**ELG1**, a yeast gene required for genome stability, forms a complex related to replication factor C

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**Results**

**Isolation of the elg1 Mutant.** Ty elements are the largest family of naturally occurring repeated sequences in yeast, comprising \( \approx 3\% \) of the genomic DNA. Despite their abundance, Ty elements spontaneously recombine at remarkably low rates (13). In addition, Ty recombination is not increased by DNA-damaging treatment, such as exposure to MMS or to UV irradiation (18), although it can be induced by directed double-strand breaks (DSBs) (19). These results suggest the existence of mechanisms that prevent recombination between repeated sequences. We screened for mutants exhibiting increased frequencies of Ty recombination. We used strain SBA1, which enables easy monitoring of recombination between Ty elements and between direct repeats (ref. 14; see Fig. 1A and Methods). Strain SBA1 was subjected to mutagenesis by randomly disrupting genes with a modified transposon (16), and individual colonies showing elevated levels of Ty recombination were isolated. One of the mutants, which exhibited a particularly high level of Ty recombination, carried a transposon insertion at ORF YOR144c.

3 RFC1-like clamps (Ctf18, Elg1 and Rad24) have nonessential and overlapping functions as seen by the increased sensitivity to DNA damage or slowed replication.
Initiation of Replication
Regulation of origin firing in E. coli by the hemi-methylated DNA binding proteins SeqA

Dam site is fully methylated

Dam sites are hemimethylated
Excess SeqA prolongs sequestration of oriC and delays nucleoid segregation and cell division

Fig. 1. Overexpression of SeqA protein extends the period of hemimethylation at oriC. (A) Schematic representation of the temperature shifts at the indicated times during the experiment. (B) Exponentially growing cultures (30°C) of MG1655 dnaC2 cells harbouring pH2102 (control) or pMAK7 (excess SeqA) were synchronized with respect to initiation for 1 h at 38°C before shifting the temperature to 30°C. IPTG (0.5 mM) was added to the cultures three mass doublings prior to synchronized initiation. Chromosomal DNA was extracted at the indicated times after returning the cultures to 30°C. The DNA was digested with XhoI and HphI, electrophoresed and hybridized to an oriC probe. The positions of cut and uncut fragments are indicated by arrows. The rightmost lane contains DNA isolated from a dam- strain which reveals the position of the cut fragment. (C) Quantification of the amount of hemimethylated oriC in (B). Squares represent dnaC2/pFH2102 and triangles dnaC2/pMAK7. The percentage of hemimethylated oriC is twice the percentage of the cut fragment in (B) since 50% of the hemimethylated fragments are digested. Time indicates minutes from shift to 30°C after 1 h synchronization at 38°C. (D) Western analyses of samples taken from the cultures at 0 and 20 min after returning the cultures to 30°C. A 1 μg aliquot of total protein of each sample was loaded.
**Fig. 2.** DNA content histograms of *dnaC2/pFH2102* (A–E), *dnaC2/pMAK7* (F–J) and *dnaC2/pSF19* (A, small panel). Samples for flow cytometry of *dnaC2/pFH2102* and *dnaC2/pMAK7* cells were collected during the temperature shift experiment in Figure 1 at the times indicated. In an identical control experiment, *dnaC2/pSF19* cells were harvested for flow cytometry, at the time indicated.
higher eucaryotic origins are not very well conserved at the sequence level. AT rich. Many possible origins, few are used, but how are they chosen?
Uninterrupted MCM2-7 Function Required for DNA Replication Fork Progression

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A (i) Replication fork movement

Chromosome III

MCM4 mcm4-td
HH HL HH HL

Relative counts

Fraction number

0 20 40 0 20 40

α

HU

Release 37°C 45'

(ii)

Fragment 1 (ARS306)
Fragment 2 (End of replicon)

MCM4

% replication

0 20 40 60 80 100

α HU 45° 90° Release at 37°C

B (i) Replication fork movement

Chromosome VI

MCM4 mcm4-td
HH HL HH HL

Relative counts

Fraction number

0 20 40 0 20 40

α

HU

Release 37°C 45'

(ii)

Fragment 3 (ARS607)
Fragment 4 (End of replicon)

MCM4

% replication

0 20 40 60 80 100

α HU 45° 90° Release at 37°C
Mechanisms of Conformational Change for a Replicative Hexameric Helicase of SV40 Large Tumor Antigen
Figure 7. A Looping Model Showing the Coupling of the $\beta$ Hairpin Movement to the dsDNA Translocation into LTag Double Hexamer for Unwinding

