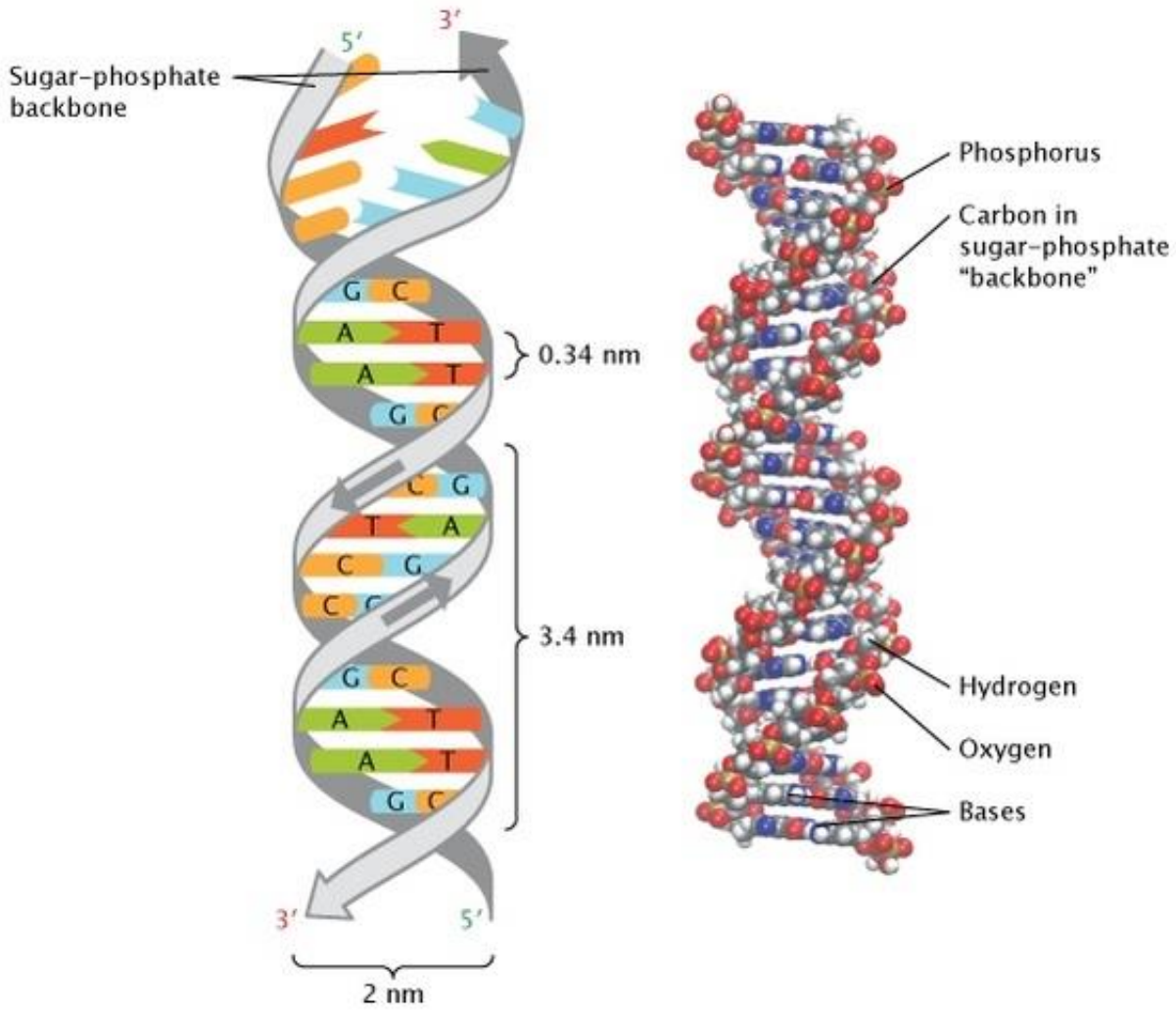
DNA Structure, Analysis & Replication

Dirk Remus

Molecular Biology Program

DNA Double Helix / B-Form DNA



First Proposed Based On Modeling



Francis Crick James Watson



No. 4356 April 25, 1953 NATURE 737

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).

[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).

[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1956).

[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the *z*-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical *z*-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J.D.W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. Watson
F. H. C. Crick

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems,
Cavendish Laboratory, Cambridge.
April 2.

[1] Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, **39**, 84 (1953).

[2] Furberg, S., *Acta Chem. Scand.*, **6**, 634 (1952).

[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).

[4] Wyatt, G. R., *J. Gen. Physiol.*, **36**, 201 (1952).

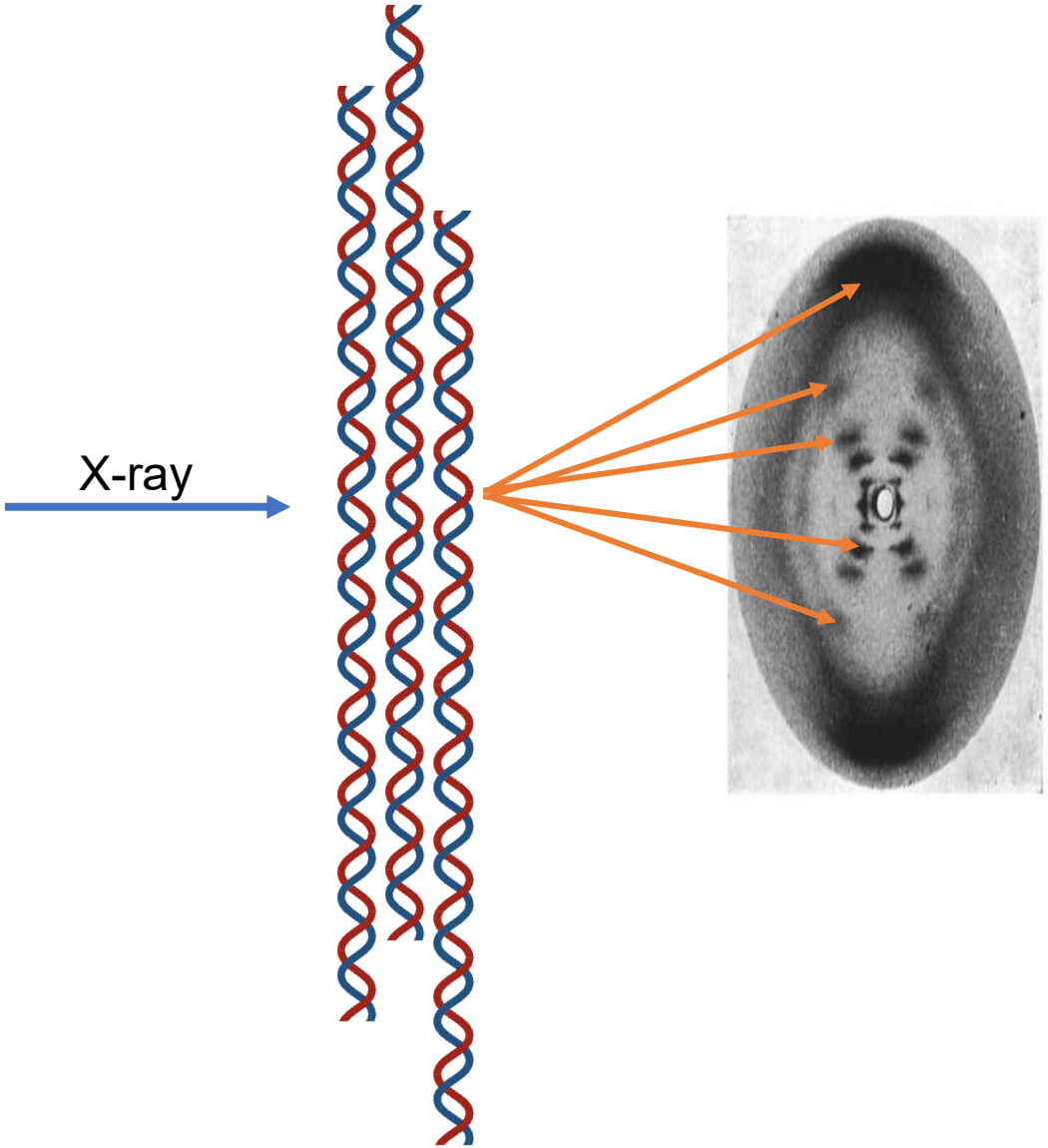
[5] Astbury, W. T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947).

[6] Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).

Experimental Evidence From X-Ray DNA Fiber Diffraction



Rosalind Franklin



Experimental Evidence From X-Ray DNA Fiber Diffraction



Rosalind Franklin

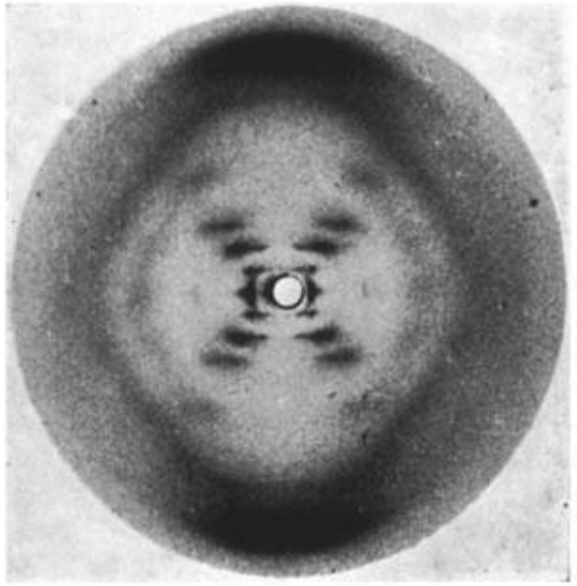


Photo 51

Franklin & Gosling, Nature (1953)

Experimental Evidence From X-Ray DNA Fiber Diffraction



Rosalind Franklin

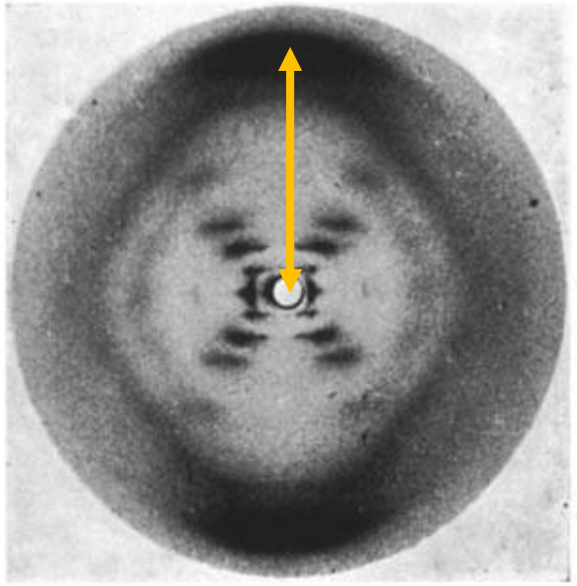
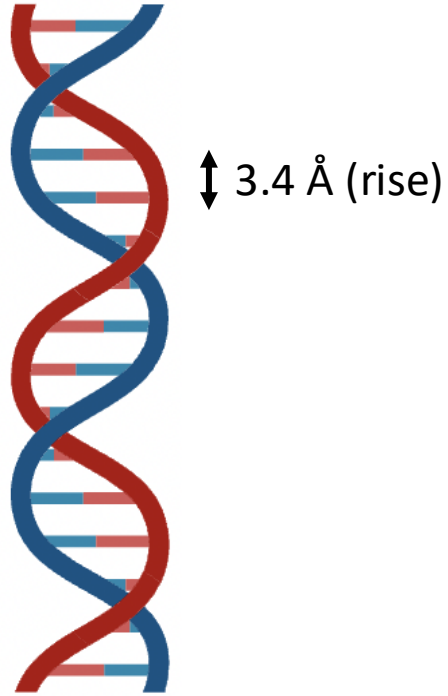


Photo 51

Franklin & Gosling, Nature (1953)



Experimental Evidence From X-Ray DNA Fiber Diffraction



Rosalind Franklin

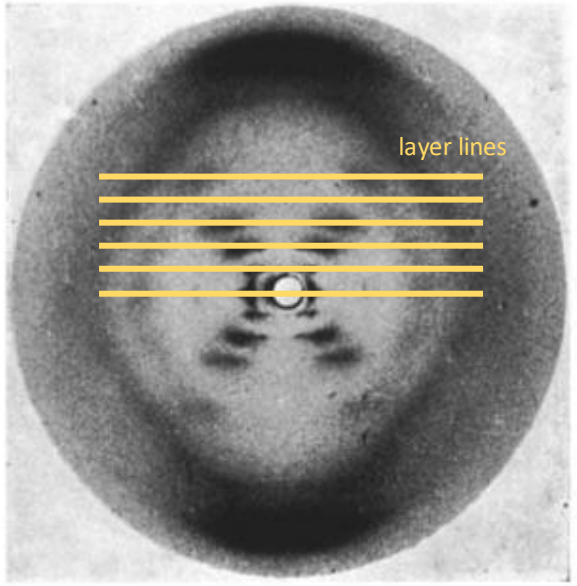
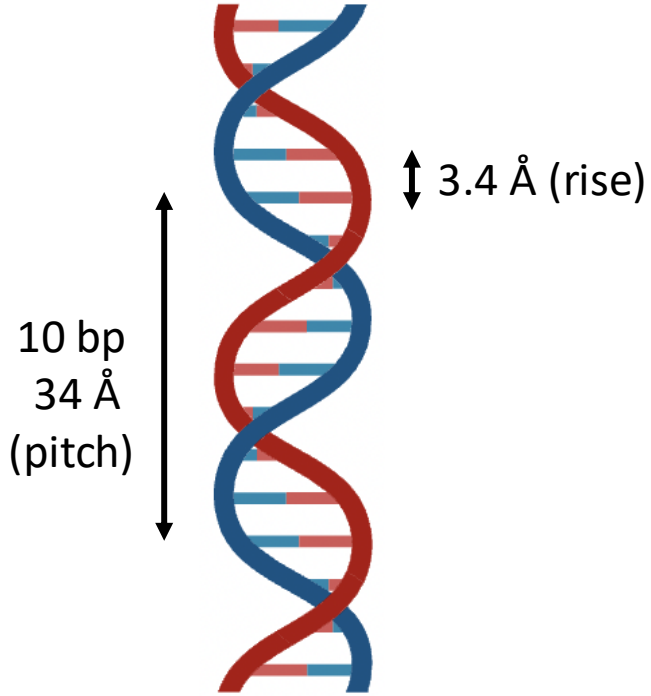


Photo 51

Franklin & Gosling, Nature (1953)



Experimental Evidence From X-Ray DNA Fiber Diffraction



Rosalind Franklin

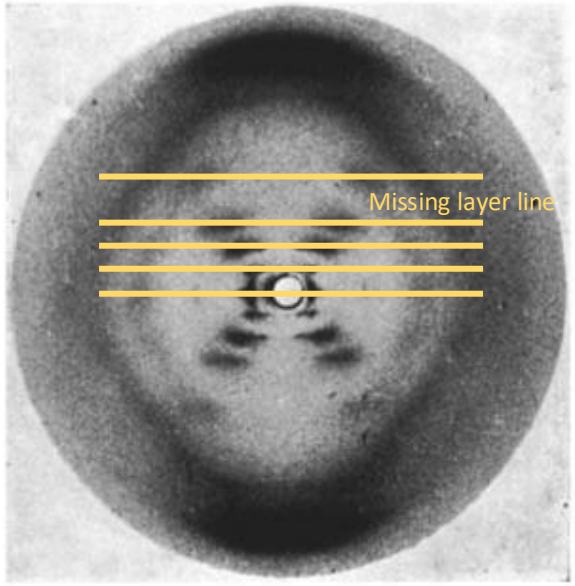
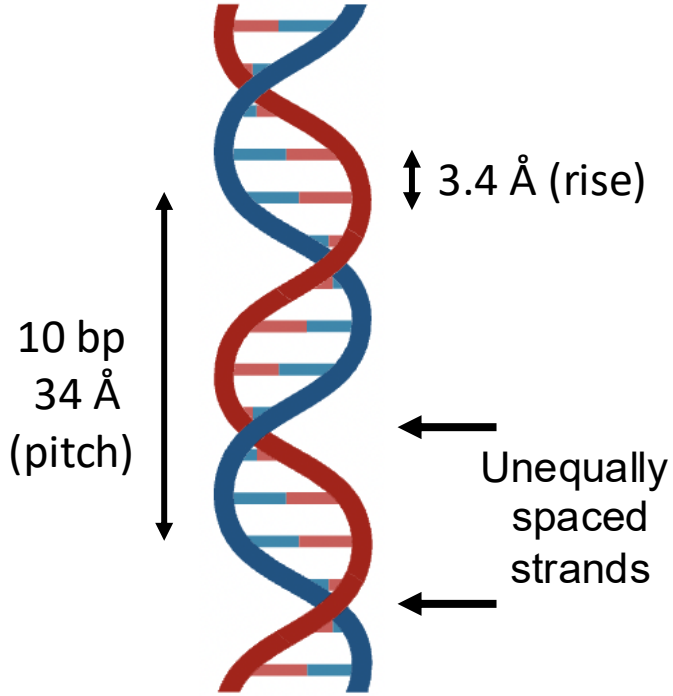


Photo 51

Franklin & Gosling, Nature (1953)



Experimental Evidence From X-Ray DNA Fiber Diffraction



Rosalind Franklin

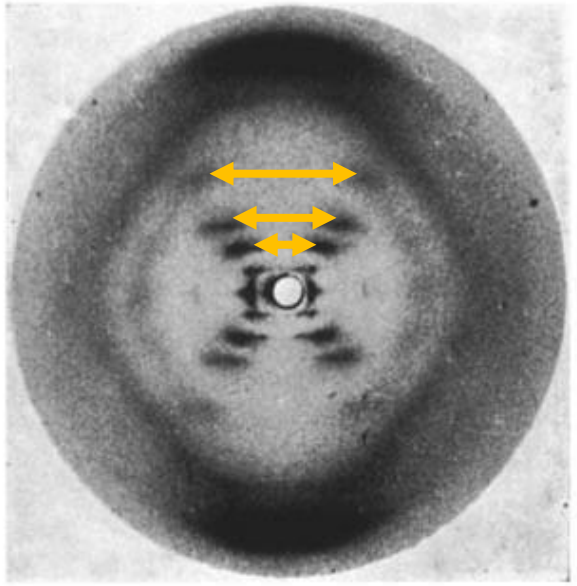
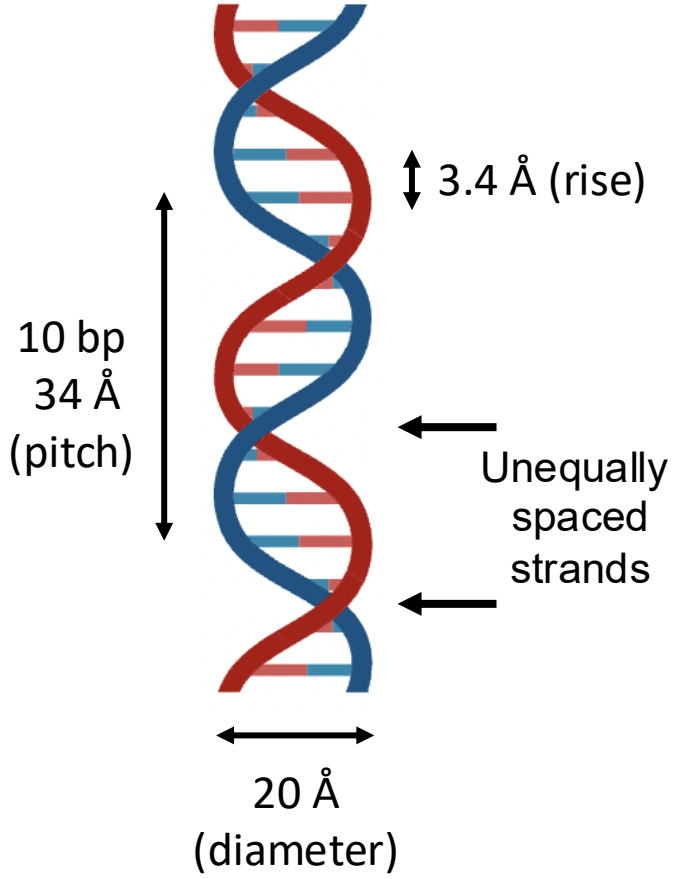


Photo 51

Franklin & Gosling, Nature (1953)



Experimental Evidence From X-Ray DNA Fiber Diffraction



Rosalind Franklin

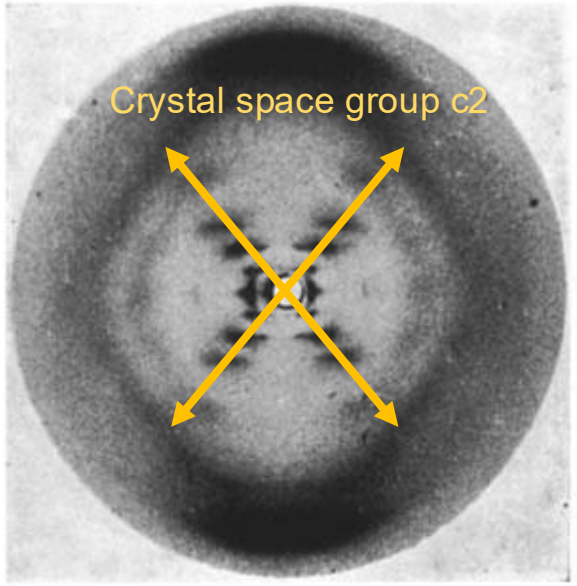
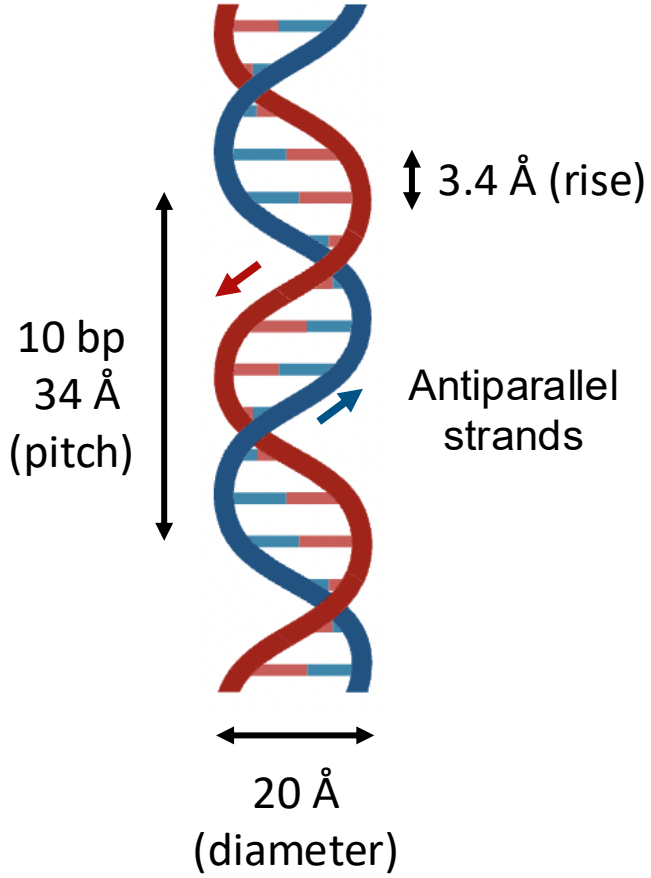
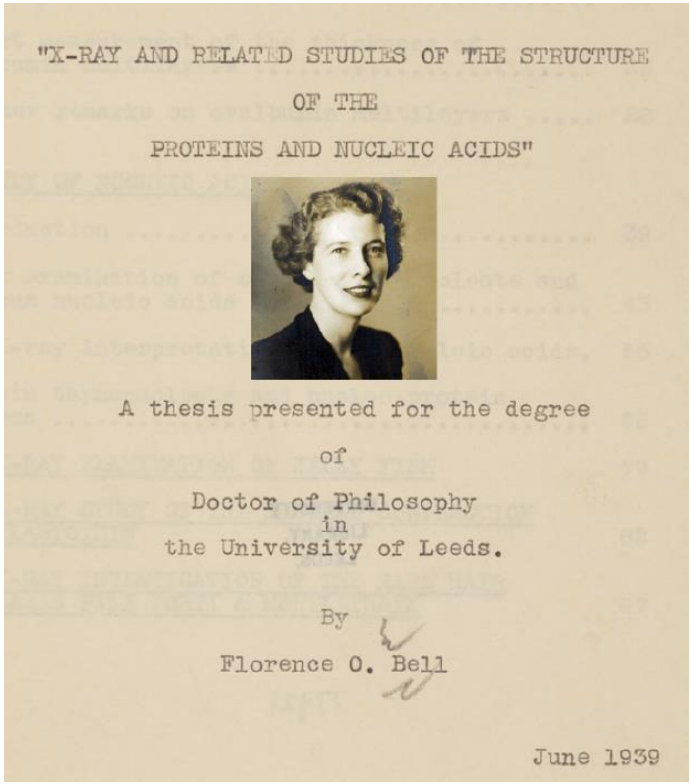


Photo 51

Franklin & Gosling, Nature (1953)



Experimental Evidence From X-Ray DNA Fiber Diffraction

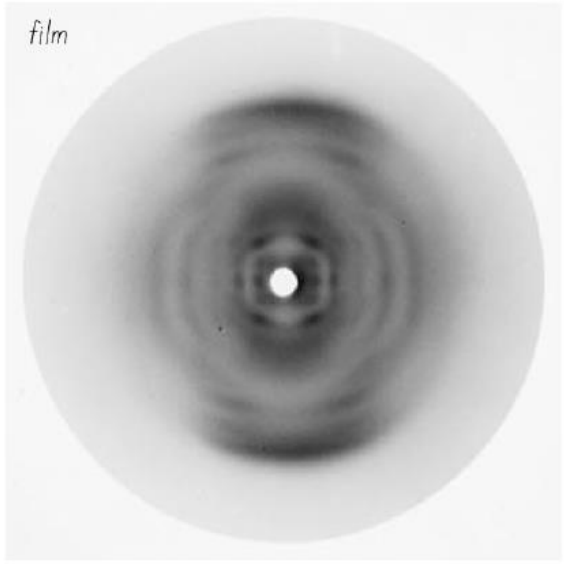


"X-RAY AND RELATED STUDIES OF THE STRUCTURE
OF THE
PROTEINS AND NUCLEIC ACIDS"

A thesis presented for the degree
of
Doctor of Philosophy
in
the University of Leeds.

By
Florence O. Bell

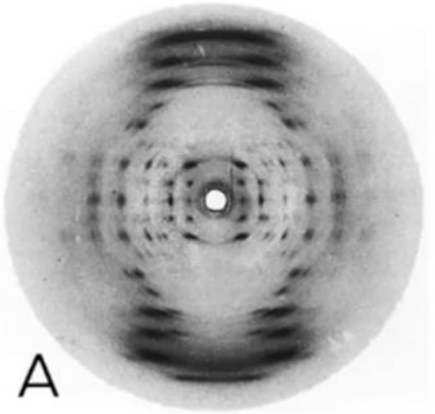
June 1939



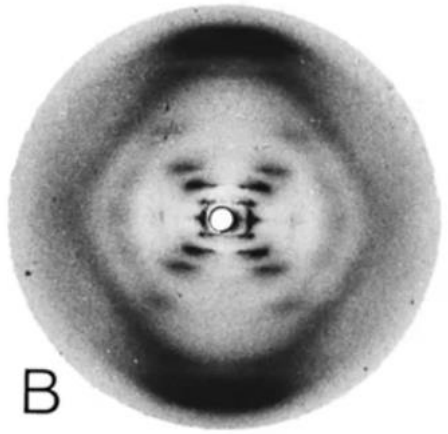
Two Forms of DNA



Rosalind Franklin

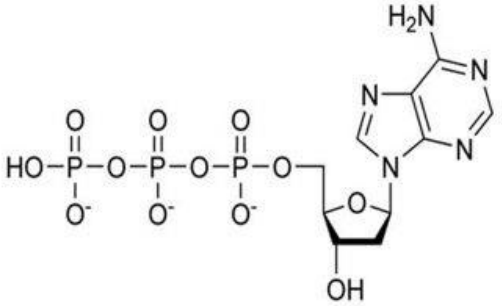


dehydrated form

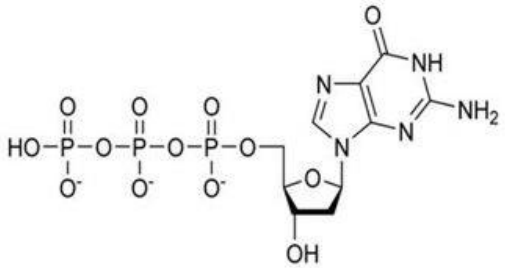


>75% humidity

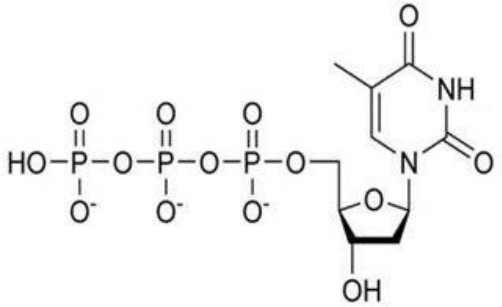
dNTPs: The Building Blocks of DNA



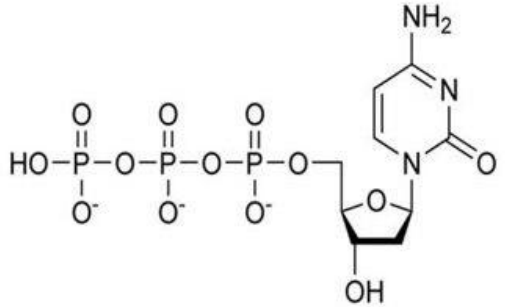
Deoxyadenosine triphosphate (dATP)



Deoxyguanosine_triphosphate
(dGTP)

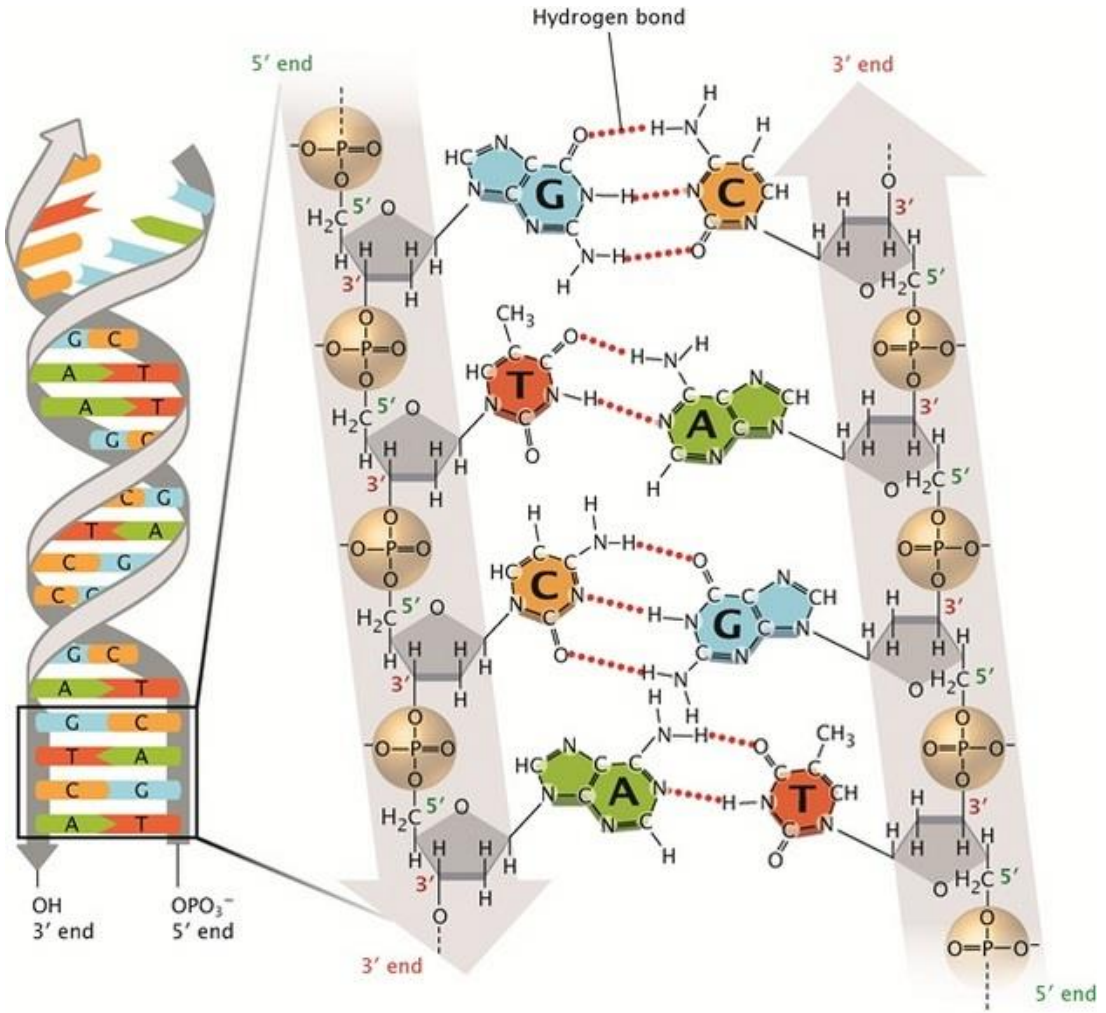


Deoxythymidine triphosphate (dTTP)

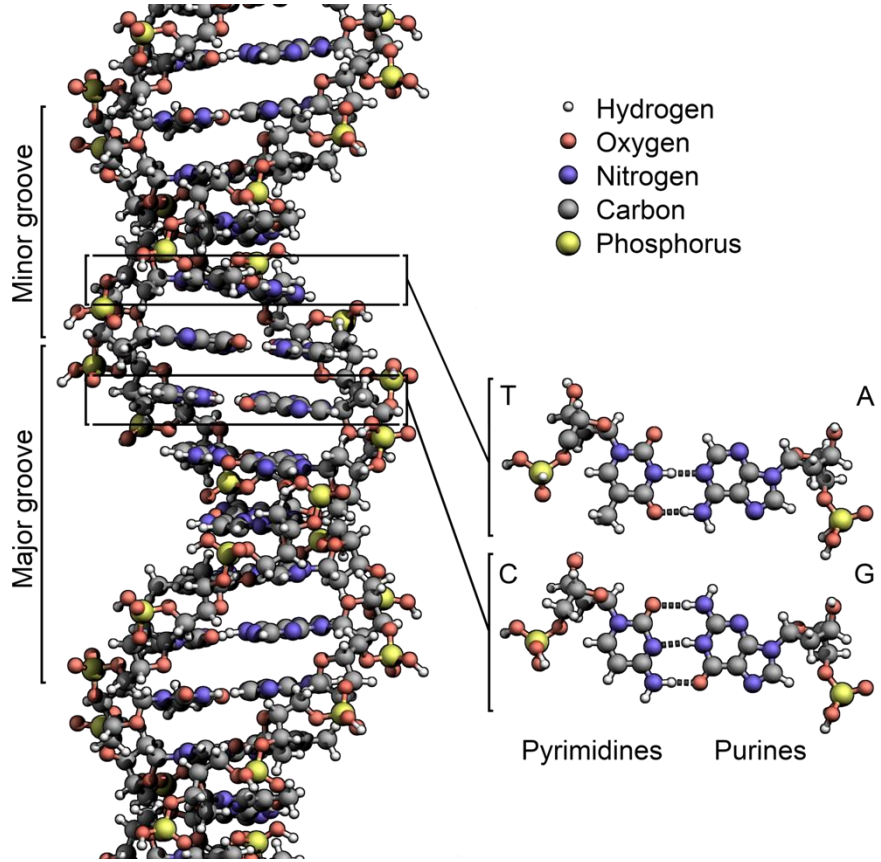


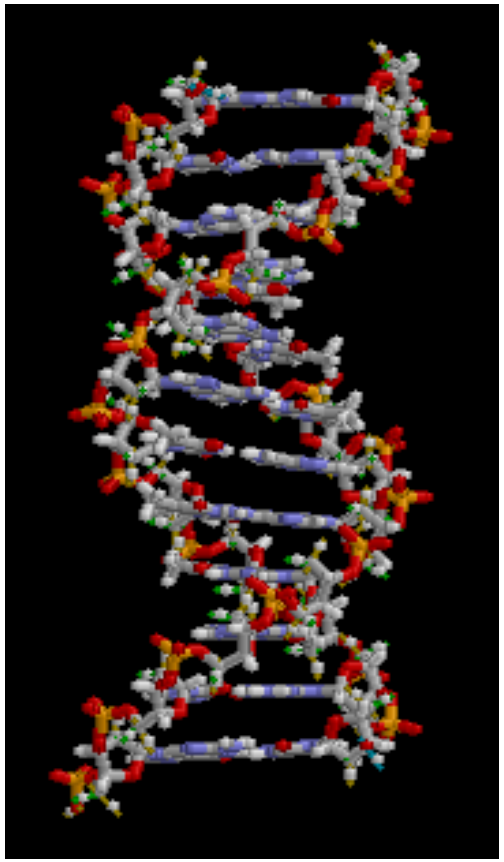
Deoxycytidine triphosphate
(dCTP)

