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 Short Research Note

First evidence of miniature transposable elements in sponges (Porifera)

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Abstract Transposable elements play a vital role in genome evolution and may have been important for the formation of the early metazoan genome, but only little is known about transposons at this interface between unicellular opisthokonts and Metazoa. Here, we describe the first miniature transposable elements (MITEs, *Queen1* and *Queen2*) in sponges. *Queen1* and *Queen2* are probably derived from Tc1/mariner-like MITE families and are represented in more than 3,800 and 1,700 copies, respectively, in the *Amphimedon queenslandica* genome. *Queen* elements are located in intergenic regions as well as in introns, providing the potential to induce new splicing sites and termination signals in the genes. Further possible impacts of MITEs on the evolution of the metazoan genome are discussed.

Keywords Early diverging Metazoa · Porifera · Transposable element · MITE · *Amphimedon queenslandica* · *Queen* elements

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The origin of animals is among the greatest enigmas in evolutionary biology. In particular, genomic evolution during the transition of unicellular protists to multicellular metazoans is still largely speculative

(Hoenigsberg et al., 2008). Most comparative approaches in genomics focus on shared genes and gene families; however, other genomic components, such as transposable elements, which have been important in genome formation (Brosius, 1999; Batzer & Deininger, 2002; Kazazian, 2004), remain understudied.

Transposable elements encompass mobile DNA sequences that can integrate into the genome at new positions. They are represented in most eukaryotes where they account for up to 90% of the genome (Bailey et al., 2003). Strand breaks during transposition and insertion can trigger various genomic rearrangements (Lim & Simmons, 1994; Zhang & Peterson, 2004). Furthermore, transposons occasionally evolve into new genes or parts of genes, including regulatory elements (Brosius & Gould, 1992; Deininger & Batzer, 1999; Feschotte et al., 2002; Krull et al., 2007; Kuang et al., 2008). Consequently, transposable elements have an enormous evolutionary potential by directly influencing phenotypes encoded in genomes, ranging from subtle regulatory perturbations to the complete loss of gene function (Feschotte & Pritham, 2007).

For the evolution of multidomain proteins in Metazoa (animals), transposable element-mediated exon shuffling may be an important mechanism to move encoded motifs and domains (Tordai et al., 2005; Feschotte, 2008). Comparative genomics of basal metazoans and choanoflagellates, the closest living relatives of animals, has recently revealed uniquely arranged combinations of metazoan domains in the choanoflagellate sister group (King et al., 2008). Novel domain architectures, for example in cnidarians and sponges (Adamska et al., 2007; Putnam et al., 2007; Larroux et al., 2008), both basal metazoan phyletic lineages, might be transposable element-mediated exon rearrangements that occurred during early metazoan evolution. Indeed, transposable elements may have played a pivotal role in the genesis of many metazoan-specific gene families prior to the divergence of Porifera (possibly the earliest diverging extant Metazoa, e.g., Philippe et al., 2009) and eumetazoan lineages. Novel domain architectures emerging prior to animal cladogenesis appear to underpin metazoan-specific regulatory and protein networks that comprise cellular, developmental, and morphological synapomorphies, as revealed by the recently published genome of the demosponge *Amphimedon queenslandica* (Srivastava et al., 2010).

In the case of sponges, physiology and evolution affords a rapidly shifting capacity to produce complex secondary metabolites, which may be modulated by transposable element-mediated changes in the genome.

While 25% of the anthozoan *Nematostella vectensis* genome consists of transposable elements (Putnam et al., 2007), only little is known about transposable elements in *Amphimedon queenslandica,* the first sponge genome to be sequenced (Srivastava et al., 2010). Our own estimations also point to a 20-30% contribution of transposable elements to the *Amphimedon queenslandica* genome (unpublished observations), but information on elements in other sponges is scarce (e.g., Arkhipova, 2001; Wiens et al., 2009). Today, the only sponge element with a published sequence is a long terminal repeat-retrotransposon from the freshwater sponge *Lubomirskia baicalensis* (Wiens et al., 2009).

