Chromatin Condensation Protects DNA from MMS Damage

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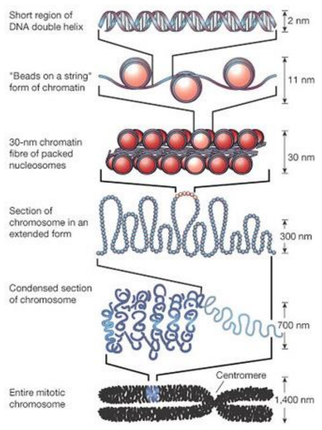
ABSTRACT

Chromatin is the complex of proteins and DNA that makes up eukaryotic chromosomes. Chromatin helps the DNA fit inside the nucleus as well as regulating what can access the DNA. Replication, repair, and transcription are all impeded by chromatin. These processes only occur efficiently when chromatin is disassembled. Just as chromatin limits how proteins can access the DNA, we hypothesized that it would also protect DNA. We used the yeast *Saccharomyces cerevisiae* to study how chromatin affects cellular sensitivity to the DNA alkylating agent methyl methanesulfonate (MMS). MMS alkylation can lead to DNA mismatches, nicks, and double strand breaks, resulting in mutation. Using growth assays, we found that active transcription significantly increases the sensitivity of a gene to MMS. Consistent with this, we found that increased chromatin compaction due to nocodazole-mediated metaphase arrest reduced the effects of MMS. Because transcription led to an increase in MMS-mediated DNA damage, we proposed that transcribed genes would be subject to increased damage under normal growth conditions, requiring recruitment of DNA repair factors. We found by chromatin immunoprecipitation that the Rad51, Rad53, and Rad18 DNA repair proteins are recruited to the *GAL1* gene during transcription, although their occupancy was not stimulated by MMS treatment. This suggests that there is enough damage occurring during normal transcription to require DNA repair factors. Together, these results support our model that chromatin helps protect DNA from damage in addition to regulating other DNA-based processes.

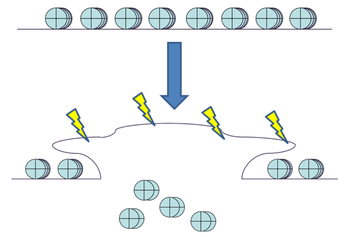


(Felsenfeld and Groudine, 2003)

INTRODUCTION

The chromosomes are constantly exposed to a barrage of damaging agents, from both within and outside the cell. Normal cellular metabolism generates reactive oxygen species that can lead to nucleotide modifications and intra- and inter-strand crosslinks. DNA is exposed to different types of damage such as UV, alkylation, and damage from metabolic byproducts. These types of damage can then cause mutations due to their effect on replication as well as imperfect repair. Damage from UV light can result in pyrimidine dimers which can formed from thymine or cytosine bases. This can result in alteration of the structure of dsDNA and can inhibit polymerase activity. Damage from alkylation can result in problems with processivity. Reactive oxygen species such as superoxide or hydrogen peroxide are metabolic byproducts and can result in double stranded breaks and DNA adducts. Intercalating agents such as Ethidium Bromide can cause inserts between bases resulting in frame shift mutations. Damage from all these factors can result in an accumulation of mutations to the DNA and eventual cell death if left unrepaired. There are many forms of DNA repair for combat the mutations such as non-homologous end binding, double stranded break repair, mismatch repair etc. One of the most common repair pathways is nucleotide excision repair. The damaged sites are identified via DNA-damage binding proteins (DDB), and using a template strand DNA polymerase will begin repairs.

