Protistology 8 (1), 8–15 (2013)

Protistology

Heat shock proteins of free-living ciliates and their impact on cell adaptation to salinity stress

Alexei Smurov[1], Yulia Podlipaeva[2], Sergei Skarlato[2] and Andrew Goodkov[2]

[1] *Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia*
[2] *Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia*

Summary

The paper describes the comparative analysis of our own experimental data concerning the chaperone activity of 70 kDa heat shock protein (HSP70) in three free-living ciliate species, *Paramecium jenningsi, Tetrahymena pyriformis, Paramecium nephridiatum*, belonging to the eufreshwater, metafreshwater and euryhaline ecological groups of unicellular organisms. These ciliates revealed different protecting strategies and adaptive potential of their chaperon defensive system regarding to the salinity stress. We infer that the constitutive level of HSP70 content in free-living ciliates reflects the environmental conditions of locations, where from these species originated. Consequently, when evaluating the ecological importance of constitutive HSP70 level in hydrobionts, it is necessary to take into account not only contemporary conditions of their existence, but the chaperone system "memory" as well.

Key words: chaperone system, free-living ciliates, heat shock proteins, HSP70, ecological groups of hydrobionts, *Paramecium nephridiatum, Paramecium jenningsi, Tetrahymena pyriformis*, salinity adaptation

Introduction

All living organisms respond to various environmental stresses by activation of different protecting mechanisms. At the cellular level, a basic and the most evolutionary conserved defensive strategy is the synthesis of stress proteins, or heat shock proteins (HSPs). These proteins are represented by a number of families, differing in molecular weight, nucleotide sequences of their genes, and functions. The best studied HSP family is characterized by the molecular weight of about 70 kDa (HSP70). This family contains highly conservative proteins with a low species-specificity (Feder and Hofmann, 1999; Margulis and Guzhova, 2000, 2009).

In free-living protists, HSP70 are rather poorly studied in comparison to those of bacteria, parasitic protozoans from homoiothermal hosts and multicellular organisms. There are just a few studies of HSP70 in the non-parasitic species of ciliates (Fink and Zeuthen, 1980; McMullin and Hallberg, 1987; La Terza et al., 2001, 2004; Hori and Fujishima,

2003; Smurov et al., 2007; Podlipaeva et al., 2008; Goodkov et al., 2010), flagellates (Drzymalla et al., 1996; Barque et al., 2000), and amoebae (Kalinina et al., 1988; Podlipaeva, 2001; Podlipaeva et al., 2006; Podlipaeva and Goodkov, 2009; Goodkov et al., 2010). Moreover, in these unicellular organisms, HSP70 were investigated mainly during adaptation to the changes in the environmental temperature.

However, as HSPs do bear the protective function, their participation in the process of adaptation to various adverse environmental factors may be hypothesized. In particular, an important topic is the adaptation of protists – the primarily aquatic creatures – to the changes in the water salinity. Salinity, alongside with temperature, is a key factor in the evolution of aquatic organisms.

There are four ecological groups of protistan species depending on their salinity preferences (Smurov and Fokin, 2001; Kudryavtseva et al., 2007). The first group – *stenofreshwate*r species – includes organisms that can survive in the medium with the salinity not higher than 2.5-3 ‰. The second group – *eufreshwater* species – includes organisms surviving in the salinity range of 0 – 6-8.5 ‰. The species of the third group – *metafreshwater* ones – can persist in more salty water, up to 12-16 ‰. And finally, the species of the fourth group may be termed as *euryhaline* ones – they can sustain the direct transfer from marine to fresh water.

Classification given above deals with the so called *potential ranges of salinity tolerance* (Smurov and Fokin, 2001), which describe the entire amplitude of salinity values, where the species can exist, or which the species might be acclimated for. It is necessary to point out that the value of *the range of salinity tolerance* of the cells acclimated to some specific salinity value is always narrower than the *potential range of salinity tolerance* of the species.

