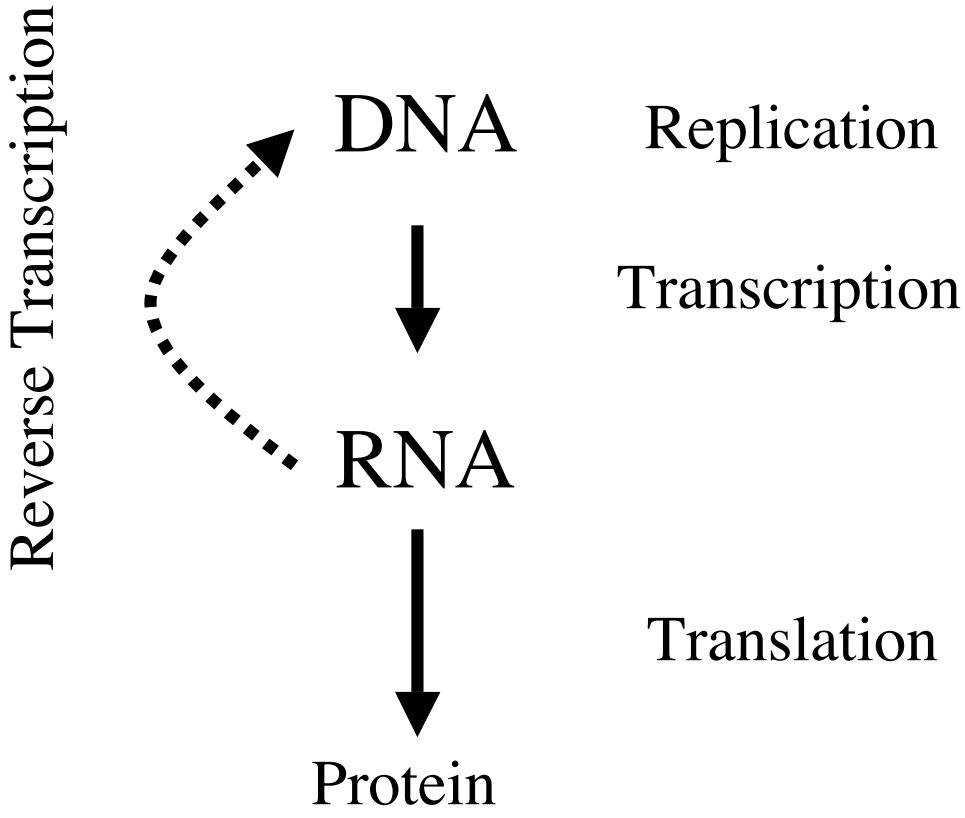
Systems Microbiology

Monday Oct 2 - Ch 7 -Brock

Information flow in biological systems

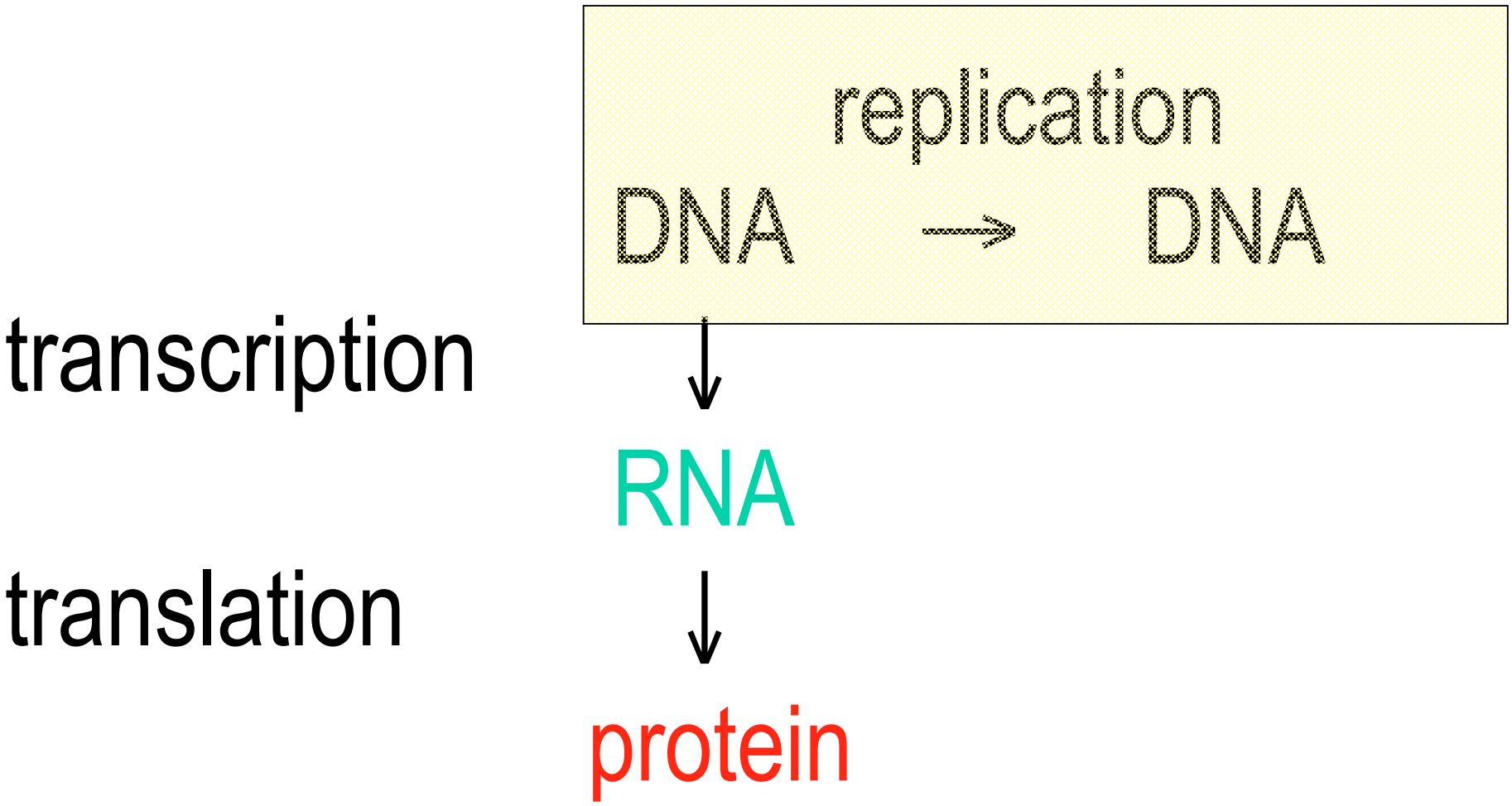
- DNA replication
- Transcription
- Translation