Because chromatin prevents other factors from accessing the DNA, we hypothesized that it would also help protect DNA from certain kinds of damage. We used the DNA-alkylating agent MMS as a source of DNA damage. DNA alkylation affects the processivity and fidelity of replication, leading to mutations. We first tested whether chromatin disassembly would lead to increased damage at transcribed genes. We induced transcription of all of the amino acid biosynthetic genes in yeast with the drug sulfometuron methyl (SM), an inhibitor of isoleucine and valine synthesis. After treatment with SM and MMS, we looked for decreased growth on minimal media due to loss of function of amino acid biosynthetic genes. While transcription leads to chromatin disassembly, the metaphase chromosome is the most compact state of chromatin. Thus, we expected metaphase chromosomes to be less sensitive to MMS than less condensed chromatin. To test this, we arrested cells in metaphase with the microtubule depolymerization inhibitor nocodazole, treated with MMS, and again looked at yeast growth, this time on rich media. Finally, we tested the hypothesis that increased DNA damage during chromatin disassembly during transcription would require recruitment of DNA repair factors by measuring occupancy of Rad51, Rad53, Rad18, and Rad9 at both uninduced and induced *GAL1* in the presence and absence of MMS. Rad51 protein is involved in double strand break repair via synthesis-dependent strand annealing and has been shown to be important for repair of MMS-mediated damage. Rad53 is involved in double strand break as well by pausing the replication fork while repairs are made. Rad18 is a ubiquitin ligase which is involved in the gap filling step of homologous recombination. Rad9 is a DNA damage dependent checkpoint protein that halts the cell at G1 by activating Rad53 until repairs are made.

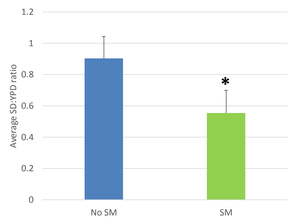


(DePamphilis, 2006)

Does transcription lead to increased DNA damage?

Yeast were grown to mid-log phase in SC media and treated with 1 µg/ml of SM for 15 min and then 0.1% MMS for 15 min. Cultures were plated on YPD and SD plates and colonies were counted. Increased damage at the transcribed amino acid biosynthetic genes was expected to lead to a decreased SD:YPD colony ratio.

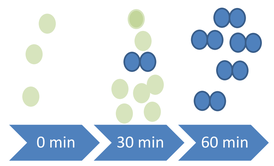
Look for reduced SD:YPD colony ratio with SM vs. without



The decreased SD:YPD ratio in SM suggests that there is increased MMS-mediated damage at transcribed genes.

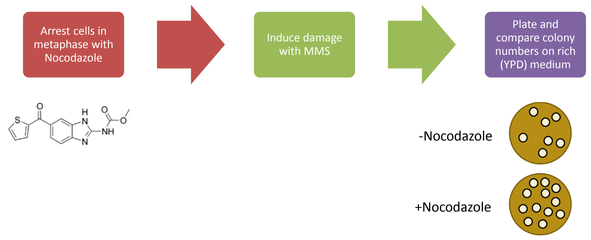
Does chromatin compaction protect DNA from damage?

To optimize the nocodazole treatment, mid-log phase cultures were treated with 1.5 mg/ml nocodazole, and samples were monitored for the presence of dumbbell-shaped cells, indicating metaphase arrest.

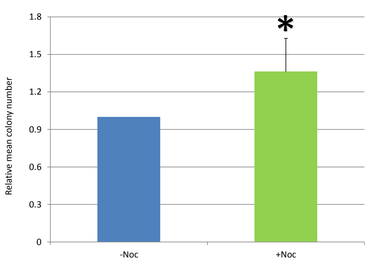


After 60 min. of nocodazole treatment, ~100% of cells were arrested in metaphase.

Yeast were grown to mid-log phase in SC media, treated with 1.5 mg/ml nocodazole for 1 h and treated with 0.1% MMS for 15 minutes. Yeast were plated on YPD and colonies were counted.



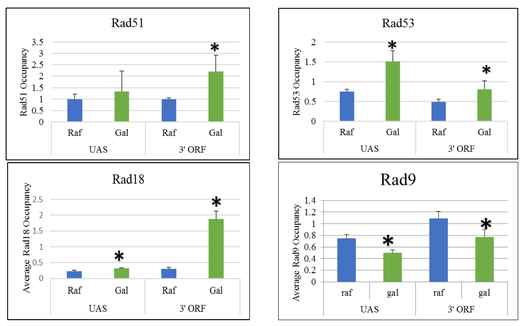
Look for reduced colony ratio number without nocodazole.