Similar ecological groups can be distinguished within the certain large taxa of multicellular invertebrate hydrobionts (Aladin, 1996). As the origin of such groups results from the prolonged evolution history, one may presume that there are some specificities of the chaperone system functioning in the species from each group. Earlier we have already shown that ciliates react to thermal and salinity shocks in different ways (Plekhanov et al., 2006), and that the reaction to shock, expressed in alterations of HSP70 level in the cells, may be different in ciliates belonging to different ecological groups (Smurov et al., 2007).

The existence of different ecological groups of protists within certain salinity ranges presumes that the species belonging to these groups are preadapted to salinity changes within these ranges; nevertheless, all of them can inhabit the same medium, i.e. the fresh water medium. If so, the physiological cell mechanisms, and the chaperone system among them, must also be preadapted to salinity changes.

The importance of salinity factor for the peculiarities of functioning of protists' chaperone system may be detected in experiments recording the constitutive level of HSP70, its expenditure, and its induction in response to the salinity stress under constant temperature conditions in protists from various ecological groups.

The main goal of this paper was to reveal the specificity of chaperone system functioning in ciliated protists with different adaptive effectiveness regarding to salinity factor. For this purpose we use experimental data about constitutive level of HSP70 in three ciliate species, acclimated to salt-water and fresh-water medium, presented in our previous publications. When possible, the comparison with the relevant published data on multicellular invertebrate hydrobionts was carried out.

Brief description of the experiments[1)] and the obtained results

The ciliates used in the study were two *Paramecium* species, namely: *P. jenningsi* (strain SR1-10) and *P. nephridiatum* (strain SR98-1), both from the culture collection of the Department of Invertebrate Zoology, St. Petersburg State University (Russia), and *Tetrahymena pyriformis* (amicronucleate strain GL) from the culture collection of the Laboratory of Cytology of Unicellular Organisms, Institute of Cytology, Russian Academy of Sciences.

The determination of salinity tolerance range of all examined ciliates was based on the method developed earlier by A. Smurov and S. Fokin (2001). Acclimation of cell cultures to the extreme values of salinity tolerance range resulted in the shift of the range borders and in getting the cultures with new characters. New cultures were tested again, and the procedure was repeated till *the potential border of salinity tolerance range* was achieved and the further increase of salinity led to cells death.

The upper limit of the salinity tolerance of *Paramecium jenningsi* cells acclimated to freshwater conditions (0 ‰) was 3.25 ‰. For the individuals acclimated to 2 ‰, the upper limit of salinity tolerance was 6 ‰, for the cells acclimated to 3.5

[1)] For the detailed description of all methodical procedures see the following publications: Plekhanov et al., 2006; Smurov et al., 2007; Podlipaeva et al., 2008; Goodkov et al., 2010.

‰ it was 8.5 ‰, being thus the potential border of salinity tolerance range of this *Paramecium* species.

The upper limit of the salinity tolerance of *Tetrahymena pyriformis* was 8 ‰ – for the cells acclimated to fresh water (0 ‰), 12 ‰ – for the cells acclimated to 2‰, and 15 ‰ (potential border of salinity tolerance range) – for the cells acclimated to 10 ‰. Cells acclimated to 10 ‰ salinity sustained direct transfer into fresh water (0 ‰), and could later adapt to it.

The upper limit of the salinity tolerance of *P. nephridiatum* cells acclimated to freshwater medium (0 ‰) was set at 20 ‰, and for the cells acclimated to 10 ‰ medium – at 34 ‰.

Thus, the potential salinity tolerance ranges were determined: for *P. jenningsi* – 0-8.5 ‰, for *T. pyriformis* – 0-15 ‰, and for *P. nephridiatum* – 0-34 ‰. Using the classification given above, the species *P. jenningsi* belongs to the ecological group of eufreshwater organisms, *T. pyriformis* – to metafreshwater, and *P. nephridiatum* to euryhaline ones.

