Tardigrades: from genestealers to space marines

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Abstract

This study is about the most tenacious organisms on the Earth - tardigrades. They are able to survive in the most extreme conditions, including under the influence of radiation. By genome analysis we will try to understand what mechanisms underlie radiation resistance.

Introduction

Tardigrades (also known as water bears, pudgy wudgies, or moss piglets) are microscopic animals capable of withstanding some of the most severe environmental conditions. These water-dwelling, eight-legged creatures can be found throughout the world, from the Himalayas (above 20,000 ft), to the deep sea (below 13,000 ft). Classified as "extremophiles", they can survive freezing (up to 1°K), total dehydration, pressure (more than 1,200 atmospheres) and radiation (1,000 times more radiation than other animals) [1].

In 2007 the grandiose project called "Tardigrades In Space" was implemented: dehydrated tardigrades were taken into low Earth orbit, and exposed to the hard vacuum of outer space and solar UV radiation. And yes they survived [2].

Of course, this stress tolerance has long attracted the attention of researches. In 2015 a group of scientists analyzed the genome of *Hypsibius dujardini* and found out that about 17% of genes were borrowed from other organisms [3]. But a year later this theory has been disproved [4].

In this work we will try to answer one question: how tardigrades acquired resistance to radiation. Considering that DNA is a major target of radiation damage, we may hypothesize that tardigrades might have unique proteins associated with their DNA to protect and/or effectively repair it. To explore this possibility, we will combine genomic and proteomic data. We will analyze the genome of *Ramazzottius varieornatus*, predict genes and try to find out which of them can be responsible for radiation resistance.

Methods

For this research we used the assembled genome of the *Ramazzottius varieornatus*, the YOKOZUNA-1 strain (sequenced in the University of Tokyo) [5]. The result of gene predictions we took from [6] (used AUGUSTUS). To extract protein sequences from prediction output we used a ready script, which you can see in Lab journal.

Peptides that were associated with the DNA we downloaded here [7]. These peptides were taken by our colleagues: using centrifugation and various techniques that disrupt membranes, they extracted chromatin fraction and analyzed the extracted proteins using tandem mass spectrometry [8].

To figure out which proteins from the *R. varieornatus* genome these peptides correspond to, we did a local blast search. We ran *makeblastdb* [9] (with parameters *-parse_seqids*, *-blastdb_version* 5, *-dbtype prot*) and created a local database from our protein fasta. Next

we ran *blastp* (version 2.9.0) [10] using our peptide sequence file as a query. To extract protein of interest from the output file we use *samtools faidx* utilite (samtools version 1.10) [11].
To predict where these proteins are found in the cell based on their sequences we used WoLF PSORT [12] and TargetP 1.1 [13] servers. For the next analysis we remove signal peptides. Then we blasted protein against the "UniProtKB/Swiss-Prot" database using BLAST [10] and predicted the proteins function using a web-version of HMMER [14].

Results

We got the assembled genome of the *Ramazzottius varieornatus* and 16435 predicted genes and proteins. After blasting on them of peptides associated with DNA, the search area narrowed down to 118 proteins. Results of prediction protein's functions gave us 12 interesting proteins. The information about best blast hit (annotation and e-value), predicted Pfam domains, probable localization(s) according to WoLF PSORT and localization according to TargetP you can see in the Table 1.

	BLAST annotation	BLAST EValue	Pfam	WoLF PSORT	TargetP
g11513.t1	Trafficking protein particle complex subunit 9	7e-83	Transport protein Trs120 or TRAPPC9, TRAPP II complex subunit	cyto: 17, cyto_nucl: 12.8333, cyto_mito: 9.83333, nucl: 7.5, E.R.: 3, mito: 1.5, plas: 1, pero: 1, golg: 1	OTHER
g11960.t1	E3 ubiquitin-protein ligase BRE1B	6e-98	Zinc finger, C3HC4 type (RING finger)	nucl: 32	OTHER
g12510.t1	No significant similarity found	-	-	plas: 29, cyto: 3	OTHER
g14472.t1	No significant similarity found	-	-	nucl: 28, plas: 2, cyto: 1, cysk: 1	OTHER
g15484.t1	Vacuolar protein sorting-associated protein 51 homolog	0.0	Vps51/Vps67	nucl: 17.5, cyto_nucl: 15.3333, cyto: 12, cyto_mito: 6.83333, plas: 1, golg: 1	OTHER
g2203.t1	Myogenesis-regulating glycosidase	2e-126	Glycosyl hydrolases family 31	plas: 29, nucl: 2, golg: 1	OTHER
g4970.t1	Enteropeptidase	4e-16	Trypsin	plas: 32	OTHER
g5237.t1	No significant similarity found	-	-	plas: 24, mito: 8	OTHER
g5510.t1	No significant similarity found	-	Membrane-associating domain	plas: 23, mito: 7, E.R.: 1, golg: 1	OTHER

