Chapter 7
Fine Tuning of the HSR in Various Organisms

It is widely assumed that heat shock response system in eukaryotes is amazingly conserved. Thus, all described organisms possess one or several genes encoding transcription factors belonging to HSF family that recognize the same sequences (HSEs) in the promoters of various *Hsps* genes (Åkerfelt et al. 2010; Morimoto 1998; Wu 1995). Furthermore, HSF family members in different organisms contain two highly similar domains responsible for DNA-binding and heat-induced trimerization as described in detail in Chap. 3. HSF recognizes practically the same simple sequences within promoters of *Hsp* genes (GAANNTTCNNGAA) that usually present in several copies at a regular distance from the transcription start. Various lines of evidence demonstrated that *Hsp70* gene promoter is able to efficiently function in the cells of phylogenetically distant organisms even belonging to different phyla. Thus, reporter constructs under the control of *Drosophila melanogaster Hsp70* gene promoter were readily expressed in the cells of mosquito *Aedes aegypti*, silkworm *Bombix mori* transgenic strains and in sea urchin embryos (Berger et al. 1985; McMahon et al. 1984; Uhlirova et al. 2002). Furthermore, constructs with *D. melanogaster Hsp70* regulatory region were efficiently transcribed in *Xenopus* oocytes, rat fibroblasts and monkey COS cells (Bienz and Pelham 1982; Burke and Ish-Horowicz 1982; Mirault et al. 1982; Voellmy and Rungger 1982). Importantly, all such constructs exhibited clear-cut heat-inducible pattern of expression in the cells of the foreign hosts, thus corroborating the presumed high conservatism of HS response in unrelated organisms.

However, subsequently several other groups described species-specific differences in the HS promoter efficiency (Kalosaka et al. 2006). These differences were attributed to the presence of specific regulatory elements (e.g. GAGA sites in *Drosophila* or ATRS in leaf-miner fly *Liriomyza*) within *Hsp* promoter regions of various species (see below).

Notably, early studies of the expression of such constructs in heterologous cells in 80th usually dealt with qualitative analysis of expression because at that time there was no technique such as Q-RT-PCR enabling to accurately measure the

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transcription levels. Therefore, it was not possible to accurately compare the efficacy of *Hsp* promoters in the homologous cells with that in the cells of a "foreign" host species.

Later, a few investigations appeared demonstrating that sometimes even in Diptera species *D. melanogaster Hsp70* promoter functions less effectively in comparison with endogenous host promoter. Thus, the heat-inducible activity of the promoter region of the *D. melanogaster Hsp70* gene, which contains motifs for HSF and GAF binding, assayed in transgenic medfly *Ceratitis capitata* germ-line carrying the *lacZ* reporter, was found to be several fold lower than the activity of the orthologous region of the medfly *Hsp70* gene with the same *lacZ* reporter (Kalosaka et al. 2006). Similarly, in transgenic Australian sheep blowfly *Lucilia cuprina* carrying reporter chloramphenicol acetyl transferase gene under *D. melanogaster Hsp70* promoter the reporter gene was expressed with 10–100-fold lower efficiency than in *Drosophila* cells and, characteristically, did not exhibit the inducible pattern of transcription (Atkinson and O'Brochta 1992).

These data indicate that certain organisms developed specific individual mechanisms underlying heat shock response in the course of divergent evolution. In this regard mechanisms that may provide the adaptation of a population or a species to highly fluctuating environments are of special interest. The recent studies demonstrated that, indeed, heat shock response system may undergo many changes providing "fine tuning" necessary for optimal expression of individual *Hsp* genes and, hence, adaptation of a given species or a population to specific environmental conditions including extreme ones.

It was shown that activity ("strength") of HS promoter is determined by the number and context of HSEs. Interestingly, it may also depend on the presence of other regulatory elements that may be specific for a give taxon or even species (Chen et al. 2011). As was shown by different studies the optimal sequence of a classical HSE (GAANNTTCNNGAA), contains three nucleotide sense units GAA/TTC. It was also demonstrated that a nucleotide before G in the unit may be also of importance, thus, it is assumed that AGAA represents an optimal combination. On the other hand within the unit itself only the first nucleotide G (or C in the case of complementary block) is strictly conserved while the second and especially the third one may be substituted by other nucleotides and these changes does not prevent HSF recognition of the modified HSEs (reviewed by Tian et al. 2010). Promoters of individual *Hsp* genes may vary in different species by the number of GAA/TTC units. Thus, in the majority of Diptera species investigated in this respect *Hsp83* promoters at least contain one HSE, consisting of six to eight elementary three nucleotides units (Astakhova et al. 2013; Tian et al. 2010). Furthermore, certain promotors may contain the so called "gap-type" and "step-type" HSEs (see Chap. 3) and such elements are also effectively recognized by HSF (Hashikawa et al. 2006; Yamamoto et al. 2005). Gap-type HSEs were detected within *Hsp* genes promoters in yeast and *Drosophila* (Tian et al. 2010; Yamamoto et al. 2005). It is of note, that surrounding nucleotides may also significantly influence individual HSE activity.

