

Abstract

The centromeric region in all eukaryotes is characterized by the presence of a histone H3 variant that replaces the canonical histone H3 in centromeric nucleosomes. In the budding yeast *Saccharomyces cerevisiae*, the centromeric histone H3 variant, termed Cse4, is present in a single nucleosome that forms the centromeric structure. The centromeric nucleosome serves as a platform for the assembly of the kinetochore, which ensures the faithful transmission of the genetic information to the daughter cell during the cell cycle. In this study, we identified posttranslational modifications on Cse4 and characterized their contribution to centromere function. We could show for the first time that Cse4 is posttranslationally modified by phosphorylation on serine 33, methylation on arginine 37 and acetylation on lysine 49. Methylation on arginine 37 as well as the acetylation on lysine 49 of Cse4 were determined both by mass spectrometry and by modification-specific Cse4 antibodies. A further analysis of mutations of the modified sites in the N-terminus of Cse4 showed no significant effect in the wild-type. Interestingly, the mutation of Cse4 R37 displayed lethality as well as growth defects in combination with mutations of genes encoding several kinetochore components. Furthermore, *cse4-R37A* caused a defect in the G2/ M-phase of the cell cycle in the absence of the Cbf1 kinetochore protein as well as a maintenance defect of plasmids and chromosome fragments lacking the Cbf1 binding sequence, CDEI. These results indicated that the methylation on arginine 37 of Cse4 contributed to the regulation of chromosome segregation. While Cse4 methylation did not affect its deposition at the centromere, the mutation of Cse4 R37 significantly reduced the recruitment of two kinetochore proteins to the centromeric region. Together with the fact that the level of Cse4 R37 methylation was increased in S-phase arrested cells, these results suggest that the methylation on arginine 37 of Cse4 supports the recruitment of kinetochore components to the centromere. Surprisingly, the additional mutation of lysine 49 to arginine led to the suppression of the growth defects of *cse4-R37A* suggesting an antagonistical effect between both modification sites. In summary, our data show that the centromeric histone H3 variant is posttranslationally modified, and that the methylation on arginine 37 of Cse4 contributes to the recruitment of kinetochore proteins to build a functional kinetochore for accurate chromosome segregation.