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Proteasomal regulation of the mutagenic translesion DNA polymerase, *Saccharomyces cerevisiae* Rev1

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ABSTRACT

Translesion DNA synthesis (TLS) functions as a tolerance mechanism for DNA damage at a potentially mutagenic cost. Three TLS polymerases (Pols) function to bypass DNA damage in *Saccharomyces cerevisiae*: Rev1, Pol ζ, a heterodimer of the Rev3 and Rev7 proteins, and Pol η (Rad30). Our lab has shown that *S. cerevisiae* Rev1 protein levels are under striking cell cycle regulation, being ~50-fold higher during G2/M than during G1 and much of S phase (Waters and Walker, 2006). *REV1* transcript levels only vary ~3-fold in a similar cell cycle pattern, suggesting a posttranscriptional mechanism controls protein levels. Here, we show that the *S. cerevisiae* Rev1 protein is unstable during both the G1 and the G2/M phases of the cell cycle, however, the protein's half-life is shorter in G1 arrested cells than in G2/M arrested cells, indicating that the rate of proteolysis strongly contributes to Rev1's cell cycle regulation. In the presence of the proteasome inhibitor, MG132, the steady-state levels and half-life of Rev1 increase during G1 and G2/M. Through the use of a viable proteasome mutant, we confirm that the levels of Rev1 protein are dependent on proteasome-mediated degradation. The accumulation of higher migrating forms of Rev1 under certain conditions shows that the degradation of Rev1 is possibly directed through the addition of a polyubiquitination signal or another modification. These results support a model that proteasomal degradation acts as a regulatory system of mutagenic TLS mediated by Rev1.

1. Introduction

Cells constantly face the challenge of maintaining genomic integrity as a result of DNA damage arising from endogenous and exogenous sources. To prevent the negative consequences of DNA damage, the cell is equipped with DNA repair and tolerance mechanisms. DNA repair restores the original state of the DNA. DNA damage tolerance, however, allows DNA lesions to remain in the genome even during replication.

When the cell employs translesion DNA synthesis (TLS) to tolerate DNA damage, specialized DNA polymerases with members from all domains of life [1] catalyze replication opposite lesions that normally prevent the replicative DNA polymerases' activity. Most TLS polymerases belong to the Y-family of DNA polymerases, which are better able to accommodate bulky DNA lesions because the active sites are less sterically constrained than those of the high-fidelity, replicative polymerases [2]. Given this structural property of TLS polymerases and their lack of any proofreading activity, TLS polymerases can exhibit high error rates. TLS across from lesions can be relatively error-free or quite error-prone depending on the lesion and polymerase involved [3–5]. Following bypass, the DNA repair pathways can later remove the DNA lesion, which remains in the DNA.

There are three known TLS polymerases in *Saccharomyces cerevisiae*: Rev1 and Pol η (Rad30) of the Y-family and the B-family member, Pol ζ, a heterodimer of Rev3 and Rev7. All three are highly conserved among eukaryotes. The *REV1*, *REV3*, and *REV7* genes were discovered in screens for *rev*ersionless mutants in yeast (a phenotype indicating loss of a mutagenic activity) [6,7]. The *rev1*Δ mutant phenotypes include an increased sensitivity to certain DNA damaging agents and a decreased damage-induced mutation frequency, indicating Rev1's instrumental role in DNA damage resistance and mutagenesis [8].

Rev1's DNA polymerase activity exhibits unique properties that include specificity for a template G and a preference for inserting dCMPs as a consequence of pairing the incoming dNTP with one of its own residues instead of with a template base [9–11]. Despite this clear and evolutionarily conserved catalytic activity (Wiltrout and Walker, In Press, Genetics), the non-catalytic functions of Rev1 appear to be more critical for DNA damage tolerance and mutagenesis *in vivo* based on known mutant phenotypes. In *S. cerevisiae*, the *rev1-1* (G193R) mutant of the BRCT domain leads

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to almost null phenotypes *in vivo*, but the mutant protein retains about 60% of the catalytic activity *in vitro* [12]. Additionally, the ubiquitin binding motif (UBM2) [13–15] and the conserved region of Rev1's C-terminus that interacts with other TLS polymerases [16–20] are critical for cellular survival and mutagenesis after DNA damage [reviewed in [21]]. Therefore, beyond its DNA polymerase function, Rev1 serves to regulate the other TLS polymerases through protein–protein interactions or direct interaction with the DNA.

The mutagenic nature of Rev1 indicates that the activity must be tightly regulated. The conservation of Rev1 in higher eukaryotes suggests that the evolutionary benefits outweigh the risks of its potentially mutagenic activity, although it is possible that all of Rev1's *in vivo* functions are not known.

Not surprisingly, disrupting the normal protein levels of TLS polymerases has negative consequences. In *S. cerevisiae*, ectopic overexpression of Pol ζ's Rev3 and Rev7 proteins leads to a greater sensitivity to UV radiation and an increase in UV-induced mutation frequency [22]. In one study in mammalian cells, a 2- to 4.5-fold overexpression of the TLS DNA Pol κ interferes with replication fork progression in CHO cell lines [23]. In another report, overexpression of human REV1 in ovarian carcinoma cells demonstrates the potential danger of misregulated Rev1 levels [24]. Therefore, understanding how the regulation of *REV1* properly balances survival and mutagenesis in the cell is crucial.

