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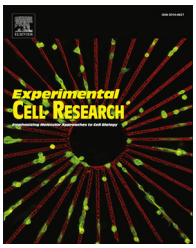

Research Article

# Roles of ChlR1 DNA helicase in replication recovery from DNA damage

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ABSTRACT

The ChlR1 DNA helicase is mutated in Warsaw breakage syndrome characterized by developmental anomalies, chromosomal breakage, and sister chromatid cohesion defects. However, the mechanism by which ChlR1 preserves genomic integrity is largely unknown. Here, we describe the roles of ChlR1 in DNA replication recovery. We show that ChlR1 depletion renders human cells highly sensitive to cisplatin; an interstrand-crosslinking agent that causes stalled replication forks. ChlR1 depletion also causes accumulation of DNA damage in response to cisplatin, leading to a significant delay in resolution of DNA damage. We also report that ChlR1-depleted cells display defects in the repair of double-strand breaks induced by the I-*Ppo*I endonuclease and bleomycin. Furthermore, we demonstrate that ChlR1-depeleted cells show significant delays in replication recovery after cisplatin treatment. Taken together, our results indicate that ChlR1 plays an important role in efficient DNA repair during DNA replication, which may facilitate efficient establishment of sister chromatid cohesion.



## Introduction

ChlR1, otherwise known as DDX11, Chl1, Ctf1, or Mcm12, is a DEAH/DEAD box-containing DNA helicase belonging to the FANCJ-like DNA helicase family [1]. The protein was first identified in a yeast genetic screen as *CHL1*/*CTF1*, whose deletion resulted in elevated levels of chromosomal loss or missegregation in the budding yeast *Saccharomyces cerevisiae* [2–4]. Subsequent studies in both budding and fission yeast demonstrated that deletion of *chl1* results in premature sister chromatid separation, and that Chl1 genetically interacts with various factors involved in sister chromatid cohesion [5–8]. These factors include Ctf7/Eco1 and RFC-Ctf18, both of which have critical roles in S phase. Ctf7/Eco1 is an acetyltranferase responsible for acetylation of the cohesin subunit Smc3, and is required for cohesion establishment specifically during S phase [9–14]. RFC-Ctf18 is an alternative replication factor C complex involved in cohesion establishment, S-phase checkpoints, and replication fork stabilization [5,7,15–22]. These results suggest that ChlR1 plays a critical role during S phase to establish proper sister chromatid cohesion.

The functions of Chl1 appear to be conserved throughout evolution. RNAi-dependent downregulation of ChlR1 causes premature sister separation and a profound delay in mitotic progression in human cells [23–25]. It is also demonstrated that ChlR1 interacts with cohesin subunits, including Scc1, Smc1 and Smc3

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[25]. Interestingly, a recent report found that the K879del mutation in ChlR1 is responsible for a cohesinopathy-related disease termed "Warsaw breakage syndrome" (WABS). The patient with WABS displays severe developmental defects, including microcephaly, growth retardation, and facial dysmorphy [26]. On the cellular level, the patient's lymphocytes show combined phenotypes of Fanconi Anemia and the cohesinopathy Robert's Syndrome, including abnormal chromosome separation or breakage, and elevated sensitivity to the interstrand-crosslinking (ICL) agent mitomycin C (MMC) and the topoisomerase inhibitor camptothecin [26]. Furthermore, ChlR1 knockout in mice results in embryonic lethality and aneuploidy due to the loss of sister chromatid cohesion [23]. These findings suggest that ChlR1 is required for normal mammalian development and preservation of genomic integrity.

Biochemical studies revealed that ChlR1 possesses a vital ATPase domain, as well as a carboxy-terminal HELICASE domain, both of which are crucial to its enzymatic function [4,27]. ChlR1 has been shown to preferentially translocate on short single-stranded DNA [27]. Further in-vitro studies showed that ChlR1 interacts preferentially with forked duplex DNA, and efficiently unwinds the 5' flap structure, a key intermediate of lagging strand processing [28]. Consistently, ChlR1 is able to stimulate the activity of the 5' flap endonuclease, Fen1 [29]. Importantly, the WABS mutation abrogates ChlR1 helicase activity [28]. These results suggest that ChlR1's helicase or unwinding activity is crucial to sister-chromatid cohesion and that ChlR1 plays an important role at the replication fork, coordinating lagging strand synthesis with sister chromatid cohesion.