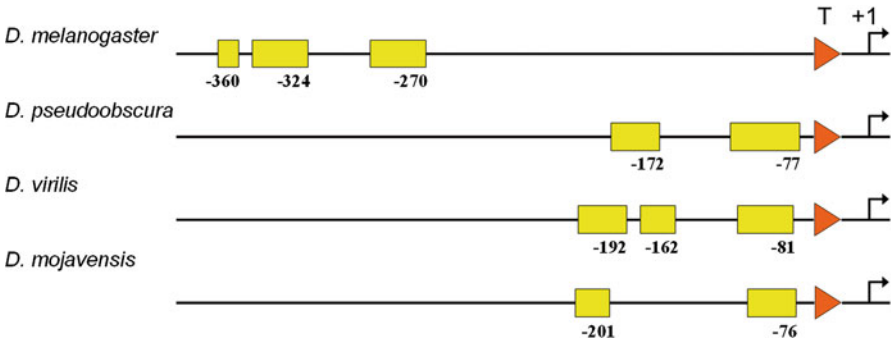

Fig. 7.1 The structure of regulatory regions of low molecular weight *Hsp* genes in *Drosophila* species. *Yellow rectangles* – HSEs, *T* TATA-box. Size of rectangles represents the amount of GAA units within HSEs (From Tian et al. 2010)

The strength of promoter is determined not only by the number of HSEs but may also depend on their distance from the transcription start site. Usually related species belonging to the same taxon comprise similar numbers of HSE copies within their *Hsp* genes promoters and HSEs have similar spacing regarding each other and transcription start. In 13 *Drosophila* species *Hsp70* promoters comprise four functional HSEs and, importantly, the position of the first two proximal HSEs regarding the transcription start is more conserved than the localization of the more distal ones (Tian et al. 2010). Furthermore, *Hsp83* promoters of *melanogaster* species group contain a single HSE, while the number of GAA/TTC units within this promoter may be species-specific. Other *Drosophila* species such as *D. mojavensis*, *D. virilis* and *D. grimshawi* have additional more distal HSE in *Hsp83* promoter. Within *Drosophila* species the *Hsp27* promoter exhibits the highest level of structural variability both in terms of HSEs number and relative position of HSEs when species of *melanogaster*, *repleta* and *virilis* groups are compared (Fig. 7.1). One may only speculate whether the observed structural polymorphism of small heat shock genes promoters resembles adaptation to specific environments or represent the result of random mutation and rearrangements in the course of species evolution. Mobile elements may be involved in the "fine tuning" of *Hsps* genes promoters as discussed in Chap. 6.

When studying heat shock genes clusters in two eurithermal Diptera species, *Stratiomys singularior* and *Oxycera pardalina*, members of the *Stratiomyidae* family (common name "soldier flies"), we described very unusual structure of *Hsp70* promoters (Garbuz et al. 2011). In contrast to all *Drosophila* species studied so far, *S. singularior* exhibits high variability of promoters of *Hsp70* genes comprising a cluster in the latter species. In *Drosophila* species high level of homology is observed not only in the promoters of neighboring *Hsp70* comprising a cluster but, is evident when promoters of different species are compared. In contrary the promoters of *S. singularior Hsp70* regulatory regions display significant similarity only within the first 50 bps upstream of transcription start site while the preceding

