# Characterization of the Five Replication Factor C Genes of Saccharomyces cerevisiae

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Received 27 February 1995/Returned for modification 28 April 1995/Accepted 26 May 1995

Replication factor C (RFC) is a five-subunit DNA polymerase accessory protein that functions as a structurespecific, DNA-dependent ATPase. The ATPase function of RFC is activated by proliferating cell nuclear antigen. RFC was originally purified from human cells on the basis of its requirement for simian virus 40 DNA replication in vitro. A functionally homologous protein complex from *Saccharomyces cerevisiae*, called ScRFC, has been identified. Here we report the cloning, by either peptide sequencing or by sequence similarity to the human cDNAs, of the *S. cerevisiae* genes *RFC1*, *RFC2*, *RFC3*, *RFC4*, and *RFC5*. The amino acid sequences are highly similar to the sequences of the homologous human RFC 140-, 37-, 36-, 40-, and 38-kDa subunits, respectively, and also show amino acid sequence similarity to functionally homologous proteins from *Escherichia coli* and the phage T4 replication apparatus. All five subunits show conserved regions characteristic of ATP/GTP-binding proteins and also have a significant degree of similarity among each other. We have identified eight segments of conserved amino acid sequences that define a family of related proteins. Despite their high degree of sequence similarity, all five *RFC* genes are essential for cell proliferation in *S. cerevisiae*. *RFC1* is identical to *CDC44*, a gene identified as a cell division cycle gene encoding a protein involved in DNA metabolism. *CDC44*/*RFC1* is known to interact genetically with the gene encoding proliferating cell nuclear antigen, confirming previous biochemical evidence of their functional interaction in DNA replication.

Replication factor C (RFC) is a multiprotein complex consisting of one large and four small subunits. The subunits of human RFC (hRFC) have apparent masses of 140, 40, 38, 37, and 36 kDa (23, 51). RFC has an associated ATPase activity that is stimulated by the binding of RFC to DNA and is further stimulated by proliferating cell nuclear antigen (PCNA) (54). RFC binds preferentially to the 3' end of a DNA primer bound to a template DNA (22, 23, 54, 55). It is a structure-specific DNA-binding protein and acts as a primer recognition factor for DNA polymerases  $\delta$  and  $\epsilon$  (Pol  $\delta$  and Pol  $\epsilon$ ) (5, 12, 22, 23, 42, 54-56). The large subunit of RFC contains a DNA-binding site, whereas at least one of the small subunits binds ATP (7, 55). PCNA recognizes and binds to the RFC-DNA complex in an ATP-dependent manner, and then DNA Pol  $\delta$  or  $\epsilon$  recognizes the RFC-PCNA complex bound to the primer-template prior to the start of DNA synthesis. In addition to stimulating polymerase loading, PCNA and possibly RFC also function as accessory proteins for Pol  $\delta$  and  $\epsilon$  by increasing their processivity (reviewed in reference 48).

RFC was shown to be essential for the simian virus 40 (SV40) in vitro DNA replication system (23, 53). It is responsible for a polymerase switch from DNA Pol  $\alpha$  to Pol  $\delta$  during initiation of leading-strand DNA replication at the SV40 origin and for the synthesis of Okazaki fragments during lagging-strand DNA synthesis (11, 52, 59). RFC binds to primers synthesized by Pol  $\alpha$ -primase, blocks them for further elongation by this polymerase, and increases the affinity of Pol  $\delta$  (or possibly Pol  $\epsilon$ ) for these primers (58). Studies with the SV40 DNA replication system, however, have not determined whether these same activities are required for replication of

the eukaryotic genome and whether RFC has additional activities in vivo such as a role in DNA repair and recombination. To address these questions, we have turned to the genetically manipulatable organism *Saccharomyces cerevisiae*. In addition to being useful for determining the role of DNA replication factors in chromosomal replication, *S. cerevisiae* provides an opportunity to attempt reconstitution of DNA replication by using well-defined eukaryotic chromosomal origins of DNA replication.

A protein complex that is functionally homologous to hRFC from *S. cerevisiae* has been identified and called ScRFC (5, 12, 26, 61). ScRFC has activities similar to those of hRFC. It binds in a structure-specific manner to primer-template junctions and is also a DNA-stimulated ATPase that can be further stimulated by *S. cerevisiae* PCNA (ScPCNA) (5, 12). ScRFC was identified as an activity that stimulated *S. cerevisiae* Pol  $\delta$  DNA synthesis by using a primed, circular single-stranded DNA template in the presence of ScPCNA and *S. cerevisiae* replication protein A (ScRPA) (12, 61). ScRFC does not cross-react with monoclonal antibodies that react with hRFC (3a), and hRFC will not cooperate with ScPCNA and ScRPA to stimulate *S. cerevisiae* Pol  $\delta$  DNA synthesis (12).

ScRFC has a subunit composition similar to that of hRFC. It is a multisubunit protein with a large subunit with an apparent mass of 103 kDa and possibly four small subunits running as a doublet at 40/41 kDa and a single band at 36 kDa on sodium dodecyl sulfate (SDS)-polyacrylamide gels (12, 26). To characterize these protein subunits, we sequenced peptides derived from them and used this information to isolate the ScRFC genes. Here we demonstrate that all five *RFC* genes are essential for cell proliferation and that the gene encoding the large subunit is identical to *CDC44*, a previously characterized gene encoding a cell division cycle protein (15). The genes encoding three of the small subunits also have recently been reported (24, 25, 34).

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ScRFC:	sequenced	peptides:
kDa kDa	103 15c	SVVVLGDEAGPK
	103 150	VRSSISRPI
200	103 31	(1)AAHLVAQELGYDILEQNA(S)DV(R)
	103 36	KIRSSEQLxSLLPLHAVLS(S)VYP(A)xk
100	103 39	EDxD(S)IMEFF
51	40/41 3	(S/G)LNALSHNEELTNFLK
- 69	40/41 4	(S/G/A)ANLPHMLFYGPPGT(GK)
- 68	40/41 5	DVRQFVTA(S)NRKL(E)xNVV
	40/41 6	SAASVVNQLHEYY(I)T(NDNFD)
1 F. 1994	40/41 9a	DAQAALRRTMEK
	40/41 9b	FRFK
- 45	40/41 28a	ELTFSLLDVETLNTTNK
	40/41 28b	SSSPIIKPD <u>D</u> IIV(I)
40		
36	36 7	ETIDRLQQIAK
	36 9	(D)xNMP
-	36 11	YRPQVLSDIVGNK
	36 12	GYSSIDIVTT <u>D</u> FRVTK
	36 13	TLSLQLP
and a second	36 15a	MLLASNLEDSIOILRTDLRK
	36 15b	IVILDEADSM(T)AGAQ
		• • •

FIG. 1. Peptide sequences obtained from the ScRFC subunits. Shown is the result of SDS-PAGE of ScRFC (left lane; preparation used for peptide sequencing) next to molecular weight markers (right lane). The 103-kDa band, the 40/41-kDa doublet, and the 36-kDa band marked were used for peptide sequencing. The contaminating bands are not present in highly purified ScRFC (12). Listed are the amino acid sequences obtained from each band. Amino acids that were not unambiguously determined are in parentheses, and amino acids that could not be determined are indicated by an x.

### MATERIALS AND METHODS

**Strains.** The S. cerevisiae strains used were BJ926 ( $MATa/MAT\alpha$  TRP1/trp1 HIS1/his1 pep4-3/pep4-3 prb1-1122/prb1-1122 prc1-126/prc1-126 can1/can1 gal2/ gal2) (17), diploid W303 ( $MATa/MAT\alpha$  ura3-1/ura3-1 his3-11,15/his3-11,15 trp1-1/trp1-1 leu2-3/leu2-3 ade2-1/ade2-1 can1-100/can1-100), its derivative W937 (as W303, but ura3-d1/ura3-d1 [43a]), YB10062 (as W303, but RFC2/rfc2\Delta:: LEU2), YB10063 (as W303, but RFC3/rfc3\Delta::HIS3), YB10064 (as W937, but RFC4/rfc4\Delta::LEU2), and YB10065 (as W937, but RFC5/rfc5\Delta::HIS3).

ScRFC purification. ScRFC was purified as described by Fien and Stillman (12) from BJ926 cells, with the following modifications. The first phosphocellulose column was loaded with the crude lysate from BJ926 cells and then washed with 1 column volume of the same buffer containing 0.66 M NaCl. RFC and other proteins were eluted from the hydroxylapatite column with a 600-ml linear gradient from 0.1 to 0.6 M KPO<sub>4</sub> HAP buffer (12). The third step, a phosphocellulose column, was eliminated from the new purification scheme. Active fractions from the glycerol gradient were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and silver staining. The RFC fractions were concentrated on a 0.1-ml Q-Sepharose column and eluted in three 0.1-ml amounts of buffer A containing 0.2 M NaCl.

Isolation of lysyl-endopeptidase fragments and protein sequencing. Purified ScRFC was fractionated by electrophoresis in an SDS-polyacrylamide gel, and four bands corresponding to the ScRFC subunits (one band at 103 kDa, a doublet at 40/41 kDa, and one band at 36 kDa) were cut out from the gel. The polypeptides were digested with *Achromobacter* protease I (lysyl-endopeptidase; Wako) (1). Peptides were separated by reverse-phase high-pressure liquid chromatography (HPLC) on a Vydac C<sub>18</sub> column and eluted with a linear gradient of 10 to 35% acetonitrile in water. Peptide fractions were sequenced directly on an Applied Biosystems model 475 automated sequencer with an on-line model 120A HPLC PTH analyzer. The sequences obtained are shown in Fig. 1.

