CHAPTER 30

MICROBIOLOGY

Doctoral Theses

01. KHANNA (Arushi)
Characterization of Elp3a Protein in the Protozoan Parasite Leishmania Donovani.
Supervisor: Prof. Swati Shah
Th 27634

Abstract

The Elongator is a multi-subunit complex composed of Elp1-6 proteins that have been identified across all domains of life. The Elp3 protein is the main catalytic subunit of the Elp1-6 complex. In trypanosomatids, only the catalytic subunit, Elp3 has been identified. Unlike other eukaryotes, two orthologs of Elp3 have been identified in these organisms- Elp3a and Elp3b. While TbElp3b has been implicated in negative regulation of rDNA transcription, the role of Elp3a has not been deciphered across any of the trypanosomatids. The work presented in this thesis attempts to elucidate the role of the Elp3a protein in Leishmania donovani. We have adopted the strategy of creating genomic knockouts of the elp3a gene, followed by analysis of the resultant phenotypes. As we were able to successfully create elp3a-nulls it appears that elp3a is not essential for cell survival under in vitro parasite culture conditions. LdElp3a depletion did not affect the growth trend and cell cycle progression of these parasites in vitro, nor affect the parasite's ability to infect and proliferate within mammalian host cells. However, elimination of elp3a increased the parasite's tolerance to certain genotoxic stress-inducing agents like methyl methane sulfonate and HU-mediated chronic stress. In working towards determining the possible reason for the increased tolerance of these parasites to genotoxic agents, it was revealed that the elp3a mutants did not elicit a hyperactive DNA damage response or trigger enhanced DNA repair, but rather, these mutants did not suffer DNA damage to the same extent as wild type cells. The mechanism by which LdElp3a mediates its effect remains to be uncovered, although our results rule out a major role for LdElp3a in transcriptional control of genes involved in DNA damage response. Our results also suggest that, unlike what is seen in other eukaryotes, reduced translational efficiency due to elimination of elp3a does not seem to be the reason for the aberrant response of elp3a mutant cells. In the search for possible mechanisms by which LdElp3a might exercise its effects, future studies will be directed towards examining the proteomes and acetylomes of the elp3a mutant.

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02. PAL (Jyoti)
Characterization of the Functional Role of the SET7 Rrotein in the Unicellular Parasite Leishmania Donovani.
Supervisor: Prof. Swati Shah
Th 27635

Abstract

Leishmania spp. causes a group of diseases known as Leishmaniases which are of three types: cutaneous, mucocutaneous and visceral leishmaniasis (VL). VL can be fatal if not treated on time. High costs, toxic side effects of current drugs as well as emerging drug resistance and threats from PKDL continue to give impetus to investigations into Leishmania biological processes as the search for new sites for therapeutic interventions continues. The present study examines the fundamental role of the SET domain protein SET7 of Leishmania donovani. We have used the approach of creating set7 genomic knockouts and characterizing the phenotypes of the knockout lines to uncover the functional role of LdSET7. We find that set7 is not essential for cell survival, though the elimination of set7 increases the generation time and hypersensitizes the cells to HU-induced G1/S block. LdSET7 is predominantly cytoplasmic throughout the cell cycle and is expressed equivalently in different forms of promastigotes. Interestingly, upon analyzing the effect of set7 elimination on parasite survival in host macrophages, we find that set7-nulls survive better than wild-type parasites. When we analyze parasite survival in in vitro H2O2-induced oxidative environment, here also we find that set7-nulls survive more proficiently than wild-type cells. The higher tolerance of set7-nulls to H2O2-induced oxidative stress is associated with an almost complete absence of detectable DNA damage in these cells upon H2O2 exposure. In exploring possible causes for this we find that set7-nulls do not display detectable ROS activation upon exposure to H2O2. Further investigations into this apparent absence of ROS activation demonstrates that there is no significant activation of peroxidase activity in set7-nulls in response to H2O2-induced oxidative environment. Instead, the basal levels of peroxidase activity are higher in set7-nulls in comparison to wild-type cells. Analysis of peroxidase expression reveals that this is due to higher txnPx transcript levels in set7-nulls. Rescue experiments carried out by ectopic expression of LdSET7 in set7-nulls reveal that wild-type SET7 partially rescues the phenotypes associated with set7 elimination. On the other hand, LdSET7-Y421A (catalytically dead mutant) doesn't do so, indicating that the cell's response to oxidative stress is controlled by LdSET7 methylation activity. Thus, LdSET7 moderates the parasite's response to the inhospitable intracellular oxidative environment experienced by these parasites within the mammalian host, allowing the establishment of infection and its persistence, without wiping out the host cell population that it needs for its survival.

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