14-3-3 Proteins Regulate Exonuclease 1–Dependent Processing of Stalled Replication Forks

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Introduction

DNA lesions can cause stalling and collapse of the replication fork and lead to chromosome breaks, mutations, genome rearrangements and eventually cancer [1]. To prevent this, a replication checkpoint has evolved as surveillance mechanism to control components of the replisome [2] and to allow coordinating replication with cell cycle progression and DNA repair. Maintenance of stable replication intermediates when DNA synthesis is impeded, requires replisome stability and checkpoint-dependent phosphorylation events. 14-3-3 proteins are able to bind phosphorylated proteins and were shown to play an undefined role under DNA replication stress. Exonuclease 1 (Exo1) processes stalled replication forks in checkpoint-defective yeast cells. We now identify 14-3-3 proteins as interaction partners of Exo1, both in yeast and mammalian cells. Yeast 14-3-3–deficient cells fail to induce Mec1–dependent Exo1 hyperphosphorylation and accumulate Exo1–dependent ssDNA gaps at stalled forks, as revealed by electron microscopy. This leads to persistent checkpoint activation and exacerbated recovery defects. Moreover, using DNA bi-dimensional electrophoresis, we show that 14-3-3 proteins promote fork progression under limiting nucleotide concentrations. We propose that 14-3-3 proteins assist in controlling the phosphorylation status of Exo1 and additional unknown targets, promoting fork progression, stability, and restart in response to DNA replication stress.

Abstract

Replication fork integrity, which is essential for the maintenance of genome stability, is monitored by checkpoint-mediated phosphorylation events. 14-3-3 proteins are able to bind phosphorylated proteins and were shown to play an undefined role under DNA replication stress. Exonuclease 1 (Exo1) processes stalled replication forks in checkpoint-defective yeast cells. We now identify 14-3-3 proteins as in vivo interaction partners of Exo1, both in yeast and mammalian cells. Yeast 14-3-3–deficient cells fail to induce Mec1–dependent Exo1 hyperphosphorylation and accumulate Exo1–dependent ssDNA gaps at stalled forks, as revealed by electron microscopy. This leads to persistent checkpoint activation and exacerbated recovery defects. Moreover, using DNA bi-dimensional electrophoresis, we show that 14-3-3 proteins promote fork progression under limiting nucleotide concentrations. We propose that 14-3-3 proteins assist in controlling the phosphorylation status of Exo1 and additional unknown targets, promoting fork progression, stability, and restart in response to DNA replication stress.
14-3-3 Regulate Exo1 at Replication Forks

Author Summary

Stalling and collapse of DNA replication forks is an important source of genome instability and has been implicated in early steps of carcinogenesis. The maintenance of stable intermediates upon stalled replication requires the coordinated action of a number of proteins and proper inhibitory control of dangerous enzymatic activities. In this study, we uncover an evolutionarily conserved mechanism through which 14-3-3 proteins modulate the checkpoint-mediated phosphorylation of, and in turn limit the activity of, an exonuclease (Exo1) previously implicated in pathological processing of stalled replication forks and other sensitive DNA intermediates. This represents an unprecedented link in the field of DNA repair and genome stability, providing a molecular rationale to the yet undefined role of 14-3-3 proteins in the maintenance of genome integrity after replication stress. In analogy to Exo1, our data suggest that additional factors at replication forks may be subjected to similar regulation, pointing to the 14-3-3 proteins as central components of the checkpoint triggered in response to replication stress.

Results/Discussion

14-3-3 proteins interact with EXO1

To identify novel interaction partners for human EXO1 we designed a yeast two-hybrid screen with GAL4-bait fusion proteins that contain either full-length EXO1 or ΔN-EXO1 (EXO1[66–340]), which lacks the entire catalytic domain. Since the former was not expressed (data not shown), we used the latter to screen a blood peripheral cDNA library. Three 14-3-3 proteins were the highest hits (Table S1), with the β- being more represented than the ε- and ζ- isoform. The presence of an established EXO1 binding protein among the hits, MLH1 (Table S1), confirmed the reliability of this screen.