Isolation of probes for the RFC genes. (i) *RFC1* probe. The amino acid sequence of peptide 103 31 (Fig. 1) was used to design four degenerate oligonucleotides: ScRFC1.4 (CGGAATTCGC HCAYCTDGTY GCHCARGA) and ScRFC1.5 (CGGAATTCGC HCAYTTRGTY GCHCARGA) from the N terminus of peptide 103 31 and ScRFC1.6 (CGGAATTCAC RTCDGADGCR TTYTGYTC) and ScRFC1.7 (CGGAATTCAC RTCRCTDGCR TTYTGYTC) from the C terminus (symbols for nucleotides according to the International Union of Biochemistry code; D = A, G, or T; H = A, C, or T; I = inosine; K = G or T; M = A or C; R = A or G; S = C or G; Y = C or T). The four primers were used in pairwise combination to generate PCR products of 75 bp, using *S. cerevisiae* genomic DNA as a template. The products were cloned in vector pBluescript KS+ (pKS+; Stratagene), using the *Eco*RI sites included in the primers, and sequenced.

(ii) RFC2 and RFC3 probes. The amino acid sequences of three peptides with

significant similarity to the hRFC subunits (35) were used to design degenerate oligonucleotides (peptide 40/41 4 [ATP+; GGICCICCIG GIACIGGIAA RAC], peptide 36 15b [DEAD-; CATISIRTCI GCYTCRTCIA RDAT] and peptide 40/41 9a [ALRR-; TYTCIATIRY ICKICKIARI GC]; Fig. 1). Oligonucleotide ATP+ in combination with either DEAD- or ALRR- was used as a primer in PCR with *S. cerevisiae* genomic DNA. Several specific PCR products were obtained. The products were reamplified and cloned in the pGEMEX-derivative pDK101 (19), using their 3' A overhangs. The predicted amino acid sequences of three clones showed strong similarity to the sequences of peptides from hRFC37 (27a/1 and 28/2 [*RFC2* probes]) and hRFC36 (29/1 [*RFC3* probe]).

(ii) *RFC4* probe. Primers DEAD+ and 15am (ATYTGRATDG ARTCYTC YAR RTT; derived from peptide 36 15a) were used for PCR with genomic *S. cerevisiae* DNA. They yielded a DNA fragment of 420 bp. Reamplification of this fragment with primers ALRR+ and 15am resulted in a fragment of 390 bp. The ALRR+/15am fragment was subcloned in pDK101 and sequenced. It showed strongest predicted amino acid sequence similarity to hRFC40 and was used as an *RFC4* probe.

(iv) *RFC5* probe. Primers derived from peptide 40/41 5 (5p [GAYGTIMGIC ARTTYGTIAC IGC]) and 40/41 28a (28A– [GTIGTRTTIA RIGTYTCIAC RTC]) were used in a PCR with genomic *S. cerevisiae* DNA and yielded a DNA fragment of 700 bp. This fragment could be reamplified with primers ALRR+ and 28A– and resulted in a 500-bp fragment. The ALRR+/28A– fragment was subcloned in pDK101 and sequenced. It showed strongest predicted amino acid sequence similarity to hRFC38 and was used as an *RFC5* probe.

**Cloning of the** *KFC* genes. The PCR-generated clones were used to screen an *S. cerevisiae* genomic DNA library in  $\lambda$  phage EMBL3A (gift of R. Young, Massachusetts Institute of Technology). A 2.1-kb *Bg*/II fragment from  $\lambda$  clone 2 and a 2.8-kb *Kpn1-SalI* fragment of  $\lambda$  clone 11 hybridized with the *RFC1* probe and were subcloned in pKS+. A 3.8-kb *Eco*RI fragment of  $\lambda$  clone 2/2/1 hybridized with *RFC3* probe 27a/1, a 3.9-kb *Eco*RI fragment of  $\lambda$  clone 3/4/1 hybridized with *RFC3* probe 29/1, a 7.6-kb *Bam*HI fragment of  $\lambda$  clone 4/5/1 hybridized with *RFC4* probe ALRR+/15am, and a 2.4-kb *Eco*RI and a 5.0-kb *Bam*HI-*KpnI* fragment of  $\lambda$  clone 5/8/1 hybridized with the *RFC5* probe ALRR+/28A-. These fragments were subcloned in pBluescript SK+ (pSK+; Stratagene). The RFC genes were sequenced by primer walking, starting from the sequences known from the cloned PCR products.

**Plasmid constructions.** Plasmid pKSRFC1-2 contains the 5' end of the *RFC1* open reading frame (ORF) on a 2.0-kb *Bg*/II fragment from  $\lambda$  clone 2 in pKS+ (Fig. 2). Plasmid pKSRFC1-11 contains the 3' end of the *RFC1* ORF on a 2.8-kb *KpnI-SaII* fragment from  $\lambda$  clone 11 in pKS+. Plasmid pKSRFC1 was constructed by ligating the pKSRFC1-2 *Bg*/II fragment with pKSRFC1-11 digested with *Ban*HI and *Bg*/II. It contains the entire *RFC1* gene. Plasmid pSKRFC2 contains the *RFC2* gene on a 3.8-kb *Eco*RI fragment from

Plasmid pSKRFC2 contains the *RFC2* gene on a 3.8-kb *Eco*RI fragment from  $\lambda$  clone 2/2/1 in pSK+ Plasmid pSKRFC3 contains the *RFC3* gene on a 3.9-kb *Eco*RI fragment from  $\lambda$  clone 3/4/1a in pSK+. Plasmid pSKRFC4B contains the *RFC4* gene on a 7.6-kb *Bam*HI fragment in pSK+. Plasmid pSKRFC4H contains the *RFC4* gene on a 1.7-kb *Hind*III fragment in pSK+. Plasmid pSKRFC5BK contains the *RFC5* gene on a 5.0-kb *Bam*HI-*Kpn*I fragment in pSK+. Plasmid pSKRFC5BK contains the *RFC5* gene on a 2.4-kb *Eco*RI fragment in pSK+. Plasmid pSKRFC5E contains the *RFC5* gene on a 2.4-kb *Eco*RI fragment in pSK+. In plasmid pRFC2ko, the *NdeI-HpaI* fragment of the *RFC2* ORF of pSKRFC2 was replaced with the *SaII* fragment of *LEU2*. In plasmid pSKRFC3Ko, the *NcoI-NarI* fragment of the *RFC3* ORF was replaced with the *Bam*HI-*ClaI* fragment of *HIS3*. In plasmid pRFC4ko, the *NcoI-NdeI* fragment of *LEU2*. In plasmid pSKRFC5E was replaced with the *Bam*HI-*ClaI* fragment of the *RFC5* ORF of pSKRFC5E was replaced with the *Bam*HI-*ClaI* fragment of the *RFC5* ORF of pSKRFC5E was replaced with the *Bam*HI-*ClaI* fragment of *HIS3*.

*RFC* gene disruptions. The inserts of plasmids pRFC2ko, pRFC3ko, pRFC4ko, and pRFC5ko were isolated by gel electrophoresis and transformed into diploid *S. cerevisiae* (pRFC2ko and pRFC3ko into yeast strain W303, and pRFC4ko and pRFC5ko into yeast strain W307). This resulted in the mutant diploid strains YB1062, YB1063, YB1064, and YB1065, respectively, in which one of the two *RFC* ORFs was replaced by the auxotrophic marker gene (Fig. 2). The disruption of one of the *RFC* alleles was confirmed by genomic Southern blot analysis and by PCR. The heterozygous diploids were then sporulated, and the tetrads were dissected (44).

**Physical mapping.** A set of overlapping *S. cerevisiae* genomic DNA clones in phage  $\lambda$  vectors and cosmids on nylon filters was provided by Linda Riles and Maynard Olson (Washington University, St. Louis, Mo.). Random hexamerlabeled probes for *RFC1* (*AccI-KpnI* fragment; Fig. 2), *RFC2* (*NdeI-HpaI* fragment), *RFC3* (*HindIII* fragment), *RFC4* (*Eco*RI-*MluI* fragment), and *RFC5* (*Eco*RI and *NdeI-SaII* fragments) were made and hybridized to the filters.

Nucleotide sequence accession numbers. The nucleotide sequences for the *RFC* genes have been submitted to GenBank under accession numbers U26027 (*RFC1*), U26028 (*RFC2*), U26029 (*RFC3*), U26030 (*RFC4*), and U26031 (*RFC5*).

#### RESULTS

**Cloning of the ScRFC genes.** ScRFC was purified as described by Fien and Stillman (12), with slight modifications (see Materials and Methods). The individual protein subunits were separated by SDS-PAGE, and peptides from the 103-,



FIG. 2. Genomic organization of the five S. cerevisiae RFC genes. Shown are restriction maps of RFC1 to RFC5 and neighboring genes. The fragments replaced by auxotrophic markers in the disruptions are indicated. ORFs are shaded, and numbering of nucleotides is relative to the RFC start codons.

40/41-, and 36-kDa bands of ScRFC were used to obtain amino acid sequences from these subunits (Fig. 1). Degenerate oligonucleotides were designed on the basis of these peptide sequences and used to amplify probes by PCR with S. cerevisiae genomic DNA as a template. The resulting PCR products were subcloned and sequenced to verify identities with peptide sequences and similarities to the hRFC subunits (3, 35). They were then used to screen an S. cerevisiae genomic DNA library. Phage  $\lambda$  clones were obtained for all of the subunits. Figure 2 shows the genomic organization of the genes RFC1, RFC2, RFC3, RFC4, and RFC5. RFC1 is located next to STE13 (accession number U08230), the S. cerevisiae dipeptidyl-aminopeptidase A. RFC2 is downstream of the phosphatidylinositol kinase gene DRR1 (6) or TOR1 (14). Downstream of RFC3 lies the *HCS26* gene (36), encoding a  $G_1$  cyclin. *RFC4* is framed by two unknown ORFs. The one downstream of RFC4 is similar in predicted amino acid sequence to DNA helicases. RFC5 is also known as YBR0810, which was sequenced as part of the S. cerevisiae genome sequencing project (accession number X78993). YBR0809 is located upstream of RFC5, and the POL30 gene corresponding to ScPCNA (YBR0811) is located downstream of RFC5 (Fig. 2).

