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# Gene Section
## Review

# RPA2 (replication protein A2, 32kDa)

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## Identity

**Other names: REPA2, RPA32**
**HGNC (Hugo): RPA2**
**Location: 1p35.3**
**Local order:** The human RPA2 gene maps on 1p35.3 between the SMPDL3B (sphingomyelin phosphodiesterase, acid-like 3B) and C1orf38 (interferon-gamma inducible gene ICB-1 (induced by contact to basement membrane)).

## DNA/RNA

### Description

The RPA2 gene is contained within 24.5 kb of chromosome 1.

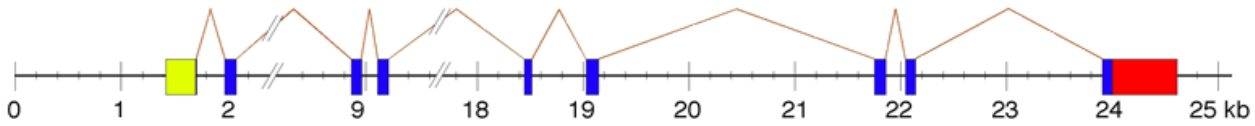
The coding sequence is contained within nine exons. There is no confirmed alternative splicing of the RPA2 gene, or differential promoter usage.

### Transcription

The RPA2 mRNA transcript is 1.5 kb. The RPA2 promoter contains four E2F consensus sequences within the region about 400 bp upstream of the mRNA start site, and putative binding sites for ATF-1 and SP-1 transcription factors. Expression is upregulated 2 to 3-fold by E2F, with mutation of the three start site-proximal E2F sites causing a loss of E2F responsiveness (Kalma et al., 2001).

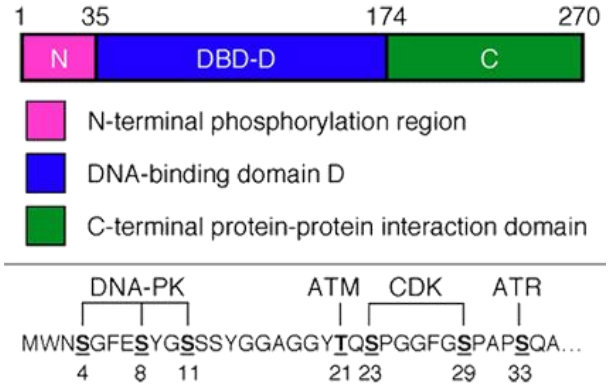
### Pseudogene

RPA2 does not have known pseudogenes.




The sequence numbering corresponds to EMBL locus DQ001128 (26.6 kb). Exon are indicated as boxes (yellow = 5' UTR, blue = CDS, red = 3' UTR), and introns with orange lines. Two lengthy introns have been truncated (indicated with parallel diagonal lines) to improve viewability.

# Protein




Upper panel: Schematic showing the key domains of RPA2. Lower panel: RPA2 phosphorylation sites are shown in bold, with the primary responsible kinases indicated above each site. Some sites can be phosphorylated by more than one kinase (e.g., T21 by ATM and DNA-PK).

## Description

RPA2 is the middle subunit of the heterotrimeric Replication Protein A (RPA; (reviewed in Binz et al., 2004)). The subunit is composed of 270 residues, and has a nominal molecular weight of 29.2 kDa. RPA2 contains an N-terminal phosphorylation region with 7 phosphorylation sites, a central DNA-binding domain (termed DBD-D), and a C-terminal region that can form a three-helix bundle. One helix of the three helix bundle is contributed by each RPA subunit, with this structure responsible for supporting heterotrimerization of the RPA complex (Bochkareva et al., 2002). At least in the non-phosphorylated state, the N-terminal region is unstructured. DBD-D is constructed from an oligonucleotide/oligosaccharide binding (OB) fold (Bochkarev et al., 1999), one of six OB folds found with the RPA heterotrimer (four OB folds are located in RPA1, and one within RPA3).

## Expression

RPA is an essential factor for DNA replication and repair, and hence is expressed in all tissues.

## Localisation

Nuclear.

## Function

**General function:** RPA is a heterotrimeric single-stranded DNA (ssDNA) binding protein that is essential for chromosomal DNA replication, homologous recombination, and particular DNA repair reactions (nucleotide excision repair). The apparent association constant of the RPA: ssDNA complex is $10^9$ - $10^{11}$ $M^{-1}$ (Kim et al., 1992). While RPA2 contains a central DBD (Philipova et al., 1996), the major effect of mutating DBD-D is to decrease the size of the ssDNA occluded by RPA binding, with only minor effects on RPA: ssDNA affinity (Bastin-Shanower and Brill, 2001). A key function of the RPA2 subunit is to regulate RPA activity in DNA replication and repair reactions, through the RPA2 phosphorylation state (see below).