Paramecium jenningsi

In the salinity shock experiments, cells grown in the freshwater medium (0 ‰) were placed for 2 h in a medium with 2 ‰ salinity. The cultures acclimated to 2 ‰ salinity were placed in fresh water also for 2 h. Protists cultivated in the media with the initial salinity values were used as the control; the culture density was the same in the control and in the experiments (Table).

In the total protein extract of the eufreshwater ciliate *P. jenningsi*, an antigen cross-reacting with anti-HSP70 antibodies was revealed. It was shown by the method of dot-blotting that the level of HSP70 in *P. jenningsi* cells grown in the freshwater medium was very low, but it increased after salinity shock (Plekhanov et al., 2006). On the contrary, ciliates acclimated to 2 ‰ demonstrated a high constitutive level of HSP70, which decreased after salinity shock (Table).

Paramecium nephridiatum

P. nephridiatum cells acclimated to fresh water (0 ‰) were placed for 1 h into water with 10 ‰ salinity; ciliates acclimated to10 ‰ were placed for 1 h into fresh water (0 ‰); then in both cases the cells were returned to the medium with the initial salinity. These cells were considered as treated by salinity shock (Table). For the control, ciliates were placed into water with the salinity usual for them, for the same period of time. Some of the cells were subjected to an analogous impact with the following difference: after the transfer into water with a different salinity, the cells were not returned to the medium with the initial salinity after an hour but were left in the water with a different salinity until the end of the experiment (up to 24 h). Those cells were considered as subjected to "adaptation".

A polypeptide antigen of ca. 70 kDa was revealed by western blotting in the total protein extract of the euryhaline ciliate *P. nephridiatum*, both in the intact (control) cells after prolonged acclimation to the freshwater medium or the salt water medium, and in the salinity-shocked cells. Besides, *P. nephridiatum* cells acclimated to 10 ‰ were shown to possess an additional polypeptide with a molecular weight of about 60 kDa, which also cross-reacted with anti-HSP70 antibodies (Smurov et al., 2007). The protein with a molecular weight of about 60 kDa was present in a considerable quantity, which was higher than that of the protein of about 70 kDa, in *P. nephridiatum* cells acclimated to 10 ‰, and was almost absent in the cells acclimated to fresh water.

The level of HSP70 in ciliates acclimated to freshwater conditions was much higher than in ciliates acclimated to 10 ‰ (Smurov et al., 2007).

In ciliates acclimated to fresh water and salt water, salinity shock elicited an asymmetric response as to the expenditure/synthesis of HSP70. The HSP70 concentration in the cells transferred from the 10 ‰ medium into fresh water was much higher than that in the cells transferred from fresh water into salt water, the time after transfer being equal (Table).

The level of 70 kDa protein after transfer from 10 ‰ medium to fresh water (10 → 0‰) was higher than that in the control. After transfer from fresh to salt water (0 → 10‰), the HSP70 staining zone was weak, the protein concentration being much less than in the control.

"Adaptation" to a new salinity (a prolonged variant of the salinity shock) in the euryhaline ciliate *P. nephridiatum* resulted in HSP70 induction in the experiments with both directions of salinity changes (Table).

Tetrahymena pyriformis

T. pyriformis cells were placed for 1 h into water with a different salinity: the cells acclimated to 2 ‰ were placed in 10 ‰, and the cells acclimated to 10 ‰, into 2 ‰. Then the ciliates were returned to the medium with the initial salinity. For the control,

Table. Ecological characters of ciliates, their acclimation to media with different salinities, and peculiarities of their reaction to salinity shock.

Characters of species / Ecological groups and species under study	Eufreshwater *Paramecium jenningsi*	Metafreshwater *Tetrahymena pyriformis*	Euryhaline *Paramecium nephridiatum*
Potential borders of salinity tolerance range	0–8.5‰	0–15‰	0–34‰
The scheme of ciliate acclimation	0→2‰	0→2→10‰	0→10‰
Salinity shock (1h, then return to initial medium)	(I) 0→2‰ (II) 2→0‰	(I) 2→10‰ (II) 10→0‰	(I) 0→10‰ (II) 10→0‰
Induction (+) or expenditure (–) of HSP70 after the stress, the absence of pronounced reaction (+/–) to the stress	(I) + (II) –	(I) +/– (II) +/–	(I) – (II) +

ciliates were placed for the same time into water with the salinity usual for them (Table).