The ScRFC genes were then subcloned and sequenced. The results are shown in Fig. 3, along with the predicted translation products. The amino acid sequences obtained by direct amino acid sequencing of peptides and the binding sites of primers used for PCR are underlined. Peptide sequences were obtained for *RFC1*, *RFC2*, *RFC4*, and *RFC5*; no peptide sequences were obtained could be accounted for in the predicted ORFs. The genes encoding ScRFC2, ScRFC3, and ScRFC4 have been

isolated independently (24, 25, 34) on the basis of the similarity in sequence to the hRFC clones, and hence only the peptide sequences of these proteins are shown in Fig. 3.

RFC1 contains one major ORF encoding 861 amino acids, and the gene was found to be identical to a recently cloned cell division cycle gene called CDC44 (15). The predicted molecular weight is 94.9 kDa, and the protein is rich in lysine (K) residues. The calculated pI is 10.0. The predicted RFC2 ORF product of 353 amino acids has a calculated molecular mass of 39.7 kDa and a pI of 8.6. The RFC3 ORF product has a length of 340 amino acids. The predicted molecular weight is 38.2 kDa, and the calculated pI is 6.4. RFC4 has an ORF encoding 323 amino acids; the predicted molecular weight is 36.2 kDa, and the calculated pI is 9.8. The RFC5 ORF product is 354 amino acids in length; the molecular weight is predicted 39.9 kDa, with a calculated pI of 8.2.

**Physical mapping.** Probes for all five *RFC* genes were hybridized to filters containing an ordered *S. cerevisiae* genomic library provided by L. Riles and M. Olson (see Materials and Methods). The *RFC1* probe detected phage  $\lambda$  clones 3556 and 4182. The gene was mapped to the right arm of chromosome XV, next to *STE13*. The *RFC2* probe detected clone 3881, and the gene was mapped to the right arm of chromosome X, between *CDC8* and *CDC11*. The *RFC3* probe hybridized with clone 5398, which is located on the left arm of chromosome XIV, between *SUF6* and *MET2*. The *RFC4* probe detected phage  $\lambda$  clones 5929 and 6006. The gene was localized to the left arm of chromosome XV between *SUF1* and *adh1*. Neither the *RFC5* probe nor the probe for the gene encoding PCNA, which is located next to *RFC5*, detected any clones in this genomic representation, which is 96% complete. Nevertheless,

CACGATACCATCAGCCTCTGGATCTGTGGACATTTTTGGAGGAACGGTGACGTTTACAGAATTGATGATTTCTTCAGGTT -401 CATCAACGTCCTCCACATGAGGATGACTCTTGGCCTTCTTACCTGTTTTCTTATTTTACCCATT -321 CTAATTAAAAAAAATGCAATTGTTTGAGGGGATCAAGTGGAAAGTATACACAAGCATCTACTATATAGGCTGTTTATACAA -241 GATTTTCTTGTTCTTCATCAATGGTCTTTCTGTTTGTTTCCATTTTTCGTATCGAAATGAGCACGAAATTCAAAAAGAAG -161 TACTGAAAAAAAAATCAACATGAGAAGGAACAGCCGATCTCTCAGCGAAAACAGTGGCTTCGTTGAATACGCTGCTTGAT -81 CCTTACACAATAAGGAATTGGGTATCTCGCAGTTAATTTTATAACTTCAAGTCACTTCATAATAAACTAAGCTGAAGAAA -1 ATG GTC AAT ATT TCT GAT TTC TTT GGT AAA AAT AAG AAA TCC GTA AGA TCG TCA ACA TCC M V N T S D F F G K N K K S V R S S T S 60 D G 20 AGA CCT ACG AGA CAG GTG GGT TCG TCT AAA CCA GAA GTT ATC GAC TTA GAT ACT GAA TCT R P T R Q V G S S K P E V I D L D T E S 120 40 180 60 TCA GAG ACA CCT GAA GGA GAA AAA AAG CTA CCT CTT CCC GCT AAG AGG AAG S E T P E G E K K L P L P A K R K GCC TCT TCA A S S 240 80 CCA ACT GTA AAG CCA GCA AGT TCA AAA AAA ACC AAA CCC TCC TCT AAA AGT TCA 300 100 Get teg ant att acg get can gat gta eta gat arg att eca tee ttg gat etg tea aat as n i t a Q d v l d k i p s l d l s n 360 120 GTT CAT GTG AAA GAG AAT GCT AAA TTT GAT TTC AAA AGC GCA AAT TCA AAT GCG GAT CCJ V H V K E N A K F D F K S A N S N A D P 420 140 480 160 gat gaa att gtt tet gaa ata ggt agt tet cea gaa gga aag cea aat tet tet tta tat dE I V S E I G S F P E G K P N C L L 540 180 CTA ACA ATT GTC TTC ACA GGT GTT CTG CCA ACC TTG GAG CGT GGT GCA TCG GAA GCT TTA L Е R Ģ A Е GCG ARA AGA TAC GGA GCA AGG GTT ACT ARA TCA ATT TCA AGC ARA ACT TCT GTA GTA AK R Y G A R V T K S I S S K T  $\underline{S}$  V V600 200 GTC 660 TTA GGT GAT GAA GCA GGG CCA AAG AAA CTA GAA AAA ATC AAG CAA TTG AAA ATC AAA GCC D G P K K L Е I ĸ Q L Ι 220 ATA GAC GAA GAA GGC TTT AAG CAA TTA ATT GCT GGG ATG CCT GCT GAA GGT GGC GAC GGG 720 240 c GAA GCT GCC GAA AAA GCA CGT CGA AAA TTA GAA GAA CAG CAT AAT ATT GCT ACC AAA GAA 780 R L Е Е Q н N 1 A т к ε 260 GCA GAA TTG CTT GTT AAG AAA GAA GAG GAA AGG TCG AAG AAG CTT GCA GCC ACT AGA GTT A E L L V K K E E E R S K K L A A T R V 840 280 TCT GGT GGC CAT CTG GAG AGA GAC AAT GTG GTT AGG GAA GAA GAT AAA TTG TGG ACA S G G H L E R D N V V R E E D K L W T 900 300 GTZ AAG TAC GCA CCA ACG AAT CTA CAA CAG GTC TGT GGT AAC AAA GGA AGT GTA ATG AAA TTA 960 320 . Arc trig ttig get ant trig gar art ter arg arr art art tit arr cat get arr N W L A N W E N S K K N S F K H A G K1020 340 GAT GGC TCT GGT GTT TTC AGG GCT GCA ATG TTA TAC GGT CCT CCT GGT ATT GGG AAG ACA D G S G V F R A A M L Y G P P G I G K T 1080 360 ACT GCT GCT CAT TTA GTT GCA CAA GAG CTT GGG TAT GAC ATT CTT GAA CAA AAC GCT 1140 TCT N 380 ٥ 1200 CGC TCT AAA ACT TTA CTT AAT GCC GGT GTT AAA AAC GCT CTT GAT AAC ATG TCT 400 т L N А G K N A L D N s ĸ GTA GTG GGA TAT TTC AAA CAC AAC GAA GAA GCT CAA AAT TTG AAC GGG AAA CAT TTT GTC 1260 420 ATT ATC ATG GAT GAA GTT GAT GGT ATG AGC GGA GGC GAT AGG GGG GGA GTT GGC CAG D Е v D G м s G G D R G G v G Q L 440 Ι GCA CAG TTT TGT CGT AAG ACG TCA ACT CCA TTG ATT CTA ATT TGT AAT GAA CGT AAT A Q F C R K T S T P L I L I C N E R N 1380 460 L 1440 CCA AAA ATG AGA CCA TTC GAT AGA GTA TGT CTT GAT ATC CAA TTT AGA AGA CCT GAT GCT D v с L D Q D 480 R R Α ARC AGT ATC AAA TCA AGA TTA ATG ACA ATT GCG ATC AGA GAA AAG TTC AAA CTT GAT CCA N S I K S R L M T I A I R E K F K L D P 1500 500 1560 AAT GTC ATT GAT AGG TTG ATA CAG ACT ACA AGA GGT GAT ATC CGC CAG GTT ATT AAT CTA р R Ι 0 т т G D T Q v 520 т τ. R R 1620 540 CTT TCA ACG ATA TCT ACG ACT ACT AAA ACC ATA AAC CAT GAA AAT ATC AAC GAG ATC TCA s т т т I N н Е N I I 1680 AAG GCA TGG GAA AAG AAT ATT GCC CTA AAA CCC TTT GAC ATT GCC CAT AAA ATG TTG GAT I к F D I А н L D 560 Ν L Ρ 1740 GGA CAA ATA TAC TCA GAC ATA GGT TCA AGG AAC TTT ACA CTG AAT GAT AAG ATC GCA TTA s п т G s R т τ. N D к Ť А t. 580 TAT TTT GAT GAC TTT GAT TTC ACA CCC CTA ATG ATT CAG GAA AAC TAT TTA TCT ACA AGA 1800 D Ð F D т L м I Q Е N Y L 600 F P s CCA AGC GTT TTG AAA CCC GGC CAG TCA CAT TTG GAA GCA GTT GCT GAG GCG GCT AAC TGT P S V L K P G O S H L E A V A E A A N C 1860 620 ATT TCA TTA GGT GAT ATT GTC GAA AAG AAG ATT CGT AGT AGT GAG CAA TTA TGG AGT CTT 1920 G Ð I v Е к W 640 к s n L s TTG CCT TTG CAT GCT GTT CTT TCA TCT GTT TAT CCA GCA TCA AAA GTT GCC GGC CAT ATG 1980 660 GCG GGA AGA ATC AAT TTC ACA GCT TGG TTG GGC CAA AAT TCT AAA TCT GCC AAA TAT A G R I N F T A W L G Q N S K S A K Y 2040 680 agg tta ctg caa gag ata cat tat cat aca aga cta ggt aca tct act gat aaa att r l c t c t s t d k i 2100 700 2160 720 cta agg ctg gat tac cta cca act ttt aga aaa aga tta ttg gac cca ttt ttg aag caa l~ r~ l~ d~ y~ l~ p~ t~ r~ r~ r~ l~ l~ d~ p~ f~ l~ k~ q~GGT GGT GGA ATT TCA TCT GTC ATA GAG GTA ATG GAC GAT TAC TAT TTG ACC AAA GAA GA D A I S S V I E V M D D Y Y L T K  $\underline{E}$ 2220 740 2280 760 GAC TGG GAT TCT ATT ATG GAG TTT TTC GTA GGT CCT GAT GTG ACC ACA GCC ATT ATC AAA v G D ANG ATA CCA GCT ACG GTT ANA AGT GGA TTC ACG CGG ANA TAC AAC AGT ATG ACA CAT CCA 2340 v ĸ s G т Y N s м т н 780 Α R ĸ GTC GCA ATT TAC AGA ACA GGT AGT ACT ATT GGT GGT GGT GGT GTT GGC ACC AGT ACT AGC V A I Y R T G S T I G G G G V G T S T S 2400 800 acc ccc gat ttc gaa gat gtc gtt gac gca gat gac aat cca gtt ccc gca gat gaa gaa tT p D f E D V V D A D D N P V P A D D E2460 820 GAA ACA CAA GAT AGT AGC ACA GAC TTG AAG AAG GAT AAA CTT ATC AAG CAG AAA GCC AAA E T Q D S S T D L K K D K L I K Q K A K 2520 840 CCT ACG AAG AGG AAG ACT GCC ACC AGT AAA CCT GGT AGC AAA AAA AGG AAA ACG AAA 2580 P T K R K T A T S K P G G S K K R K T K 860 GCA TGA TTTATTCATACACTAAGATTATAATTACACTTTTCTTCTCATTGATTTTGTGAGCCGTTCTATCACAGATAC 2658 861 GAAAACTGTGTGGTGATBATAGCTCGATGGGTATCACCGCCCTATATAAATACTCTTATTAGTATAGTACACAAAAT 2738 GAMAL TOTOTOTTATIANTAGE CONCERNMENT AND A CONCENTRATION AND A CONC