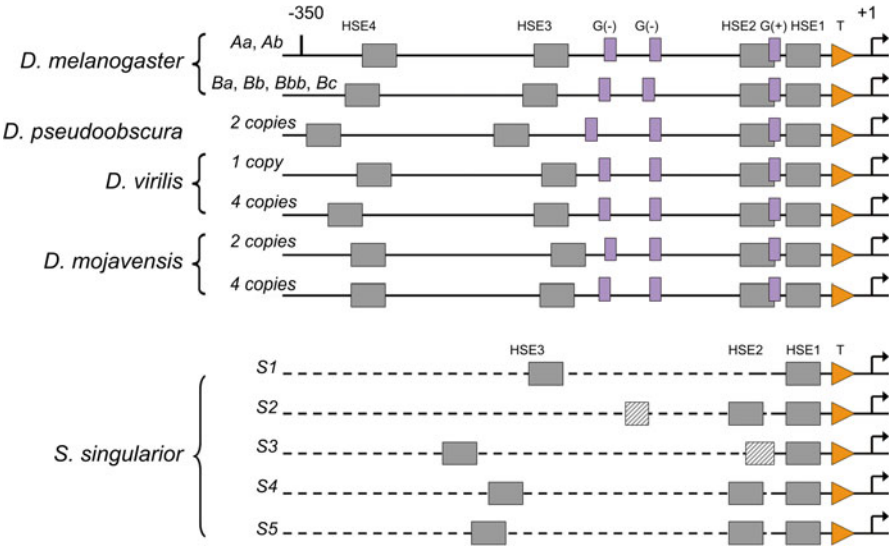

Fig. 7.2 The variability of *Hsp70* regulatory regions in *Drosophila* species and in *S. singularior*. *G* GAGA motifs in «+» or «−» orientation, *T* TATA-box (Modified from Tian et al. 2010; Garbuz et al. 2011). Lighter rectangles represent the position of putative gap-type HSEs

regions do not exhibit any significant homology (Fig. 7.2). Moreover, the number and localizations of HSEs within *S. singularior Hsp70* promoters also vary with the exception of the first HSE localized in the short (50 bps) proximal highly conserved region. Surprisingly, in *O. pardalina*, the another species of the same family (Stratiomyidae) promoters of all four sequenced *Hsp70* genes are highly homologous and differ by only a few substitutions that do not affect HSEs. The differences at 3'-regions characteristic for all five *Hsp70* genes of *S. singularior* enable to perform 3'-RACE analysis, which demonstrated that all the genes comprising *Hsp70* cluster are actively transcribed (Garbuz et al. 2011). The observed high variability of the regulatory regions of *Hsp70* genes in this species probably indicates the absence of conversion process operating in these areas and reveals a peculiar mechanism of the *Hsp70* cluster origin in this species. The observed variability in *S. singularior Hsp70* promoters may be of adaptive value providing differentiated expression of individual *Hsp70* genes after various forms of stress or at different developmental stages.

In contrast to *Hsp70* promoters, *Hsp83* promoters of *S. singularior*, *O. pardalina*, different *Drosophila* species and several other Diptera such as *Anopheles albimanus* are highly conserved not only regarding the number and positions of HSEs but share high sequence similarity as well (Fig. 7.3). There are Diptera species such as *Aedes aegypti* and *Culex pipiens* that contain additional HSEs in *Hsp83* regulatory regions localized at a greater distance from the transcription start (Astakhova et al. 2013; Tian et al. 2010).

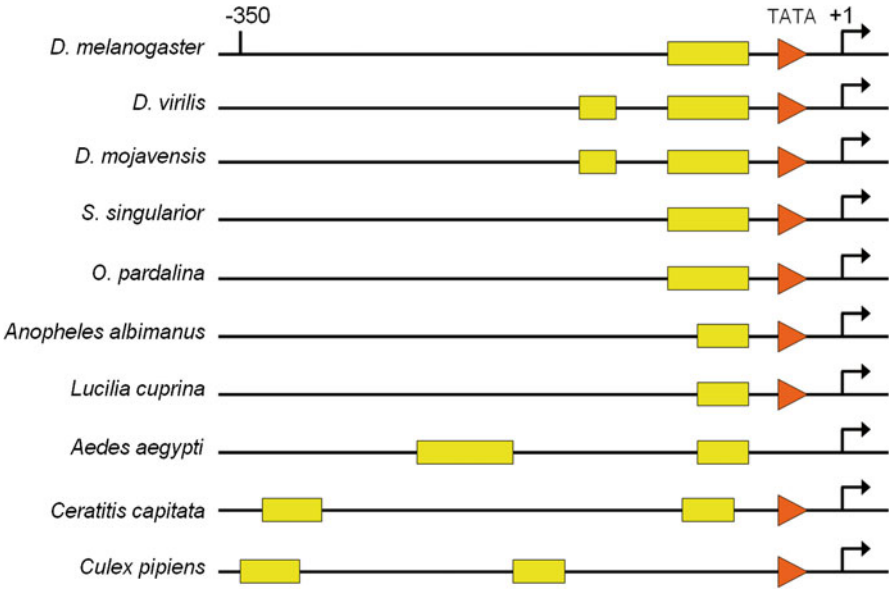

Fig. 7.3 The structure of *Hsp83* genes regulatory regions in different Diptera species. *Yellow rectangles* – HSE, *T* TATA-box. The length of rectangles resembles the number of GAA/TTC units within HSEs (Modified from Astakhova et al. 2013; Tian et al. 2010)