Hydrogen Bonds Mediate DNA Pairing

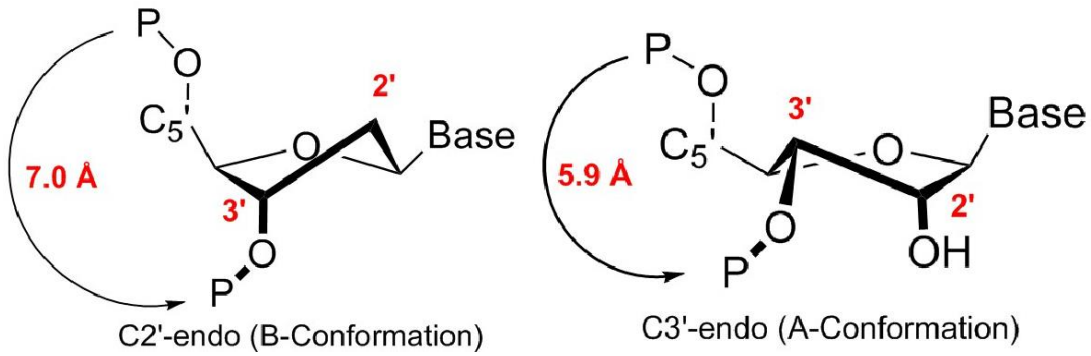


Basepair Geometry Establishes Minor/Major Grooves





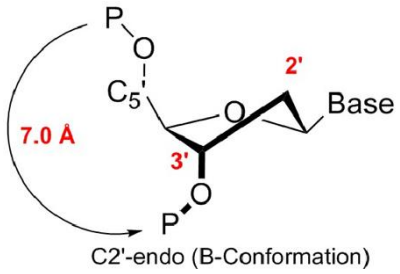
Deoxyribose Sugar Exists In Different Pucker Conformations

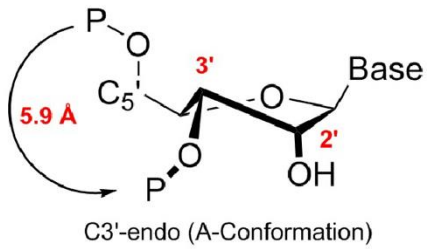


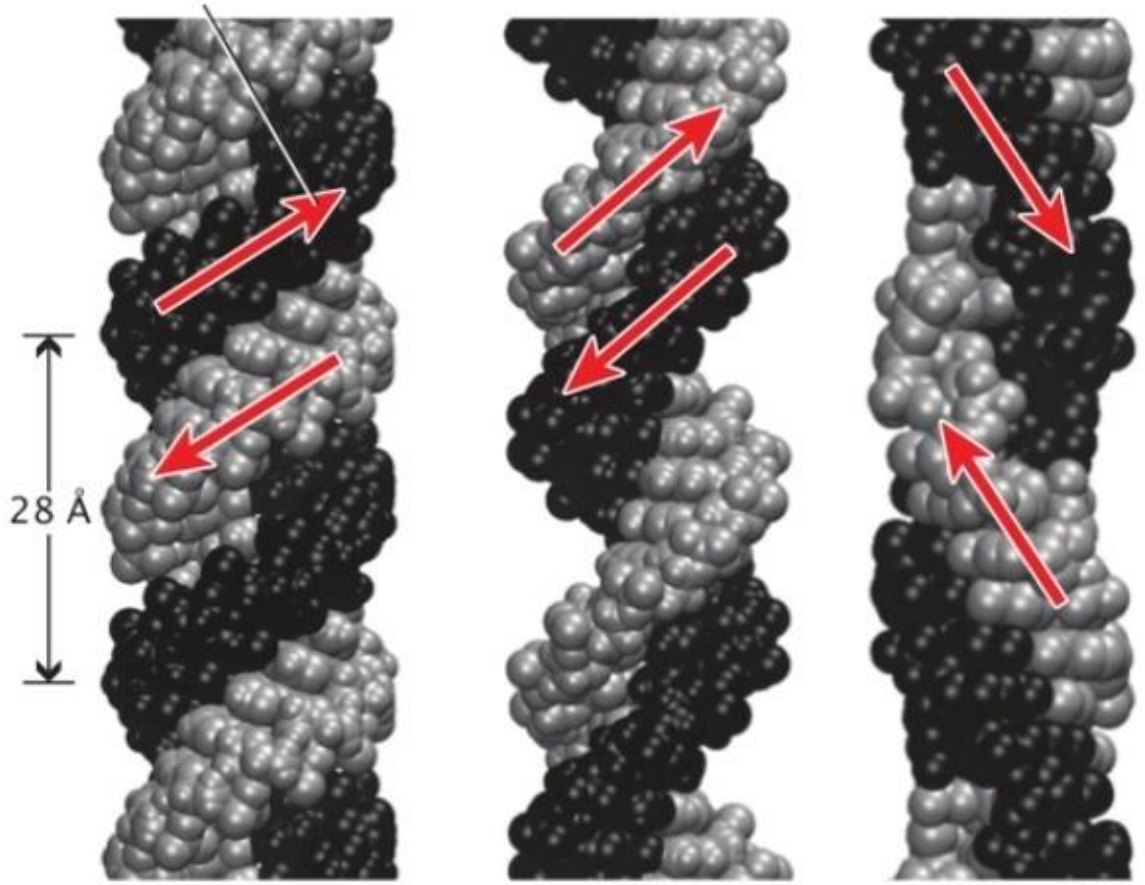
DNA

RNA

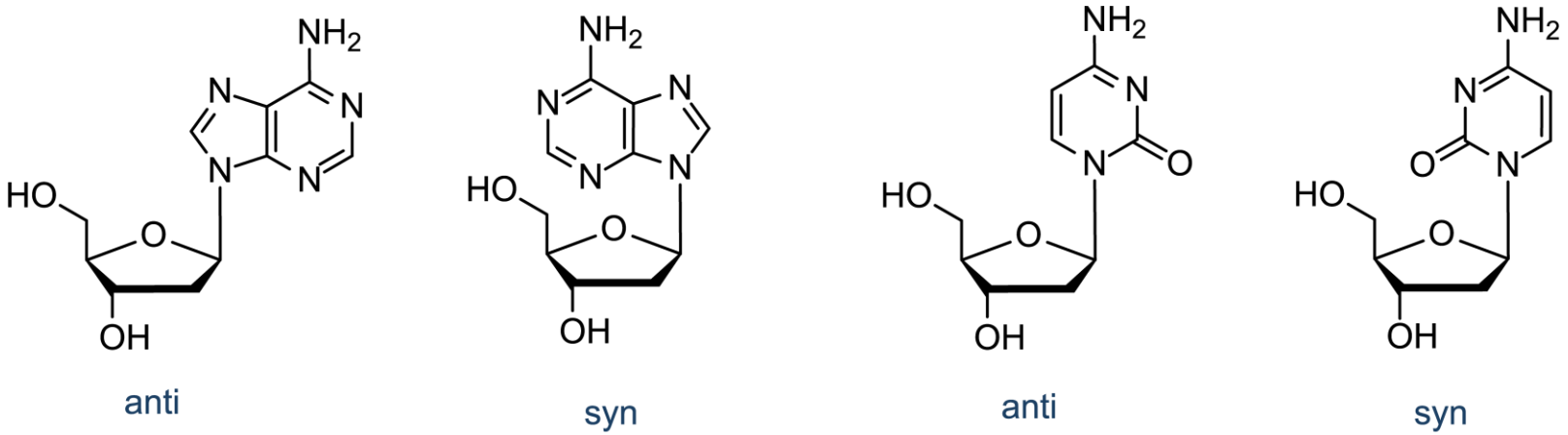
DNA Double Helix Conformations







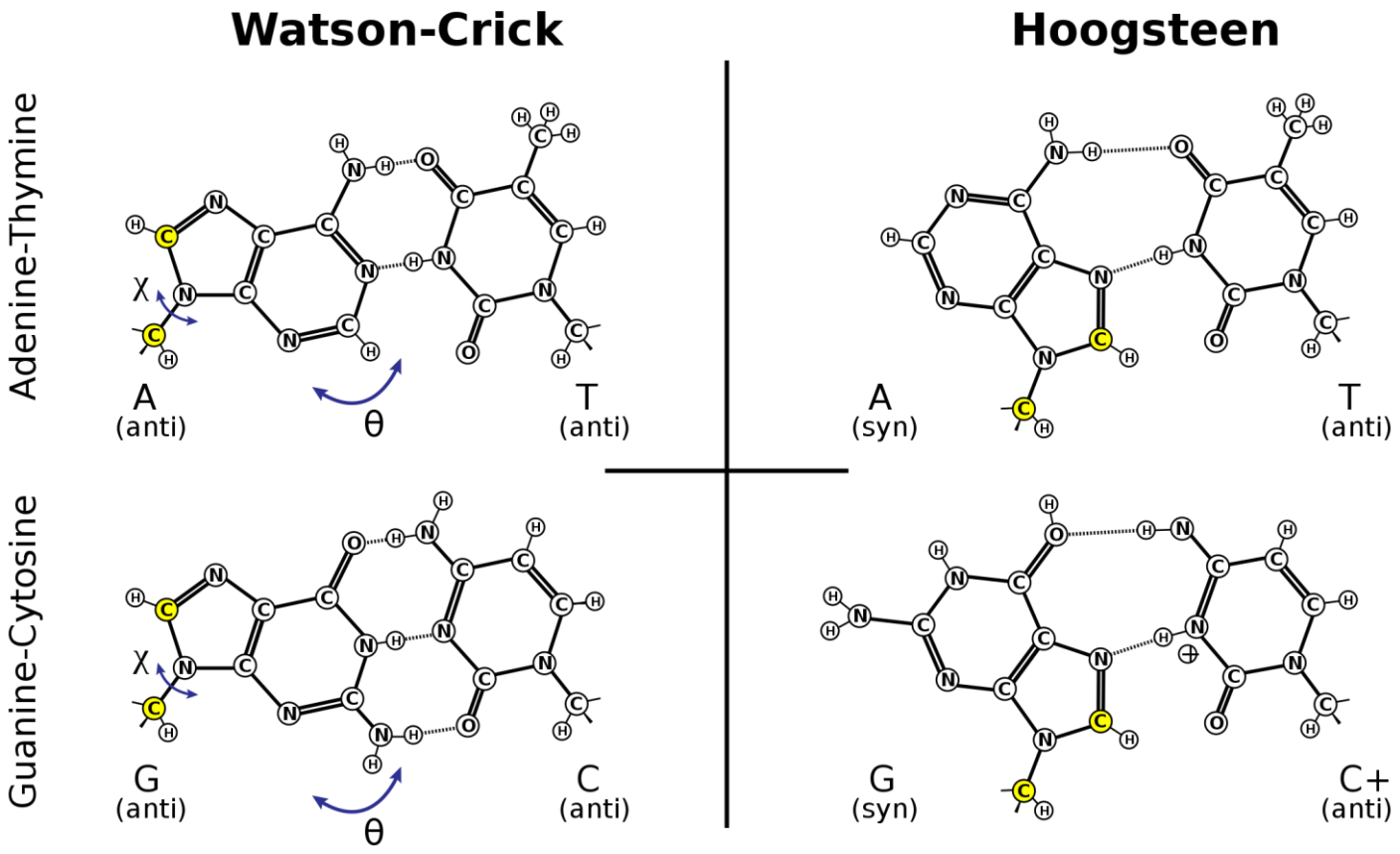
Bases Can Rotate Around The N-Glycosidic Bond



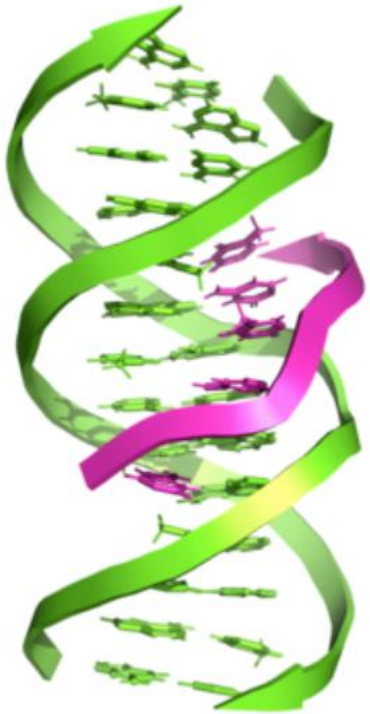
deoxyadenosine (a purine nucleoside)

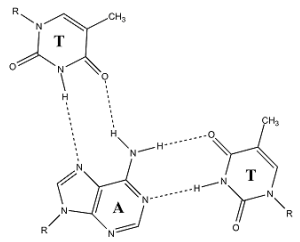
deoxycytidine (a pyrimidine nucleoside)

Alternative Hydrogen Bonds Can Mediate Base Pairing

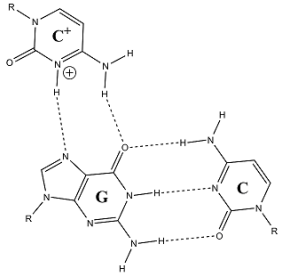


Alternative Hydrogen Bonding Occurs In Triplex DNA

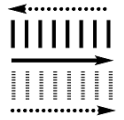




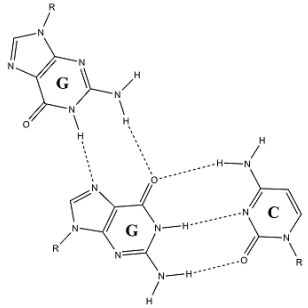
TA*T



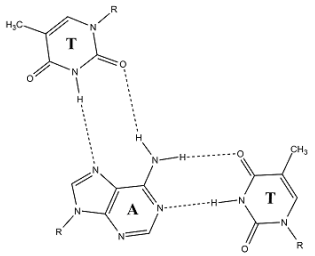
CG*C+



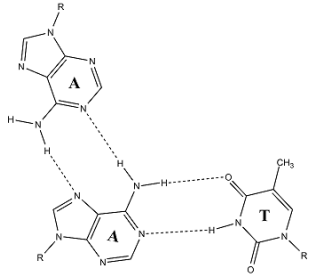
YR*Y



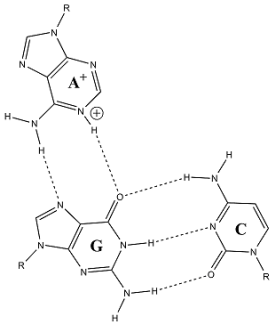
CG*G



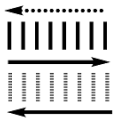
TA*A



TA*T



CG*A+

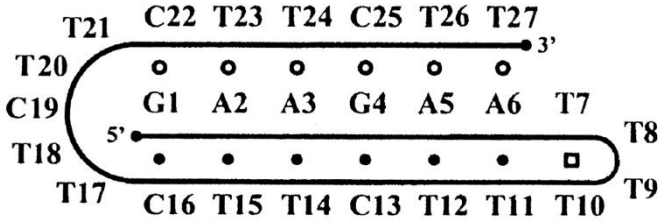


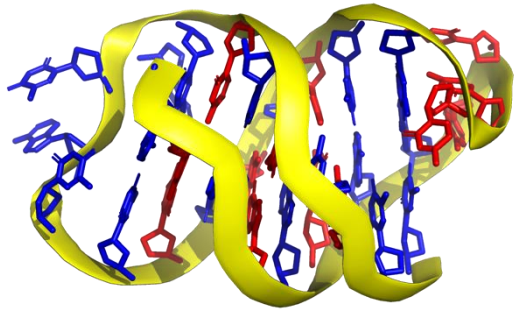
YR*R

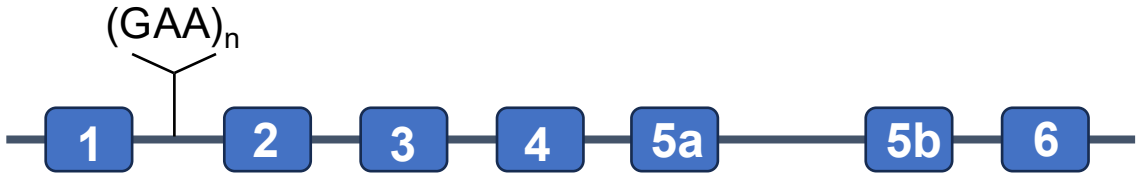
Trinucleotide Repeats Can Form Triplex Structures That Disrupt Gene Function

GAA
(Friedreich's Ataxia)



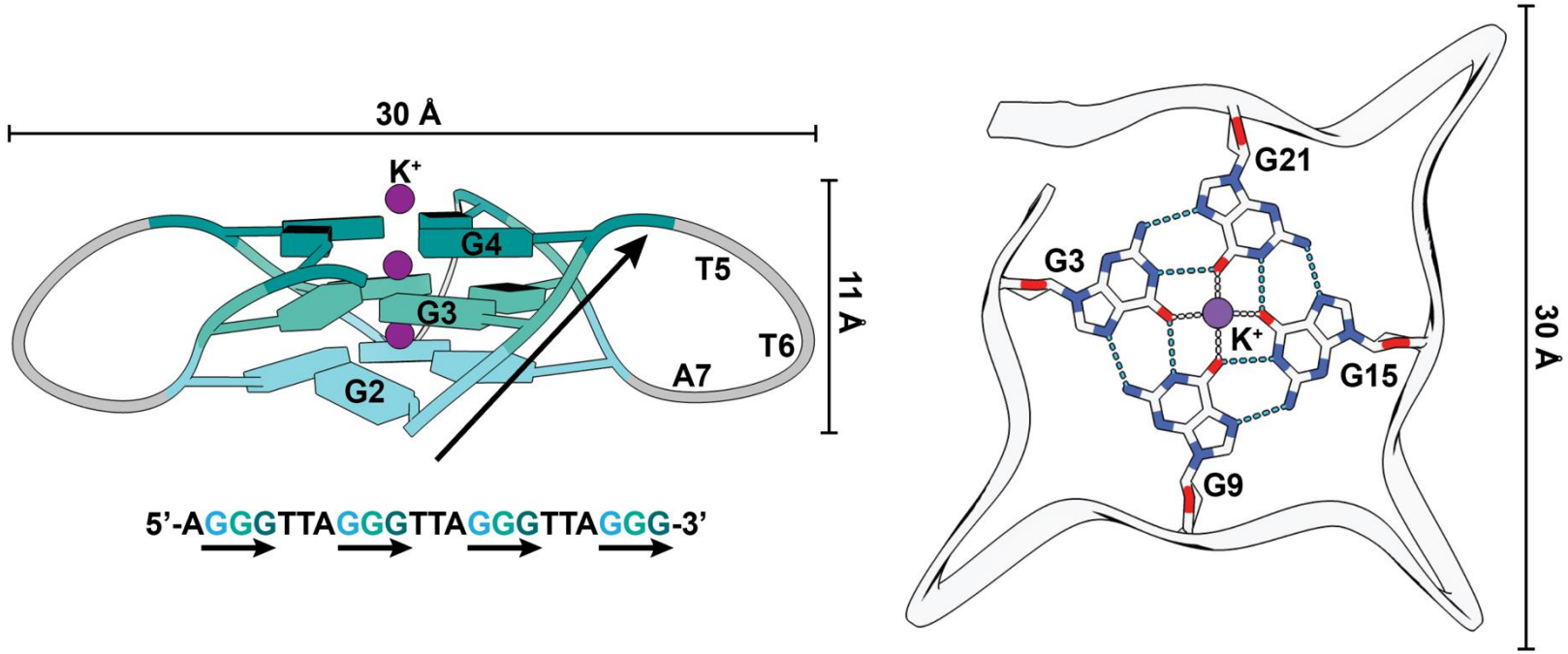






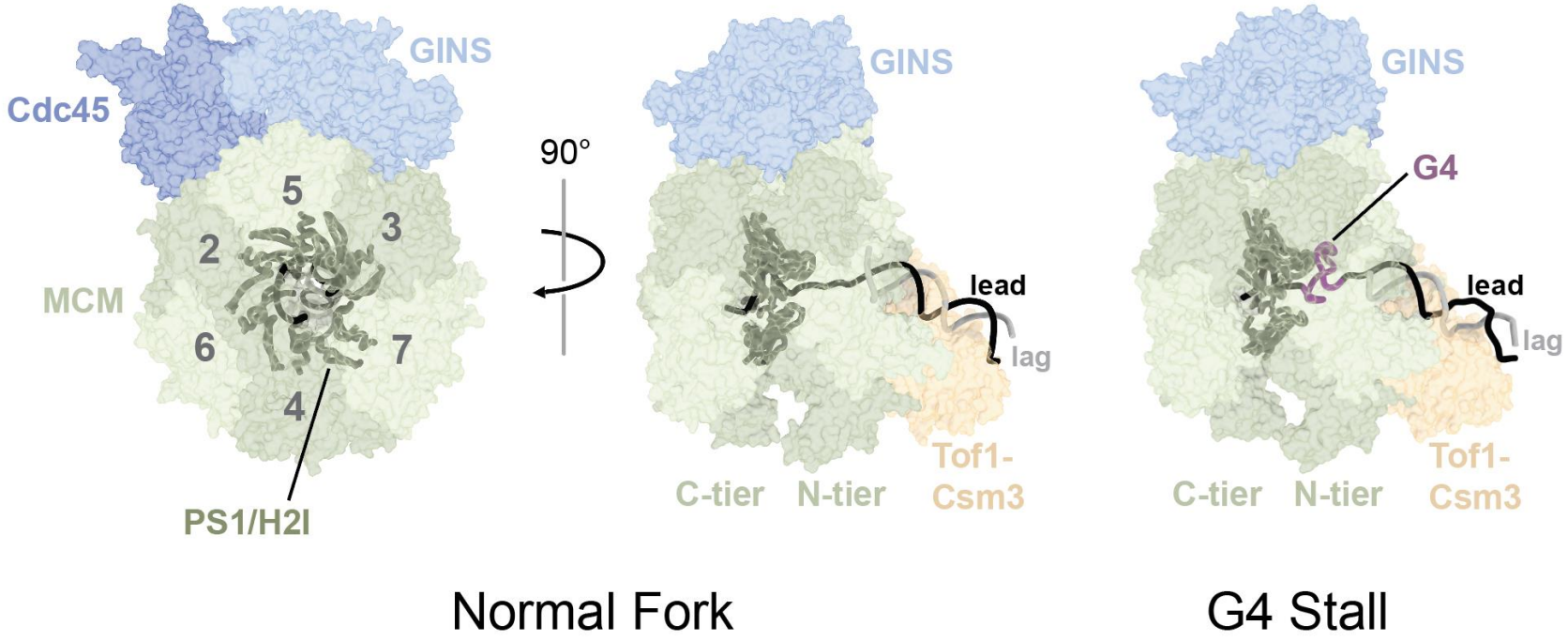
FXN (frataxin)

Alternative Hydrogen Bonds Promote G-Quadruplex Formation



G-Quadruplexes Can Stall The Replication Machinery

CMG Helicase
Stalled At Leading Strand
G-Quadruplex

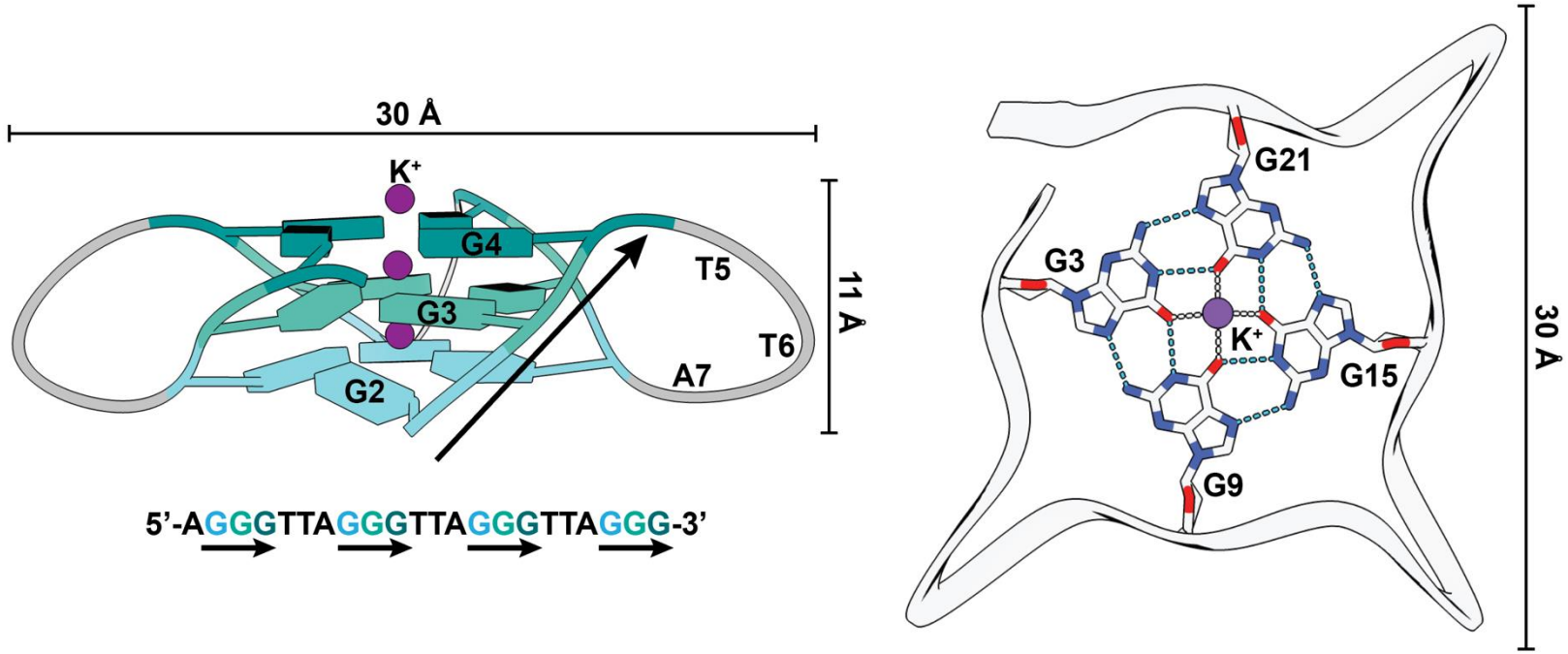


Normal Fork

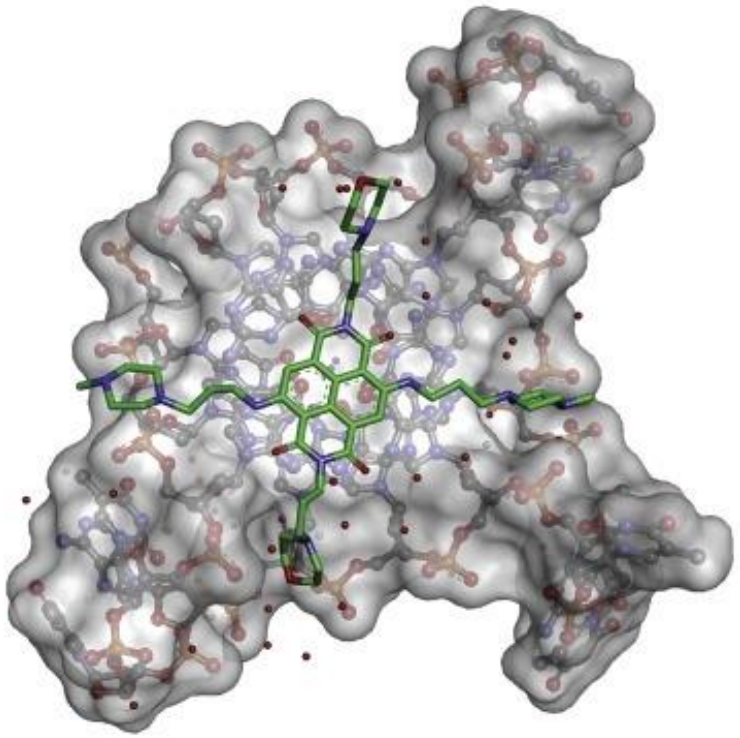
G4 Stall

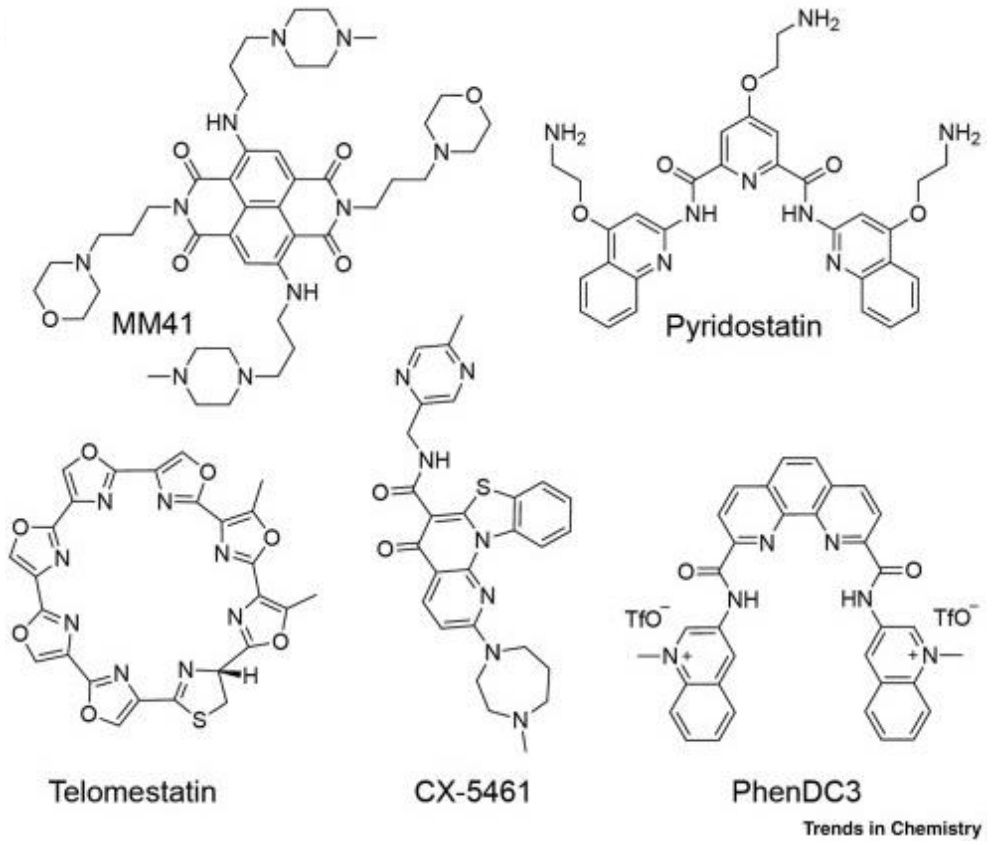
Batra S et al. (2025), PMID 40048517

How Can We Detect G-Quadruplexes In The Genome?