In the metafreshwater ciliate *T. pyriformis*, two zones were revealed in the control cells cultivated under optimal conditions (0 ‰): an intensely stained zone with a molecular weight of about 70 kDa ("72 kDa") and a zone of about 60 kDa (Podlipaeva et al., 2008). In the intact *T. pyriformis* cells acclimated to 2 ‰, a stained zone of a somewhat larger molecular weight ("73 kDa") was revealed, which remained almost unchanged in 2, 4 and 24 h after the salinity shock during 1 h at 10 ‰. Besides, both in the control samples and in the samples prepared in 24 h after salinity shock, a weakly stained zone corresponding to a protein with a molecular weight of about 65 kDa was revealed.

T. pyriformis acclimated to 10 ‰ had a constitutive HSP with a molecular weight of "72 kDa", which had the same position as in the ciliates from fresh water, and was located a little bit lower than that of the ciliates acclimated to 2 ‰. The level of this protein decreased in 2 h after salinity shock at 2 ‰. In 4 h after shock, the blot contained a protein with a slightly higher molecular weight ("73 kDa"), which substituted the protein with molecular weight about 72 kDa. Finally, in 24 h after shock both zones, "72" and "73 kDa", were distinctly stained on the blot (Podlipaeva et al., 2008). The protein with molecular weight of about 65 kDa was not found.

No induction of HSP70 synthesis in the metafreshwater ciliate *T. pyriformis* cells was registered after salinity shocks in both directions of salinity changes (Table). Moreover, in the cells acclimated to 10 ‰, there was a noticeable expenditure of the "72 kDa" protein after salinity shock. It is worth mentioning that in *T. pyriformis* the differences both in spectra and levels of content of constitutive HSP were revealed depending of the salinity of their long term acclimation.

To sum up, eufreshwater and euryhaline species of the genus *Paramecium* have different strategies of the chaperone system's response to increasing and decreasing salinity. In the eufreshwater species *P. jenningsi*, acclimation to increased salinity results in an increase of the constitutive level of HSP70 in the cell, i.e. an acclimation to such a slight shift (from 0 to 2 ‰) of salinity is a stress for this species. Acclimation of the euryhaline species *P. nephridiatum* to increased salinity does not result in any change in the HSP70 level. Decreasing salinity of the medium (from 2 to 0 ‰) is accompanied in eufreshwater *P. jenningsi* by the decreased, whereas in euryhaline *P. nephridiatum* – by the increased level of HSP70 in the cell. In *P. nephridiatum* individuals acclimated to fresh water (0 ‰), the level of HSP70 was much higher than in *P. nephridiatum* individuals acclimated to salt water (10 ‰). So, for euryhaline ciliates, a prolonged acclimation to fresh water is a stronger stress than acclimation to 10 ‰. Prolonged acclimation to fresh water appeared to result in the activation of their chaperone system.

Therefore, in fresh water these ciliates turn out to be pre-adapted to abrupt changes in the environmental salinity and respond to them by using the pool of HSP accumulated in the cell. It is, for example, expressed in the decrease of high HSP70 level in ciliates acclimated to fresh water (0 ‰) after their transfer to salt water (10 ‰).

As for the reaction of *P. nephridiatum* to salinity shock, the HSP70 concentration increased in the cells transferred from the 10 ‰ medium into fresh water and decreased in the cells transferred from fresh water into salt (10 ‰) water. Thus, the chaperone system of euryhaline ciliates, such as *P. nephridiatum*, is characterized by an asymmetric response to salinity variations, namely, by the expenditure/synthesis of HSP70 after salinity shock, depending on the direction of salinity changes.

