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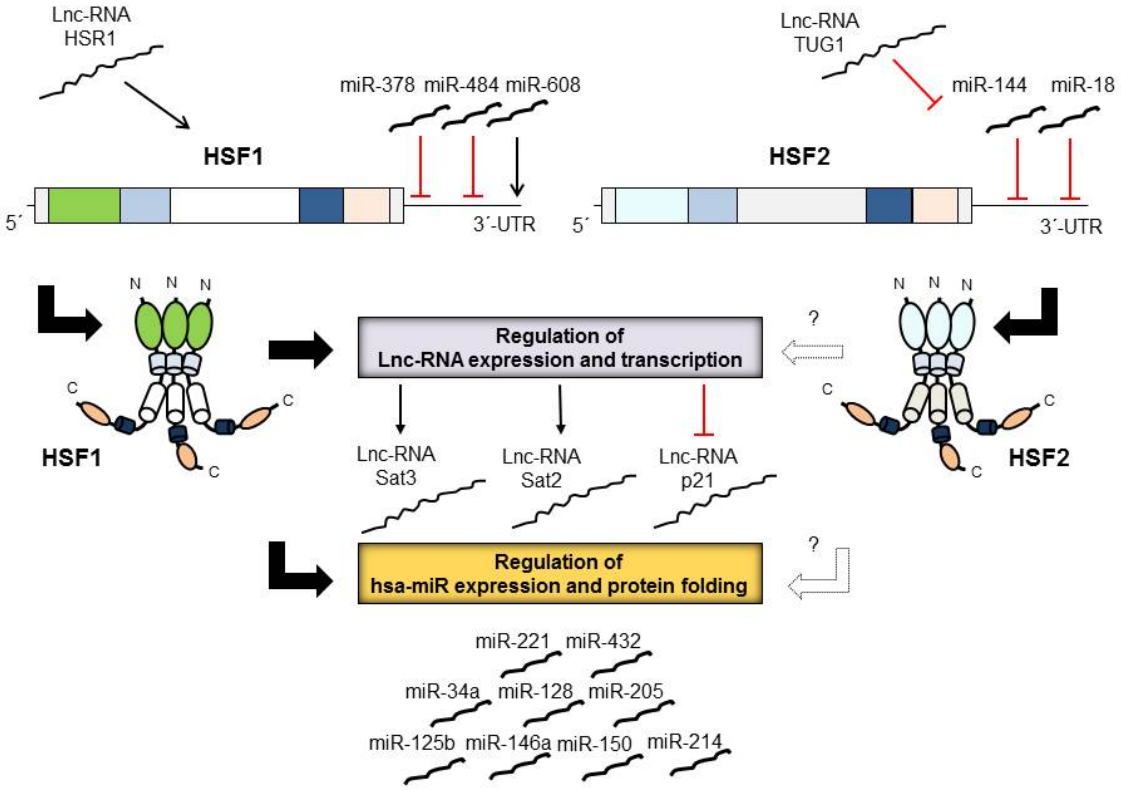
Supplementary information S1: MicroRNAs and long non-coding RNAs in the regulation of HSFs



Non-coding RNAs (ncRNAs) have been shown to be actively involved in the regulation of HSF1 and the heat shock stress response [1]. For example, different microRNAs (miRNAs) can bind in the 3´untranslated regions (3'-UTRs) of HSF1 and HSF2 regulating their expression under different conditions [2 3 4 5 6] (see Figure). Other key regulators of HSFs in human cells are the long non-coding RNAs (lncRNA). The lncRNA HSR1 (heat shock RNA-1), is up-regulated during the HSR and plays an essential role in HSF1 trimerization and subsequent DNA binding activity[7,8]. HSF2 is indirectly regulated by the lncRNA TUG1 (taurine upregulated gene 1), which is highly expressed in endothelial cells from glioma tissue and is associated with regulating

blood-tumor barrier permeability [6]. The activation of HSF1 also regulates the expression of different ncRNAs involved in global suppression of transcription, translational processes and protein aggregation [9]. Upon heat shock, HSF1 induces the expression of a class of lncRNAs known as Satellite III transcripts (Sat3) that accumulate at the site of transcription to form nuclear stress bodies (nSBs) [10-12] and are known to co-localize with several RNA binding proteins and transcription factors such as HSF1 [11,13,14]. Although knockdown of Sat3 transcripts does not affect HSF1 recruitment to the nSB-like structures, Sat3 transcripts are essential for recruitment of other transcriptional regulators to the nSBs contributing to the heat-induced transcriptional silencing [15]. Transcription of the lncRNA satellite 2 (Sat2) is also strongly up-regulated in the presence of heat shock in a HSF1 dependent manner [16] and is involved in tumor progression [16]. HSF1 also binds HSE present upstream of different human miRNA (hsa-miR) genes and activates their expression under thermal stress [17]. However, whether HSF2 plays a role in regulating these non-coding RNAs is currently unknown. Interestingly, some HSF1 regulated hsa-miRs have inhibitory effects on huntingtin protein and are significantly depleted in Huntington's disease [18] perhaps due to HSF1 depletion [19]. These studies demonstrate an integrated model of ncRNAs and HSF activity.

