Emerging field of tardigrades and their stress tolerance

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A couple of years ago, Japanese scientists thawed a frozen moss sample from Antarctica collected 30 years earlier. They observed two minute creatures crawling back to life from three decades of 'cryo-sleep' inside the moss sample[1]. These tiny time-travellers were tardigrades, also known as water bears. They have four pairs of legs and their body size is about 0.3–1 mm (Figure 1). There are more than thousand species of tardigrades which thrive in freshwater, marine and limno-terrestrial environments. Though most of them have an extraordinary ability to resist desiccation, they need water to grow and reproduce. Some tardigrades are herbivores and feed on algae, while carnivorous species feed on rotifers, nematodes and even smaller tardigrades.

Phylogenetically tardigrades are close to onychophorans and nematodes. Smith *et al.*[2] have shown that tardigrades have lost intermediate body segments that include entire thorax and most of the abdomen. This is possibly because they lack several *Hox* genes that determine these body segments during development. Therefore, the entire body of tardigrades is homologous to just the head of arthropods. Hence are rightly called as 'walking heads'[3].

Tardigrades have fascinated scientists and public alike because of their astonishing ability to resist multiple physical stresses. Depending on the habitat they are isolated from, tardigrades can resist extreme temperatures, high pressure, high dose of ionizing radiation and space vacuum which are fatal to most other life forms[4–6]. Possibly, tardigrades are the hardiest species on earth. This amazing resistance is exhibited when they undergo an anhydrobiotic and ametabolic state called 'tun' state, which is induced after desiccation[7]. Upon addition of water tuns revert back to active live form. Unfortunately, the cellular and molecular mechanism behind this tun formation and its extraordinary stress-tolerance is not known.

Despite their uniqueness, the genomic sequence of tardigrades was not available till 2015. The first report of a tardigrade genomic sequence was published last year by Boothby *et al.*[8]. This group from the University of North Carolina, USA, sequenced the genome of *Hypsibius dujardini*, a freshwater tardigrade. Their analysis showed extensive horizontal gene transfer in its genome (17.5% of all genes), which could be a reason behind their ability to resist several physical stresses[8]. However, another report challenged this and claimed that there is no unusual level of horizontal gene transfer in the genome of *H. dujardini*[9]. Microbial contamination could be a reason for the unusually high level of horizontal gene transfer observed by Boothby *et al.* (refs 9, 10).

The next breakthrough in tardigrade research came recently when Hashimato *et al.*[10] reported the genomic sequence of *Ramazzottius varieornatus*, an extremo-tolerant tardigrade, and identified a protein responsible for its radiotolerance. They too did not observe any unusual horizontal gene transfer in its genome which is about 56 M bp; the genome has less than 1.2% of putative foreign genes. Microbial cross-contamination was minimized by starving the animals and also by treating them with antibiotics for two days. The genome of *R. varieornatus* has 19,521 putative protein-coding genes, out of which 41% is unique having no similarity with any known genes. The genome is highly compact with fairly short introns (mean size 402 bp) and short inter-coding sequences.

The genome of *R. varieornatus* has more than usual copies of stress-related genes. For example, 16 superoxide dismutases (SODs) are present which are

