Tardigrades: 8-legged spacemarines

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Abstract. In this report we try to find new proteins somehow related to DNA repair mechanisms of tardigrades. As a model Ramazzottius varieornatus, the YOKOZUNA-1 strain was selected, and genome annotation data and mass spectrometry results were obtained. After analysis made, we found at least 2 candidate proteins which have nuclear localization, DNA-binding activity,as also they participate in recombination and mechanisms. Our results can partially explain the survivability of tardigrades in various harsh conditions.

1 Introduction

Greetings! Today we present you our results about searching DNA repair mechanisms in tardigrades. But before we start, let's do a quick walkthrough about Tardigrades itself and methods used in this project. Tardigrades, also known as as water bears or moss piglets, are a phylum of eight-legged segmented micro-animals. They were first described by the German zoologist Johann August Ephraim Goeze in 1773, who called them little water bears. In 1777, the Italian biologist Lazzaro Spallanzani named them Tardigrada, which means "slow steppers"[1]. They have 2 distinct traits:

1. They are almost indestructible. They could have been found everywhere, literally everywhere: in Earth's biosphere, from mountaintops to the deep sea and mud volcanoes, and from tropical rainforests to the Antarctic [1]. Tardigrades are among the most resilient animals ever known to man. They have anomalous endurance in terms of survivability of extreme conditions, and even deep dead space filled with vacuum and radiation emitted from myriads of stars could nothing to do. They are truly space marines coming from the Earth. They exist ca. 500 million years and I guess will exist for nest 500 million years. They are living grey goo in size of approx. 0.5 mm, thousands of little Thanoses. But what makes them so adapted to everything? First, they developed anhydrobiosis, cryobiosis, osmobiosis, or anoxybiosis, and so on. Depending on the environment, they simply go to sleep forming capsule around ther bodies,thus preserving resources and waiting for shiny weather. Second (and it would be our task) they seem to have some mechanisms providing DNA repair to escape the death and

different pathologies. They m u s t definitely have a galore of these methods. Let's find them! But before we start, the second trait:

2. They are extremely cute. :)

So, what about the data we have? We will be using the sequence of the Ramazzottius varieornatus, the YOKOZUNA-1 strain, its annotation provided by our team, and mass spectrometry data of chromatin of this water bear. The core idea in this project is that tardigrades might have unique proteins associated with their DNA to protect and/or effectively repair it. To put in simple, we will merge proteome data and genome data, and try to find any intersections, determine physical localization of found proteins, and try to explain and discuss our findings.

Also we need to briefly mention different tools we used. AUGUSTUS tool is a gene prediction tool running a generalized hidden Markov model (GHMM), which defines probability distributions for the various sections of genomic sequences [7]. Introns, exons, intergenic regions, etc. correspond to states in the model and each state is thought to create DNA sequences with certain pre-defined emission probabilities. Similar to other HMM-based gene finders, AUGUSTUS finds an optimal parse of a given genomic sequence, i.e. a segmentation of the sequences into states that is most likely according to the underlying statistical model.

BLAST is a gold standard alignment tool that searches for homology (i.e. similarity between sequences.For more info, please view [4].) between query sequence and desired database containing pre-analysed data. BLAST identifies homologous sequences using a heuristic method which initially finds short matches between two sequences; thus, the method does not take the entire sequence space into account. After initial match, BLAST attempts to start local alignments from these initial matches. This also means that BLAST does not guarantee the optimal alignment, thus some sequence hits may be missed. In order to find optimal alignments, the Smith-Waterman algorithm should be used [2, 3].

Brief introduction about other used tools are given in Task4 document.

2 Data

We use the assembled genome data of the Ramazzottius varieornatus, the YOKOZUNA-1 strain [5]. We also use a list of peptides obtained via tandem mass spectrometry [6].

3 Methods

We use the implemented program AUGUSTUS for gene prediction [7]. This program is specified for eukaryotic sequencing data. Usually the number of obtained proteins is

too large.

In order to detect regions, linked to DNA repair, we should use both genomic and proteomic data. We can use a list of peptides that were associated with the DNA regions. This list can be obtained via analyzis of extracted chromatin fraction using tandem mass spectrometry. To find associated proteins from the R. varieornatus genome to peptides, we use makeblastdb utilite [8].

Next, to narrow the list of proteins, we use WoLF PSORT [9]. This program allows to predict where these proteins are found in the cell based on their sequences. predicts the subcellular localization of proteins. The method is based on detection the presence of a signal peptide on their N-terminus. We also use TargetP 1.1 Server [10], which also predicts the subcellular localization.