To independently verify these data, we performed co-immunoprecipitation experiments. Given the low abundance of EXO1 in the cell [14], we transiently transfected HEK-293 cells with an Omni-tagged EXO1 construct [14] and immunoprecipitated the expressed protein using a pan-14-3-3 antibody. The data showed that Omni-EXO1 and 14-3-3 proteins could be recovered as a complex (Figure 1A).

To assess the physiological significance of the EXO1/14-3-3 interaction we selected Sac. cerevisiae, a system where only two 14-3-3 proteins are present, namely Bmh1 and Bmh2. In preliminary experiments we examined whether yeast Exo1 and 14-3-3 proteins interact. A C-terminal Myc- or a HA-tag was added to the endogenous EXO1 or BMH1/BMH2 genes, respectively. Immunoprecipitation experiments showed that Exo1 formed complexes with Bmh1 or Bmh2 in a HU-dependent manner (Figure 1B). We next explored a possible direct Bmh/Exo1 interaction by Far Western blot analysis. Exo1-Myc immunoprecipitated from control or HU-treated cells was resolved by SDS-PAGE and denatured/renatured on PVDF. Probing the membrane with purified GST-Bmh1 revealed that a direct interaction with Exo1 occurred both in the case of untreated and HU-treated cells (Figure S1). These data possibly indicate that an Exo1 domain normally hidden in non-treated cells may become available for interaction with 14-3-3 proteins upon HU-treatment. Such domain may be artificially exposed during Far Western analysis due to incomplete renaturation of Exo1.

Taken together, these data suggest that the EXO1/14-3-3 interaction is conserved from yeast to mammalian cells. While the interaction is HU-independent in mammalian cells, it requires HU in yeast. This may reflect the different modes of EXO1 regulation in the two systems [15,17].

14-3-3-deficient cells cannot restart stalled replication forks, but their recovery defect is partially suppressed by EXO1 deletion

Genetic and flow cytometric analysis evidenced the sensitivity of 14-3-3-deficient cells to DNA replication stress, with distinct bmh1 (bmh2Δ) alleles showing different defects upon nucleotide depletion (HU) or treatment with DNA damaging agents (UV or methylmethanesulfonate, MMS) [26]. However, despite the evidence that 14-3-3 proteins bind origins of replication and cruciform DNA [29], suggesting a regulatory role in DNA replication [25], the issue of possible direct involvement of 14-3-3 in fork stability or processing under genotoxic stress conditions remained to be clarified. Given the comprehensive molecular characterization of yeast Exo1 as component of the replisome and of its role, in checkpoint defective cells, in the processing of forks stalled by nucleotide depletion [4], we focused our investigations on the bmh1-280 bmh2Δ double mutant (bmh1/bmh2 hereafter). This mutant shows normal cell cycle progression in unperturbed conditions, but selective sensitivity and cell cycle recovery defects in response to HU [26]. The mutant Bmh1-280 protein carries a single point mutation (E136G) in helix αE at a residue neighboring amino acids that form salt bridges and hydrogen bonds with the ligand [21]. Interestingly, immunoprecipitation experiments showed that the mutant Bmh1-280 protein did not interact with Exo1 in untreated nor HU-treated cells (Figure 1C). Thus, we asked whether the cell cycle recovery defects of this mutant reflect a direct role of 14-3-3 proteins at replication forks and whether Exo1 is also implicated in these processes. We performed neutral-neutral bidimensional gel electrophoresis (2D gel) on the early origin of replication ARS305, which is known to be activated in HU-treated cells [3]. Although the 2D gel pattern looked normal in HU-treated bmh1/bmh2 cells, we observed that replication intermediates (RIs) in 14-3-3 defective cells were still present close to the origin 60 min after HU removal and were only restarted at 90–120 min (Figure 2A and data not shown). This suggests that misregulation of the replisome, without dramatic physical processing of the forks, might be sufficient to impair fork restart. This effect was not detectably suppressed by EXO1 deletion (Figure 2A).

