

SEMINAIRES DU DEPARTEMENT **“ Biologie du Cancer ”**

IPBS , salle de conférence n° 1 , niveau 2
205 route de Narbonne TOULOUSE CEDEX 4

Mardi 18 Décembre 2007, 11 heures

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“ Regulation of DNA replication initiation and fork progression in human cells: single molecule and single cell analysis ”

In presence of DNA damage, DNA replication origin firing is known to be inhibited in a checkpoint-dependent manner. However, the regulation of fork progression is not fully understood. Taking advantage of a single DNA molecule approach based on molecular combing we showed that replication forks emanating from neighboring origins move with nearly the same velocity and are co-regulated with the distance between those origins. We also demonstrate that the helicase BLM, whose absence in Bloom syndrome patients induces genomic instability and cancer development, is involved in the normal progression of the replication fork.

More recently, to investigate the contribution of DNA replication initiation and elongation to the intra-S-phase checkpoint, we examined cells treated with the specific topoisomerase I inhibitor camptothecin. CPT is a potent anticancer agent which inhibits DNA replication rapidly and for several hours following drug removal. That inhibition occurred preferentially in late-S, compared to early-S-phase cells, as was observed by nucleotide incorporation in replication foci into individual cells. Further analysis on single DNA molecules showed that both initiation and fork progression were inhibited. Normal kinetics of DNA replication was restored with specific Chk1- inhibitors (UCN-01 and CHI-124) or with a specific Chk1 siRNA. However, abrogation of the checkpoint markedly enhanced CPT-induced DNA damage at replication sites where histone γ -H2AX colocalized with replication foci. These data indicate that, together with initiation, fork progression is also actively regulated in a checkpoint-dependent manner relying on Chk1.

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