Pase domains and ring-shaped hexameric quaternary structure. It is distinguished by long, coiled coil M domain, absent from other AAA+ chaperones involved in proteinolysis.

In our work we postulate the importance of a certain structural aspect, namely ionic interaction network, that connects two clefs of NBD1 ATPase domain with the M domain.

It has been recently established that the M domain is essential for interaction with Hsp70 partner. Our results suggest that interaction between M-domain residue D484 and NBD1 residue K358 is pivotal for functional cycle of Hsp104. Those residues form a part of ionic interaction network that involves also D484 residue at the other clef of NBD1. The biochemical properties of point mutants in position 358 and 484 suggest that the role of those interactions is to couple ATPase activity with substrate translocation through the hexamer (and hence the disaggregation of substrate proteins). Reversal of either charge in those positions lead to the significant hyperactivity of Hsp104 accompanied by decreased Hsp70 requirement and loss of specificity.

ATPase activity and ATP affinity measurements, combined with structural analysis by molecular dynamics simulations lead us to the conclusion that the NBD1-M interdomain communication via K358-D484 interaction is important for allosteric regulation for the precise control of Hsp104 unfolding activity.

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**Exposure of human bronchial epithelial cells to hexavalent chromium [Cr(VI)] decreases the expression of heat shock protein 90 alpha (Hsp90α) and attenuates the transient growth arrest induced by an acute cold shock**

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Hexavalent chromium [Cr(VI)] has long been recognized as an occupational lung carcinogen. Awareness of this and other adverse health effects associated with exposure to Cr(VI) compounds has led to improved safety practices. On the contrary, the presence of these compounds in the environment is increasing, mostly due to industrial waste disposal, fossil fuel combustion and, possibly, tobacco smoke.

Despite numerous studies, the molecular basis of Cr(VI)-induced neoplastic transformation is still very poorly understood. Cr(VI) exposure produces several types of cellular stresses, namely oxidative and metabolic stresses, with potential relevance to carcinogenesis. Our group is currently investigating the heat shock response of human bronchial epithelial cells (BEAS-2B) to Cr(VI) exposure, as well as the impact of this response in the resistance of these cells to further stresses. Thus far, we observed that a short-term (48 h) exposure to 1 μM Cr(VI) caused a decrease on the levels of heat shock protein Hsp90α. Of note, a large number of inhibitors of this protein, which plays a critical role in the maintenance of cellular protein homeostasis, is currently undergoing clinical trials for cancer therapy.

We have also verified that the transient growth arrest induced in this cell line by an acute cold shock was attenuated in the presence of 0.1–2 μM Cr(VI). This observation suggests that BEAS-2B cells exposed to Cr(VI) were somehow better prepared to respond to this type of shock. Experiments aimed at gaining mechanistic insight into this phenomenon, including determining the effect of Cr(VI) exposure on the protein levels of heat shock factor 1 (HSF1), are under way. This transcription factor is a major player in the heat shock response, and recent evidence points to its involvement in the regulation of several other cellular processes.

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**Desulfovibrio vulgaris Peroxide Regulon Repressor (PerR)**

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Reactive oxygen species (ROS) that play an important role in the mechanisms of cell signaling and oxidations in higher organisms, are simultaneously very citotoxic. In fact they are generated by macrophages, as a weapon to eliminate pathogens. As defence cells produce protective and damage repair proteins, which are under the negative control of specific regulators. They sense the increase of ROS and transduce the signal by increasing expres- sion of defense activities. In Bacillus subtilis the adaptive response to H2O2 seems to be under the influence of a metallo-protein called Peroxide Regulon Repressor (PerR), which is a member of the Ferric Uptake Repressor (Fur) superfamily. Like other members of Fur family, PerR is a homodimer that binds two metal ions per subunit: one zinc ion, bound by four cisteines in a site that has a structural function, and one Fe or Mn ion that is bound by three histidines and two aspartates in a regulatory site, necessary for H2O2 sensing. This function involves the oxidation of two of the Fe/Mn ligands: histidine 37 and histidine 91, but only when Fe(II) is bound to the site (Fe-PerR-Zn) [1]. Histidine oxidation seems to cause the loss of the corepressor Fe (II) and inactivation of PerR to bind DNA. This results in dere- pression of the genes that are under PerR influence [3].

Although long considered an obligate anaerobe, D. vulgaris can be found in some O2 exposed environments, and even though they can survive long periods of air exposure, very small amounts of O2 can affect D. vulgaris growth negatively [2]. Furthermore, studies have revealed that the transcripts of the predicted PerR regulon, in this bacterium, were upregulated during low O2 and H2O2 exposure.

A recombinant Desulfovibrio vulgaris PerR was in E.coli, and DNA binding activity tested in the presence of Fe(II) and Zn(II).


**References**