"Adaptation" to a new salinity (that is, a case of the prolonged salinity shock) brings about HSP70 induction in *P. nephridiatum*, whatever direction the salinity changes. This observation agrees with

the reports that in some instances only a prolonged stress elicits HSP70 induction. For example, in the ciliate *Moneuplotes crassus* the level of HSP70 was the same as in the intact cells after a short heat shock (5 min) but increased after a prolonged heat shock, reaching the maximum after 180 min (Ullmann et al., 2004).

It is worth mentioning that the data about prolonged stress in unicellular hydrobionts do not totally correspond to analogous data on multicellular ones, such as marine molluscs. For example, HSP70 concentration in the cells of *Chamelea gallina* (Bivalvia) changed only in the cases of mollusc adaptation to low salinities, and in digestive gland it was increased, but in the gills – decreased (Monari et al., 2011). It was also shown that the level of HSP70 in gill epithelium of mussels *Mytilus edulis* (Bivalvia) increased after long term acclimation to both high and low salinities, but prolonged salinity stress of 24 h caused HSP70 induction only in low salinity (Podlipaeva and Berger, 2012).

As we stated above, in the metafreshwater ciliates *Tetrahymena pyriformis* no any induction of HSP70 synthesis was discovered in organisms after salinity shocks in both directions of salinity change. On the contrary, there was a noticeable expenditure of the 72 kDa protein, which substituted the 65 kDa protein 4 h after the shock. Thus, we can conclude that the chaperone system of *T. pyriformis* is less mobile than that of the two *Paramecium* species studied in response to both a prolonged salinity acclimation and a short stress.

It is worth mentioning that metafreshwater *Tetrahymena* ciliates are known to possess a heat shock protein, called HSP90, with a molecular weight of 82-85 kDa (Frankel et al., 2001). Its primary structure does not have a high homology with HSP70 of vertebrates, whereas the protein found in our study has common antigen determinants with HSP70 of vertebrates – a fact that may indicate a considerable homology in primary structure.

To sum up the results on dynamics of HSP70 level in the ciliates treated by salinity stress conditions, it may be concluded that unicellular organisms belonging to eufreshwater and euryhaline (but not to metafreshwater) groups of species respond to salinity shock at first by decreasing and then by increasing concentration of HSP70 in the cells.

Discussion

Studies of HSP70 content in the cells of invertebrates from natural populations show that slight alterations of environmental temperature do not result in changes of HSP70 level (Feder and Hofmann, 1999). Long term (about a month) trends of environmental temperature fluctuations are essential for noticeable change of constitutive HSP70 level in the cells (Menge et al., 2002). Besides, it was shown that there is a positive correlation between HSP70 level in the cells of insects and the border-values of their temperature tolerance ranges (Evgen'ev et al., 2007). The similar data were obtained when studying temperature adaptations of hydrobionts. Reliable positive correlation was revealed between the environmental temperature and the level of HSP70 content in the gills of Mediterranean mussels *Mytilus galloprovincialis*. Moreover, pronounced and reliable seasonal changes in protein content in these mussels were observed following environmental temperature changes (Hamer et al., 2004).

Our experiments differ from the investigations mentioned above where the animals mainly were obtained directly from their natural habitats; the ciliates under study underwent the same conditions in terms of medium (all of them were preliminary acclimated) and culture growth phase. Ciliates are unicellular organisms, therefore some restrictions and reservations related to the tissue specificity of multicellular organisms are not relevant to them. All these circumstances allow evaluating impartially the ecological sense of HSP70 constitutive level in the cells in connection with their different salinity tolerance (i.e. the capability to survive in the media with different salinities).

