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The Transposons of the Sea Urchin *Strongylocentrotus intermedius* Agassiz, 1863: In Silico Versus In Vitro

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Abstract—The sequenced genome data from the sea urchin *Strongylocentrotus purpuratus* were used to identify transposons (TEs, transposable elements) and test for the presence of some of them in the genome of the sea urchin *S. intermedius*. The known TEs of *S. purpuratus* from the RepBase repeats database were used to calculate the primers and identify the corresponding TEs in the *S. intermedius* transcriptome. High-coverage TE sequences from the *S. intermedius* transcriptome were assembled based on comparison with the annotated TEs of the *S. purpuratus* genome. TEs with an insufficient number of reading frames in the transcriptome were beyond the analysis. The approach applied in the present study was successful: out of the 100 assembled TEs of *S. intermedius*, 92 corresponding TEs in the *S. intermedius* genome were identified using the calculated primer pairs. The TE sequences calculated by in silico methods are present in the *S. intermedius* genome and can be useful in further research.

Keywords: repetitive DNA, transposable elements, transcriptome, sea urchins, *Strongylocentrotus intermedius*, *Strongylocentrotus purpuratus*

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INTRODUCTION

Echinoderms are deuterostome animals that are evolutionarily close to chordates. Because of their relatively early evolutionary separation, accessibility, and free development in seawater, echinoderms (particularly sea urchins) have been used as model organisms to find answers to many key questions of developmental biology, evolutionary biology, biochemistry, as well as cellular and molecular biology [32]. Many early studies of developmental biology have been performed on sea urchins of the family Strongylocentrotidae owing to their abundance, large size, and the ease of their maintenance under laboratory conditions [24, 42, 43]. Regeneration processes have been described for sea urchins and other echinoderms [52]. The fusion of gamete nuclei was first described using sea urchins as a model for egg fertilization [25, 38]. The biochemical mechanisms of the cell cycle, including cyclin regulation, were also first illustrated based on the example of sea urchins [20]. The morphology [8, 11, 22, 26, 29, 56, 57], genetics [7, 16, 47, 48, 53, 59], and ecology [4, 31] of strongylocentrotid sea urchins have been studied in detail. Due to the long lifespan in some species of sea urchins, these organisms provide a model for studying ageing processes [10, 15, 18, 19].

The genome of the sea urchin *Strongylocentrotus purpuratus* was published in 2006 [55]. This is one of the few well-annotated echinoderm genomes. The annotation of the sea urchin genome allowed structuring the information on the gene regulatory networks (GRNs) that determine embryonic development [44, 49, 50]. The GRN concept and the confirming data were generated using this sea urchin species as a model as early as in the pre-genome era. The genes involved in the GRN encode transcription factors, components of signal transduction pathways, and structural genes that serve as markers of differentiated states. The name of the concept implies that exons play a major role in gene cascades. The Human Genome project and the subsequent reading of a large number of genomes made it obvious that the bulk of the genome is comprised of sets of repetitive DNA. Exons (encoding

DNA fragments) occupy no more than 2(3)% of the genome [1]. Transposons of different classes constitute more than 50% of the best-assembled genomes. At least 80% of the genome is transcribed in different tissues and at different developmental stages [1, 28]. The TE transcripts are the major components of long non-coding RNA (lncRNA); lncRNA can regulate gene ensembles by changing chromatin conformation [3, 37]. This is a higher level of regulation compared to gene cascades postulated for GRNs.

Retrotransposons (the most numerous class of TEs in mammals) have been shown to be expressed at the early developmental stages in mouse; the expression spectrum of different classes varies at preimplantation development stages, while after implantation the transcription of most (but not all) retrotransposons is repressed. Mammalian genome reprogramming after fertilization requires a large-scale TE transcription. Such transcription is regulated in a feedback fashion through lncRNA [21]. While the first evidence has been obtained for the activity of retrotransposons during embryogenesis, the role of DNA transposons in development is unknown thus far. Non-coding DNA remains the least studied part of the genome in eukaryotes, especially invertebrates. However, due to the well-studied embryogenesis and the abundance of DNA transposons, invertebrates are a promising model for studying the lncRNA regulatory system. In this study, we used the sequenced genome of the sea urchin *S. purpuratus* in order to identify TEs and test for the presence of some of them in the genome of the sea urchin *S. intermedius*. The present research is a preliminary stage before using the selected calculated and verified primers to study the transcription of the corresponding TEs in the development of *S. intermedius*. The divergence of sea urchin species occurred approximately 10 million years ago [34, 35]. The known high level of evolutionary variability of TEs and the long period of time since speciation led to the emergence of species-specific TEs [2].