#### ScRFC2

 MFEGFGPNKKRKISKLAAEQSLAQQPWVEKYRPKNLDEVTAQDHAVTVLKKTLK<u>SANLEHMLFYGPFQTXK</u>TSTILALTK
 80

 ELYQPDLMKSRILEINASDERGISIVREKYNNFARLTVSKPSKHDLENYFCPFYKIIILDEADSMTADQSALKRMENT
 160

 SGVTRFCLICNYVTRIIDPLASRCSK<u>ERFK</u>ALDASNAIDRLRFISEQENVKCDDGVLERILDISAGLRRGITLLQSAS
 240

 GQVLGDGKNITSTQVEELAGVVFHDILISIVEKVKSGDFDEIKKYNTFMKSGW<u>SAASVVNOLHEYYITNNFFT</u>
 353

 QISWLLFTTDSRLMNGTNEHIQLLNLLVKISQL\*
 353

## ScRFC3

MSTSTEKRSKENLPWVEKYRPETLDEVYGQNEVITTVRKFVDEGKLPHLLFYGPPGTGKTSTIVALAREIYGKNYSNWL 80 ELNASDDRGIDVVRNGIKDFASTRQIFSKGFKLIILDEADAMTNAAQNALRRVIERYTKNTRFCVLANYAHKITPALLS 160 CTRFRFQPLPQEAIERRIANVLVHEKLKLSPNAEKALIELSNGDMRRVLNVLQSCKATLDNPDEDEISDDVIYECCGAP 240 PSDLKAVLKSILEDD0MGTAHYTLNKVRSAKGLALIDLIEGIVKILEDYELQNEETRVHLUTKLADIEYSISKGONDQIG 320 SAVIGAIKASFENETVKANV\* 340

#### ScRFC4

MSKTLSLQLEWVEKYRPOVLSDIVGNKETIDELOOIAKGSMPEHMIISGMPGIGKTTSVHCLAHELLGRSVADGVLELNA 80 SDDRSCIDVVRNQIKHFAQKKLHLPFOKHK<u>IVILDEADSKTAGAO</u>QALRRTMELVSNSTRFAFACNQSNKIIEFLQSRCAI 160 LRYSKLSDEDVLKRLLQIIKLEDVKVTNDGLEAIIFTAEGDMRQAINNLQSTVAGHGLVNADNVFKIVDSPHPLIVKKKL 240 LASNLEDSIOILETDLMKKGYSSIDIVTTSFRVTKNLAQVKESVRLEMIREIGLTHMRILEGVGTVLQLASMLAKIHKKN 320

#### RFC5

AAAA -321 AAAGAGAAGTTTCTCGCAGAGTGAAAAAAATGTCAAGAAAAGATCCTACAAGAACAAATTATGCGCAATAATATGCTTC -241 -161 GIGCIGITITTAGGATCTTGGAGTCAGAAATAAAATAGTTTTGTAAATTTATGTAAGATGCTGTAGTCAAATTTATGTATC GAGTCTATGCATATGTACACCGTGTTGAGGAATCTTTTCTAGAAGCACTGCGGTGTCGCCTCTTGAATATTTTAACGCGA -81 TTTTTTTATTCCTTGGAACATAAAATAGACTTTTCATTTGTATGATGGTTCATGGAAATTATAGATTAGTACAGCACAAAC -1 and the the the set of the set o 60 20 GAG TTG ACA AAT TTT CTA AAA TCG TTA TCT GAT CAG CCT CGT GAT TTA CCT CAT CTT E L T N F L K S L S D Q P R D L P H L TTA 120 40 ctg tat gga cca aat ggt aca ggt aag aaa acg cgt tgt atg gca tta ttg gag tcc ata l y g p n g t g k k t r c m a l l e s i 180 60 TT GGA CCT GGA GTC TAT AGA TGA ANA ATT GTA GTC AGA GTC TAT AGA TGA GTC TAT AGA TGA GTC AGA CTA TTT GTC ATT F G P G V Y R L K I D V R O P  $^{\circ}$   $^{\circ}$ GCT TCG AAC 240 80 VTA AGA AAA CTA GAA CTG AAT GTG GTC AGC TCG CCA TAC CAT TTA GAG ATC ACG CCA AGT GAT R K L E L N V V S S P Y H L E I T P S D 300 100 ATG GGT AAC AAT GAT AGA ATT GTC ATC CAA GAA CTA TTG AAA GAA GTG GCT CAA ATG GAA 360 G N D R Т I Q Е L L Е v А Q E 120 caa gtg gac ttt caa gat tct aag gat gga ctt gcc cat aga tat aag tgt gtt att atc Q V D F Q D S K D G L A H R Y K C V I I 420 140 AAC GAG GCG AAC TCG TTA ACA AAA GAT GCT CAA GCT GCT TTA AGA CGT ACC ATG GAA AAA 480 N s L т K D 0 RR 160 E А A А T. т м TAC TCC AAA AAC ATT AGG TTG ATA ATG GTC TGC GAT TCG ATG TCG CCT ATA ATT GCT CCT 540 180 Ĺ D TCC CGT TGT CTG TTG ATT CGT TGT CCT GCA CCA AGC GAT AGC GAA ATT TCA ACT S R C L L I R C P A P S D S E I S T 600 200 ATC TTG TCT GAT GTG GTG ACA AAT GAA AGA ATA CAA CTA GAA ACA AAG GAT ATT TTA AAA I L S D V V T N E R I Q L E T K D I L K 660 220 att get cag gea teg ant gga ane tig ega gte tee eta tta atg ett gaa tet atg i a  $\varrho$  a s N g N L R V s L L M L E s M720 õ 240 GCA CTG AAC AAC GAA TTA GCA TTG AAA AGC AGT AGC CCT ATA ATA AAA CCA GAT TGG ATT 780 260 s ATA GTG ATC CAT AAA TTA ACG AGG AAA ATC GTT AAA GAG AGA TCT GTC AAT TCT TTA ATC IVII H K L T R K I V K E R S V N S L I 840 280 900 GAA TGC AGA GCT GTC CTA TAC GAT TTA CTA GCT CAT TGT ATA CCT GCC AAT ATC ATC TTA A v L Y D L L А н с I P А N I 300 Ŕ AAG GAA CTA ACG TTT TCT TTG TTG GAT GTG GAA ACC CTG AAT 960 ACG AAT AAA TCG TCC E E N T N K S s 320 ATA ATT GAA TAC TCA AGT GTT TTT GAC GAA AGA TTA TCA CTT GGA AAC AAA GCA ATA TTC 1020 Τ s s v D Е R L s Ł G N к Α 340 Ι TTG GAA GGG TTC ATA GCA AAA GTT ATG TGC TGT CTA GAT TAA TGTAAGATATGTCATAAATA 1085 L E G F I A K V M C C L D \* 355 CAT CTGTATAAGTCACAAAAAAGCTGATATTTAACGCATCTTAGTCTTTATTTTCTTGTTATTTTTCATTTAAAAACAA 1165 CGTCAGCTACAAACTTTATTGTTTCTTTGGTGATCATGATATTAATAGAATCACTCAATTGGGACA

FIG. 3. DNA sequences of *RFC1* and *RFC5* and predicted translation products of *RFC1* to *RFC5*. The DNA primers used for amplification of RFC probes and the sequenced peptides are underlined.

