UNC PEMBROKE | MAYNOR HONORS COLLEGE

Studying the Interplay between RNA Polymerase II and Nucleosome Dynamics

Senior Project

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By

Ereny G. Gerges
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Ereny Gerges
Honors College Scholar — 5/2/18 Date

Maria Santisteban, Ph.D
Faculty Mentor — 5/2/18 Date

Teagan Decker, Ph.D.
Senior Project Coordinator — 5/2/18 Date

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Abstract

In Saccharomyces cerevisiae, histone H2A.Z is coded by the HTZ1 gene. It is a highly conserved variant of histone H2A and has many reported roles in chromosome segregation and transcriptional regulation, among others. In yeast, this gene is not essential, unlike its homologues in other species where a deletion of this gene is lethal. We previously reported that Htz1 has a role in transcription elongation, but the mechanism of this is not yet understood. It has been shown that many elongation mutants exhibit a "cryptic" initiation phenotype, i.e. initiation of transcription by RNA pol II occurs inappropriately within the protein-coding regions of genes, rather than in the proximal promoter regions. Suppression of cryptic initiation requires a repressive chromatin structure. It is then expected that mutations in chromatin structure modifying factors, as well as elongation mutants, which may fail to reassemble nucleosomes in the wake of elongating RNA polymerase II (RNAPII), would display cryptic initiation phenotypes. We tested our strains for cryptic initiation phenotypes and have found that htz1Δ cells exhibit a mild cryptic initiation phenotype and htz1ΔRPB2-2SL has a strong cryptic initiation phenotype. A plausible explanation for these results is that the Rpb2-2 mutant polymerase is prone to cryptic initiation, and when nucleosome dynamics is altered in the absence of Htz1, the effect is exacerbated.

Background:

Gene transcription and regulation does not happen in the context of naked DNA, rather it happens in the presence of chromosomal proteins such as histones. The nucleosome structure, including histones and histones variants, resemble barriers for the passage of the RNA polymerase. Although the nucleosome structure presents a physical barrier that causes an arrest/stall of the RNA Poly II from getting to the DNA templet, it modulates the process of gene transcription. In order for transcription to happen the nucleosome must disassemble, revealing 147 base pairs of DNA wrapped around the octamer histones.