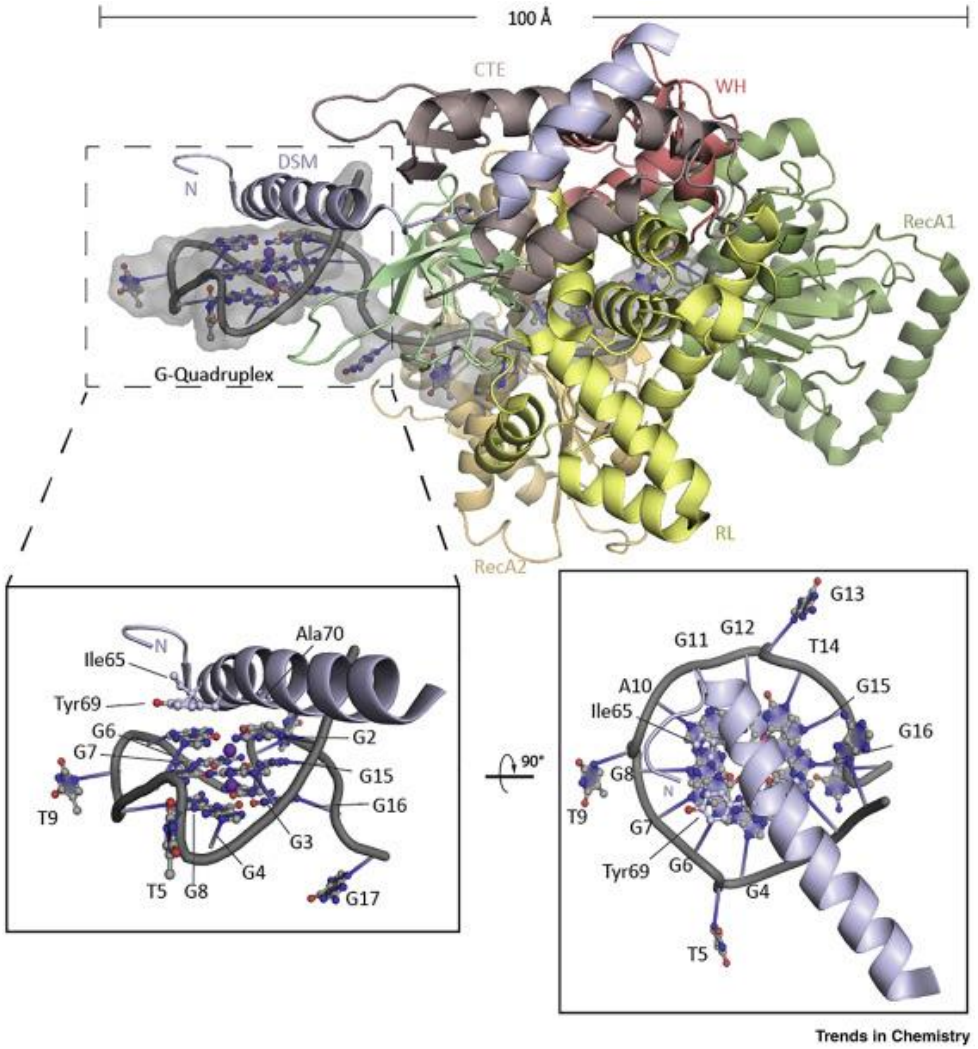


Chemical Ligands Stabilize and Detect Genomic G4s





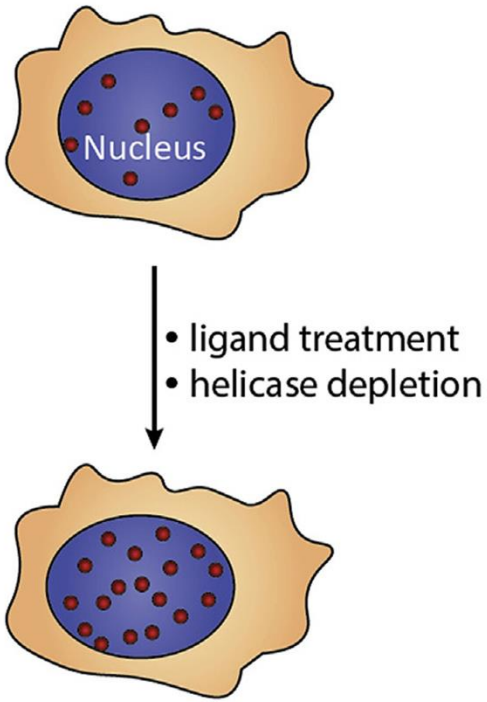
G4s Are Specifically Recognized By Proteins



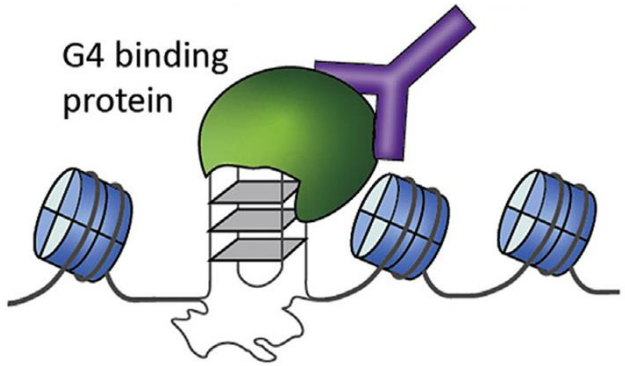
DHX36

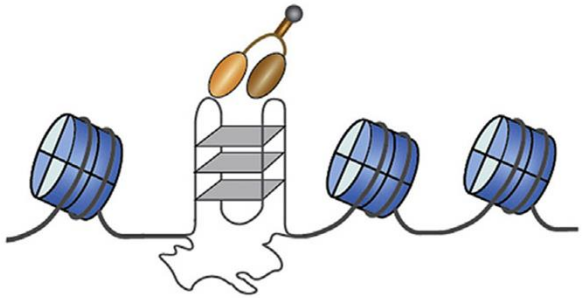
G4 Ligands To Detect Intracellular G4s

Fluorescence Microscopy

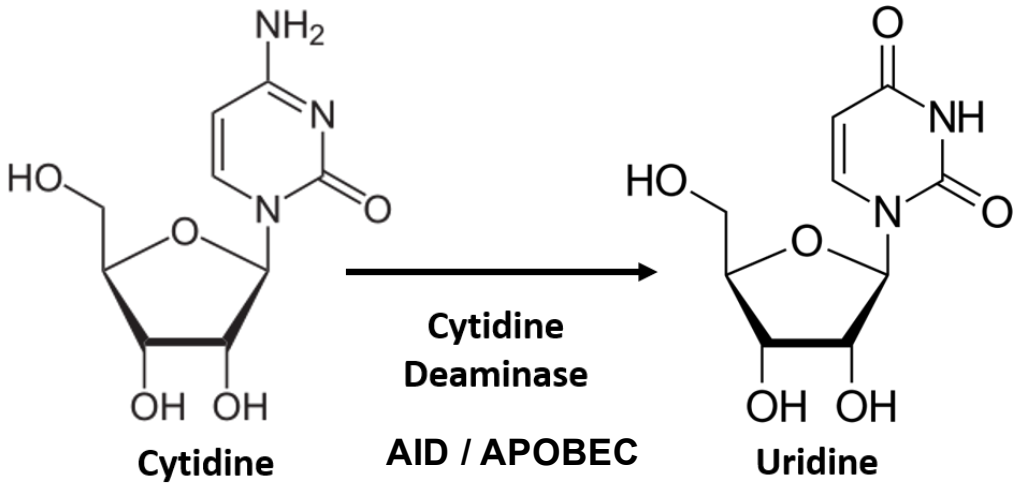


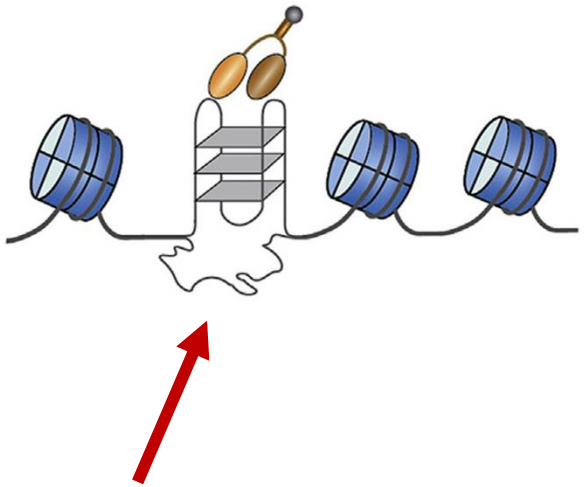
ChIP-seq





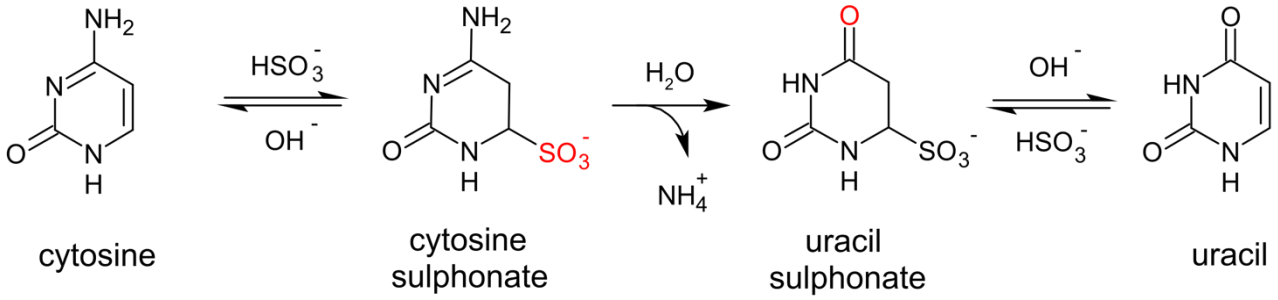
Cytosines in ssDNA Are Sensitive To Deamination

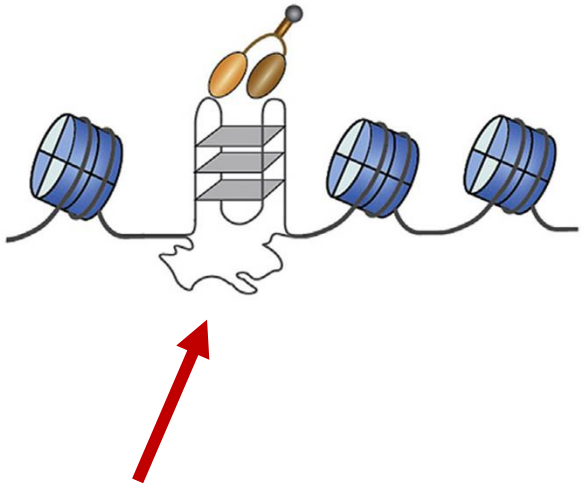




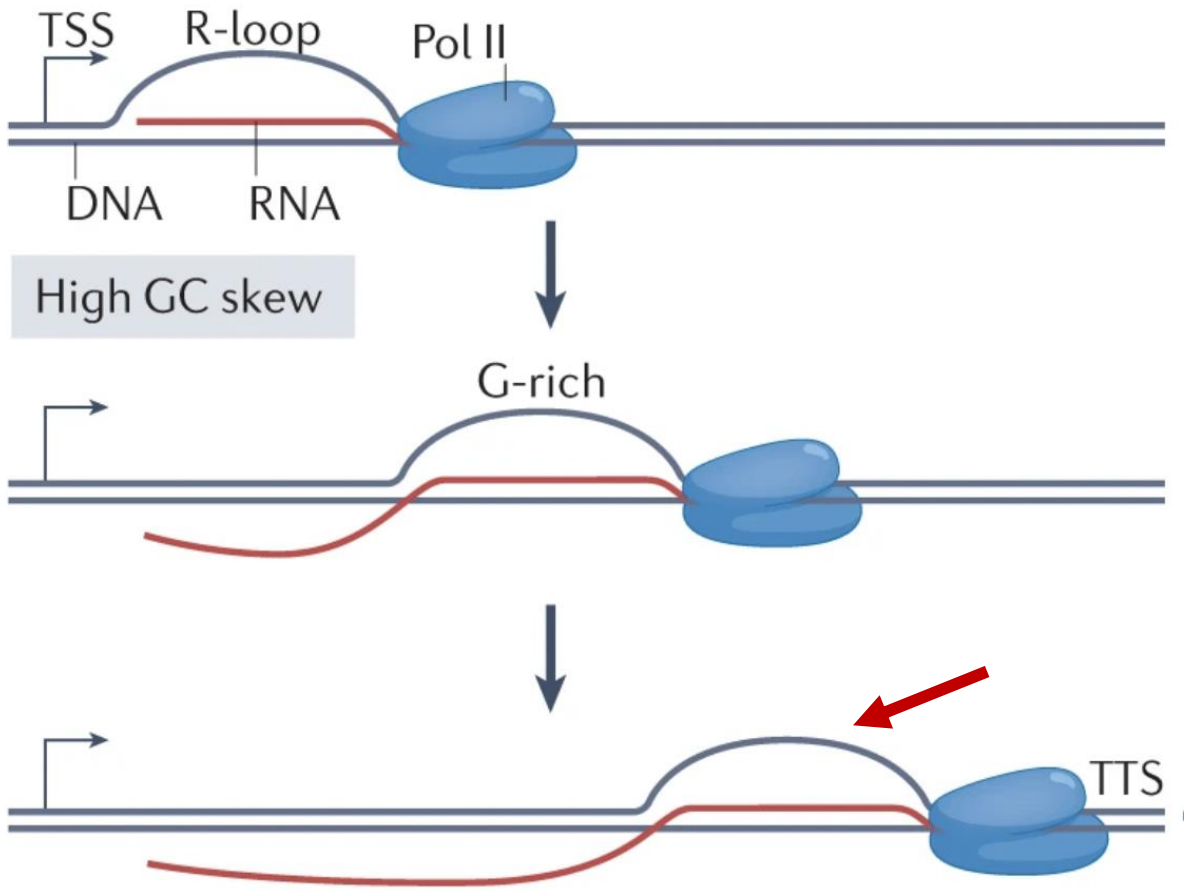
C to T transition: Mutational Signature 2
C to A/G transversion: Mutational Signature 13

ssDNA Opposite G4s Can Be Detected By Bisulfite Sequencing



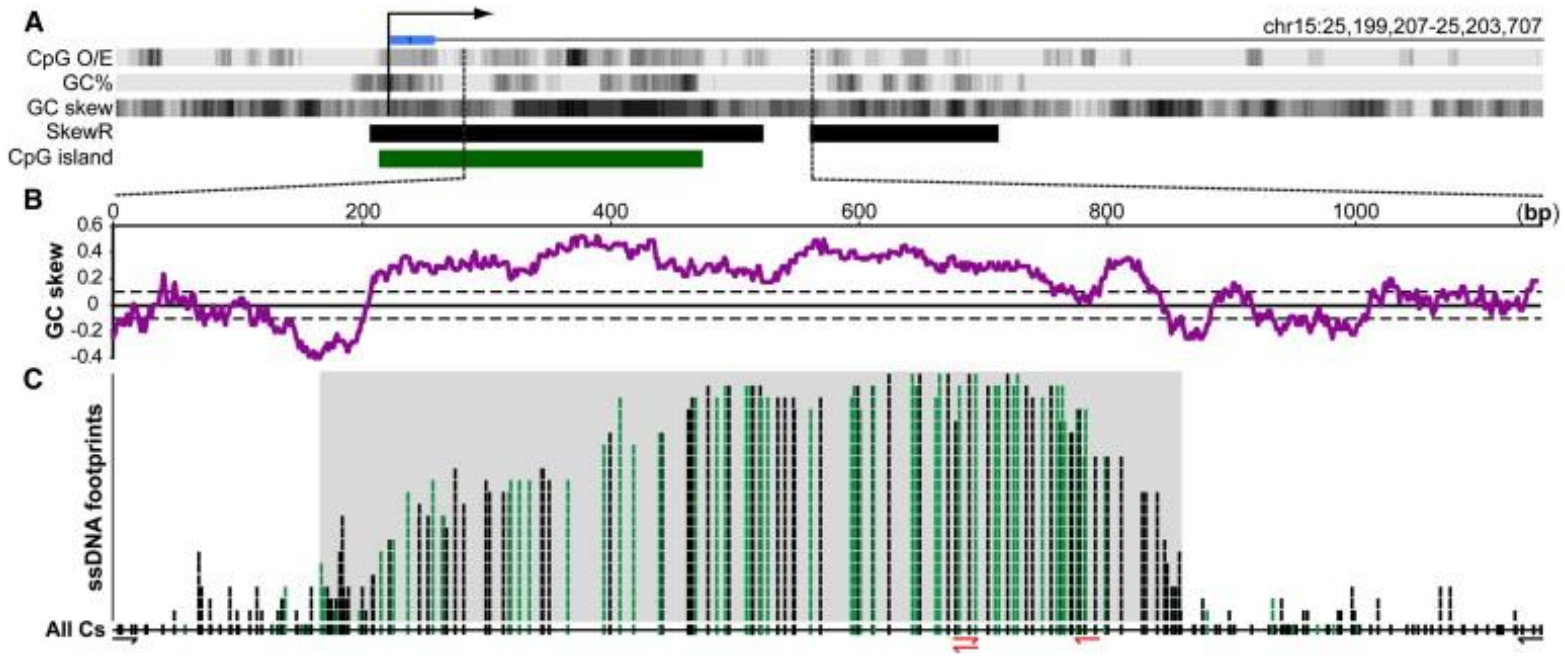


ssDNA Opposite RNA:DNA Hybrids At R-Loops Can Be Detected By Bisulfite Sequencing



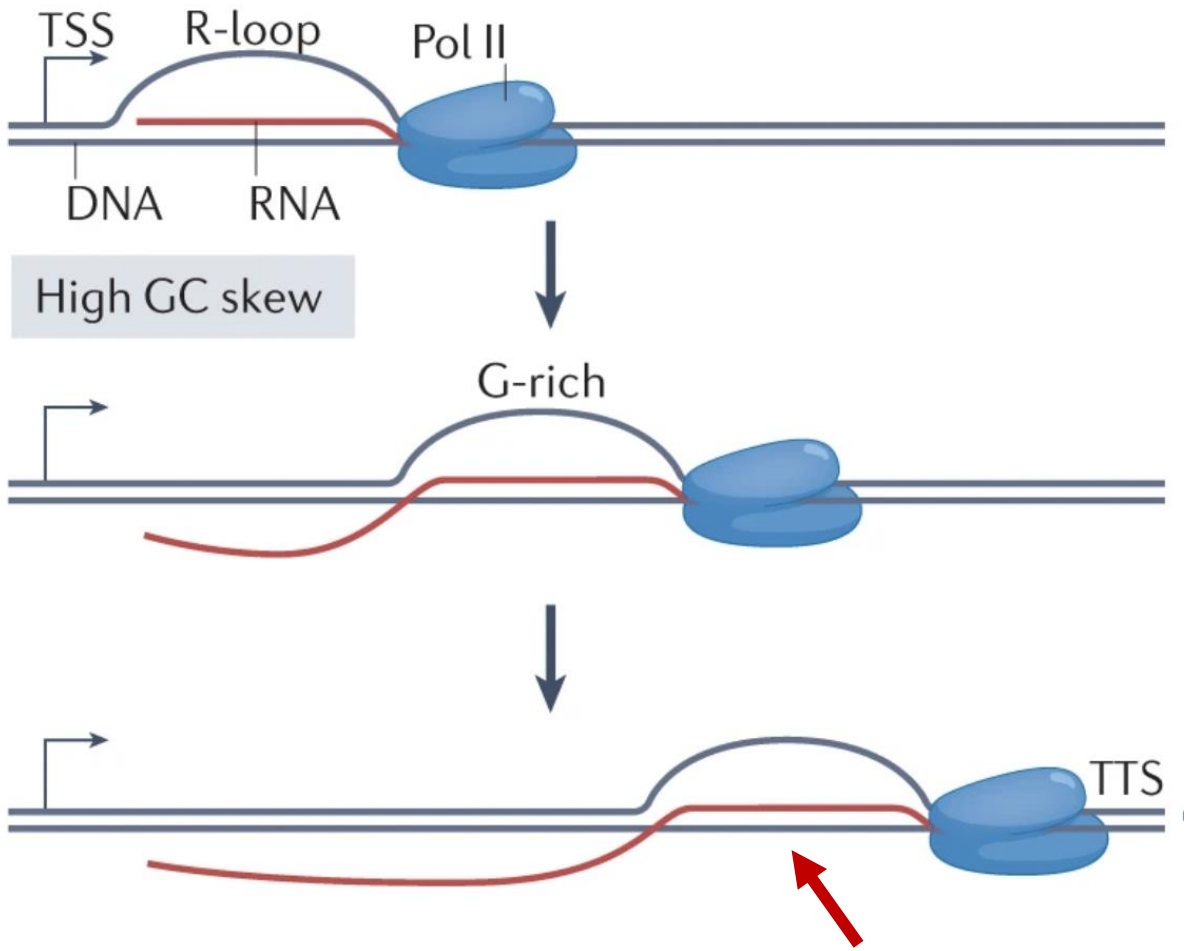
Petermann E et al., *Nat Rev Mol Cell Biol*, 2022

ssDNA Opposite RNA:DNA Hybrids At R-Loops Can Be Detected By Bisulfite Sequencing



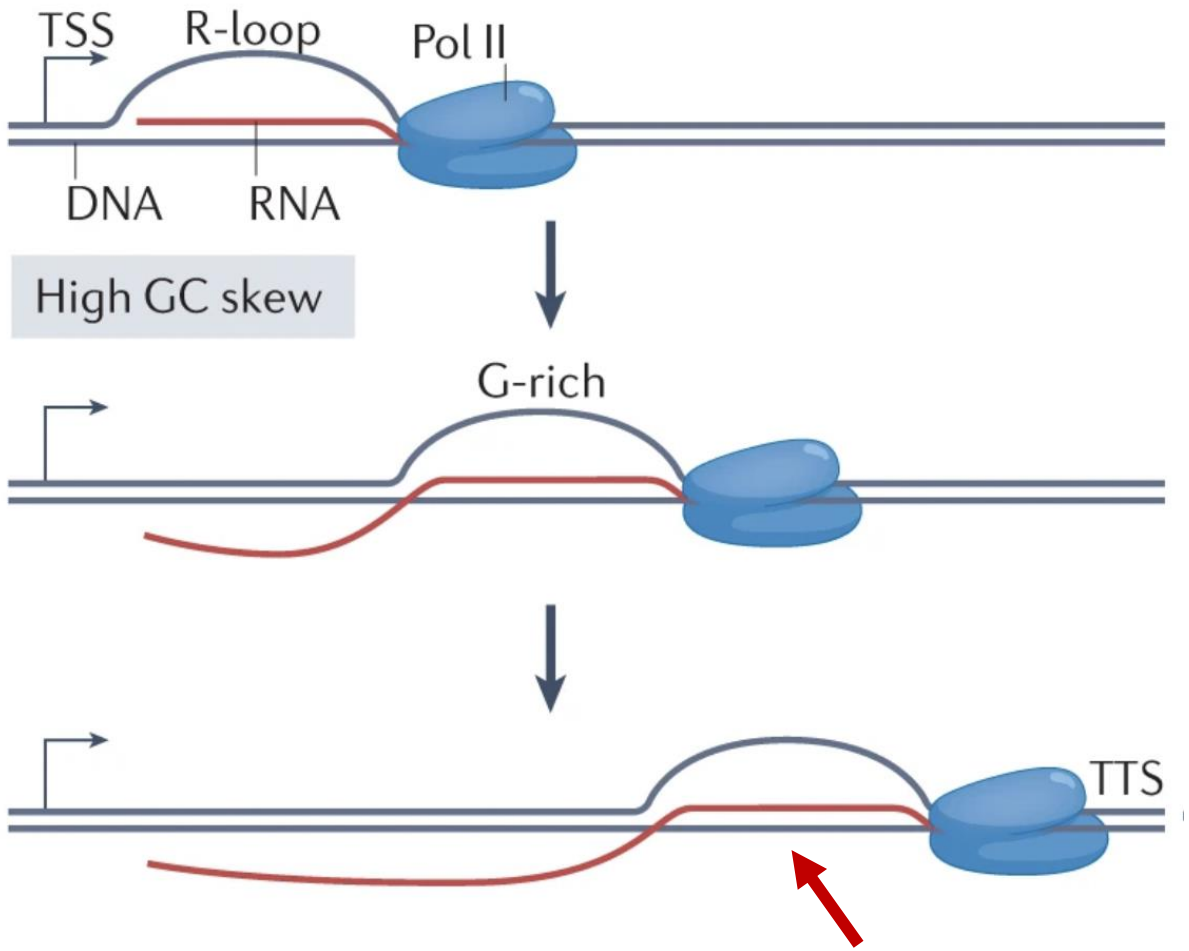
Ginno et al., *Mol Cell*, 2012, PMID 22387027

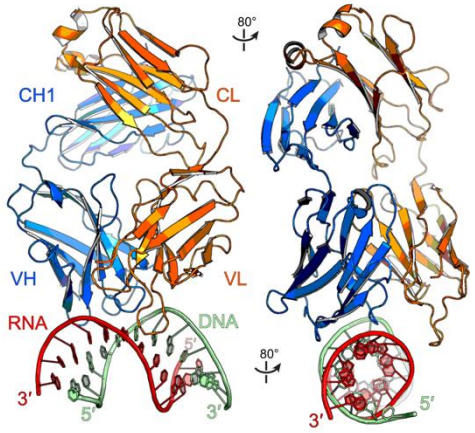
The RNA:DNA Hybrid At R-Loops Can Be Detected With The S9.6 Antibody



Petermann E et al., *Nat Rev Mol Cell Biol*, 2022

The RNA:DNA Hybrid At R-Loops Can Be Detected With The S9.6 Antibody

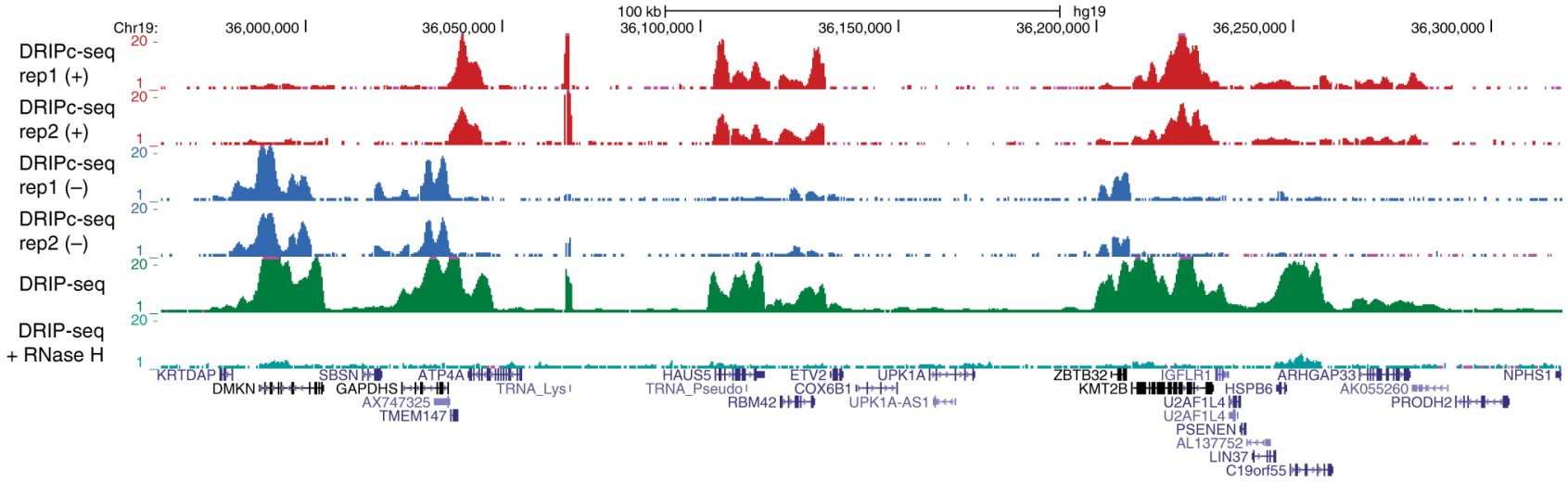




Petermann E et al., *Nat Rev Mol Cell Biol*, 2022

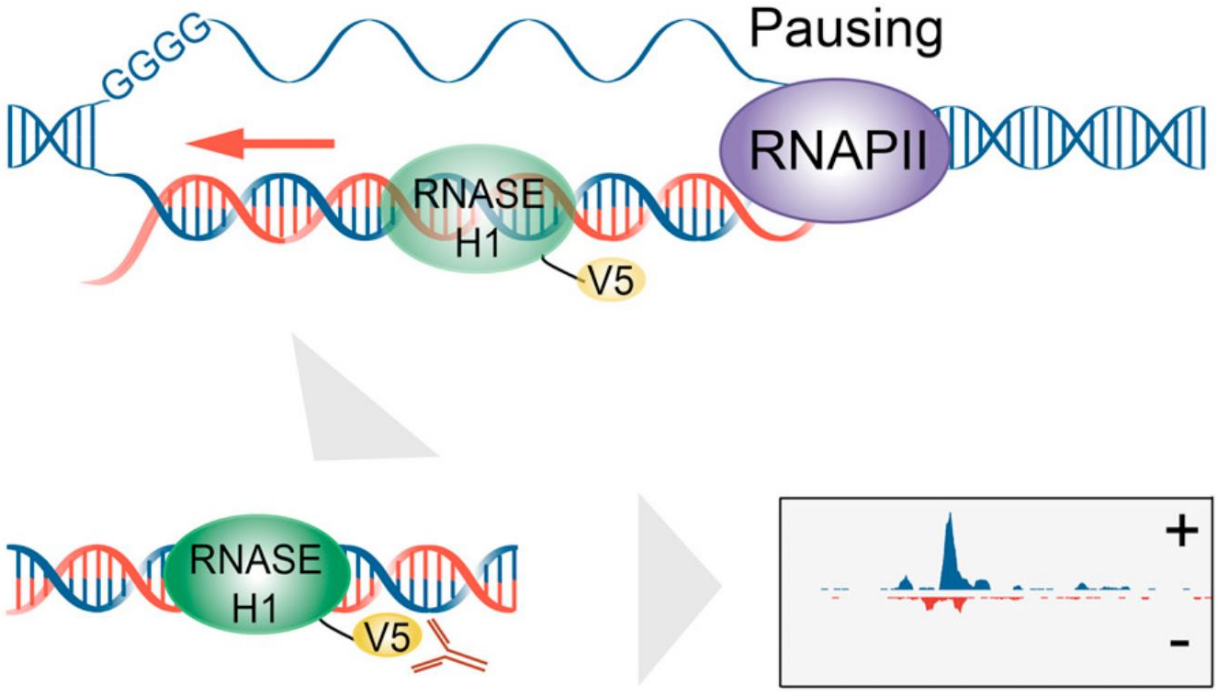
Bou-Nader C et al., *Nat Comm*, 2022, PMID 35347133

The RNA:DNA Hybrid At R-Loops Can Be Detected With The S9.6 Antibody



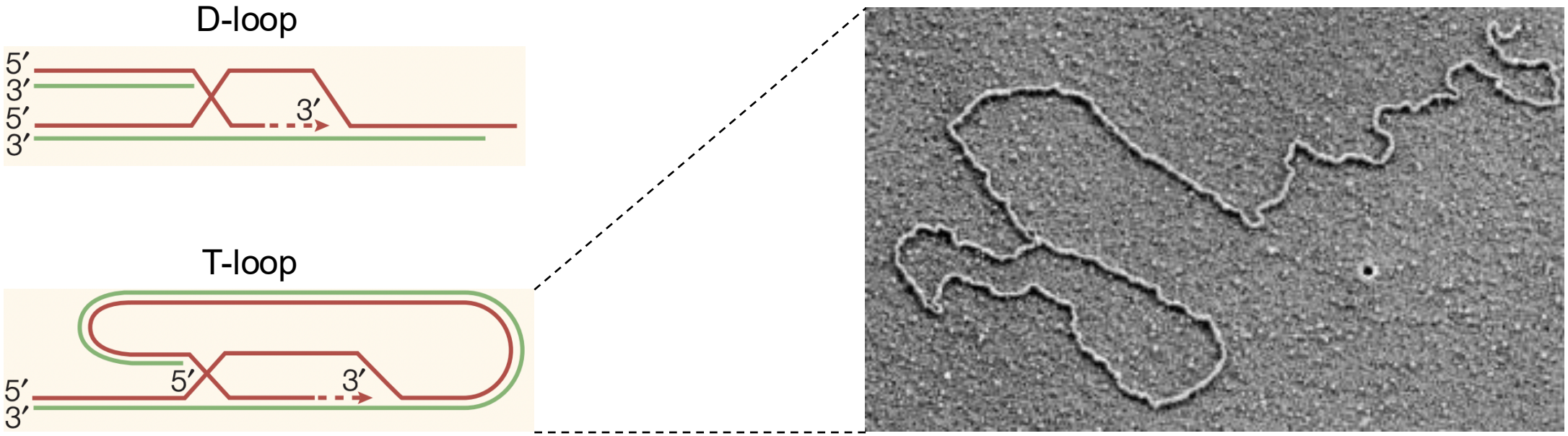
Sanz et al., *Nat Protoc.*, 2019, PMID 31053798

The RNA:DNA Hybrid At R-Loops Can Be Detected By R-Chip-seq Using Catalytically Dead RNaseH1



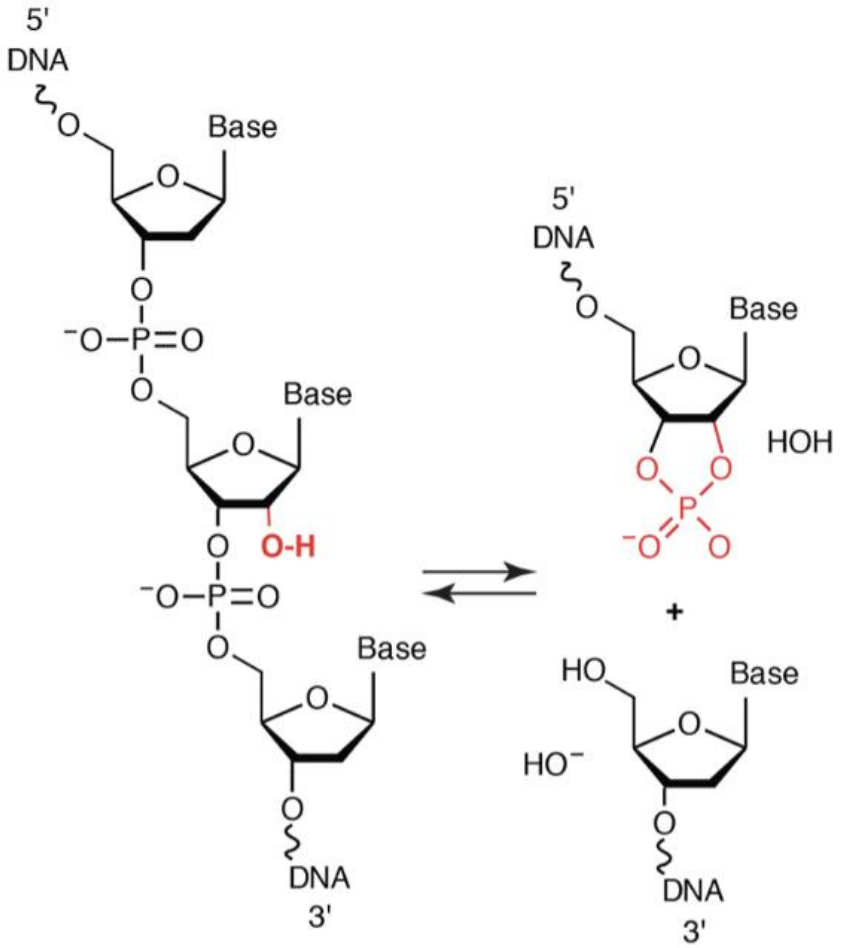
Chen et al., *Mol Cell*, 2017

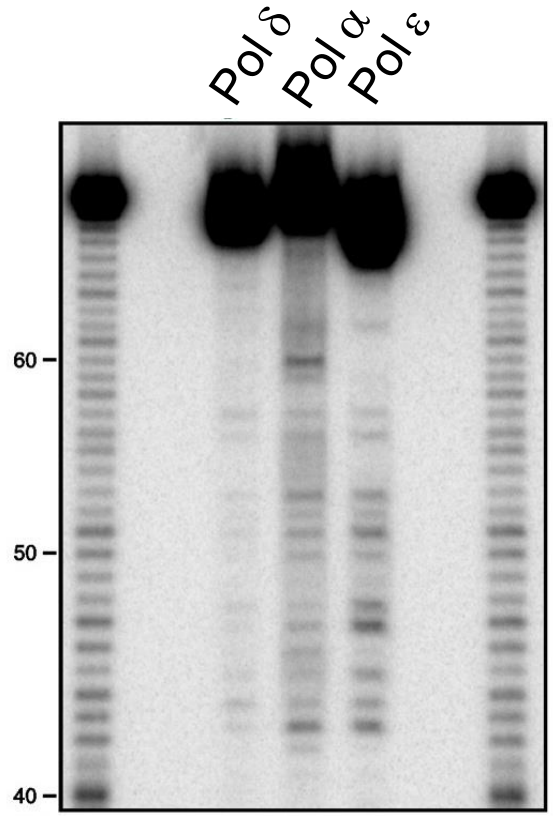
D-Loops Are Formed During Recombination And At Telomeres



Ribonucleotides Are Frequently Incorporated Into DNA By DNA Polymerases

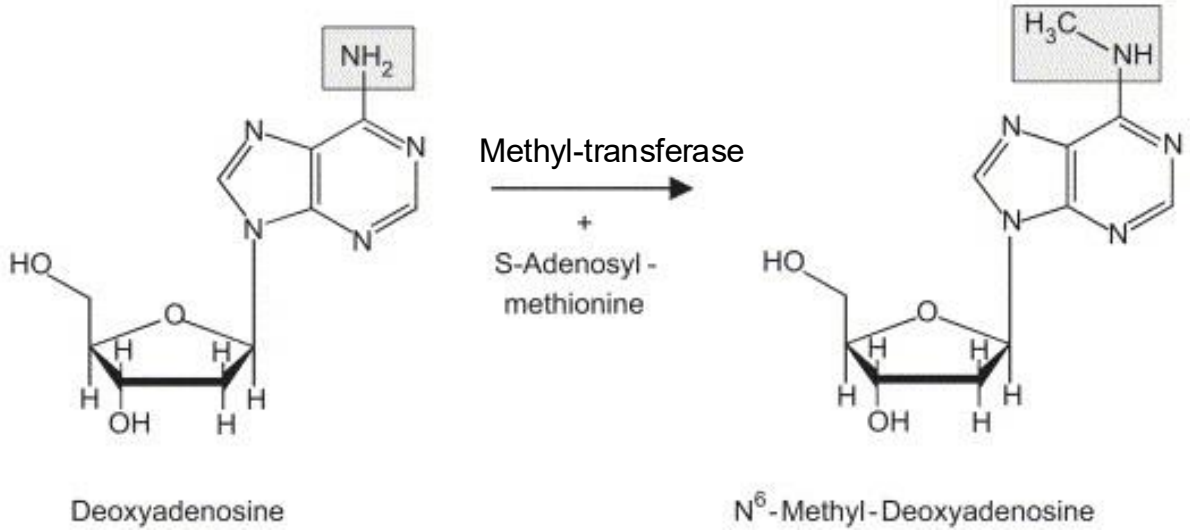




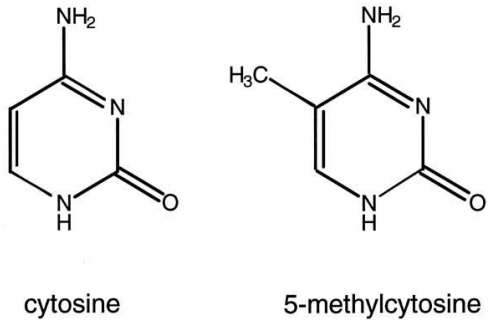


DNA Is Frequently Methylated

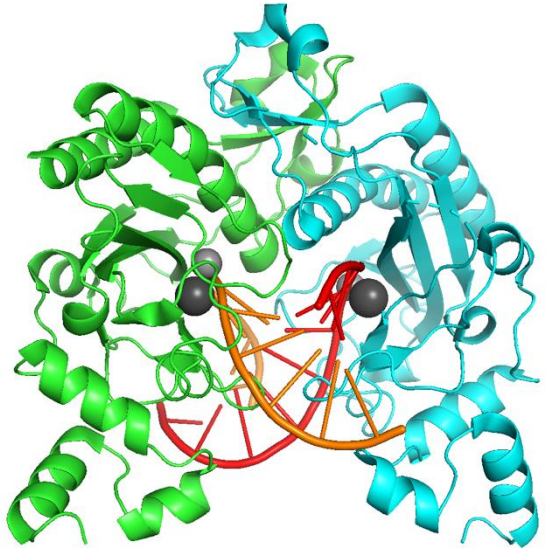
Adenine methylation



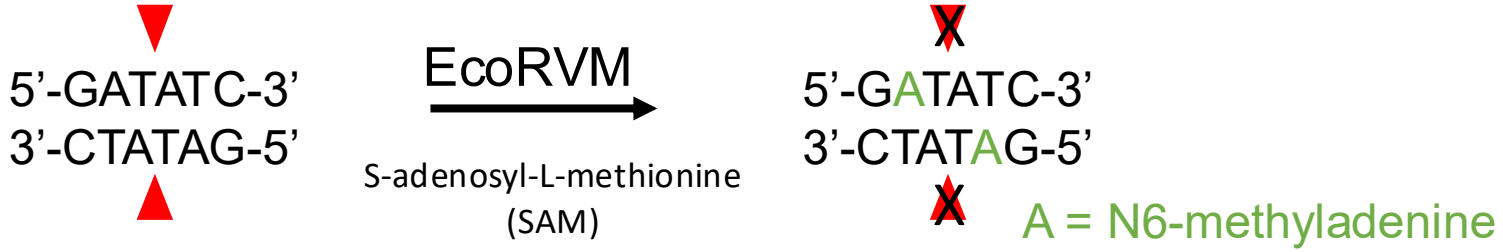
Cytosine methylation



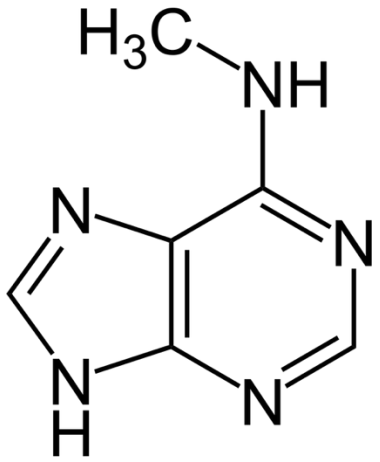
DNA Methylation Protects Bacterial Genomes From Restriction Endonuclease Digestion