Recent studies have also implicated the role of ChlR1-related proteins in DNA repair. In yeast, *chl1* deletion renders cells sensitive to S-phase stressing agents and causes a decrease in the level of DNA damage-induced recombination [5,30,31]. In human cells, ChlR1 depletion causes a lower rate of sister chromatid exchange (SCE), which is an indication of a DNA repair process that utilizes sister-chromatids for homologous recombination (HR) [23]. A study in *C. elegans* showed that the deletion of a FANCJ/ChlR1 homolog affects the ability to resolve secondary structures during replication, a process possibly involving HR [32]. Furthermore, an in-vitro biochemical study showed that ChlR1 is able to unwind a substrate representing an early intermediate of HR, as well as a substrate representing G-quadruplex DNA [28]. Thus, ChlR1's functions in DNA repair processes may play an important role in establishment of sister chromatid cohesion.

In the course of understanding how DNA replication is coordinated with sister chromatid cohesion, we previously demonstrated that the Timeless protein, which plays a central role in the maintenance of the replication fork [33], interacts with ChlR1 in human cells [24]. We also showed that Timeless depletion leads to cohesion defects, which was alleviated by overexpression of ChlR1 [24]. Furthermore, we also demonstrated in fission yeast that Chl1 overproduction suppresses DNA damage sensitivity of Swi1 (Timeless ortholog) deficient cells [5]. Considering that ChlR1/Chl1 also interacts with replication fork proteins such as PCNA and Fen1 [29,34], our findings suggested that Timeless and ChlR1 work together at the replication fork to maintain replication fork structures and promote efficient sister chromatid cohesion. In this report, we demonstrate that ChlR1 is required for cellular tolerance to an ICL agent, cisplatin, which is known to cause stalled replication forks. ChlR1-depleted cells accumulate DNA damage and have defects in repair of double stranded breaks. In addition, we show that ChlR1 is required for efficient incorporation of a nucleotide analog after cisplatin treatment. These results indicate that ChlR1 plays an important role in efficient DNA repair during DNA replication.

## Materials and methods

### Cell culture

Unless indicated otherwise, HeLa and 293 T cells were grown as described [24]. For the I-*Ppo*I DNA damage repair assay, HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum (Gemini Bio-Products, Sacramento, CA), and 100 U/ml of penicillin and 100 μg/ml of streptomycin. After retroviral infection, cells were switched into phenol red-free DMEM supplemented with 10% charcoal-stripped fetal bovine serum (Gemini Bio-Products), 110 mg/ml sodium pyruvate, 2 mM L-glutamine, 0.06 mg/ml of penicillin, and 0.1 mg/ml of streptomycin. Where indicated, cells were treated with *cis*-diammineplatinum(II) dichloride (cisplatin, P4394, Sigma, St Louis, MO) or bleomycin (bleomycin sulfate, 203401, EMD Millipore, Billerica, MA) at the indicated concentrations.

### RNAi

shRNA mediated depletion of ChlR1 and Timeless was accomplished using the lentiviral vector pLKO.1-PURO encoding shRNA sequences for scrambled control (Addgene plasmid 1684, 5'-CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG-3') [35], ChlR1/DDX11 (TRC clone ID: TRCN0000151936, 5'-CCCTTACATGATGAGAAAGATCTCGAGATCTTTCTCATCATGTAAGGG-3'), and Timeless (TRC clone ID: TRCN0000157211, 5'-GCCCACACTAACCATTGCATTCTCGAGAATGCAATGGTTAGTGTGGGC-3') [36] knockdown (Open Biosystems, Huntsville, AL). VSV-G-psuedotyped lentivirus was generated in 293 T cells using 10 μg plasmid DNA and packaging vectors as described [37]. HeLa cells were infected with viral particles overnight in medium containing 8 μg/mL polybrene, after which medium was replaced. Two days post-infection, infected cells were selected using 2 μg/mL puromycin for at least 4 days. Cells stably depleted of ChlR1 or Timeless were selected in the presence of puromycin and routinely monitored for ChlR1 levels by Western blotting.

shRNA-mediated ChlR1 depletion was also accomplished using the pGEM-based plasmid expressing an shRNA targeting ChlR1 (ChlR1-5) driven by U6 promoter as described in our previous reports [23,38].

Transfection of siRNA duplexes or shRNA vectors was performed as described [23,24,36]. The sense strands of siRNA oligonucleotides for ChlR1-#1, ChlR1-#2, and ChlR1-#3 are described in our previous report [24].

### Antibodies

Purified polyclonal ChlR1 (Hel1) and Timeless antibodies were previously generated [24,39]. Antibodies to 53BP1 (BP13) and Chk2 (clone 7) were purchased from EMD Millipore (Billerica, MA); phospho-Chk2 (Thr68, #2661) from Cell Signaling (Danvers, MA); Tubulin (alpha-tubulin, B512) from Sigma-Aldrich (St. Louis,