Central Dogma

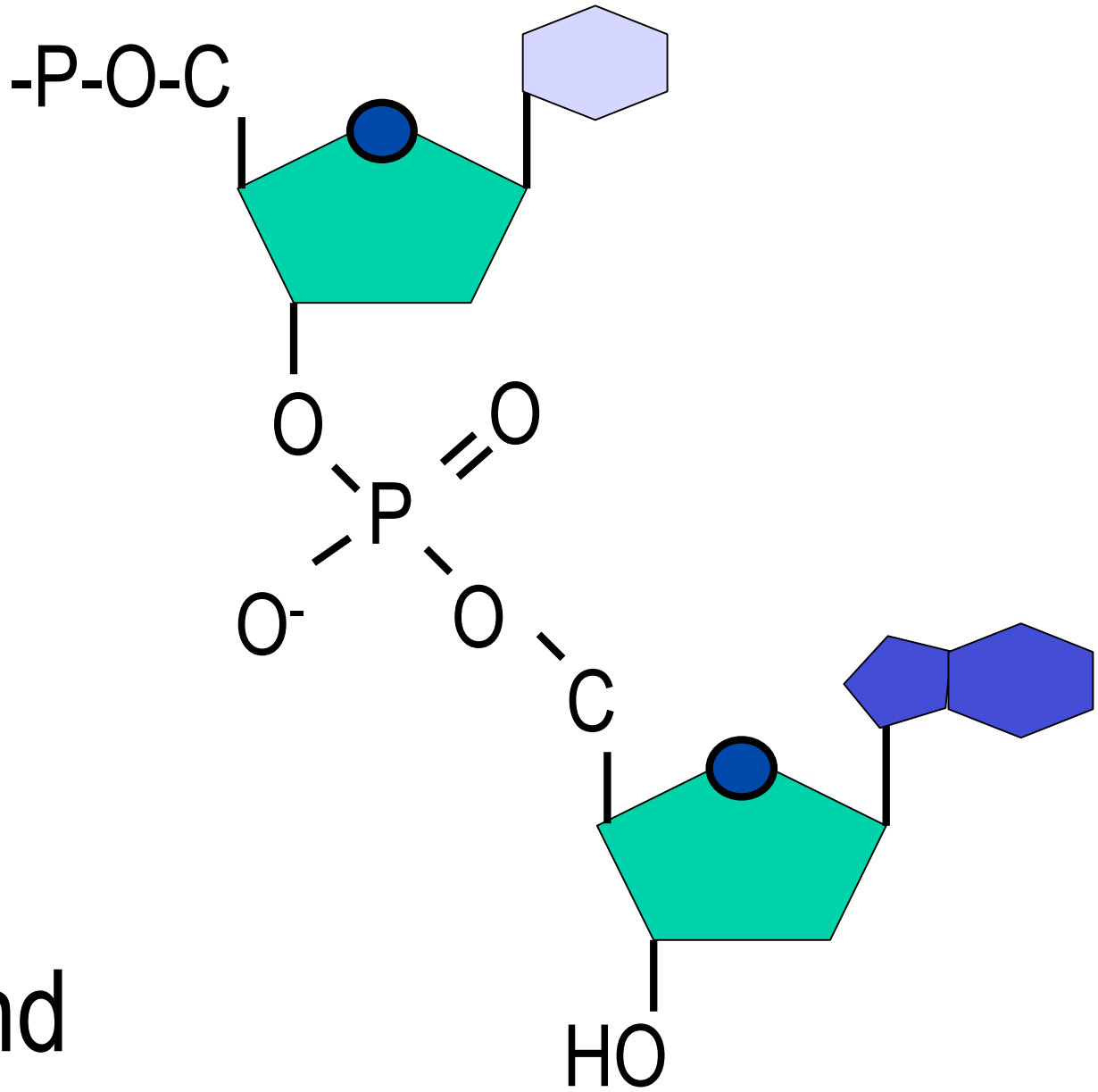


Images removed due to copyright restrictions.

