古細菌DNA複製に関わるタンパク質群の構造生物学

山梨大学 生命環境学部
大山 拓次

Structural Study of Archaeal DNA Replication Proteins

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2018 December 5
Bioinformatics Educational Seminar 2018
Tokyo University of Science

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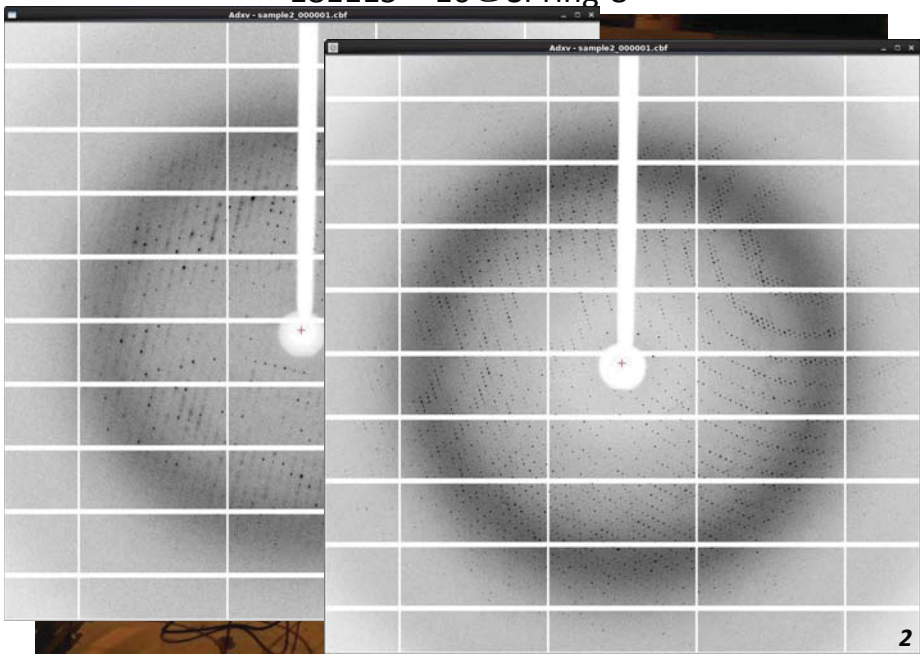


To understand the structure-function relationship

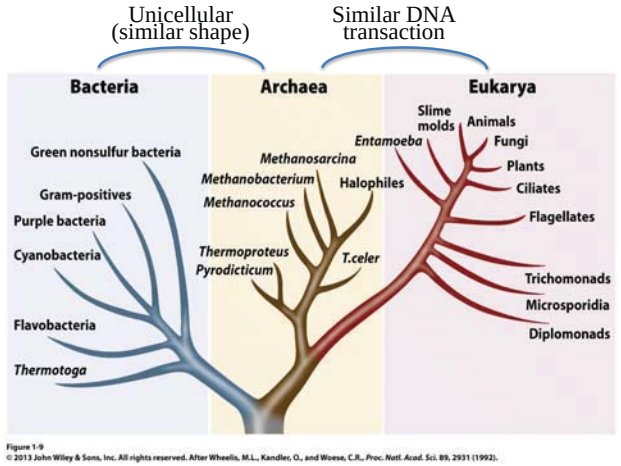
Three-dimensional Structure (at atomic level)

- Biochemistry
- Molecular Biology
- Genetics
- Cellular Biology

⋮

- Bioinformatics

Three Domains of Life



Archaea are similar to bacteria in shape, but similar to eukaryote in DNA replication system.
Archaeal DNA transacting proteins are simple and stable than those from eukaryotes.

⇒ Good model to understand the complex eukaryotic system
⇒ Interesting target to consider evolution of life and proteins

Comparison of Proteins working for DNA replication

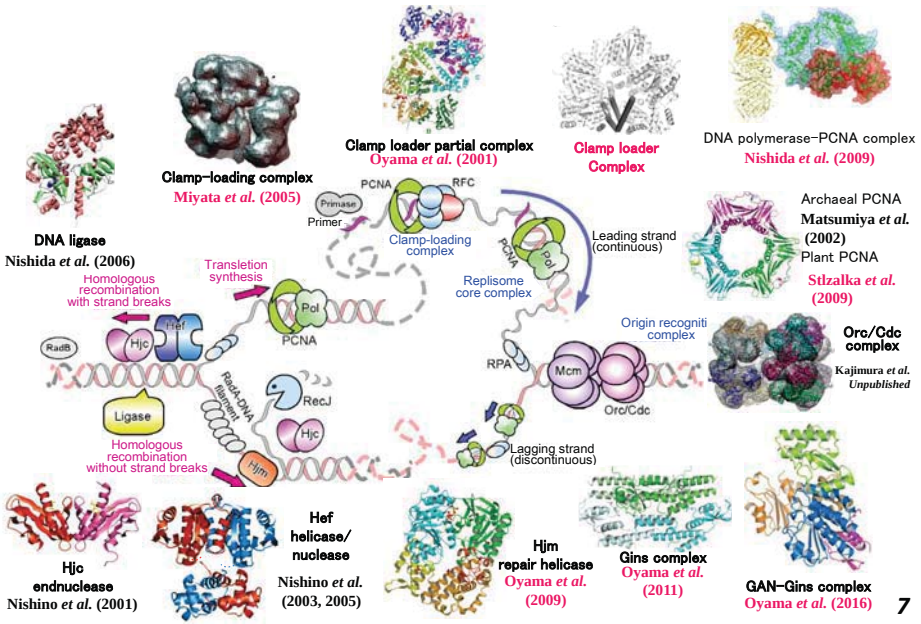
	Bacteria	Eukaryotes/Archaea
Origin Recognition	DnaA	Orc (with Cdc6)
Template unwinding	DnaB & DnaG	MCM (with Cdc45 & GINS)
ssDNA binding	SSB	RPA
Primer synthesis	RNA primase	DNA polymerase α / primase
Replication activation	β clamp dimer	PCNA trimer
Clamp-loading	γ complex	RFC
New DNA synthesis	DNA polymerase III	DNA polymerase δ/ε
Primer removal	DNA polymerase I	RNaseH (with FEN)
Strand maturation	DNA ligase	DNA ligase

