Department of Biological Sciences
Ph.D. Viva voce

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Biochemical characterization of histone H3 clipping by chicken liver H3 specific protease: Clipping is regulated by structure of histone H3 and inhibitor, stefin B

Structure of chromatin is highly dynamic as it has to allow interaction of various proteins to regulate DNA replication, transcription, repair, and recombination etc. There are various mechanisms which regulate dynamic nature of the chromatin such as DNA methylation, chromatin remodeling, histone post translational modifications, histone variants and clipping of histone tails etc. The significance of ATP-dependent chromatin remodeling and post-translational modifications of histones is well established. However, the cellular function as well as regulation of proteolytic processing of histones is still not clear. Few studies have reported clipping of histone proteins in yeast, chicken and mammals. For example; Cathepsin L dependent clipping of histone H3 occurs in mouse (1) which is required for differentiation of stem cells. In chicken, glutamate dehydrogenase has been identified as a protease for age-dependent clipping of histone H3 in liver (2) and PRB1 in yeast (3). Despite these studies, mechanism, substrate specificity and physiological significance of this epigenetic process are still not clear. We have studied the role of histone H3 and the inhibitor, stefin B in clipping of H3 by 'chicken liver H3 specific protease' (4). We have further characterized the clipping sites in histone H3 and mechanism of inhibition by stefin B. By employing site directed mutagenesis and *in vitro* biochemical assays, we have identified QVVAG region and C-terminus of stefin B protein to be very crucial for inhibition of the protease activity. In addition to stefin B, stefin A and cystatin C were found to act as weak and strong inhibitor respectively (5). We also observed that 'chicken liver H3 protease' (Glutamate dehydrogenase) creates three distinct clipping sites in recombinant human histone H3. However, post translationally modified histone H3 (isolated from chicken brain and *S. cerevisiae* wild type cells) showed different clipping pattern. Furthermore the clipping activity was also found to be regulated by N-terminal tail and globular domain of histone H3 (6). Altogether, our biochemical studies have revealed three distinct clipping sites in recombinant human histone H3 and their regulation by the structure of histone H3, histone modifications marks and stefin B.

References:

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