Molecular Mechanisms for Epigenetic Transcriptional Poised Memory State

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The major mechanism by which cells adapt to changes in their environment is by altering transcription. In some cases, the response to a stimulus is affected by the prior experiences of the cell. One such phenomenon is called *epigenetic transcriptional memory*; cell populations that have previously experienced a particular stimulus show a faster or stronger response to this stimulus in the future. Through work in budding yeast and HeLa cells, this phenomenon has been shown to be characterized by a unique transcriptional memory state that persists for 4-8 generations.

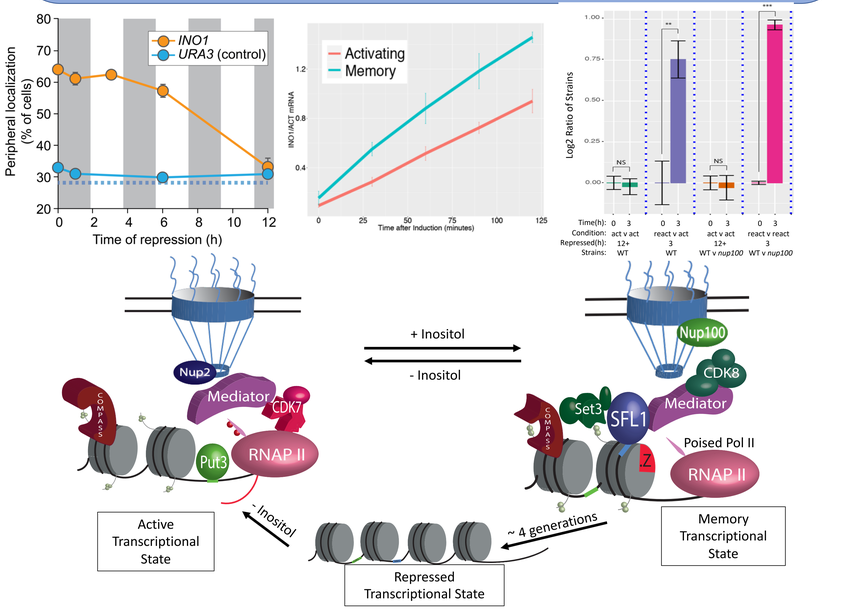

Figure 1: ***INO1* Epigenetic Transcriptional Memory** A) Percent of cells that show *INO1* at the nuclear periphery post repression of activated cells. Gray to white bars indicate generations. B) Gene expression levels of *INO1* upon activation and reactivation with Opi1-AA (after 3 hours repression) by RTq-PCR. C) Competitive fitness benefit of populations undergoing reactivation by sequencing-based competition assay. D) Model for the different transcriptional states of the *INO1* gene.

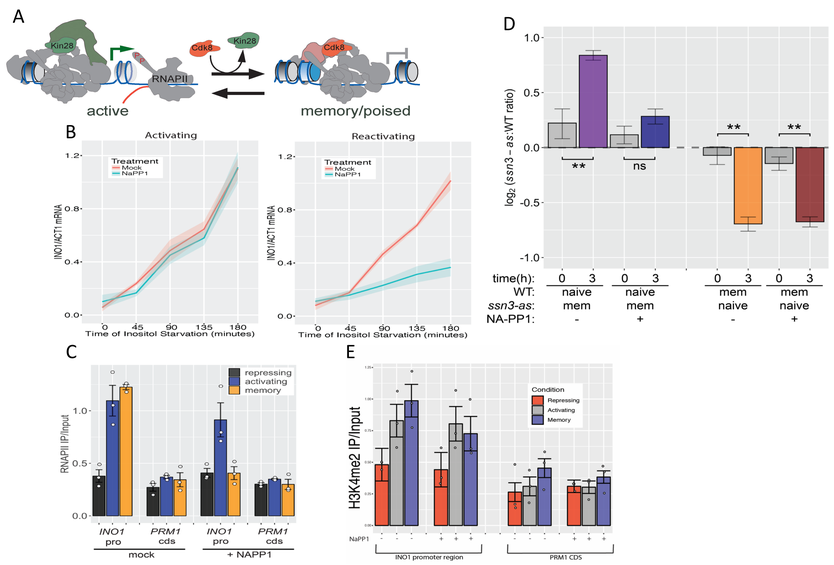

Figure 2: **Kinase Activity of Cdk8 is essential for RNA Polymerase II poising, but not chromatin changes** A) Model showing the remodeling of Mediator into the memory-specific state. B) RTq-PCR showing *INO1* mRNA levels during activating (left) and reactivating (right) transcriptional states when Cdk8 is catalytically active (red) or inactive (blue). C) ChIP-qPCR against RNA Polymerase II at the *INO1* promoter region under repressing (+inositol), activating (-inositol), and memory (overnight activating, 3 hours repressing) conditions, both while Cdk8 is active (mock) and inactive (+NAPP1). D) Sequencing-based competition assay showing the loss of the fitness benefit obtained by the 'reactivation' population if Cdk8 is inactive. E) ChIP-qPCR against H3K4me2 at the *INO1* promoter region under repressing, activating, and memory conditions (see above).