Comparison of Proteins working for DNA replication

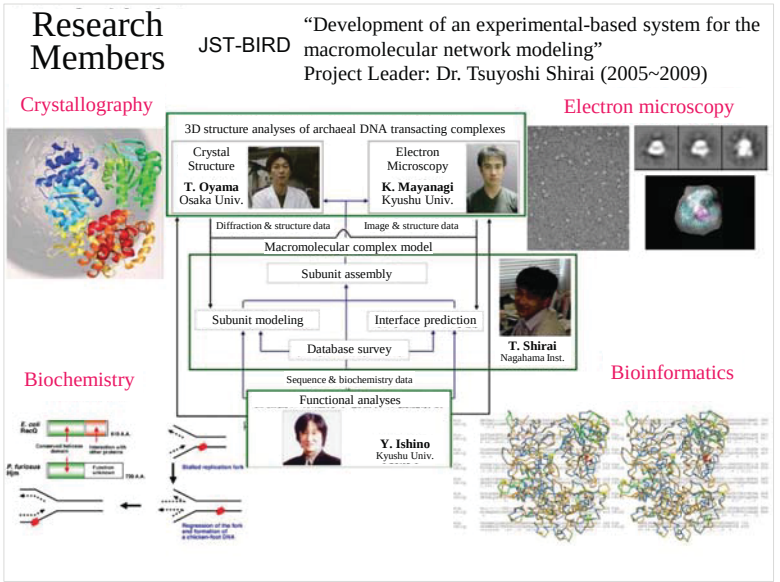
	Eukaryotes	Archaea
Origin Recognition	Orc 1~6 hetero 6mer	Orc homo 6mer
Template unwinding	Mcm2~7 hetero 6mer	MCM homo 6mer
MCM activator	GINS hetero 4mer	GINS 51:23=2:2 hetero 4mer or homo 4mer
ssDNA binding	RPA hetero 3mer	RPA hetero 3mer
Replication activation	PCNA homo 3mer	PCNA homo trimer
Clamp-loading	RFC 1-5 hetero 5mer	RFC L:S=1:4 5mer
Strand maturation	DNA ligase	DNA ligase

Eukaryotic and archaeal DNA replication proteins

(from the former institute BERI until now)

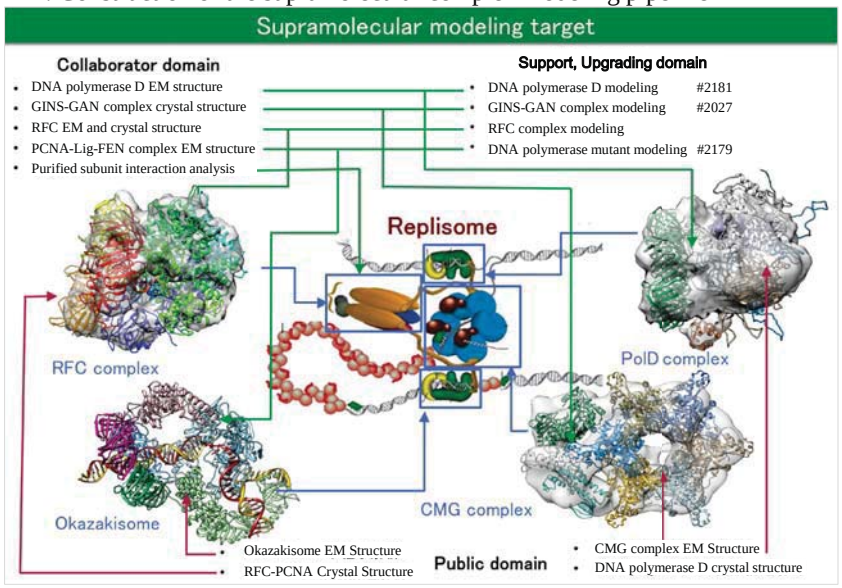


The Goal of Replisome, Still far away? or almost there?



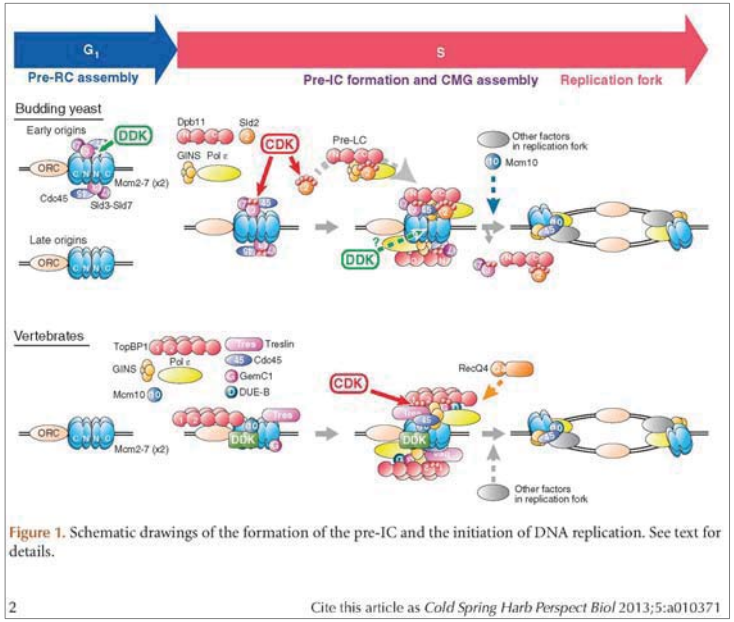
The Goal of Replisome, Still far away? or almost there?