Previously we demonstrated that all species belonging to Stratiomyidae family used in our experiments were characterized by high constitutive levels of Hsp70 and exhibited extraordinary high thermotolerance independently on the temperature of their habitats (Garbuz et al. 2008). It was of significant interest to investigate the strength of Stratiomyidae species promoters in the genome of the foreign species. To this end, we developed constructs containing sequences of *S. singularior Hsp70S3* gene for transformation of *D. melanogaster* strain where all five *Hsp70* endogenous copies were deleted (Gong and Golic 2004). In these experiments we obtained several transgenic strains carrying one or more inserts of such constructs in *D. melanogaster* genome. Surprisingly, we failed to observe puffs in polytene chromosomes at the sites of construct insertions after HS. As expected when *S. singularior Hsp70* promoter has been substituted by that of *D. melanogaster* such constructs produced puffs after HS and were strongly induced judging by Q-RT-PCR and Northern hybridization experiments. More illustrative results of Q-RT-PCR for comparison of *S. singularior* and *D. melanogaster Hsp70* promoters strength are depicts in Fig. 7.4.

To investigate the role of the observed characteristic differences in the structure of the *D. melanogaster* and *S. singularior Hsp70* regulatory regions, we investigated the ability of these highly diverged *Hsp70* promoters to drive transcription of the luciferase reporter gene in a Schneider-2 (S2) *D. melanogaster* cell culture, both at a steady state and after temperature elevation.

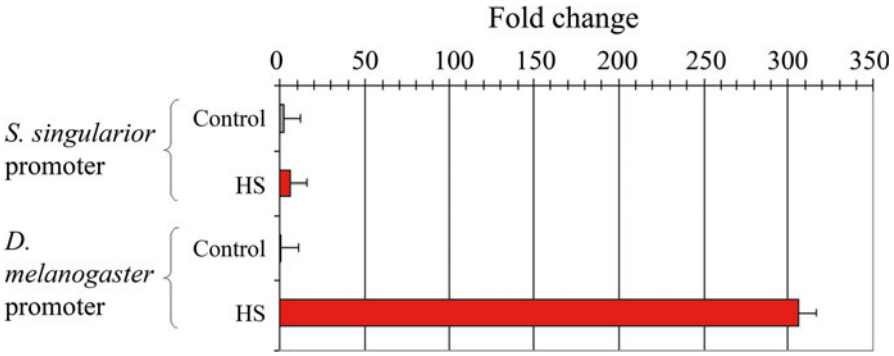

Fig. 7.4 Transcription of constructs with various *Hsp70* promoters in transformed *D. melanogaster* strains. Q-RT-PCR results exploring RNA isolated from transgenic strains transformed with constructs containing *Hsp70* genes under control of *S. singularior* or *D. melanogaster* promoters. Fold change was determined relative to the control points. *HS* heat shock. It is clearly seen that *D. melanogaster Hsp70* promoter is 100-fold more efficient, than *S. singularior* promoter

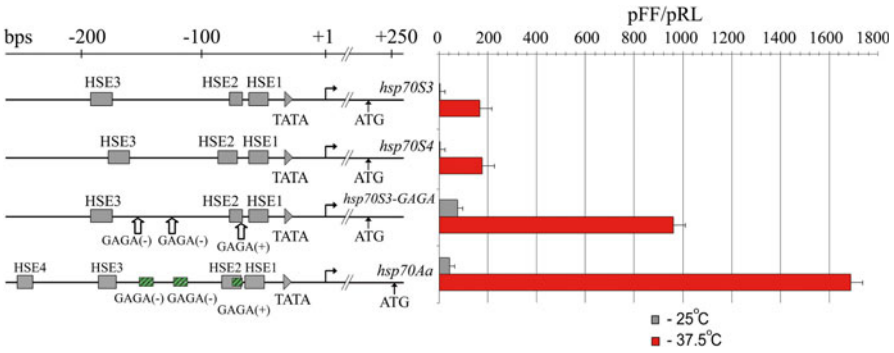

Fig. 7.5 *Left*: detailed structure of constructs used in luciferase assays. All regulatory elements within *D. melanogaster Hsp70* and *S. singularior Hsp70S3* and *Hsp70S4* promoters are shown. GAGA sites in *D. melanogaster* promoter are marked by small green boxes. Transcription start site is marked by a *bended arrow*. *ATG* – start codon of the luciferase ORF. Experimentally inserted GAGA elements in the *S. singularior Hsp70S3* promoter are indicated by open vertical arrows below the sequence of the construct. *Right*: luminescence levels as the ratio between *Firefly* (pFF) and *Renilla* (pRL) luciferase luminescence

We developed constructs with the ORF of the luciferase gene under the control of various *Hsp70* promoters (Fig. 7.5). In the construct designated *Hsp70S3*, the luciferase ORF was placed under the control of the *Hsp70S3* gene promoter used in the above experiments to obtain transgenic strains. We used a promoter of another *Stratiomys Hsp70* gene (*Hsp70S4*) to check whether the low efficiency observed in *D. melanogaster* cells was due to a specific structure of the *Hsp70S3* gene or if it represents a characteristic feature of all *Stratiomys Hsp70* genes. Our analysis indicates that promoters of *Hsp70S3* and *Hsp70S4* genes exhibited similar "strength" when tested in cell-culture based luciferase reporter system. The construct with a

promoter from the *D. melanogaster Hsp70Aa* gene served as a positive control. Except for the upstream regulatory 5'-region, all constructs contained the 5'-UTRs necessary for normal translation under HS conditions (Fig. 7.5).