To find homologous proteins we use Blast [2]. We use HMMER to predict the function of the proteins [11]. The HMMER is based on Hidden Markov Models.

4 Results

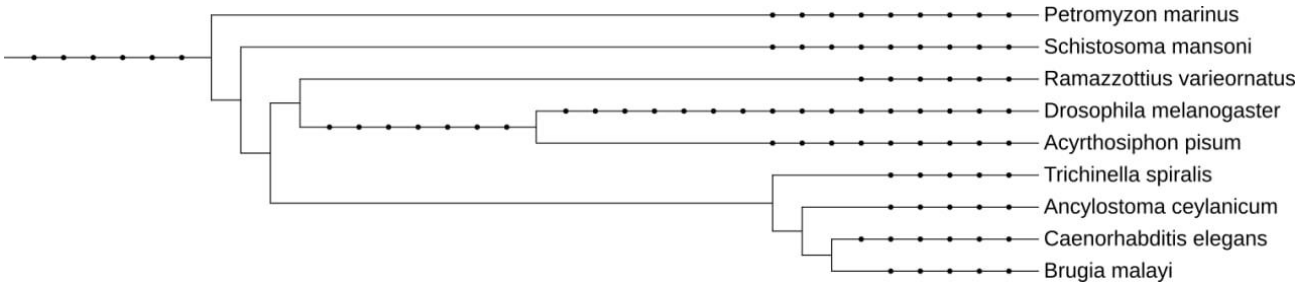

Figure 1: Phylogenetic tree of avilable pre-trained sets of annotated organisms and R.varieornatus.

First, our goal is to make functional annotation. So, we run AUGUSTUS to find homologous proteins in tardigrade genome data. We used training sets Acyrthosiphon pisum species via ugustus. We have chosen this species after fylogenetic tree construction 1. We obtained 14446 proteins.

Next, we used the list of peptides and command *makeblastdb*. After removing duplicates, we got 31 proteins. After using Blast alignment, we keeped 22 proteins. The summury information is presented in Table 1.

id	cover	E-value	Identity	WolF	TargetP	HMMER
g5214t1	32	1e-12	39.29	*nucl: 4.5, cytonucl: 5.5	Other	Chitin binding Peritrophin-A domain

g11539t1	50	3e-06	28.57		Signal peptide	
g5192t1	40	2e-14	40		Signal peptide	Chitin binding Peritrophin-A domain
g5086t1	12	2e-6	44		Signal peptide	Chitin binding Peritrophin-A domain
g5087t1	20	6e-13	40	nucl: 9	Other	Chitin binding Peritrophin-A domain
g672t1	28	1e-5	42		Signal peptide	Chitin binding Peritrophin-A domain
g7315t1	96	8e-70	38	nucl: 17.5, cytonucl: 15.5	Other	SNF2 family N-terminal domain
g2692t1	12	1e-3	26	*cytonucl: 4, nucl: 3.5	Other	Hermes transposase DNA-binding domain
g4653t1	54	1e-13	36		Other	Zinc finger
g8025t1	48	8e-104	38		Other	Cytosol amino peptidase family, catalytic domain
g3168t1	97	8e-50	48		Other	
g10626t1	72	8e-82	27		Other	Transport protein Trs120 or TRAPPC9, TRAPP II complex subunit

g1221t1	13	1e-20	43		Signal peptide	Casein kinase substrate phosphoprotein PP28
g10444t1	9	3e-3	27		Signal peptide	
g7708t1	93	2e-35	22	nucl: 15.5, cytonucl: 15.5	Other	Region in Clathrin and VPS
g5481	13	1e-11	35	nucl: 27, cytonucl: 18.3333	Other	
g7708t1	93	2e-35	22	nucl: 15.5, cytonucl: 15.5	Other	Region in Clathrin and VPS
g7784t1	83	2e-90	36	nucl: 9.5 cytonucl: 6	Signal peptide	Glycosyl transferase family 2
g11028t1	98	2e-82	26	nucl: 32,	Other	Zinc finger, C3HC4 type (RING finger)
g3380t1	51	4e-8	24	*cytonucl: 1.83333, nucl: 1.5	Signal peptide	Astacin (Peptidase family M12A)
g9785t1	29	1e-5	33	nucl: 16	Other	
g2090t1	73	2e-126	36	*nucl: 2	Other	Glycosyl hydrolases family 31

Table 1: The summary information. The star in cell WolF (localization) means that nucl and cytonucl scores are not maximum. The empty cell means that information is not presented.