Flow cytometric analysis of HU-released cells confirmed the slow recovery of the bmh1/bmh2 strain and showed that lack of Exo1 per se did not alter the pattern of cell cycle progression (Figure 2B). On the other hand, EXO1 deletion in a bmh1/bmh2 background led to a partial rescue of the recovery defect, particularly evident at late time points (>120 min) after release from HU (Figure 2B). This evidence prompted us to ask whether EXO1 deletion in this background may affect Rad53 activity. Western blot analysis with
total and phosphospecific Rad53 antibodies [30] showed that, compared to wild type cells, Rad53 was hyperphosphorylated in HU-treated \( bmh1bmh2 \) cells and that its dephosphorylation was retarded during the HU-recovery phase (Figure 2C and 2D), thus correlating with the described replication restart defect. Importantly, deletion of \( EXO1 \) in 14-3-3-deficient cells re-established to a great extent the pattern of rapid Rad53 dephosphorylation in the recovery phase (Figure 2C and 2D), substantiating the flow cytometry data (Figure 2B).

Overall these data suggest that 14-3-3 proteins are directly implicated in the effective restart of stalled DNA replication forks. Alternatively, they may assist rapid Rad53 dephosphorylation, which is in turn required for fork restart upon HU removal [31]. The latter interpretation is however unlikely as \( EXO1 \) deletion, which markedly restores Rad53 dephosphorylation upon HU removal, does not detectably improve the defective fork restart observed in 14-3-3 deficient cells (Figure 3B, 120 min). Defective Exo1 phosphorylation in HU-treated 14-3-3-deficient cells is not an indirect consequence of defective checkpoint activation, as under these conditions Rad53, another Mec1-dependent checkpoint target, is promptly phosphorylated (Figure 2C and 2D). Since phosphorylation restrains yeast Exo1 activity [17], we propose that 14-3-3 proteins play an important role in the dynamic control of Exo1 activity upon DNA replication stress and may act as platform for the control of Exo1 phosphorylation. In this respect, attempts to assess the phosphorylation status of Bmh-bound Exo1 were unfortunately inconclusive, as - differently from TCA extracts (Figure 3) - the extracts used for immunoprecipitation fail to be resolved in discrete bands by Phos-tag SDS-PAGE (data not shown). Given that 14-3-3 proteins bind ligands in phosphorylation-dependent and -independent manner [21], it will be important to overcome these technical limitations to address the role of 14-3-3 proteins in controlling the phosphorylation of Exo1 and, possibly, additional targets in the DNA damage response (see below).

Reversible Exo1 phosphorylation in response to HU

Exo1 is responsible for the accumulation of ssDNA gaps behind the fork in \( bmh1bmh2 \) cells

As Exo1 activity and Rad53 phosphorylation have been linked to the processing of stalled DNA replication forks, we decided to assess whether defective Rad53 and Exo1 phosphorylation in 14-3-3-deficient cells could reflect changes in the fine architecture of stalled forks. To answer this question, we synchronized the cells in G1, released them for 1 h in YPD medium containing 0.2 M HU
and examined RIs by electron microscopy (EM) under non-denaturing conditions [33]. For each strain, about 100 RIs were analyzed in duplicate. 14-3-3-deficient cells showed a dramatic accumulation of ssDNA gaps behind the replication fork (Figure 4A). Statistical analysis indicated that approximately 50% of all RIs analyzed contained one or more ssDNA gaps (Figure 4B). Interestingly, deletion of EXO1 in the bmh1bmh2 background completely suppressed this phenotype, leading to a reduction of the ssDNA gaps behind the fork to a level similar to wild type or exo1D cells (Figure 4B). The comparison of ssDNA gaps length scored by EM evidenced a striking difference: whereas bmh1bmh2 cells displayed a significant number of large size gaps (>0.5 Kb), the latter were absent in bmh1bmh2exo1D cells (Figure 4C). The resolution limit of 50–70 nucleotides may have impaired detection of nicks/small gaps in this as well as in previous EM studies with HU [34]. Such structures, however, become visible in 14-3-3-deficient cells, where the unleashed Exo1 activity would enlarge gaps above the detection limit.

