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Review

# Elg1, a central player in genome stability

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ABSTRACT

*ELG1* is a conserved gene uncovered in a number of genetic screens in yeast aimed at identifying factors important in the maintenance of genome stability. Elg1's activity prevents gross chromosomal rearrangements, maintains proper telomere length regulation, helps repairing DNA damage created by a number of genotoxins and participates in sister chromatid cohesion. Elg1 is evolutionarily conserved, and its mammalian ortholog (also known as ATAD5) is embryonic lethal when lost in mice, acts as a tumor suppressor in mice and humans, exhibits physical interactions with components of the human Fanconi Anemia pathway and may be responsible for some of the phenotypes associated with neurofibromatosis. In this review, we summarize the information available on Elg1-related activities in yeast and mammals, and present models to explain how the different phenotypes observed in the absence of Elg1 activity are related.



**Contents**



## 1. Genome stability and the DDR

A stable genome is a pre-requisite for the survival and proper functioning of all organisms. Genome stability is compromised

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constantly by external insults (such as DNA damaging agents) and may be even threatened by the normal metabolism of the cell. In addition, in each cell division it is necessary to duplicate the genetic material; during DNA replication the double stranded helix must be open to allow its copying, a situation that renders DNA prone to chemical modifications and alterations. Several surveillance and repair mechanisms operate in eukaryotic cells to ensure the stability of the genome, and dire consequences to the cell ensue when they fail to act properly. Indeed, genomic instability is a hallmark of cancer cells. Most human cancer cells show signs of genome instability, ranging from elevated mutation rates, to gross chromosomal rearrangements, including deletions and translocations.

The current view is that most spontaneous chromosomal rearrangements and mutations in the genome arise during DNA replication. The activity of the DNA polymerases may be impaired by the presence of secondary structures, bound proteins or DNA lesions; they may also be halted by collisions with other DNA-interacting proteins (such as RNA polymerases, or topoisomerases). These encounters may lead to stalling or even collapse of replication forks, creating single-stranded gaps or double-strand breaks (DSBs). In response, cellular mechanisms are activated that arrest cell cycle progression, induce DNA repair or lesion bypass, and restore replication, in what is commonly called the "DNA damage checkpoint" or the "DNA damage response" (DDR). The checkpoint pathways are activated by delays in DNA replication (replication stress pathway) or by DNA lesions that obstruct replication (DNA damage pathway) [1,2]. This protective cellular response is conserved, which underscores its centrality and importance. Because of this conservation, simple organisms, such as budding yeast, are extremely useful for studying the basic principles of genome stability and maintenance. Not surprisingly, genes that were found to affect genome stability in yeast were later found to be tumor suppressors in human cells. Unless otherwise specified, we will hereafter refer to the vast knowledge on the mechanisms that maintain genome stability accumulated in yeast.

PCNA is a homotrimeric ring that encircles the double stranded DNA and plays a central role in DNA replication. It acts as a processivity factor for the replicative DNA polymerases, and serves as a moving platform during DNA replication to which DNA interacting proteins can bind. Many proteins are known to interact with PCNA, including factors involved in DNA replication, DNA repair, chromatin remodeling and other DNA-related activities that are important for cell viability, cell division, and genomic stability. Many of these proteins interact with PCNA *via* a PCNA-interacting peptide (PIP) or motif [3].