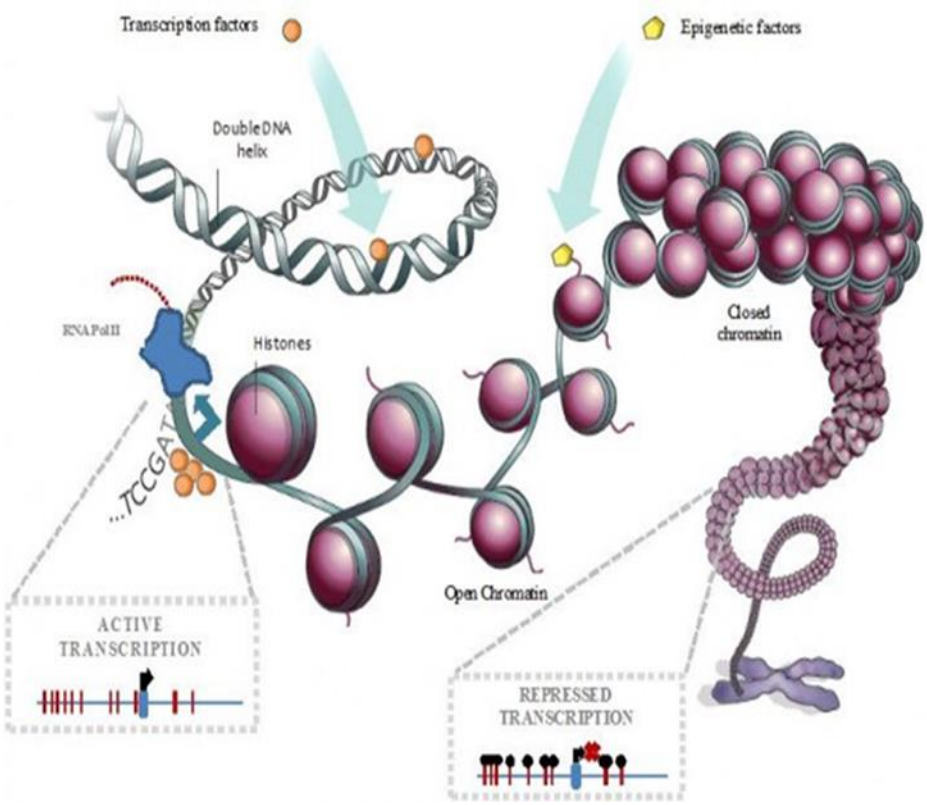


Figure 1. Chromatin structure and transcriptional proteins

There are many factors that impact the level of chromatin condensation and potentially alter the access of the DNA templet by the RNA polymerase. For example nucleosome remodeling complexes disassemble or displace the nucleosomes to facilitate the passage of the RNA polymerase. Histone modifications (methylation and acetylation) affects the level of chromatin compaction close vs. open. Also histone variants provide alternatives for nucleosome structures and access to DNA. In our lab we study one of these histone variant H2A.Z and its role in gene transcription and regulation.

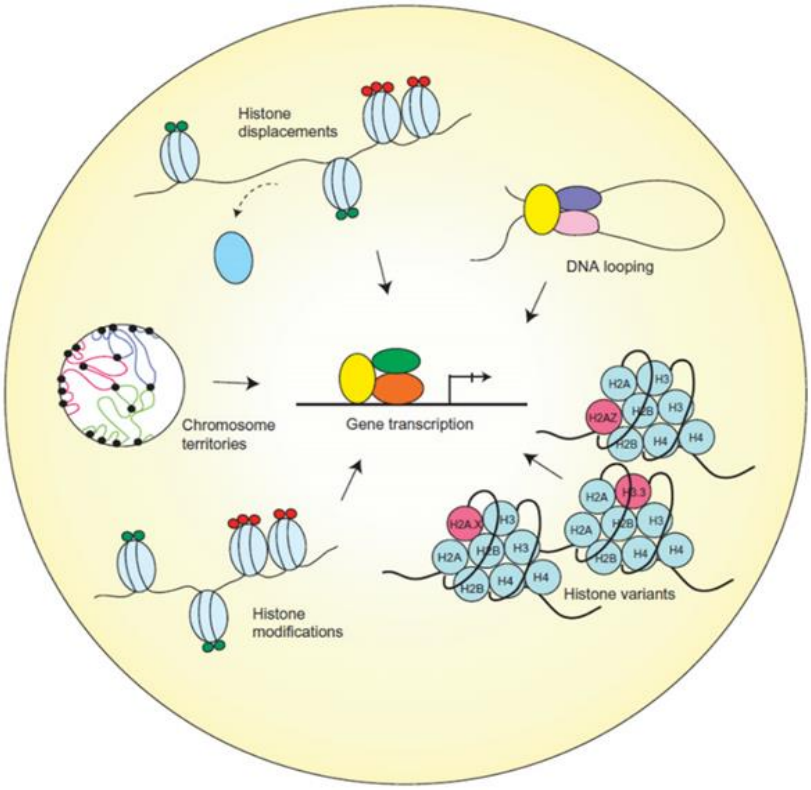


Figure 2. Various architectures of the chromatin, histone displacement, DNA looping, histone variants, histone modification, and chromosome territories regulate gene transcription.

H2A.Z, belongs to one of the most conserved family of histone variants (H2A.Z/F). Members of this family are more similar to each other across species than they are to the canonical H2A in their same species. In Saccharomyces cerevisiae H2A.Z is coded by the HTZ.1 gene. Unlike its homologues in other species,

the protein is not essential in yeast, as deletions of this gene are not lethal. Htz1 plays important roles in chromosome segregation, maintenance of heterochromatin-euchromatin boundaries, DNA repair, cell cycle control and resistance to genotoxic stress, among others. One of the most studied roles of Htz1 is in transcription regulation. In the early 2000 my PI's lab showed for the first time that Htz1 was present at the promoter regions of genes mostly in the repressed state and was displaced upon activation. This early observation was generalized in genome wide studies by several labs and today the role of Htz1 in transcription initiation is well established. In yeast, general regulatory factors and chromatin remodelers promote the formation of nucleosome-depleted regions (NDRs). SWR-C binds to DNA in the NDR and replaces H2A–H2B with H2A.Z–H2B on NDR-flanking (+1 and −1) nucleosomes. H2A.Z recruits RNA polymerase II (Pol II) and H2A.Z is displaced from promoters by binding the pre-initiation complex.