AMED: Construction of the supramolecular complex modeling pipeline

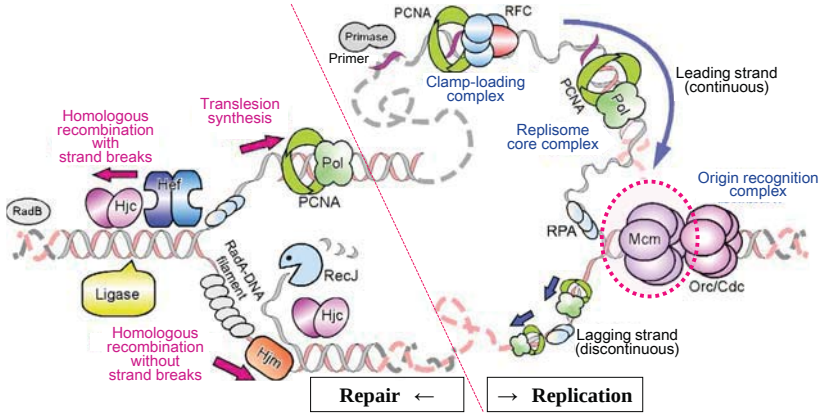


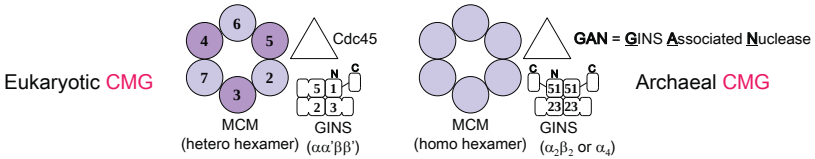
Unwindosome (CMG helicase holo-enzyme)

Replication Fork Formation (Long Long Road)



Eukaryotic and archaeal DNA replication





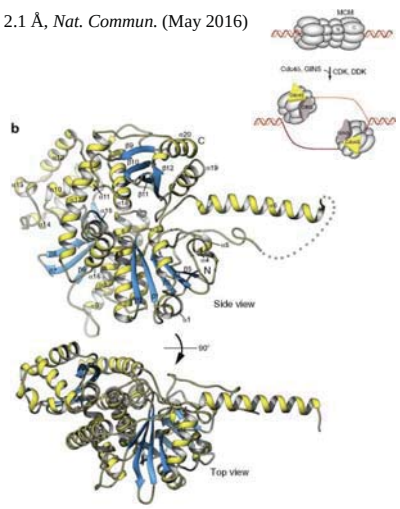
Cdc45, GAN and RecJ are homolog

ARTICLE

Structure of human Cdc45 and implications for CMG helicase function

Aline C. Simon[1], Vincenzo Sannino[2], Vincenzo Costanzo[2] & Luca Pellegrini[1]

2.1 Å, *Nat. Commun.* (May 2016)



Structural basis for DNA 5′-end resection by RecJ

2.3 Å, *eLife*, (Apr 2016)

Kaiying Cheng, Hong Xu, Xuanyi Chen, Liangyan Wang, Bing Tian, Ye Zhao*, Yuejin Hua*

Key Laboratory of Chinese Ministry of Agriculture for Nuclear-Agricultural Sciences, Institute of Nuclear-Agricultural Sciences, Zhejiang University, Hangzhou, China

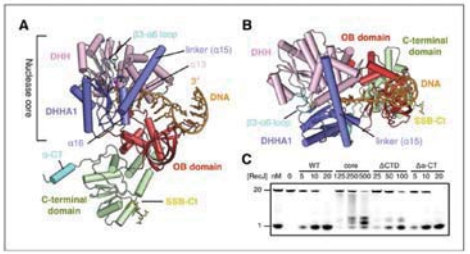
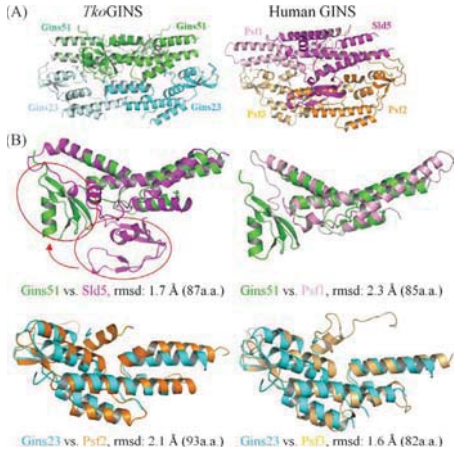


Figure 2. Structure of drRecJ complex. (**A**) Overall structure of drRecJ complex viewed from the side. Protein domains of drRecJ are labeled and shown in distinct colors. The DNA and SSB-Ct are colored orange and yellow respectively. Two Mn^{2+} in the active site are shown as magenta spheres. Two regions that are disordered in the ttRecJ structures (PDB code: 2ZXP) are highlighted in cyan. Three helices that form a helical gateway are also labeled. (**B**) Overall structure of the drRecJ complex viewed from the top of the DNA. The downstream nucleotides stack well to mimic the double-stranded DNA. (**C**) Denaturing PAGE gel showing the nuclease activities of different truncations of drRecJ. 3′-Fluorescence-labeled 20 nt ssDNA (100 nM) was incubated with various concentrations of different truncations of drRecJ proteins (see methods).