The $\beta$ hairpins move along the central channel in response to ATP binding (step 1), hydrolysis (step 2), and ADP release (step 3). (A) LTag double hexamer with two ssDNA loops coming out from the side channels. Each hexamer contains a helicase domain (represented by two squares in pink) and an OBD (oval in light blue). The $\beta$ hairpin structure is represented by two bars (in blue) within the helicase domain. The colored dots on the DNA (red, orange, black, and blue) are position markers for translocation. (B) A LTag hexamer corresponding to the left half of the double hexamer in (A) in the Nt-free state. (C) The movement of $\beta$ hairpins upon ATP binding, which serves to pull dsDNA into the helicase for unwinding. The unwound ssDNA extrudes from the side channels. For clarity, only one hexamer is shown. (D) The $\beta$ hairpins move back about halfway toward the Nt-free position after ATP hydrolysis. (E) The ADP is released from the LTag hexamer, and the $\beta$ hairpins return to the original Nt-free position.
In a ts cdc9 strain where there is no ligase activity, low-MW nascent DNA was purified. The length of the Okazaki fragment pointing to the origin was determined by primer extension using a 32P-labelled primer that is homologous to sequences at the origin. This shows that the size of the initial Okazaki fragment is quite uniform.
Okazaki fragments are “chased” into higher MW DNA.
number of additional ARS seqs.

adding more ARS sequences stabilizes a plasmid that is otherwise unstable in a cdc6 mutant where the probability of initiation of replication is lowered.
Budding yeast replication

Note that not all origins fire at the same time and some origins don’t fire at all while others fire in some cells and not others.

Location of ARS sequences on yeast chromosomes
Release from G1-arrest

Appearance of ARS1 or R14 DNA in a HL fraction after S phase is initiated in synchronized cells.
Early DNA replication in human chromosomes, hybridized with metaphase chromosomes

Cyrus Vaziri
Identifying origins of replication 2-D gels

- size
- shape
- autorad of gel
- double bubble
- simple Y
- closed bubble
- double Y
- simple Y

Images of autorad with rad53 Δasir4 ARS301 and ARS305 probes.
bubble arc

Y-arc in the adjacent restriction fragment

Y-arc in the next adjacent restriction fragment arrives later

20  25  30  45  60  90 min
ribosomal DNA: 140 repeated copies in yeast
rDNA replication in budding yeast has a Replication Fork Blocking sequence regulated by FOB1

\[ fob1^\Delta \]

replication is quasi-unidirectional

this origin doesn’t fire
Each eukaryotic origin fires once (or not at all) during one cell cycle

“Licensing” of DNA replication

Sc Cdc6
Cdc6 and Cdt1 are destroyed or exported from the nucleus to ensure no re-initiation.
Yeast ORC binds to an origin at all times in the cell cycle but is assembled into a replication complex (RC) in G1. Once fired, the ORC complex can’t fire again until the next G1.

The complex in G2-arrested cells is modified by the time cells are ready to begin replication.
Figure 3. MCM2-7 proteins are essential for pre-RC formation.

1 = G2 arrest
2 = release to G1 arrest
Maintenance of genome stability

**G₁**
- RAD17
- RAD24
- MEC3

**S**
- RFC5
- POL2
- DPB11
- DRC1
- RAD17
- RAD24
- MEC3
- SGS1

**G₂**
- RAD17
- RAD24
- MEC3

**M**
- BUB1,3
- MAD1,2,3
- BUB2
- BFA1/BYR4

**G₁** arrest in response to DNA damage

DNA damage checkpoint (G₁)

Replication checkpoint (S)

S phase arrest in response to replication blocks

Slowing of replication in response to DNA damage in S phase

S phase arrest in response to DNA damage

Mitotic checkpoint (M)

Block of exit from mitosis

**Transducer and effector functions**
- MEC1
- TEL1
- MRE11-RAD50-XRS2
- RAD9
- RAD53
- DUN1
- RAD55
- PDS1

**Human homologs**

<table>
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<th>Yeast</th>
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<tr>
<td>MEC1/TEL1</td>
<td>ATR/ATM</td>
<td>Ataxia telangiectasia</td>
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<td>MRE11</td>
<td>MRE11</td>
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Figure 4. Efficient Activation of Rad53 in Response to MMS (an alkylating agent that stalls replication) Occurs in S Phase

http://www.molecule.org/content/article/fulltext?uid=PIIS1097276503001692
HU (hydroxyurea) inhibits ribonucleotide reductase hence deoxynucleotide pools are depleted.

*rad53* mutant cells are unable to recover even when cells are removed from HU.
Rad53 protects stalled replication forks from “collapse” as seen by the conversion of bubble arcs to Y structures (breakage of the fork or other alternations).
Fig. 3. RIs containing four-way junctions from HU-treated rad53 cells. In most of the Holliday junctions formed at the replication forks, the four single strands involved in the branch point are visualized [(A), (B), (C), (G)]. (A to F) Samples were prepared under nondenaturing conditions. Arrows indicate single-stranded regions [(C) and (D)]. (G) and (H) Samples were prepared under denaturing conditions. In (H), the fourth arm is organized in single-stranded bubbles characteristic of nucleosomal DNA.

RI = Replicaiton Intermediate

http://www.sciencemag.org/cgi/content/full/297/5581/599
loss of fork integrity.
In HU, early origins still fire, producing Okazaki fragments, but late origins don’t
But in *rad53* or *mec1* mutants, in the presence of HU, late origins can now fire, though they are still “late” you should know what this means