

HSP90 : more than just a heat shock response

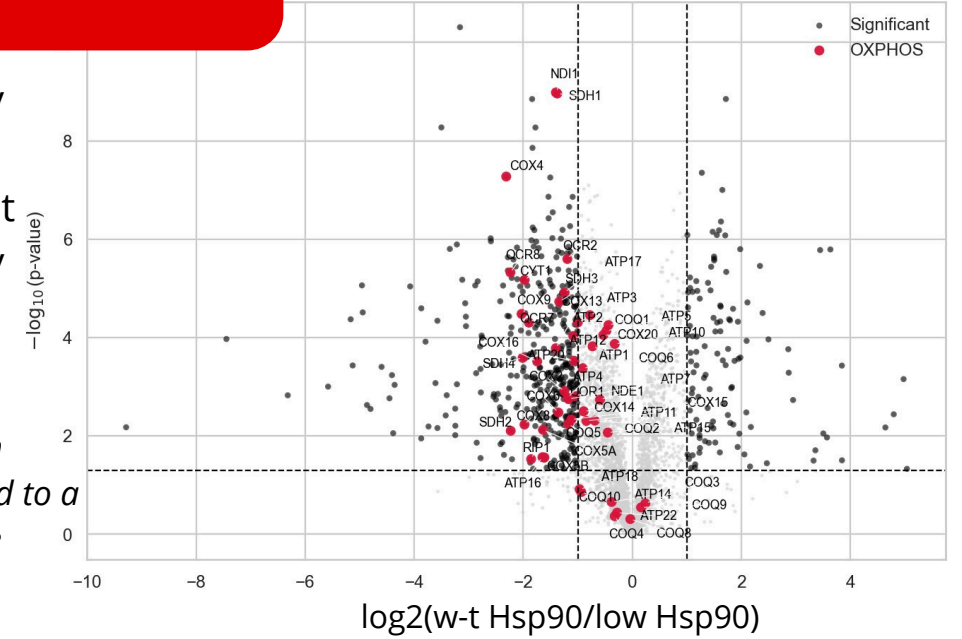
Background

HSP90 is a molecular chaperone highly conserved among eukaryotes and is one of the most abundant proteins in our cells^{1,2}. Beyond heat shock resistance Hsp90 is implicated in controlling folding, stability and degradation of client proteins³. Not surprisingly, it is a major target of novel cancer therapies, but its actual interactions and its role in cell physiology are not fully understood^{4,5}. **This study addresses a surprising role of Hsp90 in controlling expression levels of mitochondrial OXPHOS proteins.**

Hsp90 regulates OXPHOS

Budding yeast cells with constitutively low (~7%) levels of HSP90 expression are temperature sensitive. However at normal temperatures they specifically overexpress mitochondrial OXPHOS proteins.

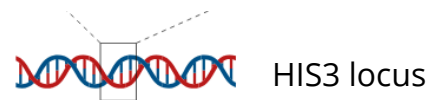
Figure 1: Mass spectrometry analysis of protein abundances in low Hsp90 yeast strain compared to a w-t strain. Note that OXPHOS proteins (red) are overexpressed.



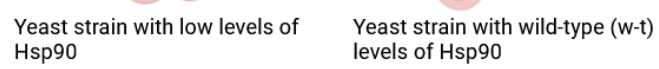
Does Hsp90 regulate transcription of OXPHOS genes?

Transcriptional effects of Hsp90 were probed using promoters of OXPHOS genes highly overexpressed in the Hsp90 deprived cells (Figure 1) driving expression of a fluorescent protein mScarlet from HIS3 genomic locus.

promoter of a gene of interest is fused with mScarlet ORF and this construct is integrated in the HIS3 locus in yeast chromosome



yeast clones expressing a single copy of the reporter construct are selected based on mScarlet fluorescence levels



Fluorescence signal reflects the number of mScarlet gene transcripts in a cell: more transcripts → more mScarlet molecules produced → higher fluorescence