Currently, limited data exists regarding the regulation of *REV1* gene expression. Unlike some other genes encoding DNA repair proteins, *S. cerevisiae REV1* transcription is not inducible by DNA damage or heat shock [25]. *REV1* transcript levels are, however, upregulated during sporulation in *S. cerevisiae* [26–28]. At the protein level, previous work from our lab has shown that Rev1 is under striking cell cycle control with protein levels peaking during G2/M rather than S phase when the bulk of replication occurs [29]. Despite the approximately 50-fold change at the protein level, *REV1* transcript levels only increase 3-fold during G2/M relative to G1. Interestingly, Rev1 is phosphorylated in a similar cell cycle-dependent manner, demonstrating another potential method of regulation [30]. The molecular means controlling the unexpected cell cycle regulation of Rev1, however, are not yet fully understood.

More recent studies support the hypothesis that cell cycle regulation of Rev1 is functionally important. For example, the action of Rev1 and Pol ζ is key for bypass of ultraviolet-induced DNA damage during and after S phase of the cell cycle and can occur separately from bulk genomic replication [31]. In another study, the use of G2-specific promoters to express Rev3 and Rad30 complemented the deletion of the TLS polymerases with respect to survival and mutagenesis phenotypes in response to specific types of DNA damage [32]; a G2-specific promoter was not used to express Rev1 in this study.

Several genetic studies indicate that TLS may be subject to regulation by the proteasome. These studies took advantage of the *ump1*Δ strain, which is a viable mutant of a gene encoding a maturation factor for the 20S catalytic core of the 26S proteasome [33]. The spontaneous and UV-induced mutator phenotype of the *ump1*Δ strain is dependent on the TLS polymerase gene, *REV3*, which is generally placed in the same genetic pathway as *REV1* [34,35]. The *ump1*Δ strain is hypermutable, whereas *rev3*Δ and *ump1*Δ *rev3*Δ strains are hypomutable, suggesting that Ump1 may act as a negative regulator of Rev3 activity, possibly through Rev1's interaction with Pol ζ. The authors of this study, however, did not examine *REV1*'s genetic interactions with *UMP1*. In an *ump1Δ* strain, short-lived proteins are stabilized and ubiquitin–protein conjugates accumulate [33]. Therefore, we hypothesized the involvement of proteasomal degradation in TLS regulation as a means for control of this potentially mutagenic process.

Selective protein turnover through ubiquitination and subsequent proteasomal degradation represents an essential regulatory mechanism in eukaryotic cells. The irreversibility of protein degradation ensures both spatial and temporal control and eliminates improper reactivation of the protein. The attachment of monoubiquitin or polyubiquitin chains to specific proteins is critical for a variety of cellular processes from DNA repair and replication to gene silencing, in addition to protein degradation [36,37].

Here, we studied the role that proteasomal degradation has in regulating the mutagenic TLS polymerase Rev1, the levels of which are cell cycle regulated. We show that Rev1 is a moderately short-lived protein throughout the cell cycle but is degraded more rapidly during G1 than during G2/M. Our data indicate that Rev1 undergoes proteasome-mediated degradation during both G1 and G2/M arrests that is potentially targeted through a polyubiquitin modification. Overall, these results indicate that proteasomal degradation serves as an efficient and irreversible mechanism of regulating the potentially mutagenic effects of Rev1's action.

2. Materials and methods

2.1. Yeast strains

A strain list for this study is described in Table 1. All strains are derivatives of the W1588-4C (*MAT***a** *leu2-3,112 ade2-1 can1-100 his3-11,15 ura3-1 trp1-1 RAD5*) [38] parent strain. The Rev1 protein was tagged at its native locus with a C-terminal—TEV-ProA-His_7 epitope tag (marked with *HIS3*) using pYM10 [39], similar to that previously described [17,29]. *UMP1* and *ERG6* (also called *ISE1*) were each separately deleted via a one-step replacement, amplifying the *ump1::kanMX4* or *erg6::kanMX4* cassette from the deletion library and transforming the product into the appropriate strain background [40]. The *BAR1* gene was disrupted by a one-step gene replacement using digested pZV77 to aid in arresting cells with α factor (gift from S. Bell). The multicopy vector, pMRT7 (pCK322), contains the P_{CUP1}-*myc-UBI* expression cassette and the *URA3* marker [41] (gift from C. Kaiser). All cassettes and plasmids were introduced through a standard lithium acetate protocol [42]. Oligonucleotide sequences that were used in strain construction are available up request.

2.2. Cell cycle arrest

Cells were grown in YEPD media at 30 °C with the exception of *ump1*Δ strains that were grown at 25 °C. When the culture reached an OD of 0.5, the cells were split into two cultures for arrest, one G1 arrested with α factor (50 ng/ml) and the other G2/M arrested with nocodazole (15 μg/ml). Cells were treated for 3–4 h prior to starting the assays.

2.3. Immunoblot

Protein extracts were made using a trichloroacetic acid (TCA) procedure similar to that published [39]. TCA precipitations were run on 7.5% SDS-PAGE gels (Lonza), and the immunoprecipitation samples were run on NuPAGE 3–8% tris-acetate gels (Invitrogen) before being transferred to polyvinylidene difluoride membranes (PVDF, Immobilon-P; Millipore). PVDF membranes were probed with rabbit peroxidase-anti-peroxidase soluble complex (PAP, Sigma) for ProA-tagged proteins and anti-3-phosphoglycerate kinase (yeast), mouse IgG, monoclonal antibody (anti-PGK, Molecular Probes) with mouse secondary for the Pgk1 control.