Archaeal and Eukaryotic GINS share the common architecture

	Tetramer form	Subunits α (AB-type)	Subunits β (BA-type)	Note
Human	$\alpha\alpha'\beta\beta'$	Sld5 Psf1	Psf2 Psf3	References [14-16]
T. kodakaraensis	$\alpha_2\beta_2$	Gins51	Gins23 (B domain, A domain)	Present structure
T. acidophilum	α_4	*Tac*Gins (A domain, B domain)	-	Present modeling

Different subunit composition of GINS in the different species



Comparison of the overall tetramer assembly

Comparison among the corresponding subunits

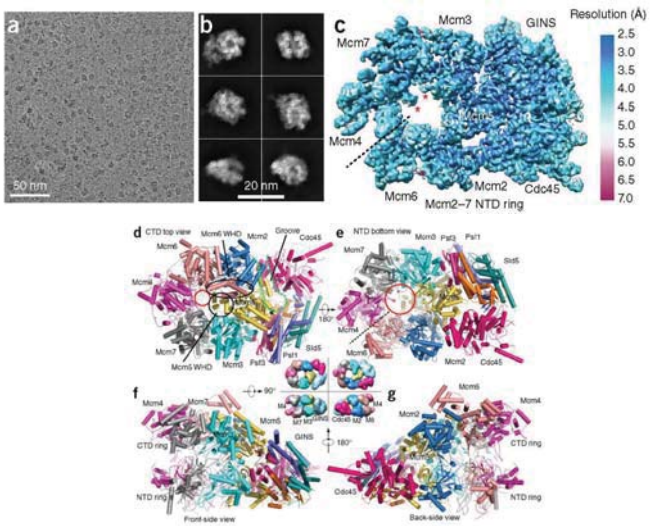
Oyama et al. *BMC Biol.* (2011)

High resolution CMG EM structure

Structure of the eukaryotic replicative CMG helicase suggests a pumpjack motion for translocation

3.7 Å, *Nat. Struct. Mol. Biol* (Feb 2016)

Zuanning Yuan[1,2], Lin Bai[2], Jingchuan Sun[2], Roxana Georgescu[3,4], Jun Liu[3], Michael E O'Donnell[3,4] & Huilin Li[1,2]



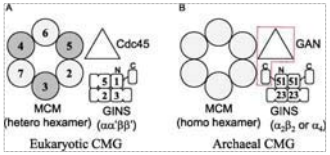
GAN could be an evolutionary intermediate between RecJ and Cdc45

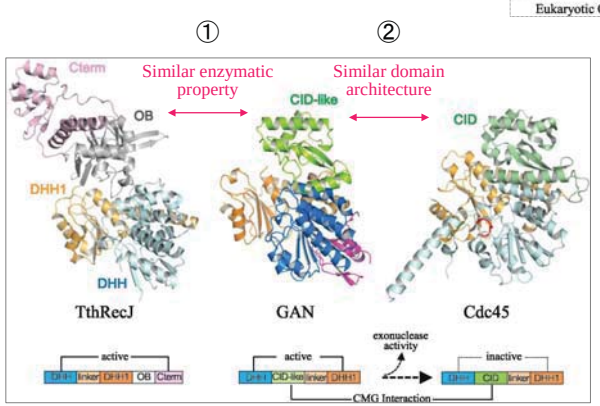
Nucleic Acids Research Advance Access published September 5, 2016

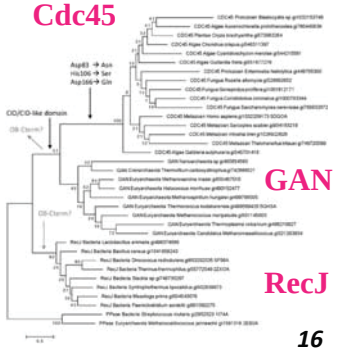
Nucleic Acids Research, 2016 1
doi: 10.1093/nar/gkw706

Atomic structure of an archaeal GAN suggests its dual roles as an exonuclease in DNA repair and a CMG component in DNA replication

Takuji Oyama[1,*], Sonoko Ishino[2], Tsuyoshi Shirai[3], Takeshi Yamagami[2], Mariko Nagata[2], Hiromi Ogino[2], Masami Kusunoki[1] and Yoshizumi Ishino[2,*]







Clamp-loading Complex (RFC-PCNA-DNA)

Identification of the Replisome Components

THE JOURNAL OF BIOLOGICAL CHEMISTRY
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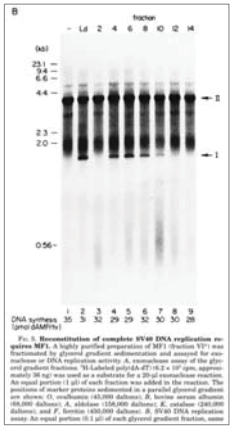
Vol. 269, No. 14, Issue of April 8, pp. 10923–10934, 1994
Printed in U.S.A.

Reconstitution of Complete SV40 DNA Replication with Purified Replication Factors*

(Received for publication, September 20, 1993, and in revised form, January 26, 1994)

Shou Waga, Glenn Bauer‡, and Bruce Stillman§
From the Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

The identification and purification of human cell proteins required for the production of form I DNA following DNA replication from the simian virus 40 (SV40) origin is described. Using these proteins, complete SV40 DNA replication was reconstituted with only purified DNA replication factors: SV40 large tumor antigen (TAg), replication protein A (RPA), DNA topoisomerases I and II, DNA polymerase α-primase, replication factor C (RFC), the proliferating cell nuclear antigen (PCNA), DNA polymerase δ, maturation factor 1 (MF1), and DNA ligase I. MF1, a 5′ to 3′ exonuclease and DNA ligase I were both identified as essential components for production of covalently closed circular relaxed (form I) DNA. MF1 is probably the same exonuclease previously shown by others to function during DNA synthesis on artificial DNA templates or in conjunction with DNA polymerase α from the SV40 origin. Combined with these previous studies, our results suggest that MF1 functions to remove an RNA primer attached to every Okazaki fragment during lagging strand DNA synthesis. Interestingly, whereas mammalian DNA ligase I functioned in the reconstituted replication system, mammalian DNA ligase III did not substitute and the phage T4 DNA ligase functioned inefficiently, suggesting that DNA ligase I has a specific role as a replicative DNA ligase in eukaryotic cells.