EcoRV



DNA Methylation Can Inhibit Restriction Enzymes

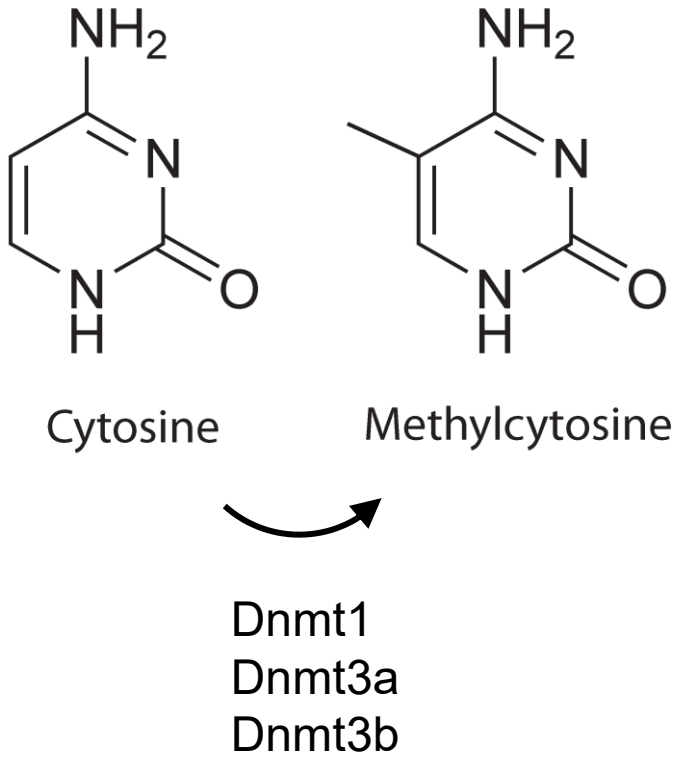


N6-methyladenine

NEW ENGLAND BioLabs inc. be INSPIRED drive DISCOVERY stay GENUINE

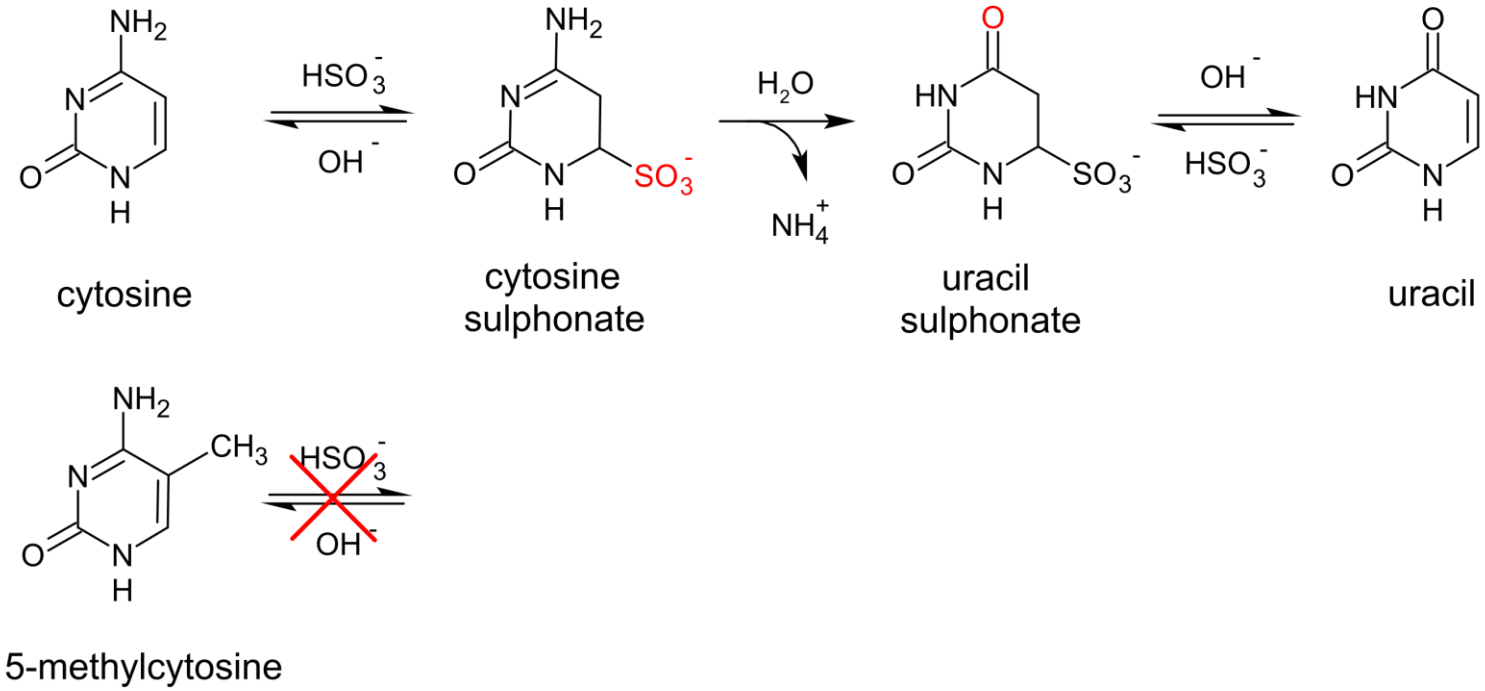
Dam Methylation: G^{m}ATC	
Blocked by Overlapping Dam	
AlwI	**GGATC**
BcgI[1]	**CGATCNNNNTGC**
BclI	**TGATCA**
BsaBI	**GATCNNNATC**
BspDI	**ATCGAT**C
BspEI	**TCCGGA**TC
BspHI	**TCATGA**TC
ClaI	**ATCGAT**C
DpnII	**GATC**
HphI	**GGTGA**TC
Hpy188I	**TCNGA**TC
Hpy188III	**TCNNGA**TC
MboI	**GATC**
MboII	**GAAGA**TC
NruI	**TCGCGA**TC
Taq$^{\alpha}$ I	**TCGA**TC
XbaI	**TCTAGA**TC

CpG Methylation Is An Important Epigenetic Regulator In Eukaryotes

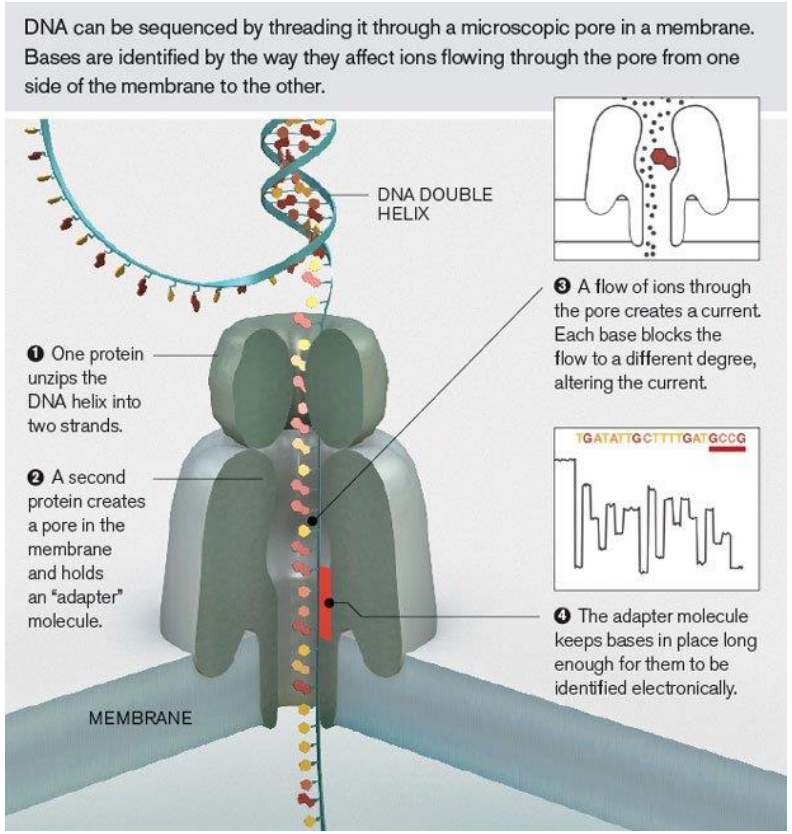




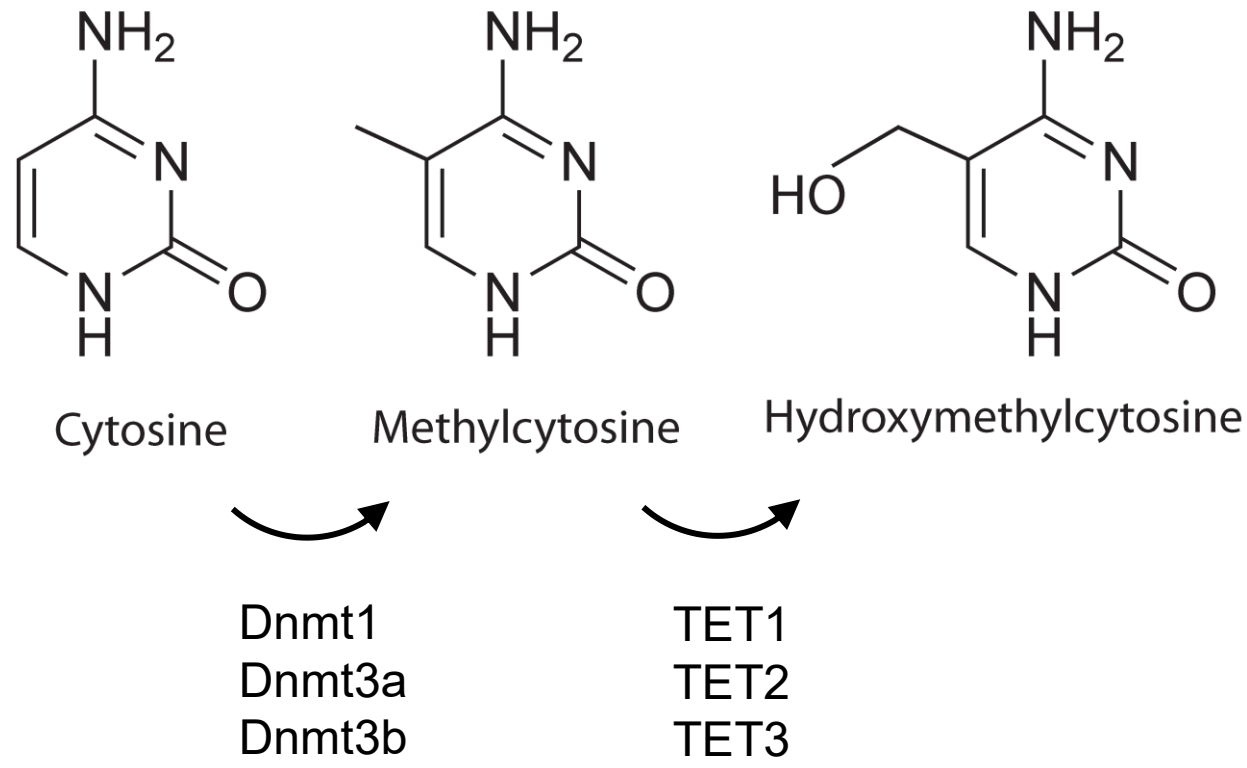
Bisulfite Sequencing Can Be Used To Map Sites Of Cytosine Methylation



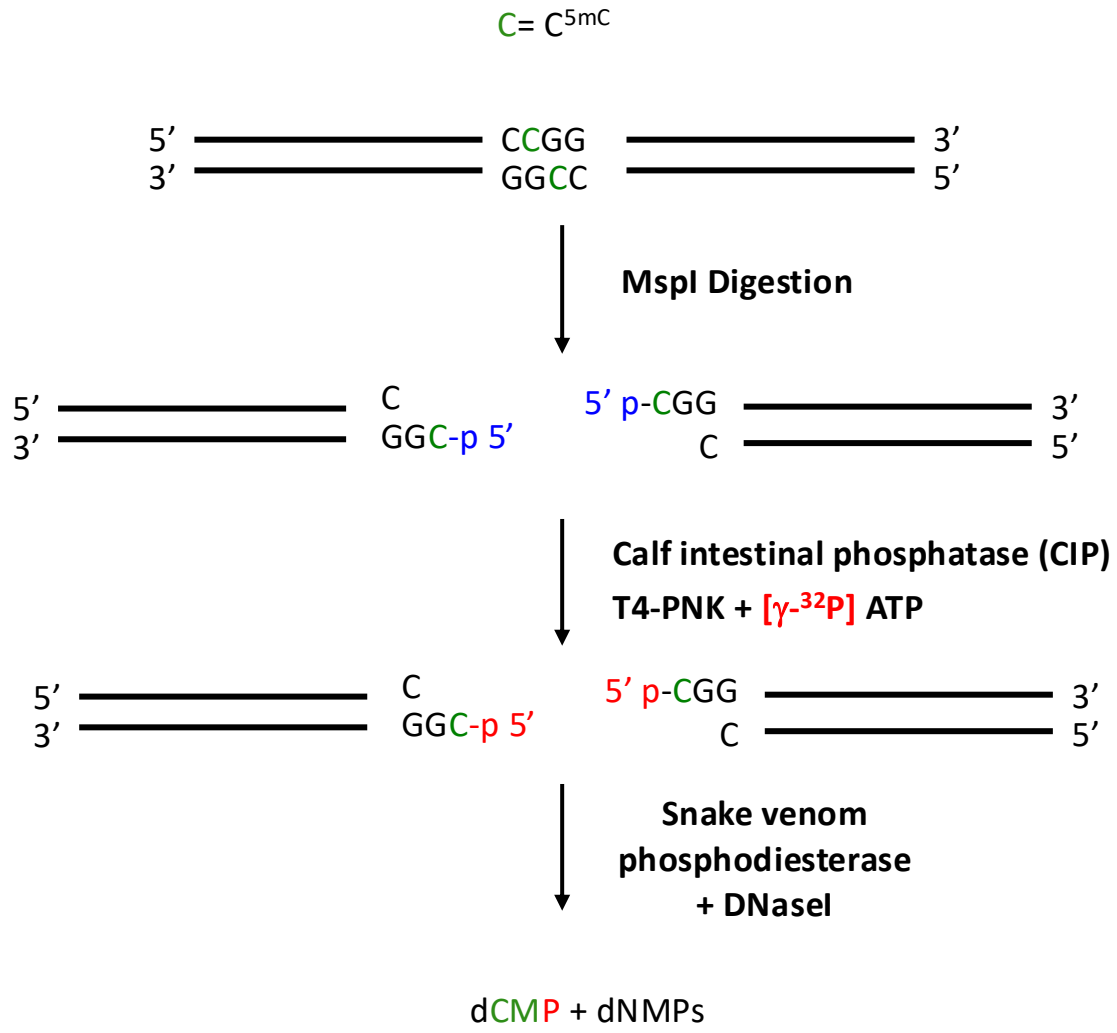
Long-Read Sequencing Can Be Used To Map DNA Modifications

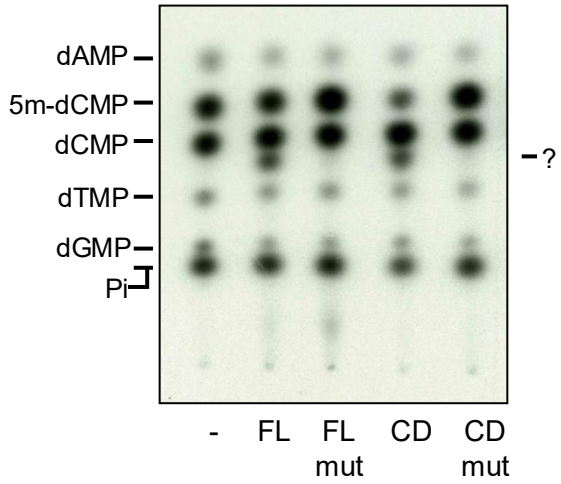


TET Enzymes Regulate CpG Methylation



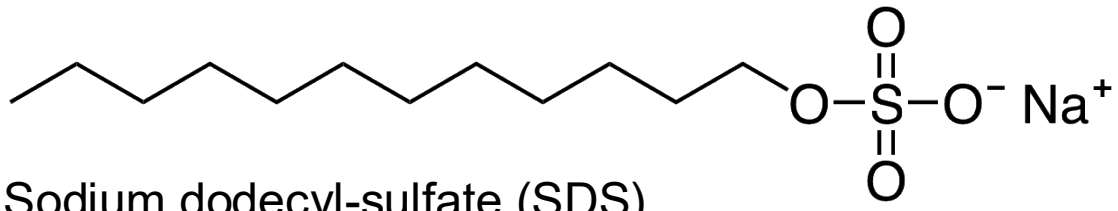
Biochemical Detection Of CpG Methylation In Genomic DNA





Tahiliani et al. Science 2009;324:930-935

How To Extract DNA From Cells: Alkaline Lysis

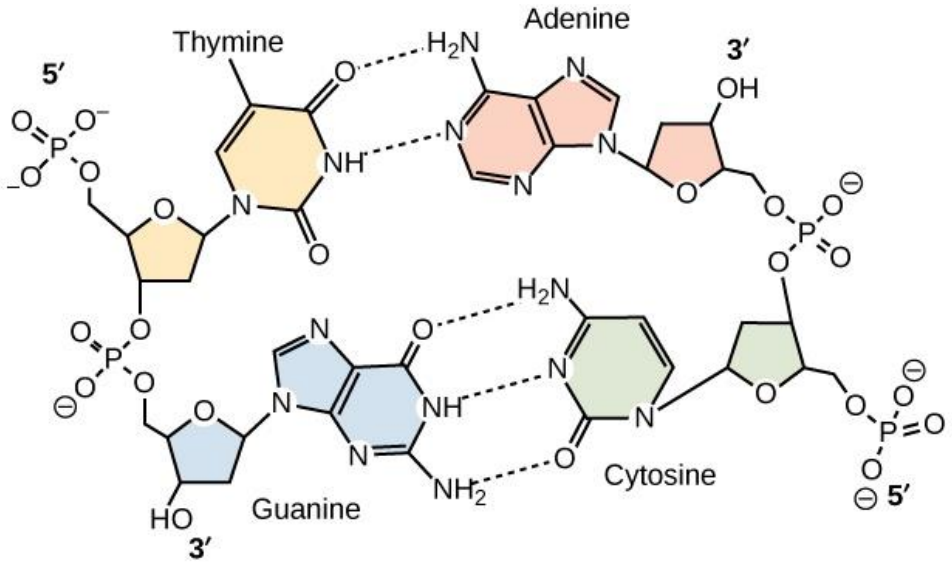


Sodium dodecyl-sulfate (SDS)

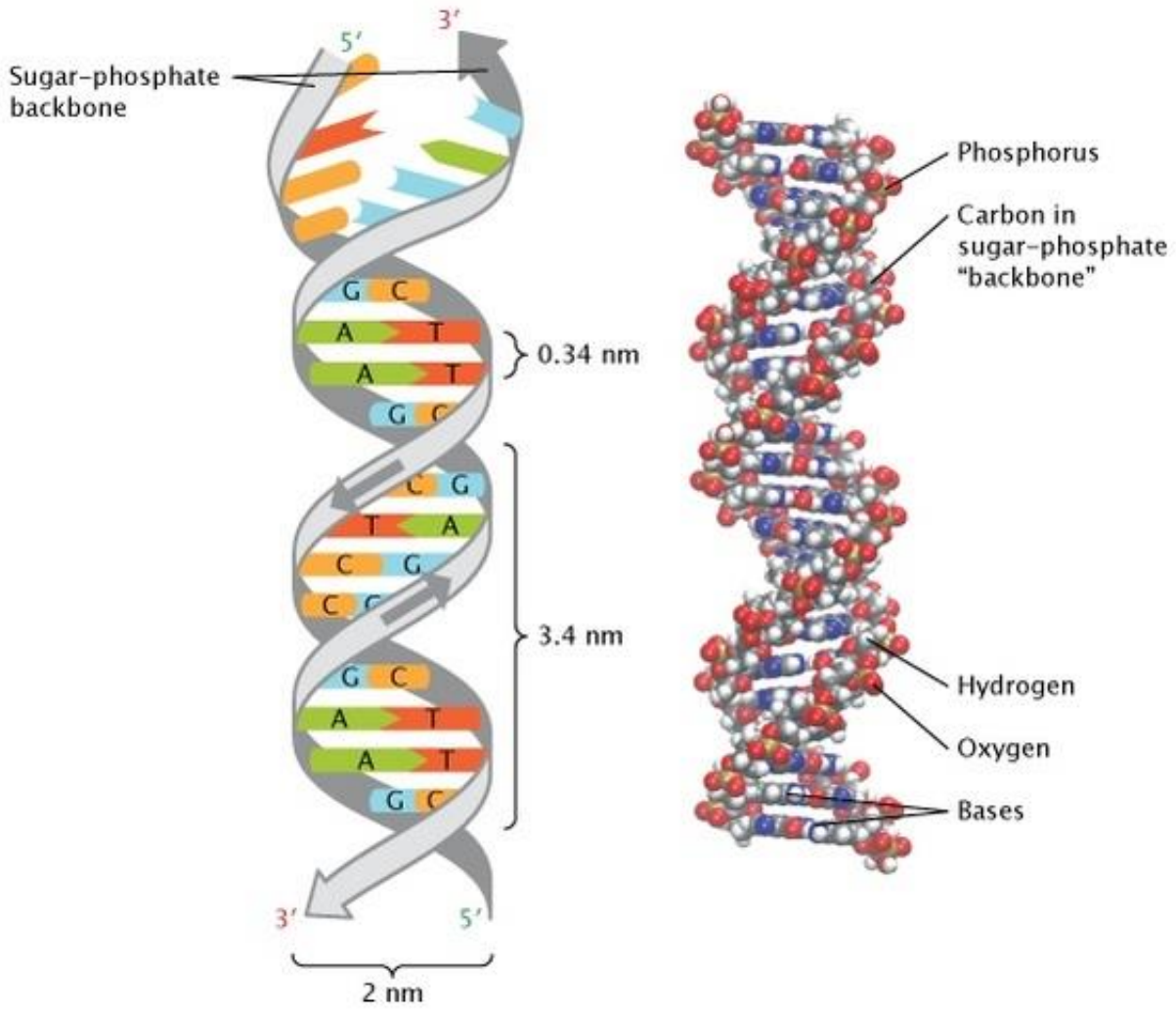
Alkaline lysis solution I
50 mM glucose
25 mM Tris-Cl (pH 8)
10 mM EDTA
(RNase)

Alkaline lysis solution II
0.2 N NaOH
1% SDS

Alkaline lysis solution III
3 M KOAc pH 5.2



The Scale Of DNA Replication



The Scale Of DNA Replication

Bacterial genome

~ $5x10^6$ bp = 1.6 mm

Largest human chromosome

~ 245 x 10^6 bp = 8.3 cm

Human genome: 23 chromosomes

~ 3 x 10^9 bp = 1 m (diploid: 2 m)

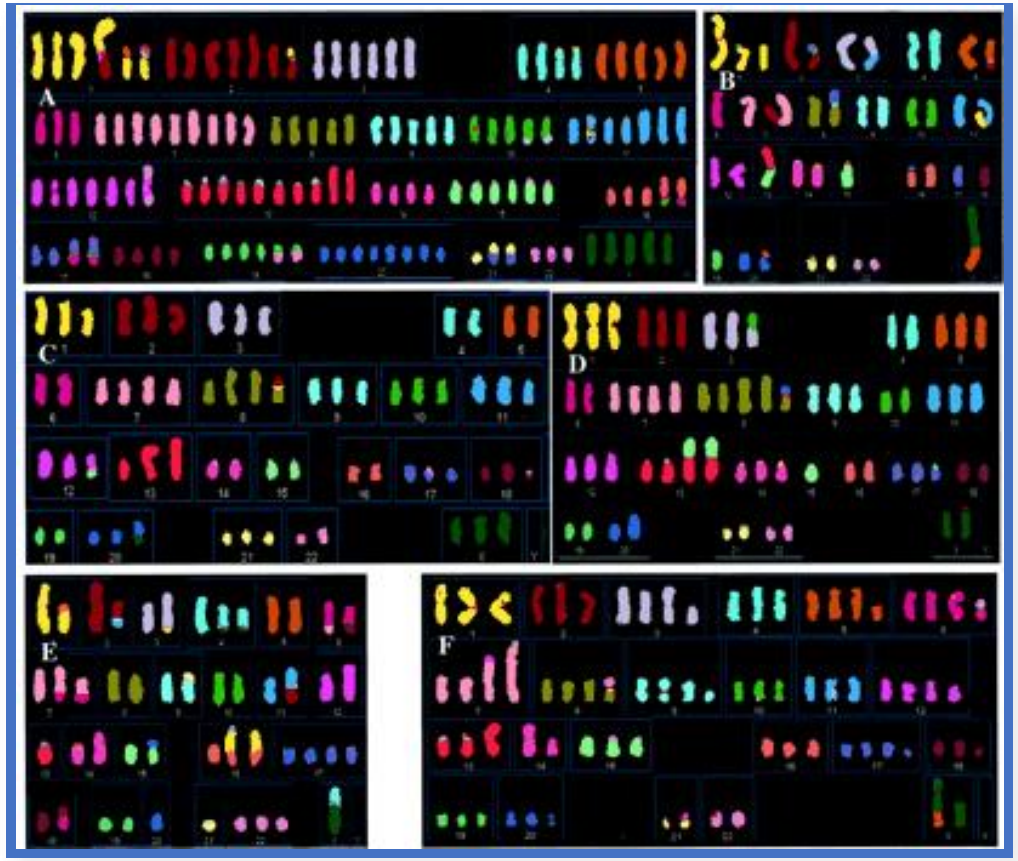
Every minute: 500 – 700 million new white blood cells

~ 10^6 km DNA (25 times around equator, ~ 2.5 x distance to moon)

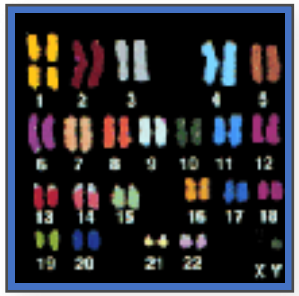


Replication Defects Drive Genome Instability

Colon Cancer Cells



Normal Cell



Abdel-Rahman et al., PNAS, 2001

Replication Defects Drive Genome Instability

articles

DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis

Jirina Bartkova[1], Zuzana Hořejší[1,5], Karen Koed[2], Alwin Krämer[1], Frederic Tort[1], Karsten Zieger[2], Per Guldberg[1], Maxwell Sehested[3], Jahn M. Nesland[4], Claudia Lukas[1], Torben Ørntoft[2], Jiri Lukas[1] & Jiri Bartek[1]

[1]Institute of Cancer Biology and Centre for Genotoxic Stress Research, Danish Cancer Society, Strandboulevarden 49, DK-2100 Copenhagen, Denmark
[2]Department of Clinical Biochemistry, Aarhus University Hospital, Skejby, DK-8200 Aarhus N, Denmark
[3]Department of Pathology, University Hospital, Frederik V's Vej 11, DK-2100 Copenhagen, Denmark
[4]Department of Pathology, The Norwegian Radium Hospital, University of Oslo, Ullernchausseen 70-0310 Oslo, Norway
[5]Institute of Molecular Genetics, Czech Academy of Sciences, Flemingovo nam. 2, Praha 6, CZ-16637, Czech Republic

Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions

Vassilis G. Gorgoulis[1]*, Leandros-Vassilios F. Vassiliou[1]*, Panagiotis Karakaidos[1], Panayotis Zacharatos[1], Athanassios Kotsinas[1], Triantafillos Liloglou[2], Monica Venere[3,4], Richard A. DiTullio Jr[3,4], Nikolaos G. Kastrinakis[1], Brynn Levy[6], Dimitris Kletsas[7], Akihiro Yoneta[3], Meenhard Herlyn[3], Christos Kittas[1] & Thanos D. Halazonetis[3,5]

[1]Department of Histology and Embryology, School of Medicine, University of Athens, Athens GR-11527, Greece
[2]Roy Castle Lung Cancer Research Programme, Cancer Research Center, University of Liverpool, Liverpool L3 9TA, UK
[3]The Wistar Institute, Philadelphia, Pennsylvania 19104-4268, USA
[4]Graduate Group in Biomedical Sciences and
[5]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA
[6]Department of Human Genetics, Mount Sinai School of Medicine, New York, New York 10029, USA
[7]Institute of Biology, National Centre of Scientific Research 'Demokritos', Athens GR-15310, Greece

* These authors contributed equally to this work

LETTERS

Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints

Jirina Bartkova[1]*, Nousin Rezaei[2]*, Michalis Liontos[3]*, Panagiotis Karakaidos[3], Dimitris Kletsas[4], Natalia Issaeva[5], Leandros-Vassilios F. Vassiliou[3], Evangelos Kolettas[6], Katerina Niforou[3], Vassilis C. Zoumpourlis[7], Munenori Takaoka[8], Hiroshi Nakagawa[8], Frederic Tort[1], Kasper Fugger[1], Fredrik Johansson[5], Maxwell Sehested[9], Claus L. Andersen[10], Lars Dyrskjot[10], Torben Ørntoft[10], Jiri Lukas[1], Christos Kittas[3], Thomas Helleday[5,11], Thanos D. Halazonetis[2,12], Jiri Bartek[1] & Vassilis G. Gorgoulis[3]

LETTERS

Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication

Raffaella Di Micco[1], Marzia Fumagalli[1], Angelo Cicalese[2], Sara Piccinin[3], Patrizia Gasparini[1], Chiara Luise[1], Catherine Schurra[4], Massimiliano Garre'[1], Paolo Giovanni Nuciforo[1], Aaron Bensimon[5], Roberta Maestro[3], Pier Giuseppe Pelicci[2] & Fabrizio d'Adda di Fagagna[1]

DNA Replication Is A Chemotherapeutic Target

Antimetabolites:

These drugs mimic the molecules that cancer cells need to synthesize DNA, disrupting the process by depleting nucleotides.

• *5-fluorouracil (5-FU):* A synthetic analog of uracil that inhibits thymidylate synthase, limiting the availability of thymidine nucleotides for DNA synthesis

• *Methotrexate*: An antifolate that inhibits the dihydrofolate reductase enzyme, blocking the synthesis of nucleotides

Nucleoside analogs:

These drugs are incorporated into replicated DNA or RNA strands, inhibiting replication or transcription elongation.

• *Gemcitabine*

• *Fludarabidine*

• *Cytarabine*

DNA damaging agents:

These drugs modify the composition and structure of the nucleic acid substrate to indirectly inhibit DNA synthesis.

• *Cisplatin*

• *Chlorambucil*

Topoisomerase inhibitors:

These drugs induce replication stress by physically hindering replication forks or inducing fork reversal.