Disrupted gene	No. with indicated dissection pattern (viable <sup><i>a</i></sup> :nonviable)			Total no. of
	2:2	1:3	0:4	tetrads
RFC2	15	5		20
RFC3	7	3		10
RFC4	19	9	2	30
RFC5	32	7	1	40

TABLE 1. Disruption of the RFC genes shows they are essential

<sup>*a*</sup> All viable spores tested negative for the auxotrophic marker used for the disruption and thus contained an intact copy of the *RFC* gene.

*RFC5* could be mapped to chromosome II, since this chromosome was sequenced as part of the *S. cerevisiae* genome project (accession number X78993). It is located on the right arm, 38 kb proximal from *CMD1*.

RFC genes are required for S. cerevisiae viability. To determine whether all of the ScRFC subunits perform an essential function, the coding regions of one of the alleles of the RFC2 to RFC5 genes were replaced by auxotrophic markers in diploid yeast strains (see Materials and Methods). The RFC1 gene, which is identical to CDC44, was shown recently to be essential (15), and hence we did not disrupt this gene. Sporulation and tetrad dissection predominantly yielded a 2:2 segregation pattern, as shown in Table 1. All of the viable spores exhibited a phenotype negative for the relevant auxotrophic marker. If the diploid strains carried a plasmid with the respective RFC gene prior to sporulation and dissection, the segregation pattern was switched to 3:1 or 4:0 (data not shown). This finding confirmed that the lethality was due to the disruption of the respective RFC gene. Cells with the deleted RFC gene typically arrested as microcolonies at the 2- to 32cell stage. Most of the cells were budded, which is consistent with an arrest in S phase.

**Comparison of** *S. cerevisiae* and human RFC subunits. Figure 4 shows a comparison between the amino acid sequences of the hRFC subunits and the ScRFC subunits. The amino acid identity among all the subunits is high (24 to 37%), but amino acid comparisons indicate that there are homologous pairs of human and yeast subunits with distinctly higher amino acid



FIG. 4. Comparison between the hRFC and ScRFC subunits. Indicated are calculated masses and pIs as well as the levels of amino acid identity and similarity between the homologous subunit pairs connected by bars. Differences in nomenclature lead to a somewhat different order of the subunits in human and *S. cerevisiae* proteins.



FIG. 5. Evolutionary tree for the hRFC and ScRFC subunits. Although the amino acid identity among all subunits is high (24 to 37%), there are homologous pairs with distinctly higher amino acid identity and similarity.

identity and similarity (Fig. 4 and 5). The large S. cerevisiae subunit, ScRFC1, is somewhat smaller than the human subunit, hRFC140. This variation in size is mostly due to differences in the N terminus. The pI of this protein is very basic in both species. Traditionally, the small human subunits have been ordered according to their apparent sizes in SDS-PAGE, and this ordering does not coincide with their calculated molecular masses. The ScRFC subunits are numbered according to decreasing calculated molecular mass, except for ScRFC5. This causes a slightly different order of the homologous subunits. ScRFC2 and hRFC37 have virtually identical calculated masses and basic pIs. ScRFC3 is slightly shorter than hRFC36 and has a slightly acidic pI, compared with the neutral pI of hRFC36. ScRFC4 and hRFC40, despite being the most homologous pair, show the biggest difference in mass and pI. ScRFC5 and hRFC38 are the least homologous small subunits but have similar masses and pIs.

**Comparison of amino acid sequences of RFC subunits.** Figure 6 shows an alignment of all RFC subunits from *S. cerevisiae* and humans (summarized in Fig. 8). They were compared with the functionally homologous protein gp44 from phage T4 (47) and the prokaryotic DNA polymerase III holoenzyme subunits *Escherichia coli*  $\gamma/\tau$  (13, 60) and  $\delta'$  (10) and *Bacillus subtilis* DnaH (37), an *E. coli*  $\gamma/\tau$  homolog ( $\gamma$  consists of the same amino acid sequence as  $\tau$  but ends as a result of a translational frameshift at amino acid 431).

In addition to these functionally related proteins, a search of the sequence databases revealed sequence similarity to a predicted protein sequence from the *CHL12* gene (18) of *S. cerevisiae*. There is a significant similarity between the *CHL12* gene product and the RFC subunits, ranging from 20 to 25% amino acid identity and up to 50% similarity. The *CHL12* ORF predicts an 84.3-kDa protein with a pI of 8.5. RFC boxes II to V, VII, and VIII are conserved, but CHL12 is not known to be a component of ScRFC. *CHL12* was isolated as a chromosome loss mutation (18) and is identical to *CTF18* (46). Mutants in *CHL12* are incapable of stable maintenance of circular and linear artificial chromosomes. Mitotic recombination frequency and sensitivity to UV and  $\gamma$  irradiation are normal, suggesting that DNA repair is not affected by *CHL12* mutations.

There is an overall similarity between the prokaryotic and eukaryotic proteins, as has been noted previously for the human sequences (7, 8, 35) and some of the *S. cerevisiae* sequences (24, 25, 34). The small RFC subunits and the bacterial and phage T4 polypeptides align with the central part of the large RFC subunits from different species. We named the similar regions RFC boxes I to VIII, numbered from the N terminus toward the C terminus (Fig. 6; see also 8). The most obvious feature of all the sequences is a conserved ATP/GTP-







FIG. 6. Alignment of the amino acid sequences of all RFC subunits from *S. cerevisiae* (this report and references 24, 25, and 34) and human (3, 7, 8, 15a, 35) proteins in comparison with sequences of the functionally homologous proteins gp44 from bacteriophage T4 (T4gp44) (47), *E. coli*  $\gamma/\tau$  (EcTau) (13, 60), *E. coli*  $\delta'$  (EcDelta') (10), and *B. subtilis* DnaH (BsdnaH) (37) and the sequence of the product of a chromosome loss mutation from *S. cervisiae*, *CHL12* (CHL12) (18). Similar amino acids are assigned similar colors so that conserved regions become obvious. Indicated are RFC box I (ligase homology) and boxes II to VIII, which are conserved in all RFC subunits. Box VIa is conserved in the large RFC subunits, and box VIb is conserved in the other proteins. Amino acids affected by mutations in the *CDC44* gene, resulting in a cold-sensitive phenotype, are boxed in black in the ScRFC1 protein sequence.

binding region. It consists of several motifs in the N-terminal half of the small RFC subunits and the equivalent region of the large subunit.

The most conserved motif is within box III and is the phosphate-binding loop (P loop) with the consensus sequence GxxxxGK(S/T). This loop usually contains additional glycines and prolines, and we know from analyses of  $p21^{ras}$ , for which the tertiary structure has been solved, that it is involved in the binding of the phosphate groups of the nucleotide (39, 40). In the case of the RFC subunits, for which we named this domain RFC box III, it has the consensus sequence phUUuyGPPGt GKT(S/T)t (where U stands for a bulky aliphatic residue such as I, L, V, or M).

The second-most-conserved domain is RFC box V, with the consensus sequence (F/H/Y)kUUUUDE(V/A)D for the RFC subunits. It bears similarity to the DEAD-box proteins, a family of putative RNA helicases which also have P loops and are ATPases (27, 41). There is no further similarity between the RFC subunits and the DEAD-box proteins, and no helicase activity could be found for RFC.

hRFC140 DmRFC140 ScRFC1 Tthligase Ecligase	350 178 105 540 539	ETKTPKKTKSSPAKKESVSPEDSEKKRTNYQAYRSYLNREGPKALGSKEIPKGAENCLEGLIFVITGVLESIERDI TTPRVKKEKPAADLESSVLTDEERHERKRASAVLYQKYKNRSSCLNPGSKEIPKGSPDCLSGLTFVVTGVLESMEREI TAQDVLDKIPSLDLSNVHVKENAKFDFKSANSNADPDEIVSEIGSFPEGKPNCLLGLTIVFTGVLPTLERG LEASLEELLEVEEVGELTARAILETLKDPAFRDLVRRLKEAGVEMEAKEKGGEALKGLTFVITGELSR.PREI EAASIEELQKVPDVGIVVASHVHNFFAEESNRNVISELLAEGVHWPAPIVINAEEIDSPFAGKTVVLTGSLSQMSRDD	EAK EAE ASE EVK DAK
zmiigase	595	VDRELISFHIPNMGGRIIRSLLDFFÆTHNSDVVSDLLQEVQIEPLIFELASSPLSGRIIVFTGSLQRITRD	EAK
HSPARP	332	EWVTPREFREISYLKKLKVKKQDRIFPPET.SASVAATPPPSTASAPAAVNSSASADKPLSNMKILTLGKLSR.NKD	EVK
GINU	551	DWVIINDITTIIUTICKQDKIFFEER.RIVNOAFFFFRORFIIETVIRFQDKFLINDKIIIIGKISK.NKE	LVK
hRFC140	428	SLIERYGGKVTGNVSKKTNYLVMGRDSGQSKSDKAAALGTKIIDEDGLLNLIRTMPGKKSKYEIAVETEMKKES	501
DmRFC140	258	SVIKEYGGKVMTVVGKKLKYLVVGEEAG PKKLAVAEELNIPILSEDGLFDLIREKSGIAKQVKEEKKSPKKEHS	331
ScRFC1	179	ALAKRYGARVTKSISSKTSVVVLGDEAGPKKLEKIKQLKIKAIDEEGFKQLIACMPAEGGOGEAAEKARRKLEE	252
Tthligase	614	ALLRRLGAKVTDSVSRKTSYLVVGENPG,SKLEKARALGVPTLTEEELYRLLEARTGKKAEELV	676
Ecligase	619	ARLVELGAKVAGSVSKKTD.LVIAGEAAGSKLAKAQELGIEVIDEAEMLRLLGS	671
Zmligase	671	RQAENLGAKVASSVSKKTN.LVVAGEAAGSKLSKAFELDISIIDEDRWHRIVENGGQESIKI	731
HSPARP	410	AMIEKLGGKLTGTANKASLCISTKKEVEKMNKKMEEVKEANIRVVSEDFLODVSASTKSLOELFLAHILSPWGAEV	485
GaPARP	407		192

FIG. 7. RFC box I (ligase homology) compared with prokaryotic DNA ligases from *Thermus thermophilus* (Tthligase) (21), *E. coli* (Ecligase) (accession number M24278), and *Zymomonas mobilis* (Zmligase) (accession number Z11910) and PARPs from humans (HsPARP) (57) and chickens (GgPARP) (16). Shading of the amino acids is according to conservation in one, two, or three of the protein families.