These data suggest that 14-3-3 proteins are required to prevent unscheduled Exo1 activity behind stalled replication forks in a checkpoint-proficient background. The implications of these observations are of great significance. A loose control of Exo1 activity may render DNA synthesis more discontinuous in conditions of replicative stress, either promoting additional uncoupling/repriming events or enlarging existing ssDNA gaps via 5’-3’ exonucleolytic processing. Although additional work is required to directly address this point, it is conceivable that continuous polymerase stall due to insufficient deoxynucleotide levels might per se lead to increased repriming events, thus raising the number of 5’-ends available for processing by Exo1. In this setting, a strict control of Exo1 activity would be needed to limit damage. We observed no bias for the presence of gaps in leading vs. lagging strands - whenever these could be identified [35] - and we could occasionally detect gaps on opposite strands within the same molecule (Figure S3), suggesting that unscheduled Exo1 activity in 14-3-3 defective cells is not restricted to leading or lagging strand.

Defective fork progression in 14-3-3-defective cells is independent on EXO1

Replication recovery defects have been previously described and usually reflect replication fork collapse detectable by 2D gel analysis [3]. On the contrary, stalled replication forks in 14-3-3 deficient cells, albeit unable to restart DNA synthesis and abnormally processed by Exo1 activity, upon prolonged HU

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**Figure 2. Pattern of HU recovery in wild-type, exo1Δ, bmh1-280 bmh2Δ, and bmh1-280 bmh2Δ exo1Δ strains.** (A) Representative 2D gels of replication intermediates (RIs) at ARS305 analyzed after 90 min HU-treatment and upon HU removal (recovery, 60 and 90 min). (B) Time-course flow cytometric analysis of the DNA content in the indicated strains upon recovery from a HU-arrest. (C) Western blot analysis showing in the indicated strains the phosphorylation-dependent mobility shift of Rad53 during the HU-arrest and the recovery phase. (D) Western blot analysis performed with monoclonal antibody F9 to phosphorylated Rad53 showing in the indicated strains the status of Rad53 activation in HU-arrested cells and during the recovery phase.

doi:10.1371/journal.pgen.1001367.g002
treatment show a 2D gel pattern indistinguishable from that of wild type cells. We thus decided to investigate in more detail the structure and progression of these forks, performing 2D gel analysis at different time points after HU addition. To this end, cells synchronized in G1 by α-factor were released into medium containing HU and RIs were examined by 2D gels. Figure 3B shows the probes designed to visualize replication fork progression in a region of Chromosome III that contains, besides the early active origin ARS305 [36], a contiguous passively replicated region (Part A) and a region including the dormant origin ARS301 (Part D) [3]. As compared to wild type, bmh1bmh2 cells showed the same kinetics of origin firing, albeit with slightly lower efficiency as revealed by the intensity of the bubble arc at 30 min (Figure 5C). Progression of the forks in HU from ARS305 across the region of Part A (~5 Kb to the left of ARS305) was completed after 2–3 h in wild type cells, with the peak of intermediates detectable after ~1 h. In bmh1bmh2 cells the first intermediates appeared on this region with 30 min delay, whereas the peak of intermediates was delayed of ~2 h as compared to wild type cells (Figure 5C), indicative of a significant decrease in the rate of the replication fork progression in HU. Slow RIs disappearance from the origin and delayed invasion of adjacent chromosomal regions may in principle result also from asynchronous firing of ARS305 during the HU arrest. However we consider this alternative interpretation unlikely for the following reasons: a) by budding experiments, 14-3-3 defective cells do not display asynchronous entrance in S-phase (data not shown); b) the comparable intensity of the Y arc on fragment A in the wt (60 min) and in the 14-3-3 mutant (180 min) suggests that forks progress synchronously but slower from the early origin ARS305; c) accordingly, the progressive accumulation of the Y signal on fragment A in 14-3-3 defective cells strictly correlates with the disappearance of the bubble arc on the