References

1 Place, R. F. & Noonan, E. J. Non-coding RNAs turn up the heat: an emerging layer of novel regulators in the mammalian heat shock response. *Cell Stress Chaperones* **19**, 159-172, doi:10.1007/s12192-013-0456-5 (2014).

2 Jie Yuan , H. M., Hui Gong , Ning Zhou , Yanyan Liang , Yuhong Niu , Yunzeng Zou. in *Congress of Cardiology* (Beijing, 2010).

3 Huang, A. J., Yu, K. D., Li, J., Fan, L. & Shao, Z. M. Polymorphism rs4919510:C>G in mature sequence of human microRNA-608 contributes to the risk of HER2-positive breast cancer but not other subtypes. *PLoS One* **7**, e35252, doi:10.1371/journal.pone.0035252 (2012).

4 Li, Q. L. *et al.* A SNP in the 3'-UTR of HSF1 in dairy cattle affects binding of target bta-miR-484. *Genet Mol Res* **14**, 12746-12755, doi:10.4238/2015.October.19.18 (2015).

5 Björk, J. K., Sandqvist, A., Elsing, A. N., Kotaja, N. & Sistonen, L. miR-18, a member of Oncomir-1, targets heat shock transcription factor 2 in spermatogenesis. *Development* **137**, 3177-3184, doi:10.1242/dev.050955 (2010).

6 Cai, H. *et al.* The long noncoding RNA TUG1 regulates blood-tumor barrier permeability by targeting miR-144. *Oncotarget* **6**, 19759-19779, doi:10.18632/oncotarget.4331 (2015).

7 Shamovsky, I., Ivannikov, M., Kandel, E. S., Gershon, D. & Nudler, E. RNA-mediated response to heat shock in mammalian cells. *Nature* **440**, 556-560, doi:10.1038/nature04518 (2006).

8 Shamovsky, I. & Nudler, E. Isolation and characterization of the heat shock RNA 1. *Methods Mol Biol* **540**, 265-279, doi:10.1007/978-1-59745-558-9_19 (2009).

9 Lindquist, S. The heat-shock response. *Annu Rev Biochem* **55**, 1151-1191, doi:10.1146/annurev.bi.55.070186.005443 (1986).

10 Rizzi, N. *et al.* Transcriptional activation of a constitutive heterochromatic domain of the human genome in response to heat shock. *Mol Biol Cell* **15**, 543-551, doi:10.1091/mbc.E03-07-0487 (2004).

11 Jolly, C. *et al.* Stress-induced transcription of satellite III repeats. *J Cell Biol* **164**, 25-33, doi:10.1083/jcb.200306104 (2004).

12 Sengupta, S., Parihar, R. & Ganesh, S. Satellite III non-coding RNAs show distinct and stress-specific patterns of induction. *Biochem Biophys Res Commun* **382**, 102-107, doi:10.1016/j.bbrc.2009.02.137 (2009).

13 Cotto, J., Fox, S. & Morimoto, R. HSF1 granules: a novel stress-induced nuclear compartment of human cells. *J Cell Sci* **110 (Pt 23)**, 2925-2934 (1997).

14 Metz, A., Soret, J., Vourc'h, C., Tazi, J. & Jolly, C. A key role for stress-induced satellite III transcripts in the relocalization of splicing factors into nuclear stress granules. *J Cell Sci* **117**, 4551-4558, doi:10.1242/jcs.01329 (2004).

15 Goenka, A. *et al.* Human satellite-III non-coding RNAs modulate heat-shock-induced transcriptional repression. *J Cell Sci* **129**, 3541-3552, doi:10.1242/jcs.189803 (2016).

16 Tilman, G. *et al.* Cancer-linked satellite 2 DNA hypomethylation does not regulate Sat2 non-coding RNA expression and is initiated by heat shock pathway activation. *Epigenetics* **7**, 903-913, doi:10.4161/epi.21107 (2012).

17 *Srijit Das, N. P. B.* Widening HSF1 horizon: the microRNA connectionWidening HSF1 horizon: the microRNA connection. *RNA & DISEASE* **2**, doi:10.14800/rd.596 (2015).

18 Das, S. & Bhattacharyya, N. P. Heat Shock Factor 1-Regulated miRNAs Can Target Huntingtin and Suppress Aggregates of Mutant Huntingtin. *Microrna* **4**, 185-193 (2015).

19 Gomez-Pastor, R. *et al.* Abnormal degradation of the neuronal stress-protective transcription factor HSF1 in Huntington's disease. *Nat Commun* **8**, 14405, doi:10.1038/ncomms14405 (2017).