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Suppression of gross chromosomal rearrangements by a new alternative replication factor C complex

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Abstract

Defects in DNA replication fidelity lead to genomic instability. Gross chromosomal rearrangement (GCR), a type of genomic instability, is highly enhanced by various initial mutations affecting DNA replication. Frequent observations of GCRs in many cancers strongly argue the importance to maintain high fidelity of DNA replication to suppress carcinogenesis. Recent genome wide screens in *Saccharomyces cerevisiae* identified a new GCR suppressor gene, *ELG1*, enhanced level of genome instability gene 1. Its physical interaction with proliferating cell nuclear antigen (PCNA) and complex formation with Rfc2–5p proteins suggest that Elg1 functions to load/unload PCNA onto DNA during a certain DNA metabolism. High level of DNA damage accumulation and enhanced phenotypes with mutations in genes involved in cell cycle checkpoints, homologous recombination (HR), or chromatin assembly in the *elg1* strain suggest that Elg1p-Rfc2–5p functions in a fundamental DNA metabolism to suppress genomic instability.

Keywords

Genomic instability; DNA replication; ELG1; RFC; PCNA

DNA is constantly challenged by intracellular as well as environmental stress. Inaccurate repair of DNA damage by such stress can result in genomic instability that can manifest as gross chromosomal rearrangements (GCRs). GCRs include translocations, deletions, inversions, amplifications, chromosome end-to-end fusions, and aneuploidy [1]. These genetic malformations often lead to cell death or carcinogenesis. Frequent observations of genomic instability in tumor cells support the argument for the importance of proper DNA repair to suppress carcinogenesis.

Multiple mutations in cancer normally exceed the expected number of mutations that cells are able to obtain with normal mutation rates. In order to generate multiple mutations, cells must develop specific initial mutations that can facilitate further mutagenesis [2]. These initial mutations, known as mutator mutations, have been suggested to facilitate multiple mutations in many oncogenes and tumor suppressor genes during carcinogenesis. Mutator mutations were first proposed from cases of the hereditary nonpolyposis colorectal cancer

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(HNPCC) and sporadic cancers where the mismatch-repair defects underlie cancer susceptibility [3]. Inherited cancer susceptibility syndromes including Ataxia Telangiectasia (AT), Nijmegen Breakage Syndrome (NBS), Bloom's Syndrome (BS), Ataxia Telangiectasia-Like Disorder (ATLD), and familial breast cancer predisposition with a mutation in the BRCA1 or BRCA2 genes show increased numbers of chromosomal breaks and other GCRs suggesting that similar to HNPCC, certain mutator mutation could facilitate GCRs and carcinogenesis [1;4]. Recent discoveries of multiple pathways involved in suppressing GCRs using yeast *Saccharomyces cerevisiae* and their evolutionary conserved mechanisms across species start to elucidate detail mechanisms that could lead to uncover clues for new therapeutic approach against tumorigenesis.

Genome stability is ensured by complex mechanisms that coordinate DNA replication, DNA repair, and chromosome segregation. GCRs are highly induced by various initial mutations affecting DNA replication. During DNA replication, DNA replication forks stall at damaged DNA or at naturally occurring sequences such as replication barriers. If not properly repaired, stalled DNA replication forks collapse and produce double strand breaks (DSBs) that are intermediates of GCRs. Extensive genetic interaction studies using various yeast GCR assays have identified eight pathways that suppress and six pathways that promote GCR in yeast (Table 1).

1. A new member of RFC family (ELG1-RFC)

Processing of stalled replication forks are usually modulated by a homo-trimeric DNA sliding clamp called PCNA in eukaryotes. Differential post-translational modifications in PCNA have been documented as a regulatory mechanism for many different DNA metabolisms including GCR [5]. Replication factor C (RFC) complex is a heteropentameric complex that catalyzes the loading of PCNA [6]. There are four different RFC complexes (Fig. 1). All RFC complexes share a core complex consisting of four subunits (Rfc2p, Rfc3p, Rfc4p, and Rfc5p) and differ by a specific subunit. A RFC complex having the specific subunit Rfc1p loads PCNA onto DNA during general DNA replication [7]. The Rad24p (yeast homolog of human RAD17)-Rfc2-5p complex loads a PCNA-like heterotrimeric clamp consisting of Rad17p, Ddc1p and Mec3p (yeast homologs of human RAD9, RAD1, and HUS1, respectively) onto damaged DNA for activation of DNA damage checkpoint [8]. The third RFC complex is Ctf18p-Rfc2-5p and unloads PCNA from DNA where sister chromatids are held by cohesion complex [9]. The fourth complex that was recently added to the RFC family contains Elg1p as a specific subunit [10;11;12]. Although the function of Elg1p-Rfc2-5p RFC is unclear, increases in GCR and prolonged S phase progression in the *elg1 Δ* strain suggest a function in genome stability [10;11;12;13;14]

