

University of Maryland Baltimore Graduate School

# Announcement of Doctoral Dissertation Defense\*

Candidate: Wei Song

Date, Time, and Place: December 5<sup>th</sup>, 2007, 2pm, BRB, 9 floor conference room

Dissertation Title: Interplay among DNA ligase I, DNA Clamp Loaders and DNA Clamps

## Dissertation Abstract\*\*:

Human DNA ligase I joins Okazaki fragments during DNA replication and completes certain excision repair pathways. The participation of DNA ligase I in these transactions is directed by physical and functional interactions with proliferating cell nuclear antigen (PCNA), a DNA sliding clamp, and, replication factor C (RFC) that loads the PCNA clamp onto DNA. PCNA and RFC are the prototypic members of an emerging family of DNA sliding clamps and clamp loaders that are involved in cell cycle checkpoints, sister chromatid cohesion, and genome stability in addition to DNA replication. In the cell cycle checkpoint response activated by either replication blockage or DNA damage, the DNA sliding clamp is a heterotrimer composed of hRad9, hRad1, and hHus1 proteins. The hRad9-hRad1-hHus1 (9-1-1) clamp is loaded onto DNA by the heteropentameric hRad17-RFC clamp loader. Both the checkpoint and replicative clamp loader complexes contain the same four small RFC subunits, p36, p37, p38, and p40, but are distinguished by their large subunits, hRad17 and RFCp140, respectively. The present study focuses on characterizing the interplay of DNA ligase I with DNA sliding clamps and clamp loaders, in particular the 9-1-1 clamp complex and the hRad17-RFC. Here we show that DNA ligase I interacts with the hRad17 subunit of the hRad17-RFC cell cycle checkpoint clamp loader, and with each of the subunits of its DNA sliding clamp, 9-1-1 complex. In agreement with published studies, we show that 9-1-1 stimulates DNA joining by DNA ligase I. Although DNA ligase I is progressively phosphorylated during cell cycle progression, the phosphorylation status of DNA ligase I does not affect its interaction with either 9-1-1 or PCNA. A conserved motif at the amino terminus of DNA ligase I plays a major role in its physical interaction with PCNA and is required for the participation of DNA ligase I in DNA repair and DNA replication *in vivo*. Here we show that the DNA binding domain (DBD), one of three domains within the DNA ligase I catalytic fragment, also interacts with PCNA. Interestingly, the DBD preferentially interacts with the PCNA trimer, suggesting that it may bind across the subunit/subunit interface of PCNA monomers within the PCNA trimer. In addition, the DBD of DNA ligase I also participates in the interaction with the 9-1-1 clamp. The results of these studies suggest mechanisms by which the DNA clamp may contribute to the conversion of DNA ligase I from its elongated structure in the absence of DNA to the compact ring formed on nicked DNA. Unlike the replicative clamp loader, which inhibits DNA joining, the checkpoint clamp loader weakly stimulates DNA joining. Similar results were obtained with the homologous *S. cerevisiae* proteins indicating that the interaction between the replicative DNA ligase and checkpoint clamp loader is conserved in eukaryotes. Furthermore, we show that *in vitro* hRad17-RFC preferentially interacts with and specifically stimulates dephosphorylated DNA ligase I and that *in vivo* there is an increased association between DNA ligase I and hRad17 in S phase following DNA damage and replication blockage that occurs concomitantly with DNA damage-induced dephosphorylation of chromatin-associated DNA ligase I. These results indicate that the *in vivo* interaction between DNA ligase I and the checkpoint clamp loader is probably regulated by post-translational modification of DNA ligase I and suggest that this interaction may be involved in recovery following checkpoint activation. In support of this hypothesis, we found that DNA ligase I-deficient cell lines are defective in checkpoint recovery.

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Dr. Alien Lu-Chang, Professor

Dr. Teresa Wilson, Assistant Professor

Dr. Tom Ellenberger, Professor

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*\*The Open Presentation is open to the university community and invitees of the candidate. Any member of the Graduate Faculty may observe the Final Examination. Only committee members may vote. For more information, see **Procedures for Examination of the Doctoral Dissertation.***

*\*\*You must type your abstract on this form in the space provided.*