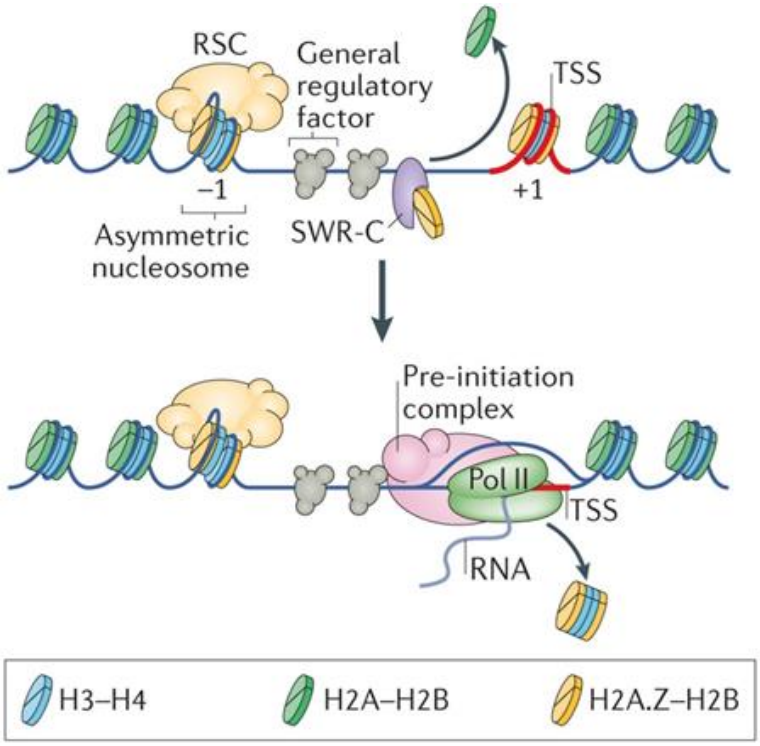


Figure 3. The role of histone variant Htz.1 in transcription initiation

More importantly is the role of the histone variant Htz.1 in transcription elongation which is less understood. In our lab the first connection to elongation came from the finding of the synthetic lethal case between a double mutation of the htz1 gene and the gene that encodes of the second largest subunit of the RNA polymerase, rpb2-2.

Materials and Methods:

In the Santisteban lab, we use a yeast strain called W303 that has a double mutation in ade3ade2 genes, causing the yeast to appear white. Ade2 mutation alone causes a red pigment to accumulate but because ADE3 is epistatic to ade2, which means that it functions upstream of ade2, ade3 mutants do not allow for accumulation of the red pigment. If a plasmid with a wild type ADE3 gene is present, then the cells are again red because the wild type ADE3 rescues the ade3 mutations. For our synthetic lethal screen we use an ADE3 plasmid that also carries a wild type copy of HTZ1. Starting with an htz1 null, the strain is randomly mutagenized and red colonies were selected which could harbor a synthetic lethal mutation, since they must retain the HTZ1 plasmid to survive. This system had allowed us to monitor the cells ability to survive after the mutation by expressing the sectoring phenotype indicating that the Htz1 plasmid is disposable.