According to our data on eufreshwater and euryhaline unicellular organisms, different strategies of the chaperone system response to increasing and decreasing salinity may be observed. Acclimation to elevated salinity results in an increase of the constitutive level of HSP70 in cells of eufreshwater ciliates, whereas in the euryhaline ones it does not result in any change in the HSP70 level. Decreasing salinity of the medium is accompanied by an increased level of HSP70 in both euryhaline and eufreshwater species of *Paramecium* ciliates, and the metafreshwater species *Tetrahymena pyriformis* which is "intermediate" according to its ecological characters, reacts weekly to salinity changes in the medium. The level of HSP70 content in *T. pyriformis* cells practically does not allow presuming that its chaperone system is less reactive than that of eufreshwater and euryhaline ciliate species. We cannot exclude that constitutive level of HSP70 in the cells of these ciliates, even belonging to different ecological groups of species depending on their salinity tolerance, may reflect, in some respect, the conditions in the habitat of their origin: fresh water

bodies in the case of *P. jenningsi*, fresh or brackish water bodies in the case of *T. pyriformis*, brackish-water estuaries or seas in the case of *P. nephridiatum*. Differences in the salinity tolerance limits of the species studied suggest that, even though they are pre-adapted to salinity changes within these limits, all of them may live in the same medium – fresh water.

Thus, we presume that when evaluating the ecological importance of constitutive HSP70 level in the unicellular hydrobionts it is reasonable to take into account not only the current conditions of their habitat, but also the "memory" of their chaperone system. The experiments with preliminary acclimation represent a convenient method to reveal such a "memory".

One of the examples of chaperone system "memory" is the induction of HSP synthesis (HSP70 among them) in the trypanosomatid flagellate *Phytomonas characias*, a parasite of plants, after temperature shift from 22 to 37 °C (Sanchez-Moreno et al., 1997). Nevertheless, these trypanosomatids do not have warm-blooded host in their life cycle, HSP70 synthesis induction in *Phytomonas* cells occurs namely at 37 °C, which is a characteristic feature of heteroxenous trypanosomatids (*Trypanosoma* spp.), parasitizing mammals. This fact may be considered as a sort of chaperone system "memory" of flagellates from the genus *Phytomonas*, which is phylogenetically connected with ancestral trypanosomatids – parasites of homoiothermal hosts, and as one more confirmation of the presence of common ancestors in all representatives of this family including those turned to parasitizing insects and plants (Maslov et al., 2001).

It is known that in the multicellular organisms which inhabit highly variable environments, constitutive HSP70 level is elevated (Ulmasov et al., 1992; Evgen'ev et al., 2005; Garbuz et al., 2008). At the same time, it was shown that the deep-sea animals adapted to thermally stable conditions should be highly sensitive to temperature change and should not have inducible heat-shock responses; it concerns the cases when high constitutive levels of HSP70 are present (Berger and Young, 2006).

We obtained similar data for various protists (Podlipaeva, 2001; Plekhanov et al., 2006; Podlipaeva and Goodkov, 2009); the hypothesis was put forward that high constitutive HSP70 level may be accounted as the protists' preadaptation not only to temperature changes, but to other factors as well (Plekhanov et al., 2006; Podlipaeva and Goodkov, 2009). The presence of similar ecological groups for uni- and multicellular organisms, distinguished according to their relation to environmental salinity changes (Smurov and Fokin, 2001), allow presuming the universality of their chaperone system reactions.

We have also shown that after certain environmental salinity changes, eufreshwater and euryhaline ciliate species may demonstrate pronounced HSR (heat shock response). Species with intermediate adaptive capacities (e.g. *T. pyriformis*), on the contrary, possess initially high HSP70 level and lack HSR. It may be hypothesized that high level of HSP70 content and the absence of HSR allow the species to exist in the extremely changeable conditions. However, in order to be able to colonize new habitats (e.g. salt water for freshwater species and vice versa) the presence of HSR is essential.

Aknowledgements

This study was funded by the Russian Foundation for Basic Research (grant 13-04-00703) and the German Federal Ministry of Education and Research 01DJ12107.

References

Aladin N.V. 1996. Salinity adaptations in Ostracoda and Branchiopoda. Proceedings of the Zoological Institute RAS, St. Petersburg. 265, 1–208 (in Russian).