We used the known TEs of *S. purpuratus*, for which data are available from the RepBase repeats database, for primer design and TE identification in the *S. intermedius* transcriptome. For comparison of the TE transcription, the coelomocyte transcriptomes of *S. intermedius* and *S. purpuratus* were analyzed.

MATERIALS AND METHODS

Repetitive Element Analysis of the Strongylocentrotus Purpuratus Genome

The genome of the sea urchin *S. purpuratus* contains 284 TE sequences, which are annotated in the RepBase repeats database (http://www.girinst.org/repbase/update/search.php?query=purpuratus&querytype=Taxonomy). The genomes of *Caenorhabditis elegans, Drosophila melanogaster, D. pseudoobscura, Anopheles gambiae, Ciona intestinalis, C. savignyi,* and *S. purpuratus* were retrieved from a public database (www.ncbi.nlm.nih.gov/assembly) and were analyzed for repeat element distribution using the Repeat Masker software (http://www.repeatmasker.org) with default parameters. A dataset of repeats characteristic of eukaryotes was used for the search.

Identification of Repetitive Sequences of the Sea Urchin Strongylocentrotus intermedius

The TEs of the *S. purpuratus* genome and *S. intermedius* transcriptome were compared to level the possible differences in TE sequences between the sea urchin species that separated millions of years ago. The reading frames of the *S. intermedius* transcriptome were mapped against the corresponding mobile elements of *S. purpuratus*. The sequences of the first 100 transposable elements, in terms of percent coverage, were assembled from them using a SPADES genome assembler with default parameters.

Reading frames from the *S. intermedius* and *S. purpuratus* coelomocyte transcriptome sequencing are available in open access at the Sequence Read Archive (http://trace.ddbj.nig.ac.jp, accession ID SRP034740 and SRS353141, respectively). In the original experiment, the polyA-transcripts were enriched. The initial reading frames were processed with the Trimmomatic software [9]. The reading frames were filtered for quality (medium-quality reading frames less than 20 and reading frames containing Illumina sequences). The filtered reading frames were mapped to the mobile element sequences of *S. purpuratus* (RepBase). Mapping was carried out using the Bowtie2 sortware with a local-sensitive mode. For each TE, the percentage ratio of the mapped reading frames to the total number of reading frames that were mapped to TE was calculated (Fig. 1).

Primer Design

Based on the assembled TE sequences, specific primers were designed. Design of the primers was accomplished using the Primer-BLAST software (www.ncbi.nlm.nih.gov/tools/primer-blast), with a primer annealing temperature in the range of 59–61°C and a flanking region no larger than 300 bp.

Extraction of the Sea Urchin S. intermedius Genomic DNA

DNA was obtained by the phenol–chloroform extraction method [54]. DNA concentration and purity were assessed from the optical density of the solution on a Nanodrop ND-2000. The sample concentration varied from 250 to 390 μg/mL (A260/280 was 1.8–2). The DNA samples of *S. intermedius* were diluted to a concentration of 1 μg/μL and used as the matrix for the polymerase chain reaction (PCR).

