Functional characterisation of the histone H2A variant, H2A.F/Z.

by

Michael John Clarkson

A thesis submitted for

DOCTOR OF PHILOSOPHY

of the

THE UNIVERSITY OF ADELAIDE

UNIVERSITY OF ADELAIDE

December 2006
ABSTRACT

Since the DNA in eukaryotes is packaged into chromatin it is perhaps not surprising that cellular activities that involve DNA, such as replication, recombination, transcription and mitosis also intimately involve chromatin. In each of these cellular activities, particular chromatin structures have been identified that have characteristic biochemical properties. These different properties can be generated by altering the composition of chromatin and/or by the action of specialised enzymes on chromatin constituents.

The basic subunit of chromatin is the nucleosome which packages 147bp of DNA by wrapping it twice around an octameric protein complex consisting of two molecules each of histones H2A, H2B, H3 and H4. The functional and biochemical properties of chromatin can be altered at the nucleosome level by post-translational modifications of histones, ATP-dependent remodelling of histone-DNA contacts in the nucleosome or by incorporation of histone variants.

This thesis details the characterisation of a histone H2A variant, H2AF/Z. A unique and important role has been ascribed to this protein on the basis of demonstrations that null mutations in the H2A.F/Z gene are lethal in mouse (Thonglairam, 1996), *Tetrahymena thermophila* (Liu et al., 1996a) and *Drosophila melanogaster* (van Dam and Elgin, 1992). Although the actual function of this histone is unknown, an enrichment of H2AF/Z in transcriptionally competent chromatin has led to the hypothesis that it is involved in the establishment or maintenance of transcription in the nucleus (Gabrieli et al., 1981; Wenkert and Allis, 1984; Allis et al., 1986; Huang et al., 1986; Ridsdale and Davie, 1987; Stargell et al. 1993).

In chapter 3, experiments were conducted in *Drosophila* to identify domains of His2AvD that functionally distinguish it from the core H2A histone in vivo. Prior to the commencement of this project, it had been demonstrated that null mutant lethality in *Drosophila* could be rescued with a transgene derived from a 4.1kb genomic DNA fragment containing the His2AvD gene (van Dam and Elgin, 1992). Based on this result, a strategy was employed here where regions or "cassettes" encoding amino acids in the His2AvD rescue fragment were mutated, in vitro, to the equivalently positioned H2A.1 residues. Lines of flies containing stably integrated wild type and mutant His2AvD transgenes were generated by *P*-element mediated transformation of *Drosophila*. These transgenes were then tested for their ability to rescue His2AvD null mutant lethality. Interestingly, this experiment demonstrated that unique features of His2AvD reside in a C-terminal region of the protein not in the histone fold. This C-terminal region is part of a short α-helix that, in H2A, is buried deep inside the nucleosome core and appears to
be important for the stability of the histone octamer (Luger et al., 1997). To characterize the extent of rescue afforded by the mutant transgenes, the null mutant phenotype was characterized using phenotypic and molecular developmental markers. Analysis of the phenotype of His2AvD null mutants found that these individuals undergo a protracted third instar and then die without entering pupation. Consistent with this observation, transcripts from developmental genes activated site in third instar are not detected in His2AvD null mutant individuals. Interestingly, heat shock genes can still be induced after this developmental block.

In chapter 4, the distribution of His2AvD was characterized in Drosophila using a transgene that encoded His2AvD with green fluorescent protein (GFP) fused to the C-terminus. It was demonstrated that the transgene could provide functional His2AvD protein by being able to rescue null mutant lethality. During early embryonic development, the appearance of His2AvD in nuclei coincided with the onset of transcription. Subsequently, GFP associated fluorescence was observed in all nuclei at all stages of embryonic and larval development and in adult somatic tissues. In nuclei, His2AvD was widely, but not homogeneously, distributed. His2AvD-GFP fusion protein remained associated with chromatin throughout the cell cycle, including during mitosis when transcription is shut down.

The tissue specific expression and protein distribution of mouse H2A.Z was also examined. Mice were used to examine H2A.Z expression and protein concentration in different tissues because tissue samples from mice are more convenient to obtain and are less subject to contamination by other tissues on dissection than they are from Drosophila. In adult mice, the amount of H2A.Z transcript varies by up to two orders of magnitude between tissues and is directly proportional to the rate of cell turnover. H2A.Z protein is present at the same concentration, relative to the core histones, in all tissues examined. The subcellular location of mouse H2A.Z was also investigated on tissue sections and cell culture monolayers using antibodies directed against the C-terminus of the protein. Histone H2A.Z containing chromatin is generally distributed throughout nuclei but is not associated with transcriptionally silent satellite DNA sequences.

In summary, experiments conducted in this thesis identified that His2AvD provides its unique function through a region at the C-terminus of the protein. In addition, results presented here support the temporal and spatial association of histone H2A.F/Z with transcriptional activity.
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Note added in proof

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