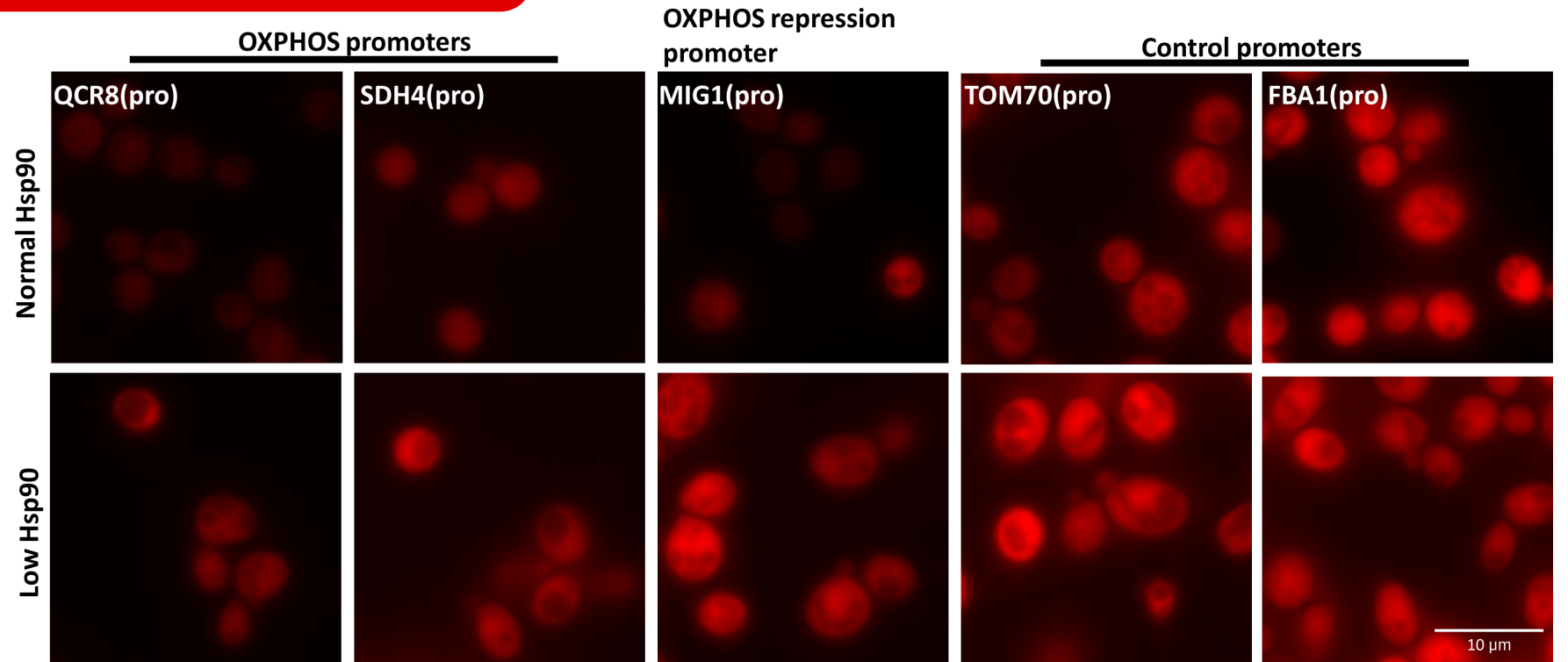


Figure 2: Expression levels conferred by the OXPHOS (QCR8, SDH4) and non-OXPHOS (TOM70, FBA1, MIG1) gene promoters in cells with normal and reduced content of Hsp90. Yeast cells with w-t (Normal Hsp90) or reduced (Low Hsp90) levels of Hsp90 were modified to express a single copy of mScarlet gene driven by the respective promoters and analysed side-by-side by fluorescence microscopy.

Quantification by flow cytometry:

