NUCLEAR LAMINS: MAJOR DETERMINANTS OF NUCLEAR ARCHITECTURE

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The Type V family of intermediate filament (IF) proteins, the nuclear lamins, are found primarily in the nucleus of mammalian cells. The lamins are divided into two subtypes, A and B, the expression of which is developmentally regulated. Within the interphase nucleus, the lamins are concentrated in the lamina, a complex polymeric network located at the interface between chromatin and the nucleoplasmic face of the nuclear envelope.

For many years it was thought that nuclear lamin networks were static throughout interphase, and were altered only with respect to their molecular organization during the processes of nuclear envelope breakdown preceding mitosis and during nuclear reassembly in daughter cells. However, we now have evidence that the lamins are dynamic and change their organizational states throughout the cell cycle. This has been determined by immunofluorescence confocal microscopy and a variety of in vivo labeling methods including the expression of GFP-tagged A- and B-type lamins, fluorescence recovery after photobleaching (FRAP), and the microinjection of mutant lamins designed to disrupt the structure of the lamina. The results of our studies of GFP-lamin transfected cells demonstrate their dynamic properties throughout the cell cycle. For example, the lamina frequently alters its shape, and nucleoplasmic lamin foci appear, disappear and are translocated within the nucleoplasm during interphase. During S-phase, these foci are closely associated with DNA replication sites, or replicons, in cultured mammalian cells. Using the *Xenopus* interphase extract system we have been able to demonstrate that the function of these replicons is reversibly inhibited when N-terminal deleted mutants of human lamin A are introduced into nuclei during and following their assembly in vitro. This disruption appears to take place at the interface between the initiation and elongation phases of DNA replication as suggested by the use of inhibitors such as CIP and AraC. Recently we have also been studying the role of lamins in the in vitro assembly of *Xenopus* nuclei. This involves the use of *Xenopus* lamin B mutants which disrupt nuclear envelope assembly, probably through the inhibition of membrane vesicle fusion around chromatin.

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