Coordinated protein and DNA remodeling by human HLTF and comparison of its activity with Bloom Syndrome helicase protein

Summary of the Ph.D thesis

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Introduction

Human HLTF (Helicase Like Transcription Factor) is a member of the Swi2/Snf2 family of ATP-driven molecular motor proteins. Like many members of Swi2/Snf2 family, HLTF does not exhibit a canonical DNA helicase activity but has an ATP hydrolysis-driven double stranded (ds) DNA translocase activity. The biological importance of HLTF is indicated by the finding that it has a role in replication of damaged DNA and preventing genome rearrangement, and in accord, it is considered as tumor suppressor. The discovery of the replication fork remodeling activity of HLTF, particularly its fork reversal activity, provided a mechanistic explanation for its role in template switch dependent error-free DNA damage bypass. However, a stalled replication fork contains several single-stranded (ss) DNA- and dsDNA-bound proteins such as RPA, RFC, PCNA, and replicative polymerase. How these proteins are displaced before the DNA remodeling occurs has been unknown.

Here we examine whether proteins bound to replication fork like DNA structures inhibit fork remodeling by two distinct fork reversal enzymes, namely HLTF, a Swi2/Snf2 family protein, and Blooms syndrome helicase (BLM), a RecQ family helicase.

We provide evidence that HLTF can specifically remodel replication forks bound by either dsDNA- or ssDNA-binding proteins, which is associated with a novel protein remodeling activity of HLTF. These observations shed light on how masses of proteins surrounding the stalled replication fork can become displaced from the DNA providing thereby access to new damage bypass players.

Goals and objectives

The main goal of the thesis is to compare the two proteins that have been shown to carry out fork regression activity, namely BLM and HLTF. Although both the enzymes have been shown to carry out fork regression activity on a modeled replication fork, we hypothesized that they might possess different mechanisms to regress a stalled replication fork.

Based on this idea we formulated the specific objectives which are listed below.

I. Generation of different kinds of model replication fork like structures to compare the activity of BLM and HLTF

II. To understand the mechanism how a replication fork regressing enzyme overcomes the inhibitory effect of a protein complex at the site of DNA replication.

III. To investigate if HLTF possesses any protein remodeling activity in addition to its DNA remodeling activity.

Methods

Proteins

- Purification of HLTF
- Purification of BLM
- Purification of *E. coli* E111Q EcoRI
- Purification of human replication protein A (RPA)

Generation of Oligo based replication fork-like structures

Protein bound replication fork-like structures

Fork reversal assay

Protein displacement assay

Gel retardation assay

Results and conclusions

HLTF can regress a modeled replication fork bound by dsDNA binding protein

We investigated if HLTF DNA remodeling activity is inhibited by a protein bound to stalled replication fork-like DNA structures. First, we wanted to rule out any specific protein-protein interactions between HLTF and the DNA-bound protein, therefore we chose the *E. coli* E111Q EcoRI endonuclease mutant protein that is selectively defective in DNA cleavage but retained its sequence specific dsDNA binding activity. We generated various homologous replication fork-like DNA substrates containing EcoRI binding sites in the arms, to which we stoichiometrically bound E111Q EcoRI proteins as our gel mobility shift experiment confirmed. The remodeling of these protein-bound DNA structures can be followed by the appearance of 75/75 or 30/30 nucleotide-long double stranded DNAs that would arise upon fork reversal as described. In a control experiment, we used the Bloom helicase (BLM) for which a fork reversal activity has also been reported, and we detected that it was completely inhibited by binding of single E111Q EcoRI proteins to both arms. In contrast, we found that HLTF retained its fork reversal activity on such a substrate and only weak inhibition occurred. In addition, when the fork DNA contained only single EcoRI binding site in one of its arms, HLTF processed the leading or lagging strand-bound protein containing substrates equally well.