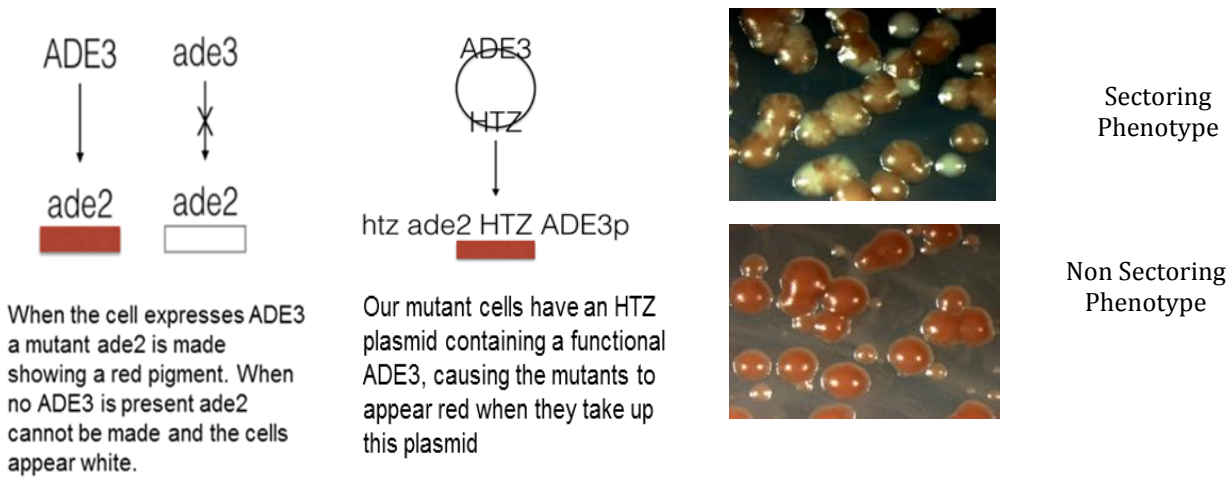


Figure 4. Sectoring vs. non sectoring phenotypes and Htz.1 plasmid

In our lab, we have uncovered a synthetic lethality between a mutation in the second largest subunit of RNA Poly 2 (rpb2-2) and htz1. Yeast cannot survive with both mutations but can function with either of them. The double mutation is synthetic lethal, so without the HTZ1 plasmid the cells die. The solid red colonies remaining are the ones with the double mutation and the HTZ1 plasmid, and they have survived. Our interpretation is the cause of the synthetic lethality case is that only on the deficient chromatin template (without htz1), the mutant polymerase stalls on the template thus blocking transcription by wild type polymerase, resulting in a dominant negative phenotype.

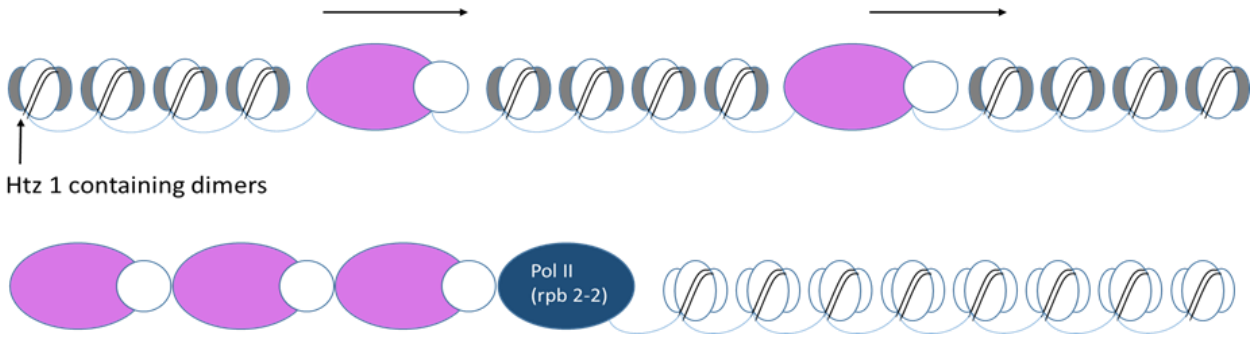
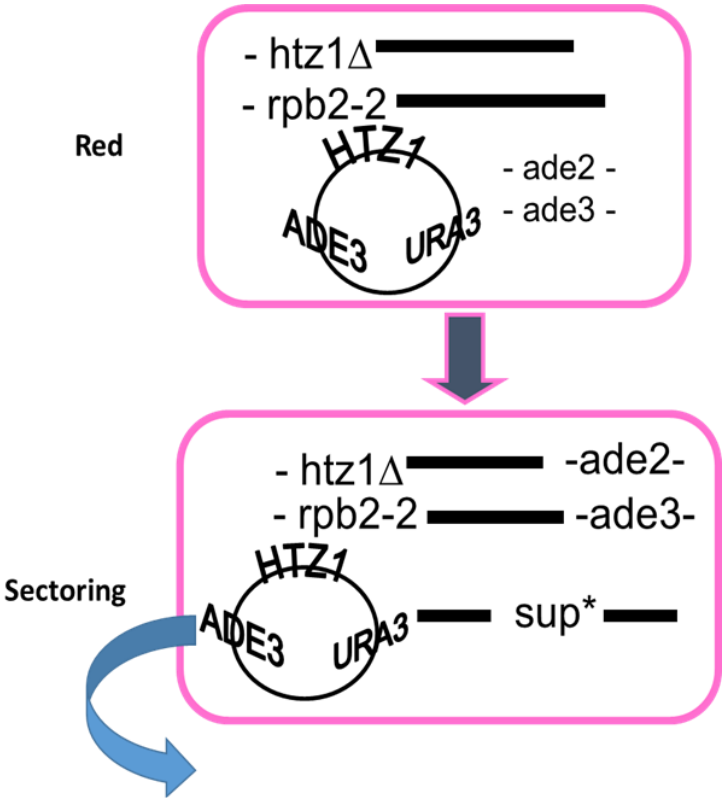
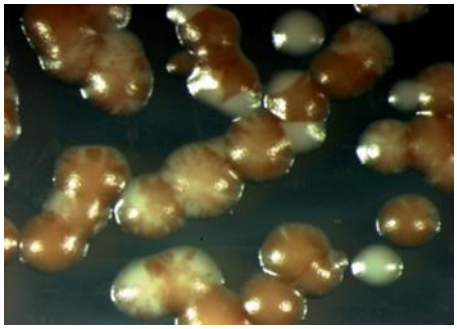