When DNA is damaged, PCNA may be mono-ubiquitinated at lysine 164 by the E2/E3 pair Rad6 and Rad18 [4]. This modification of PCNA activates the DNA Damage Tolerance pathway (DDT, also known as post-replication repair or PRR). Mono-ubiquitination of lysine 164 allows the binding of special DNA polymerases able to replicate damaged DNA molecules, at the expense of accuracy. These translesion synthesis polymerases participate in an error-prone bypass mechanism that results in the creation of mutations. Alternatively, PCNA can be further poly-ubiquitinated on the same lysine residue by an alternative DDT mechanism that also requires an E2 heterodimer composed of the Ubc13 and Mms2 proteins and the E3 protein Rad5 [5]. This poly-ubiquitination coordinates an error-free repair mechanism, whose details are still unclear [6]. Interestingly, the same residue of PCNA (lysine 164) can be modified by the ubiquitin-like molecule SUMO. This modification takes place during S-phase or after high doses of DNA damage and requires the SUMO-specific E2 Ubc9 and the SUMO ligase Siz1 [4]; short SUMO chains are sometimes seen, but their significance is still unclear [7]. An additional residue, lysine 127, can also be SUMOylated, but not ubiquitinated. In contrast to mutations in lysine 164, those in lysine 127 do not lead to DNA damage sensitive phenotypes [4]. SUMOylation of PCNA strongly affects the choice of pathway used for processing the lesions. SUMOylation seems to prevent homologous recombination, favoring ubiquitin-dependent lesion bypass [8,9]. Thus, PRR mutants, such as *Δrad5*, *Δmms2* or *Δubc13* exhibit a high level of spontaneous mutations, caused by channeling of lesions to the trans-lesion synthesis pathway. This mutagenesis can be reduced by preventing PCNA SUMOylation, for example, by deleting the E3 SUMO ligase *SIZ1*. In these PRR defective backgrounds, the lesions are channeled to error free recombinational pathways and mutation levels are reduced [10,11].

## 2. The RFC-like complexes (RLCs)

Replication factor C (RFC) is a 5-subunit complex in charge of loading PCNA onto DNA to allow its replication. RFC is composed of a large subunit, Rfc1, and four small subunits (Rfc2–5). *In vitro*, RFC is capable of both loading and unloading PCNA onto DNA, although it is believed that its main function is to load the clamp, a function required for accurate replication and repair of the genome [12].

Mutations in the yeast *ELG1* gene lead to a variety of genomic instability phenotypes, including, among others, hyper-recombination, hyper-transposition, chromosome loss, gross chromosomal rearrangements, elongated telomeres and increased telomeric silencing [10–24]. The Elg1 protein resembles Rfc1, the large subunit of replication factor C, and forms an RFC-like complex (RLC) together with the four small RFC proteins, Rfc2–5 [11–13] (Fig. 1). Elg1 RLC, as will be discussed below, can unload PCNA from DNA during replication. Two additional RLCs can be detected in most organisms: both are composed of the small RFC subunits, but have the large Rfc1subunit replaced by the proteins Rad24 or Ctf18 (Fig. 1). The canonical RFC complex is essential, as it is required for polymerase loading during DNA replication. In contrast, none of the alternative RLCs is essential, alone or in combinations. The triple *Δelg1 Δrad24 Δctf18* mutant is viable, although it grows very poorly and shows extremely high levels of genome instability and sensitivity to DNA damaging agents [12].

### 2.1. Rad24 RLC

The first RLC to be found was Rad24 RLC [13]. Interestingly, this evolutionarily conserved complex loads an alternative clamp composed of three different proteins, Rad17/Mec3/Ddc1, that forms a PCNA-like heterotrimer (the clamp in humans is called the 9-1-1 complex) [14]. The Rad24-RLC loads the alternative clamp on partial duplex DNA and activates the DNA damage checkpoint activation and repair. The loading mechanism of Rad24 RLC/9-1-1 is similar to that of RFC/PCNA, although the DNA substrate requirements are different [15,16]. *In vitro* experiments demonstrated that only after proper loading of the 9-1-1 clamp by Rad24, Mec1 (the yeast ortholog of ATR) binds and phosphorylates the clamp subunits, as well as the Rad53 protein (the yeast ortholog of Chk2), thus eliciting the DNA damage response [15]. Thus, the Rad24 RLC seems to be responsible for the first step in checkpoint activation [17]. No actual DNA damage is required: in fact, it is enough to load the 9-1-1 complex and Mec1-Ddc2 to the chromatin to obtain a full DDR in the absence of DNA damage [18]. In addition, loading of the 9-1-1 clamp seems to be necessary for proper induction of the error-free DDT pathway [19].

### 2.2. Ctf18 RLC

In the second RLC, the four small Rfc subunits (Rfc2–5) interact with Ctf18 [20,21]. Mutations in *CTF18* were found in two independent screens for genes important for prevention of chromosome loss [22,23], and later, in a specific screen for mutants affecting origin of replication firing [24]. *Δctf18* mutants are