Protein displacement from modeled fork requires the DNA translocase activity of HLTF

These results suggested that during fork reversal, HLTF can displace bound E111Q EcoRI proteins from both leading and lagging arms. To confirm the actual removal of the E111Q protein and that it was dependent on the dsDNA translocase activity of HLTF we monitored the displacement of E111Q EcoRI protein from model replication fork by capturing displaced E111Q EcoRI protein on a labeled duplex DNA containing single EcoRI binding site. The displaced E111Q EcoRI was monitored by the appearance of protein bound trap DNA in gel mobility shift experiment. The appearance of band shift only observed with wild type HLTF but its absence in ATPase mutant HLTF supported that HLTF can actively remove E111Q EcoRI from modeled fork and this function is dependent on ATP dependent double strand DNA translocase activity.

HLTF can remodel gapped replication forks bound by ssDNA-binding proteins

Uncoupling of leading and lagging strand synthesis can frequently occur if the replication machinery encounters DNA lesions, which can lead to fork structures containing a ssDNA region that can become covered by ssDNA binding proteins such a RPA. Previous data indicated that some helicases such as BLM has the ability to remove the Rad51 protein bound to ssDNA, but one can not expect ssDNA-bound protein removal, which requires translocation on ssDNA, by a dsDNA translocase such as HLTF. To verify if these kind of ssDNA bound proteins can challenge the fork remodeling activity of HLTF, we generated a 15nt ssDNA gap region in the leading strand of modeled fork where a ssDNA binding protein like RPA or E.coli SSB can be successfully bound. Fork reversal assay on these kinds of substrates reveal that HLTF and BLM can successfully regress a fork even in the presence of ssDNA binding protein. On this special substrate, RPA displacement can be explained by the dsDNA translocase activity of HLTF, and this is consistent with a model that during fork reversal HLTF translocates on the parental duplex DNA when concertedly unwinds the arms of the fork and zips the parental stands and the nascent strands. RPA displacement can not be attributed to a potential interaction between HLTF and RPA since HLTF was also able to remove equally well the E. coli SSB protein from such a substrate. This finding indicates that on some complex DNA substrate, HLTF double-stranded DNA translocase action can also remodel a ssDNA binding protein. Taken together, these experiments provide evidence that HLTF can carry out coordinated protein displacement/DNA remodeling dual function at stalled replication forks.

HLTF can dislodge PCNA & RFC complex from DNA replication fork

To provide evidence that HLTF can indeed remodel proteins expected to be present at a stalled replication fork, we examined if HLTF can overcome the inhibitory effect of the complex of PCNA and RFC bound to a model replication fork substrate. In control experiment, these DNA binding proteins completely inhibited fork reversal by BLM helicase. Importantly, however, HLTF was able to remodel the fork DNA substrate bound by these protein factors of the replication machinery.

Discussion

In general, proteins in the Swi2/Snf2 family have been considered as chromatin remodeling enzymes for nucleosome displacement. However, for some members a special function was found such as for Mot1, which can displace the TATA box-binding protein; for HARP, which can rewind a stably unwound DNA; and for FancM, HLTF, and yeast Rad5, which exhibit fork reversal activity. The discovered coordinated protein displacing/DNA remodeling activity of HLTF further extends the repertoire of the enzymatic ability of this intensively examined protein family, and raises the question if other Swi2/Snf2 proteins exhibit similar activities. Moreover, we suggest that the protein displacing/DNA remodeling activity of HLTF can be important for genome stability as indicated by that in high percentage of cancers HLTF expression is either silenced or various Swi2/Snf2 domain deletion mutant HLTF proteins are expressed.

Publications:

1. ***Yathish Jagadheesh Achar****, David Balogh, and Lajos Haracska*

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Presentations at conferences

- **DNA Repair Meeting**, **Brno**, Czech Republic 2010 “ Rescuing stalled replication fork in a helicase way; different approaches of DNA helicases”

- **Bruno F. Straub memorial conference, Szeged**, Hungary 2009 “Roles of the Bloom’s syndrome helicase in the maintenance of genome stability”

- **Bruno F. Straub memorial conference, Szeged**, Hungary 2005 “Choreography of DNA polymerases; A new model for polymerase switching at the stalled replication fork”