Figure 5. RPB2-2^{SL} is synthetic lethal with htz1Δ

In order to learn about the defect causing the synthetic lethality in the double mutant. We screened for suppressor of the synthetic lethality. The double mutant strain was randomly mutagenized and sectoring colonies were selected, because these have regained the ability to live without the HTZ1 plasmid. So they are no longer synthetic lethal. The suppressor gene is allowing for the double mutation colonies to stay alive.

We used a yeast genomic high copy library to complement the suppressor and in two separate instances. we isolated a clone containing SET2 gene among others. Sub cloning demonstrated that SET2 gene was responsible for the complementation. SET2 is histone methyl transferase that methylates histone 3 in lysine 36 and has been shown to have roles in transcription elongation.





Phenotype of the suppresser strain

Figure 6. Suppressed gene fixes the synthetic lethal case by disposing the plasmid

In order to check if the mutant gene responsible for the suppression of the synthetic lethality is actually SET2, we constructed a set2 null mutation in htz.1-rbp2-2 strain and this was crossed to the suppressor mutant strain. The diploid strain was sporulated and the tetrads were dissected. Two outcomes can be expected from the dissection of this diploid strain. If the suppressor mutation is

indeed in the SET2 gene, then all four spores of the progeny would be sectoring, because they each spore would carry one version (sup*) or the other set2::KAN. This of course, assumes that a null mutation on the gene is equivalent to the mutation harbored in the suppressor strain. On the other hand, If SET2 is not gene mutated under the sup* identity, at the suppressor locus, two of the spores would receive mutant sup* (sectoring) and two would have wild type SUP (non-sectoring). The set2::KAN would segregate independently from sup* and so the 2:2 KAN resistance would randomly distribute in the four spore.