The ATP/GTP-binding region also includes RFC boxes II, IV, and VI, which are not present in other ATP/GTP-binding proteins but are quite unique to RFC and the related proteins. RFC box II shows a high degree of similarity among the RFC subunits and a conserved RP dipeptide in the related proteins. The consensus sequence for the RFC subunits is (L/P)WV(E/D)KYrPxxU.

RFC box IV is only weakly conserved in the prokaryotic proteins, but it is found in T4 gp44 and the CHL12 protein with the consensus sequence LEUNaSD.

RFC box VI is different in the small and the large RFC subunits. RFC box VIa, present in the large subunits, has the consensus sequence gMaGneDRGGUqeL and is not conserved in other proteins. RFC box VIb, present in the small subunits, is somewhat conserved in the prokaryotic accessory proteins and in T4 gp44. The consensus sequence among the small RFC subunits is s(M/L)TxxAQxALRRtmE.

RFC box VII, SRC, is conserved within the small subunits, the prokaryotic accessory proteins, and T4 gp44, but only the Cys is present in the large RFC subunits and CHL12. Between box VII and box VIII, we can align single amino acids present in most of the proteins, but they do not cluster as a conserved box. RFC box VIII has the consensus sequence gdURxx(L/ I)xxlq, and mutations in the codons for G and D have been shown to cause a cold-sensitive phenotype in *cdc44* mutants.

Box I is present only in the large RFC subunits (3, 4, 29). It consists of about 90 amino acids, and similar boxes can be found in all three known prokaryotic DNA ligases and, to a lesser extent, in all known poly(ADP-ribose) polymerases (PARPs) (Fig. 7). This region has been designated the ligase homology domain and has been recognized previously in the mammalian RFC (3, 4, 29).

## DISCUSSION

The DNA replication apparatus is conserved in function, structure, and amino acid sequence from yeasts to mammals among all eukaryotes investigated so far. Most of the factors also seem to be functionally conserved in bacteria like *E. coli* and bacteriophages such as T4 (48). Some of these DNA replication proteins, such as the DNA polymerases, even show amino acid similarity (2), but this is not always the case (e.g., PCNA and *E. coli*  $\beta$  subunit).

RFC is functionally similar to the bacteriophage T4 accessory protein complex gp44-gp62 and to the *E*. *coli*  $\gamma/\delta$  complex (35, 48). Each of these proteins binds to primer-template structures and loads the respective donut-shaped sliding DNA clamp, gp45 for T4, β for *E. coli*, and PCNA for the eukaryotes, onto the DNA template. Besides their similar functions, there also exists considerable amino acid sequence similarity between subunits of these protein complexes (3, 4, 24, 25, 29, 34, 35) (Fig. 6). Nevertheless, the subunit compositions of the respective complexes are quite different. T4 gp44-gp62 seems to be composed of four gp44 subunits and one gp62 subunit (62), with gp62 having no similarity to any RFC subunit. The E. *coli* proteins can be found in two different complexes. The  $\tau$ homodimer has a DNA-dependent ATPase activity (50) and might work as a bridge between two Pol III core DNA polymerases (49).  $\gamma$  binds ATP in a similar fashion, which is expected since  $\gamma$  consists of the N-terminal three-fourths of the  $\tau$ subunit, including the ATPase consensus domain. But  $\gamma$  becomes an ATPase only as part of the  $\gamma$  complex with  $\delta$ ,  $\delta'$ ,  $\Psi$ , and  $\chi$  (38). This ATPase activity is, like  $\tau$ , stimulated by DNA but can be further stimulated by the  $\beta$  clamp. ATPase activity stimulated by DNA can be reproduced by  $\gamma\delta,~\gamma\delta',~or~\gamma\delta\delta'$ complexes, but only the activity of complexes containing  $\gamma$  and δ can be further stimulated by the β clamp (38).

We used peptide sequences obtained by sequencing the purified ScRFC subunits and the sequence similarity of hRFC to T4 gp44 and *E. coli*  $\gamma/\tau$  and  $\delta'$  (35) to design degenerate oligonucleotides. PCR yielded probes for all five *RFC* genes, which were then used to clone *RFC1* to *RFC5* from a genomic *S. cerevisiae* library. All peptide sequences could be accounted for by the amino acid sequences of *RFC1*, *RFC2*, *RFC4*, and *RFC5* (Fig. 3). No peptide sequences were obtained from *RFC3*. However, *RFC2* (34), *RFC3* (25), and *RFC4* (24) were cloned recently by others, and Li and Burgers (25) have shown that a polyclonal antiserum directed against bacterially expressed ScRFC3 reacts with a band of about 40 kDa in an immunoblot with biochemically isolated ScRFC. These data and our peptide sequencing data indicate that ScRFC, like hRFC, is a protein complex of five different subunits.



FIG. 8. Summary of the ScRFC subunits. The small subunits align to the middle part of the large subunit. There are eight conserved RFC boxes numbered consecutively from N terminus to C terminus. Box I is the DNA ligase homology domain, and boxes II to VIII contain within them an ATP-binding region.

The RFC1 gene is identical to the CDC44 gene. Cold-sensitive cdc44 mutations isolated by Moir et al. (32) and Howell et al. (15) show a cell division cycle arrest phenotype and arrest as large-budded cells with the nucleus at the neck between mother and daughter cells. The phenotypes observed in cdc44 mutants, while complicated, are consistent with a role for this protein in DNA metabolism in cells (15) and thus consistent with previous biochemical characterization of both human and yeast RFCs. The amino acid changes in cdc44 mutants are all due to defects in or near the ATP/GTPase consensus domain in RFC boxes V and VI or in box VIII, which is also conserved in all five RFC subunits (Fig. 6; summarized in Fig. 8). These mutations are likely to affect the ATPase activity of ScRFC1 (since GTP is not essential for RFC activity and substitutes only poorly for ATP [23], we assume that those sites bind primarily ATP). RFC most likely acts as a protein topoisomerase in that it opens up the donut-shaped PCNA trimer and loads it onto partially duplex DNA in an energy-consuming step. The RFC1 mutations can be suppressed by point mutations in the POL30 gene, which encodes PCNA (31). Because of their positions in the protein, the amino acid changes are likely to affect the interaction between the PCNA monomers within the trimer structure in a way that compensates for the reduced activity of the RFC complex (20). It is not clear from these data whether the interaction between ScRFC1 and PCNA is direct or exerted through the small RFC subunits, but the data show that this interaction is essential in vivo.

Some investigators cloned RFC1 from human (28, 29), mouse (4, 29), and *Drosophila melanogaster* (accession number 17340) cells in attempts to screen expression libraries with double-stranded oligonucleotides as a probe for sequence-specific DNA-binding proteins. In none of these cases, however, was DNA sequence specificity of the RFC large subunit demonstrated. It is most likely that the RFC large subunit bound to nicks or single-stranded–double-stranded DNA junctions present in the probe. Nevertheless, these results clearly show that the RFC large subunit has a DNA-binding activity by itself. This was demonstrated directly (55), and the DNAbinding activity was mapped to a region containing the ligase homology domain (4).

RFC box I shows similarity to a region in prokaryotic DNA ligases and in procyclic acidic repetitive proteins (PARPs) from eukaryotes (Fig. 7). All three protein groups bind to primer-template or nick structures in DNA, and box I is the only conserved region in the fragment mapped by Burbelo et al. (4) as the DNA-binding domain of the large RFC subunit. The DNA-binding domains of the ligases have has not been mapped. In the PARPs, the region of similarity is contained in the automodification domain and not in the known DNA-

binding domain (9). More work is necessary to characterize the DNA binding by these proteins.

Because of the high similarity between the five ScRFC subunits, it was surprising that each one is essential for cell proliferation, and we therefore assume that each one has a unique function in DNA replication, repair, or recombination. This could be accounted for by significant differences in the ATPase domains of the five subunits. In ScRFC5 and the homolog hRFC38, for example, we can see divergences from the consensus sequence of RFC boxes III (P loop), IV, and V (DEAD box) that affect residues that are important for the GTPase activity of p21<sup>ras</sup> (39, 40). Homologous RFC subunits also show good conservation outside the ATP/GTPase domain, where there is little similarity among the five subunits. This finding indicates that these regions (e.g., the part C terminal of RFC box VIII) are important for subunit-specific functions.

Biochemical data so far provide little clue as to what the specific functions of the four small RFC subunits might be. It is not clear whether all of the RFC subunits actually have an ATPase activity. An ATPase activity that is stimulated most efficiently by single-stranded DNA or primed single-stranded DNA was shown for ScRFC3 (25). There are also reports about ATP cross-linking to ScRFC2 (34), to a ScRFC 40-kDa band (26), and to one of the small hRFC subunits that runs as 41 kDa (55). But even if not all subunits have an ATPase activity, the question remains of why these domains (II to VIII) are conserved in all subunits. We can speculate that some subunits might have regulatory functions. We also do not know whether all of the RFC subunits associate in a single complex or whether several complexes with different subunit composition, e.g., the large subunit with different small subunits, may act in different processes such as DNA replication and repair. PCNA has been demonstrated to play a role in both DNA replication (43) and DNA repair (30, 33, 45). Although RFC has a clear role in DNA replication, its role in DNA repair remains to be determined.

