UTR) of 80 bp, a 3'-UTR of 55 bp with a short polyA tail, and an open reading frame (ORF) of 951 bp encoding a polypeptide of 316 amino acids with the predicted molecular mass of 35.18 kDa and theoretical isoelectric point of 8.14. The deduced amino acid sequence of *DjPRPS* shared the eukaryotic PRPS family conserved motif characteristic of the PRPP and divalent cation binding sites, in which the signature of DLHASQIQGFFNVPVD at the position of 128-143 was the most conserved region (Fig. 2 and Fig.3). DjPRPS had no signal peptide and located in the cytoplasm. The predicted protein domain of DjPRPS, as identified by the SMART program, consisted of a Pribosyltan_N and a PRTases_type I domain (Fig. 4). Fig. 5 showed the predicted three-dimensional structure of phosphoribosyl pyrophosphate synthetase of *D. japonica*. It was a hexamer and very similar to that of human phosphoribosyl pyrophosphate synthetase 1 (hPRS1).

Homology and phylogentic relationship analysis

Sequence homology search using BLASTn and BLASTp revealed that *DjPRPS* sequence had high homology with other eukaryotic PRPS sequences, among them the highest similarity of 85.7% with *D. ryukyuensis* (GenBank accession No. AB284777), 61.8%, 52.0% and the lowest of 44.7% with *Schistosoma mansoni* (XM_002573602), *Hydra magnipapillata* (XM_002157771) and *Nomascus leucogenys* (XM_003262165), respectively. The protein encoded by *DjPRPS* shared the highest identity of 95.6% with *D. ryukyuensis* (AB284777), the lowest of 49.1% with *Hydra magnipapillata* (XM_002157771), and 51.6% with *Schistosoma japonicum* (FN319250) and *Schistosoma mansoni* (XM_002573602) at the amino acid level, respectively.

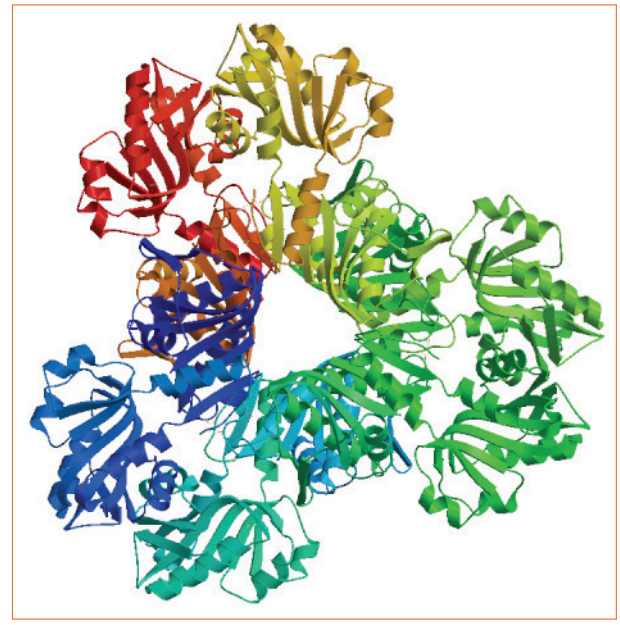

Figure 5. Predicted three-dimensional structure of phosphoribosyl pyrophosphate synthetase from *Dugesia japonica*.

To shed light on the evolutionary position of *DjPRPS*, a phylogentic tree was reconstructed using the amino acid sequences of *DjPRPS* and other animals by NJ and UP-

