served. H2A.Z is deposited into nucleosome as H2B-H2A.Z histone heterodimer, and this deposition is mediated by an ATP-dependent chromatin-remodeling complex termed SWR1 in budding yeast and SRCAP in vertebrates. The deposition of H2A.Z regulates transcription, DNA damage repair, and chromosome segregation. While the presence of H2A.Z is conserved among eukaryotes, its functions in eukaryotes have not been analyzed. To approach the molecular evolution of H2A.Z, we firstly expressed vertebrate H2A.Z (vH2A.Z) in yeast H2A.Z (yH2AZ) deletant. However, vH2A.Z was not deposited into yeast nucleosome and did not rescue phenotypic defects in the yH2A.Z deletant. When yH2A.Z and vH2A.Z structure are compared, they are very similar in the sequence recognized by SWR1 and SRCAP. On the other hand, the sequence interacting with H2B histone is not conserved between them. This observation raises a possibility that the intermolecular interaction mode between H2A.Z and H2B, which is required for the deposition of H2A.Z into nucleosome, is not conserved between yH2A.Z and vH2A.Z. To test this hypothesis, we expressed a vH2B-vH2A.Z fusion histone in the yH2A.Z deletant. This vertebrate fusion histone was successfully deposited into yeast nucleosome in a SWR1 complex-dependent manner, and it partly complemented phenotypic defects in the yH2A.Z deletant. This observation suggests that the deposition machinery and functions of yH2A.Z and vH2A.Z are evolutionarily conserved. We expect that the vH2B-vH2A.Z fusion histone contributes to further analysis of epigenetic regulation by H2A.Z and its evolutionary conservation.

doi: http://dx.doi.org/10.7124/bc.0009E8

K-4. UVA irradiation strengthened an interaction between UBF1/2 proteins and H4K20 di-/tri-methylation

L. Stixová, D. Komůrková, A. Svobodová Kovaříková, E. Bártová

Institute of Biophysics of the Czech Academy of Sciences, Královopolská 135, CZ-612 65, Brno, Czech Republic.
komurkova@ibp.cz

Repair of ribosomal DNA (rDNA) is a very important nuclear process due to the most active transcription of ribosomal genes. Proper repair of rDNA is required for physiological biogenesis of ribosomes. Here, we analyzed the epigenetics of the DNA damage response in a nucleolar compartment, thus in the ribosomal genes studied in nonirradiated and UVA-irradiated mouse embryonic fibroblasts (MEFs). Using advanced microscopic techniques, we analyzed the distribution pattern and interactions of transcription factors UBF1/2 and histone modifications H3K9me3, H4K20me2 and H4K20me3. Interactions of these proteins were confirmed by co-immunoprecipitation. Furthermore, we used ChIP-PCR analysis to examine an abundance of UBF1/2, H3K9me3, H4K20me2 and H4K20me3 at the rDNA promotor and rDNA encoding 28S rRNA. We found that the promoter of ribosomal genes is not abundant on H4K20me2, but it is densely occupied by H4K20me3. Ribosomal genes, regulated via UBF1/2 proteins, were characterized by an interaction between UBF1/2 and H4K20me2/me3. This interaction was strengthened by UVA irradiation that additionally causes

a focal accumulation of H4K20me3 in the nucleolus. No interaction has been found between UBF1/2 and H3K9me3. Interestingly, UVA irradiation decreases the levels of H3K9me3 and H4K20me3 at 28S rDNA. Altogether, the UVA light affects the epigenetic status of ribosomal genes at 28S rDNA and strengthens an interaction between UBF1/2 proteins and H4K20me2/me3. Based on these results, we conclude that the injury in the region encoding 28S rRNA is likely recognized via H4K20me3-dependent repair mechanism. Studied histone signature in this genomic region was, contrary to histone markers at the rDNA promoter, significantly affected by UVA light.

This work was supported by Czech Science Foundation (grant number: 18-07384S). Work was also supported by Strategie AV21, programme Qualitas, the Center for Epigenetics (68081707).

doi: http://dx.doi.org/10.7124/bc.0009E9

K-5. Investigation the role in mRNA export of the actin binding protein, Moesin

Ildikó Kristó[1], Csaba Bajusz[1], Péter Borkúti[1], Zoltán Kovács[1], Aladár Pettkó-Szandtner[2], Péter Vilmos[1]

[1] HAS BRC, Institute of Genetics, 6726 Szeged, Temesvári krt. 62., Hungary; [2] HAS BRC, Institute of Biochemistry, 6726 Szeged, Temesvári krt. 62., Hungary
kristoi@brc.hu

Accurate and precise control of gene expression is critical for cell survival in order to respond to cellular stress and environmental stimuli. Gene activity is tightly regulated at the level of transcription and translation but mRNA export which links the two processes also plays key role in gene regulation. During RNA export, several specific proteins are recruited to the transcribed RNA molecule where they form an RNA-protein complex, called messenger Ribonucleoprotein Particle (mRNP). In our laboratory we are studying the nuclear function of Moesin, the single cytoskeletal actin-binding ERM protein in Drosophila melanogaster. ERMs (Ezrin, Radixin and Moesin) compose a highly conserved group of proteins and carry out many crucial cytoplasmic functions including reorganization of the actin cytoskeleton, cell survival, membrane dynamics or cell migration. Previously we demonstrated that the Moesin protein is present also in the nucleus where it shows clear co-localization with mRNA export factors. In a functional assay we observed the accumulation of total mRNA in the nucleus upon RNAi against moesin in cultured cells and *in vivo* as well, demonstrating that the inhibition of Moesin's function impairs mRNA export. As the detailed molecular mechanism underlying the nuclear activity of Moesin is still not known, we aim to identify the nuclear protein interaction partners of Moesin in order to get a deeper insight into the role and significance played by the actin-binding ERM proteins in nuclear mRNA export. Methods: Immunostaining of larval polytene giant chromosomes, Drosophila cell culture, mass spectrometry analysis, protein co-immunoprecipitation Results: In the mass spectrometry analysis Moesin pulled down almost the entire Mediator complex which is a multisubunit protein complex function in gene expression regulation and mRNA export as well. Moesin also showed colocalization with Mediator proteins on Drosophila larval giant