The experiments demonstrated that the *D. melanogaster Hsp70A*a promoter exhibits at least a ten-fold higher efficiency, both in steady-state conditions and after HS (37.5 °C) in comparison with *Hsp70S3* and *Hsp70S4* promoters in the luciferase reporter system (Fig. 7.5).

Although HSE sequences differ slightly in *D. melanogaster* and *S. singularior Hsp70* promoters *D. melanogaster* HSF efficiently recognizes them in both species as was demonstrated by *in vitro* experiments. Therefore, the observed drastic differences in the strength of *Drosophila* and *Stratiomys Hsp70* promoters in *D. melanogaster* cells are independent of the differences in the number and/or structure of HSEs, but rather associated with the presence of other recognition sites. It is well known that the presence of HSEs is necessary but not sufficient for high efficacy of *Hsp* promoters after HS and other factors should be involved. Thus, in the case of *Drosophila Hsp70* and small *Hsps* genes the presence of GAGA elements necessary for binding of GAGA-binding factor (GAF) is prerequisite for rapid and efficient induction of protein synthesis. The position and number of GAGA elements is also very conserved in *Drosophila* species. It was shown that experimental deletion of GAGA elements leads to dramatic decrease of *D. melanogaster Hsp70* expression after HS (Georgel 2005). Functional GAGA element in *Drosophila* usually looks like GAGAG or GAGAGAG or consists of several trinucleotides units (GAG), separated by spacers comprised by uneven number of nucleotides (one, three or five). In some cases the changes in the GAG units are admitted (Georgel 2005; Omelina et al. 2011). In contrast to *Drosophila* species promoters of *Hsp70* in *S. singularior* and *O. pardalina* contain only single trinucleotides GAG (or CTC). Although there are experimental evidences that such units may also bind GAF with low efficiency (Wilkins and Lis 1998), EMSA analysis performed in our laboratory failed to reveal significant binding of *Drosophila* recombinant GAF with *Stratiomys* promoters sequences containing GAG *in vitro*. We cannot exclude, however, that endogenous Stratiomyidae GAF is able to bind with such short units more efficiently.

In order to investigate the role of GAGA elements in the efficiency of *Stratiomys* promoters in *D. melanogaster* S2 cells we developed constructs containing one or several consensus GAGA elements in different orientation in *S. singularior* promoters (Fig. 7.5). These experiments exploring luciferase reporter system demonstrated that insertion of three GAGA elements into *Hsp70S3* promoters resulted in the pronounced increase (five to sixfold) in the level of reporter gene expression which is close to the efficiency of endogenous *D. melanogaster Hsp70* promoters (Fig. 7.5). Therefore, it is evident that the absence of functional GAGA sites in *Stratiomys Hsp70* regulatory regions represents the key factor responsible for dramatic differences in *Hsp70* promoters strength of the two species in *D. melanogaster* cells. It is noteworthy, that *Hsp70* genes in both species (*S. singularior* and *D. melanogaster*) in spite of the drastic differences in their constitutive expression are strongly induced by temperature elevation (Garbuz et al. 2008; Garbuz et al. 2011). Therefore, it is not clear what additional transcriptional factors similarly to GAF in *Drosophila* are

involved in the regulation of *Hsp70* genes in thermotolerant Stratiomyidae species. Importantly, in other Diptera species e.g. *Ceratitis capitata*, just like in the case of *S. singularior Hsp70* genes promoters do not contain GAGA elements (Kalosaka et al. 2006). Furthermore, in other Diptera species such *Liriomyza sativae* and *L. huidobrensis*, there is only one complete GAGA element located at −135 position in relation to transcription start site (Chen et al. 2011). However, it was shown that efficient function of *Hsp70* promoter in *Drosophila* cells requires the presence of at least three full-size GAGA elements within the first 150 bps of regulatory region, with the first proximal GAGA element in direct orientation and two more distal elements in inverse orientation (Georgel 2005). Interestingly, even in *D. melanogaster* functional GAGA elements were detected in the promoters of small *Hsp* genes and *Hsp70* copies while the induction of *Hsp83* and *Hsp68* genes apparently does not require the presence of these motifs and these loci are induced by HS and produce large puffs. The search of various databases using MEME and MatInspector gene analisis programs failed to identify any motifs for binding of known transcriptional factors besides HSEs and TATA-boxes in *S. singularior Hsp70* promoters. The demonstrated involvement of GAF in heat-induced induction of *sHsps* and *Hsp70* genes is probably unique for *Drosophila* and other Diptera species evolved other mechanisms for *Hsp70* genes induction. It is also possible that the induction of *Hsp70* genes in other Diptera species may require long distance interactions with not yet identified sequences similar to scs/scs' elements described in *D. melanogaster* (Hart et al. 1997; Petesch and Lis 2008).