**1) RPA2 phosphorylation.** The N-terminal 33 residues of RPA2 contain seven phosphorylation sites. In interphase cells, genotoxic stress (e.g., caused by chromosomal double-strand DNA breaks or DNA replication stress) induces RPA2 phosphorylation by members of the phosphatidylinositol 3-kinase-like kinase (PIKK; ATM, ATR, and DNA-PK) and cyclin-dependent kinases (CDK) families (reviewed in Binz et al., 2004). Mutation of particular RPA2 phosphorylation sites causes defects in homologous recombination (Lee et al., 2010), and Rad51 recruitment to nuclear repair foci (Anantha et al., 2008; Lee et al., 2010). Mutation of these sites also causes genomic instability in response to DNA replication stress induced by cellular treatment with hydroxyurea (Vassin et al., 2009). RPA phosphorylation also increases cell viability in response to DNA damage arising during mitosis (Anantha et al., 2008). Modification of sites in the phosphorylation region of RPA2 proceeds in a favored order in response to genotoxic stress (Anantha et al., 2007). The phosphorylation of individual RPA2 residues is dependent on the type of DNA damage or replication stress encountered (Anantha et al., 2007; Vassin et al., 2009). RPA2 is a substrate both for PP2A and PP4 phosphatases (Feng et al., 2009; Lee et al., 2010).

**2) Involvement of RPA2 in protein-protein interactions.** RPA2 interacts with the nucleotide excision repair factor XPA (He et al., 1995), base excision repair enzyme UNG2 (Mer et al., 2000), homologous recombination (HR) factor Rad52 (Mer et al., 2000), replication checkpoint protein Tipin (Unsal-Kacmaz et al., 2007), and the annealing helicase HARP/SMARCAL1 (Bansbach et al., 2009; Ciccia et al., 2009; Yuan et al., 2009). These interactions likely aid the multiple roles of RPA in facilitating DNA repair.

## Homology

A close homolog of RPA2, termed RPA4, is located on Xq21.33 (Haring et al., 2010).

# Mutations

**Note**

Naturally-occurring mutations of human RPA2 have not yet been described. A small number of genetic polymorphisms have been described in SNP datasets (Y14S, G15R, and N203S), but these have not yet been reported to have any biological effects (NIEHS SNPs program).

# Implicated in

## Colorectal adenocarcinoma

**Disease**

Overexpression of the RPA2 (and RPA1) proteins have been found to be prognostic indicator of colon cancer. Strong associations between RPA2 expression and disease stage, lymph node metastasis, and the histological grade of carcinomas have been observed.

**Prognosis**

In addition, RPA2 protein expression correlates with poor survival of stage II and III patients (Givalos et al., 2007).

### *Ductal breast carcinoma*

**Disease**

Levels of anti-RPA2 antibodies was observed to be significantly higher in sera from breast cancer patients (10.9%; n = 801) as compared to normal controls (0.0%; n = 221). Examining individuals with early stage intraductal in situ carcinomas, 10.3% (n = 39) similarly showed the presence of high levels of anti-RPA2 antibodies. Even so, follow-up studies indicated that there were no apparent differences in mean survival, occurrences of a second primary tumor, or metastasis frequency between breast cancer patients that were positive or negative for anti-RPA2 sera. Although RPA is a nuclear protein, RPA was seen to be localized to both nuclei and cytoplasm in the cells of at least one breast tumor, with RPA also over-expressed (Tomkiel et al., 2002).

### *Non-small cell carcinoma*

**Disease**

A fraction of individuals with squamous cell lung cancer were found to have significant levels of anti-RPA2 antibodies (9.1%; n = 22) (Tomkiel et al., 2002).

### *Laryngeal tumors*

**Disease**

One patient (out of 35; 2.9%) with head and neck tumors tested positive for the presence of anti-RPA2 sera (Tomkiel et al., 2002).

### *Promyelocytic leukemia*

**Disease**

A derivative of the human HL-60 promyelocytic leukemia cell line (HL-60/P1), selected for its decreased sensitivity to undergo apoptosis in response to TNF-related apoptosis-inducing ligand (TRAIL), was found to have decreased (2-fold) expression of RPA2 (Petrak et al., 2009).

### *Sjögren syndrome*

**Disease**

Serum from a patient with Sjögren syndrome was found to have high levels of anti-RPA2 antibodies. A higher rate of non-Hodgkin lymphoma, and lymphoid malignancies, is seen in individuals with Sjögren syndrome, compared to normal individuals (Garcia-Lozano et al., 1995).

### *Systemic lupus erythematosus (SLE)*

**Disease**

One out of 55 individuals with autoimmune disorders was found to test positive for anti-RPA2 antibodies (1.8%). This individual had SLE, and secondary Sjögren syndrome (Garcia-Lozano et al., 1995).

### *Rheumatoid arthritis (RA)*

**Note**

Fibroblast-like synoviocytes (FLSs) are a cell type whose invasive properties provide an indicator of RA severity. Microarray studies from FLSs in DA rats (arthritis-susceptible inbred model) show a modest increase in the level of RPA2 mRNA, compared to back-crossed arthritis-resistant DA.F344 (Cia5d) congenic strains (Laragione et al., 2008).

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