Flow of information

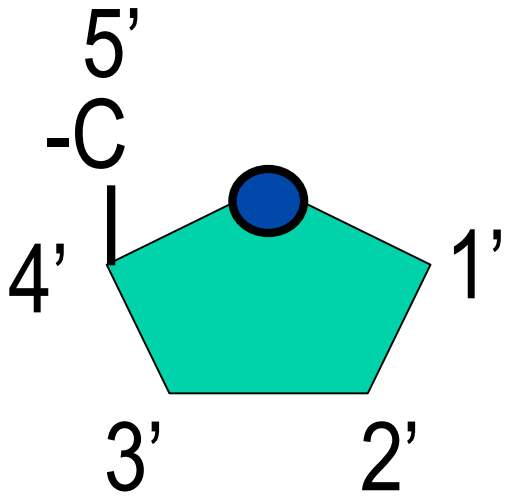


5' end



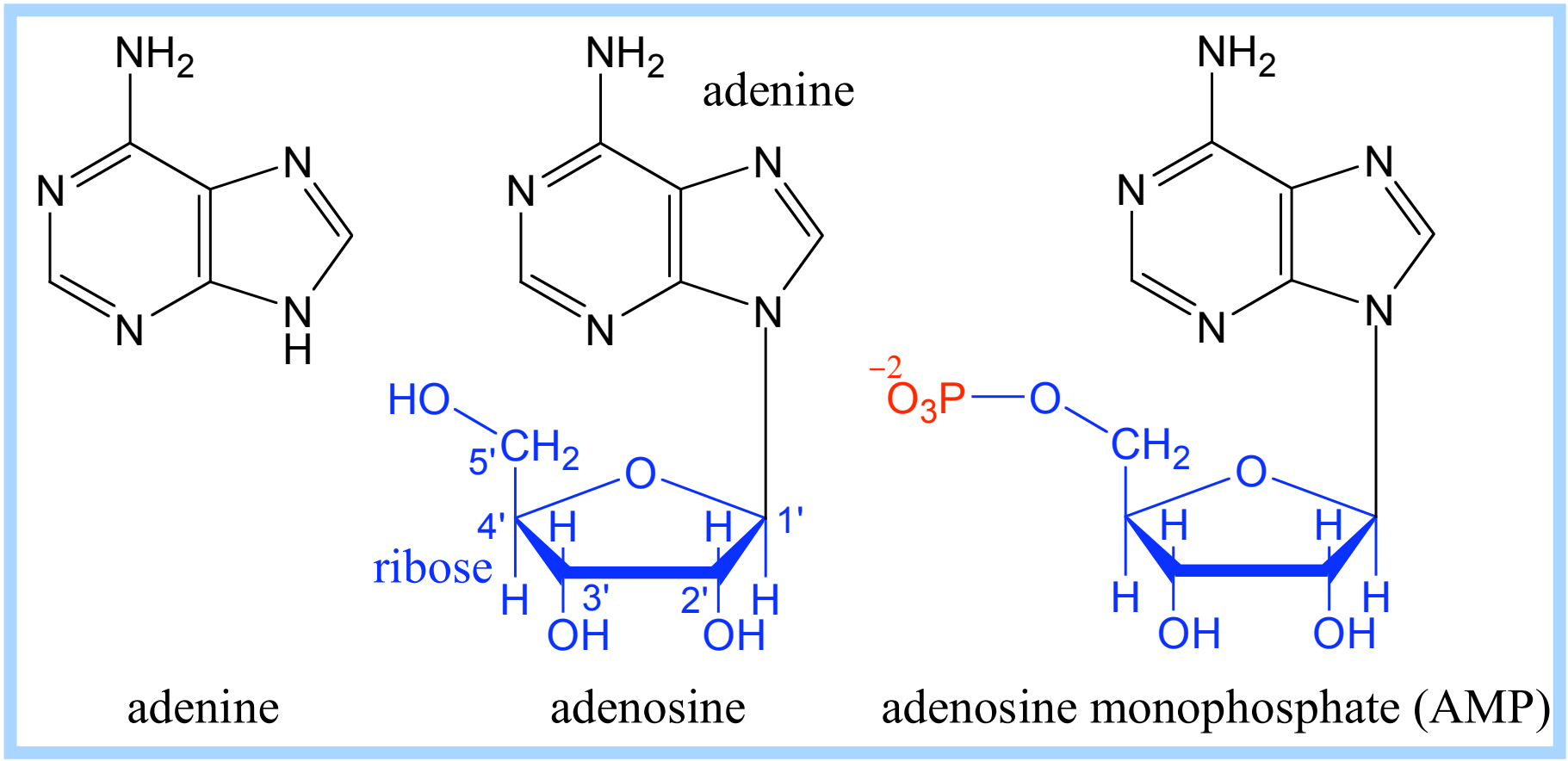
3’ end

ring numbering system for deoxyribose



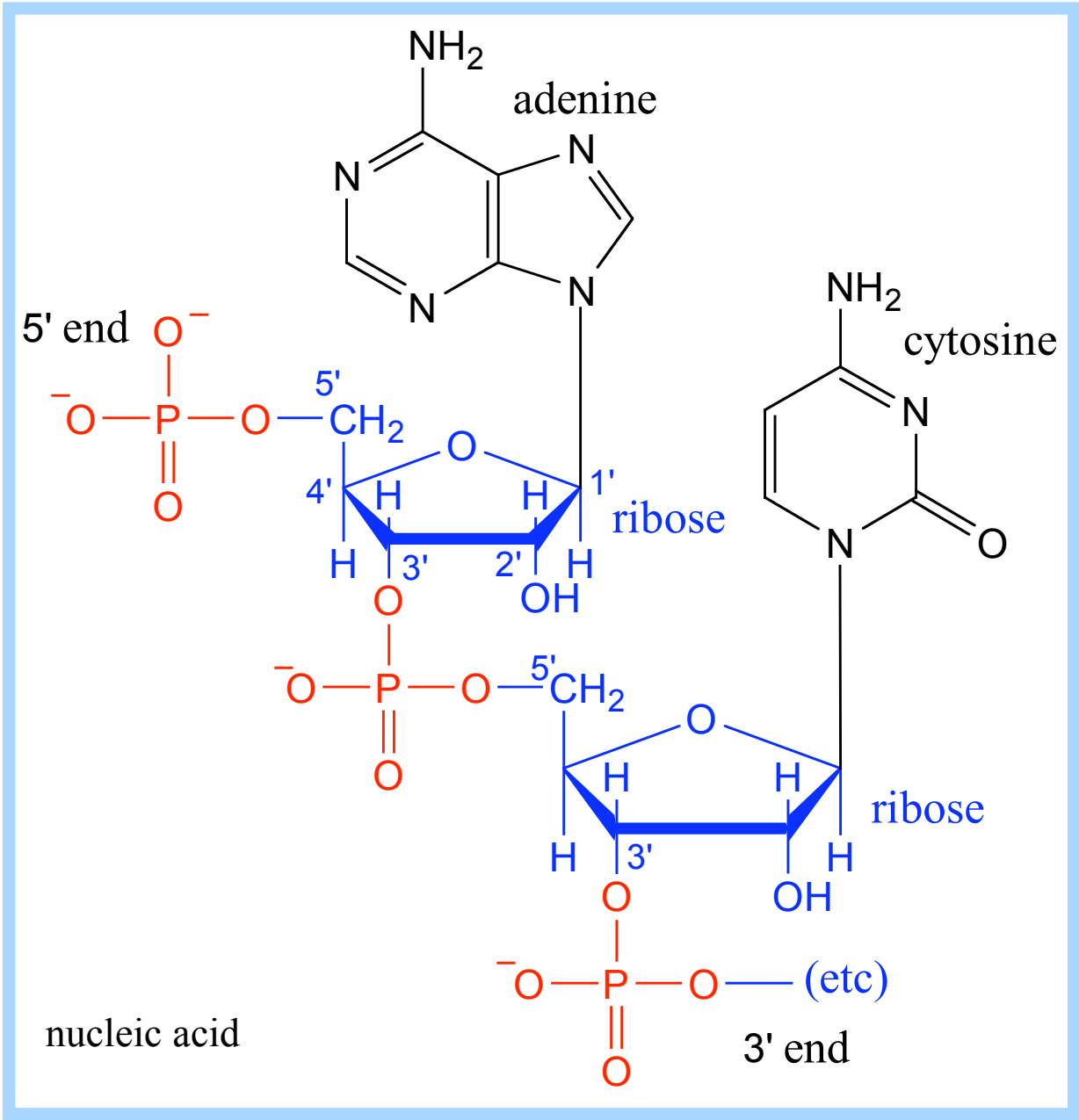
ssDNA

In a **nucleotide**, e.g., adenosine monophosphate (AMP), the base is bonded to a ribose sugar, which has a phosphate in ester linkage to the 5' hydroxyl.



Nucleic acids have a backbone of alternating P_i & ribose moieties.

Phosphodiester linkages form as the 5' phosphate of one nucleotide forms an ester link with the 3' OH of the adjacent nucleotide.