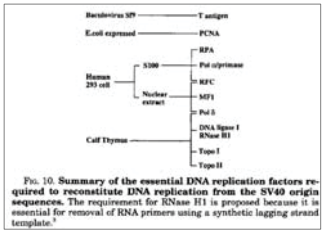
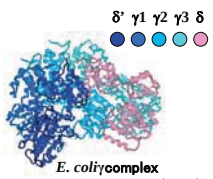


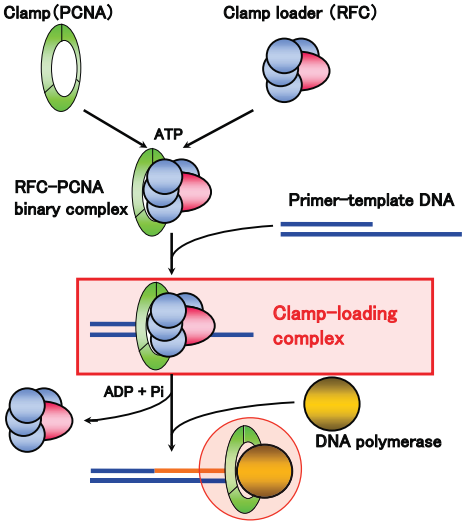
FIG. 10. **Summary of the essential DNA replication factors required to reconstitute DNA replication from the SV40 origin sequences.** The requirement for RNase H1 is proposed because it is essential for removal of RNA primers using a synthetic lagging strand template.[3]

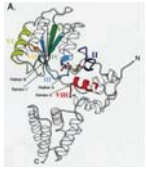
Structural study of clamp-loading complex

PCNA
Matsumiya *et al.* (2002)

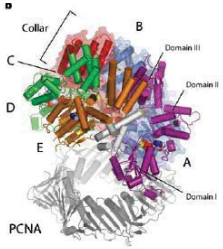


*E. coli*γcomplex
Jeruzalmi *et al.* (2001)





RFCS
Oyama *et al.* (2001)

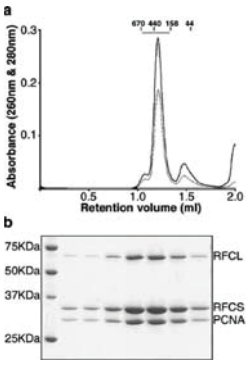


Yeast RFC–PCNA complex
Bowman *et al.* (2004)

Processivity of DNA polymerase is conferred by attachment to the sliding clamp loaded onto DNA

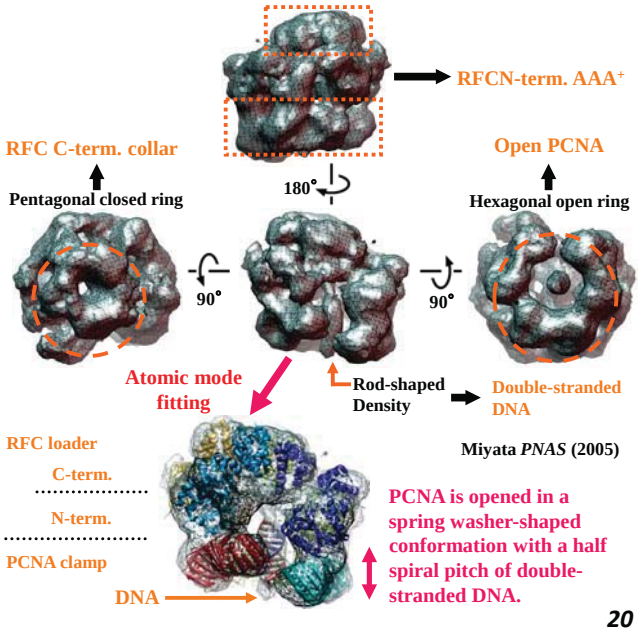
EM 3D Structure of RFC-PCNA-DNA

In vitro reconstruction of the RFC-PCNA-DNA complex



- Use of ATP analog (ATPγS)
- Purification by gel-filtration column chromatography

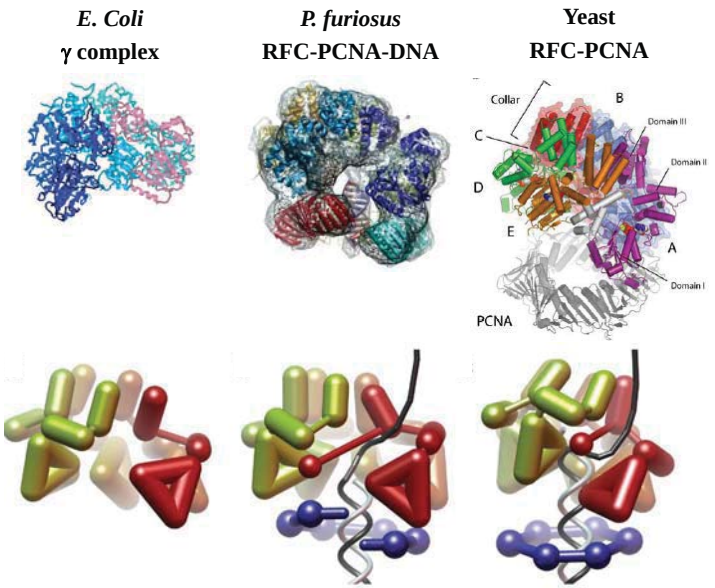
Miyata *NSMB* (2004)



Miyata *PNAS* (2005)

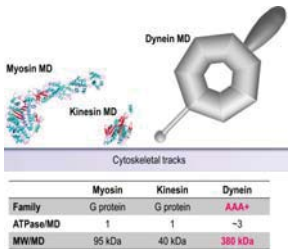
PCNA is opened in a spring washer-shaped conformation with a half spiral pitch of double-stranded DNA.