Species-specific features in heat shock response mechanisms seem to be characteristic for *Hsp70* family. Thus, when we compared the expression of constructs containing luciferase reporter gene under *S. singularior* or *D. melanogaster Hsp83* promoters in S2 cells we observed even higher expression of constructs with *Stratiomys Hsp83* promoter both in normal conditions and after HS (Fig. 7.6). *Hsp83* gene in the genome of most Diptera species is represented by only one or two copies that are involved in the regulation of multiple vital signaling pathways both

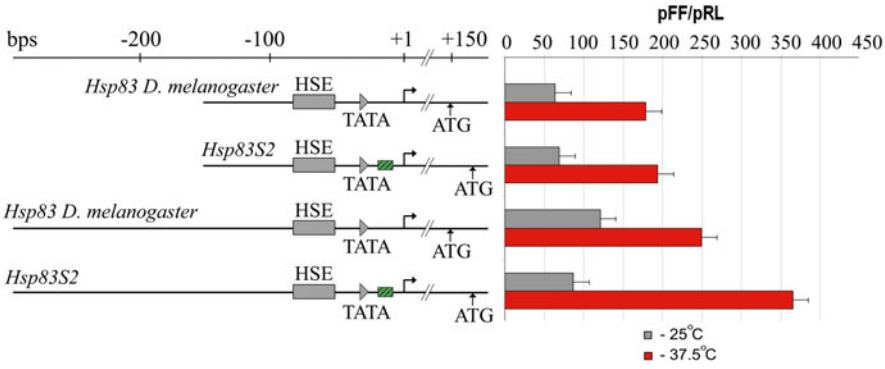

Fig. 7.6 Different efficiency of *S. singularior* and *D. melanogaster Hsp83* promoters in S2 cells. Transcription start site is marked by a *bended arrow*. Putative GAGA site in *Hsp83S2* promoter is marked as *green box*. *ATG* – start codon of the luciferase ORF

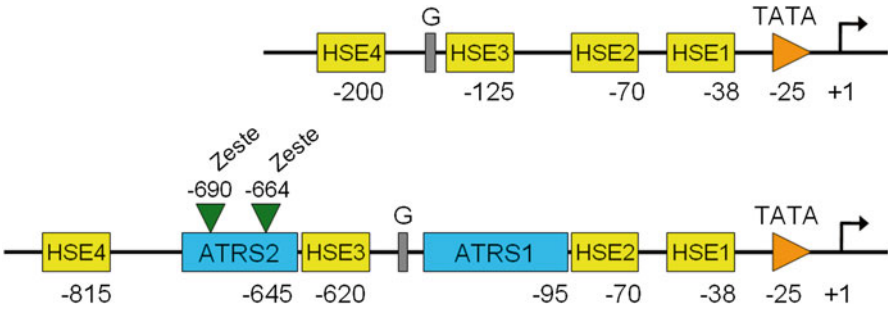

Fig. 7.7 The structure of *Hsp70* promoters in two close related species of flies – *Liriomyza huidobrensis* (*top* panel) и *Liriomyza sativae* (*bottom panel*). *G* – putative GAGA box (From Chen et al. 2011)

under normal conditions and after stress (see Chap. 3) which probably requires high conservatism of *Hsp90* genes including regulatory regions.

Besides GAGA elements described in a few *Drosophila Hsp* genes other regulatory elements were detected in the promoters of *Hsps* genes of other Diptera species that may be involved in modulation of environmental stress response.

Multiple studies have shown that GAGA elements are not the only sequences that may play an important role in *Hsp70* gene induction by HS. In fact, *Hsp70* promoters of the leaf-miner fly *Liriomysa sativa* harbour AT-rich sequence elements (ATRS) that are absent in the congeneric species.