• *Irinotecan*

• *Topotecan*

• *Etoposide*

• *Doxorubicin*

DNA Structure Suggests A Mechanism For Replication



equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).
[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).
[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik.* (*Stockholm*), **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

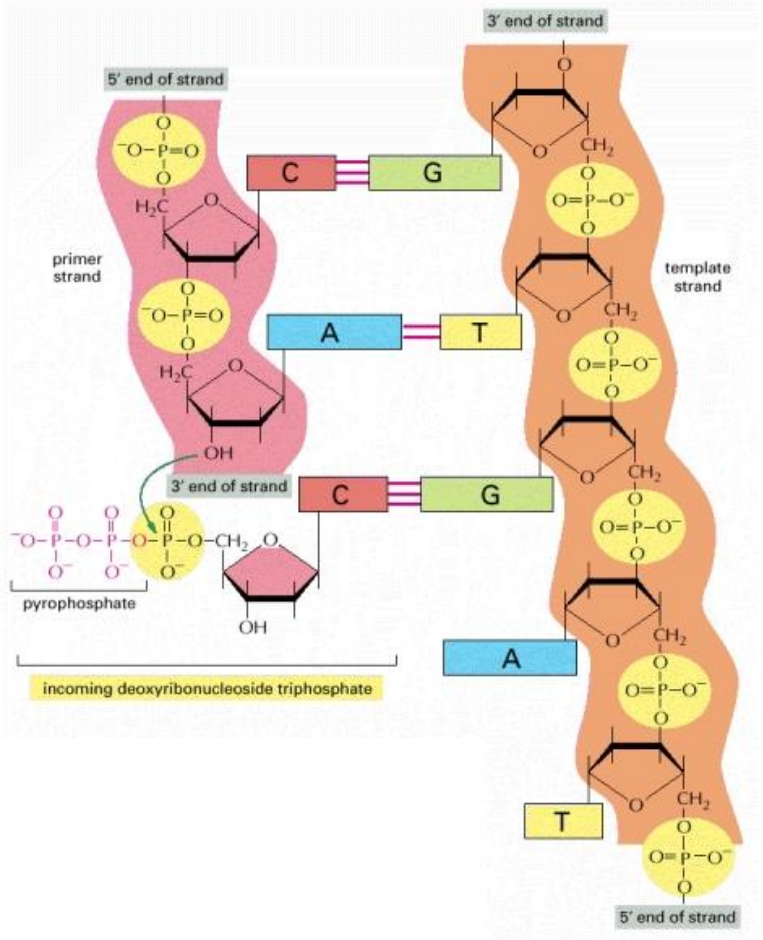
Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

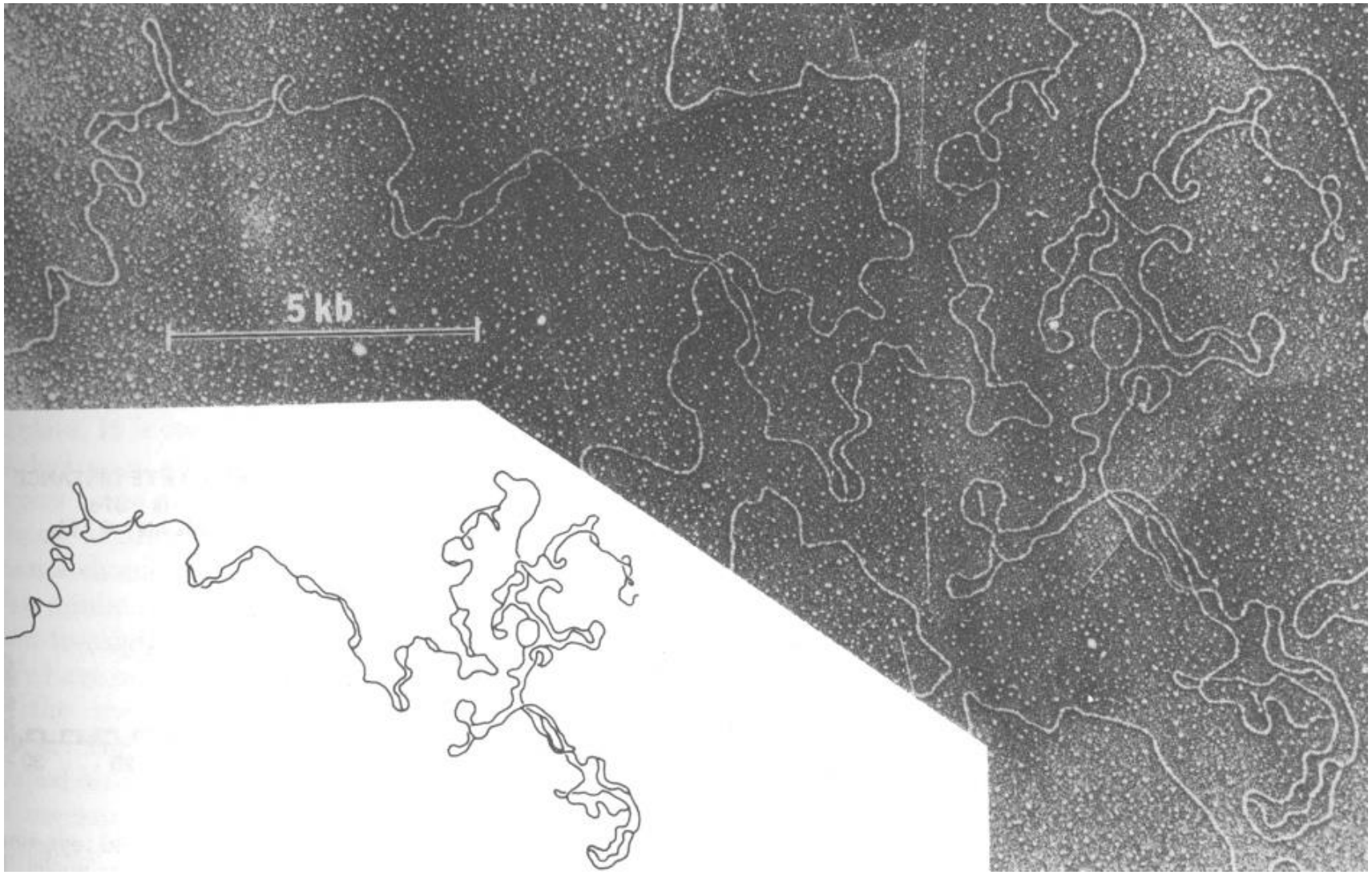
"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

DNA Polymerases Synthesize DNA In 5’ to 3’ Direction

- Addition of deoxyribonucleoside monophosphate to the 3’-end of a growing DNA strand
- The substrates are the four deoxyribonucleoside triphosphates
- Formation of the phosphodiester bond is accompanied by the release of pyrophosphate
- Requires template strand
- Newly synthesized strand is complementary in sequence to template strand
- Cannot start DNA synthesis de novo, requires primer with 3’ OH terminus



DNA Replication Initiates From Many Origin Sites



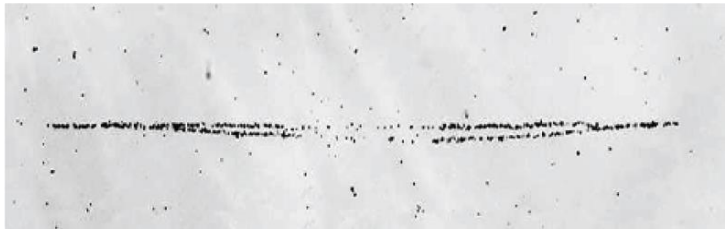
Blumenthal et al, CSHSQB 1974; Kriegstein & Hogness, PNAS 1973

DNA Replication Initiates Bidirectionally

^{3}H-thymidine at low specific activity

↓

Shift to ^{3}H-thymidine at high specific activity



Autoradiograph

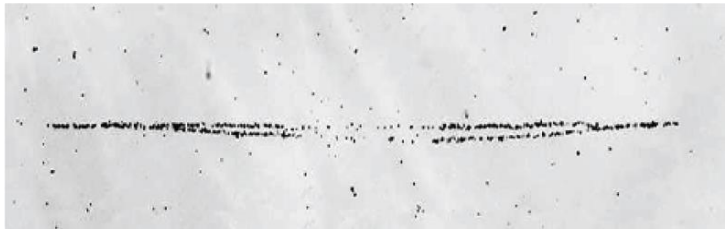


DNA Replication Initiates Bidirectionally

^{3}H-thymidine at low specific activity

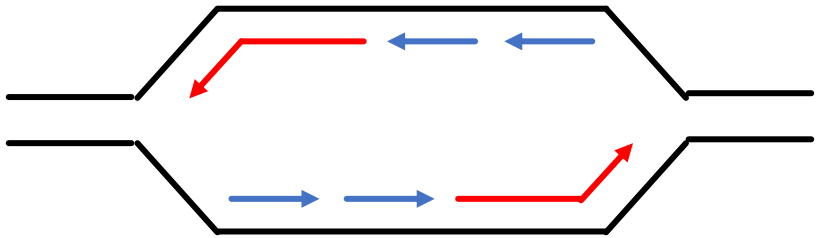
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Shift to ^{3}H-thymidine at high specific activity



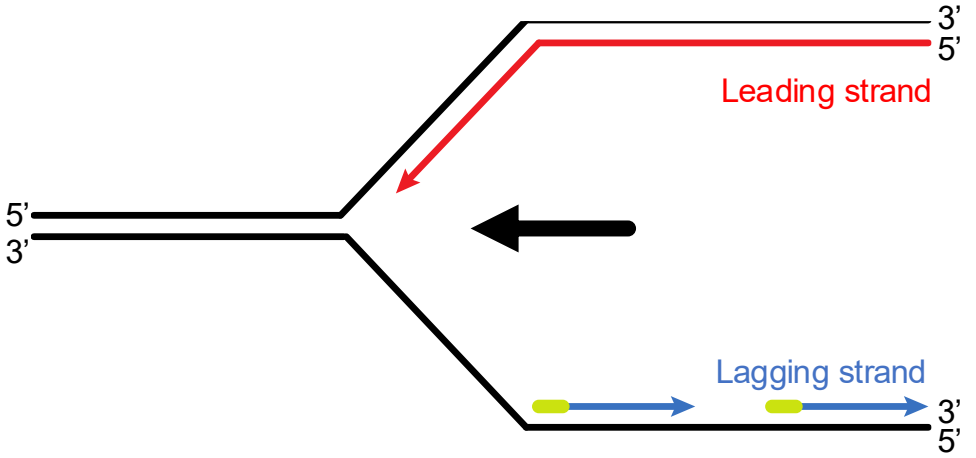
Autoradiograph



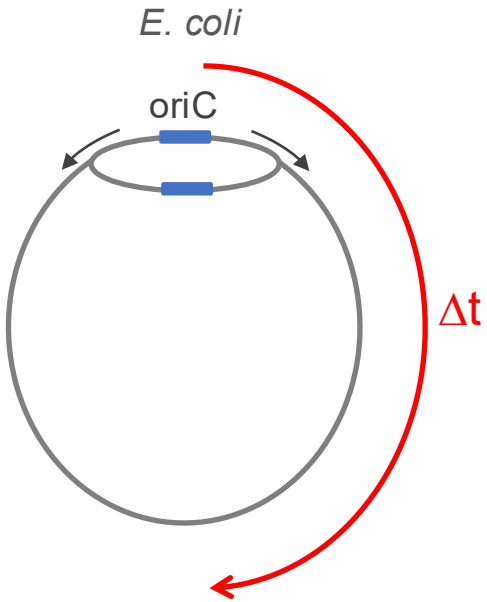


DNA Replication Fork

- Helicase
- Primase
- Polymerase

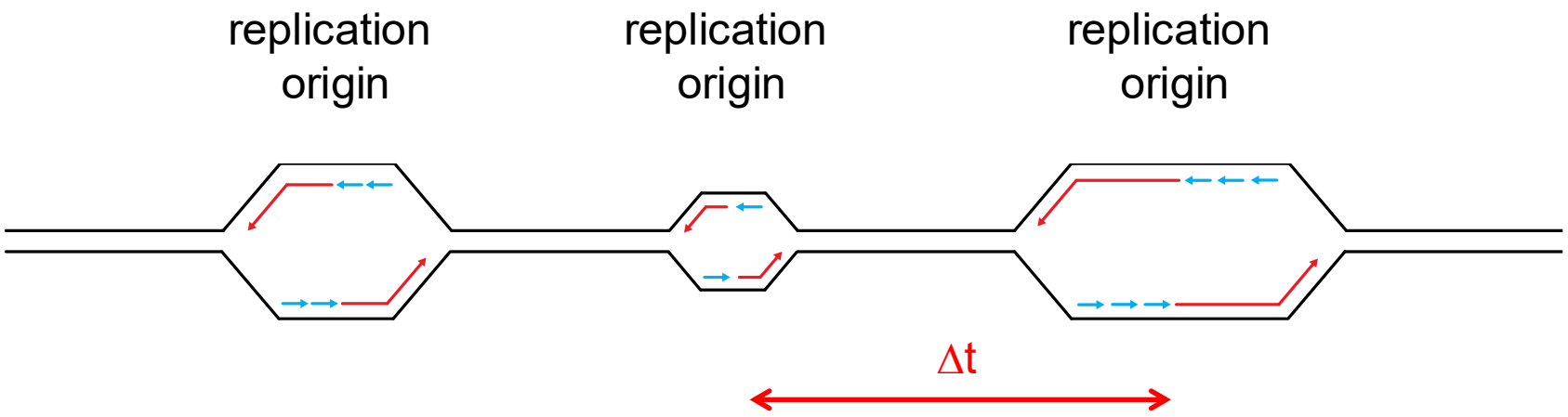


Replicon Size Determines Time Of Replication



Time of replication proportional to genome size

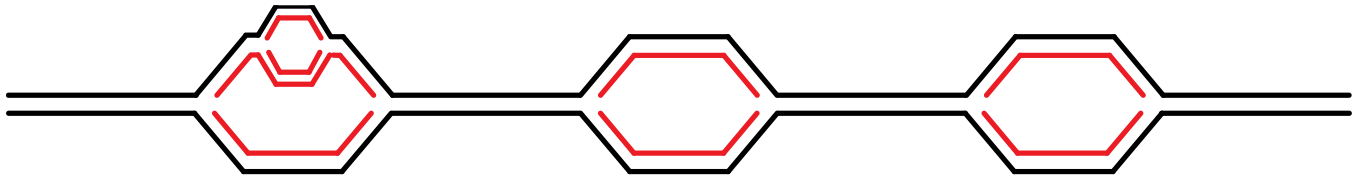
Replicon Size Determines Time Of Replication



Time of replication proportional to inter-origin distance

Once-And-Only-Once Replication

Origin re-firing



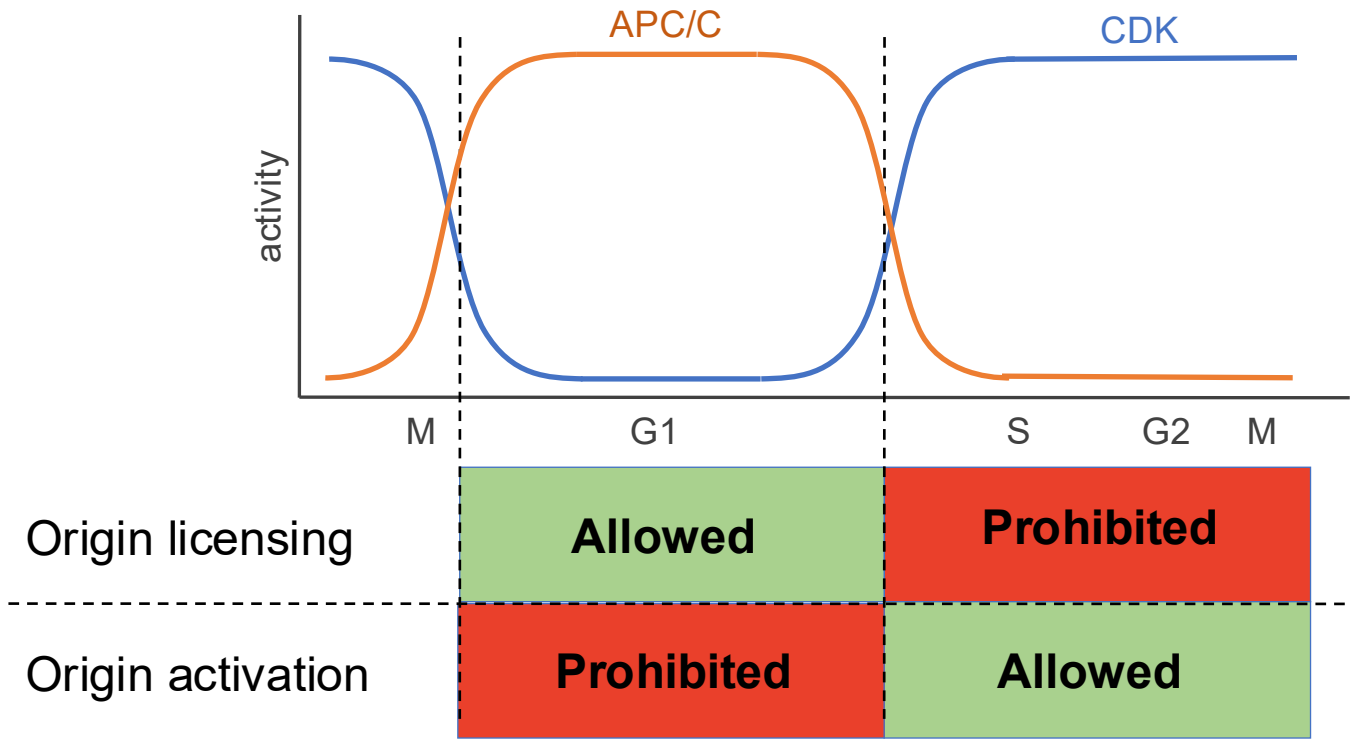
Once-And-Only-Once Replication

- In a human cell, approx. 50,000 replication origins (1 per 70 kb) per S phase.

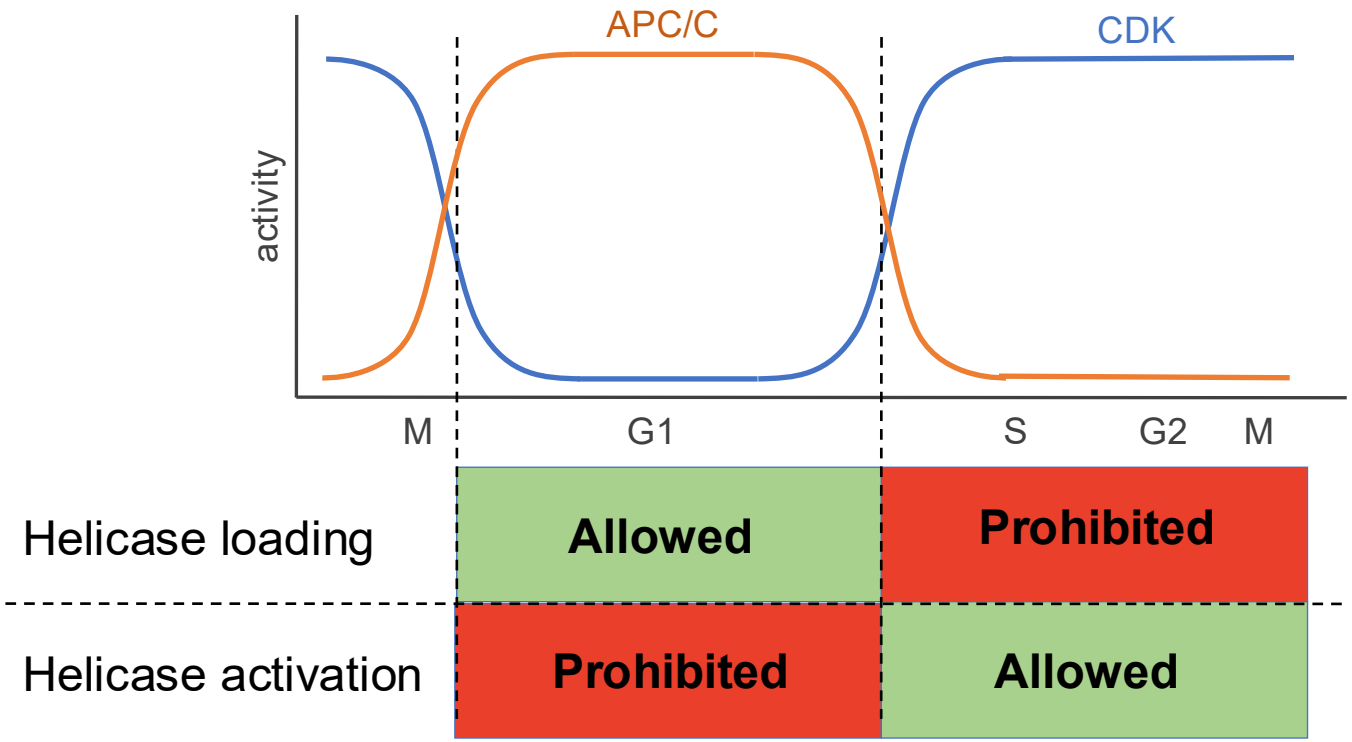
- If block to re-replication were 99.99% efficient (per origin), the probability of completing a cell cycle without re-initiating replication from any origin is $0.9999^{50,000} = 0.06$ (i.e. 6%).

- To reach 99% probability of completing S phase without re-initiating, the block to re-initiating replication from any individual origin needs to be approximately 99.99999% efficient (i.e. 'error rate' $\leq 10^{-7}$)

Once-And-Only-Once Replication

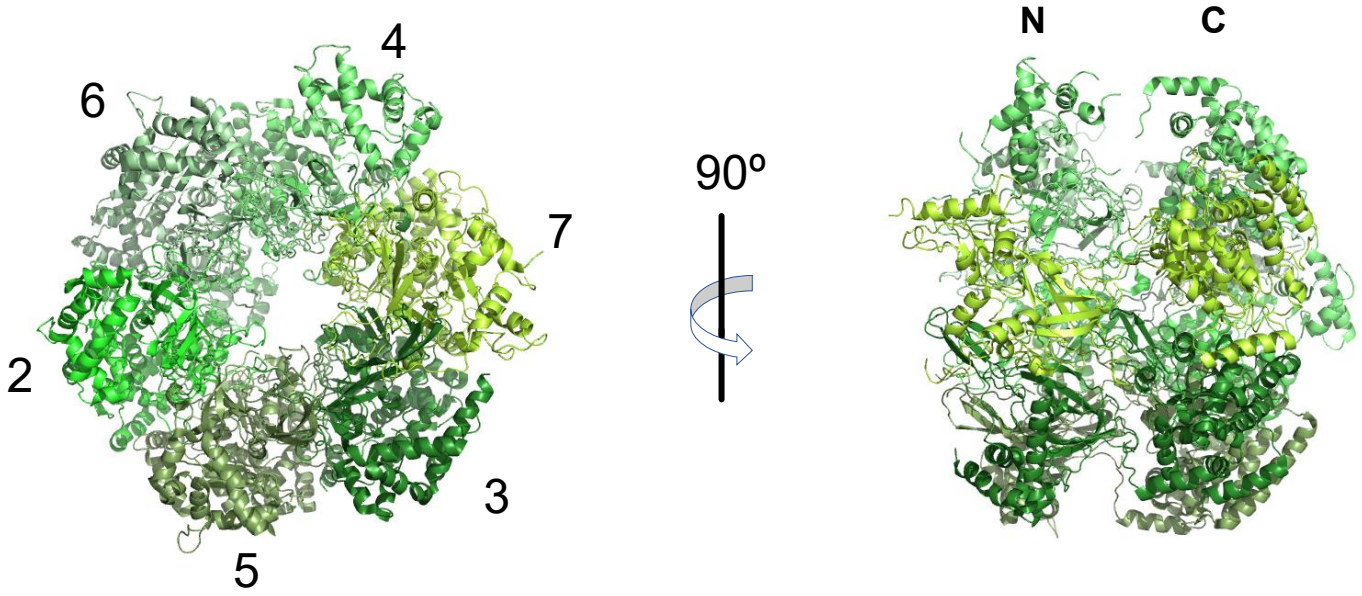


Once-And-Only-Once Replication



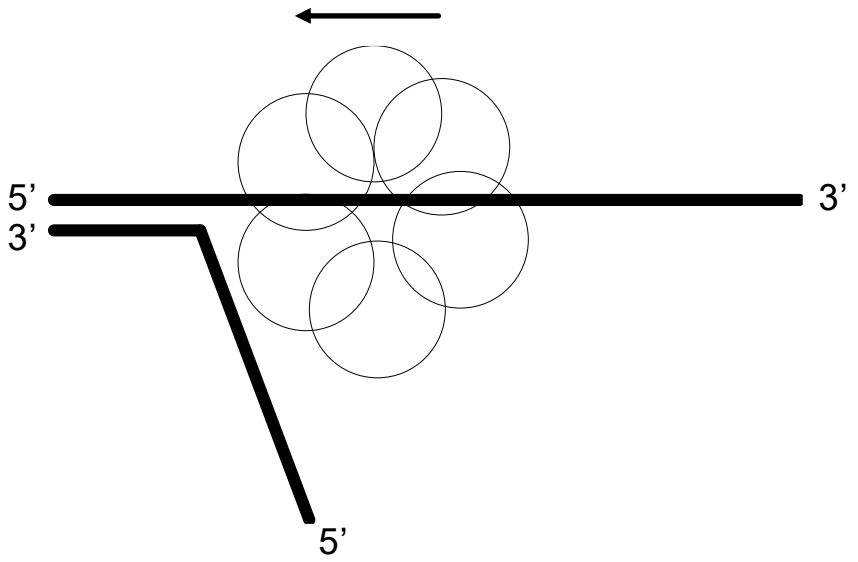
The Eukaryotic Replicative DNA Helicase: MCM2-7

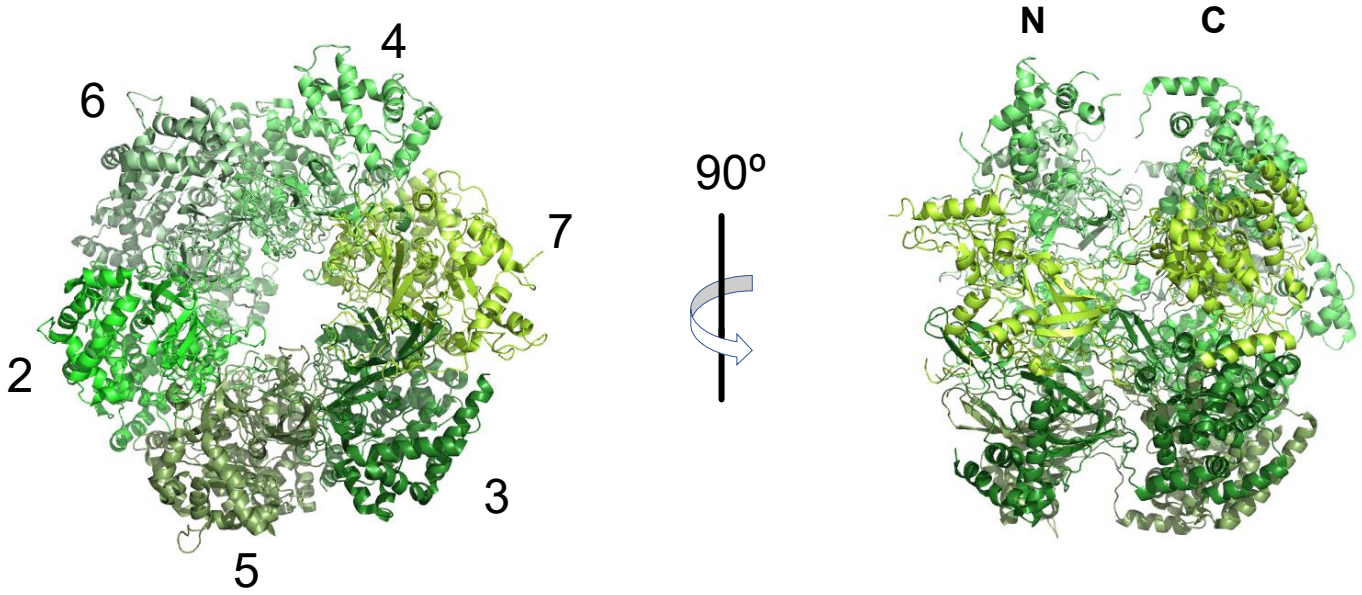




PDB: 5U8S

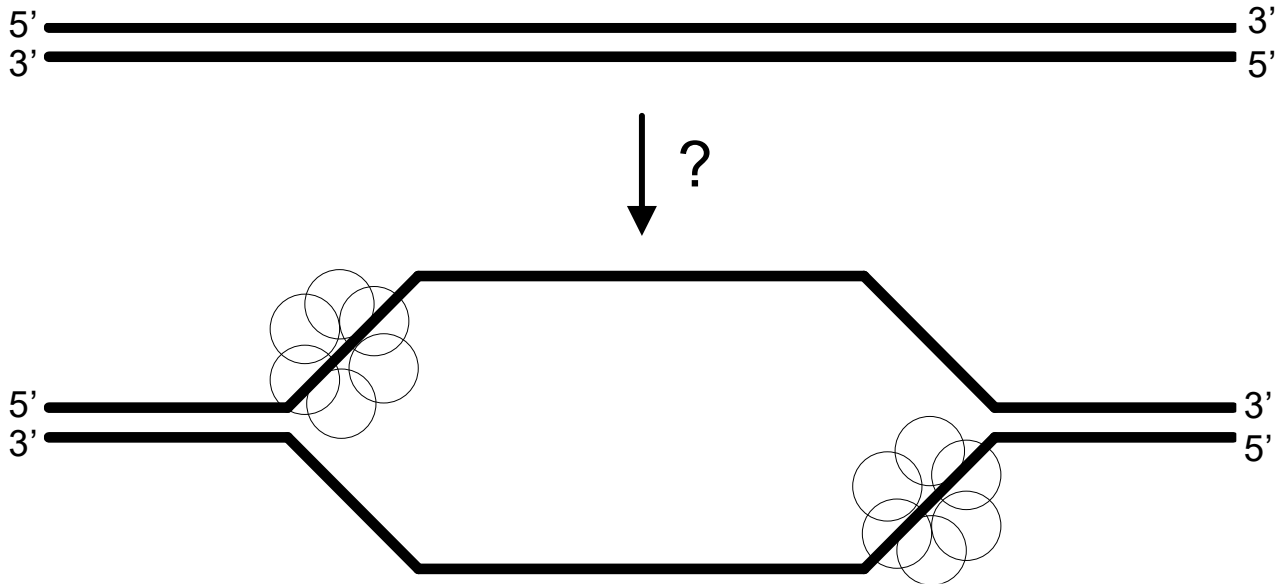
The Eukaryotic Replicative DNA Helicase: MCM2-7



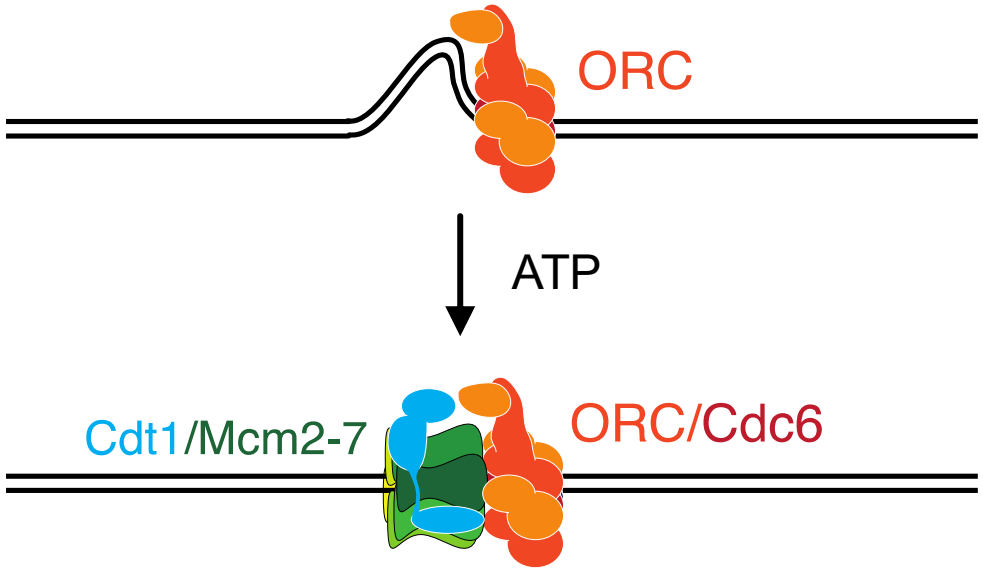


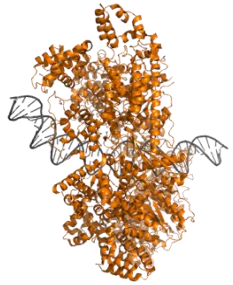
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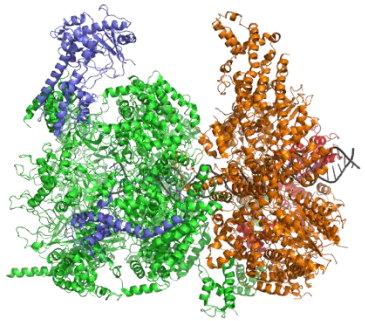
The Eukaryotic Replicative DNA Helicase: MCM2-7



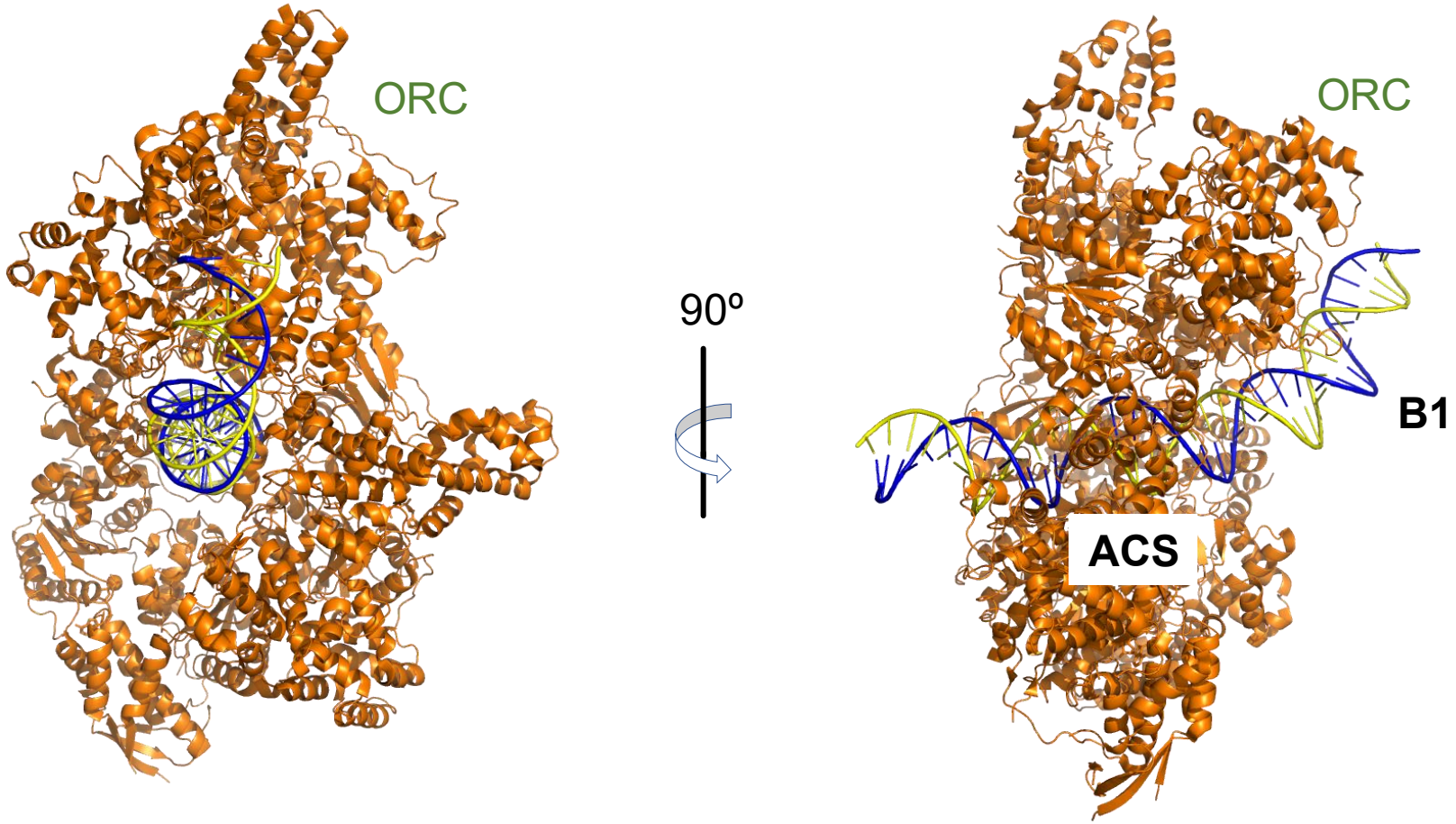
MCM Loading Mechanism





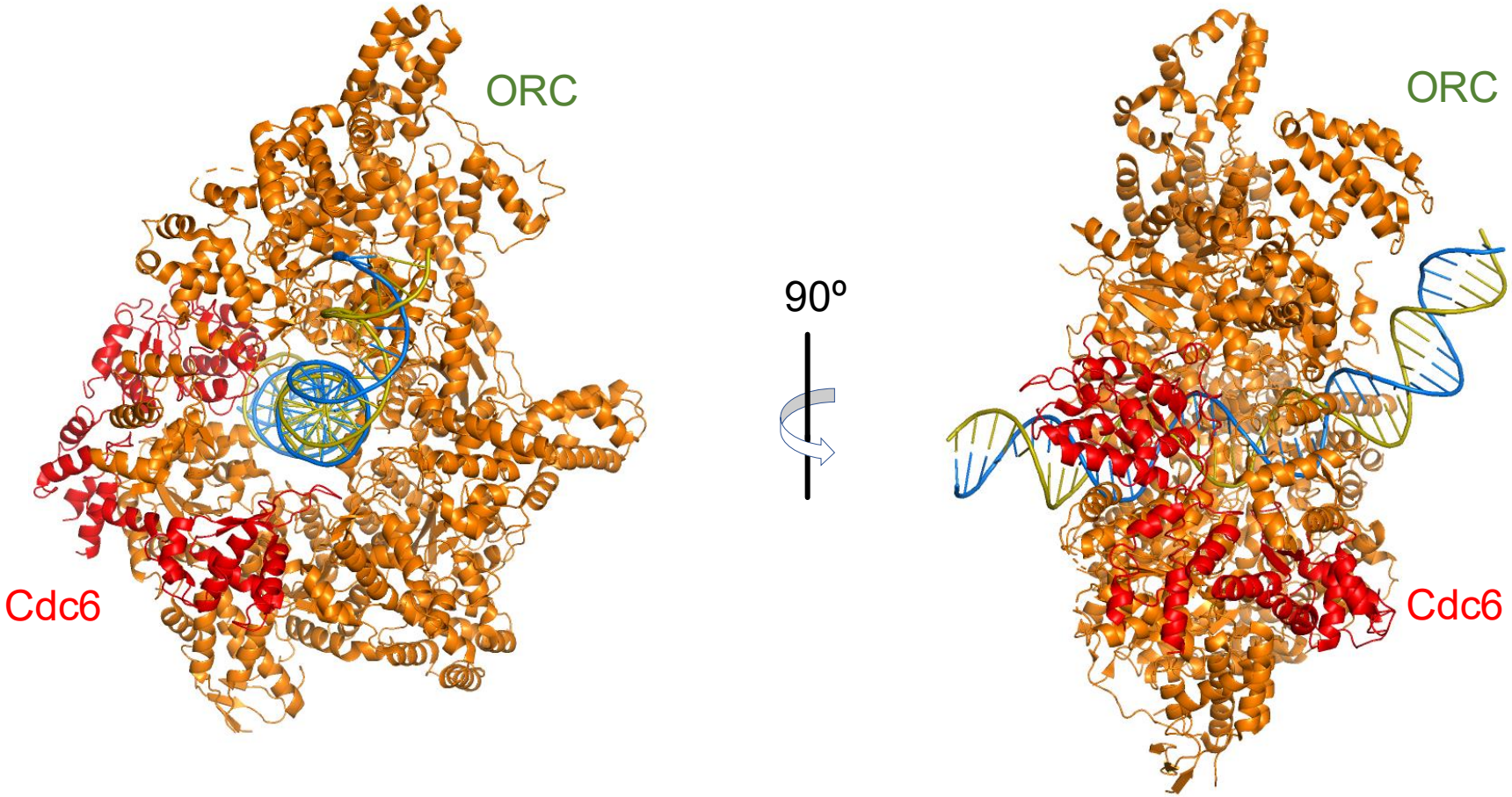


ORC: Origin Recognition Complex



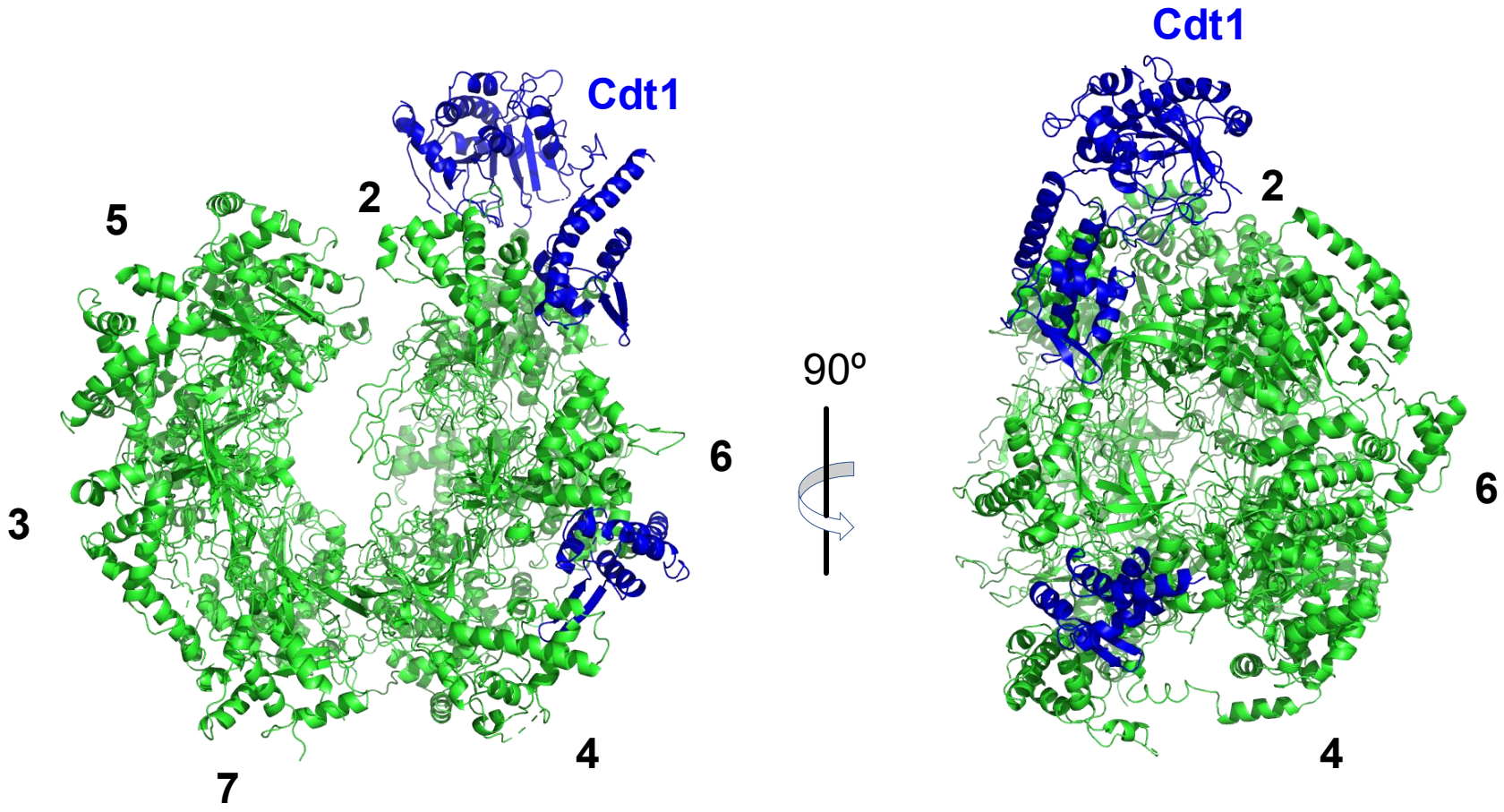
Li N et al. Nature. 2018. PMID: 29973722

