

Molecular Interplay between the S-phase Checkpoint and FA Core Complex

S.J. Collis¹, A. Ciccia¹, M. O'Driscoll², P. Jeggo², S. West¹, S.J. Boulton³

¹LRI, Clare Hall Laboratories, South Mimms, United Kingdom; ²Genome Stability Centre, Sussex, United Kingdom; ³Cancer Research UK, South Mimms, United Kingdom

Objective: We have recently identified and characterised a novel component of the mammalian S-phase checkpoint: HCLK2. Similar to ATR-defective cells, decreased levels of HCLK2 leads to defective activation of the FA pathway and subsequent increased sensitivity to DNA interstrand cross-linking agents. We were therefore interested in determining how HCLK2, and the S-phase checkpoint, regulates the FA pathway.

Methods: siRNA, immunoprecipitation and Western blotting techniques were used to study the molecular interplay between the mammalian S-phase checkpoint and FA core complex.

Results: We have determined through the use of endogenous co-immunoprecipitation experiments that HCLK2 interacts with both FANCM and its newly identified binding partner FAAP24. Interestingly, depletion of HCLK2 or FAAP24 severely affects the stability of the other protein, suggesting that they might form and function as a heterodimer within the cell. Decreased stability of each protein in the absence of the other protein becomes more apparent following replication stress, and FAAP24-depleted cells share several phenotypes with HCLK2-depleted cells. In contrast, depletion of FANCM appears to stabilise cellular levels of HCLK2, particularly following replication stress. We have previously shown that HCLK2 interacts with ATR and Chk1 as part of the S-phase checkpoint. Consistent with this and the hypothesis that HCLK2 and FAAP24 may function as a heterodimer, immunoprecipitation and gel filtration analyses suggests that ATR, HCLK2, Chk1, FANCM and FAAP24 form a multi-protein complex within the cell. Furthermore, cells treated with either FANCM or FAAP24 siRNA show defects in activation of the S-phase checkpoint.

Conclusions: To our knowledge, this is the first report of direct molecular interactions between components of the mammalian S-phase checkpoint and the FA core complex. Our data suggest that the FA pathway and downstream repair mechanisms are intimately linked with and directly coordinated by the S-phase checkpoint.

Translational Applicability: Our data suggests that components of the S-phase checkpoint may be dysfunctional in a sub-set of FA and/or FA-like disorders. We will present preliminary data to suggest that this indeed may be the case.