#### ACKNOWLEDGMENTS

We thank J. Hurwitz for communication of the unpublished sequence from the hRFC38 cDNA.

This research was supported by grants AI20460 and CA13106 from the National Institutes of Health. G.C. was supported by a joint postdoctoral fellowship from the Swiss National Science Foundation and the Deutsche Forschungsgemeinschaft.

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hRFC140	MDIRKFFGVIPSGKKLVSETVKKNEKTKSDEETLKAKKGIKEIKVNSSRKEDDFKQKQPSKKKRIIYDSDSESEETLQVKNAKKPPEKLPVSSKP	95
hRFC140	GKI SRQDPVTYI SETDEEDDFMCKKAA SKSKENGRSTNSHLGTSNMKKNEENTKTKNKPLSPI KLTPTSVLDYFGTGSVQRSNKKMVA SKRKELS	190
hRFC140 ScRFC1	QNTDESGLNDEALAKQLQLDEDAELERQLHEDEE FARTLAMLDEEPKTKKARKDTEAGETFSSVQANLSKAEKHKYPHKVKTAQVSDERKSYSPR MVNLSDFFGKNKKSVRSSTSRPTRQVGSSKPEVIDLDTESDQESTN	285 46
hRFC140 ScRFC1	KQSKYESSKESQQHSKSSADKIGEVSSPKASSKLAIMKRKKESSYKEIEPVASKRKENAIKLKGETKTPKKTKSSPAKKESVSPEDSEKKRTN KTPK	378 127
hRFC140 ScRFC1	I (Ligase-homology) YQAYRSYLNREGPKALGSK., EIPKGAENCLEGLIFVITGVLESIERDEAKSLIERYGCKVTGNVSKKTNYLVMGRDSGQSKSDKAAALGTKIID KFDFKSANSNADPDEIVSEIGSFPEGKPNCLLGLTIVFTGVLPTLERGASEALAKRYGARVTKSISSKTSVVVLGDEAGPKKLEKIKOLKIKAID	471 222
CHL12	WUDTAPYIG	9
hRFC140 ScRFC1	EDGLLNLIRTMPGKKSKYEIAVETEMKKE, SKLERTPQKNVQGKRKISPSKKESESKKSRPTSKRDSLAKTIKKETDVFWKSLDFKEQVAEETSG EEGFKQLIAGMPAEGGDGEAAEKARRKLE.EQHNIATKEAELLVKKEEERSKKLAATRVSGGHLERDNVV	565 291
hRFC37 ScRFC2 hRFC40	MQAFLKGTSISTKPPLTKD MFEGFGPNKKRKISK. MEVEAVCGGAGEVEAQD	19 15 17
hRFC36 ScRFC3	M	1
CHL12	SLGRSSLFDTGDIEQAPGNNAI,GINEEDIHAFVSSTGETVQLKKKPAKLATGNISLYTNPDTVWRSDDTYGININYLLDKIEASGD	95
Bschaff EcTau EcDelta' hRFC140 ScRFC1 hRFC38 ScRFC37 ScRFC37 ScRFC37 ScRFC4 hRFC40 ScRFC4 hRFC36 ScRFC4 hRFC36 ScRFC3 T4gp44 CHL12 Bschaff EcDelta' hRFC140	II         III           MSYQALYRVFRPORFEDVCOEHT TKTLONAL         LOKKFS.HAYLFSSPRGTGKT           MSYQVLARKWRPOTFADVGOEHVLTALANGL         SLGRIH, HAYLFSSTRGVGKT           MSYQVLARKWRPOTFADVGOEHVLTALANGL         SLGRIH, HAYLFSSTRGVGKT           MRYPPULRPD, FEKLVA         SY           DSKARNLADDSSENKVENILLWVDKYKPTSI KTTI GQOGDQSCANKLLRWLRNWQKSSSEDKKHAAKFGKFSGKDDGSSFK, AALLS, GPPGVGKT           MSLWVDKYRPTSI KTTI GQOCDQSCANKLLRWLRNWQKSSSEDKKHAAKFGKFSGKDDGSSFK, AALLS, GPPGVGKT           MSLWVDKYRPTSI KTTI GQOCDQSCANKLLRWLRNWDNSKKNSFKHA           MSLWVDKYRPTSI KTTI GQOCDQCCNKG.           MSLWVDKYRPTSI KATSI GANAQUURL           MSLWVDKYRPTSI KATSI GANAQUURL           MSLWVDKYRPTSI GANAQUURL           MSLWVDKYRPTSI MELTNELTNELTNELTNEL           SDPAPAFSKAPGSAGHVEKYRPTKUNE VENKRETDENLQIA           MSTLS. LQLPWVEKYRPTI DEVIGONEVITTURKET           MSTLS. LQLPWVEKYRPTDI DEVIGONEVITTURKFV           DE. GKLP, HILLY, GPPGTGKT           MITVNEKEHT I LQXYRPSTI DECILPAFDKETFKSIT           MITVNEK	52 52 38 658 360 49 50 85 72 83 56 67 67 190 125 125 114 7192
SCRFC1 hRFC38 SCRFC5 hRFC37 SCRFC2 hRFC40 SCRFC4 SCRFC4 SCRFC3 T4GP44 CHL12	TAAHLVAQELGYDILEQAA.SDVRSKTLLNAGVKNALDNMSVVGYF.KHNEEAQNLNCKHFVIIM         TRIMCILRELYGVGVEKLRIEHQTITPSKKKIEISTIASNYHLEVNP.SDAGNSD.RVVIQEMLKTVAQSQQL.ETNSQRDFKVVLI         TRIMAILESIFGEGVYRLKIDVRQEVTASNRKLELNVVSSPYHLEITP.SDMGNND.RVVIQEMLKTVAQSQQL.ETNSQRDFKVVLI         STILAARELFGPELFRLRV.         LEINA.SDERGIQ.VVREKVNFAQLTVSGSRS.DGKPCPFFKIVIL         STILALTKELYGPDLMKSNI.         LEINA.SDERGID.VVREKVNFAQLTVSGSRS.DGKPCPFFKIVIL         TSILCLARALL.G.ALKDAM.         LEINA.SDERGID.VVRNKIKMFAQLTVSKPSKHDLENYPCPFYKIVIL         TSILCLARALL.GRSYADGV.         LEINA.SDDRGID.VVRNKIKMFAQKVTLPK.GRHKIIIL         STILACAKQLYKDKEFGSMV.         LEINA.SDDRGID.VVRNQIKHFAQKVLHLPP.GKHKIVIL         STILACAKQLYKDKEFGSMV.         LEINA.SDDRGID.VVRNQIKHFAQKVLHLPP.GKHKIVIL         STILACAKQLYKDKEFGSMV.         LEINA.SDDRGID.VVRNQIKHFAQKVLHLPP.GKHKIVIL         STIVALAREIY.GKNYSMV.         LEINA.SDDRGID.VVRNQIKHFAQKVLHLPP.GKKLVIL         STVALAREIY.GKNYSMV.         LEINA.SDDRGID.VVRNQIKHFASASF.         STVALAREIY.GKNYMV.         SGFSVSEINA.SDERAGPMVKEKIYNLENTFASAASF.         VELVA	423 134 140 149 139 139 113 124 116 106 240
BsdnaH	V VI a/b VII DEVH.MLSIGAFNALLKTLEEPPIVGR	186
EcTau EcDelta' hRFC140 ScRFC1 hRFC38 ScRFC5 hRFC37 ScRFC2 hRFC40 ScRFC4 hRFC36 ScRFC4 hRFC36 ScRFC3 T4gp44 CHL12	DEVH. MLSRHSFNALLKTLE. EPP. EHVKFLLATTDPQKLPVTILSRCLQFHLKALDVEQ. IRHQ TDAA JLITDAAANALLKTLE. EPP. EHVKFLLATTDPQKLPVTILSRCLQFHLKALDVEQ. IRHQ DEVDCMACNEDRGGIQELIGLIKHT. KIPIICNCNDRNHPKIRSLMHVCFDLRPQRPRVEQ. IKGA DEVDCMACNEDRGGIQELIGLIKHT. KIPIICNCNDRNHPKIRSLMHVCFDLRPQRPRVEQ. IKGA DEVDCMACNEDRGGIQELIGLIKHT. KIPIICNCNDRNHPKIRSLMHVCFDLRPQRPRVEQ. IKGA DEVDCMACNEDRGGIQELIGLIKHT. KIPIICNCNDRNHPKIRSLMHVCFDLRPQRPRVEQ. IKGA DEVDCMACNEDRGGIQELIGLIKHT. KIPIICNCNDRNHPKIRSLMHVCFDLRPQRPRVEQ. IKGA DEVDCMACNEDRGGIQELIGLIKHT. KIPICNEDRGULAVRVPAPSIED. ICHV NEAN.SITKDAQAALRRTMEKYSKN. IRLINVCDSMS.PIIAPIKSRCLLIRCPAPSDSE. ISTI DEAD.SMTSAAQAALRRTMEKYSKN. IRLINVCDSMS.PIIAPIKSRCLLIRCPAPSDSE. ISTI DEAD.SMTSAAQAALRRTMEKYSKN. TRFCLICNYVT.RIIEPLTSRCSKFRFKRLDSKI QQQR DEAD.SMTDGAQQALRRTMEIYSKT. TRFALACNASD.KIIEPICGSRCATLRYKKLDAQ. ILTR DEAD.SMTGAQQALRRTMELYSNS. TRFAFACNQSN.KIIEPICGSRCATLRYSKLSDED. VLKR DEAD.SMTGAQQALRRTMELYSNS. TRFCLICNYLS.KIIPALGSRCTRFRFGPLTPEL MVPR DEAD_AMTNAAQNALRRVIEKFTEN. TRFCLICNYLS.KIIPALGSRCTRFRFGPLTPEL MVPR DEAD_AMTNAAQNALRRVIERYTNN. SIIITANNID.GIIKPLQSRCRVIFGQPDEDKIEMKKMRSKILRFM	186 175 783 486 195 201 210 200 200 200 174 185 177 175 328