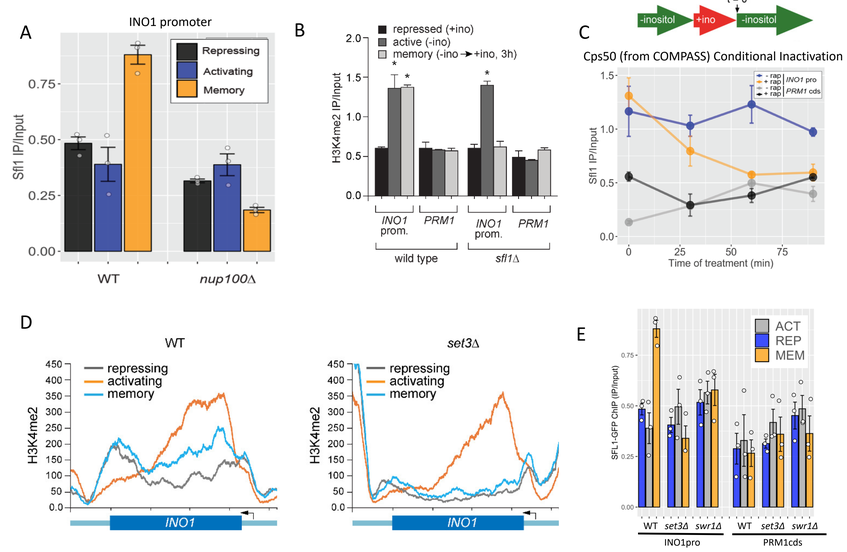

Figure 3: **The essential components of Epigenetic Transcriptional Memory form a feedback loop** A) Sfl1 ChIP at the INO1 promoter region in WT and *nup100Δ* strains under repressing (+inositol), activating (-inositol), and memory (-inositol overnight, then 3 hours repressing) conditions. B) H3K4me2 ChIP in repressed, activating, and memory conditions (see above) at the *INO1* promoter in WT, *Sfl1Δ,* and *mrsmut* strains. C) ChIP-qPCR of SFL1 at the *INO1* promoter region upon conditional inactivation of Cps50 by Anchor Away in memory conditions D) H3K4me2 ChIP-seq experiment focused over the *INO1* gene in WT and *set3Δ* strains. E) Sfl1-GFP ChIP-qPCR at the INO1 promoter region in WT, *set3Δ,* and *swr1Δ* strains, showing that SFL1 binding in memory conditions is dependent on chromatin modifiers.

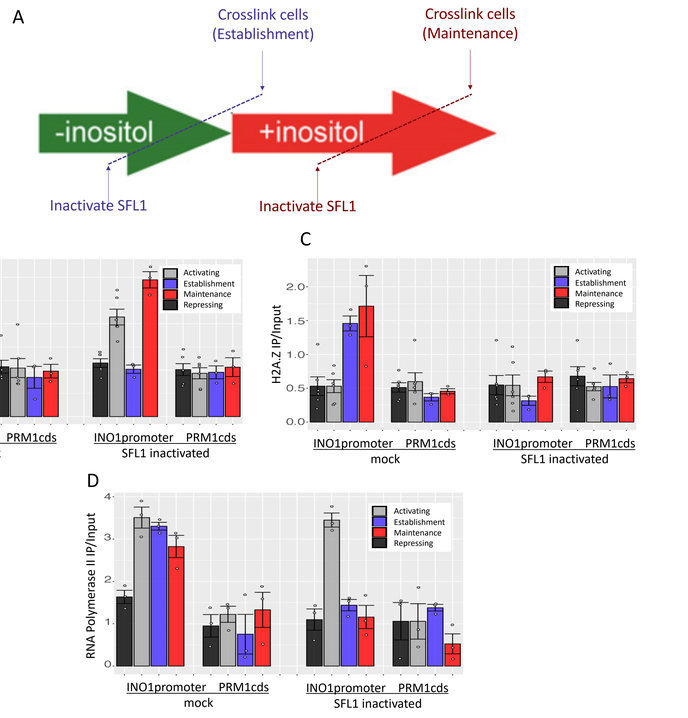

Figure 4: **SFL1 plays roles in both the establishment and the maintenance of the memory-specific chromatin state** A) Schematic for investigating the separate roles of SFL1 in the establishment and the maintenance of the memory-specific chromatin marks and presence of poised RNA Polymerase II. B) H3K4me2, C) H2A.Z, and D) RNA Polymerase II ChIP at the *INO1* promoter region in the conditions of repressing (+inositol), activating (-inositol), and 2 hours with SFL1 removed either before (establishment) or after (maintenance) the switch into the memory state.