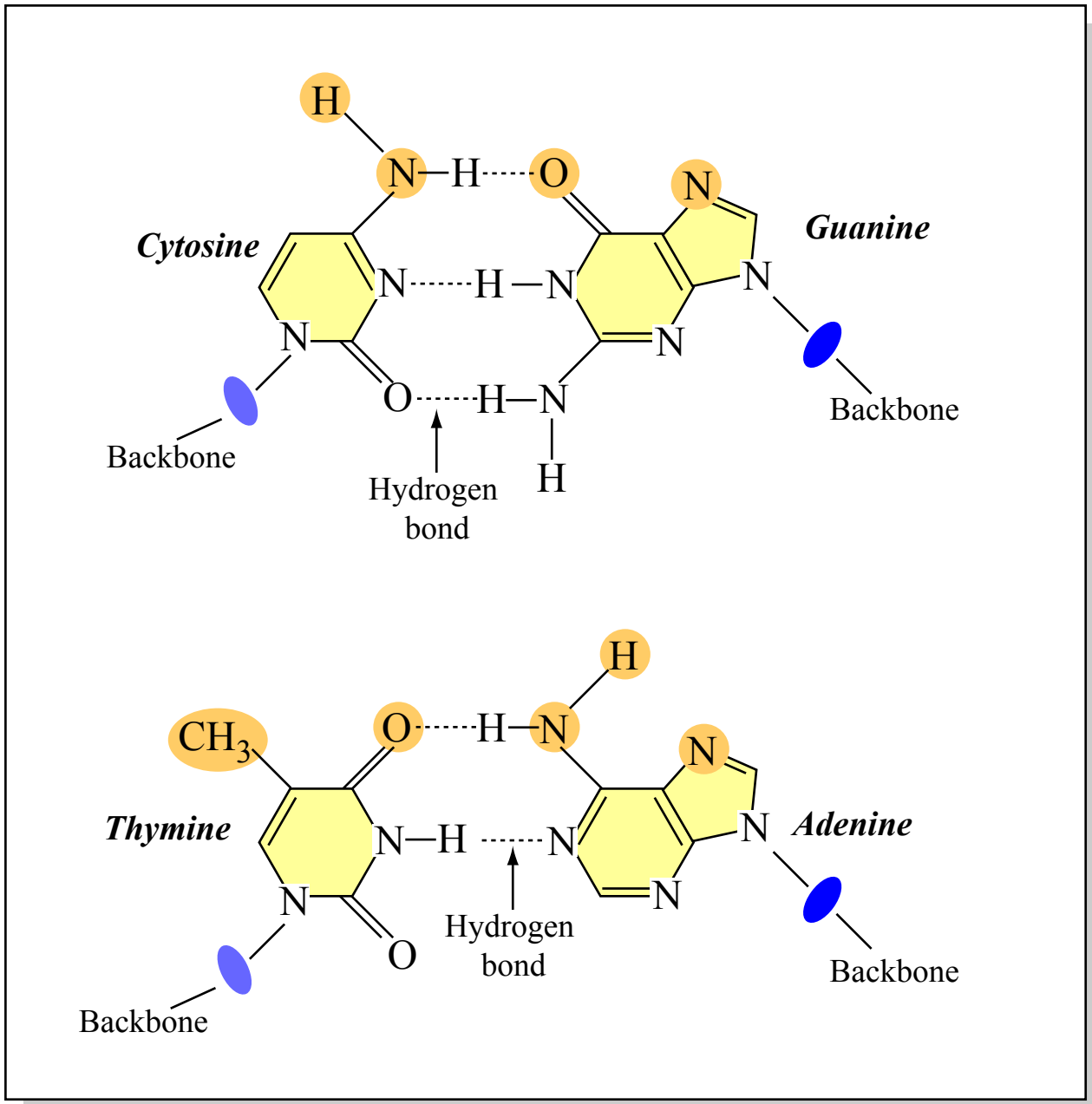
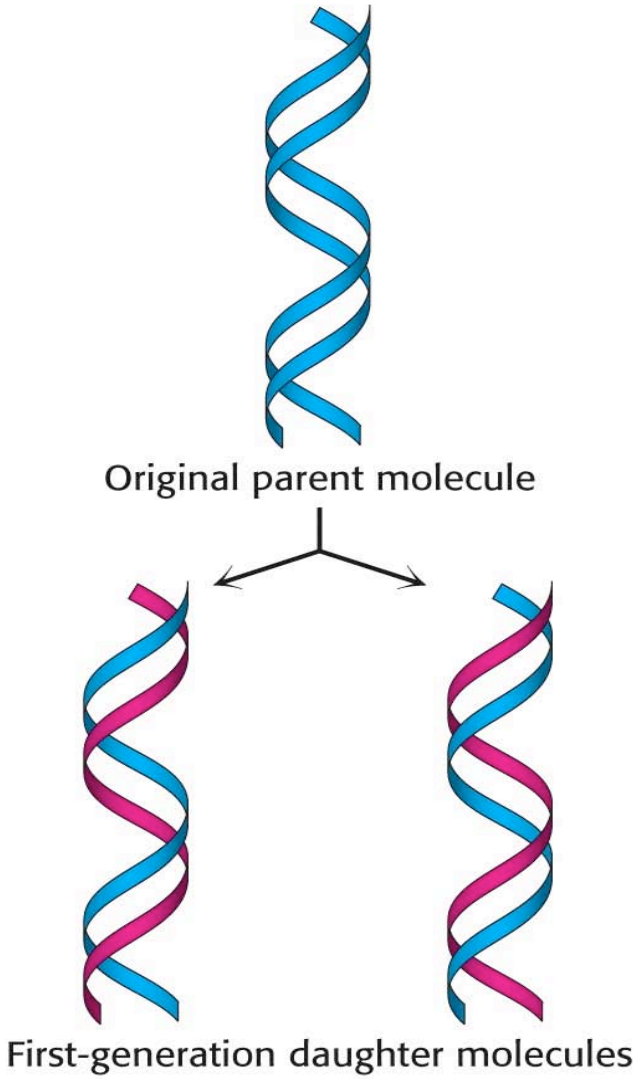


Figure by MIT OCW.

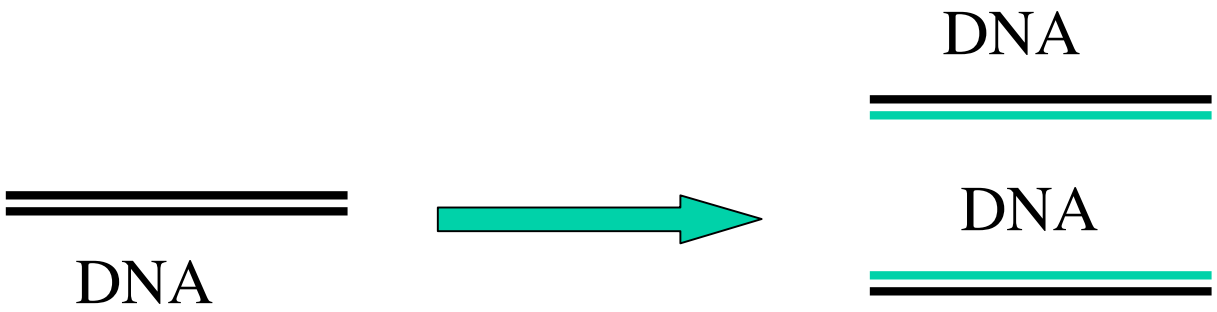
Diagram of genetic structure removed due to copyright restrictions.
See Figure 7-4 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Replication