Cdc6



Yuan et al. PNAS. 2020. PMID: 32669428

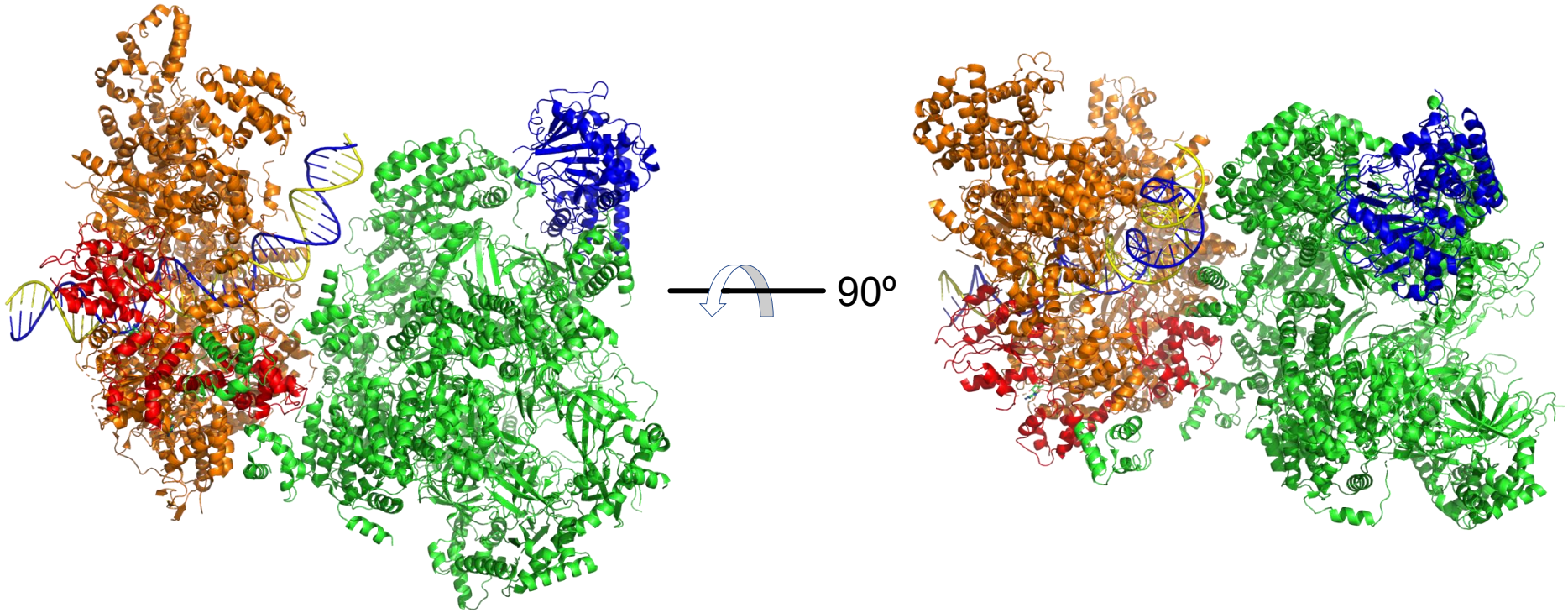
Cdt1-MCM



Zhai Y et al. NSMB. 2017. PMID: 28191894

ORC:Cdc6:Cdt1:MCM (OCCM)

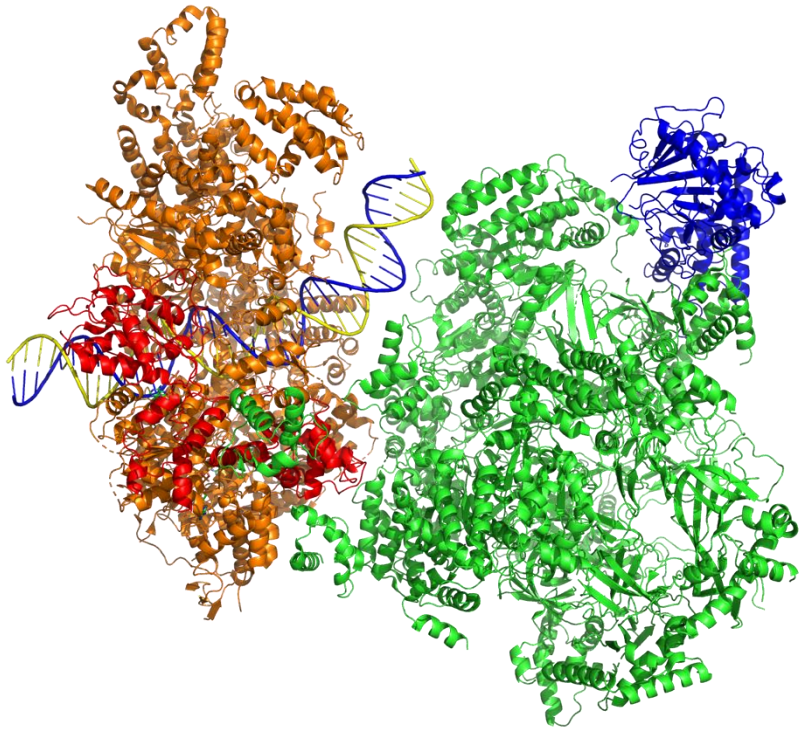
Pre-insertion state



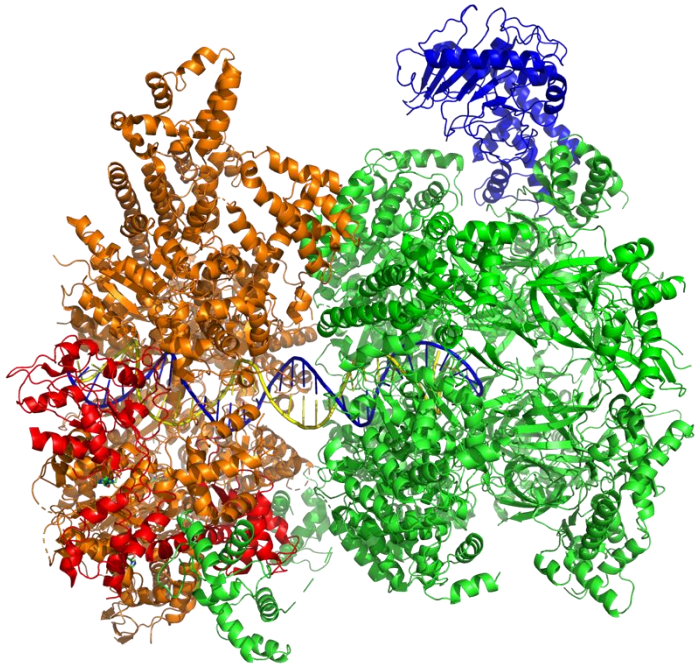
Yuan et al. PNAS. 2020. PMID: 32669428

ORC:Cdc6:Cdt1:MCM (OCCM)

Pre-insertion state



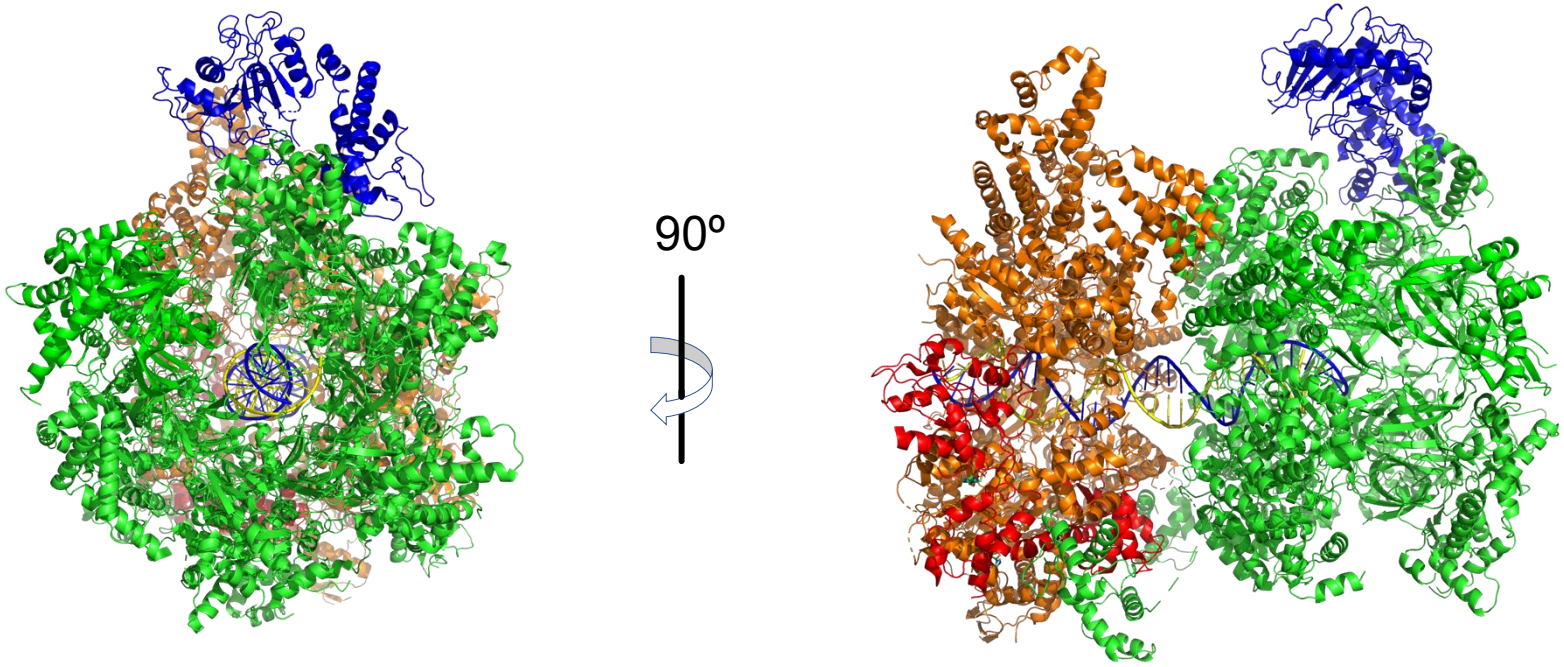
Post-insertion state



Yuan et al. PNAS. 2020. PMID: 32669428

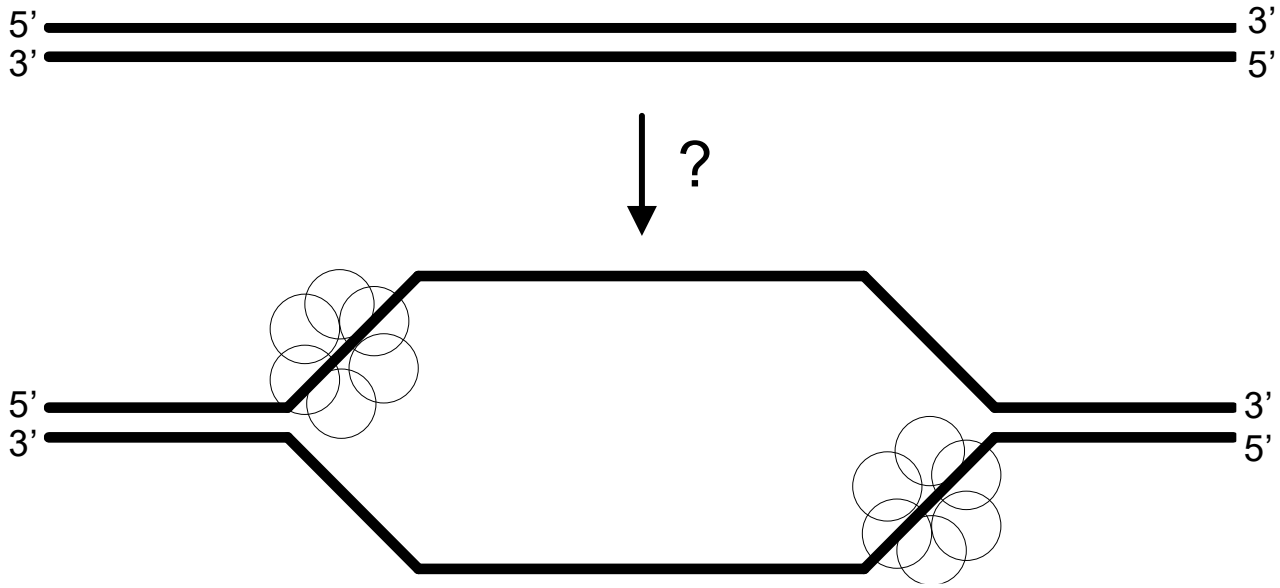
ORC:Cdc6:Cdt1:MCM (OCCM)

Post-insertion state

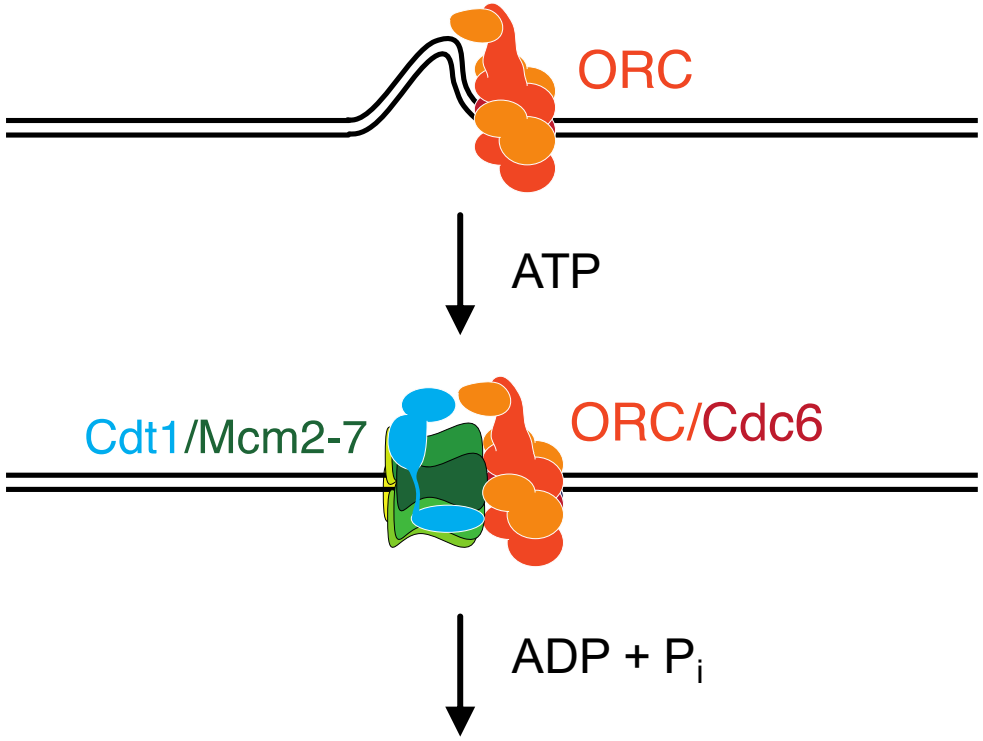


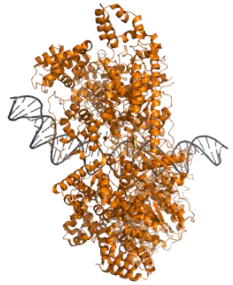
Yuan et al. PNAS. 2020. PMID: 32669428

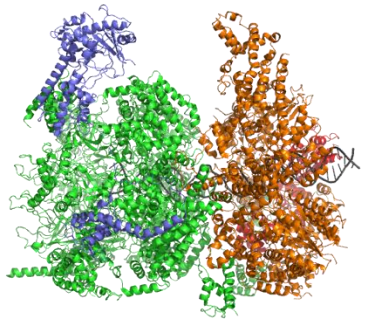
The Eukaryotic Replicative DNA Helicase: MCM2-7



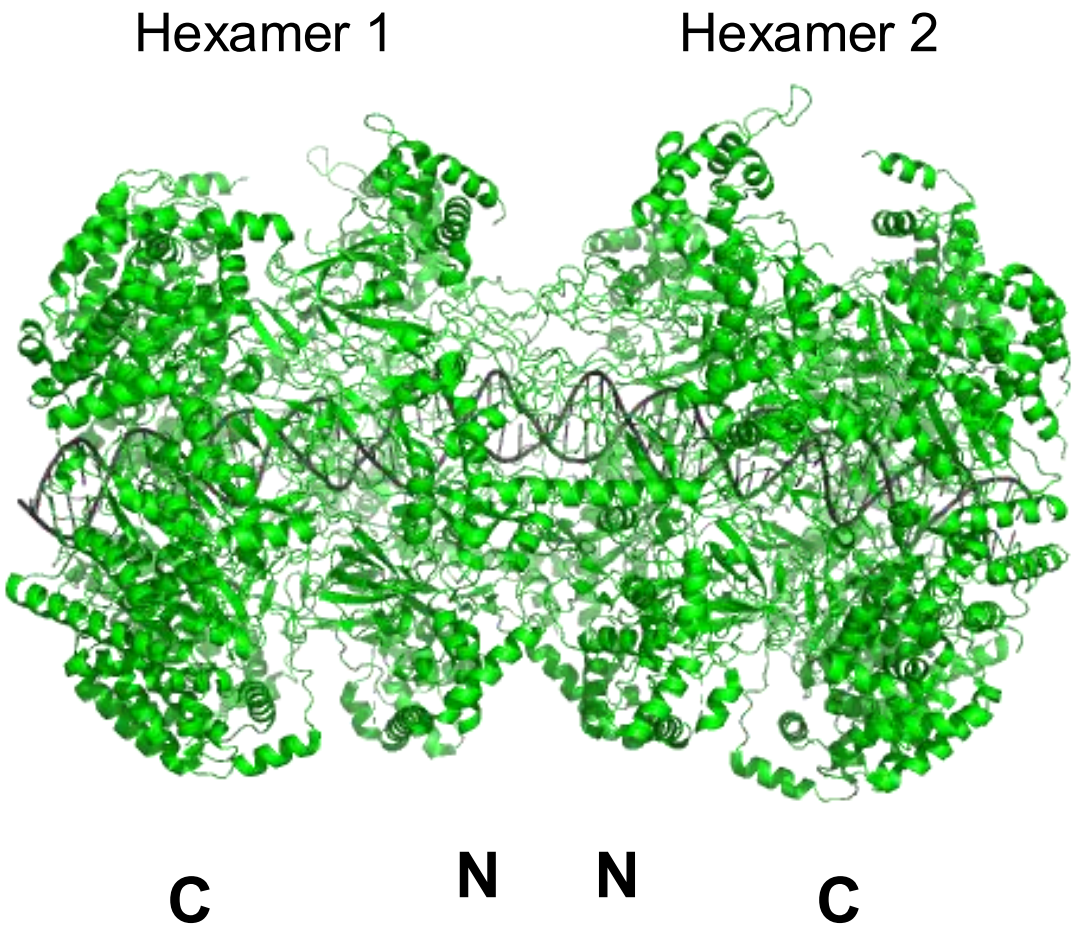
MCM Loading Mechanism





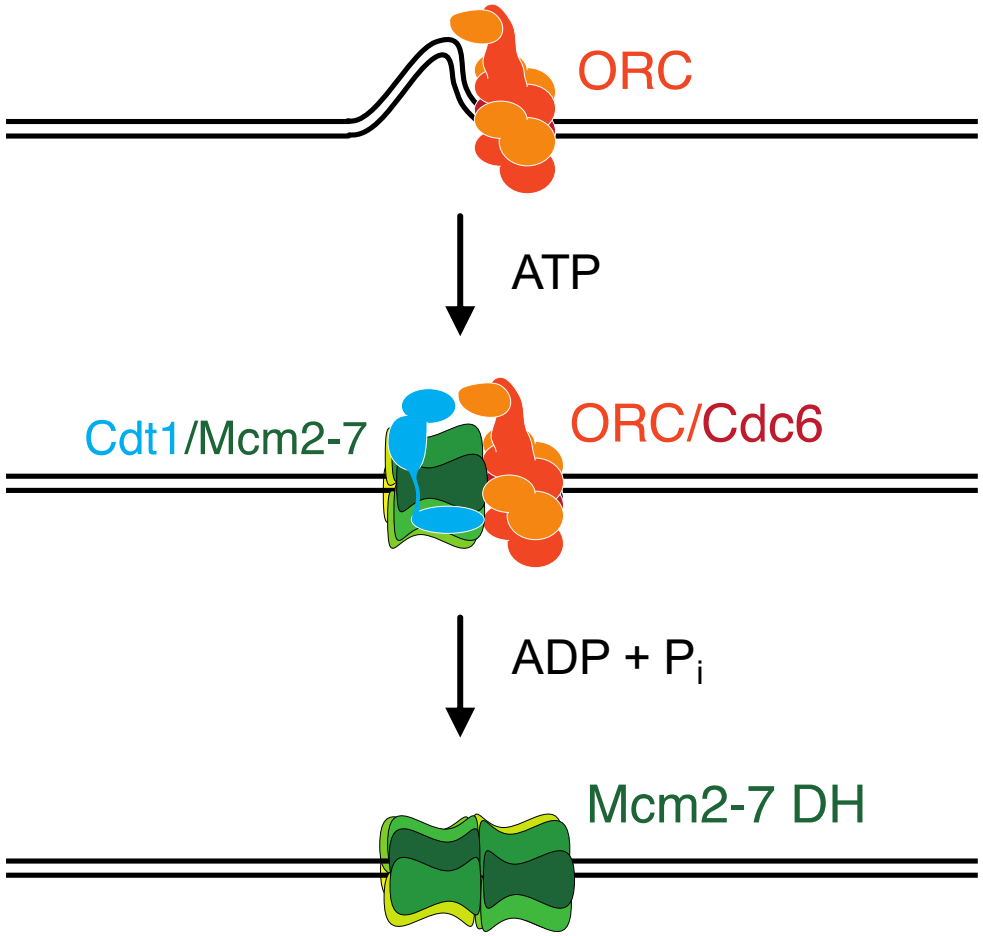


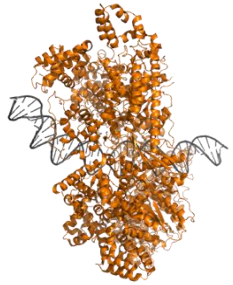
MCM2-7 Are Loaded As Double Hexamers

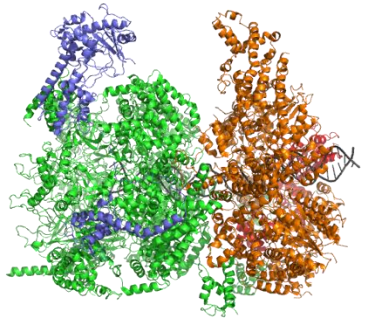


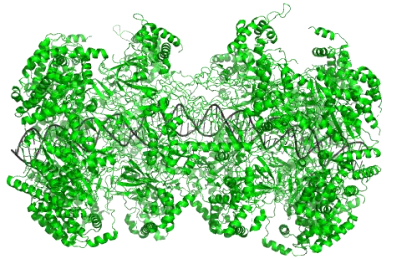
Abid Ali F et al. Nat Comm. 2017. PMID: 29269875

MCM Loading Mechanism

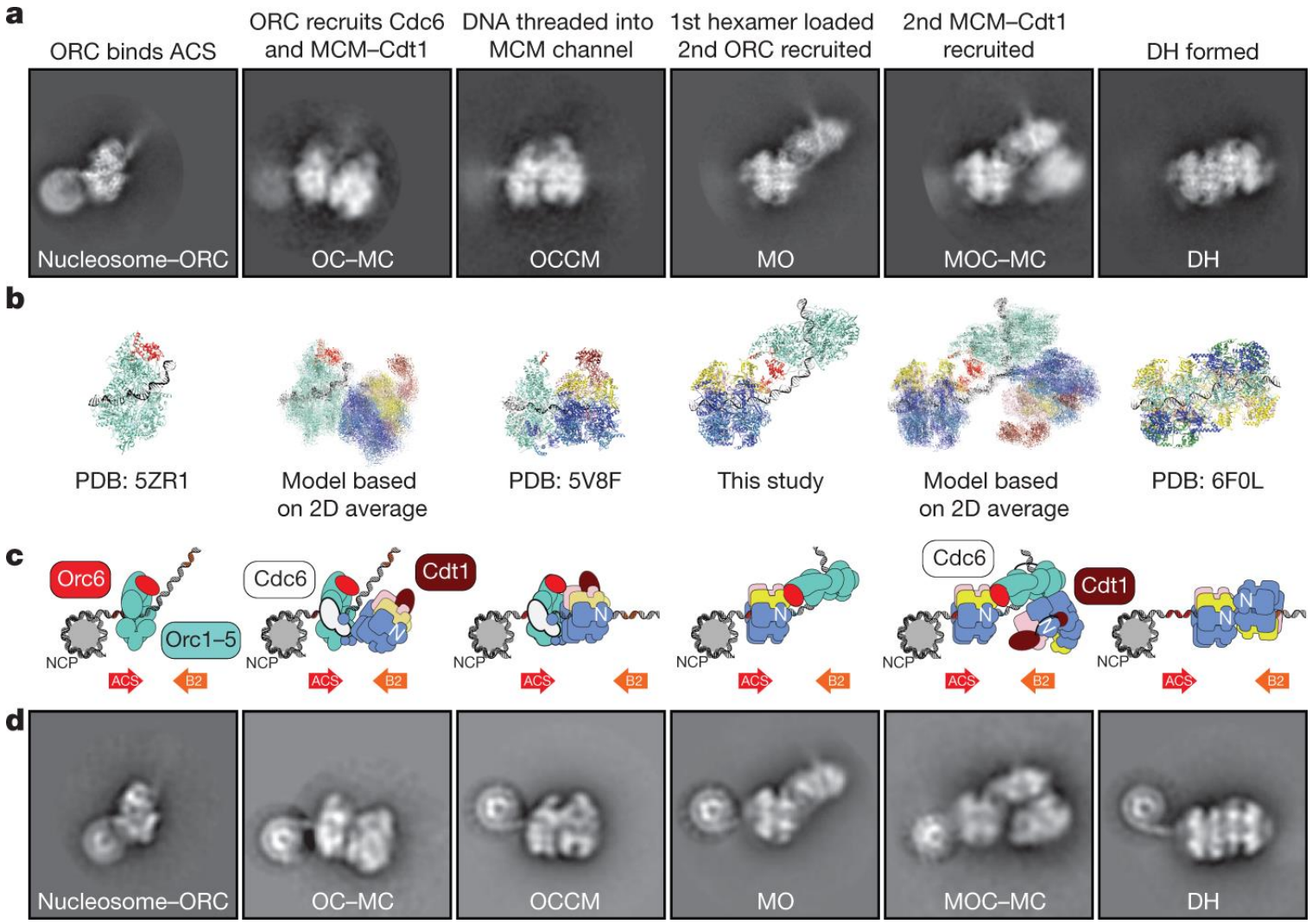






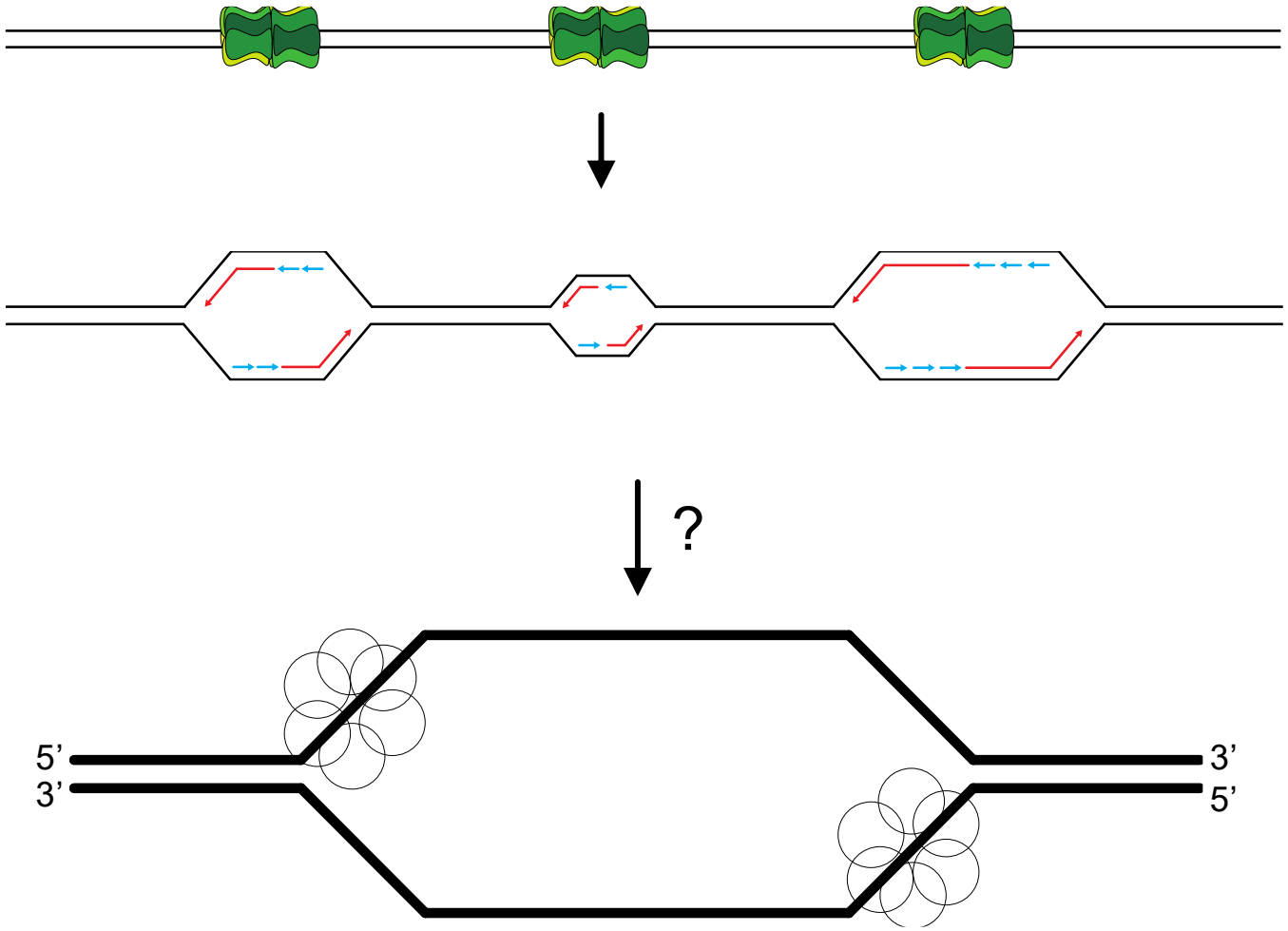


An ORC Flip Enables MCM DH Loading



Miller et al., Nature, 2019, PMID 31748745

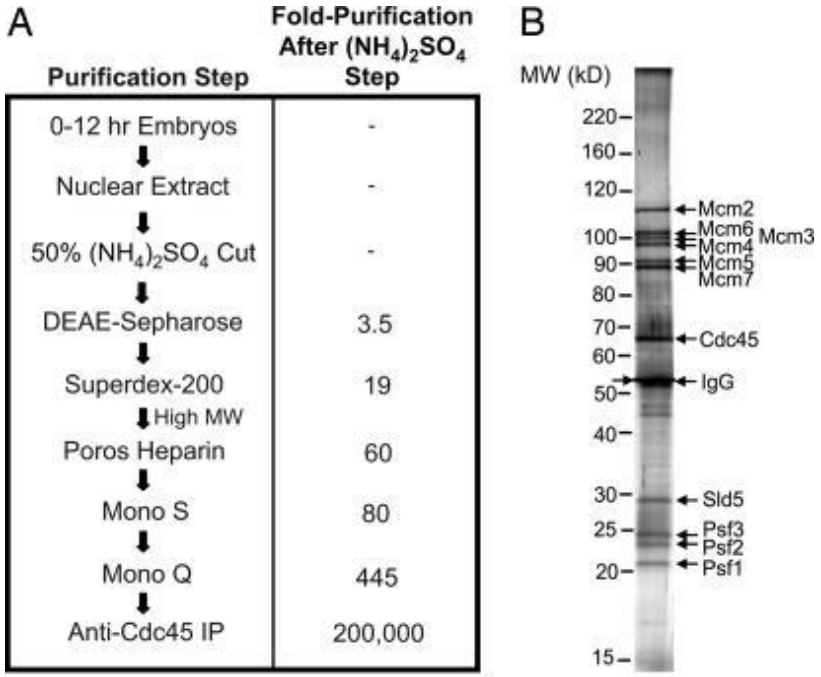
MCM Double Hexamers Mark Replication Origins