	VIII	
BsdnaH EcTau EcDelta' hRFC140 ScRFC1 hRFC38 ScRFC3 hRFC37 ScRFC40 ScRFC4 hRFC40 ScRFC4 hRFC36 ScRFC3 T4gp44 CHL12	MNKIVDAEQIQVEEGSL.EIIASAADGGMRDAISIIDQAISFSGDILKVEDALLITGAVSQLYIGKLAKSLHDKNVSDALETL LEHILNEEHIAHEPRAL.QLLARAAEGSLRDAISLTDQAIASGDGQVSTQAVSAMLGTLDDDQALSLVEAMVEANGERVMALI LSREVTMSQDALLAALRISAGSPGAALALEQGDNWQARETLQALAYSVPSGDWYSLLAAL MMSIAFKEGLKIPPPAM.NEIILGANQDIRQVIHNISMWCARSKALTYDQAKADSHRAKKDIKMGPFDVARKVFAAGETAHMSL LMTIAIREKFKLDPNVI.DRLIQTTR IRQVINILSTISTTKTINHENINEISKAWEKNIALKPFDIAHKMIDGQIYSDIGSRNFL LSTVCKKEGLNL.PSQLAHRLAEKSCRNLRKALLMCEA.CRV.QYPFTADQEIPETDWEVYLRETANAIVSQQTPQRLLEVRGRL LSDVVTNERIQLETKDILKRIAQASNGNLRVSLIMLES.MALNNELALKSSSPIIKVDWIIVIHKLTRKIVKERSVNSLIECRAVL LLDIAKKENVPISHRGI.AYLVKVSECDLRKAITFLQS.ATR.LTGGKEITEKVTHIAGVIPAEKIDGFAACQSGSFDKLEAVV LKRISEQENVKCDGVL.ERIIDISACDLRRGITLQS.SKGAQYLCDGKNITSTQVEELAGVVPHDILIEIVEKVKSGDFDEIKKYVL LLDIAKKENVPISHRGI.AYLVKSGDMRQAINNLQS.TFSGFGFINSENVFKVCDEPHPLLVKEMIQHCVNANIDEAYKIL LQIIKLEDVKYTNDGL.EAIIFTAGCDMRQAINNLQS.TVACHGLVNADVVFKIIDSPHPLIVKKMLLASNLEDSIQIL LEHVVEEKKVDISEDGM.KALVISGDMRRAINIQS.TNMAFGKVTEETVYTCTGHPLKSDIANILMNINDFTAYRNI IANVIVHEKLKLSNAE.KALIELSNGDMRRVINVLQS.CKAATDNPDEDEISDDVIYECCGAPRPSDLKAVLKSILEDSQGTAYCNI LNUVHEKLKLSNAE.KALVILSSGDMRRVINVLQS.CKAATDNPDEDEISDDVIYECCGAPRPSDLKAVLKSILEDSQGTAYRNI IANVIVHEKLKLSNAE.KALVILSSGDMRRVINVLQS.CKAATDNPDEDEISDDVIYECCGAPRPSDLKAVLKSILEDSQGTAYRNI LNLICHKENNNIPIKAI.NDLIDLAQGDVRCINNIQFLASNVDSRDSSASDKPACKNTWASSNKDSPISWFKIVNQLFRKDPHPDIKEQFYEL	268 268 191 867 278 286 293 287 281 252 266 263 254 422
BschaH EcTau EcDelta' hRFC140 ScRFC1 hRFC38 ScRFC5 hRFC37 ScRFC2 hRFC40 ScRFC4 hRFC36 ScRFC3 T4gp44 CHL12	NELLQQGKDPAKLIEDMIFYFRDMLLYKTAPGLEGVLEKVKVDETFRELSEQIPAQALYEMIDIL.NKSHQEMKWTNHPRIFF NEAAARGIEWEALLVEMLGLLHRIAMVQLSPAALCN.DMAAIEIRMRELARTIPPTDIQLYYQTL.LIGRKELPYAPDRMGV NHEQAPARLHWLATLLMDALKRHH GAAQVTNVDVPGLVAELANHLSPSRLQAILGDVCHIREQIMSVTGIRNELL VDKSDLFFHDYSIAPLFVQENYIHVKPVAAGGDMKKHLMLSRAADSICDGDLVDSQIR.SKQNWSLLPAQAIYASVLPCELMRGYMTQFPTFPS NDKIALYFDDFDFTPIMIQENYLSTRPSVLKPG.QSHLEAVAEAANCISLGDIVEKKIRSSEQLWSLLPLAVISSVYPASKVAGHMAGRINFTA YELLTH.CIPPEIIMKGLLSELL.HNCDGQLKGEVAQMAAYYEHRLQLGSKAIYHLEAFVAKFMALYKKFIQDGLEGMMF YDLLAH.CIPPANIILKELTFSLLDVETINTTNKSSIIEYSSVFDERLSLGNKAIFHLEGFIAKVMCCID KDLI.DEGHAATQLVNQLHDVVVEN.NLSDKQKSIITGELAEVDKCLAEGADEHLQLISLCATVMQQLSQNC NTFM.KSGWSAASVVNQLHEYYITNDNFDTNFKNQISWLLFTTDSRLNNGTNEHIQLLNLLVKISQL .AHUWHLGYSPEDIIGNIFRVCKTF.QMAEYLKLEFIKEIGIT.HMKIAEGVNSLLQMAGLLARLCQKTMAPVAS RTDLWKKGYSSIDIVTSFRVTKNLAQVKESVRLEMIKEIGLT.HMKIAEGVNSLLQASLAMLAKIHKLNNKA TELKTLKGLALHDIITEIHLFVHRVDFPS.SVRLEMIKEIGLT.HMRILEGVGTYLQLSSLIAAFQVTRDLIVAEA NKVRSAKGLALIDLIEGIVKILEDYELQNEETRVHLLTKLADIEYSISKGGNDQIQGSAVIGAIKASFENETVKANV FKYAADYSWFVGKLAEEIYSAVTPQSIIRMYEIVGEN.NQYHGLAANTELHLAYIFIQLACEMQWK LNQVE.LNGNSDRILQGCFNIFFVVKSDNGIRKPANISDWLFFHDLMYQSMYAHNGELLRYSALVPLVFFQTFGDIANKDDIRMKNSEYEQREL	350 349 311 961 356 354 353 353 353 323 340 340 319 516
BsdnaH EcTau EcDelta' hRFC140 ScRFC1 CHL12	EVAVVKICQTSHQSAADLEEVDMIMKKIQDLEQEVERLKTTGIKAAAESPKKEAPRVPKGGKSNYKAPVGRIH EMTLLRALAFHPRMPLPEPEVPRQSFAPVAP.TAVMTPTQVPPQPQSAPQQAPTVPLPETTSQVLAARQQLQRVQGATKAKKSE.PAAATRAR ITDLLIRIEHYLQPGVVLPVPHL WLGKHSSTGKHDRIVQDLALHMSLRTYSSKRTVNMDYLSLLRDALVQPLTSQGVDGVQDVVALMDTYYLMKEDFENIME.ISSWGGKPSPFSKLD WLGQNSKSAKYYRLLQEIHYHTRLGTSTDKIGLRLDYLPTFRKRLLDPFLKQGADAISSVIEVMDDYYLTKEDWDSIMEFFVGPDVTTAIIKKIP KRANSDIVSLIMRHISVQSPLMASFTDRKSLIFEILPYLDSMISSDFNKIRNLKLKQAIMEELVQLLKSFQLNLIQNRSEGFDVRGGLTIDPPID	423 440 334 1055 763 611
BsdnaH EcTau hRFC140 ScRFC1 CHL12	EILKEATRPDI.DI.LRNSWGKILLAHLKQQNKVSHAALI.NDSEPVAAGSAAFVI.KFKYEIHCKMVAE PVNNAALERLASVTDRVQARPVPSALEKAPAKKEAYRWKATTPVMQQKEVVATPKALKKALEHEKTPELAAKLAAEATERDPWAAQVSQLSLPKL PKVKAAFTRAYNKEAHLTPYSLQAIKASRHSTSPSLDSEYNEELNEDDSQSDEKDQDAIETDAMIK.KKTKSSKPSKPEKDKEP ATVKSGFTRKYNSMTHPVAIYRTGSTIGGGGVGTSTSTPDFEDVVDADDNPVPADDEETQDSSTDLKKDKLIKQKAKPTKRKTATSKPGGS EVVLLNPKHINEVQHKRANNLSSLLAKIEENRAKKRHIDQVTEDRLQSQEMHSKKVKTGLNSSSSTIDFFKNQYGLLKQTQELEETQKTIGSDET	488 535 1138 854 706
BsdnaH EcTau hRFC140 ScRFC1 CHL12	DNNGVRTNLEQILESMLGKRMDLIGVPEAQWGKIREEFLEDHQQANEGSNEPAEEDPLIAEA VEQVALNAWKEESDNAVCLHLRSSQRHLNNRGAQQKLAEALSMLKGSTVEL.TIVEDDNPAVRTPL.EWRQAIYEEKLAQARESIIADNNIQTL RKGKGKSSKK KKRKTKA NQADDCNQTVKIWVKYNEGFSNAVRKNVTWNNLWE	550 627 1148 861 741
BsdnaH EcTau	KKLVGADLIEIKD	563