Figure 3. Exo1 phosphorylation pattern in response to HU in wild-type, mec1Δ, and bmh1-280 bmh2Δ strains. (A) Western blot analysis of Exo1 phosphorylation in HU-arrested cells of wild type and mec1Δ smt1Δ strains. (B) Western blot analysis of Exo1 phosphorylation in HU-arrested cells and during the recovery phase of the indicated strains. Both in (A) and (B) proteins were resolved on an 8% Phos-tag SDS-polyacrylamide gel. doi:10.1371/journal.pgen.1001367.g003

Figure 4. Exo1–dependent generation of ssDNA gaps in bmh1-280 bmh2Δ cells. (A) Representative RIs visualized by EM in bmh1-280 bmh2Δ cells released synchronously from G1 phase in 0.2 M HU for 1 h: the magnified inset (asterisk) shows a representative ssDNA gap located behind the replication fork. Black arrows: ssDNA gap at the fork; White arrows: internal ssDNA gap located behind the fork. (B) Statistical analysis of ssDNA gap number. The number of analyzed molecules is indicated in brackets. (C) Statistical analysis of ssDNA gap length. The number of analyzed gaps/molecules is indicated in brackets. doi:10.1371/journal.pgen.1001367.g004
ARS305 fragment, further suggesting slow but synchronous progression of replication forks on the ARS305 replicon.

It was previously shown that yeast 14-3-3 proteins bind to the checkpoint kinase Rad53 and directly influence its DNA damage-dependent functions [27]. Therefore, we asked whether the slow fork progression in \textit{bmh1bmh2} cells might be solely due to checkpoint defects. To address this issue, we used checkpoint defective Rad53-mutant cells (\textit{rad53-K227A}). The latter displayed striking differences when compared to \textit{bmh1bmh2} cells. Both the destabilization of RIs (ARS305 and Part A) and the uncontrolled firing of dormant origins displayed by \textit{rad53-K227A} cells (Part D) [3], were absent in \textit{bmh1bmh2} cells (Figure 5C). Furthermore, EM did not display any fork reversal or accumulation of ssDNA at replication forks, typical of HU-treated \textit{rad53} cells [34] (data not shown). Finally, 2D gel analysis (Figure S4C, S4F and S4H) and drop assays (Figure S5) revealed synergistic effects of 14-3-3 and Rad53 on both fork stability and survival. Overall, these data indicate that the phenotype observed in 14-3-3 deficient cells reflects a genuine role of 14-3-3 proteins at replication forks and that 14-3-3 and Rad53 have crucial but distinct roles at HU-challenged forks.

Deletion of \textit{EXO1} partially rescued the HU-sensitivity of \textit{rad53-K227A} cells, but not of a \textit{bmh1bmh2} strain (Figure S5). Furthermore, in contrast to checkpoint defective cells, where stability of RIs could be rescued by \textit{EXO1} deletion [4], fork progression defects of \textit{bmh1bmh2} cells were not rescued by loss of \textit{EXO1} (Figure S4G). Thus, while the processing defect that leads to accumulation of ssDNA gaps in 14-3-3-deficient cells was completely suppressed by \textit{EXO1} deletion, this did not reflect in suppression of HU sensitivity nor of defective fork progression in HU-treated 14-3-3 deficient cells. Altogether this evidence suggests that 14-3-3 proteins might regulate additional targets during replication stress, possibly through modulation of their phosphorylation. This is not unexpected, given the role of 14-3-3 as integrators of signaling pathways [19] and considering the multiplicity of 14-3-3 targets [37,38]. Our data implicate 14-3-3 proteins as possible central regulator of the checkpoint response.