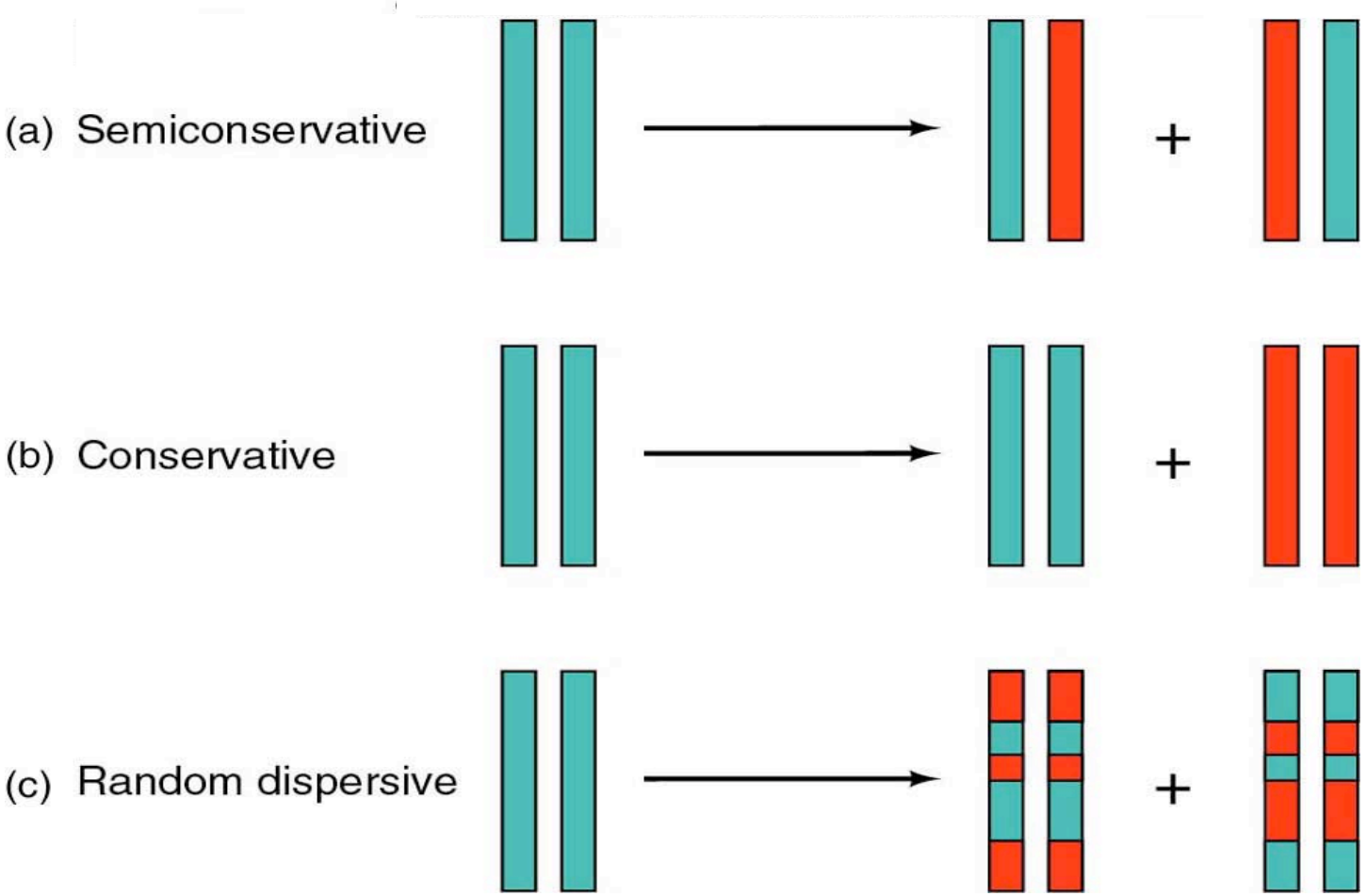


DNA Replication

- A fundamental process
- Experimentally demonstrated

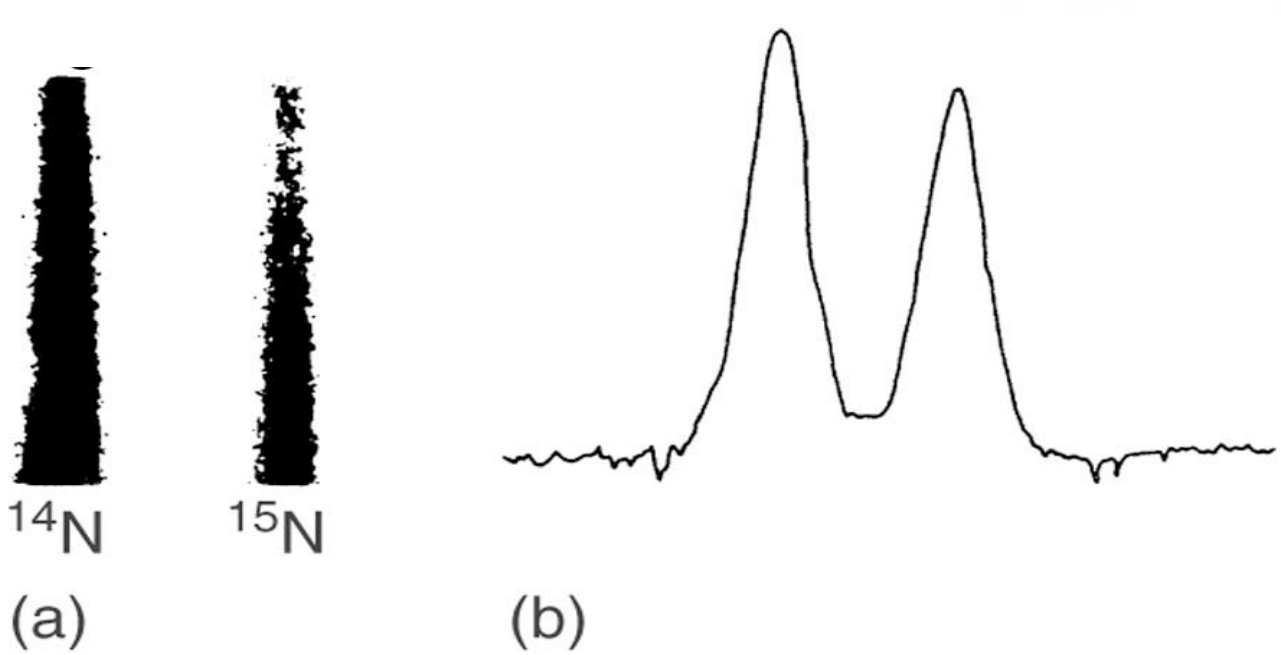


DNA Replication



THREE HYPOTHESES FOR DNA REPLICATION

Stable isoptopes in biology



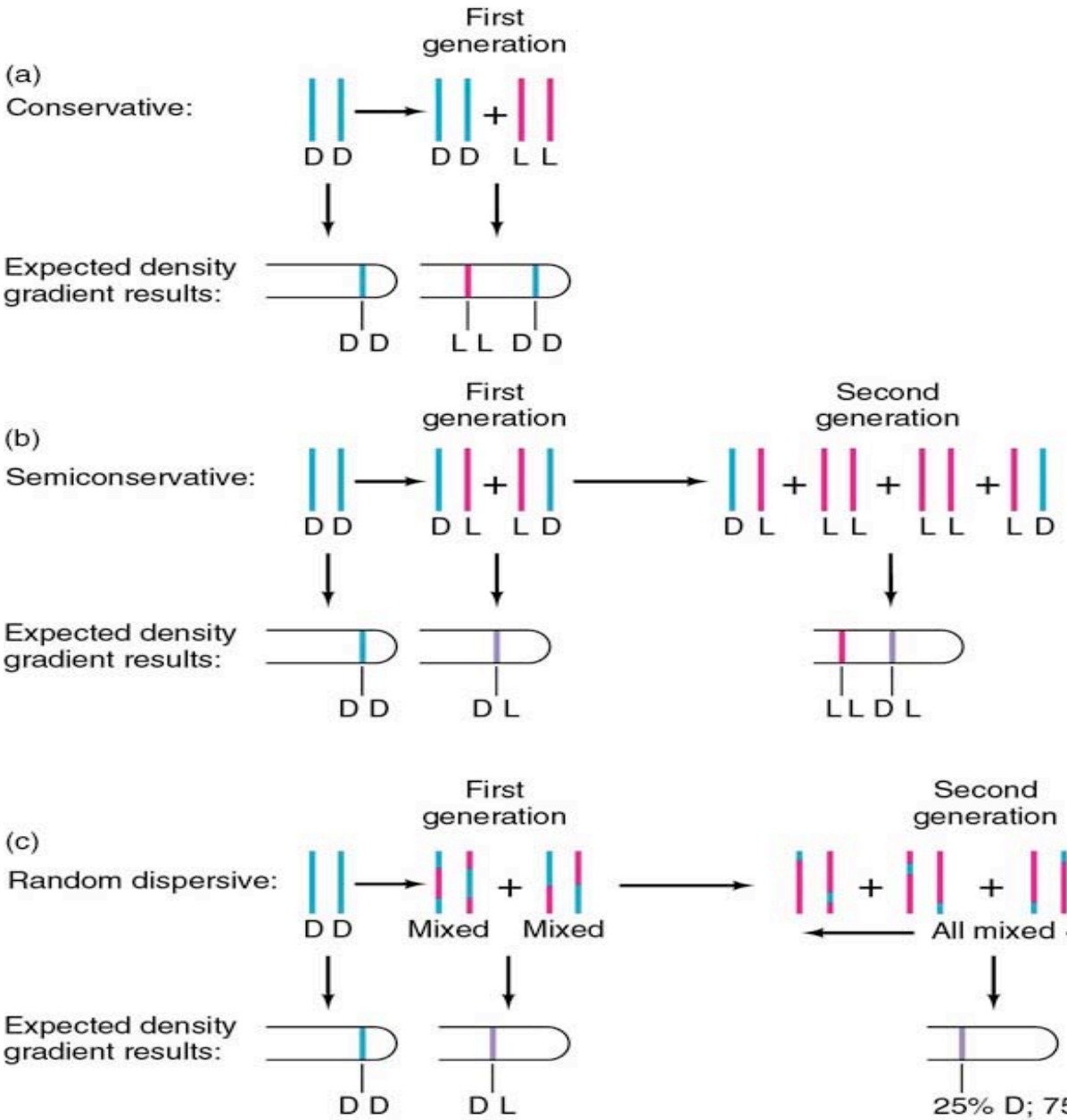
SEPARATION OF DNAS BY CESIUM CHLORIDE DENSITY GRADIENT CENTRIFUGATION
(a) Photo of DNA in ultracentrifuge rotor made with UV light
(b) Densitometric trace of UV scan

D= ^{15}N labeled