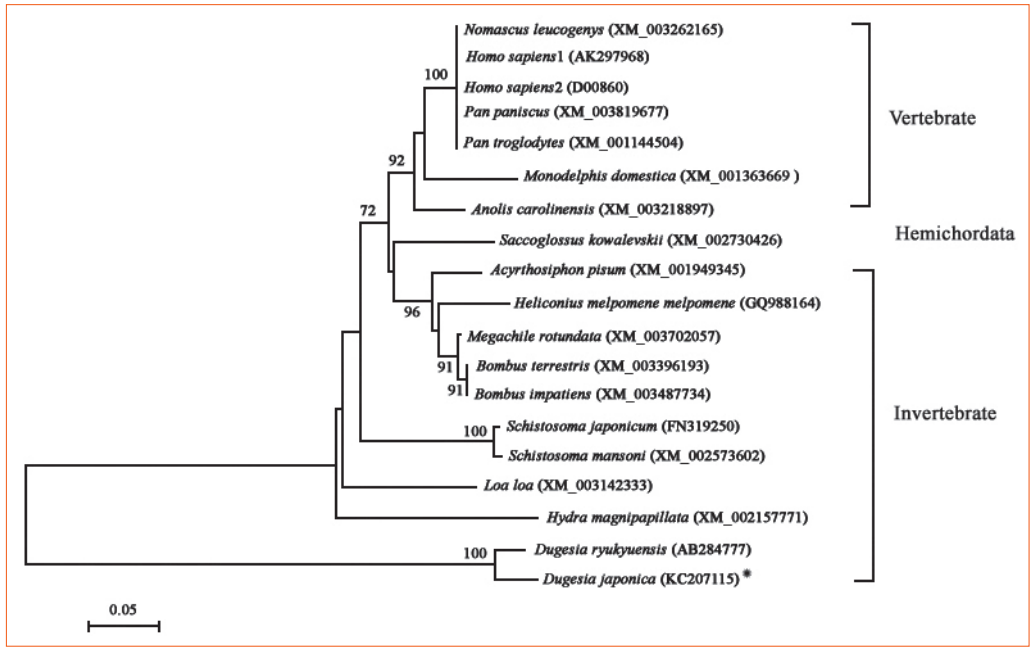

Figure 6. Phylogenetic tree of PRPS proteins constructed by the NJ method. The DjPRPS is asterisked. Numbers at the nodes denote the bootstrap percentages of 1,000 pseudoreplicates (only those>60% are shown).

GMA method, respectively. The results suggested that the two trees were very similar in topology (UPGMA tree not shown). Both trees showed that vertebrate PRPS/PRPS-like proteins clustered together and lay at the terminal of the phylogenetic tree, while invertebrates used in this analysis were all at the base of the tree. *Saccoglossus kowalevskii* belonging to Hemichordata branched out at the midst. This evolutionary pattern was in accordance with the concept of traditional taxonomy. Not surprisingly, DjPRPS clustered with that of planarian *D. ryukyuensis* with robust support (bootstrap value 100%). But interestingly, these two species of planarians did not cluster with *Schistosoma japonicum* (FN319250) and *Schistosoma mansoni* (XM_002573602) which also belong to phylum Platyhelminth but lay at the most basal position of the phylogenetic tree, more ancestral than hydra and schistosome (Fig. 6).

The spatial expression pattern of DjPRPS in sexual intact and regenerating planarians

To determine the spatial expression pattern of *DjPRPS*, we performed whole-mount in situ hybridization (WISH) on sexual intact planarians using a DIG-RNA probe of 600 bp in the encoding region of *DjPRPS*. Positive hybridized signals were found ubiquitously in the whole body except the pharynx. Intense signals were detected as bilateral lines of patches in the ventral side where the ovaries and yolk glands located (Fig.7). In order to analyze the expression of *DjPRPS* during regeneration, WISH was also performed on regenerating planarians after being cut behind the auricles at different time points. Not surprisingly, the expression pattern of *DjPRPS* in the head and tail segments was same as that in the intact planarian.

