Critical Review:

The 26 S proteasome negatively regulates the level of overall genomic nucleotide excision repair

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Nucleic Acids Research, 2000, Vol. 28, No. 24 4839-4845

Lori Lommel’s paper clearly presents data that implicates a role of the 26S proteasome in negatively regulating DNA repair in yeast. The abstract and introduction provide a brief background of proteasome function and the ubiquitin-mediated protein degradation pathway. The hypothesis of their project is derived from a goal to further understand the role of the proteasome in cellular processes. They state that the 26S proteasome is known to regulate cell cycle progression, stress responses and cell differentiation. The introduction also presents some evidence prior to their study that suggests a correlation between ubiquitylation and proteolysis in the cellular response to damaged DNA. From this evidence Lommel hypothesized that DNA repair proteins may be degraded by the proteasome after nucleotide excision repair. They also postulate that proteasome-dependent degradation of DNA repair machinery may be essential in preventing naturally occurring DNA structures from being incised during routine cellular processes.

They test this hypothesis by quantifying transcription-coupled repair and genomic nucleotide excision repair in the presence and absence of proteasome function. Their methods are appropriate to answer the posed question although additional experiments could have further reinforced their data. Lommel utilized yeast strains with conditional proteasome mutations. The strains can degrade proteins at the permissive temperature, but are deficient
in proteolysis at the non-permissive temperature. These cells were irradiated with 60 J/m² at 254 nm. After isolating the DNA, efficiency of repair was assessed by calculating the quantity of cyclobutane pyrimidine dimers (CPDs). This number was determined by employing the enzyme T4 endonuclease V which is a CPD-specific DNA glycosylase which produces a single-stranded break at each CPD. Gel electrophoresis of the fragmented DNA, subsequent hybridization to ³²P-labeled RNA probes generated by \textit{in vitro} and audioradiodiagrams allowed Lommel to calculate the number of CPDs per fragment from the ratio of intensities of bands from the T4 treated samples and control samples. Successful DNA repair is then reflected by the restoration of the full-length DNA restriction fragment, following T4 endonuclease-treatment. Although this is a common method of quantifying DNA repair, an additional assay using the immuno slot-blot technique and monoclonal antibodies may have provided more precise data. This method employs antibodies which can specifically bind CPDs and be visualized via a secondary antibody and chemiluminescent substrate.

Lommel’s results clearly demonstrate that the proteasome mutants possess faster repair compared to the parental strains. By examining the removal of CPDs from the reporter gene RPB2, they observed that the repair of the transcribed and non-transcribed strands in the mutant strains is more efficient than in the parental strain. More than 90% of the CPDs were removed from the transcribed strand of the mutant strains in the first 30 minutes following UV irradiation compared to the parent strain which removed only 75-80% of the CPDs. Even more striking, in the non-transcribed strand of RPB2 the mutants removed nearly 67% of CPDs in the first 30 minutes whereas the parental strain achieved less than 15%. Their assays show that in the absence of proteasome function, repair is increased in both
transcribed and non-transcribed strands. The specific interaction and pathways between the proteasome and repair regulation, however, were not thoroughly examined. The second part of the experiment (see next paragraph) tested only one conjecture regarding this interaction.

Lommel also examined the possibility that the stability of a specific repair protein, Rad4, could be regulated by 26S-mediated degradation. They chose Rad4 because it has been shown to form a high-affinity interaction with Rad23, a protein which interacts with the active 26S proteasome. Because Rad23 inhibits the synthesis of multiubiquitin chains, Lommel hypothesized that Rad23 may protect Rad4 from proteolysis. This hypothesis was tested by quantifying repair following overexpression of Rad4 in yeast. They found that overexpressing Rad4 resulted in approximately 55% removal of CPDs at 30 minutes post-irradiation in the non-transcribed strand compared to only 25-30% in the parent strand. The similar rates in repair between the Rad4 overexpressing cells and conditional proteasome mutants at the non-permissive temperature suggests that Rad4 may be a target of proteasome degradation. Lommel also proposes that in the absence of proteasome function, the repair protein (or proteins) accumulate and enhance repair throughout the genome. This study did not, however, unequivocally prove that Rad4 is degraded via the 26S proteasome.

The paper overall is written clearly and the discussion is very thorough. Lommel presents and considers contradictory evidence from prior studies. Weeda et al proposed that the proteasome may actually process the inactive precursors of proteins and suggest a model correlating loss of proteasome function with defective repair. In another published report, Russell et al inhibited the 19S regulatory subunit of the proteasome and found that nucleotide excision repair was impaired. Lommel skillfully explores possible explanations for these discrepancies such as the difference in repair assays used by each study and the
theoretical questions not considered by contradicting research groups. The paper also elaborates on the possible functions that intertwine the proteasome and repair. For example, they discuss the theory that Rad23 may prevent premature degradation of specific proteins, escort proteins to the 26S proteasome and also function independently to assemble the nucleotide excision repair complex. Despite these theoretical suppositions, the discussion section of the paper blatantly lacks direction for further specific experiments. The only “future experiment” mentioned is to assess the levels of Rad4 following UV irradiation in order to determine if the amount of Rad4 in the cell is reduced to pre-irradiation levels as repair is completed. I agree that this is the next logical step in pursuing their hypotheses but this research group has the potential to elucidate much more about the proteasome and DNA repair. This study should have included a western blot to determine if Rad4 is in fact accumulated in the absence of proteasome function. This would have filled in one piece of the puzzle relating proteolysis and repair. Another simple way to further our understanding of the interaction between these two cellular processes would be to run a series of Western blots for well known DNA repair proteins in yeast (not just limited to Rad4) in the absence of proteasome function. This would shed light on which specific proteins are accumulated in the cell and possibly conferring more efficient repair following inhibition of proteolysis. Lommel also does not mention the importance of examining the hypotheses of this study in mammalian cells (this is what I am currently researching for my honors project). It is essential to examine questions of repair in mammalian (including human) cells to work toward goals of understanding cancer in humans and to target repair as a chemotherapeutic agent.
The paper is organized succinctly and logically. The title of the paper was well chosen and effectively conveys the principal message of the paper. The abstract is also well written and appropriately summarizes their data and conclusions but fails to mention the strain of yeast used in the study. This is a small detail but I felt it should be included. The paper succeeded in providing a thorough context of the research and theory that led up to their hypothesis and methodology. All though the paper as a whole is thorough, more text could have been devoted to explaining the significance of the contribution presented by the paper. The concluding sentences of the discussion do not leave the reader with a concise synopsis of what they have just read nor an understanding of why the study is important to the larger fields of biology and medicine.