In analogy with previously reported cases [22] and according to structural data on the dynamic nature of 14-3-3 dimers [39], one may envisage a role for 14-3-3 proteins as docking clamp tethering \textit{Exo1} - and other unknown targets - with the kinase controlling its activity. Notably, 14-3-3 proteins were reported to bind Rad53 [27], one of the candidate checkpoint kinases required for \textit{Exo1} phosphorylation [17].

In conclusion, this work sheds further light on processes occurring at stalled replication forks, proposing 14-3-3 proteins as central integrators of signals that regulate fork stability and processing. Challenges lying ahead consist in the identification of components of the replisome, or proteins controlling them, that may be 14-3-3 targets, as well as in the elucidation of the exact mechanism by which 14-3-3 modulate \textit{Exo1} phosphorylation and activity.
Materials and Methods

Materials

The antibodies used in this study were: goat polyclonal anti-Omni-probe (M21, sc-499, Santa Cruz Biotechnology); rabbit polyclonal anti-pan 14-3-3 (SA-483, Biomol); mouse monoclonal anti-HA (12CA5, Sigma) and anti-Myc (9E10, Santa Cruz Biotechnology); rabbit polyclonal anti-pan 14-3-3 (SA-483, Biomol); mouse monoclonal 1-Mating Factor (Sigma). The chemicals and peptides used in this study were: 14-3-3 Regulate Exo1 at Replication Forks

Saccharomyces cerevisiae strains

The yeast strains used in this study are isogenic to W303-1A (wild type) [40] and are listed in Table 1. All strains have been obtained by one step replacement using the indicated markers and tags that have been generated by PCR. The isolated clones have been verified by colony PCR and Southern Blot or Western Blot.

Yeast two-hybrid screen

The yeast two-hybrid screening was performed with ΔN-EXO1 (EXO1Δ66-416) as bait on a cDNA library generated from human peripheral blood mRNA (a kind gift of I. Staggljar, Toronto, Canada) as described previously [41] and using THY AP4 as reporter strain.

Protein extraction, Western and Far Western blotting, immunoprecipitation

Western blot analysis of yeast proteins was carried out upon TCA extraction [42]. To visualize Exo1, an optimized Phos-tag system (50 μM Phos-tag reagent) was employed according to [32]. Proteins were transferred to nitrocellulose (poreblot NCP, 0.45 μm pore size, Macherly-Nagel) overnight at room temperature applying constant amperage (200 mA). Far Western blotting [43] was performed using purified recombinant GST-Bmh1 [44] to probe Exo1 that was immunoprecipitated from control or HU-treated yeast cells.

HEK293T cells were grown and lysed as described [14] and protein concentration was determined using the Bio-Rad Protein Assay Reagent (Bio-Rad). Immunoprecipitation of Omni-EXO1 or 14-3-3 proteins from 2.5 mg total cell extracts with specific antibodies was performed as previously described [14]. For immunoprecipitation of yeast proteins, cells were lysed using ice-cold lysis buffer (25 mM Tris-HCl pH 7.4, 15 mM NaCl, 15 mM EGTA, 1 mM NaF, 1 mM Na orthovanadate, 4 mM p-Nitro-Phenyl-Phosphate (pNPP), 0.1% Triton X-100, 1 mM PMSE, complete protease inhibitors cocktail (Roche)). 14-3-3-HA was immunoprecipitated from 10 mg total yeast cell extracts using the monoclonal HA antibody.

2D gel electrophoresis and electron microscopy

DNA extraction with the CTAB method and neutral-neutral two-dimensional gel electrophoresis were performed as described [45]. EM analysis was performed as described [33].

Supporting Information

Figure S1 Far Western blot analysis. Exo1-Myc immunoprecipitated from untreated or HU-treated cells was resolved by SDS-PAGE, proteins were transferred to PVDF and denatured/
renatured as described in Materials and Methods. The membrane was probed with purified, recombinant GST-Bmh1 (2 µg) (middle), stripped and reprobed with monoclonal antibody 9E10 to the Myc-tag (top). Wt = control; 1 = Bmh1-HA Exo1-Myc; 2 = Bmh2-HA Exo1-Myc. Ponceau Red (PR) is shown in the lower panel as loading control.