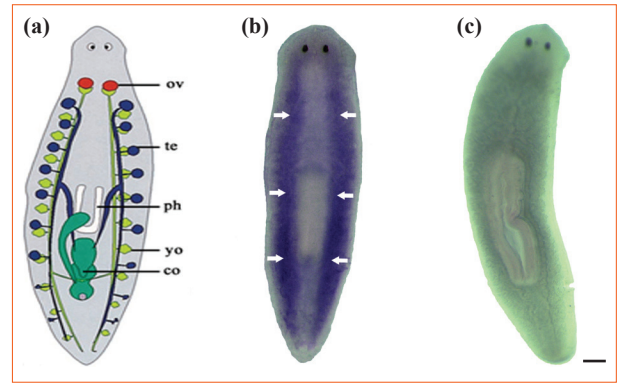

Figure 7. Spatial expression of DjPRPS in the sexual planarian by whole-mount in situ hybridization. **(a)** Scheme of dorsal view of a sexually mature planarian (from [30]). A pair of ovaries (ov) is present in the neck region of the ventral side. A lineal pair of testes (te) is present in the dorsal side from neck to tail. At the ventral side corresponding to the position of testes lie bilateral lines of patches of yolk glands (yo). The copulatory organ (co) is located in the region posterior to the pharynx. **(b)** Ventral view of the intact sexual planarian. DjPRPS is expressed as bilateral lines along the anterior-posterior axis of the planarian (white arrows). **(c)** Ventral view of the intact sexual planarian by WISH without probes as a negative control. In A-C anterior is to the top. The scale bar represents 300 μm.

No intense transcipt signals were detected in the blastema regardless of regenerating at 1, 3 or 5 days (Fig. 8).

Discussion

Phosphoribosyl pyrophosphate synthetase (PRPS) can catalyze ribose-5-phosphate and MgATP to form phosphoribosyl pyrophosphate (PRPP) in the synthesis of virtually all nucleotides, as well as being an important regulator of

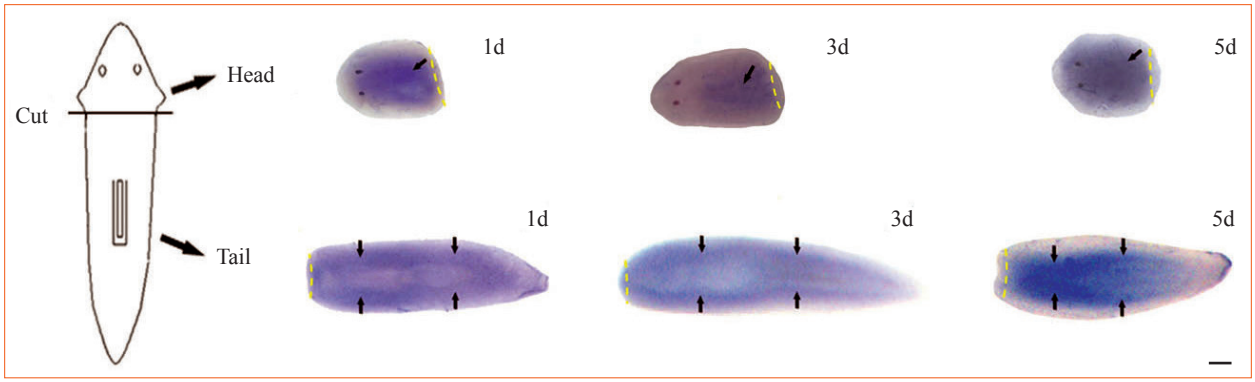

Figure 8. Expression of DjPRPS by WISH in regenerating planarians. The upper shows the head segments, and the lower shows the tail segments. All of them are ventral views and anterior to the left. DjPRPS expressed as bilateral lines along the anterior-posterior axis of the planarian in the tail segments and ubiquitously in the head segments (black arrows). There are no intense signals detected in the regenerating blastema. The yellow dash lines indicate the border between the regenerating region and the stump. 1d, 3d, 5d: 1, 3, 5 days after amputation. The scale bar represents 400μm.

