Investigating histone deubiquitination: Xenopus manipulations

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Method Article

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Abstract

Introduction

Posttranslational histone modifications play important roles in regulating chromatin structure and function. One example of such modifications is histone ubiquitination, which occurs predominately on H2A and H2B. Although the recent identification of the ubiquitin ligase for histone H2A has revealed important roles for H2A ubiquitination in Hox gene silencing as well as in X inactivation, the enzyme\(s) involved in H2A deubiquitination and the function of H2A deubiquitination are not known. Here we report the identification and functional characterization of the major deubiquitinase for histone H2A, Ubp-M. Ubp-M prefers nucleosomal substrates in vitro, and specifically deubiquitinates histone H2A but not H2B in vitro and in vivo. Importantly, knockdowns of Ubp-M result in slow cell growth rates, which are due to defects in the mitotic phase of the cell cycle. Furthermore, we demonstrate that Ubp-M regulates Hox gene expression through H2A deubiquitination and that blocking the function of Ubp-M results in defective posterior development in Xenopus laevis. Therefore, this study identifies the major deubiquitinase for histone H2A and demonstrates that H2A deubiquitination plays a critical role in cell cycle progression and gene expression.

Reagents

Xenopus RNA probes Ubp-M antigen and antibody

Procedure

1. Inject 300-600 pg IgG or anti-Ubp-M antibody into both blastomeres of two-cell stage embryos. 2. Collect the embryos at tailbud stages for _in situ_ hybridization with HoxD10, Sox2, Otx2, En2 and Krox20 antisense RNA probes, or at tadpole stages for morphological assessment of the effects. 3. For specificity control, mix an equal amount of Ubp-M antigen peptide with the antibody on ice for at least 30 min before coinjection into the embryos. 4. Perform whole-mount _in situ_ hybridization as described \(Harland, 1991).

References

Harland, R. _In situ_ hybridization: an improved whole-mount method for _Xenopus_ embryos. _Methods Cell Biol_. **36**, 685-695 \(1991).