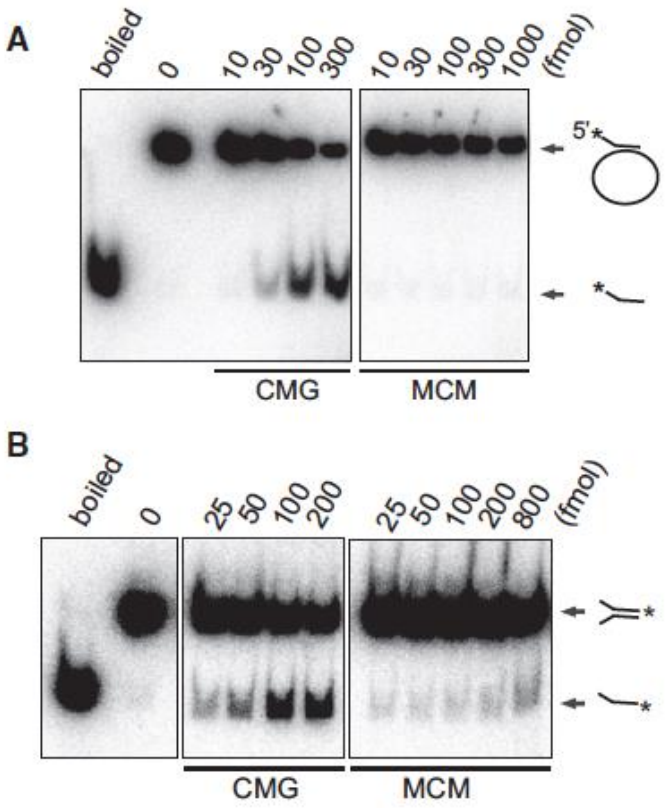


The CMG (Cdc45:MCM:GINS) Helicase

A

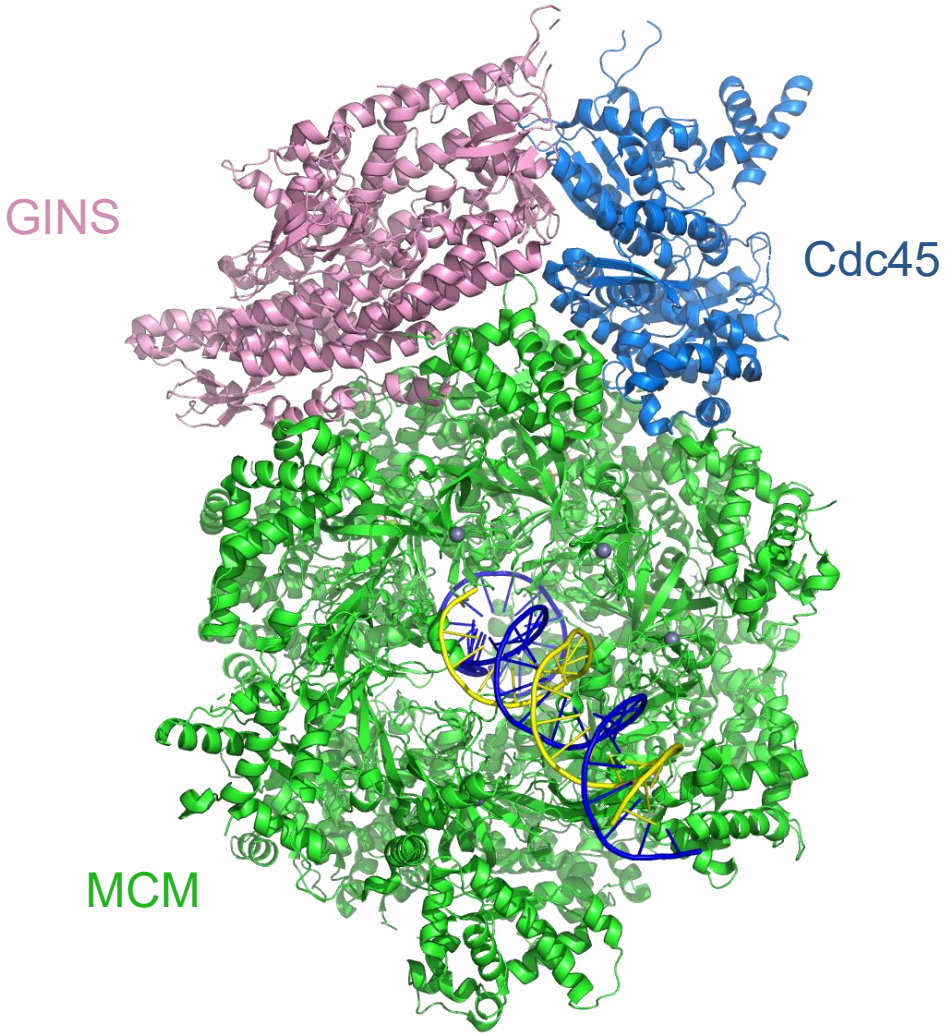
Purification Step	Fold-Purification After $(NH_4)_2SO_4$ Step
0-12 hr Embryos	-
⬇ Nuclear Extract	-
⬇ 50% $(NH_4)_2SO_4$ Cut	-
⬇ DEAE-Sepharose	3.5
⬇ Superdex-200	19
⬇ High MW Poros Heparin	60
⬇ Mono S	80
⬇ Mono Q	445
⬇ Anti-Cdc45 IP	200,000



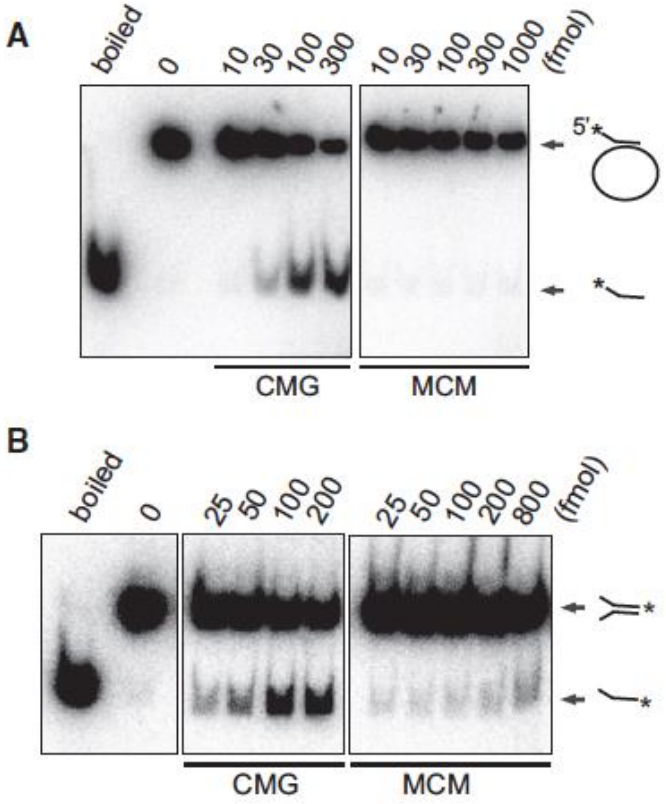


SE Moyer et al. PNAS 2006

The CMG (Cdc45:MCM:GINS) Helicase

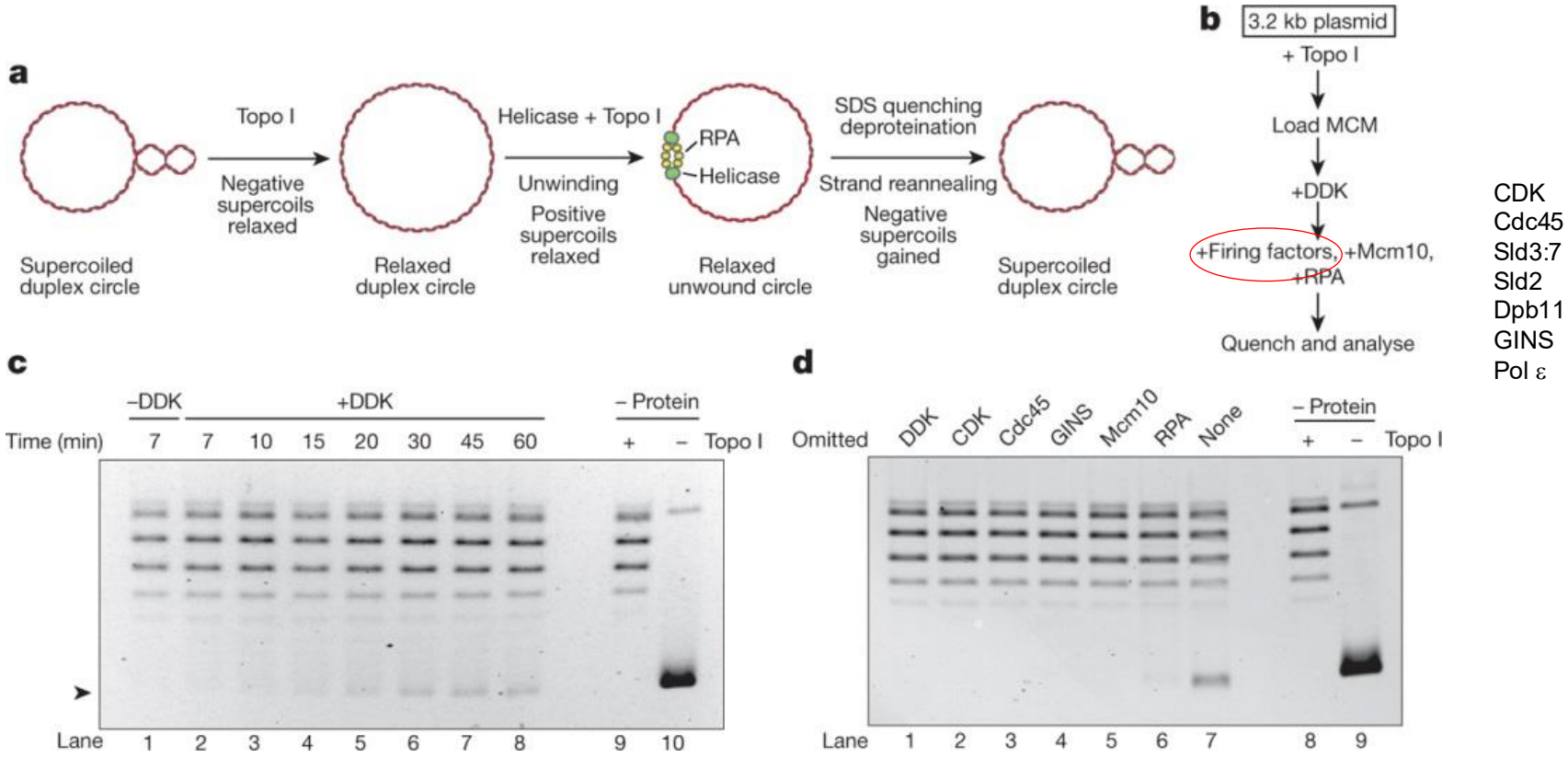


Baretic et al. Mol Cell 2020



SE Moyer et al. PNAS 2006

Activation Of The CMG Helicase

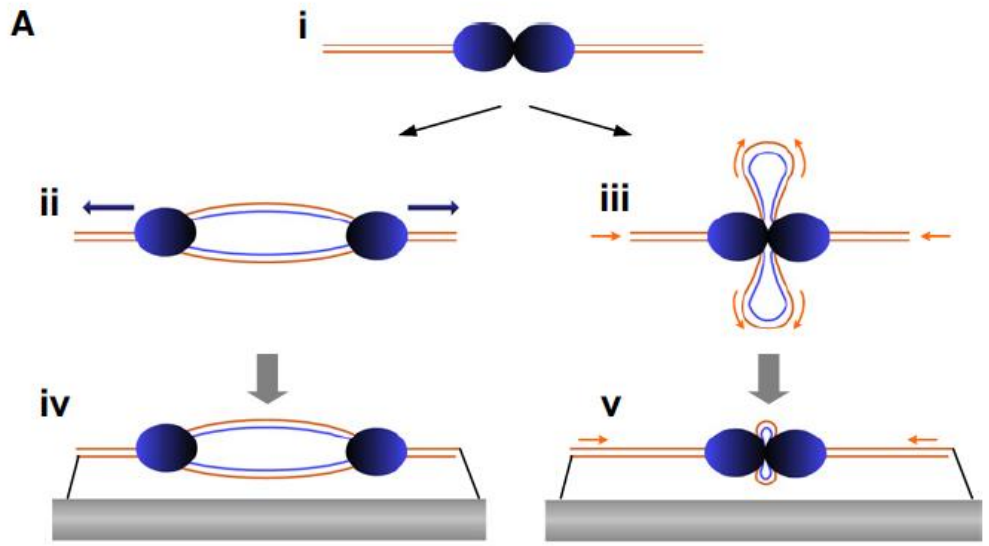


CDK
Cdc45
Sld3:7
Sld2
Dpb11
GINS
Pol ε

M E Douglas *et al. Nature* **555**, 265–268 (2018) doi:10.1038/nature25787

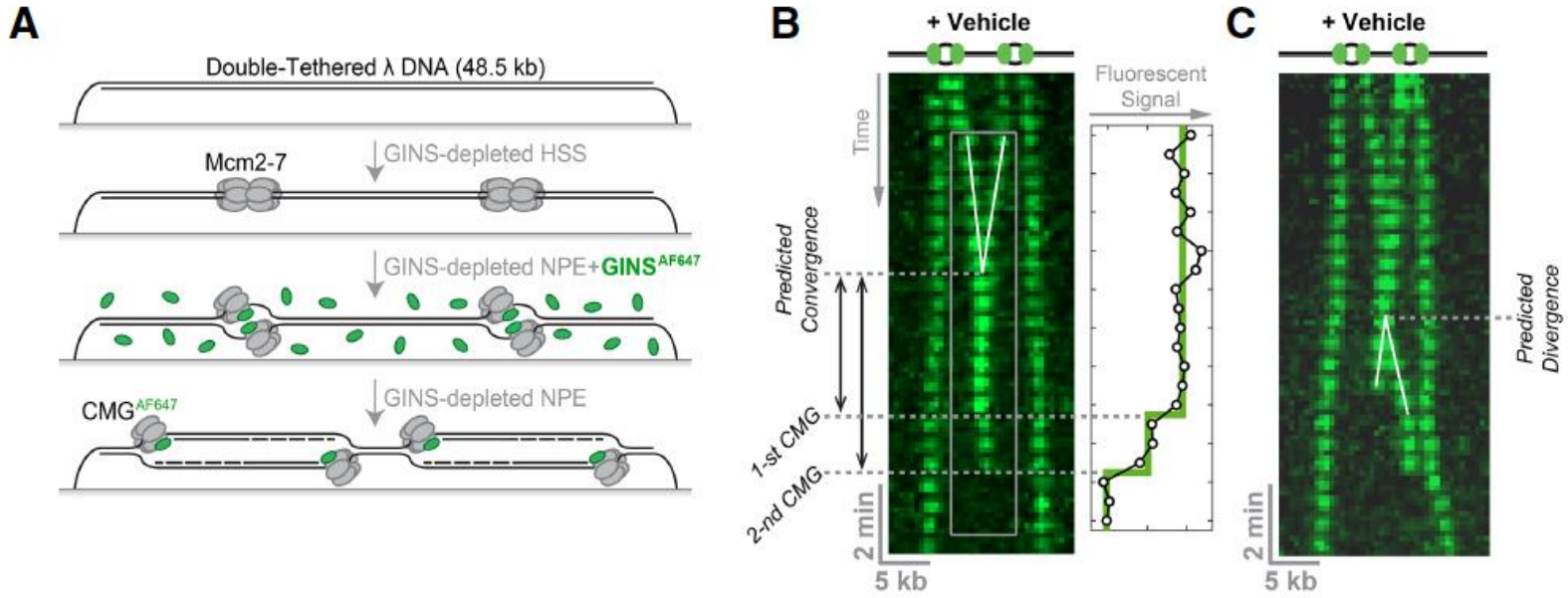
MCM DHs Separate During Origin Firing

Eukaryotic replisomes can progress uncoupled from each other



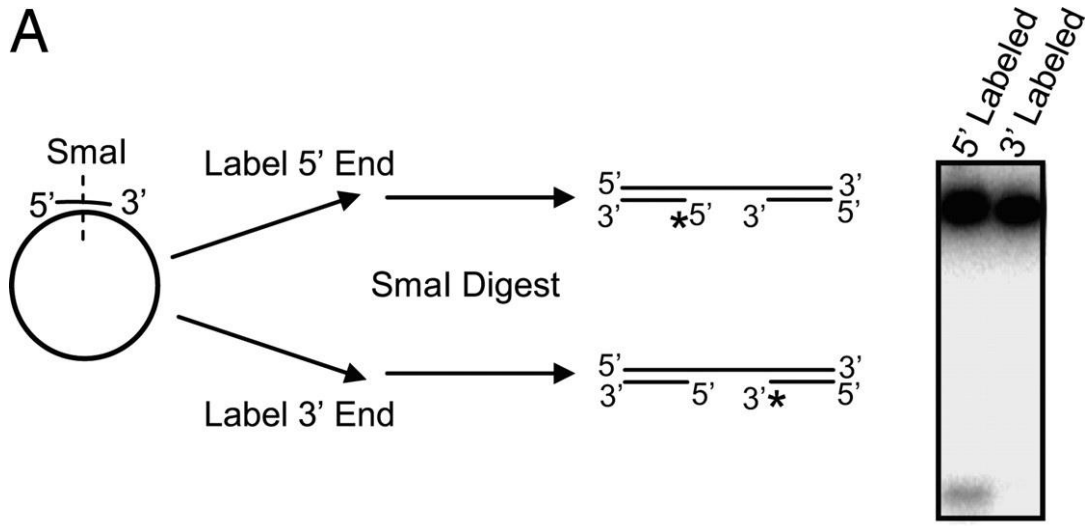
Yardimci H et al., Mol Cell, 2010

MCM DHs Separate During Origin Firing



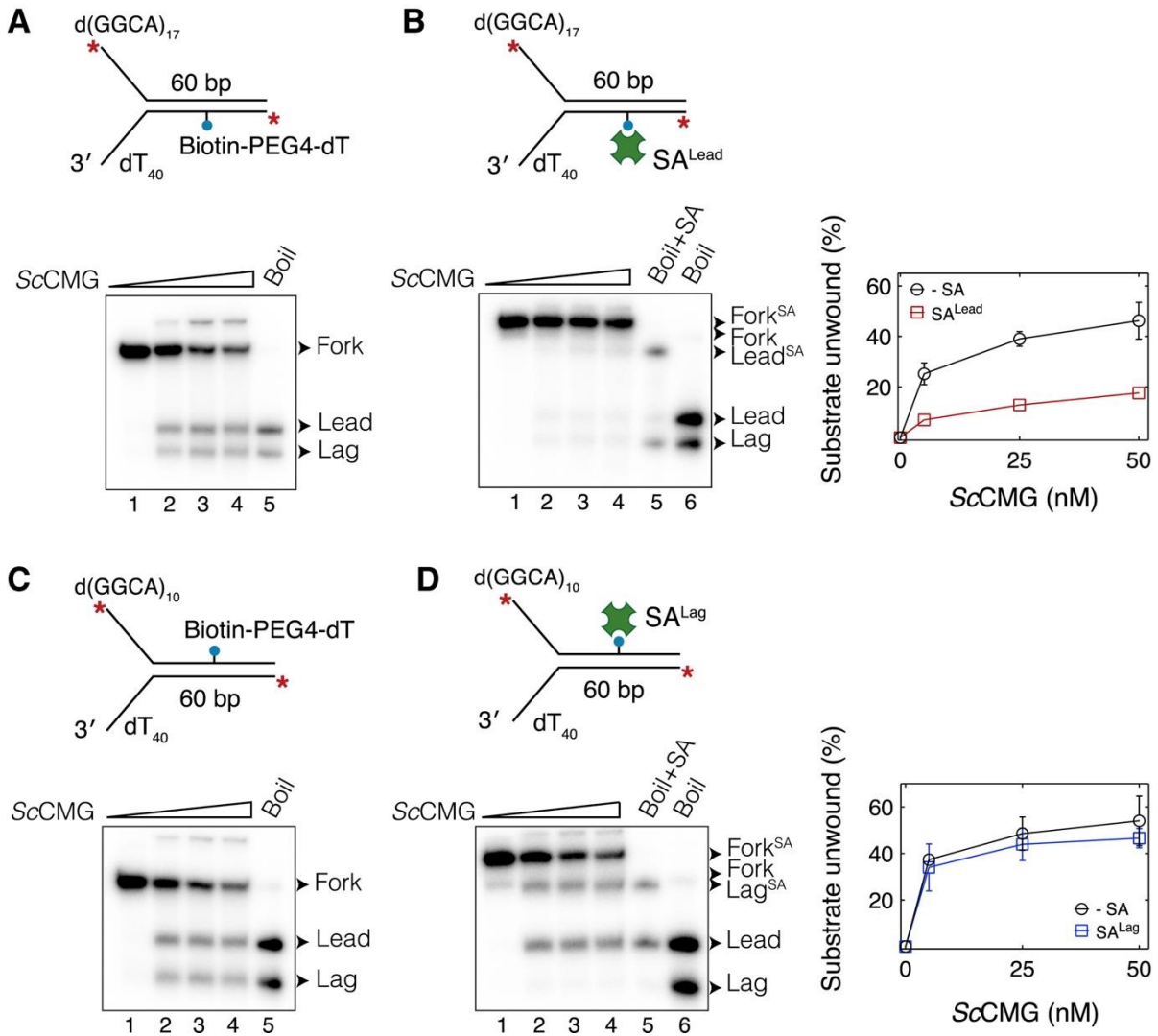
Low et al., G&D, 2020, PMID 32943574

The CMG Is A 3’-5’ DNA Helicase



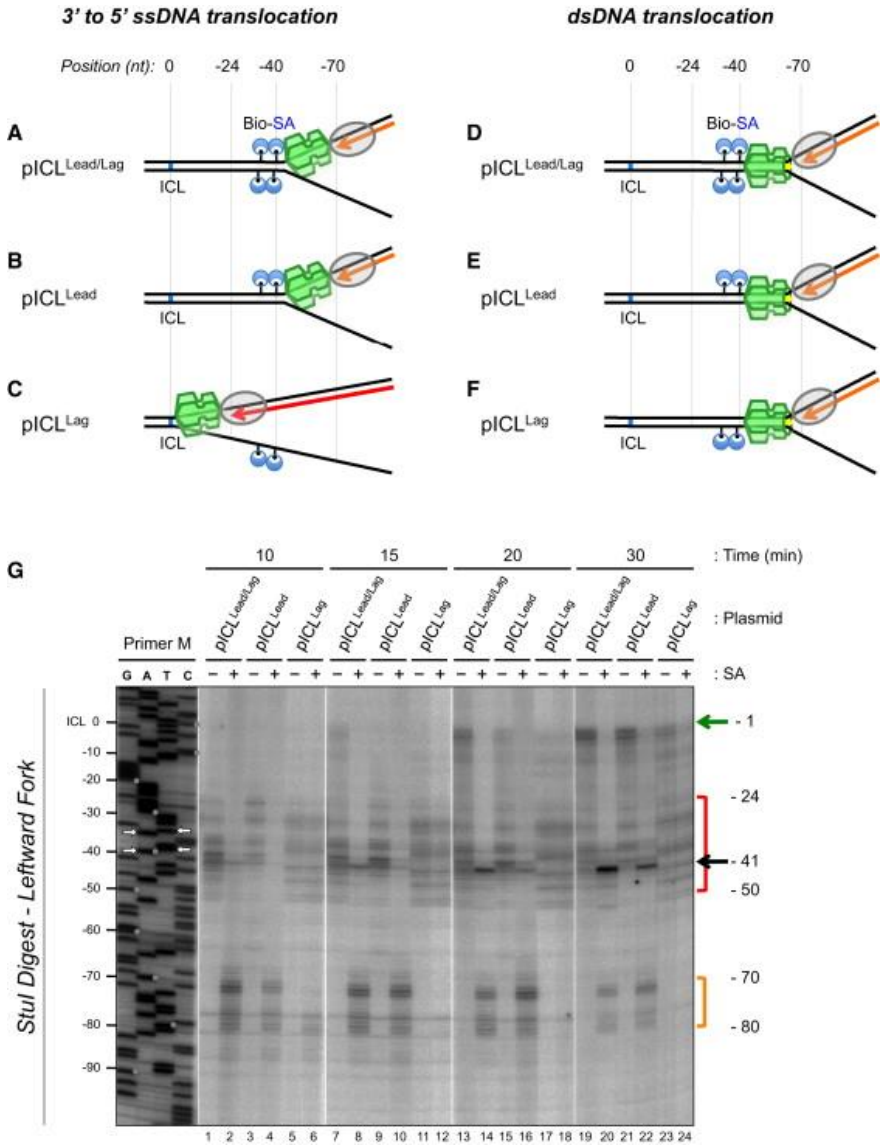
SE Moyer et al. PNAS 2006

CMG Encircles The Leading Strand



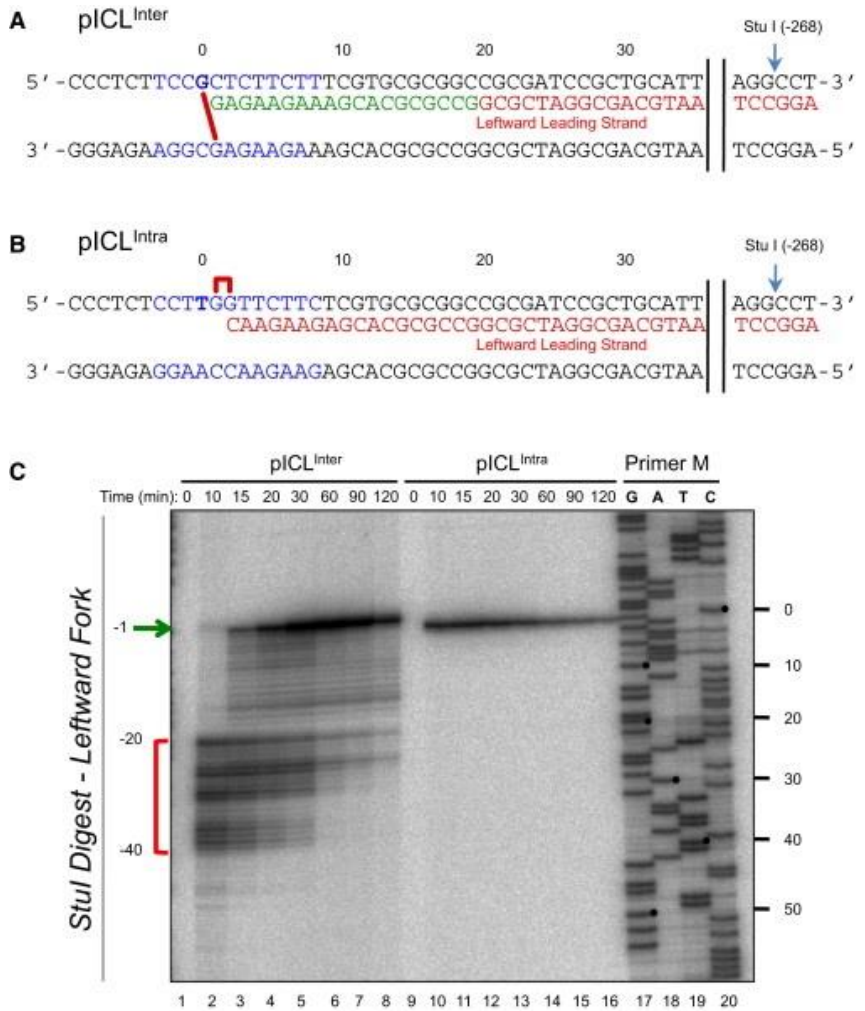
Kose et al., Cell Reports, 2019, PMID 30784593