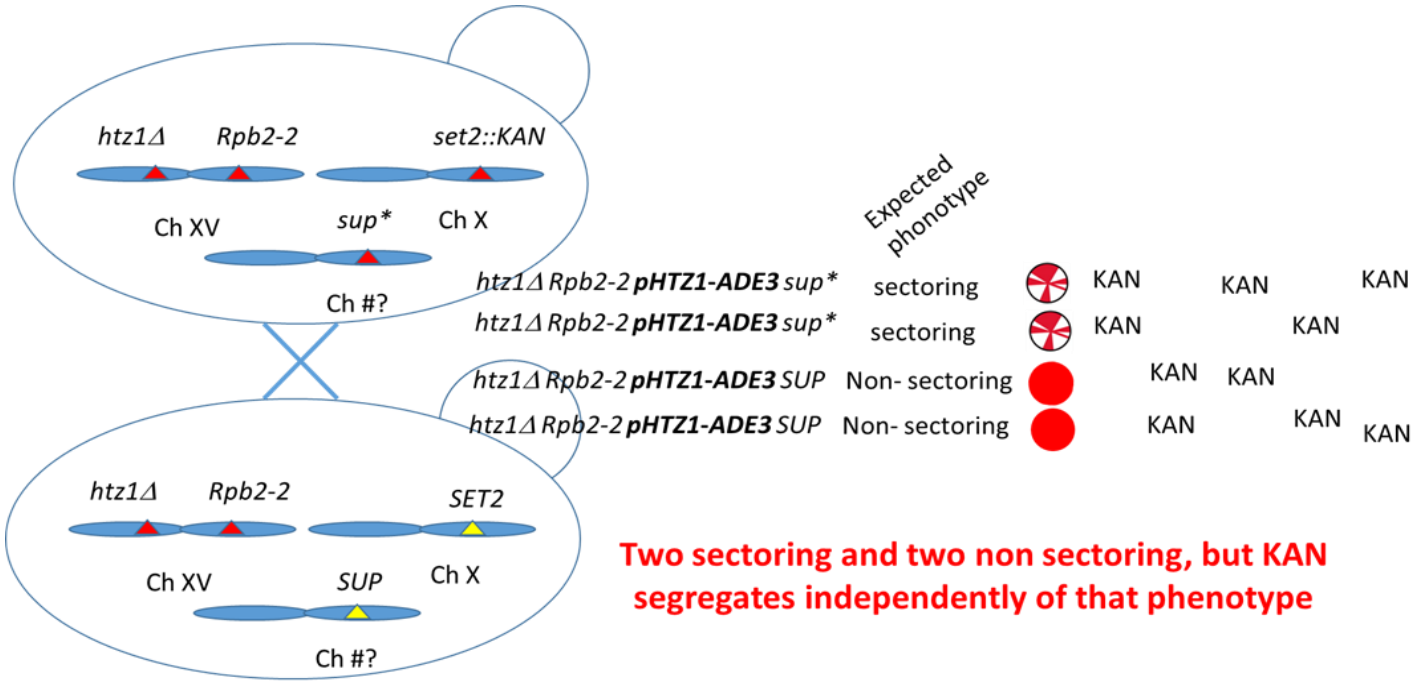


Figure 7. Progeny outcomes If suppressor gene (sup*) is different from SET2

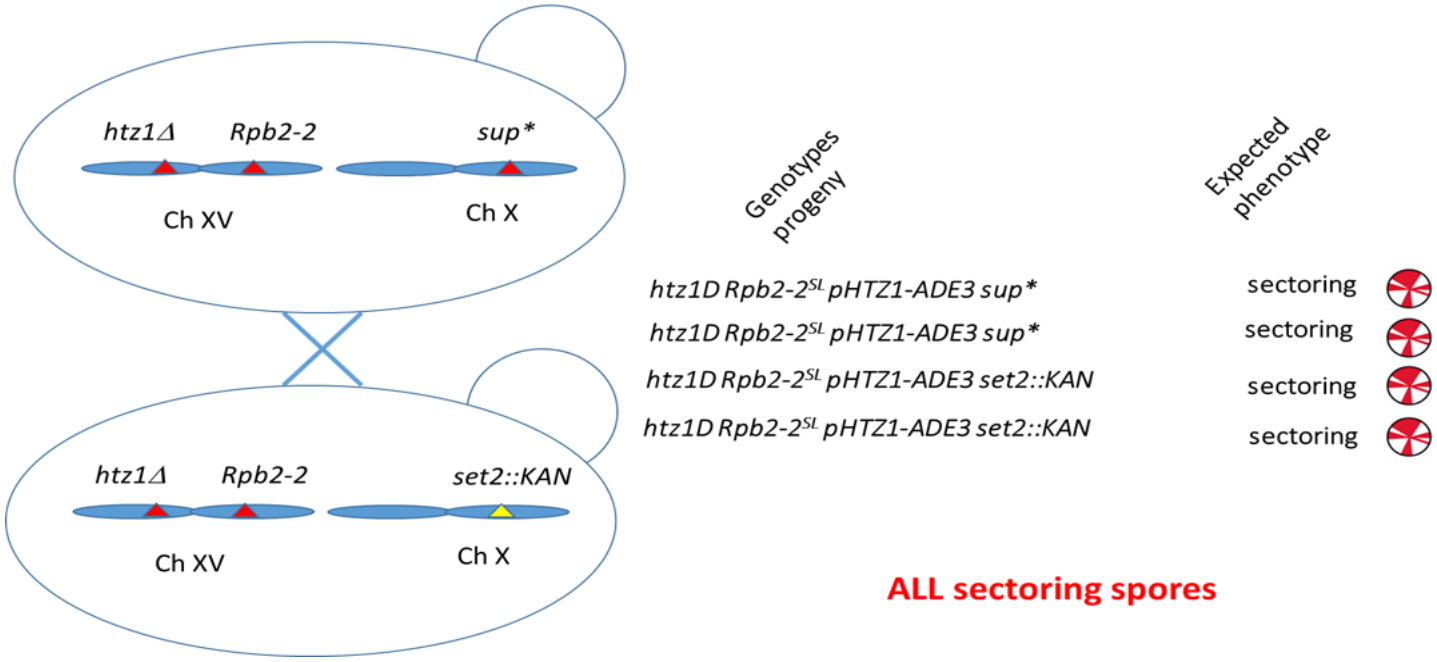


Figure 8. Progeny outcomes If suppressor gene (sup*) is the same as SET2

Results:

Our diploid cross, shown in the table below, shows the sectoring phenotype segregating in a 2:2 ratio in tetrads. Although these results would seem to indicate that SET2 is not the mutaed gene that is responsible for the suppression of the synthetic lethality of htz1 rpb2-2, we noticed that the KAN marker of the set2 null did not segregate independently of the sectoring phenotype associate with the suppressor. In other words, we detected a preferential segregation of KAN marker with the non-sectoring spores, which indicates that the set2: KAN does not segregate away from the suppressor gene and they are likely the same. Then the

question remains as why the null mutant does not sector as well as the suppressor mutant. One possibility is that the mutant is a gain of function. Consistent results with our hypothesis that SET2 mutation is a gain of function, is when SET2 gene is knocked out using a KAN marker in the suppressor strain the strain shows a solid red phonotype suggesting that the gene that is causing the sectoring is linked to SET2. More profound were the results of the diploid between the suppressor strain with SET2 knock out and suppressor strain that showed a minimal sectoring phonotype suggesting that SET mutation is dominant which is consistent for a gain of function mutation.

Tetrads	A	B	C	D
3	Died	Non	Sectoring	Non
4	Non	Sectoring	Sectoring	Non
5	Sectoring	Non	Sectoring	Non
6	Non	Sectoring	Non	Sectoring
7	Non	Sectoring	Sectoring	Non
8	Sectoring	Died	Non	Non
9	Sectoring	Sectoring	Non	Non