rates of the *de novo* and salvage pathways of purine and pyrimidine nucleotide synthesis [27,31]. This allosteric enzyme is inhibited by both ADP and GDP, and can be repressed by uridine compounds [32]. *PRPS* genes and their products are of interest not only for their importance to our understanding of biochemistry but also for their medical significance. Overexpression of X-chromosome linked *PRPS* in humans correlates with gouty arthritis and in some cases, disorders of the nervous system, such as sensorineural hearing loss [33,34]. Study of *PRPS* in protozoan parasites may lead to the development of new, more specific anti-parasitic drugs [18]. As one of the comparatively conservative genes during evolution, *PRPS* are found ubiquitously from bacteria to human. It has been reported that *PRPS* genes have been cloned and sequenced from a variety of organisms, including bacteria [19], yeast [20], protozoa [21], nematodes [22], rat [24] and human [25]. However, except for Hase and co-workers [27], this important gene has not been explored in planarians so far.

In this study, we successfully cloned a full-length *PRPS* cDNA from the freshwater planarians *D. japonica* for the first time. A *DjPRPS* homologue does not exist in planarian S*chmitdea mediterranea* by Blast retrieving the *S. mediterranea* genome database. *DjPRPS* was likely acquired in an ancestor of *Dugesia* or a related species and subsequently became diversified [14]. By multiple alignment of *PRPS* of *D. japonica* and other eukaryotic organisms, we found *DjPRPS* protein possesses a very conserved region corresponding to the PRPS motif characteristic of the PRPP- and divalent cation-binding sites. The phylogenetic relationship based on PRPS supports the traditional concept of zoological taxonomy in the large scale. However, the systematic position of planarian has not been solved in this molecular tree. This is probably due to the sparse sampling or the less evolutionary information in PRPS molecules.

It has been reported that there is a single-copy *PRPS* gene in *E. coli* [35], *Bacillus subtilis* [36] and *Salmonella typhimurium* [37], two in rat [38,24], at least three in man [39], and five members are included in PRPS gene family of *Saccharomyces cerevisiae* [18,20]. All these genes have been cloned and sequenced. In mammals, two PRPS isoforms (*PRPS1* and *PRPS2*) were detected in almost all tissues and an additional *PRPS1*-related gene was found specifically expressed in testis [26,38]. Whether there is a *PRPS* multigene family or whether there are other *PRPS* isoforms in *D. japonica* still needs further study.

The spatial expression pattern of *DjPRPS* was analysed by whole-mount in situ hybridization. In the sexual intact planarian, *DjPRPS* mRNA was mainly found as bilateral lines of patches in the ventral side corresponding to the region of ovaries and yolk glands. This expression pattern was well consistent with that in planarian *D. ryukyuensis* [27] and indicated the reproduciton-related function of *PRPS* in planarians. But interestingly, dispersible positive signals were also detected ubiquitously in the whole body except the pharynx. This may suggested other functions of *PRPS* in *D. japonica*.

Freshwater planarians exhibit powerful regenerative ability and are viewed as a well-known model for studying regeneration [16]. After amputation, the neoblasts adjacent to the wound proliferate, giving rise to the regenerative blastema where new structures will differentiate [40,41]. Distribution of *DjPRPS* transcripts were also examined in regenerating planarians by in situ hybridization. Results showed that the expression pattern in the amputated body fragments was similar to that of intact animals, suggesting *DjPRPS* was not activated in the blastema during regeneration, independently on the regenerating time prolonged.

In summary, we successfully cloned a full-length *PRPS* cDNA from the freshwater planarian *D. japonica* for the first time. The *DjPRPS* possessed the conserved motif similar to other eukaryotic PRPS proteins. The *DjPRPS* gene did not mainly express in the blastema but in the ovaries and yolk glands of sexual planarians, indicating that its function was probably reproduction-related and

maybe not involved in regeneration. This work will lay the foundation for further functional studies of *DjPRPS*.

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Conflict of Interest

There are no conflicts of interest among the authors.

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