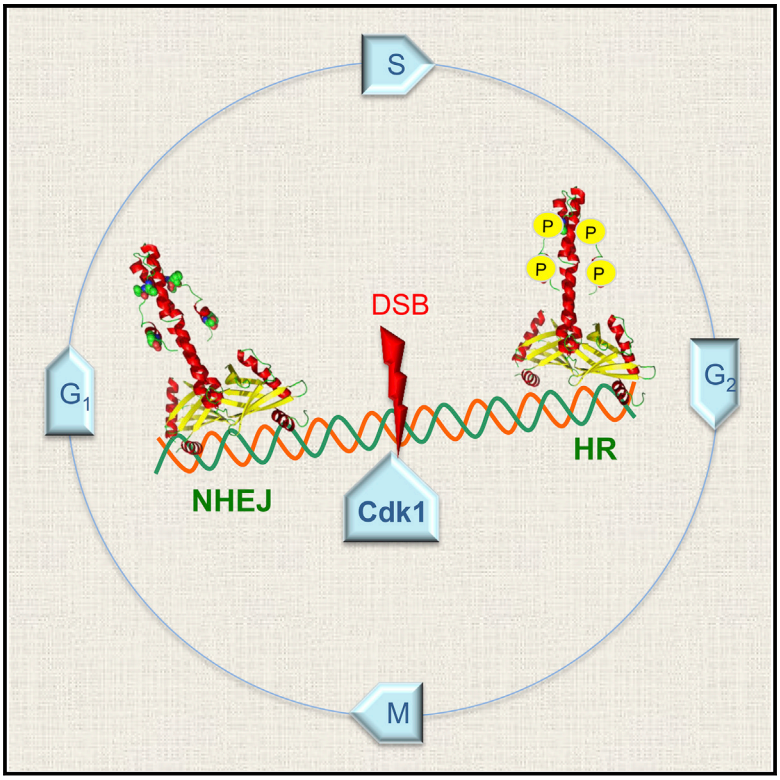
Report

Cell Reports

Cdk1 Restrains NHEJ through Phosphorylation of XRCC4-like Factor Xlf1

Graphical Abstract



Authors

Pierre Hentges, Helen Waller, ...,
Miguel Godinho Ferreira, Aidan J. Doherty

Correspondence

ajd21@sussex.ac.uk

In Brief

Repair of DNA double-strand breaks (DSBs) by homologous recombination is activated by the cell cycle kinase Cdk1. Hentges et al. now find that the NHEJ factor Xlf1 is phosphorylated by Cdk1 and that this modification restrains end-joining in cycling cells. Removal of this regulation alters DSB pathway selection in vivo.

Highlights

- Cdc2^{Cdk1} phosphorylates the core NHEJ factor Xlf1 in fission yeast
- Phosphorylation of Xlf1 inhibits nonhomologous end-joining (NHEJ)
- Cells with phospho-null Xlf1 have elevated levels of NHEJ repair
- NHEJ repair can predominate over HR when Cdc2^{Cdk1} regulation of Xlf1 is lost

Hentges et al., 2014, Cell Reports 9, 2011–2017
December 24, 2014 ©2014 The Authors
http://dx.doi.org/10.1016/j.celrep.2014.11.044

Cell Reports
Report

Cdk1 Restrains NHEJ through Phosphorylation of XRCC4-like Factor Xlf1

Pierre Hentges,[1] Helen Waller,[1] Clara C. Reis,[2] Miguel Godinho Ferreira,[2] and Aidan J. Doherty[1,*]
[1]Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Brighton BN1 9RQ, UK
[2]Instituto Gulbenkian de Ciência, Oeiras 2781-901, Portugal
*Correspondence: ajd21@sussex.ac.uk
http://dx.doi.org/10.1016/j.celrep.2014.11.044

SUMMARY

Eukaryotic cells use two principal mechanisms for repairing DNA double-strand breaks (DSBs): homologous recombination (HR) and nonhomologous end-joining (NHEJ). DSB repair pathway choice is strongly regulated during the cell cycle. Cyclin-dependent kinase 1 (Cdk1) activates HR by phosphorylation of key recombination factors. However, a mechanism for regulating the NHEJ pathway has not been established. Here, we report that Xlf1, a fission yeast XLF ortholog, is a key regulator of NHEJ activity in the cell cycle. We show that Cdk1 phosphorylates residues in the C terminus of Xlf1 over the course of the cell cycle. Mutation of these residues leads to the loss of Cdk1 phosphorylation, resulting in elevated levels of NHEJ repair in vivo. Together, these data establish that Xlf1 phosphorylation by $Cdc2^{Cdk1}$ provides a molecular mechanism for downregulation of NHEJ in fission yeast and indicates that XLF is a key regulator of end-joining processes in eukaryotic organisms.