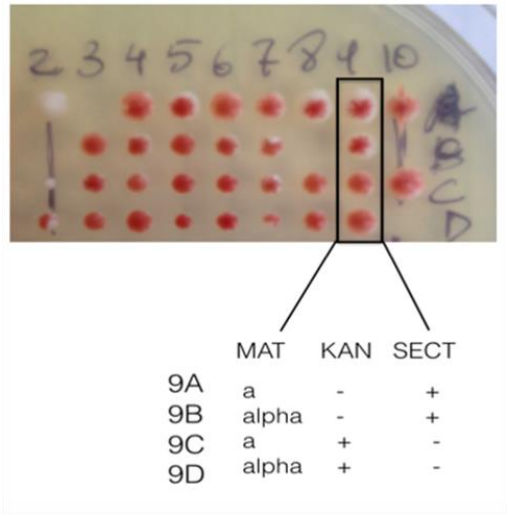


Figure 9: Actual dissection results

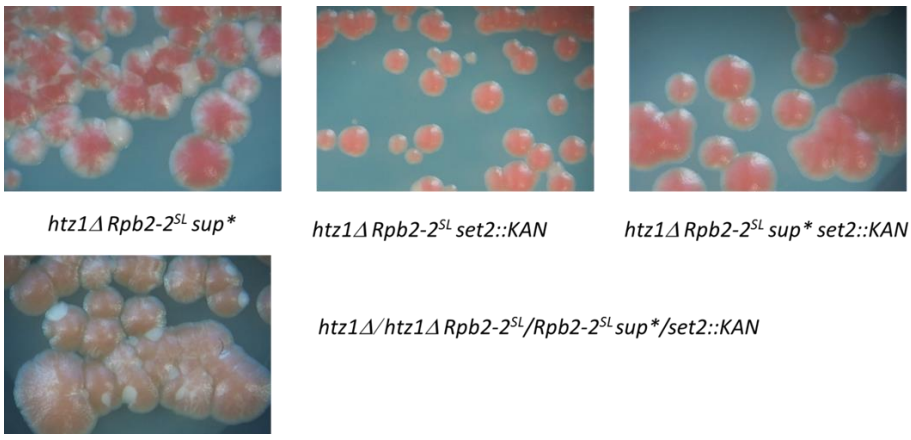


Figure 10. Knock-out (KO) of SET2 in the suppressor strain abolishes the sectoring phenotype

Conclusion:

We propose that in the absence of Htz1 the defective RNA polymerase stalls on the DNA template and blocks wild-type polymerase from elongation. Support for this hypothesis comes from our own previous work showing that Htz1 deletion in budding yeast led to slower RNAPII elongation (Santisteban et al., 2011). Additionally the Henikoff's lab (Weber et al 2014) has shown that H2A.Z enrichment at the +1 nucleosome correlates with decreased RNAPII stalling, suggesting that its incorporation reduces the high-energy barrier to RNAPII progression. Because Set2 has been shown to be involved in establishing repressive chromatin after the polymerase passage; deletion of this gene could suppress the stalling of the polymerase in cells lacking Htz1 because the chromatin template remains open.

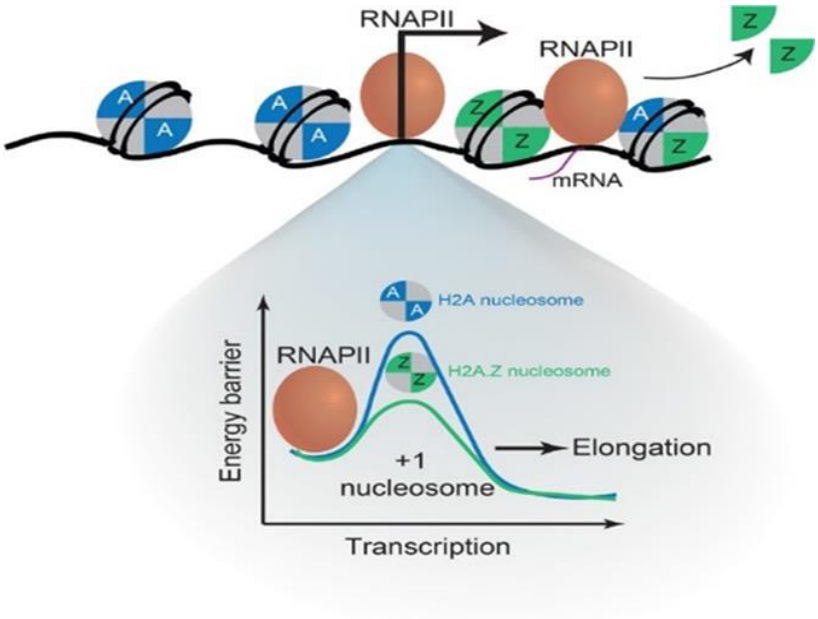


Figure 11. Htz.1 enriched nucleosome overcomes the energy barrier more efficiently

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