Structural comparison between clamp loaders and clamps



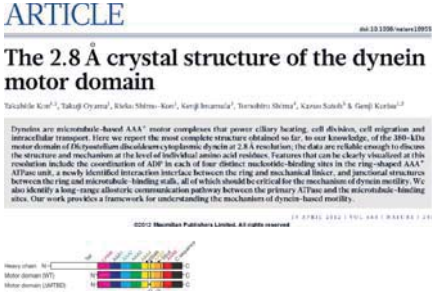
Miyata et al., *Proc Natl Acad Sci* (2005)

Dynein, Another AAA+ ATPase Protein

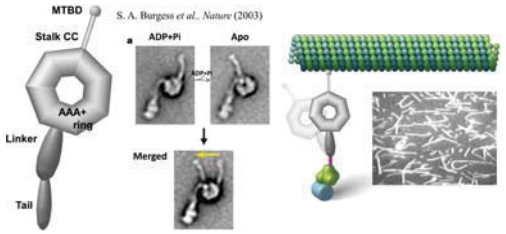


	Myosin	Kinesin	Dynein
Family	G protein	G protein	AAA+
ATPase/MD	1	1	~3
MW/MD	95 kDa	40 kDa	380 kDa

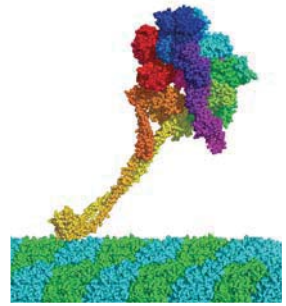
Major motor proteins in the cell


ARTICLE

The 2.8 Å crystal structure of the dynein motor domain



Electron microscopic analysis has proposed the "power-stroke model" of walking of Dynein on microtubules.



Clamp loaders and clamps are required throughout the cell cycle

(1) Various proteins working cooperatively with the clamp

PCNA interacting proteins

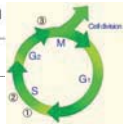
protein	function	sequence	total aa
Eukarya			
human p21	cell cycle regulation	143 RQTSMTDFYHSKRRLIF 159	164
human Fen1	flap endonuclease	336 TQGRLDDFFKVTGSLSS 352	380
S. pombe Cdc27	DNA polymerase d subunit	360 QQKSIMSFFQKK 371	371
human DNA ligase I	replication-specific DNA ligase	1 MQRSIMSFFHPKKEGKA 17	919
human RFC140	clamp loader large subunit	1 MDIKKFFQVPSPGKK 15	1148
human MSH3	mismatch repair	20 RQAVLSRFFQSTGSLKS 36	1128
human MSH6	mismatch repair	3 RQSTLTSFFKPSPALSD 19	1360
murine XPG	excision repair endonuclease	988 TQLRIDSFFKLAQQSKKQ 1004	1170
human MCMT	5' cytosine methyltransferase	163 RQTTITSHFAKGPAKKK 179	1616
human UNG2	uracil DNA glycosilase	3 QQKTLTSFFSPSPARKK 19	313
human WRN	helicase	73 DQWKLLNDFDIKLKNFV 89	1362
P. furiosus			
Pol I	DNA polymerase	762 RQVGLTSWLNIKKES 775	775
Pol II DP2	DNA polymerase large subunit	1252 KVISLDDFFSKR 1263	1263
RFCL	clamp loader large subunit	469 KQATLFDFLKK 479	479
Fen1	flap endonuclease	330 KQSTLKNMFKK 340	340
Hjc	holiday junction resolvase	115 IQKTLKGKS 123	123
PIP-box	consensus sequence	Qxxhxxas	PIP (PCNA-Interacting Protein) Box peptide

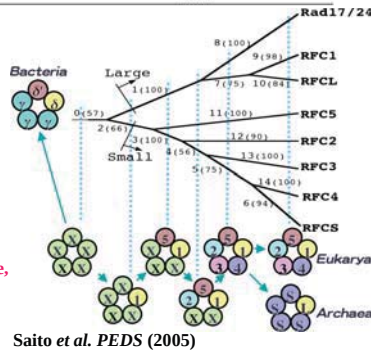
h = moderately hydrophobic residues (e.g. L, I, M);
a = highly hydrophobic / aromatic residues (e.g. F, Y, W); x= any residue.

Biomolecular Engineering Research Institute, *Unpublished data*

(2) Alternative clamp loader/clamp systems

Function	① Replication	② Repair	③ Sister chromatid segregation
Clamp loader	RFC1 RFC2-5	Rad17 RFC2-5	Ctf18 RFC2-5
Clamp	PCNA x3	Rad9 Rad1 Hus1	?



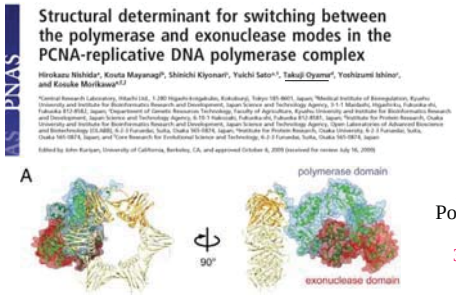


Archaeal RFC is not a preserved ancestral type, but a degenerated version of eukaryotic RFC.