2. ELG1 functions in DNA replication to suppress GCR

We identified Elg1p-Rfc2-5p as a new suppressor of GCR in *Saccharomyces cerevisiae* through a genome wide screen in identifying additional GCR suppression pathways [13]. Independently, Elg1p-Rfc2-5p was discovered as a suppressor of the Ty1 mediated recombination and a synthetic lethality mutation with holiday junction resolvase mutations, *mus81* or *mms4* [10;11;12].

In the absence of *ELG1*, spontaneous intra- or inter-allelic recombination frequencies are elevated [10]. In addition to enhanced GCR, high level of chromosomal loss was observed in the *elg1* strain [11;12]. Thus, Elg1p-Rfc2-5p functions to maintain genome stability during normal cell growth. The synthetic growth defect of the *elg1* strain by the mutation of homologous recombination (HR) repair genes (*rad51*, *rad52*, *rad54*, *rad55*, *rad57*, and *mre11*) suggests that the lack of Elg1p-Rfc2-5p would create DSBs that HR proteins need to repair [12]. Although Elg1p's function is not essential for viability in yeast, the replication

fork could be stalled more frequently in the absence of Elg1p and ultimately generate DSBs. In this situation, cell cycle checkpoints could be activated and alternative repair machinery such as HR can be elicited. Strong synergistic increase of GCRs by the inactivation of cell cycle checkpoints in the *elg1* deletion strain supports the notion that cell cycle checkpoints indeed suppress GCR from DNA damage initiated by the *elg1* mutation [14]. However, hetero allelic recombination frequency after DNA damaging agent treatment was reduced in the *elg1* strain [15].

3. Possible molecular functions of Elg1p-Rfc2–5p for maintaining genome stability

Elg1p interacts with PCNA and Rad27p (yeast homolog of human FEN1, FLAP endonuclease) that removes the flap structure at the lagging strand during DNA replication [12]. Therefore, Elg1p-Rfc2–5p may play an important role during DNA replication to protect the genome by loading or unloading a clamp similar to other RFCs' roles. Despite negative *in vitro* PCNA loading or unloading PCNA by Elg1p-Rfc2–5p [9], the physical interaction of Elg1p with PCNA [12] strongly suggests Elg1p-Rfc2–5p as a PCNA loader or unloader. The PCNA loading/unloading activity of Elg1p-Rfc2–5p seems to overlap with other RFCs because synergistic sensitivities to DNA damaging agents were observed by inactivation of other RFCs with Elg1p-Rfc2–5p [11;12]. However, Elg1p-Rfc2–5p seems to have a unique function because the *elg1* mutation itself made cells sensitive to DNA damaging agents and grow slowly [12;14]. Elg1p-Rfc2–5p could also function as a platform for polymerase switching in the post replication repair (PRR) pathway. When the DNA replication machinery encounters a DNA lesion that blocks further replication, a PRR pathway called translesion synthesis (TLS) is initiated [5]. TLS is largely dependent on the mono-ubiquitination of PCNA by Rad6p–Rad18p. The mono-ubiquitination of PCNA triggers the switch of replicative polymerase δ or ϵ to a specialized TLS polymerase such as pol ζ or pol η that is extremely efficient in extending terminal mismatch or inserting A opposite to T-T dimers produced by UV irradiation, respectively [5]. In S phase, these TLS polymerases bypass DNA lesion and when DNA replication ends, several DNA repair pathways repair it. Co-localization of human ELG1 and human DNA polymerase η and the interaction between ELG1 and PCNA suggest that human ELG1-RFC could function in a PRR pathway (Banerjee, S., Sikdar, N. and Myung, K. unpublished data). PCNA is further poly-ubiquitinated by Ubc13p-Mms2p-Rad5p and directs another PRR pathway called template switching [16;17]. The exact mechanism of template switching is not clearly understood. However, their shared role in GCR suppression by template switching and Elg1p, suggests that Elg1p could function in template switching.