Thus, when comparing the *Hsp70* regulatory regions of two related leaf-miner fly species *Liriomyza huidobrensis* and *Liriomyza sativae*, it was shown that the latter species is characterized by the presence of two AT-rich blocks designated "ATRS1" and "ATRS2". Apart from this difference the species exhibited highly conserved localization of HSEs in their promoters (Fig. 7.7). Intriguingly, the authors detected sites for the binding of *Zeste* transcription factor within these ATRSs, which may play a role of transcription activator in this particular Diptera species, somehow enhancing the strength of the *Hsp70* promoter (Chen et al. 2011). It was shown that this protein plays an important role in the transcription activation in *Drosophila* (Kostyuchenko et al. 2009). Furthermore, the deletion of the whole ATRS2 or *Zeste* recognition sites significantly decreased the *Liriomyza sativae Hsp70* promoter strength in luciferase reporter system while cotransfection of the construct with Zeste overexpression vector enhanced the promoter efficiency.

It can be concluded, that in the case of *Hsp70* promoters HSEs represent highly conserved basic elements, described in all Diptera species studied so far. However, mechanisms underlying fine regulation of *Hsp70* in response to stress are highly variable and usually in addition to HSF, require the participation of several other factors, providing chromatin modifications and other changes in transcription machinery after stress. Genome-wide studies have shown that evolution of regulatory regions of *Hsp* genes in relevance to HSE sequences mostly included the duplications of the GAGA motifs and single nucleotide substitutions within HSEs most of which as we showed above for *D. melanogaster* and *S. singularior* promoters did

not significantly affect their binding activity. On the other hand, taxon divergent evolution may involve dramatic changes within *Hsp* genes promoters that do not necessary involve HSF but may depend on other transcriptional factors.

In Chap. 5 we consider the major trends in the evolution of *Hsp70* genes in flies (Diptera) and mammalian species. Generally speaking, high conservatism demonstrated for genes comprising *HSPA1* cluster is also evident when regulatory regions of these three genes are aligned. We carried out comparative analysis of regulatory regions of *HSPA1A*, *HSPA1B* and *HSPA1L* genes in eight unrelated mammalian species belonging to different families and demonstrated practically identical pattern and localization of major regulatory elements in all species studied (Garbuz et al. 2011).

The demonstrated interspecific differences were represented by nucleotide substitutions and a few deletions. Previously we demonstrated that HS-induced expression of *HSP70* genes in Arabian camel *Camelus dromedarius* characteristically differ from that in human cells (Ulmasov et al. 1993) and, hence, it was of significant interest to compare the regulatory regions of camel's *HSP70* with orthologous sequences of other mammalian species. It is well known that camel is highly adapted to desert conditions and one may expect to find some specific features in the structure of its heat shock genes and in particular in their regulatory regions. The results of sequence comparison of camel's *HSPA1* cluster with the orthologous genes from other mammals are depicted in Fig. 7.8.

At the next step we developed several constructs where a reporter gene (firefly luciferase) was placed under the control of promoters of different origin. Thus we used promoters of all three genes comprising *HSPA1* cluster in humans and in camel and investigated the expression of such constructs in human culture cells

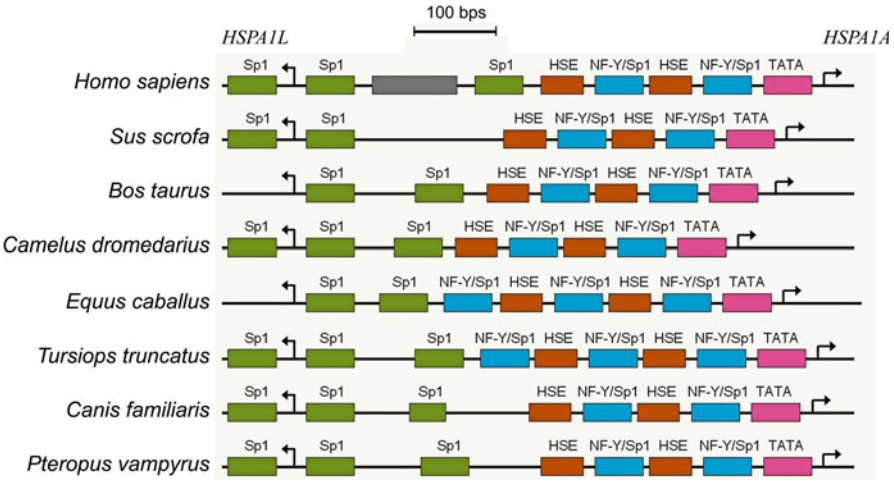

Fig. 7.8 The arrangement of *HSPA1L* и *HSPA1A* regulatory regions in different mammalian species. *Grey rectangles* represent a sequence with unknown functions which is present only in primates

