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Actin genes of the White Sea sponge *Halisarca dujardinii*: structure and regulation

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Motivation and Aim: Sponges (Porifera) are the oldest living multicellular organisms. The body of sponges is formed by several different cell types that do not form tissues *sensu stricto*. Cell plasticity is a one of the key features of sponges: their cells constantly perform movement, remodeling, and reorganization. Most of these cells have ability to transdifferentiate. Such plasticity of sponge cells is fully manifesting after dissociation of the sponge body, when cells are actively reaggregating in order to form multicellular aggregates and eventually rebuild the body.

Actin is one of the main components of the cytoskeleton of eukaryotic cells. It creates mechanical support, participates in cell division, intracellular transport, and cell movement. The contribution of actin to the maintenance of plasticity of sponge cells, especially during reaggregation is undeniable. In the present work, we studied the structure of actin genes, the regulation of their expression and the functional features of protein products in the White Sea sponge *H. dujardinii*.

Methods and Algorithms: Whole-genome sequencing of the *H. dujardinii* was performed using Oxford Nanopore and Illumina. A draft assembly was made and contigs containing actin genes were identified and examined. Expression in different physiological states was studied using RNA-seq of intact sponges, cell suspension, and aggregates at the 24 h after reaggregation. 5'UTRs of actin genes were determined by RACE analysis. Protein products were separated in the native or SDS-containing polyacrylamide gels and stained with Coomassie blue. Mass spectra of tryptic peptides were obtained on the matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometer Ultraflextreme (Bruker), equipped with UV laser (Nd) and reflectron.

Results: Whole-genome study of the *H. dujardinii* sponge revealed seven actin genes. Six out of seven genes lacked introns, some of the genes are likely formed as a result of a recent duplication. Phylogenetic analysis classified the actins of *H. dujardinii* as beta-actins. Transcriptomic data confirmed the expression of products of all genes and mass spectrometry showed the presence of their protein products. Actins 1–5 have high sequence similarity to human beta-actin (Table 1). The most variable actin 7 lacks the sequence required for polymerization, which may indicate its special molecular function. All *H. dujardinii* actins have binding motifs for profilin and gelsolin – the key protein regulators of actin filament assembly and disassembly. The analysis of the upstream genomic regions of the actin genes revealed the diversity of actin gene promotors in *H. dujardinii*, which may indicate the cell type-specific mode of expression for these genes. This is indirectly confirmed by the changes in actin gene expression during reaggregation, when numerous events of dedifferentiation and transdifferentiation occur.

Table 1. GenBank accession numbers (ANs) for mRNA sequences of actins in *H. dujardinii* and accession numbers of their homologs / best blastp hits in *Homo sapiens* and *Amphimedon qeenslandica*

Protein name	*H. dujardinii*	Homologs / best blastp hits			
		H. sapiens		*A. queenslandica*	
	AN	AN	Identity, %	AN	Identity, %
actin 1	MT451954	NP_001092.1	97.33	XP_019853827.1	62.13
actin 2	MT451955	NP_001092.1	97.33	XP_019853827.1	62.13
actin 3	MT451956	NP_001092.1	97.33	XP_019853827.1	62.13
actin 4	MT451957	BAD96645.1	97.06	XP_019853827.1	61.60
actin 5	MT451958	NP_001092.1	96.26	XP_019853827.1	61.33
actin 6	MT451959	NP_005150.1	73.60	XP_019853827.1	51.06
actin 7	MT518195	7P1H_B	88.20	XP_003384376.1	59.26

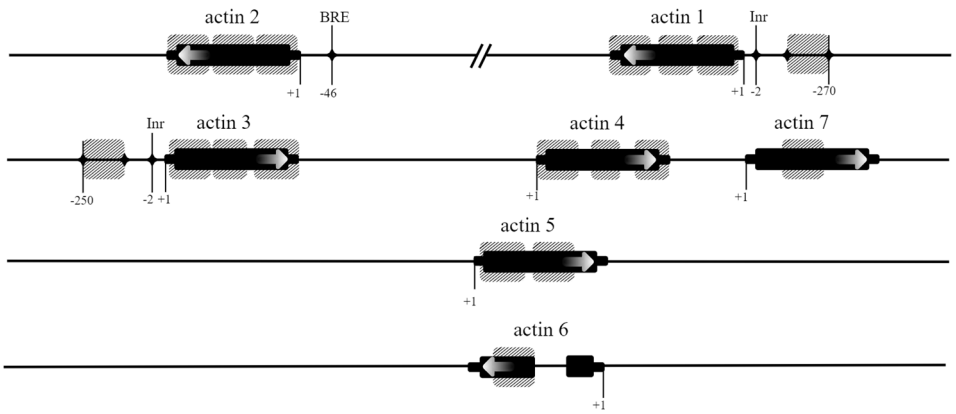

Fig. 1. Actin genes of *H. dujardinii*. Arrows indicate the direction of the transcription; CpG islands are marked with grey shading; Inr, initiator of transcription; BRE, B recognition element. The representation of the regulatory elements and genes is not to scale

Conclusion: High level of identity between sponge and human actins indicates that the structure of these proteins was formed in great antiquity. Despite its simple organization, *H. dujardinii* has seven actin genes featuring a variety of regulatory elements. Five of the actins are conserved, while two other have distinct structural features. We hypothesize that the differential expression of actin genes contributes to the distinct features of sponge cell types.

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