In the assembled genome of the demosponge *Amphimedon queenslandica* we have identified the first miniature transposable elements (MITEs) that we call "*Queen1*" and "*Queen2*" (see Supplementary Methods). MITEs are short, nonautonomous DNA transposons with high copy numbers and homogeneous lengths (Bureau & Wessler, 1992; Zhang et al., 2000).

Queen1 is 210 bp long with an inverted terminal repeat (TIR) of 28 bp, including a potential dinucleotide (TA) target site duplication (TSD), a 3-bp linker, and 14 bp of a sub-TIR sequence (Fig. 1). We estimated that there are more than 3,800 *Queen1* elements in the *A. queenslandica* genome (0.28% of the genome). Thirty-four percent of full-length *Queen1* elements display 90% identical inverted repeats (IRs).

Queen2 comprises more than 1,700 elements of 245 bp with a 25-bp TIR, a 2-bp linker, and 11 bp of sub-TIR (0.15% of the genome). Fifty-seven percent of full-length elements display 90% perfect IRs, indicating a relatively recent activity period. *Queen1* and *2* appear to be novel MITEs, probably deriving from the widespread Tc1/mariner transposase superfamily, as they also possess characteristic 5′-TA-3′ target sites (Fig. 2). Except for this target site preference, there is no sequence similarity between *Queen1* and *2*. Adjacent *Queen* elements are significantly often of the same type ($P < 0.01$).

Queen elements have not been detected in other organisms thus far, including in EST data of other sponges, and the high copy numbers of these MITEs

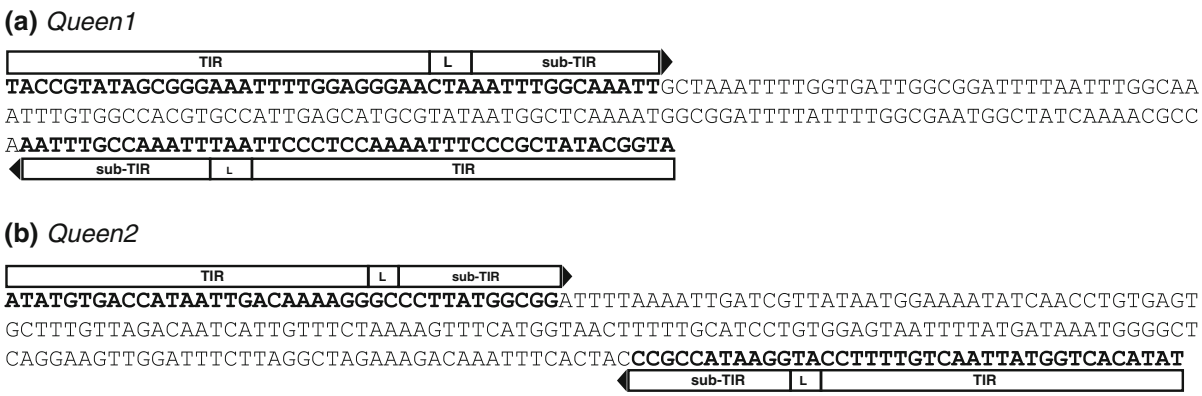

Fig. 1 *Queen1* and *Queen2* as detected in *A. queenslandica*. Arrows indicate location and direction of terminal inverted repeat (TIR), linker (L), and sub-TIR (sub-TIR) sequences

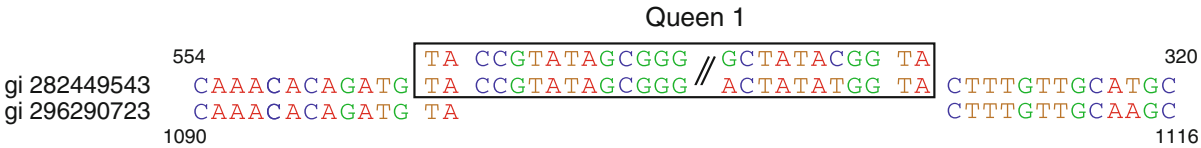

Fig. 2 Presence of *Queen1* (boxed region) in an EST sequence (gi282449543) and its absence at the genomic level (gi296290723). The discrepancy between EST and genome is probably caused by different experimental sources for the cDNAs and DNA extraction, and is also an indication that this specific MITE element inserted recently and/or is polymorphic. The coordinates of the sequences are indicated. The upper sequence in the box represents the *Queen1* consensus sequence (65%). The slashes replace 196 nucleotides of the MITE element

