ABSTRACT

Fanconi anemia is an autosomal recessive disorder caused by mutation in one of Fanconi genes and it is manifested by developmental abnormalities, bone marrow failure, predisposition to cancer, cellular sensitivity to cross-linking agents and many other symptoms. Proteins encoded by Fanconi genes and some other proteins are part of Fanconi anemia pathway (FA pathway), which is responsible for DNA repair of an interstrand cross-link (ICL). The repair by this pathway requires monoubiquitination of FANCD2, which is induced and regulated by ATR dependent FANCI phosphorylation. The FANCI phosphorylation initiates the FA pathway but the molecular mechanism of this initialization is not known. Furthermore the proper function of entire pathway requires both: sequence of phosphorylation events of FANCI and monoubiquitination of FANCI:FANCD2 complex .

The principle of this work was to study molecular mechanism of initiation and regulation of FA pathway by FANCI phosphorylation. Therefore phosphomimetic mutants of FANCI have been created to investigate their role in processes leading to FANCD2 monoubiquitination. The main aim was to reveal how the phosphorylation of FANCI affects DNA binding and also DNA binding of FANCI:FANCD2 complex. Since both DNA and FANCI phosphorylation are required for proper FANCD2 monoubiquitination, individual phosphorylation mimetic and phosphorylation dead mutants of FANCI were measured and compared in DNA binding assays and by fluorescence anisotropy. The effect of FANCI phosphorylation on monoubiquitination of FANCD2 was studied using monoubiquitination assays.

Key words: Fanconi anemia, Fanconi anemia pathway, DNA repair pathway, FANCI, FANCD2, phosphorylation of FANCI, monoubiquitination of FANCD2, DNA binding assays

(In Czech)