Barque J.P., Schedler P., Floch E. and Bonaly J. 2000. In *Euglena gracilis*, a heat-shock protein related to hsc73 is constitutive and stress inducible. Arch. Biochem. Biophys. 378, 1–5.

Berger M.S. and Young C.M. 2006. Physiological response of the cold-seep mussel *Bathymodiolus childressi* to acutely elevated temperature. Marine Biology. 149, 1397–1402.

Drzymalla C., Schroda M. and Beck C.F. 1996. Light-inducible gene HSP70B encodes a chloroplast-localized heat shock protein in *Chlamydomonas reinhardtii*. Plant Mol. Biol. 31, 1185–1194.

Evgen'ev M.B., Garbuz D.G. and Zatsepina O.G. 2005. Heat shock proteins: functions and role in adaptation to hyperthermia. Ontogenez. 36, 265–273 (in Russian, with English summary).

Evgen'ev M.B., Garbuz D.G., Shilova V.Y. and Zatsepina O.G. 2007. Molecular mechanisms underlying thermal adaptation of xeric animals. J. Biosci. 32, 489–499.

Feder M.E. and Hofmann G.E. 1999. Heat-shock proteins molecular chaperones, and the stress

response: evolutionary and ecological physiology. Ann. Rev. Physiol. 61, 243–282.

Fink K. and Zeuthen E. 1980. Heat shock proteins in *Tetrahymena* studied under growth conditions. Exp. Cell Res. 128, 23–30.

Frankel J., Williams N.F., Nelsen E.M. and Keeling P.J. 2001. An evaluation of Hsp90 as a mediator of cortical patterning in *Tetrahymena*. J. Eukaryot. Microbiol. 48, 147–160.

Garbuz D.G., Zatsepina O.G., Przhiboro A.A., Yushenova I., Guzhova I.V. and Evgen'ev M.B. 2008. Larvae of related Diptera species from thermally contrasting habitats exhibit continuous up-regulation of heat shock proteins and high thermotolerance. Mol. Ecol. 17, 4763–4777.

Goodkov A.V., Smurov A.O. and Podlipaeva Yu.I. 2010. Free-living protists as a model for studying heat shock proteins in the cell. In: Handbook of molecular chaperones: roles, structure, and mechanisms (Eds: Durante P. and Colucci L.). Nova Science Publishers, New York, pp. 293–312.

Hamer B., Hamer D. P., Muller W. E. and Batel R. 2004. Stress-70 proteins in marine mussel *Mytilus galloprovincialis* as biomarkers of environmental pollution: a field study. Environ. Int. 30, 873–882.

Hori M. and Fujishima M. 2003. The endosymbiotic bacterium *Holospora obtusa* enhances heat-shock gene expression in the host *Paramecium caudatum*. J. Eukaryot. Microbiol. 50, 293–298.

Kalinina L.V., Khrebtukova I.A., Podgornaya O.L., Wasik A. and Sikora J. 1988. Heat shock proteins in *Amoeba*. Eur. J. Protistol. 24, 64–68.

Kudryavtseva A.V., Smurov A.O. and Goodkov A.V. 2007. Salinity tolerance of lobose amoebae and ciliates. Protistology. 5, 46.

La Terza A., Papa G., Miceli C. and Luporini P. 2001. Divergence between two antarctic species of the ciliate *Euplotes, E. focardii* and *E. nobilii*, in the expression of heat-shock protein 70 genes. Mol. Ecol. 10, 1061–1067.

La Terza A., Miceli C. and Luporini P. 2004. The gene for the heat-shock protein 70 of *Euplotes focardii*, an Antarctic psychrophilic ciliate. Antarctic Science. 16, 23–28.

Maslov D.A., Podlipaev S.A. and Lukes J. 2001. Phylogeny of the kinetoplastida: taxonomic problems and insights into the evolution of parasitism. Mem. Inst. Oswaldo Cruz. 96, 397–402.