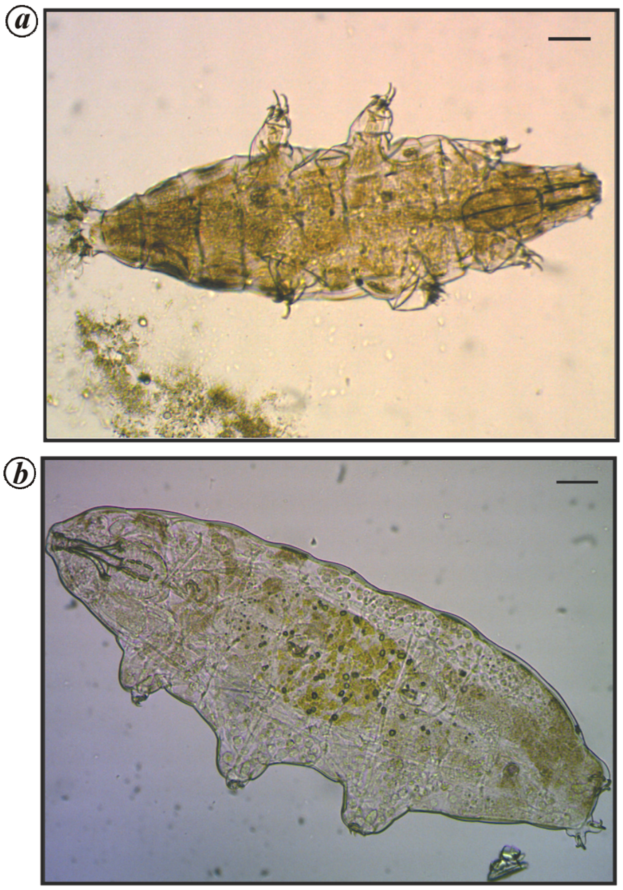

Figure 1. Tardigrades isolated at the Indian Institute of Science, Bengaluru campus. (***a***) *Milnesium* sp. and (***b***) *Paramacrobiotus* sp. Scale bar represents 50 μm.

involved in detoxifying superoxide radicals. Similarly, *R. varieornatus* possesses four copies of the *MRE11* gene which is involved in repair of double-strand breaks. Most metazoans have less than 10 SODs and only one *MRE11* in their genome. More number of SODs might help this tardigrade effectively resist oxidative stress and similarly, *MRE11* might help repair its DNA during prolonged anhyrdobiotic and ametabolic state. Thus, selective expansion of these two stress-related gene families could be a reason behind their extraordinary stress-tolerance.

Intriguingly, there is selective loss of peroxisomal oxidative pathway in *R. varieornatus*. Several genes encoding oxidative enzymes of the β-oxidation pathway and factors involved in peroxisome biogenesis are absent. In this light, it will be interesting to know the morphology and function of peroxisomes in tardigrades. This observation also questions the very existence of peroxisomes in *R. varieornatus*. Furthermore, the genes which connect stresses such as hypoxia, oxidative stress and DNA damage to mTORC1 (mammalian target of rapamycin complex) are also absent in *R. varieornatus*. The significance of these losses of selective pathways is not clear. Authors speculate that absence of the β-oxidation pathway might lead to decreased hydrogen peroxide production, which would preserve the crucial antioxidant ability of tardigrades.

Hashimato *et al.*[10] compared the transcriptome (mRNA-seq) of active adults with the anydrobitotic tuns. Surprisingly, they detected minor differences which were not biologically insightful. This indicates that transcriptional regulation might not play a major role in inducing the tun state and therefore in stress tolerance which is counter-intuitive. This also suggests that the genes responsible for their stress-tolerance are constitutively expressed. Their analysis revealed that many of the tardigrades-unique genes are present in multiple copies in *R. varieornatus* and are abundantly expressed. For example, there are 16 *CAHS* (cytoplasmic abundant heat soluble) and 13 *SAHS* (secretory abundant heat soluble) genes. These two classes of genes have been reported earlier in tardigrades. Their protein products maintain solubility even after heat treatment, which might protect the biomolecules during desiccation. Interestingly, proteins homologous to CAHS and SAHS are not present in other metazoans.

R. varieornatus can resist high doses of radiation which damage DNA[10,11]. Hashimato *et al.*[10] identified a chromatin-associated, tardigrade-unique nuclear protein, which they called damage suppressor (Dsup). The most fascinating result of this study is the demonstration of suppression of radiation-induced DNA damage by Dsup when expressed in human embryonic kidney (HEK293) cells. This was shown by comet assay as well as by testing γ-H2AX formation (indicator of double-strand breaks) after exposing Dsup-expressing HEK293 cells to 10 Gy X-ray irradiation or hydrogen peroxide which induces single-strand breaks. Furthermore, Dsup improves the viability of irradiated HEK293 cells up to 12 days after irradiation.

Though they were discovered more than 200 years ago, tardigrades are not as popular as *Drosophila* or *Caenorhabditis elegans* as a model system. Recent surge in the reports of genome and transcriptome analysis of tardigrades shows that the situation is changing. More and more laboratories, including ours are using tardigrades as a model system to study development as well as stress-tolerance. In spite of these developments, tardigrades research is still in its infancy. We still do not understand the mechanism of their stress-tolerance. In this light, discovery of Dsup protein in extremotolerant *R. varieornatus* by Hashimato *et al.*[10] is a major step forward. Understanding the molecular mechanism of their stress tolerance can potentially lead to technological breakthroughs. For example, it might help to safely store and transport vaccines and other biological samples without refrigeration. Definitely, this is an exciting period for those interested in tardigrades and their extraordinary stress-tolerance.

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