5 Discussion

So, we run AUGUSTUS to find homologous proteins in tardigrade genome data. To detect conserved domains, we used training sets Acyrthosiphon pisum species via AUGUSTUS. We obtained 14446 proteins. After creating local BLAST database and doing a BLAST, we obtained 31 unique hits, which were used in downstream analysis.

Using multiple tools, we successfully combined results from various sources, thus allowing to find only desired proteins: Selection criteria were:

1. WoLFPROT: predominantly localisation in nucleus/cytonucleus(the bigger score, the better its confidence in localisation in one compartment)

2. hmmscan: desired top hits (with minimum of E-value). Although useful in common sense, here we're not interested in carbohydrates metabolism or Golgi complex proteins.

3. TargetP: not signal proteins, because we need proteins that are constantly present in cell nucleus to maintain chromatin stability. 4. BLAST: max score/

All the data are in Table 1.

According to these criteria, at least 2 proteins passed this filtration. First we present the top hits for each protein. There are g7315_t1, which is SNF2 family N-terminal domain(Pfam PF00176.23) and HepA-related protein (HARP)(Pfam PF07443.13), and g11028_t1(Zinc finger, C3HC4 type (RING finger)(Pfam PF00097.25)). 1 more protein, g4563_t1, could be potentially interpreted as Zinc knuckle(Pfam PF00098.23), although it's not the best hit for this protein. Fig.2,3,4 provides more detailed data about best hits. BLAST against SwissProt/UniProt database partially confirms our results. For g7315_t1, best BLAST hit is HepA-related protein(Uniprot B4F769) for g11028_t1, best BLAST hit is RING-type E3 ubiquitin transferase rad18(Uniprot Q4WZJ6).

According to the article provided with Task, we found neither MRE11 protein, nor Dsup protein, which is confusing. Perhaps if we use pre-computed AUGUSTUS annotation, we will find them. Although we didn't find specific proteins related to tardigrades 'genetic endurance', we still found proteins related to DNA repair and chromosome maintenance. Thinking that only 2 genes wiil do all the magic is naive and suitable only for newcomers in biology, so I think we reached a partial, but success. To better explain this, I would like to provide some description for each protein we found. For other details please use provided Pfam codes. Thanks to tight integration with Uniprot database, you can easily access additional info! WARNING! Some parts of the text below are taken without changes from its Pfam/UniProt articles. It's not a plagiarism–I just can't write it better than it is.

To be honest, not all of our findings are proteins. Some of them are just their domains, like our first hero–SNF2 family N-terminal domain. As far as I know, one

protein domain performs 1 specific function. SNP NTD domain is a part of proteins which involved in many functions transcription regulation, DNA repair (The most important for us!This domain is a part pf e.g. ERCC6, protein that conduct excision repair. This protein has ATPase activity thanks to this domain. Damaging this domain could ruin functioning of ERCC6 as the whole. More info about ERCC6 here [12]), DNA recombination and chromatin unwinding. Our second candidate for g7315 is a protein–HepA-related protein, also known as SMARCAL1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A-like1), also known as DNA-dependent ATPase A. Its function is maintaining genome integrity during DNA replication, working as helicase and stabilisng replication forks in right orientation. And we are very glad that BLAST confirms this protein too!

The last candidate for g7315_t1...it's not much to say. Presumably it's Type III restriction enzyme, res subunit, and that's all. Without exact knowing of which is this restrictase we can only assume its(g7351) participation in DNA-related activity, such as participation in recombination.

For g11028 we did find only domain, so we can only speculate about which specific protein this domain belongs. A RING (Really Interesting New Gene) finger domain is a protein domain of zinc finger type which contains a C3HC4 amino acid motif which binds two zinc cations (seven cysteines and one histidine arranged non-consecutively).This protein domain contains from 40 to 60 amino acids. Many proteins containing a RING finger play a key role in the ubiquitination pathway [13]. Whatever the exact protein is, we know its fuctions–ubiquitination and/or DNA-binding activity.

What is ubiquitination? Mainly it is the defensive mechanism providing fast and harmless degradation of proteins too damaged to be refolded with chaperones. This process consists of 5 stages [14]:

1) The unique E1 "ubiquitin-activating" enzyme activates ubiquitin by binding it to one of its own cysteins.