L= ^{14}N labeled

1. Grow E coli so DNA uniformly ^{15}N labeled

2. Add ^{14}N labeled to growth media and observe result over several generations of growth



PREDICTED DENSITIES OF NEWLY REPLICATED DNA MOLECULES ACCORDING TO THE THREE HYPOTHESES ABOUT DNA REPLICATION

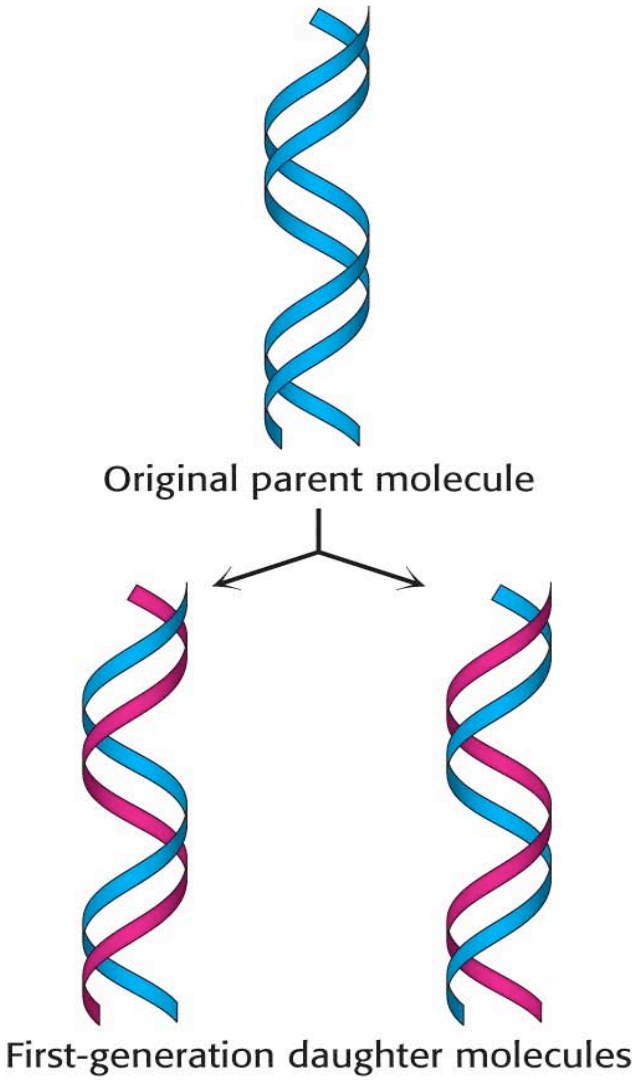
Image of experimental results removed due to copyright restrictions.
See Meselson, and Stahl. "The Replication of DNA in Escherichia coli." *PNAS* 44 (1958): 674, f. 4.