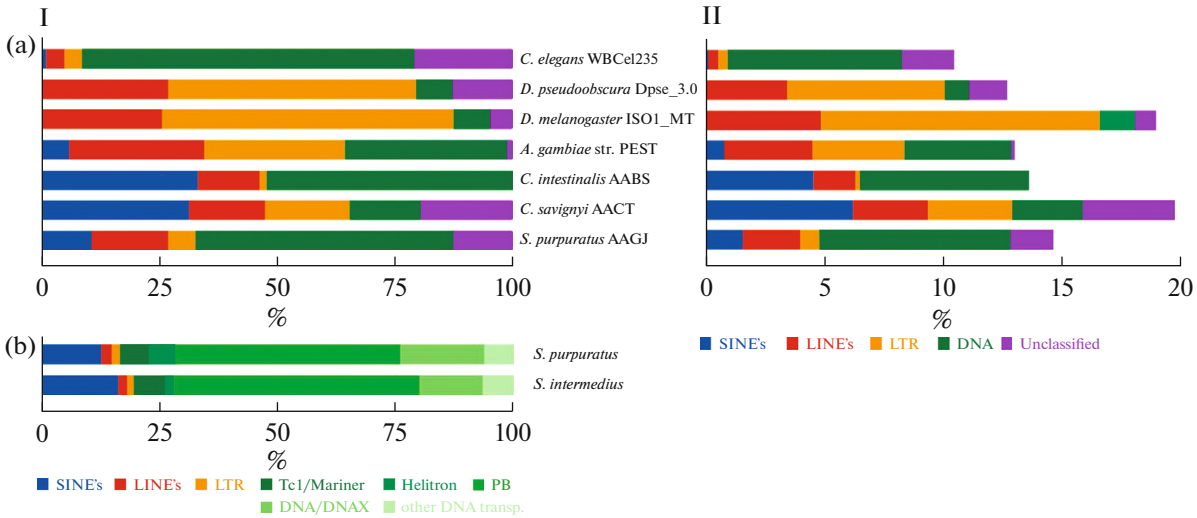

Fig. 1. TEs in the genome of the sea urchin *Strongylocentrotus purpuratus*, compared to invertebrate genomes (a), and in the coelomocyte transcriptomes of the sea urchins *S. purpuratus* and *S. intermedius* (b). (a) TE distribution in the genomes of *Drosophila melanogaster*, *D. pseudoobscura*, *Anopheles gambiae*, *Ciona intestinalis*, *C. savignyi*, *Caenorhabditis elegans,* and *Strongylocentrotus purpuratus*. TE classes are marked with a color: nonLTR SINE, blue; LINE, red; LTR TE, yellow; DNA transposons, green; nonannotated repeats, lilac. I, relative distribution of all TE sequences in genomes (the sum of all TEs masked by RepeatMasker was taken as 100%); II, the percentage content of TEs in invertebrate genomes. (b) The relative coverage of TE classes in the coelomocyte transcriptomes of *Strongylocentrotus purpuratus* and *S. intermedius* by representatives of different TE classes (the number of reading frames of a particular TE × read length/sequence length of the same TE were considered). The number of TE classes is expressed as the percentage of all TEs of the transcriptome. Color markings are the same as in (a); types of DNA transposons are designated with shades of green (scale under panel (b)). Nonannotated repeats were disregarded.

PCR Analysis

The amplification of the target fragments was performed in an Eppendorf Mastercycler Personal 5332 thermal cycler (Eppendorf, Germany). For each run, a 25 μL reaction mixture contained 1× Taq-Red Buffer (Evrogen, Russia), 2 mM $MgCl_2$, 5 pM of each primer, 0.2 mM dNTP, 0.25 units of Taq-polymerase (Evrogen, Russia), and 1 μg of the DNA matrix. The reaction parameters were as follows: primary denaturation at 95°C for 3 min, 94°C for 20 s, 59°C for 20 s, and 72°C for 60 s; 30 cycles. Control PCR was carried out under identical conditions using as the matrix (a) salmon genomic DNA, (b) human genomic DNA (HeLa cells), and (c) the matrix was replaced with an equal volume of ddH_2O.

The amplification products were analyzed by electrophoresis in 1% agarose gel in TAE (tris-acetate) buffer. The gel was stained with ethidium bromide. For printing, the images were processed with the Adobe Photoshop software.

RESULTS

Based on the analysis of the 284 TE sequences of the sea urchin *S. purpuratus* available from the RepBase repeats database, we plotted a diagram of the distribution of their major classes: non-LTR, LTR–TE, and DNA-transposons (Fig. 1a). Up to 12.5% of the sequences remained unidentified, which is not surprising due to the insufficient annotation of the genomes [1, 51].

For each mobile element in the transcriptome, the coverage of reading frames (the percentage of reading frames), which indicates the representativeness of a given element, was calculated. The coelomocyte transcriptomes of both species of sea urchins were similar in terms of the major TE class distribution: as is the case for the genome, DNA transposons were predominant. Non-LTR TEs made up no more than 20% of the TE transcriptome. DNA transposons piggyBac (PB), Helitron, and Tc/Mariner were represented at high percentages (Fig. 1b). SINE elements were dominant among non-LTR TEs. The proportions of LTR retrotranspasons in the transcriptome were lower than in the genome and varied from 1 to 2% (Fig. 1b).