2) It then passes ubiquitin onto one among several dozens of E2 "ubiquitin-conjugating" enzymes. Here again, the C-terminus of ubiquitin is bound via a cystein.

3) One among hundreds of E3 "ubiquitin ligase" enzymes transfers ubiquitin to its final protein target. The C-teminus of ubiquitin is linked to the NH2 side chain of a lysine in the target, forming a covalent peptide bond.

4) Because ubiquitin itself contains 7 lysines, it is possible to build chains of ubiquitins onto the target protein. This may require the same or a different E3, and possibly the help of an E4 enzyme.

5) Specialized enzymes known as de-ubiquitinases (DUB) can shorten the chains or even remove all ubiquitins from the target protein.

To put in simple, possible mechanism of participation of this protein with RING

on board is: something damaged DNA-binding protein $\Longrightarrow$ to negate unwinding or wrong folding of chromatin this protein Ub-degraded $\Longrightarrow$??? $\Longrightarrow$ PROFIT. Maybe this finding is not so groundbreaking as a Dsup discovery, but these unnamed soldiers do their routine job, and without them even a superhero would be defeated.

Last candidate–g4563_t1. One more Zinc finger (we discussed its role in the last paragraph), one more unnamed soldier. Either in Ub degradation, or just keeping DNA tight and compacted via its binding, it also does their job.

Finally I want to discuss 2 possible sources of flaws made in this project. First, is DIY annotation of R.varieornatus made by picking nearest 'neighbor'. But going further in this project I realized that maybe we should have been tried to use annotation made by professionals or changed training set to C.elegans or tried to do de novo annotation. But lack of time and resources hindered these ideas. Second is not to use default parameters when doing a BLAST search. In everyday use default parameters is the go-to variant, but if we lessen the threshold, maybe we could find more proteins and domains.

To sum up: we found some proteins and domains which are participants in DNA maintaining activity. None of them are specific, none of them are major discovery, but like without Imperial Guard the Astartes would be drowned in horrors of the galaxy (WH40k reference), so without these proteins our 8-legged space marine would be melten/frozen/irradiated/insert_everything to death. Oh, and working with DNA data data and protein data simulteneously was a very interesting experience! Thank you for your attention!

6 Supplementary

References

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[12] https://www.uniprot.org/uniprot/Q03468

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[14] http://www.unige.ch/medecine/nouspikel/ubiquitin.html

✔ disorder ✔ coiled-coil ✔ tm & signal peptide

Pfam Matches

Advanced

	Family Id	Accession	Clan	Description	Cross-references	Start	End	Domain E-values Ind.	Cond.
>	zf-C3HC4	PF00097.25	CL0229	Zinc finger, C3HC4 type (RING finger)		927	965	3.8e-05	2.1e-09

Your search took: 0.43 secs

Download your results in various formats.

Figure 2: HMMER search results for g11028.

✔ disorder ✔ coiled-coil ✔ tm & signal peptide

Pfam Matches

Advanced

	Family Id	Accession	Clan	Description	Cross-references	Start	End	Domain E-values Ind.	Cond.
>	SNF2_N	PF00176.23	CL0023	SNF2 family N-terminal domain		268	566	7.6e-26	1.3e-29
>	HARP	PF07443.13	n/a	HepA-related protein (HARP)		173	225	3.0e-11	5.0e-15
>	ResIII	PF04851.15	CL0023 ✖	Type III restriction enzyme, res subunit		251	413	5.2e-06	8.6e-10

Your search took: 0.05 secs

Figure 3: HMMER search results for g7315.

✔ disorder ✔ coiled-coil ✔ tm & signal peptide

Pfam Matches

Advanced

	Family Id	Accession	Clan	Description	Cross-references	Start	End	Domain E-values Ind.	Cond.
>	Trypsin	PF00089.26	CL0124	Trypsin		810	1054	2.3e-21	3.8e-25
>	zf-CCHC	PF00098.23	CL0511	Zinc knuckle		51	68	1.7e-07	2.8e-11
>	zf-CCHC	PF00098.23	CL0511	Zinc knuckle		134	151	1.3e-07	2.2e-11
>	Ldl_recept_a	PF00057.18	n/a	Low-density lipoprotein receptor domain class A		520	565	1.1e-05	1.9e-09
>	Ldl_recept_a	PF00057.18	n/a	Low-density lipoprotein receptor domain class A		631	670	3.1e-05	5.1e-09

Figure 4: HMMER search results for g4653.