

## Interplay between Human FANCD2 and the MRE11-RAD50-NBS1 Complex

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**Background:** Fanconi anemia is a recessive disorder with congenital abnormalities, progressive anemia and increased risk of malignancy. There are 13 FA complementation groups (A, B, C, D1, D2, E, F, G, I, J, L, M and N). Eight of these proteins assemble in a nuclear core complex required for monoubiquitination or activation of the FANCD2 protein. FANCD2 monoubiquitination occurs during S phase progression or in response to DNA damage such as DNA crosslinking agents. The MRE11-RAD50-NBS1 (MRN) complex is also a critical component of the cellular response to DNA double-strand breaks during S-phase. Mutations in the MRE11 gene are responsible for the ataxia-telangiectasia-like disorder, characterized by defective checkpoint responses and high level of chromosomal abnormalities. Several evidences connect FANCD2 to the DNA damage signaling and the MRN complex: (i) two parallel pathways downstream of ATR lead to interstrand crosslink-induced S-phase checkpoint. One branch depends on Chk1 activity and the other on the FANC-MRN complex; (ii) FANCD2 interacts with NBS1 and (iii) MRE11 and FANCD2 co-localize during S-phase and ionizing radiation treatment. However, the control of FA proteins by MRE11-RAD50-NBS1 remains unclear at the biochemical level.

**Objective:** Our main objective is to characterize the FANCD2 protein and its relationship with the MRE11-RAD50-NBS1 complex at the biochemical and cellular level.

**Methods:** Biochemical assays, immunoprecipitations, ChIP analysis as well as cellular approaches such as immunofluorescence were used.

**Results:** To monitor whether FANCD2 could be activated by a single DNA damage event, we developed immunofluorescence and chromatin immunoprecipitation methods on human cells where a unique DSB can be created *in vivo* (Rodrigue *et al*, 2006). Using this system, we observed a single focus of FANCD2 which co-localized with 3B3-H2AX and MRE11. Chromatin immunoprecipitations revealed that endogenous FANCD2 localizes in the vicinity of the DSB. Moreover, the localization of FANCD2 was dependent on arginine methylation of human MRE11. Co-localization between MRE11 and FANCD2 were performed during each phase of the cell cycle. In order to characterize the relationship between MRE11, NBS1, RAD50 and FANCD2, baculoviruses were generated for expression in insect cells. GST pull-down assays and immunoprecipitation analyses revealed that FANCD2 interacts with both human MRE11 and NBS1. The FANCD2 protein was purified from insect cells using a novel protocol. Experiments assessing the biochemical effect of FANCD2 on MRN will be presented.

**Conclusion:** Our data indicate that FANCD2 may regulate MRE11 during DSB repair.

**Translational Applicability:** The establishment of a biochemical model for the Fanconi pathway will facilitate the elucidation of the mechanism of the FA pathway and its interplay with DNA repair pathways.