The TE sequences obtained using in silico methods were tested for their presence in the genomic DNA of *S. intermedius*. For this, using the Primer-BLAST we selected a primer pair that flanked a region not exceeding 300 bp of each mobile element. This limitation is necessary for convenient electrophoresis and the subsequent stage of research: the synthesis of labeled probe for fluorescence in situ hybridization (FISH). In the PCR analysis, in 92 (out of 100) tests, we obtained a product of the calculated length. In other cases, either there was no product, or the reaction was nonspecific (smear or ladder) (Fig. 2, line *10*). In all 92 tests, the calculated primers yielded no prod-

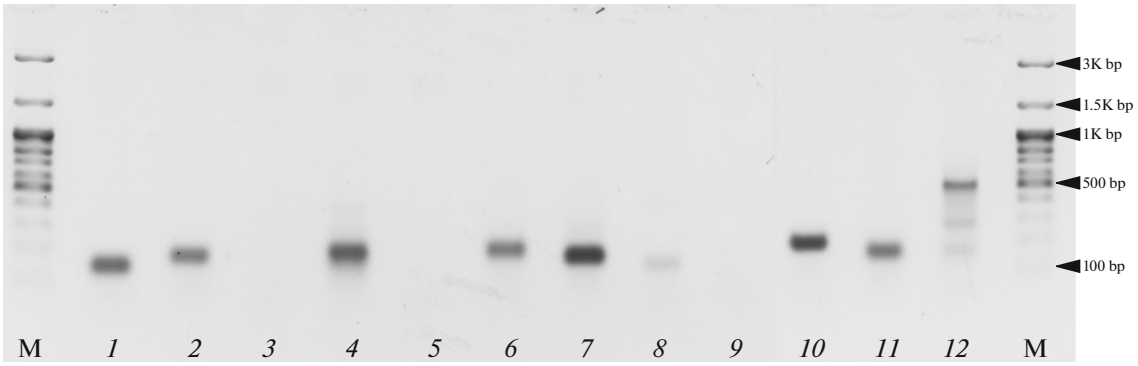

Fig. 2. PCR analysis of genomic DNA with calculated primers for TEs of the sea urchin *S. intermedius*. (*1*) PB-N5; (*2*) PB-N7; (*3*) DNA-1-2; (*4*) DNA-1-1; (*5*) **Sola2-3N1**; (*6*) **Tc1-N1**; (*7*) DNAX-12; (*8*) **Mariner-2N**; (*9*) **SINE2-3**; (*10*) RTE-12; (*11*) **Gypsy10-LTR**; (*12*) **DNAX-8**. M, molecular mass marker [100bp + 1.5Kb + 3Kb (Evrogen)]. (*3*, *5*, and *9*) Primers were unsuitable; (*10*) element underwent species-specific changes; (*1*, *2*, *4*, *6*, *7*, *8*, *11*, and *12*) positive result.

uct in the control PCRs where the genomic DNA of *S. intermedius* was replaced with human or salmon DNA.

The PCR analysis for the presence of TEs in the genomic DNA of *S. purpuratus* was performed under standardized conditions without changing the number of cycles (see Materials and Methods). As a consequence, the intensity of the zone in electrophoretic separation of the amplification products reflected, to some extent, the relative content of TEs in the genome (Fig. 2). The content of DNAX-12 (Fig. 2, line *7*) was probably higher than that of Mariner-2N (Fig. 2, line *8*), while the quantity of Gypsy10-LTR and DNAX-8 did not differ (Fig. 2, lines *11*, *12*).

The approach we used in this study was fruitful: out of the 100 assembled TEs of *S. intermedius*, the presence of 92 TEs in its genomic DNA was proved using the calculated primers. The in silico identified TE sequences are present in the *S. intermedius* genome and can be used in further research.

DISCUSSION

Repetitive non-coding DNA remains the least studied part of the eukaryotic genome. TE activity may be deleterious for the host genomes and cause various diseases; however, TEs are the major source of genetic changes producing the diversity of genomes [58]. Recently, a few works have been published that show the importance of TEs for cell functioning: a role in the genome structure [46], size [23], and rearrangements [6], as well as their evolutionary contribution to the genome [41].