g5927.t1	Glucosamine 6-phosphate N-acetyltransferase	1e-18	-	nucl: 30.5, cyto_nucl: 16.5, cyto: 1.5	OTHER
g7861.t1	WI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein 1	2e-71	SNF2 family N-terminal domain	nucl: 16, cyto_nucl: 14, cyto: 8, plas: 5, pero: 1, cysk: 1, golg: 1	OTHER
g8312.t1	Vacuolar protein sorting-associated protein 41 homolog	0.0	Region in Clathrin and VPS	nucl: 15.5, cyto_nucl: 15.5, cyto: 12.5, mito: 2, plas: 1, golg: 1	OTHER

Table 1. Best blast hit (annotation and e-value), predicted Pfam domains, probable localization(s) according to WoLF PSORT and localization according to TargetP for 12 "interesting" proteins.

Discussion

We found 12 candidate proteins to be involved in the protection or repair of tardigrades DNA. In our opinion, the most suitable protein for this is WI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein (yellow in the Table 1). This is an ATP-dependent annealing helicase that binds selectively to fork DNA relative to ssDNA or dsDNA and catalyzes the rewinding of the stably unwound DNA. Rewinds single-stranded DNA bubbles that are stably bound by replication protein A (RPA). Acts throughout the genome to reanneal stably unwound DNA, performing the opposite reaction of many enzymes, such as helicases and polymerases, that unwind DNA. This protein may play an important role in DNA damage response by acting at stalled replication forks [15].

Citation

1. Ricardo Cardoso Neves, Lykke K. B. Hvidepil, Thomas L. Sørensen-Hygum, Robyn M. Stuart, Nadja Møbjerg. (2020). Thermotolerance experiments on active and desiccated states of Ramazzottius varieornatus emphasize that tardigrades are sensitive to high temperatures. *Sci Rep*. **10**;
2. K. Ingemar Jönsson, Elke Rabbow, Ralph O. Schill, Mats Harms-Ringdahl, Petra Rettberg. (2008). Tardigrades survive exposure to space in low Earth orbit. *Current Biology*. **18**, R729-R731;
3. Thomas C. Boothbya, Jennifer R. Tenlena, Frank W. Smitha , Jeremy R. Wanga, Kiera A. Patanella, Erin Osborne Nishimur, Sophia C. Tintori , Qing Li, Corbin D. Jones, Mark Yandell, David N. Messina, Jarret Glasscock , and Bob Goldstein (2015). Evidence for extensive horizontal gene transfer from the draft genome of a tardigrade. Proc Natl Acad Sci USA (112:15976–15981)
4. Georgios Koutsovoulos, Sujai Kumar, Dominik R. Laetsch, Lewis Stevens, Jennifer Daub, et. al.. (2016). No evidence for extensive horizontal gene transfer in the

genome of the tardigrade Hypsibius dujardini. *Proc Natl Acad Sci* USA. **113**, 5053-5058

5. Assembled genome: http://kumamushi.org/data/YOKOZUNA-1.scaffolds.fa
6. Gene prediction (AUGUSTUS): http://public.dobzhanskycenter.ru/mrayko/BIMM185/augustus.whole.gff
7. Peptides that were associated with the DNA : http://public.dobzhanskycenter.ru/mrayko/BIMM185/peptides.fa
8. Tandem mass spectrometry: https://en.wikipedia.org/wiki/Tandem_mass_spectrometry
9. Makeblastdb: https://www.ncbi.nlm.nih.gov/books/NBK279688/
10. BLAST: https://blast.ncbi.nlm.nih.gov/Blast.cgi
11. Samtools: http://www.htslib.org/
12. WoLF PSORT: https://wolfpsort.hgc.jp/
13. TargetP 1.1: http://www.cbs.dtu.dk/services/TargetP/
14. HMMER: https://www.ebi.ac.uk/Tools/hmmer/
15. https://www.uniprot.org/uniprot/Q9NZC9