INTRODUCTION

The ability to repair DNA damage is critically important for the preservation of genomic integrity. DNA double-strand breaks (DSBs) can be repaired by two different cellular pathways: homologous recombination (HR) and nonhomologous end-joining (NHEJ) (Symington and Gautier, 2011). HR processes use undamaged homologous DNA sequences—typically from the sister chromatid—as a repair template, thus enabling error-free repair. NHEJ can also restore chromosome integrity by religation of DSB ends (Chiruvella et al., 2013) in the absence of homologous sequences but is potentially more error prone. While core factors such as Ku, XRCC4, XRCC4-like Factor (XLF), and DNA ligase 4 are required for all NHEJ repair reactions, accessory factors, including polymerases and nucleases, are also needed to process termini of imprecise DSBs into ligatable substrates.

The relative preference for break repair pathways differs between eukaryotes. Mammalian cells use NHEJ as the predominant DSB repair mechanism, where the pathway is available throughout the cell cycle. Yeast prefer to repair DSBs by HR (Manolis et al., 2001). Nevertheless, most eukaryotes utilize both NHEJ and HR; therefore, the choice of repair pathway is crucial for cell survival. DSB repair pathway selection is regulated in the cell cycle, with NHEJ predominating in G_1 phase and HR restricted to the G_2 and S phases of the cell cycle (Ferretti et al., 2013). Cyclin-dependent kinase 1 (Cdk1) plays a key role in regulating end resection during HR. Resection is strongly inhibited by low Cdk1 activity in G_1 and can be reduced in G_2 by Cdk1 inhibition (Aylon et al., 2004; Ira et al., 2004). In mammalian cells and budding yeast, the main target of CDK phosphorylation is CtIP/Sae2, which facilitates DSB end resection (Huertas et al., 2008). Cdk1 phosphorylation also influences later steps in HR, as well as expression levels of HR proteins.

In budding yeast, the initiation of DSB resection is normally suppressed in G_1 due to low Cdk1 activity and depends on the MRX complex (Clerici et al., 2008). However, this dependence on Cdk1 activity can be overcome by deletion of Ku, suggesting that it induces indirect control over NHEJ by affecting HR instead. Several potential Cdk1 phosphorylation sites have been found in budding yeast Ku70/Ku80; however, their mutation did not affect NHEJ activity (Zhang et al., 2009). Thus, direct Cdk1 targets for NHEJ regulation have not yet been identified. In fission yeast, there is a reciprocal relationship between the deployment of the two major DSB pathways with NHEJ functioning during G_1 and HR predominant in G_2 cells (Ferreira and Cooper, 2004). It has been proposed that Cdk1 may influence this pathway selection, but a mechanism has not been identified.

Xlf1 is the fission yeast homolog of XLF/Cernunnos (Hentges et al., 2006; Cavero et al., 2007), a core NHEJ factor that binds to DNA and stimulates end-joining. In the present study, we identify Xlf1 as a key regulator of NHEJ activity in the cell cycle. We report that Cdk1 phosphorylates specific residues in the C terminus of Xlf1 over the course of the cell cycle. Using phospho-null and phosphomimic mutant strains, we demonstrate that Xlf1 phosphorylation inhibits the NHEJ pathway. We also identify effects on the checkpoint response and cellular events related to DSB resection. Together, these data establish that Xlf1 phosphorylation by $Cdc2^{Cdk1}$ provides a molecular mechanism for the downregulation of NHEJ in fission yeast and offers insights into how this pathway may be regulated in other eukaryotic organisms.

RESULTS AND DISCUSSION

Cdk1 Phosphorylates Xlf1 In Vitro

NHEJ is tightly regulated in fission yeast, but the mechanism is unknown (Ferreira and Cooper, 2004). To identify if posttranslational