Transposons, also known as "jumping genes", are a widespread class of repetitive sequences, which until recently were mainly considered "egoistic", parasitic within-genomic sequences [17, 45]. However, TEs occupy a substantial part of eukaryotic genomes: no less than 50% in the assembled part of the human and mouse genome, but no greater than 15–20% among the relatively well-annotated genomes of invertebrates (Fig. 1a, II). The larger amount of TEs in evolutionarily more advanced taxa may suggest their importance for progressive evolution. Thus, mammals were shown to obtain an evolutionary advantage due to the activity of endogenous retroviruses (ERV, class LTR). The carrying of a fetus involves more than 1000 genes, which previously performed other functions in different parts of the organism. These genes acquired sensitivity to the female hormone progesterone, which, in its turn, was due to ERVs [3]. The ERV probably integrated its DNA into a spermatozoon or egg cell [39]. The ERVs are now considered parts of the regulatory system of the genome: transcripts and components of lncRNA.

In recent years, TE transcripts were found to be major components of lncRNA. At early developmental stages, almost the entire genome in the few studied eukaryotes is transcribed [27, 40]. This results in the formation of RNA, while most of the RNA is not expressed in protein [12, 14]. LncRNAs are components of a new regulatory system that is different from the well-studied GRS [1, 5, 28]. The identification and annotation of TEs is becoming important; however, this is considered to be one of the most complicated objectives of genomics [30, 36]. In the present research, we used a set of the known software and algorithms to identify and systematize TEs of the sea urchin *S. intermedius*, for which there is no annotated genome. In the case of *S. intermedius*, none of the known methods for TE searching could be used. Therefore, we used an approach based on the sequenced transcriptome analysis of the species studied and the genomes of closely related species.

This study confirmed the idea that sea urchins have a high content of DNA transposons, which occupy almost half of all TEs. The content of nonLTR TEs of the class SINE was substantially lower than in vertebrate genomes [13]. In the *S. purpuratus* genome, DNA transposons (~55% versus all TEs), non-LTP TEs, nonannotated TEs, and LTR TEs occurred

(Fig. 1a). This composition rather markedly differs from the averaged mammalian genome; the TE amount is given in the decreasing order: non-LTR (not less than ~50% SINE and LINE), LTR (ERVs ~10%), DNA transposons (~1%) [33, 60]. In terms of coverage, the major TEs that constitute the coelomocyte transcriptomes in both species of sea urchins were mainly representatives of two classes: DNA transposons (piggyBac, Helitron, and Tc/mariner) and non-LTR retrotransposons (SINE). TE sequences from the *S. intermedius* transcriptome, which were most numerous (major) in terms of coverage values, were assembled based on comparison with the annotated TEs of the *S. purpuratus* genome. Many TEs had an insufficient number of reading frames in the transcriptome and therefore were beyond our analysis. Successfully assembled TE sequences allowed us to assess the degree of similarity compared to genes between species with an evolutionary distance of 10 million years. The comparison showed that the TE similarity does not exceed 87%, while the similarity of gene nucleotide sequences (Hsp70, Actin, and GAPDH) reaches 97%.

In summary, the results of this study justify the approach we employed. Experimentation with primers that were constructed from *S. purpuratus* TEs to identify TEs of *S. intermedius* would lead to erroneous results. As an example, the lack of PCR product could be considered an artifact based on the dissimilarity of sequences. Ninety-two out of the 100 assembled TE sequences of *S. intermedius* that were tested and yielded positive results are without doubt suitable for subsequent research. Eight primer pairs that either produced no amplicon of the calculated mass or produced a set of products (smear) were calculated for sequences with a small number of reading frames in the transcriptome, which led to assembly errors. We hope to correct the errors after the sequenced genome of the sea urchin *S. intermedius* is assembled.

The spatial and temporal changes in the transcriptional activity of the genome are accompanied by the alteration of the nucleus architecture, which, in turn, plays a role in regulating gene expression. The ordered, but dynamic organization of chromatin is particularly necessary for morphogenesis during early embryogenesis. Revealing the roles of particular TEs in regulating chromatin conformation, which is impossible without their detailed classification and annotation, is an important future objective.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare no conflict of interests.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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