LAW AND ORDER IN THE NUCLEUS: DYNAMICS OF REPLICATION FACTORIES IN LIVING CELLS

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Several biological processes within the eukaryotic nucleus occur in discrete subnuclear compartments which, in contrast to cytoplasmic organelles, are not separated by membranes. Different factors involved in a particular process are found concentrated together at the subnuclear sites where the respective process takes place, which is designated "functional organization of the nucleus". DNA replication occurs in discrete subnuclear foci during S-phase. We are studying the mechanism and timing of the assembly of these higher order structures. As a first step towards this goal, we and others have identified a number of proteins that specifically redistribute to replication foci during S phase. These include cell cycle factors, DNA replication, repair and methylation enzymes. We could also show that DNA ligase I as well as DNA methyltransferase (Dnmt1) contain at their N-terminus a distinct targeting sequence, that is necessary and sufficient for association with replication foci. These targeting sequences are dispensable for enzyme activity in vitro but are most likely required for the efficient ligation of Okazaki fragments and maintenance of the methylation pattern in vivo and may thus ensure genome integrity and correct duplication of the epigenetic information in mammalian cells. In order to investigate the dynamics of these replication foci, we used GFP as an in vivo label to tag the proliferating cell nuclear antigen (PCNA), a central component of the DNA replication machinery. The fusion protein localized as the endogenous PCNA at subnuclear replication foci and could be expressed in stable cell lines albeit only at very low levels. We have performed time lapse studies and FRAP analysis using these cell lines to test the hypothesis whether replication machines are stably anchored in the nucleus or whether they move throughout the nucleus during S-phase. Our results show that individual replication foci assemble at a particular nuclear site, keep this position for a given period, then disassemble and reassemble at new sites. Assembly and disassembly of different foci occur asynchronously suggesting that replication origins also fire asynchronously within these microscopically visible clusters. We are currently testing whether DNA is pulled into replication factories for replication and afterwards expelled.