Metaphase arrest increased resistance to MMS, suggesting decreased DNA damage of the condensed chromosomes.

Are Rad DNA repair proteins recruited to *GAL1*?

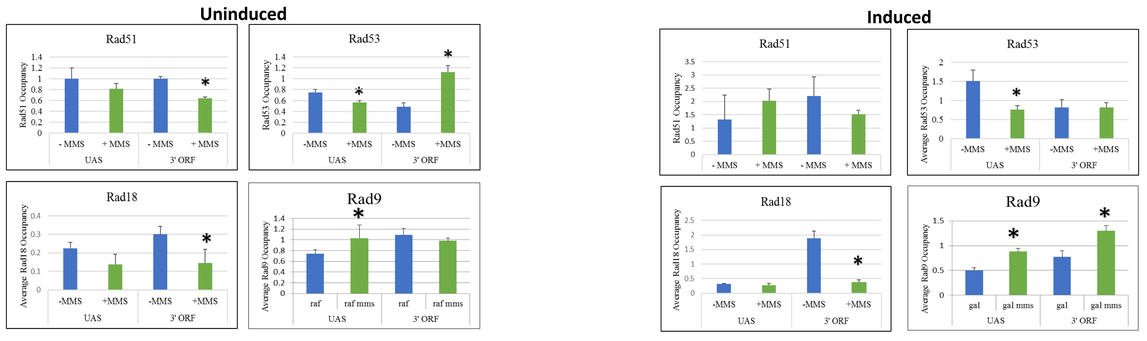
Chromatin immunoprecipitation (ChIP) of Rad51, 53, 18, and 9. *RAD51-Myc* and *Rad53/18/9-TAP* yeast were grown in SC_{Raf} to early log phase, induced with 2% galactose for one hour, and processed for ChIP with anti-Myc and anti-TAP antibodies and primers for the *GAL1* UAS and 3' ORF and an intergenic region on chromosome V (Ch V). Occupancy was calculated as the *GAL1*:Ch V band intensities for the IP samples divided by the same ratio for the input samples.



Rad51/53/18 are recruited to *GAL1* during transcription, suggesting DNA damage during normal transcription. However, Rad9 has reduced recruitment suggesting a separate function.

Does MMS treatment increase Rad protein recruitment to *GAL1*?

Chromatin immunoprecipitation (ChIP) of Rad51, 53, 18, and 9. *RAD51-Myc* and *Rad53/18/9-TAP* yeast were grown in SC_{Raf} to early log phase, induced with 2% galactose for one hour, treated with 0.1% MMS for the last 15 min., and processed for ChIP with anti-Myc antibodies and primers for the *GAL1* UAS and 3' ORF and an intergenic region on chromosome V (Ch V). Occupancy was calculated as the *GAL1*:Ch V band intensities for the IP samples divided by the same ratio for the input samples.



With MMS treatment, Rad51,53, and 18 may be diluted away from *GAL1* to repair other parts of the genome. Increased recruitment of Rad53 to the ORF without transcription, and increased recruitment of Rad9 in the absence and presence of transcription suggest that they may work separately from Rad51 and Rad18.

CONCLUSIONS

- Decreased chromatin condensation during transcription leads to increased MMS-mediated DNA damage.
- Increased chromatin condensation during metaphase protects DNA from MMS-mediated damage.
- Increased damage to transcribed DNA leads to increased recruitment of repair factors.

FUTURE DIRECTIONS

- Are other DNA repair factors recruited during transcription?
- Are DNA repair proteins recruited to other genes?
- Does chromatin protect DNA from other types of damage?
- Does cell age affect chromatin compaction?

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