CE-MS analysis of epigenetically active metabolites during *in vitro* chromatin assembly

Marco Borso, PhD

Ludwig-Maximilians-Universität, Munich

Abstract

Histone modifying enzymes frequently use key metabolites to set up specific histone modification patterns, which in turn affect the structure and function of chromatin. The local concentration and availability of specific metabolites is therefore a crucial parameter to determine levels of gene expression and epigenetic inheritance. However, the molecular mechanisms underlying the generation of these metabolites within the nucleus and their precise influence on histone modifications, have been poorly understood.

We use a well-established *in vitro* model (drosophila preblastodermic embryo extracts) which can assemble chromatin *in vitro* and set certain histone modifications. To vary the concentration of important metabolites and to assess their synthesis in situ, extracts were dialyzed overnight and then used for the *in vitro* chromatin assembly experiment. Samples were extracted using a liquid-liquid extraction and then analyzed with CE-MS to measure the levels of key metabolites that were generated during chromatin assembly.

The intrinsic sensitivity of CE-MS allowed us to inject only a few nanoliters, corresponding to approximatively 5 fly embryos and allowed us to detect a wide range of key epigenetic metabolites, including different amino acids (SAM and SAH). Our results showed that most of the metabolites were generated *in situ* during chromatin assembly which can then be used by metabolic enzymes or to set histone modifications.