Chromatin assembly is a fundamental biological process that is essential for the replication and maintenance of the eukaryotic genome [18]. In dividing cells, newly synthesized DNA is rapidly assembled into chromatin and packaged into nucleosomes that form the fundamental repeating subunits of chromatin. The nucleosome assembly is initiated by the deposition of a tetramer composed of two of each histone H3 and H4 to DNA. Subsequently, another tetramer comprised of two of each histone H2A and H2B is recruited to complete the nucleosome assembly. The synthetic lethality of the *elg1* mutation with the *htb1* or *bre1/bre5* mutations that creates defects in histone levels or histone modification suggests a function for Elg1p in chromatin assembly through PCNA interaction [19]. Disruption of proper chromatin assembly by the inactivation of chromatin assembly factor 1 (CAF1) blocks DNA synthesis and induces DNA damage that activates checkpoints [20]. Intriguingly, the inactivation of each subunit of CAF1 including Cac1p, Cac2p, and Cac3p increased GCRs [21] and Cac1p physically interacts with PCNA [22]. Therefore, it is possible that Elg1p-Rfc2–5p could function in chromatin assembly by assisting proper chromosome condensation by PCNA loading or unloading.

4. Concluding Remarks

Elg1p is conserved throughout eukaryotic evolution with clear orthologues in *C.elegans*, Zebra fish, mouse, Rat and human. Mammalian ELG1 seems to have similar functions. Human ELG1 gene is mapped to Chr17q11.2 loci, proximal to Neurofibromatosis (NF) 1 gene. Intriguingly, the human ELG1 is often deleted in NF patients who are prone to cancer development [23]. It is possible that these cancer prone NF patients develop cancer as a result of ELG1 defect. High induction of GCRs and, faulty DNA replication presumably caused by stalled replication fork and abnormal HR in the *elg1* strain suggest that Elg1 has a fundamental role of Elg1 to in maintaining genomic stability.

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Figure 1.

There are four different replication factor C (RFC) complexes. Each RFC is composed of four common subunits (Rfc2p, Rfc3p, Rfc4p, and Rfc5p) in addition to a specific subunit for each RFC. Rfc1p makes a general RFC complex with common subunits and function in general DNA replication for loading PCNA. Rad24p with common subunits functions specifically to load DNA damage checkpoint sensor Rad17p-Mec3p-Ddc1p to DNA damage. Ctf18p makes another RFC to unload PCNA where chromosome cohesion is established. The function of a RFC carrying Elg1p as a specific subunit is not clearly understood, yet.

Table 1.
Pathways that suppress or promote GCR formation in *Saccharomyces cerevisiae*

There are more proteins participating pathways listed here. Only proteins tested by GCR assay are listed. The “/” between Rad51 and Rad59 or Rad54 and Rhp54 means that both proteins are redundantly required for break induced replication. Detailed studies to identify pathways listed here are discussed in other reviews [1;24].

Pathways	
GCR suppression	Proteins in pathways
1. S phase checkpoints	
a. Replication checkpoint	Rfc5, Pol2, Dpb11, Drc1, Mec1, Ddc2, Dun1
b. Intra-S checkpoint	Rad9, Rad17, Mec3, Ddc1, Mec1, Rad53, Dun1, yKu70, yKu80
c. Intra-S checkpoint	Sgs1, Chk1, Mre11, Rad50, Xrs2, Tel1, yKu70, yKu80
2. Break induced replication	Rad52, Mre11, Rad50, Xrs2, Rad51/Rad59, Rad54/Rhp54
3. Telomerase inhibition	Pif1
4. Chromatin assembly	Cac1, Asf1
5. Chromosome fusion suppression	Tlc1, Est1, Est2, Est3, Cdc13, Mec1, Tel1
6. Mismatch repair pathway	Msh2, Sgs1
7. Detoxification of oxygen species	Tsa1, Ato1
8. Template switching	Rad18, Rad5, PCNA
GCR promoting	
1. <i>de novo</i> telomere addition	Est1, Est2, Est3, Cdc13, Tlc1, Tel1, yKu70, yKu80
2. Non-homologous end joining (NHEJ)	Lig4, Lif1, yKu70, yKu80
3. Mitotic checkpoints & Mitotic exit network	Bub1, Bub3, Mad1, Mad2, Mad3 and Bub2
4. Endonuclease	Rad1, Rad10
5. PCNA sumoylation	Siz1, Srs2, PCNA
6. Histone ubiquitination	Bre1, H2B