Margulis B.A. and Guzhova I.V. 2000. Stress proteins in eukaryotic cells. Tsitologiya. 42, 323–342 (in Russian, with English summary).

Margulis B.A. and Guzhova I.V. 2009. Dual role of chaperones in the response of a cell and of a whole organism to stress. Tsitologiya. 51, 219–228 (in Russian, with English summary).

McMullin Th.W. and Hallberg R.L. 1987. A normal mitochondrial protein is selectively synthesized and accumulated during heat shock in *Tetrahymena thermophila*. Mol. Cell Biol. 7, 4414–4423.

Menge B.A., Olson A.M. and Danilohoff E.P. 2002. Environmental stress, bottom-up effects, and community dynamics: integrating molecular-physiological and ecological approaches. Integ. Comp. Biol. 42, 892–908.

Monari M., Foschi J., Rosmini R., Marin M.G., Serrazanetti G.P. 2011. Heat shock protein 70 response to physical and chemical stress in *Chamelea gallina*. J. Exp. Mar. Biol. Ecol. 397, 71–78.

Plekhanov A.Yu., Smurov A.O., Podlipaeva Yu. I., Ivanova L.O. and Goodkov A.V. 2006. Heat shock proteins of freshwater protists and their involvement in adaptation to changes in salinity of environment. Tsitologiya. 48, 350–354 (in Russian, with English summary).

Podlipaeva Yu. I. 2001. Heat shock protein of 70 kDa in *Amoeba proteus*. Protistology. 2, 123–129.

Podlipaeva Yu. I., Shmakova L.A., Gilichinski D.A. and Goodkov A.V. 2006. Heat shock protein of hsp70 family revealed in some contemporary freshwater amoebids and in *Acanthamoeba* sp. excysted from cysts isolated from permafrost samples. Tsitologiya. 48, 691–694 (in Russian, with English summary).

Podlipaeva Yu.I., Smurov A.O. and Goodkov A.V. 2008. Expression of heat-shock protein 70 kDa in *Tetrahymena pyriformis* during cell adaptation to salinity changes in the medium. Cell and Tissue Biology. 2, 373–375.

Podlipaeva Yu.I. and Goodkov A.V. 2009. Heat shock proteins of 70 kDa family in the cells of free living and amphizoic amoeboid organisms. Tsitologiya. 51, 1019–1024 (in Russian, with English summary).

Podlipaeva Yu.I. and Berger V.Ya. 2012. Changes of heat shock proteins content in the gill epithelium cells of mussel *Mytilus edulis* L. depending upon the salinity of medium. Tsitologiya. 54, 580–584 (in Russian, with English summary).

Sanchez-Moreno M., Rodriguez-Cabezas N., Fernandez-Becerra C., Mesa-Valle C. and Osuna A. 1997. Induction of stress proteins in the plant trypanosome *Phytomonas characias*. Parasitol. Res. 83, 771–775.

Smurov A.O. and Fokin S.I. 2001. Use of salinity tolerance data for investigation of phylogeny of *Paramecium* (Ciliophora, Peniculia). Protistology. 2, 130–138.

Smurov A.O., Podlipaeva Ju.I. and Goodkov A.V. 2007. Heat shock protein of the Hsp70 family

in the euryhaline ciliate *Paramecium nephridiatum* and its role in adaptation to the salinity changes. Cell and Tissue Biology. 1, 244–247.

Ullmann J., Reidt U. and Klein A. 2004. The expression of the HSP70 gene in *Moneuplotes crassus* is controlled by a two-step process at the transcript level. J. Eukaryot. Microbiol. 51, 344–350.

Ulmasov Kh.A., Shammakov S., Karaev K. and Evgen'ev M. 1992. Heat shock proteins and thermoresistance in lizards. Proc. Natl. Acad. Sci. USA. 89, 1666–1670.

Address for correspondence: Andrew Goodkov. Institute of Cytology, Russian Academy of Sciences, Tikhoretsky Ave. 4, St. Petersburg, 194064, Russia; e-mail: *pelgood1@gmail.com*