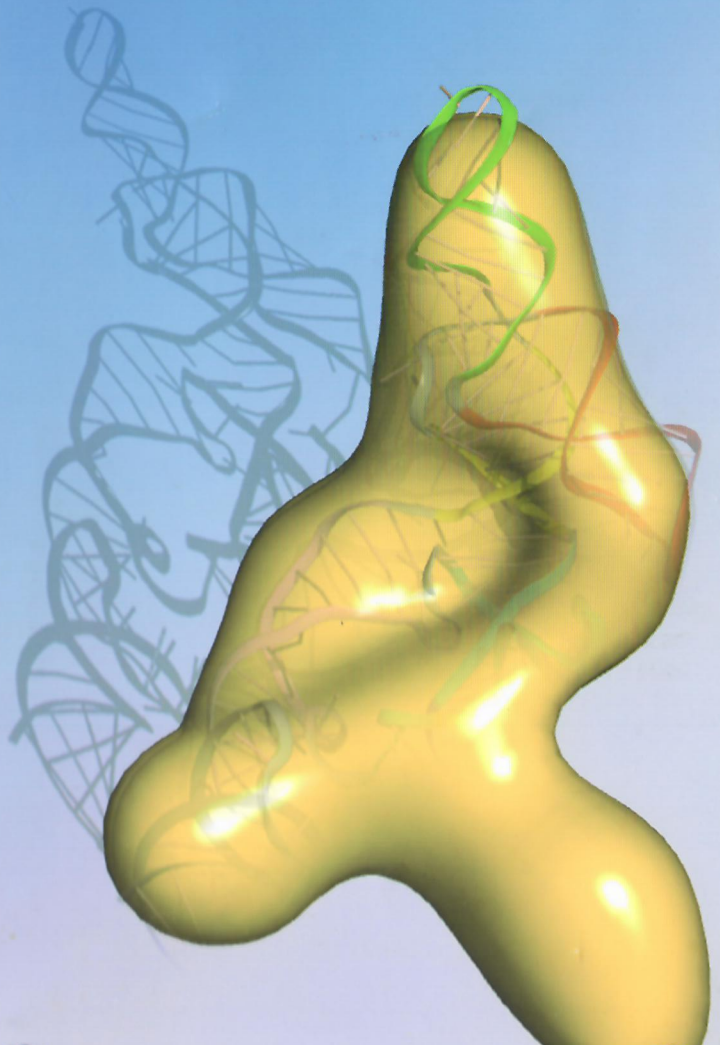


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HSP104 MEDIATED POSTTRANSCRIPTIONAL REGULATION OF GENE EXPRESSION

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Heat shock proteins are ubiquitous and very ancient proteins with critical functions in protecting cellular integrity upon proteotoxic insult. Traditionally, heat-shock proteins also known as molecular chaperones are believed to be involved in protein folding, trafficking and rescuing of improperly folded polypeptides. But it has recently become evident that many molecular chaperones establish molecular interactions with RNA and some of them were found to be involved in the translation regulation. We presume that upon stress conditions and very low basal transcriptional level the chaperones mediate precise regulation of particular mRNAs translation increasing amount of stress coping proteins.
We have created reporter system for analysis of yeast *Saccharomyces cerevisiae* shaperone Hsp104 mediated posttranscriptional regulation of gene expression under control of *CUP1* promoter. In experiments with regular Hsp104 production, its overproduction and absence of Hsp104 we have found that the chaperone positively regulates reporter gene expression controlled by the *CUP1* promoter without any change in the transcription rate of the corresponding mRNA. Appling transcription inhibition assay with Actinomycin D we have found that Hsp104 is not involved in regulation of *CUP1* mRNA stability. Using system for visualization of particular mRNA in living cells we have shown that Hsp104 level increase does not change the dynamic of *CUP1* mRNA nucleocytoplasmic trafficking. Gel-shift experiments clearly demonstrate strong binding of the Hsp104 with single strand *CUP1* mRNA leader sequence containing tandem repeat $(CAAU)_4$. Similar results were reported for Hsp101, plant ortholog of yeast chaperone Hsp104, which binds degenerate tandem repeat $(CAA)_n$ in the 5'-UTR of the tobacco mosaic virus mRNA and positively regulates its translation via interaction with the eukaryotic translation initiation factor 4F (eIF4F) complex. We have found that variation in the level of Hsp104 production in tif4632Δ yeast strain with imperfect eIF4F does not affect the rate of reporter gene translation. To confirm our results we have designed new reporter system with fused *GPD* promoter bearing *CUP1* 5'-UTR sequence upstream initiation codon. Addition of this particular sequence was enough to turn the reporter gene translation into Hsp104 dependent way.
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CHROMATIN MODIFICATION BY TRIMETHYLATION OF LYSINE 36 OF HISTONE 3 IS SPLICING-DEPENDENT

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Modifications to histone tails by lysine methylation provide an important mechanism to regulate chromatin structure. Among well-characterized histone modifications, trimethylation of lysine 36 of histone 3 (H3K36me3) is preferentially found within the body of active genes, and very recently it was found that H3K36me3 chromatin marks are enriched in exons relative to introns (1-3). The mechanism underlining this new mode of communication between chromatin and splicing remains elusive. One possibility is that modifying chromatin structure through methylation facilitates splicing. Alternatively (or concomitantly), intron removal by co-transcriptional splicing may trigger histone methylation.
Here, we used chromatin immunoprecipitation (ChIP) followed by deep-sequencing to determine the genome-wide association of H3K36me3 in mouse T cells. In agreement with previous reports (1-3), we observed first, that the H3K36me3 mark is preferentially associated with exons and second, that the levels of this mark increase along the gene body in a transcription-dependent manner. However, we also found that the increase in H3K36me along the body of highly transcribed genes correlates with the exon/intron structure. Moreover, H3K36me levels are much lower in intronless genes, irrespectively of their transcriptional activity. To investigate the temporal link between transcription, splicing and the H3K36me3 mark, we performed ChIP experiments after inhibiting splicing, either by treating cells with spliceostatin A or RNAi-mediated depletion of an essential splicing factor. In the absence of splicing, the exonic levels of H3K36me3 decreased significantly, and this effect was reverted upon removal of the drug. In contrast, increased levels of this histone modification were found on CD44 alternative exons in HeLa cells treated with a phorbol ester that promotes their inclusion. Altogether, these data suggest that ongoing transcription and splicing enhance H3K36me3 modification.

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