is unusual for nonbilaterian Metazoa. In the Placozoa *Trichoplax adhaerens*, MITEs are very rare; genome-wide, only a single family with about 20 copies is represented (Wang et al., 2010b). Likewise, the number of *CMITE* elements found in two stony coral genomes (Wang et al., 2010a) is far less than the number of *Queen* copies in *A. queenslandica*.

Generally, MITEs are located in low-copy-number genomic regions and in gene-rich environments (Bureau & Wessler, 1992; Zhang et al., 2000). Their frequent insertions close to genes indicates a significant potential for generating allelic and genomic diversity (Feschotte & Pritham, 2007). Consequently, regulatory and coding mutations are a frequent side effect of MITE insertion (Bureau & Wessler, 1992; Nakazaki et al., 2003; Xu et al., 2007; Kuang et al., 2008). We detected several *Queen* elements located in introns, where they might potentially influence the splicing of a pre-mRNA by introducing new splice sites, resulting in intron retention, exon skipping, or the creation of new exon/intron boundaries (Feschotte, 2008). This may result in new protein isoforms with different functions and fitness advantages; especially, because *Queen* elements might induce new splicing sites.

In this context, we detected what appeared to be *Queen* elements in the transcriptome of *A. queenslandica*; *Queen2* elements were present in many ESTs. However, currently the amount of EST data for *A. queenslandica* is very limited (63,542 EST sequences compared to the half million such sequences in *C. elegans* or several millions in human). So far no case of MITE inclusion in protein-coding sequence regions (in the sense orientation to genes) could be determined. In contrast, a detailed analysis of the predominantly computationally predicted annotation of the *A. queenslandica* genome did indicate such cases. However, careful inspections and RT-PCR analyses could not confirm any of the potential protein-coding MITE cassettes (data not shown).

Our results shed light on the occurrence and abundance of miniature transposable elements in sponges. The genetic features of *Queen1* and *Queen2* corroborate hypotheses that such transposable elements might have contributed to the evolution of early

Metazoa and the formation of the early-branching metazoan genome (Hoenigsberg et al., 2008). MITE insertions in introns and exons of (vital) genes, such as *Queen* element insertions in *A. queenslandica*, might have a wide range of effects at the transcriptional and posttranscriptional levels, as summarized by Feschotte (2008): (1) *Queen* insertions in the untranscribed region of genes might disrupt existing promoters, transcription start sites, and regulatory elements. (2) An intronic insertion of a *Queen* element might trigger antisense transcription and inhibition of sense transcription, as was shown for MITEs in Solanaceae (Kuang et al., 2008). (3) Once inserted into an intron, *Queen* elements might trigger formation of heterochromatin, leading to transcriptional inhibition of adjacent genes (Cam et al., 2008). (4) An intronic *Queen* element may interfere with the normal splicing pattern of a pre-mRNA, leading to various forms of alternative splicing (e.g., intron retention and exon skipping). (5) A *Queen* element that has inserted into an intron and contains cryptic splice sites may be incorporated as an alternative exon, which may result in the translation of a new protein isoform or in the destabilization or degradation of the mRNA by the nonsense-mediated decay (NMD) pathway.

The sequence divergence observed among *Queen1* and *Queen2* elements indicates that MITE integrations may be an ongoing process shaping the *A. queenslandica* genome. The full gambit of evolutionary effects caused by MITEs and other transposable elements will be evident with the discovery and characterization of a more complete set of TEs in *A. queenslandica* and other sponge classes and a comprehensive transcriptome analysis. Genome sequencing of calcareous, hexactinellid, and homoscleromorph sponges is therefore eagerly needed.

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