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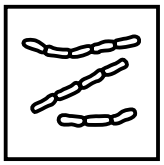

Conference Review

The heat shock response of *Mycobacterium tuberculosis*: linking gene expression, immunology and pathogenesis

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Abstract

The regulation of heat shock protein (HSP) expression is critically important to pathogens such as *Mycobacterium tuberculosis* and dysregulation of the heat shock response results in increased immune recognition of the bacterium and reduced survival during chronic infection. In this study we use a whole genome spotted microarray to characterize the heat shock response of *M. tuberculosis*. We also begin a dissection of this important stress response by generating deletion mutants that lack specific transcriptional regulators and examining their transcriptional profiles under different stresses. Understanding the stimuli and mechanisms that govern heat shock in mycobacteria will allow us to relate observed *in vivo* expression patterns of HSPs to particular stresses and physiological conditions. The mechanisms controlling HSP expression also make attractive drug targets as part of a strategy designed to enhance immune recognition of the bacterium. Copyright © 2002 John Wiley & Sons, Ltd.

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Background

The classical heat shock proteins (HSPs) are stress-inducible molecular chaperones which represent the most conserved proteins in cellular life. In prokaryotes and eukaryotes their main role is to maintain a correctly folded and assembled protein component of the cell [14]. The essentiality of this function is reflected in the ubiquity of these proteins throughout cellular organisms. Indeed, it is the ancient nature of these highly conserved molecules, combined with the utility of their peptide-binding function, that has allowed evolution to engender many HSPs with functions additional to those of simple chaperones. Of greatest importance to the pathogen biologist are the roles of HSPs in the immune response. As host molecules whose expression is induced during stress conditions, such as infection with a pathogen, they are in perfect position to act as facilitators of the immune response, and nature has made no mistake in taking advantage of circumstance. Mammalian/host HSPs act as signals to the immune system through recognition by cell-surface receptors triggering inflammatory responses [2,8,9,33]. In addition, their functional role as chaperones has been utilized such that exogenous HSP carrying peptides from damaged or infected cells can, in an exquisitely efficient pathway, be taken up by receptor-mediated endocytosis into antigen presenting cells and processed for MHC class I-restricted presentation to T cells [1,3,4,10,29]. Thus, HSPs provide an important link between the innate and acquired arms of the immune response [27].

What makes the expression of HSPs so interesting to the pathogen biologist is that pathogen HSPs are also recognized by specific receptors on host immune cells, triggering an inflammatory immune response [19,21,23,32]. In addition to this, the conservation between host and pathogen HSPs means that pathogen HSPs can be utilized by the host to shuttle peptides into the HSP-mediated antigen presentation pathway [17,30].

Thus, while the pathogen needs to increase expression of its HSPs in response to the stresses induced by host defenses [7,20,22,24], it must temper this need so as not to alert the host immune response to its presence. It is in the context of this dilemma that we are interested to learn how pathogens control the expression of their heat shock proteins. We have chosen to study *Mycobacterium tuberculosis*, an intracellular bacterial pathogen whose infection profile is critically governed by a dynamic relationship with the host immune response. In a recent study we demonstrated the vital importance of HSP regulation to *M. tuberculosis* by generating a mutant strain lacking the HspR repressor protein thus effecting dysregulation of the Hsp70 response [28]. The mutant strain constitutively overexpressed Hsp70 and associated HSPs, and during murine infection its survival was emphatically reduced. The underlying cause for this attenuation was enhanced immune recognition of the bacterium.

HSPs are so called because their expression is, in most cases, easily inducible by elevation of temperature causing denaturation of proteins. However, the expression of many is also determined by other stresses or environmental stimuli, such as oxidative stress, reactive nitrogen and micro-aerophilic conditions [13,18,26], to name several with particular importance to an intracellular pathogen. It is our aim to be able to describe under which conditions the mycobacterial HSPs are expressed and identify the molecular mechanisms behind their regulation.

Strategy

We have embarked upon an approach based on whole genome expression profiling, using DNA microarrays to characterize the wild-type transcriptional response under relevant stresses/stimuli. These responses will then be dissected into their constitutive regulatory circuits by generating specific mutants in different regulatory pathways and comparing the expression profiles of resultant strains in stressed and unstressed conditions with the wild-type. Such an approach has only been made possible by the recent development of whole genome microarrays for *M. tuberculosis* and also by the acquisition of techniques to manipulate the genetics of the bacterium.