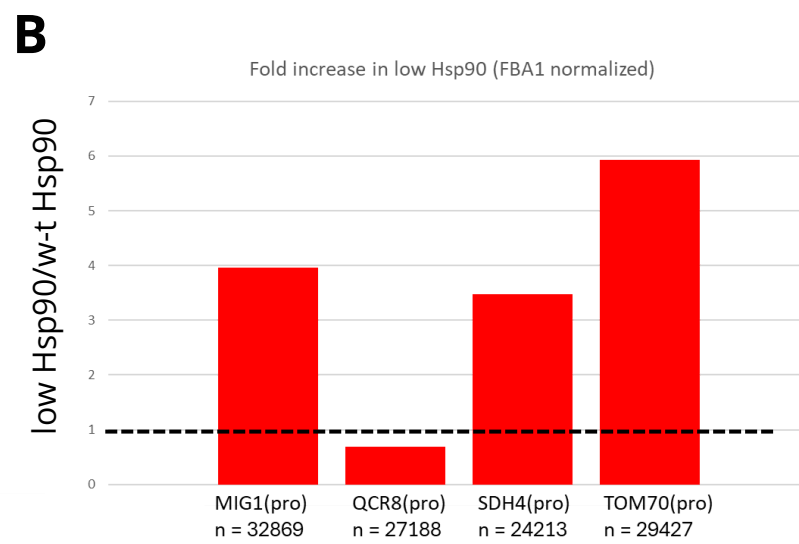
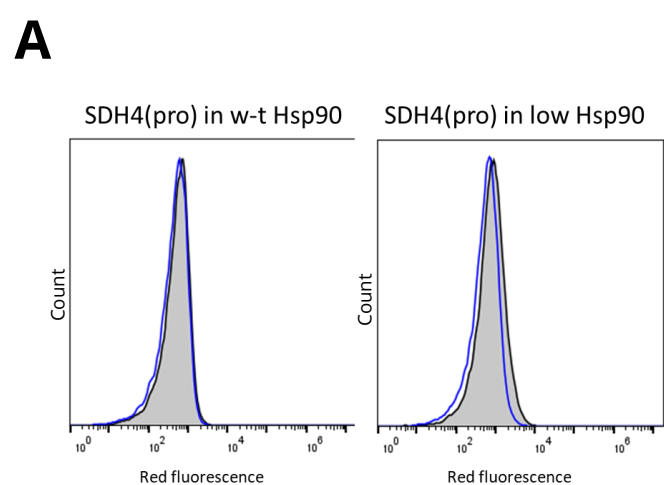


Figure 3: Effects of Hsp90 on the reporter expression levels quantified by flow cytometry. (A) Exemplary flow cytometry readout. Reporter signal is quantified by subtracting background fluorescence of cells without reporter (non-tinted) from fluorescence of the reporter-expressing cells (tinted). (B) Fold difference in the median reporter signal in cells with low vs. w-t levels of Hsp90. The signal is normalized for the control FBA1(pro) reporter.

Quantification by qPCR:

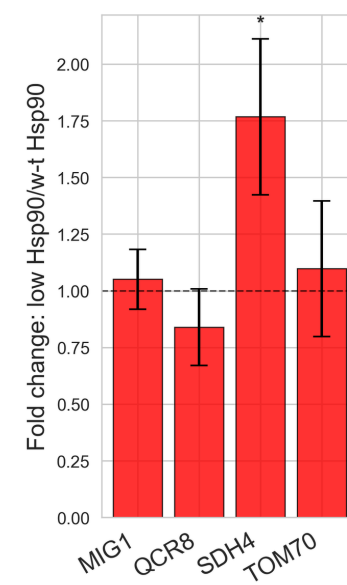


Figure 4: Effects of Hsp90 on the native mRNA expression levels. Fold differences in the native mRNA levels for the genes and conditions described in Figure 3 were determined by RT-qPCR and normalised for the FBA1 control. Error bars: SD (N= 4 biological replicates). * - p < 0.05.

Table 1: Summary of Hsp90 effects on gene expression based on three different assays.

fold-change	Protein levels (MS):	Reporter levels (FC):	mRNA level (RT-qPCR):
MIG1	~32-fold	~ 4-fold	no change
SDH4	~ 4-fold	~3.5-fold	~2-fold
QCR8	~ 4-fold	~ 0.6-fold	~0.8-fold
TOM70	no change	~ 5-fold	no change

Analysis of Hsp90 effects on the OXPHOS gene expression shows discrepancies between fluorescent reporter-based, mass-spectrometry-based and qPCR-based readouts.

Conclusions:

Control of the OXPHOS gene expression by Hsp90 may extend beyond transcriptional regulation.

- SDH4 overexpression can be well explained by the transcriptional up-regulation of the SDH4 gene in Hsp90-deprived cells.
- Absence of transcriptional up-regulation contrasts with the elevated levels QCR8 protein in Hsp90-deprived cells and hints at possible protein stabilisation effects of Hsp90.

Outstanding questions and future perspectives:

- Why are the effects of Hsp90 on the OXPHOS gene expression seemingly more pronounced at the protein rather than the transcript level?
 - Analysing protein stability using live stability reporters may shed light on this discrepancy.
- What is the origin of discrepancy between the fluorescent reporter-based and RT-qPCR-based readouts of gene expression effects observed for some genes?
 - More data are needed to verify the copy number of the reporter genes, for example, by using qPCR analysis of genomic DNA.

References:

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