RESULTS OF CsCl GRADIENT ULTRACENTRIFUGATION EXPERIMENT SHOWING DISTRIBUTION OF DNA DENSITY IN *E. coli* CELLS AFTER 0 TO 4.1 GENERATIONS OF GROWTH

THIS EXPERIMENT ESTABLISHED THAT DNA REPLICATION IS SEMICONSERVATIVE

Conclusion

1. DNA replication is semi-conservative



DNA Replication Process

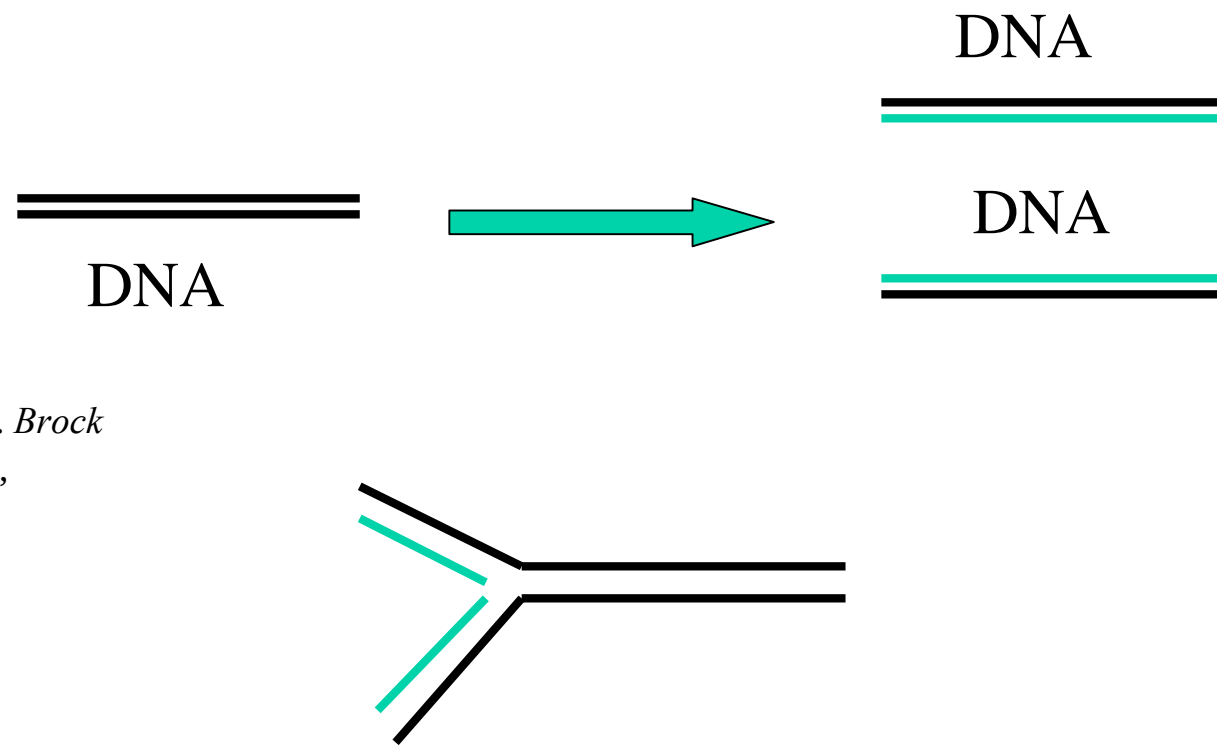


Diagram removed due to copyright restrictions.
See Figure 7-12 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

All DNA polymerases require a primer

DNA is synthesized 5' to 3'

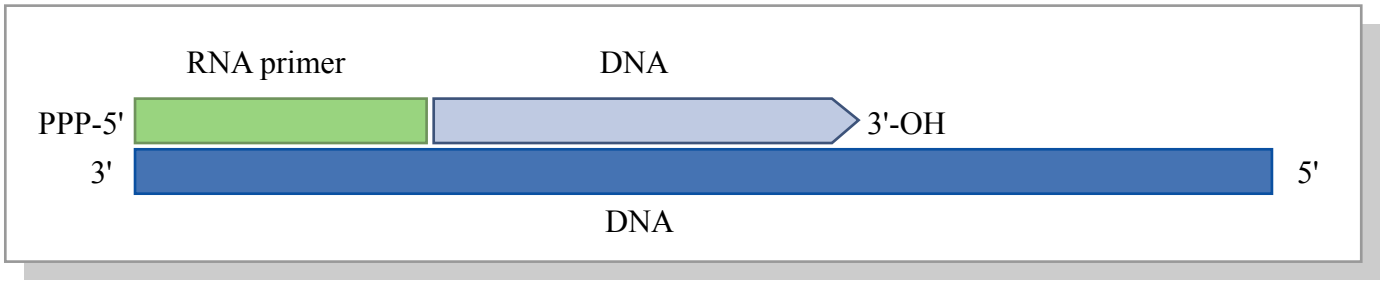
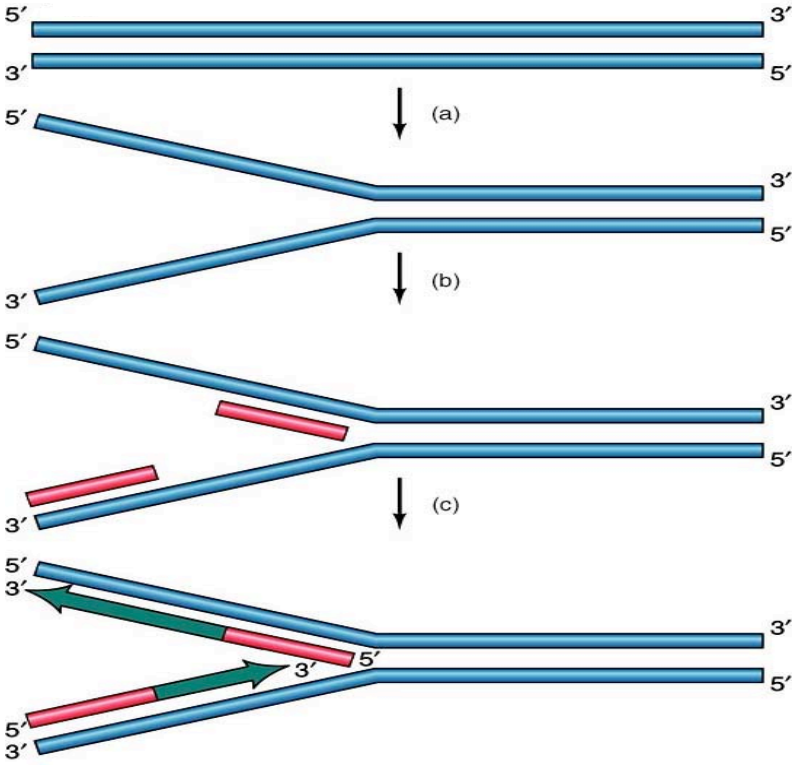


Figure by MIT OCW.