**Figure S2**
Analysis of Exo1 stability. Western blot analysis of Exo1 during HU-arrest and release of the indicated strains. The extracts used in Figure 3 were loaded on a standard (no Phos-tag) SDS-polyacrylamide gel, where Exo1 appears as one compact band. This allows visualizing stable and similar levels of total Exo1 protein in wild type and bmh1-280 bmh2A strains during HU-arrest and release. Ponceau Red is shown in the lower panel as loading control.

Found at: doi:10.1371/journal.pgen.1001367.s001 (0.28 MB TIF)

**Figure S3**
ssDNA gaps arise on both leading and lagging strands in HU-treated bmh1-280 bmh2A cells. Two representative replication bubbles visualized by EM in bmh1-280 bmh2A cells synchronously released from G1 phase in 0.2 M HU for 1 h. The molecules are shown at the same magnification. A scale bar is included in the lower panel. Black arrows: ssDNA gaps at the fork. White arrows: internal ssDNA gap located behind the forks. In the top panel, length measurements show that two internal gaps on opposite replicated duplexes cover the same distance from the replication forks: by definition, one must have resulted from leading strand and the other from lagging strand DNA synthesis. Similarly, in the bottom panel, the two internal ssDNA gaps lay very close to opposite forks on the same replicated duplex, marking by definition opposite strands (leading and lagging) of DNA synthesis.

Found at: doi:10.1371/journal.pgen.1001367.s002 (0.59 MB TIF)

**Figure S4**
2D gel analysis of RIs from wild-type and mutant strains. Wild-type (B), rad53-K227A (C), exo1A (D), rad53-K227 and exo1A (E), bmh1-280 bmh2A (F), bmh1-280 bmh2A exo1A (G), bmh1-280 bmh2A rad53-K227A (H), bmh1-280 bmh2A rad53-K227A exo1A (I) strains were used for 2D gel analysis as described in Figure 5. Additional genomic fragments (B and C) were visualized by Southern blot on the same filters, as depicted in panel (A).

Found at: doi:10.1371/journal.pgen.1001367.s003 (7.60 MB TIF)

**Figure S5**
HU-sensitivity assay of wild-type and mutant strains. Wild-type, rad53-K227A, exo1A, rad53-K227A exo1A, bmh1-280 bmh2A, bmh1-280 bmh2A exo1A, bmh1-280 bmh2A rad53-K227A, and bmh1-280 bmh2A rad53-K227A exo1A cultures were grown exponentially. Serial dilutions (1:10) were spotted on YPD plates containing different HU concentrations and grown for 3 days before scoring.

Found at: doi:10.1371/journal.pgen.1001367.s005 (3.12 MB TIF)

**Table S1**
Identification of novel EXO1 interacting partners by two-hybrid-screen in yeast. List of the most prominent proteins found to interact with human EXO1, with indication of the overall hit representation.

Found at: doi:10.1371/journal.pgen.1001367.s006 (0.14 MB TIF)

**Acknowledgments**
We are grateful to I. Stagljar (Toronto, Canada) for providing yeast-two-hybrid screen reagents; M. P. Longhese (Milan, Italy) for the YLI909, YLI1090 strains; P. Schaer (Basel, Switzerland) for reagents; C. Santocanale (Galway, Ireland) for the Rad53 antibody; M. Foiani (Milan, Italy) for the phospho-Rad53 monoclonal antibody; L. Demmel (Vienna, Austria) for the pGEX-Bmh1 construct; and the Center for Microscopy of the University of Zurich for assistance in electron microscopy.

**Author Contributions**
Conceived and designed the experiments: KE ML SF. Performed the experiments: KE MG. Analyzed the data: KE ML MMF ML SF. Contributed reagents/materials/analysis tools: MMF. Wrote the paper: KE ML SF.

**References**