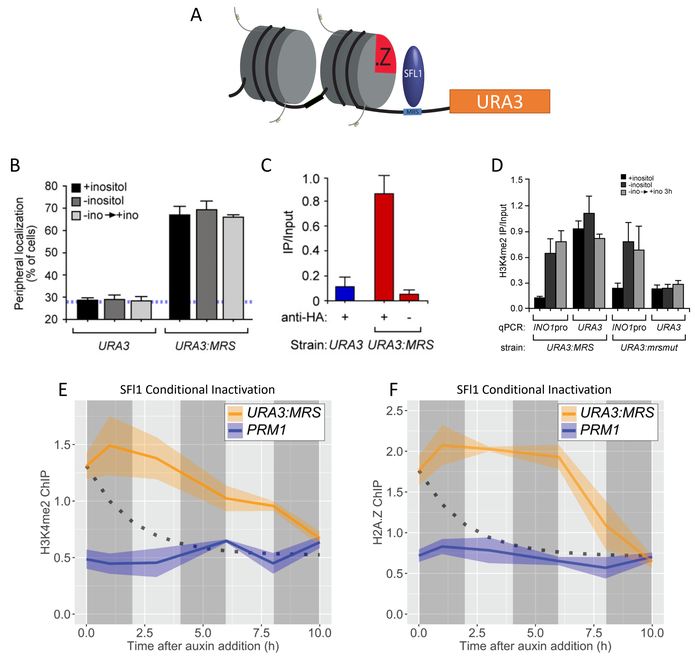

Figure 5: **Memory-specific chromatin marks are epigenetic when induced at ectopic locus by Memory Recruitment Sequence (MRS)** A) Model for integration of MRS at uninduced URA3 locus. B) Percent of cells that show URA3 at the nuclear periphery when in repressing (+inositol), activating (-inositol), and memory (overnight –inositol, then 3 hours repressing) conditions with or without MRS integration at URA3. C) ChIP-qPCR against HA-H2A.Z in strains either with or without MRS integrated at URA3. D) ChIP-qPCR against H3K4me2 at INO1 promoter region and URA3 under repressing, activating, and memory conditions (see above) when either the MRS or *mrsmut* is integrated at URA3. ChIP-qPCR against E) H3K4me2 or F) H2A.Z at URA3 when the MRS is integrated at URA3 after conditional inactivation of SFL1 by Auxin-Induced Degron (AID) technique.

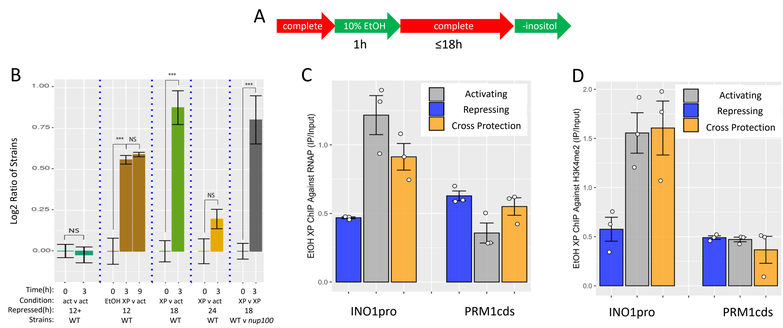

Figure 6: **Heat Shock Mimic Ethanol Treatment leads to Cross Protection for inositol starvation similar to the memory state** A) Schematic of 10% Ethanol cross protection treatment. B) Competitive fitness benefit of the Ethanol Cross Protection treatments in WT and *nup100Δ* strains with 12, 18, and 24 hour repression times. C) RNA Polymerase II ChIP-qPCR at the INO1 promoter region in activating (-inositol), repressing (complete), and XP (10% Ethanol 1 hour -> 18 hours complete). D) H3K4me2 ChIP-qPCR at the INO1 promoter region in activating (-inositol), repressing (complete), and XP (10% Ethanol 1 hour -> 18 hours complete).

The INO1 gene experiences a phenomenon known as epigenetic transcriptional memory which is characterized by movement to the nuclear periphery, interaction with Nup100, a memory-specific chromatin state and PIC. This results in more rapid mRNA production and a competitive fitness benefit. The establishment and maintenance of these chromatin changes across generations are seen to be controlled by differing mechanisms and are self-perpetuating when induced at an ectopic site by incorporation of a Memory Recruitment Sequence. Components of this phenomenon are also seen when INO1 is induced by a heat shock mimic showing variety in maintenance ability depending on how the gene is induced.

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