The microarray we are using consists of 3924 PCR amplicons (size range 60–1000 bp) derived from regions of all the predicted coding sequences of the sequenced strain of *M. tuberculosis* H37Rv [11], spotted onto poly-L-lysine-coated glass slides. RNA was extracted from bacteria grown in different conditions or from different strains, and cDNA was labelled with Cy3 or Cy5 dCTP during reverse transcription and competitively hybridized to the microarray. The analysis of microarray data is of critical importance and, like the technology itself, remains a largely developing science. We utilized two methods to analyse the data comparisons (e.g. wild-type vs. mutant strain or heat-shocked vs. non-heat-shocked cells). Firstly we used an ANOVA analysis, which took into account three main effects — the array effect, the gene effect and the variety (strain or environmental condition) effect, along with the pairwise interactions between these effects. The second method utilized was significance analysis of microarrays (SAM) [31], which is based on generating pseudo-datasets and assimilating sets of gene-specific *t*-tests in order to assign each gene a score on the basis of its change in expression relative to its own standard deviation. We are fortunate that heat shock is one of the best-studied prokaryote gene expression responses, as this endowed us with at least some biological knowledge to assess the performance of these analysis methods. In the wild-type heat shock vs. non-heat shock comparison, both analysis methods were able to identify all the known heat-inducible HSP genes as upregulated. There were other genes identified as upregulated and, although ANOVA and SAM largely concurred, there were a number of differences. Further experiments will be required to finally determine which analysis best reflects the true changes in gene expression.

Heat shock proteins as part of a wider stress response

To date we have examined the transcriptional response of *M. tuberculosis* to heat shock at 45 °C

for 30 min. Of course, heat shock is a complex response which varies with both time and temperature, but this snapshot of the response provides a reference with which to compare transcriptomic changes in defined regulatory mutants. One general observation on the heat shock transcriptional profile is that the response is not simply the elevated transcription of the known HSPs but encompasses genome-wide changes in gene expression. Our first experiments to dissect this response involved gene knockout of two likely heat shock repressor proteins in *M. tuberculosis*, HspR and HrcA, which were identified by homology to regulators in *Streptomyces* and *Bacillus* [6,15]. We made mutants of both of these regulators using the pSMT100 gene replacement system [28]. Comparison of the expression profiles of strains lacking these regulators with wild-type, combined with identification of repressor binding sites in promoter regions, established the HspR and HrcA regulons. All members of these regulons were also found to be upregulated during heat shock and amongst them were many of the classical heat shock chaperones. Comparison of our results with those obtained in studies examining the regulation of other arms of the mycobacterial stress response reveals a high degree of crosstalk and overlap between the different stress regulons For example, the Hsp70 regulon forms a central element of the heat shock response and is under negative regulation by HspR in complex with Hsp70 itself [5,28]. However, the Hsp70 operon is also under control of the heat-inducible alternative sigma factor σH [12], which in addition promotes transcription at the stress response sigma factors σB and σE [25]. Further to this, the functional activity of these sigma factors is under the control of anti-sigma factor pathways [16].

Future work and conclusions

Thus, while we have identified how many of the major HSPs are regulated under heat shock, we have, in the process, revealed a greater complexity and extensiveness of the heat shock response than originally expected. Further disassembly of the response will require more extensive transcriptional profiling at different temperatures for different lengths of time and the generation of other regulatory mutants. It will also require the use of techniques to analyse post-transcriptional and post-translational control mechanisms of expression. It also remains to investigate the different types of stresses, such as reactive oxygen and nitrogen, which may stimulate HSP expression. Further identification of when during infection and in which tissues these proteins are expressed should enlighten us about what physiological stresses the bacteria are exposed to in these situations. HSPs make excellent candidates for such a study because they are extremely responsive to changes in stimuli, producing transcriptional changes of great amplitude. In addition, their qualities of high immunogenicity allow the possibility of using host immune recognition of different HSPs as a surrogate marker of HSP expression and in turn the physiological stresses imposed on the bacteria. Such knowledge could inform whether an infection was predominantly under aerobic or anaerobic conditions and would help greatly in the choice of treatment. Most importantly, we envisage that understanding the regulatory mechanisms behind mycobacterial HSP expression may allow the development of novel strategies for the treatment of tuberculosis. We have already demonstrated that dysregulation of the *M. tuberculosis* Hsp70 response allows the host to mount a more effective immunological response against the bacterium [28]. Thus, drugs that disrupt HSP regulation by interfering with specific regulators make an attractive mechanism by which to enhance host immunity.

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