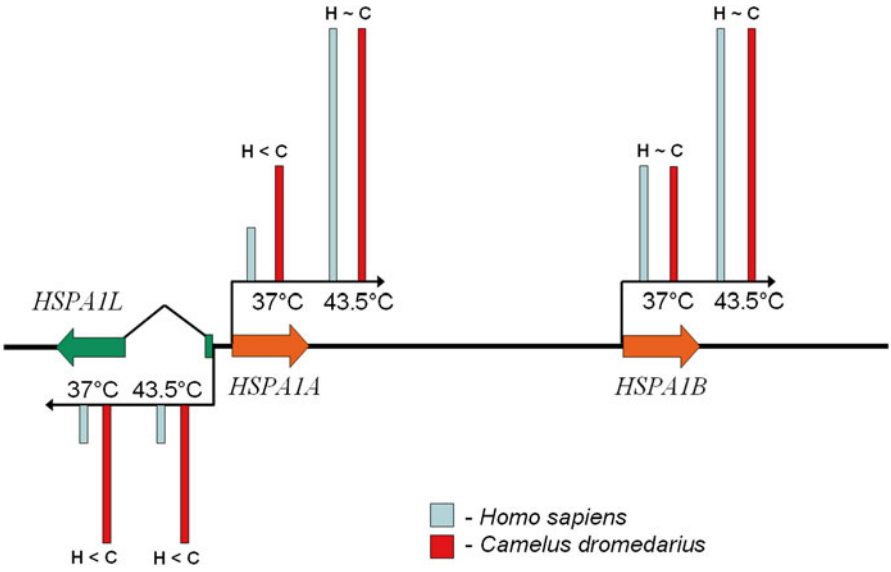

Fig. 7.9 The comparison of expression levels of camel and human genes *HSPA1A*, *HSPA1B* and *HSPA1L* at different temperature regimes. *H Homo sapiens*, *C Camelus dromedarius*

HEK293. In spite of the fact that in general the architecture of human and camel *HSP70* promoters was rather similar we demonstrated significant differences in the activity of *HSPA1A* and *HSPA1L* regulatory regions studied. Characteristically, under normal physiological conditions the activity of camel's *HSPA1A* promoter significantly exceeded (two to three times) the activity of orthologous human promoter (Fig. 7.9). However, after temperature elevation both promoters were equally efficient. Furthermore, in the case of *HSPA1L* gene the camel's promoter exhibited seven to eightfold higher efficiency that the orthologous human sequence, both under normal conditions and after heat shock (Fig. 7.9). At the present time we cannot determine with confidence the exact sequence responsible for the observed differences. In principle, the presence of ~100 bps sequence characteristic for only primates *HSP70* genes promoters (Fig. 7.8) may explain the observed differences if this sequence serves as transcription attenuator.

Actually, several lines of evidence indicate that evolution of mechanisms underlying the regulation of stress response may also act at the level of translation. Thus, soon after the discovery of heat shock response it was demonstrated that in *Xenopus* oocytes translation of *D. melanogaster Hsp70* mRNA is strongly inhibited by HS while endogenous mRNAs are efficiently translated (Bienz and Pelham 1982). On the other hand, it was shown that in *D. melanogaster* cells translation of *Hsp70* mRNAs is strongly activated by HS (Hernández et al. 2004). Therefore, it is thought that sophisticated regulatory mechanisms exist in different organisms operating at the translation level.

Along these lines, when camel *HSPA1* cluster was investigated we detected the presence of upstream silenced start (AUG) codon in the 5′-UTR of correspondent mRNA with stop codone (UAG) following right after it. Moreover, this additional start codon was found in the context not optimal for effective translation initiation (Garbuz et al. 2011). As a rule such codons are missed in the process of scanning 5′-UTR by 40S ribosomal subunit, but occasionally may be involved in translation initiation exploring non-canonical mechanisms (Kozak 1987; Sheikh and Fornace 1999). In the bull *Bos taurus* we also detected upstream AUG in a few *HSPA1A* alleles which is included into a short upstream ORF before *HSP70* coding ORF (Garbuz et al. 2011). At the present time the functional significance of such additional start codons is not clear, because as we demonstrated its substitution in the camel's promoter did not affect the translation of correspondent construct mRNA in the HEK293 cells either under normal conditions or after HS. Possibly, the revealed upstream start codons are involved in tissue-specific translation regulation or just represent the result of random mutations that predated the divergence of these species (*Camelus dromedarius* and *Bos taurus*) and do not affect the *HSPA1A* mRNA translation efficiency.

7.1 Conclusions

In contrast to high conservatism of heat shock genes coding sequences and heat shock response system as a whole, promoters of many *Hsp* genes may exhibit a high degree of variability in different organisms, even including members of phylogenetically close forms. Besides highly conservative heat shock elements (HSEs) present in heat shock promoters of all organisms, in certain species the *Hsp* promoters may contain specific regulatory elements (motifs) which are recognized by special regulatory factors restricted to the particular species or species group. This variability in terms of *Hsp* promoters structure and function may play an important role in the fine tuning of Hsps expression in response to rapidly changing environmental conditions.

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