CMG Encircles The Leading Strand



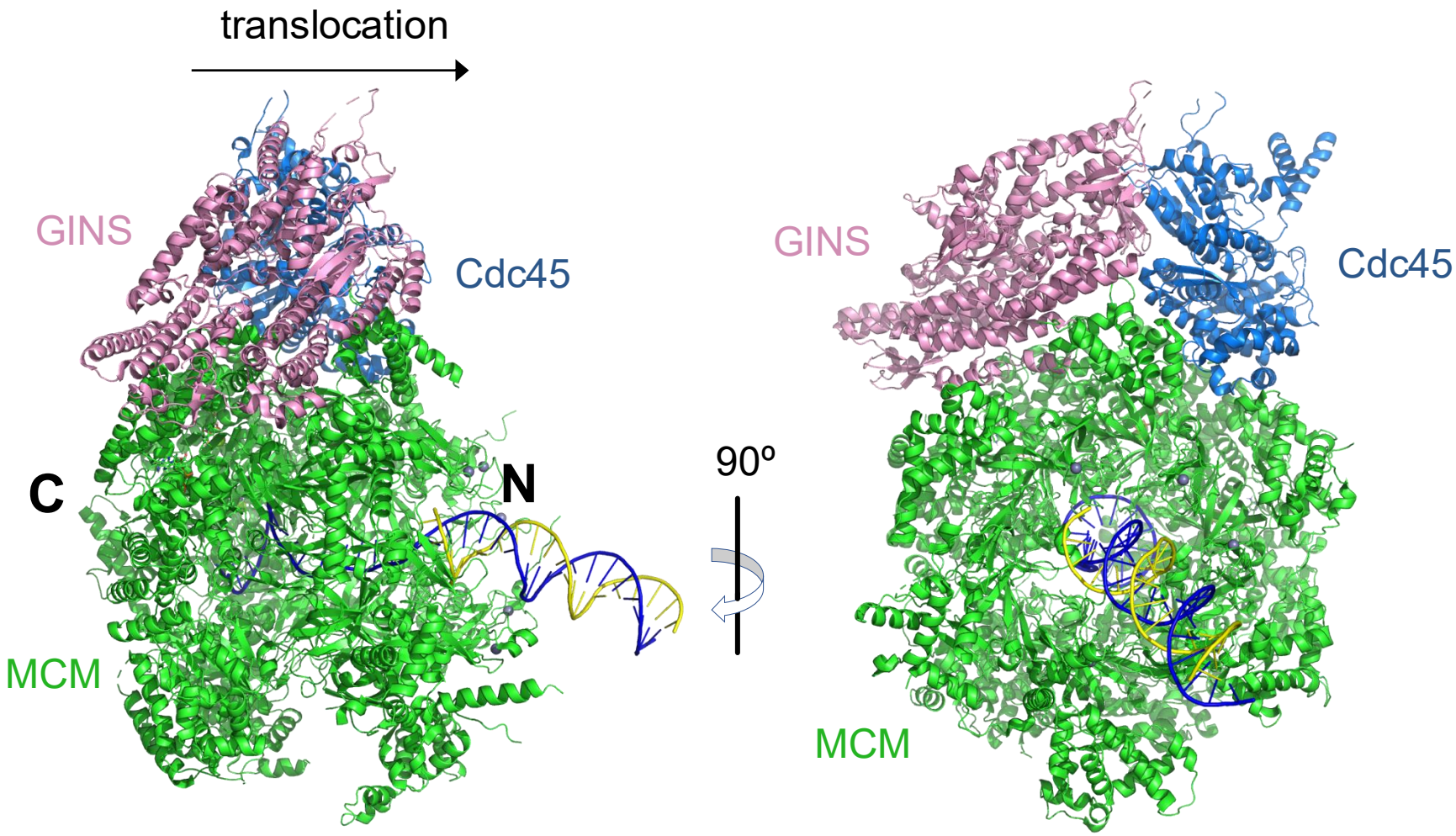
Fu YV et al., Cell 2011. PMID: 21925316

CMG Encircles The Leading Strand



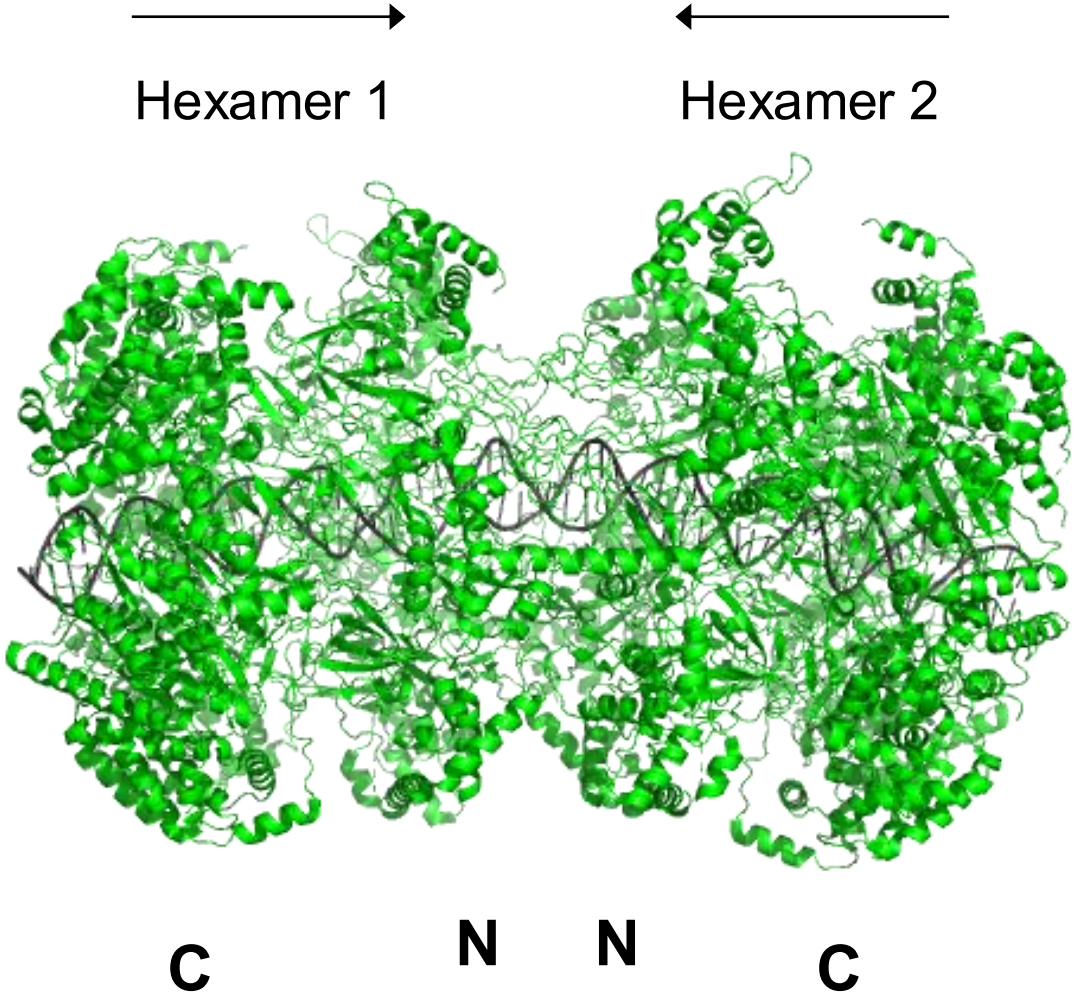
Fu YV et al., Cell 2011. PMID: 21925316

The CMG (Cdc45:MCM:GINS) Helicase

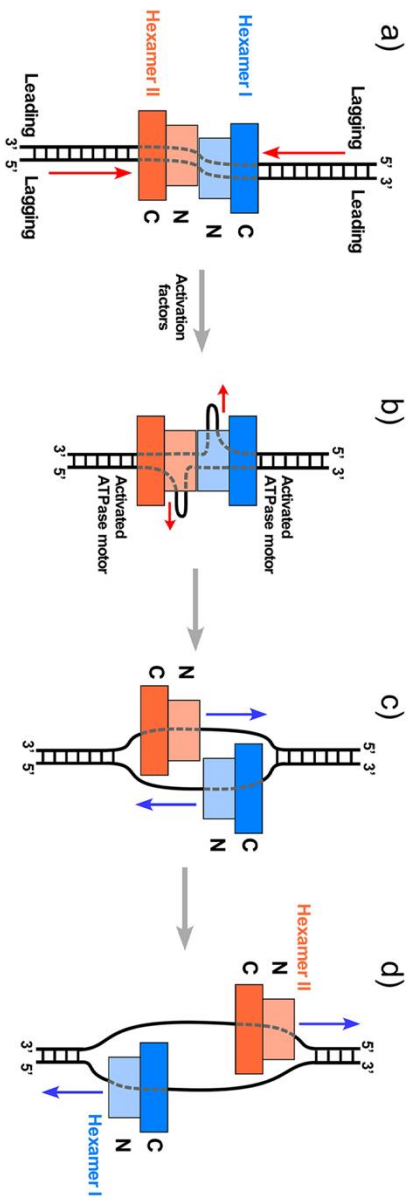


Baretic et al., Mol Cell, 2020

MCM2-7 Are Loaded As Head-To-Head Double Hexamers

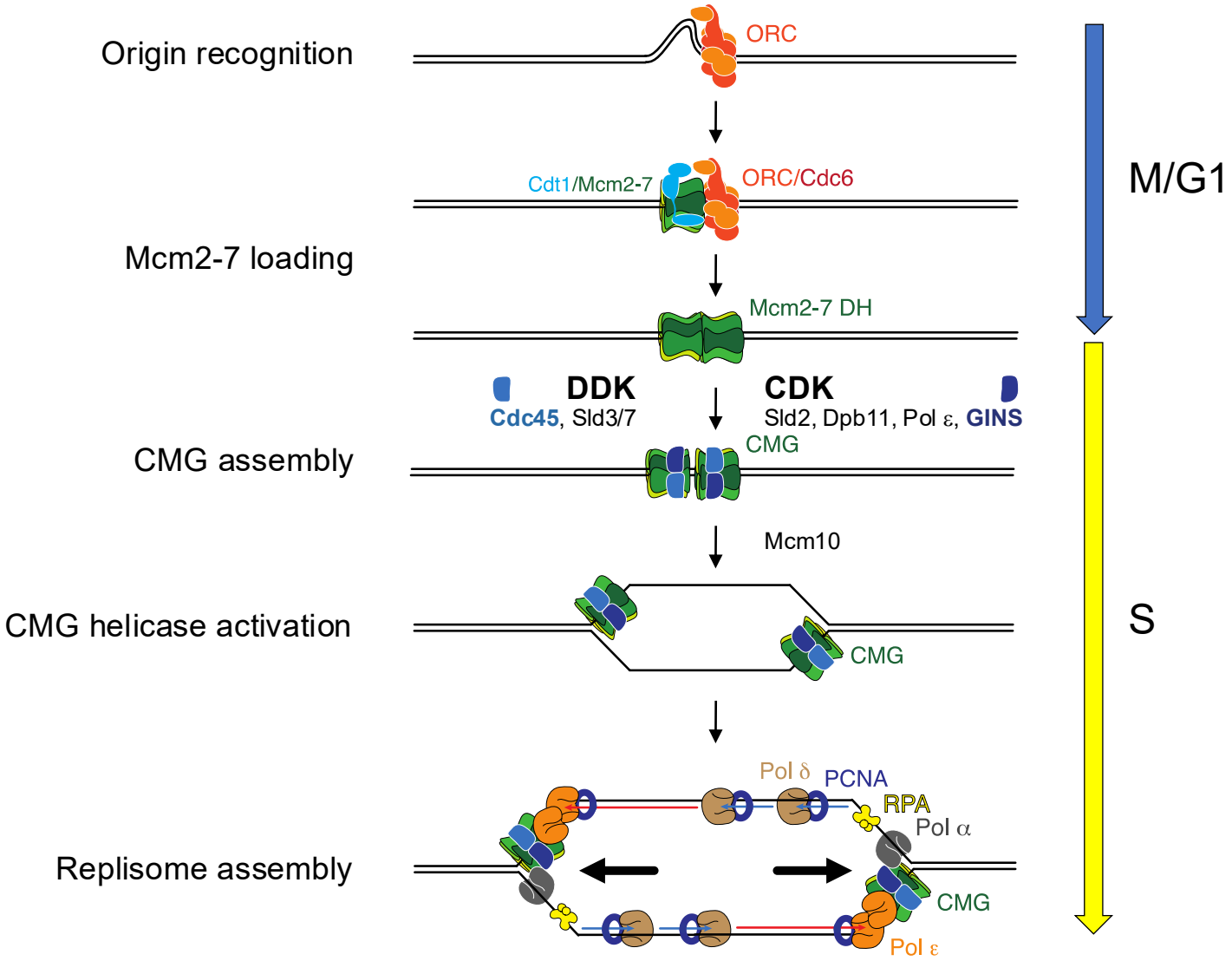


Mechanism To Ensure Bidirectional Origin Firing

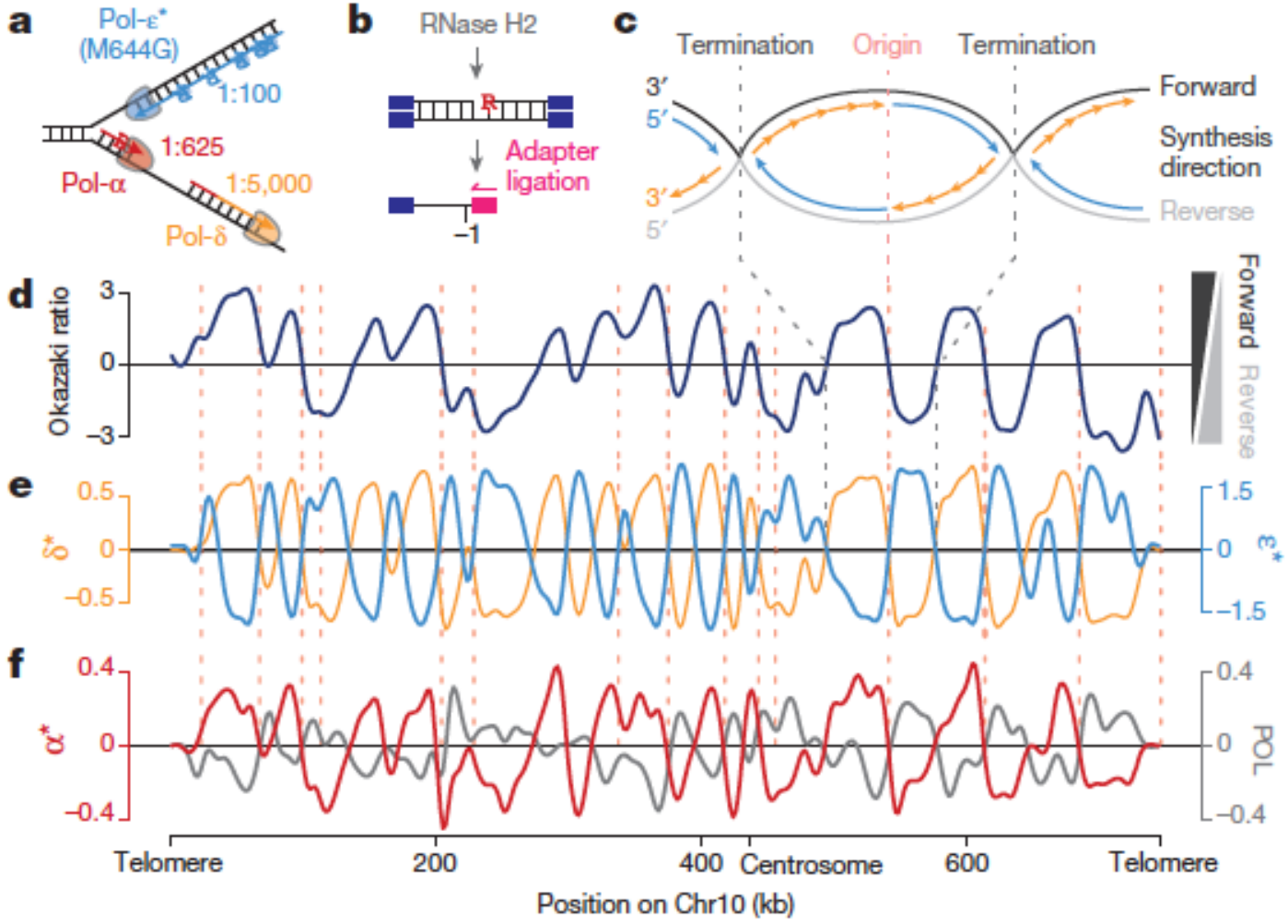


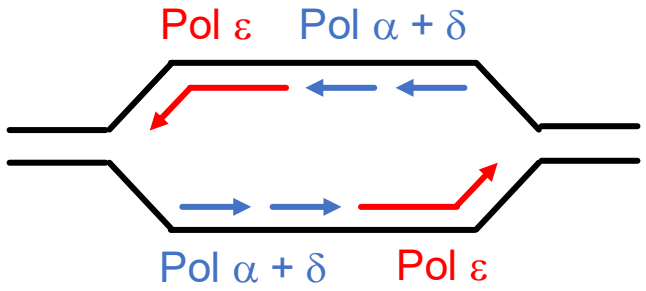
Li H et al, Bioessays, 2018, PMID 29405332

Mechanism Of DNA Replication



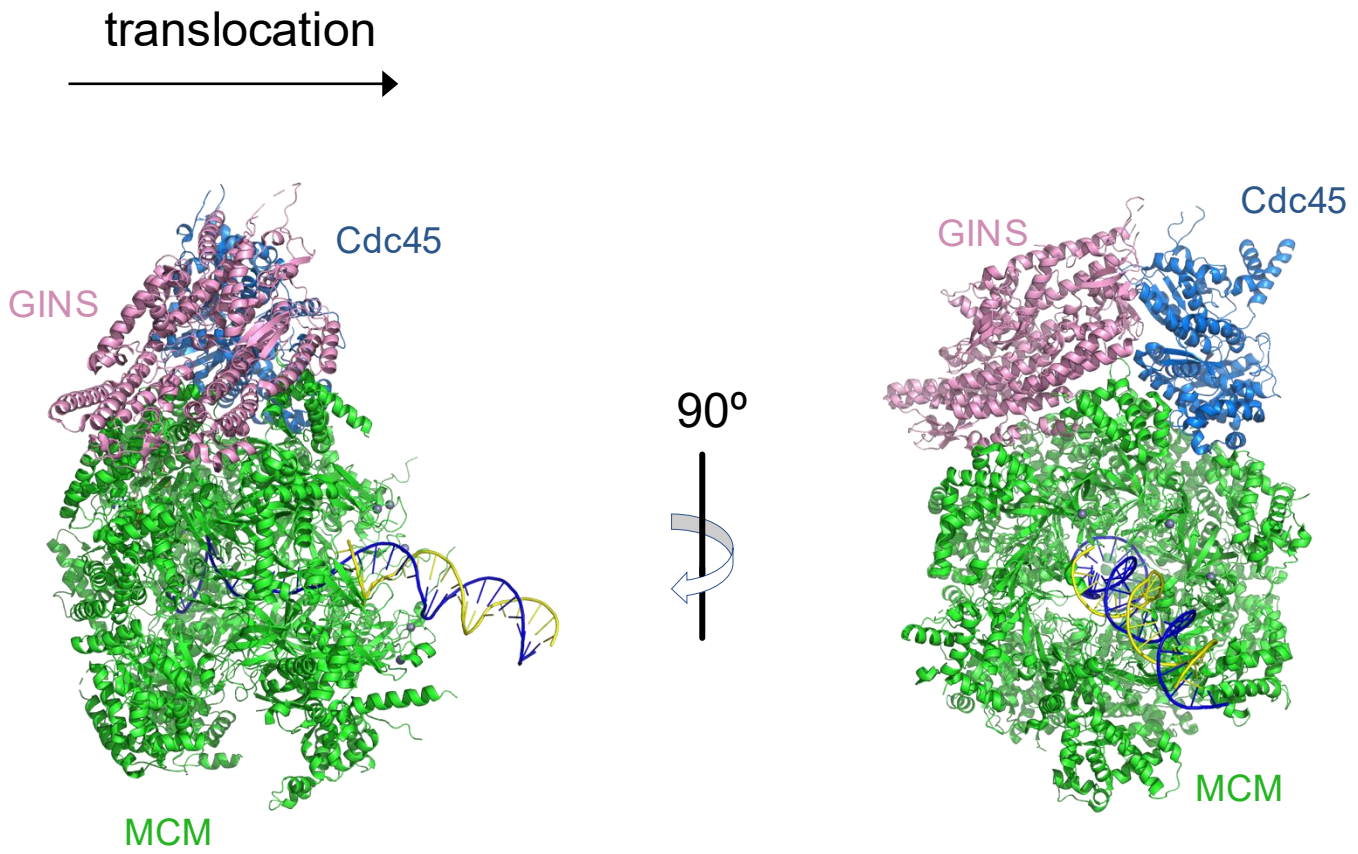
Division Of Labor At Replication Forks





Reijns MAM et al, Nature 2015, PMID **25624100**

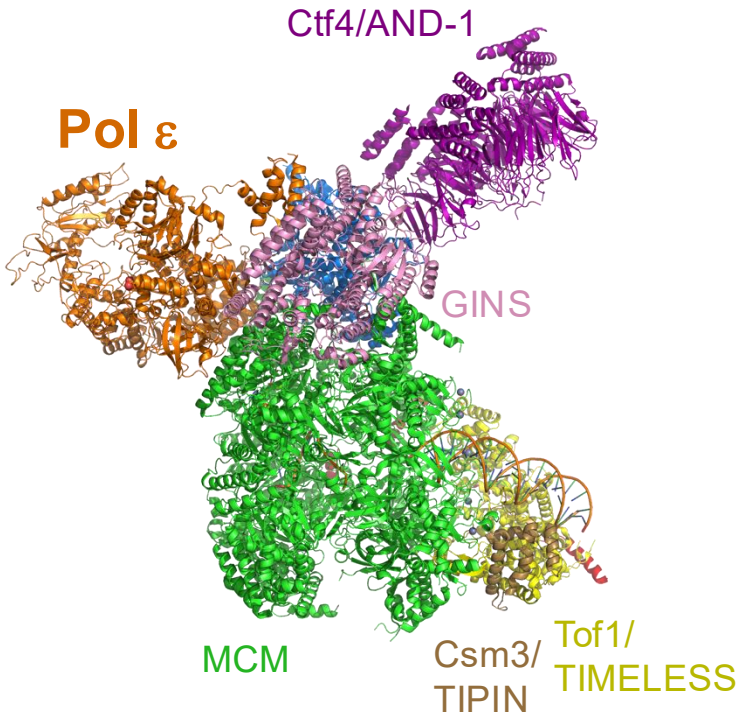
CMG (Cdc45:MCM:GINS) Helicase



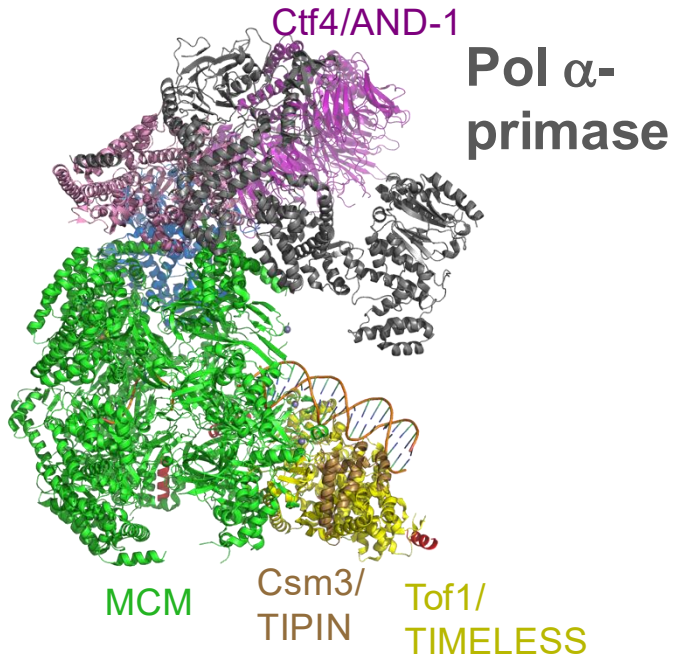
Baretic et al., Mol Cell, 2020

CMG Forms The Core Of The Replisome

translocation

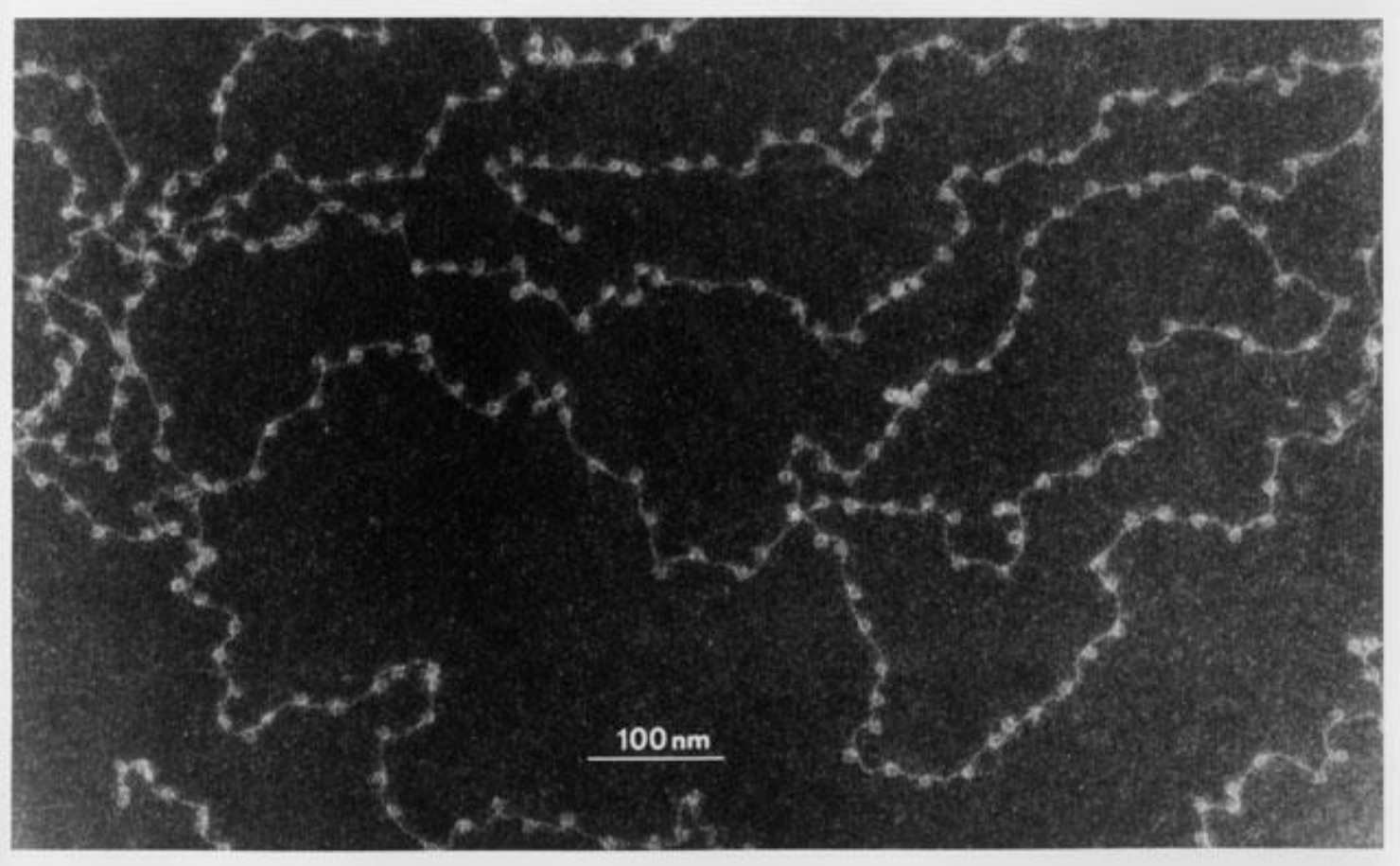


Jones et al., EMBO J, 2023



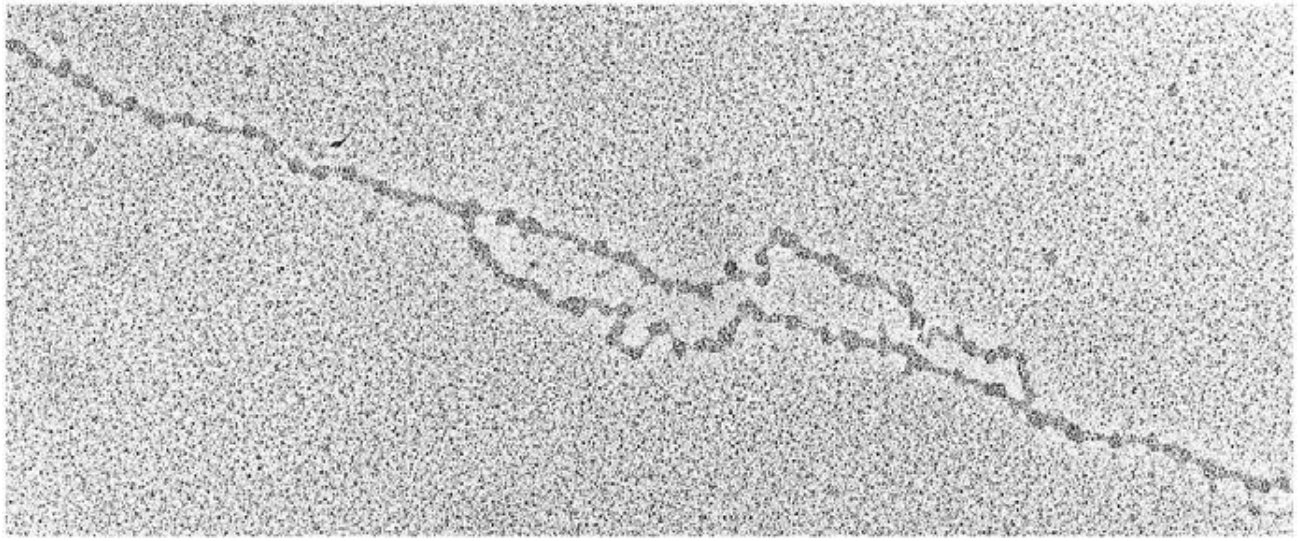
Jones et al., Mol Cell, 2023

Nuclear DNA Is Packaged Into Chromatin



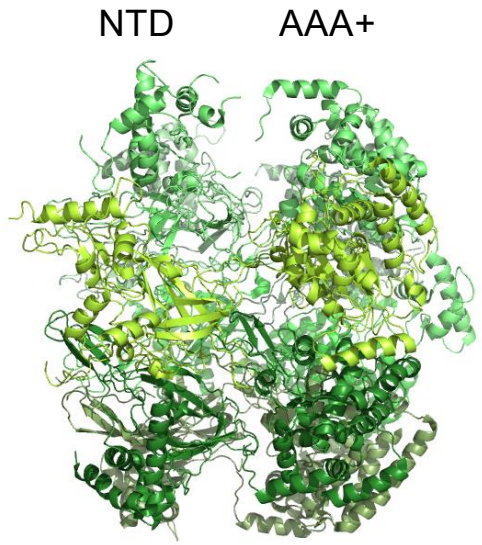
AL Olins and DE Olins. Science 1974; 183:330-2

Chromatin Is Rapidly Reassembled At Replication Forks

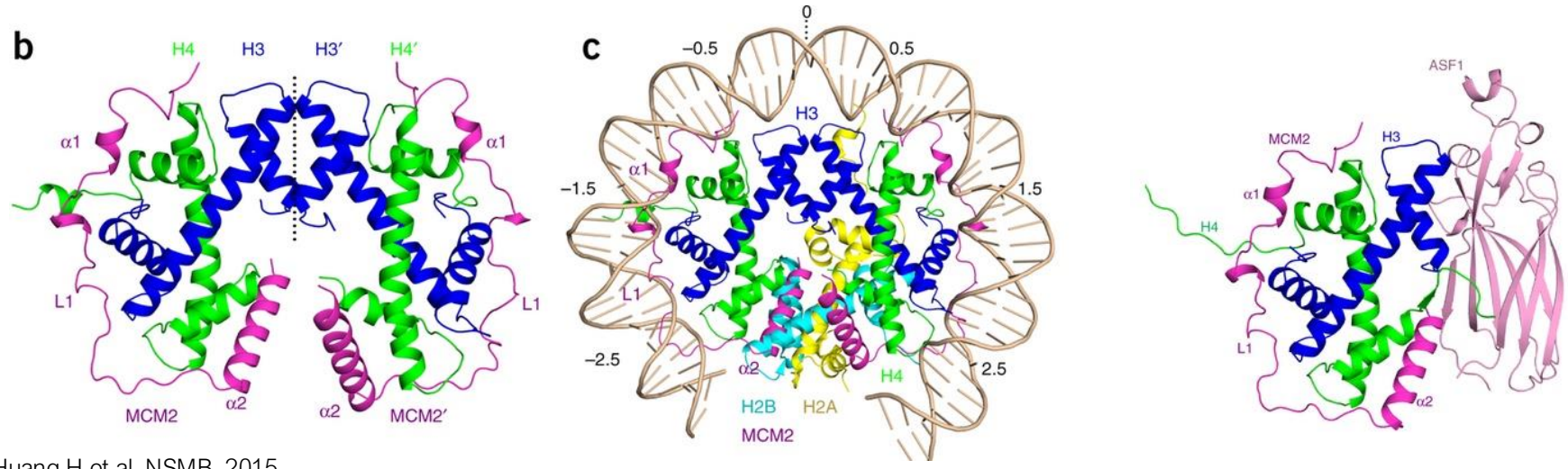


SL McKnight and OL Miller, Jr. Cell 1977; 12:795-804

The Mcm2 N-Terminal Tail Is A Histone H3/H4 Chaperone

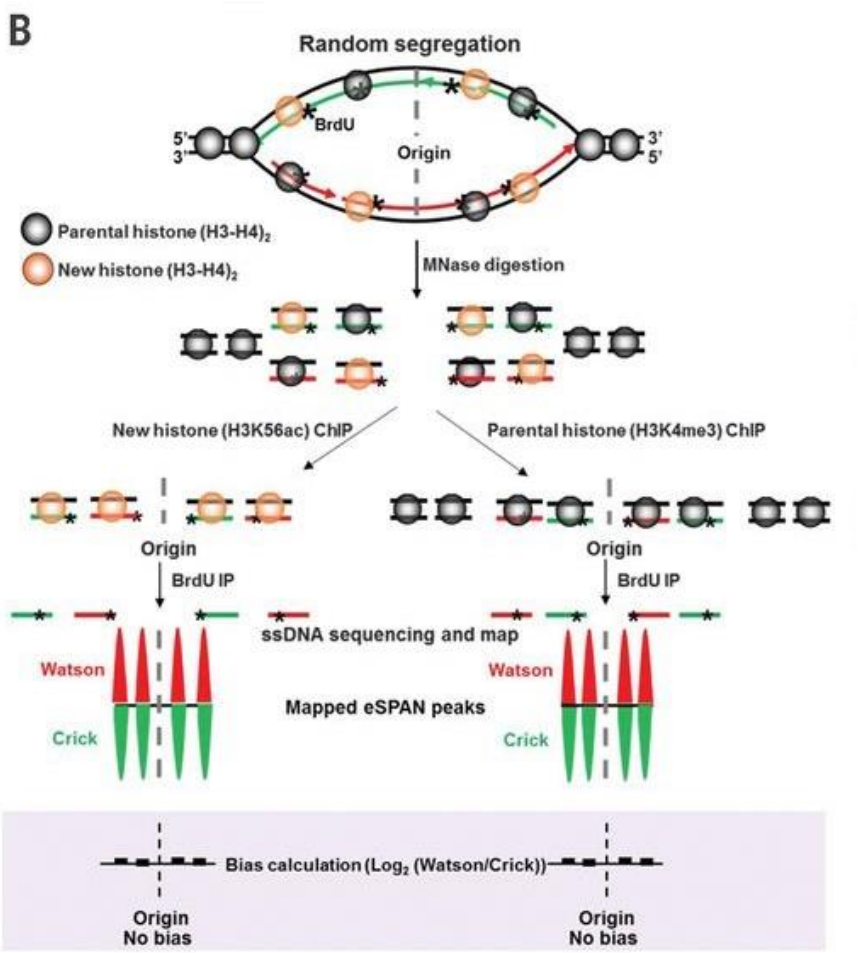


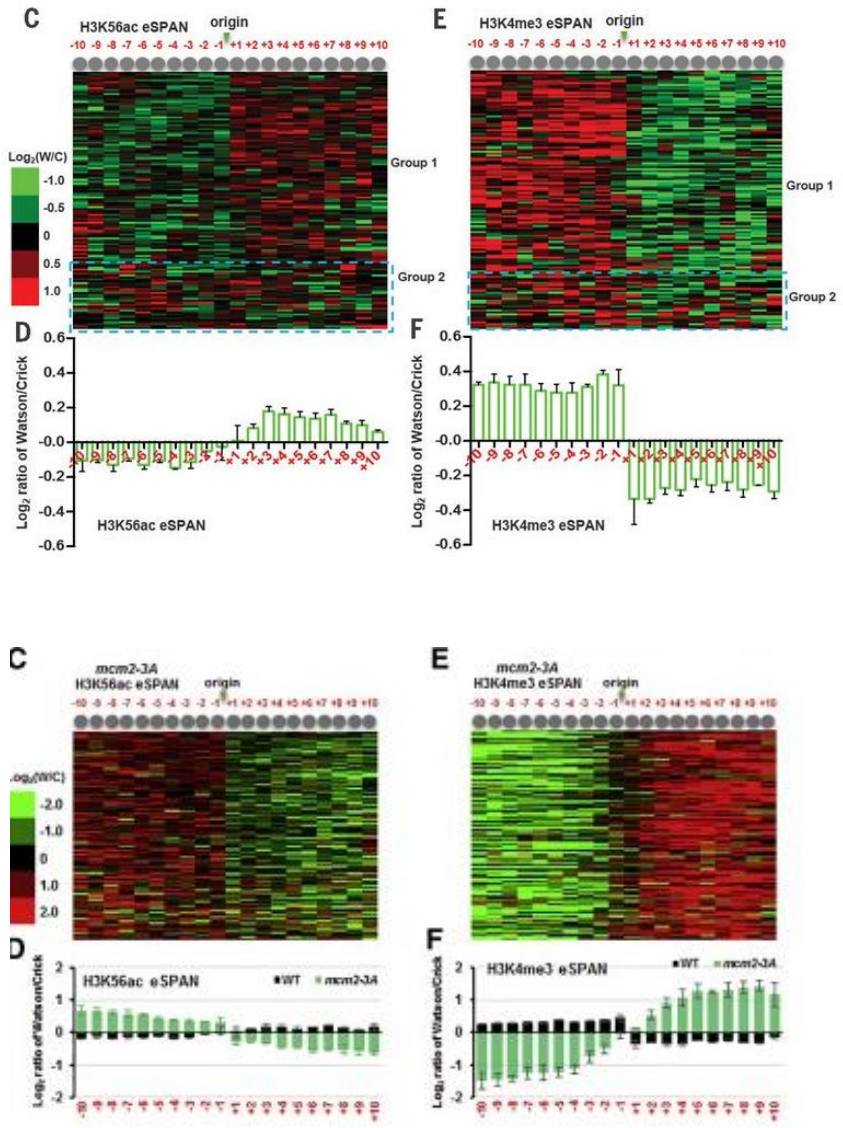




Huang H et al, NSMB, 2015

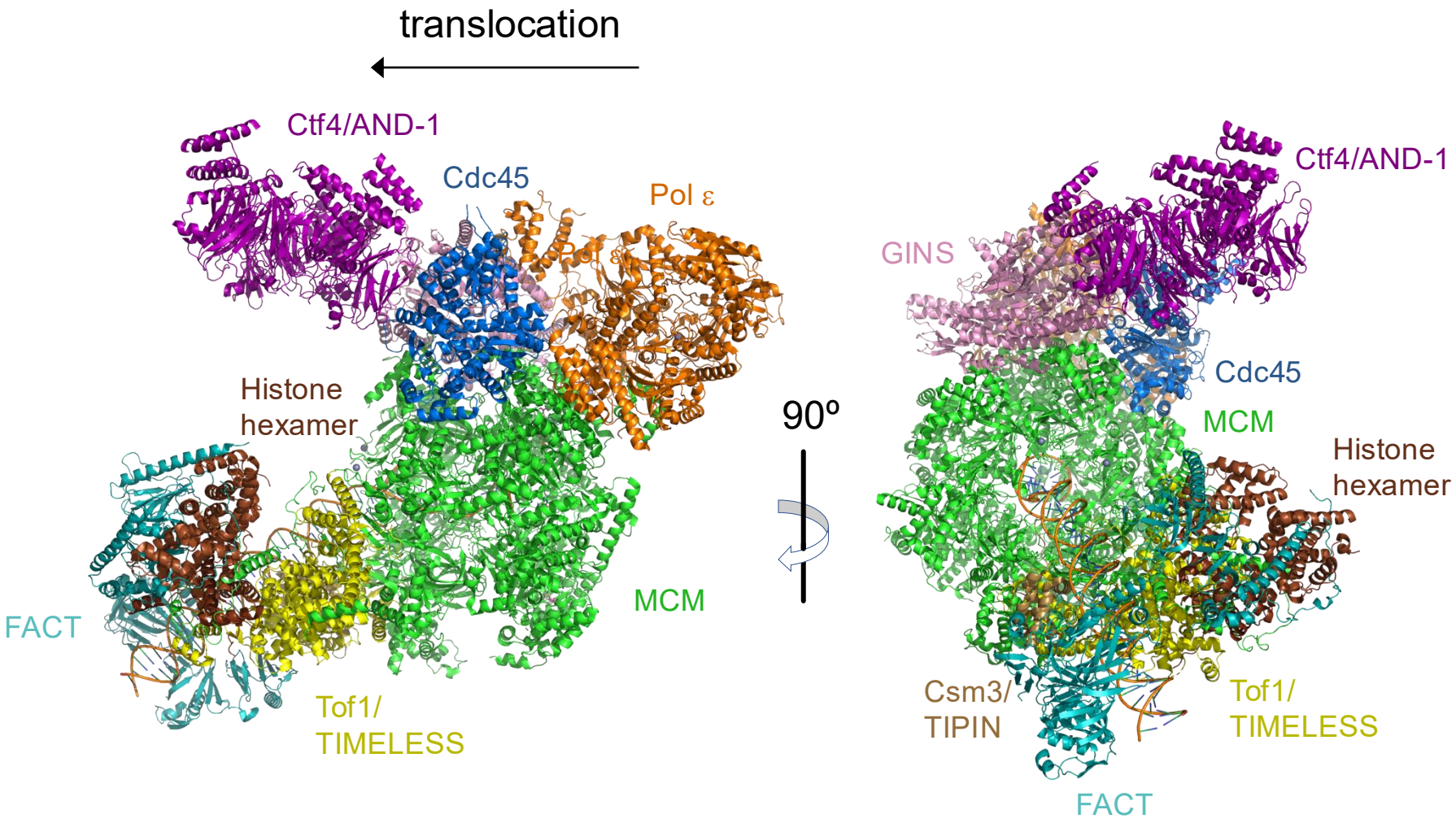
Mcm2 Promotes Parental Histone Segregation To The Lagging Strand





Yu C et al, Science, 2018, PMID 30115745
Gan et al, Mol Cell, 2018, PMID 30244834

CMG Cooperates With Histone Chaperones At The Fork



Li et al., Nature, 2024