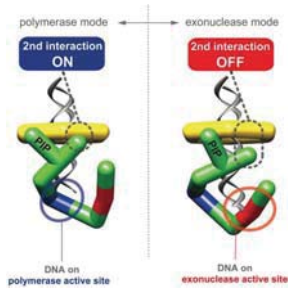
Saito *et al. PEDS* (2005)

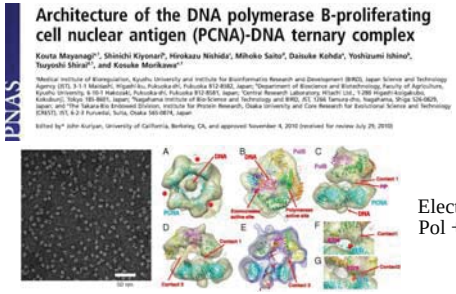
Archaeal DNA polymerases in complex with PCNA and DNA

Pyroccus furiosus DNA polymerase B

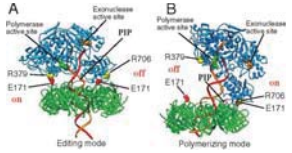


X-ray 2.6Å
Pol + 1/3 PCNA
↓
3mer modeling





Electron microscopy
Pol + PCNA + DNA



Okazakisome
(FEN-PCNA-DNA, FEN-LIG-PCNA-DNA)

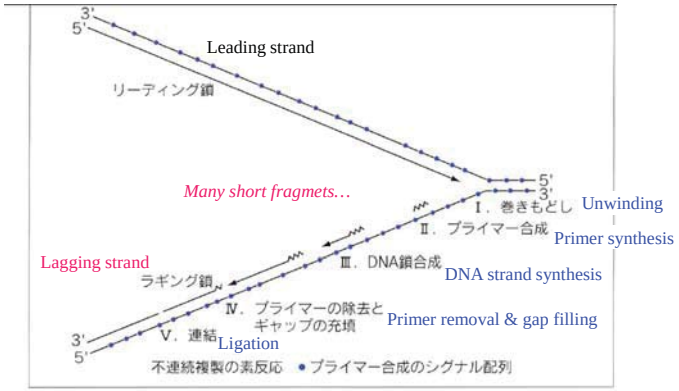
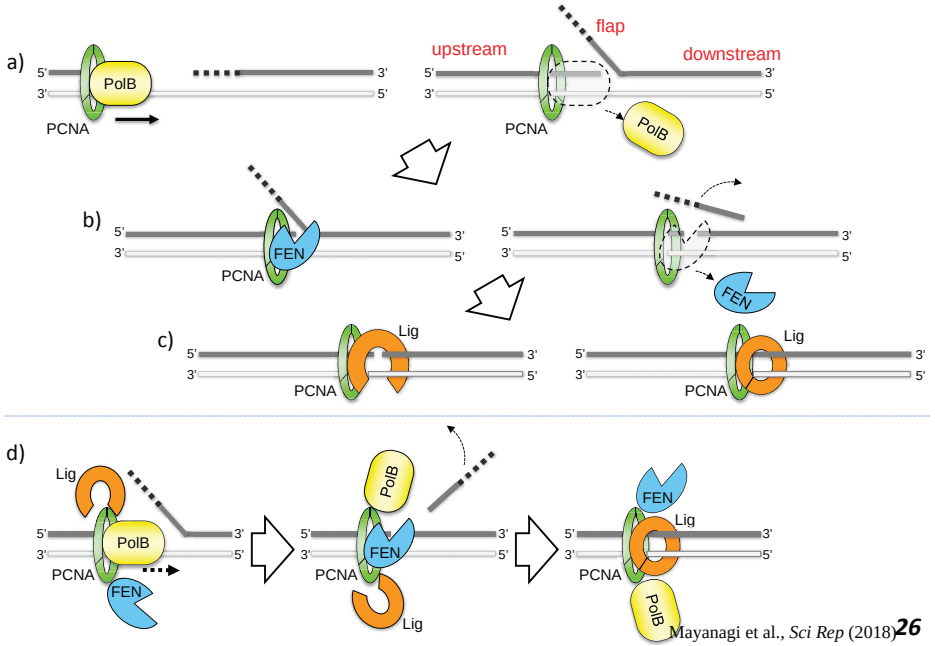


図 16　不連続複製の素反応と片鎖不連続複製モデル
●はプライマー合成のシグナルで，鋳型上に存在する多数のシグナルからフォークの進行に合わせて適当なものがプライマー合成の開始部位に使われる．プライマー RNA を使って DNA が合成され，プライマーが除去されて，ギャップが埋められ，成熟した岡崎フラグメントは DNA ライゲースによりつながれる．

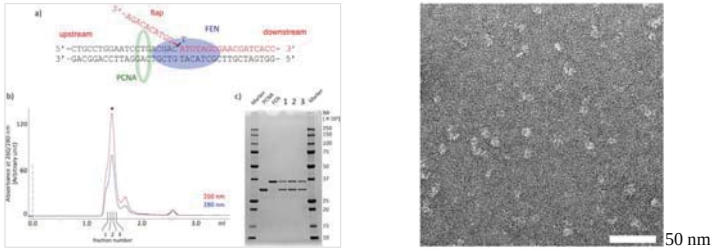
T. Okazaki, *Tanpakushistu-Kakusan-Kouso* (2002)

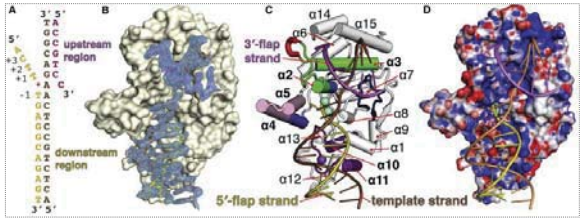
Sequential model? Too-belt model? or another?



