Just the Facts about Regulating Cenh3 Localization

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Accurate chromosome segregation relies upon a protein machine known as the kinetochore, which attaches to specific sites on chromatin known as centromeres. The identity of centromeres is specified at least in part by the replacement of histone H3 in nucleosomes with a structurally distinct variant known as CenH3 (also called CENP-A in humans and Cse4 in yeast). Incorporation of CenH3 outside of centromeric chromosomes can lead to the formation of dicentric chromosomes and subsequent chromosome breakage, so cells have multiple mechanisms in place to control CenH3 distribution. One such mechanism depends on the histone chaperone complex Facilitates Chromatin Transactions (FACT), which occludes CenH3 from chromatin by promoting the reassembly of H3-containing nucleosomes following transcription. Postdoctoral fellow Gary Deyter, in the laboratory of Dr. Sue Biggins (Basic Sciences Division), further investigated the regulation of CenH3 localization by the FACT complex and found a surprising link between FACT function and proteolytic degradation of CenH3.

Previous studies of FACT have suggested that it interacts with the ubiquitin ligase Psh1, which the authors have previously shown to control the degradation of CenH3 (Ranjitkar et al., 2010). The authors thus tested if loss of FACT affected CenH3 levels. They found that conditional depletion of Spt16, a component of FACT, in conjunction with inhibition of translation, resulted in stabilization of CenH3. This indicates a connection between FACT and targeting of CenH3 to the proteasome. Overexpression of CenH3 was also toxic to both Psh1 and FACT mutant cells. Furthermore, depletion of Spt16 in a Psh1 mutant background resulted in increased levels of CenH3 in chromatin.

Co-immunoprecipitation experiments revealed that it was the Spt16 subunit of FACT that interacts with Psh1, so the authors next texted if the interaction between FACT and Psh1 was necessary for the ability of Psh1 to target CenH3 for degradation. Removal of the FACT-binding domain of Psh1 impaired CenH3 degradation both in vitro and in vivo, and overexpression of CenH3 in the yeast strain expressing the Psh1 FACT-binding mutant severely impaired its growth, perhaps due to increased chromatin incorporation of CenH3 in this strain.

FACT promotes nucleosome disassembly, leading the authors to speculate that this role of FACT might facilitate Psh1-mediated degradation of CenH3 by evicting CenH3 from chromatin. Indeed, nucleosome structure prevented CenH3 ubiquitination by Psh1, indicating that undisrupted nucleosome structure is an impediment to Psh1-mediated degradation of CenH3. Additionally, Psh1 association with CenH3 was also impaired in yeast expressing the FACT-binding deficient Psh1 mutant.

In summary, in this study the authors discovered a new connection between a chromatin-regulating complex and a ubiquitin ligase, leading to the elucidation of a novel molecular mechanism for the maintenance of genome integrity. It will be interesting to determine if this mechanism of regulation of CenH3 localization is conserved in humans, where its dysregulation might lead to the formation of ectopic centromeres and chromosome breakage.

Deyter GMR, Biggins S. 2014. The FACT complex interacts with the E3 ubiquitin ligase Psh1 to prevent ectopic localization of CENP-A. *Genes Dev* 28(16):1815-1826.

See also: Ranjitkar P, Press MO, Yi X, Baker R, MacCoss MJ, Biggins S. 2010. An E3 ubiquitin ligase prevents ectopic localization of the centromeric histone H3 variant via the centromere targeting domain. *Mol Cell* 40(3):455-464.

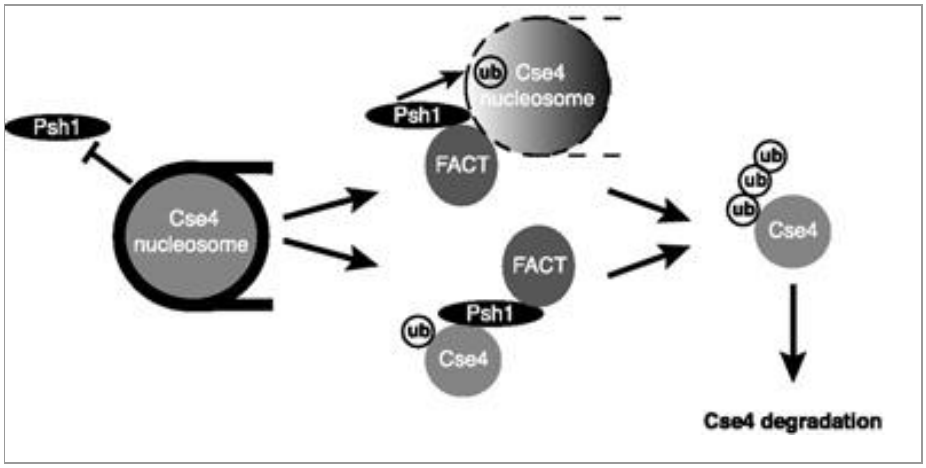

Image provided by Dr. Gabriel Zentner.

Model for the regulation of Cse4 localization by FACT. (Left) Cse4 mislocalizes to euchromatin, where it is resistant to ubiquitination by Psh1. (Center, top) FACT increases the accessibility of Cse4 to Psh1, allowing it to be ubiquitinated. (Center, bottom) FACT may also evict Cse4 from chromatin, allowing it to be ubiquitinated by Psh1. (Right) Ubiquitinated Cse4 is targeted for proteasomal degradation.