Mayanagi et al., *Sci Rep* (2018)

EM structure of FEN-PCNA-DNA

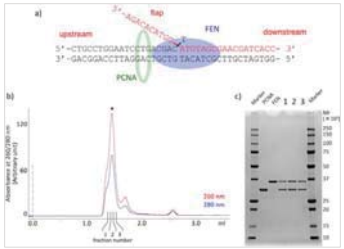


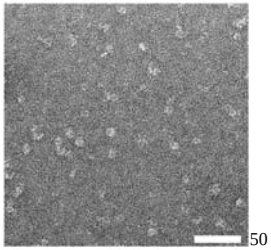


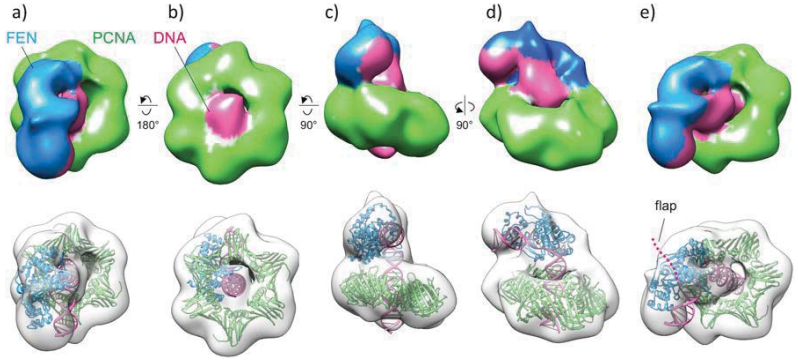
***P. Furiosus* FEN-1**
Hwang et al., *Nat. Struct. Biol.* (1998)

Human FEN1-DNA complex
Tsutakawa et al., *Cell.* (2011)

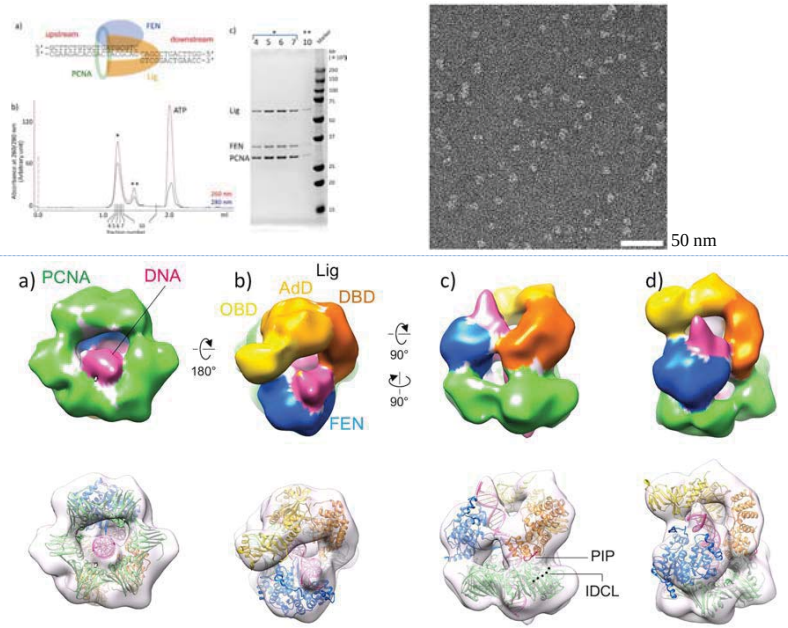
EM structure of FEN-PCNA-DNA



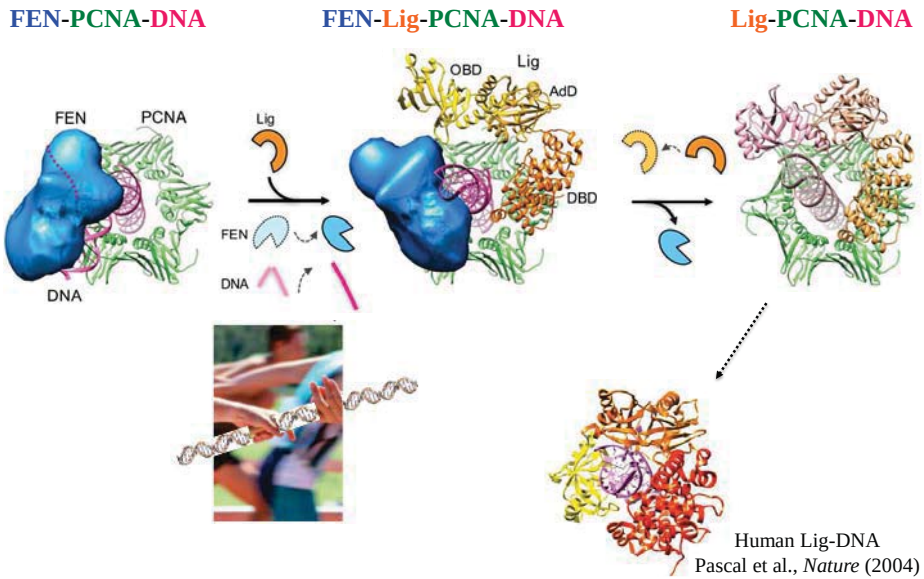




EM structure of FEN-Lig-PCNA-DNA



The DNA button pass, handing-over mechanism



Summary

We have studied archaeal Replisome (DNA replication machinery) by the combined approach of structural biology with X-ray crystallography and electron microscopy, molecular biology, and bioinformatics.

1. At the initial stage, CMG unwinds the template DNA for the new DNA strand synthesis. We determined the crystal structure of MCM activator GINS and GAN. GAN might be an evolutional intermediate from RecJ to Cdc45.
2. At the middle stage, PCNA acts as a universal activator for the DNA transacting enzymes such as the replicative DNA polymerase, and we proposed a clamp-loading mechanism based on the electron microscopy RFC-PCNA-DNA ternary complex.
3. At the late stage, Okazaki fragment maturation is essential in particular on the lagging strand. We determined EM FEN-PCNA-DNA and FEN-Lig-PCNA-DNA complexes and proposed a new “DNA button handing over mechanism” from FEN to Lig on PCNA.