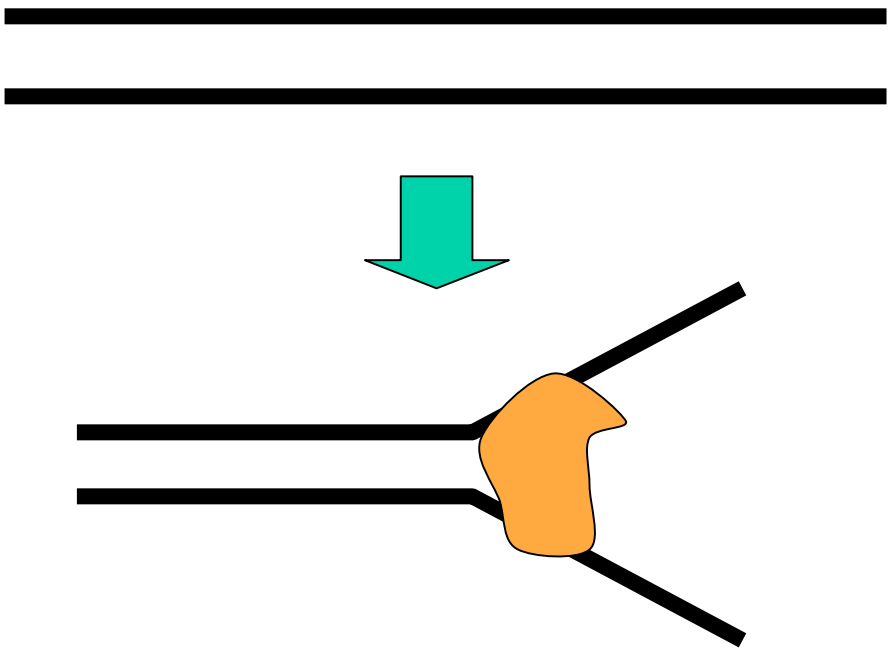
PRIMING OF DNA SYNTHESIS BY SHORT SEQUENCES OF RNA (RED)

DNA POLYMERASE USES THE PRIMERS AS STARTING POINTS TO SYNTHESIZE PROGENY DNA STRANDS (GREEN ARROWS)

Table of the major enzymes involved in DNA replication in bacteria removed due to copyright restrictions.
See Table 7-3 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms.*
11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

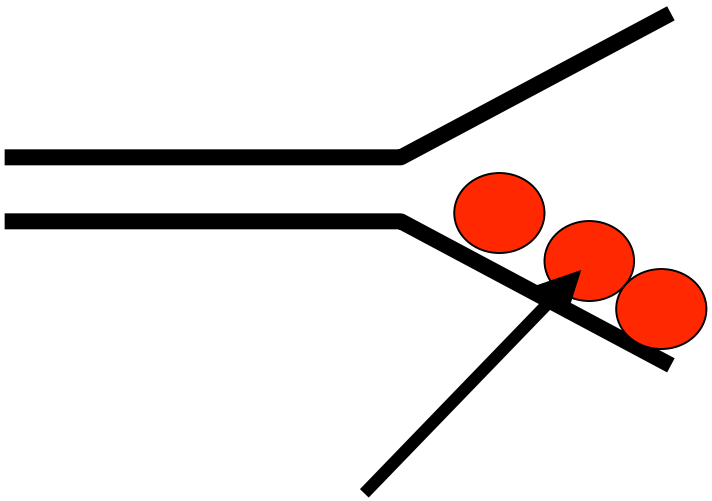
Helicase & topoisomerase

Unwind & remove supercoils in duplex DNA



ssDNA binding protein

binds to and stabilizes ssDNA

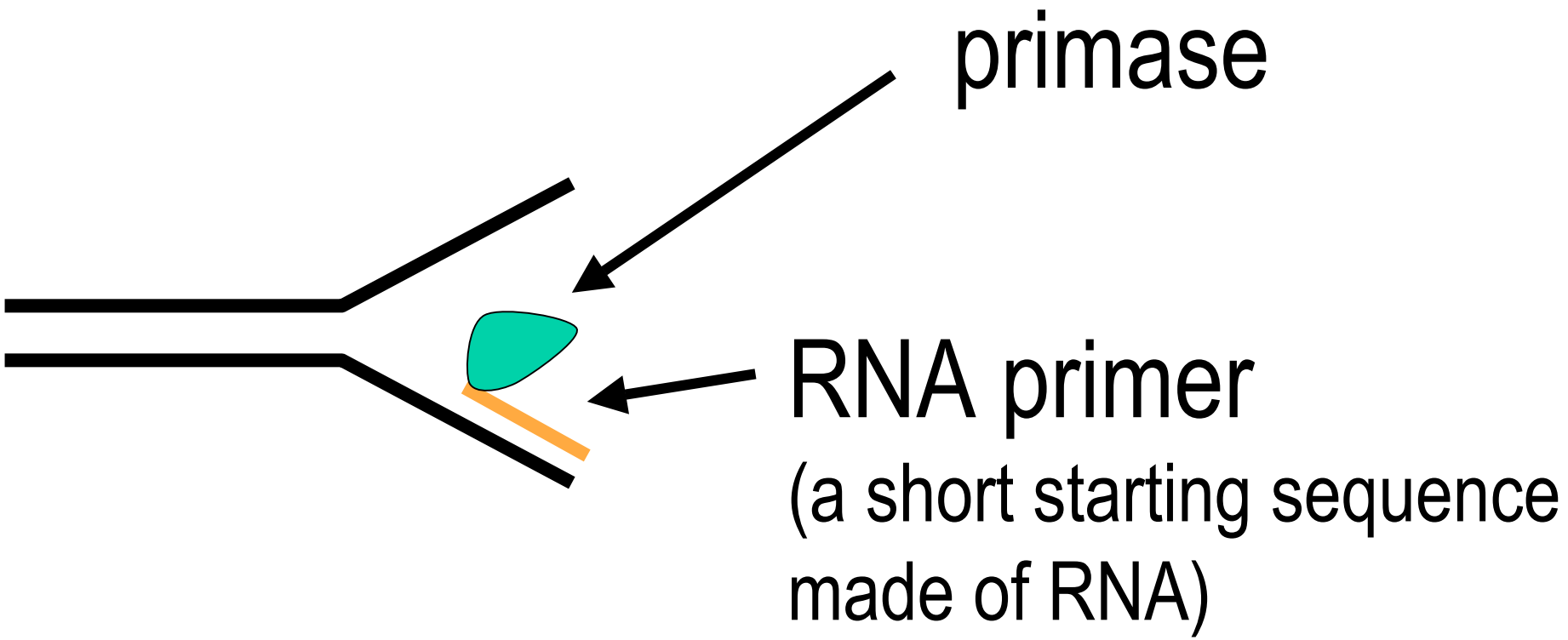


prevents base pairing

ssDNA binding protein

primase

synthesizes a short RNA primer using a DNA template



DNA polymerase III

Synthesizes DNA 5'->3', by priming off the RNA primer on the lagging strand template.

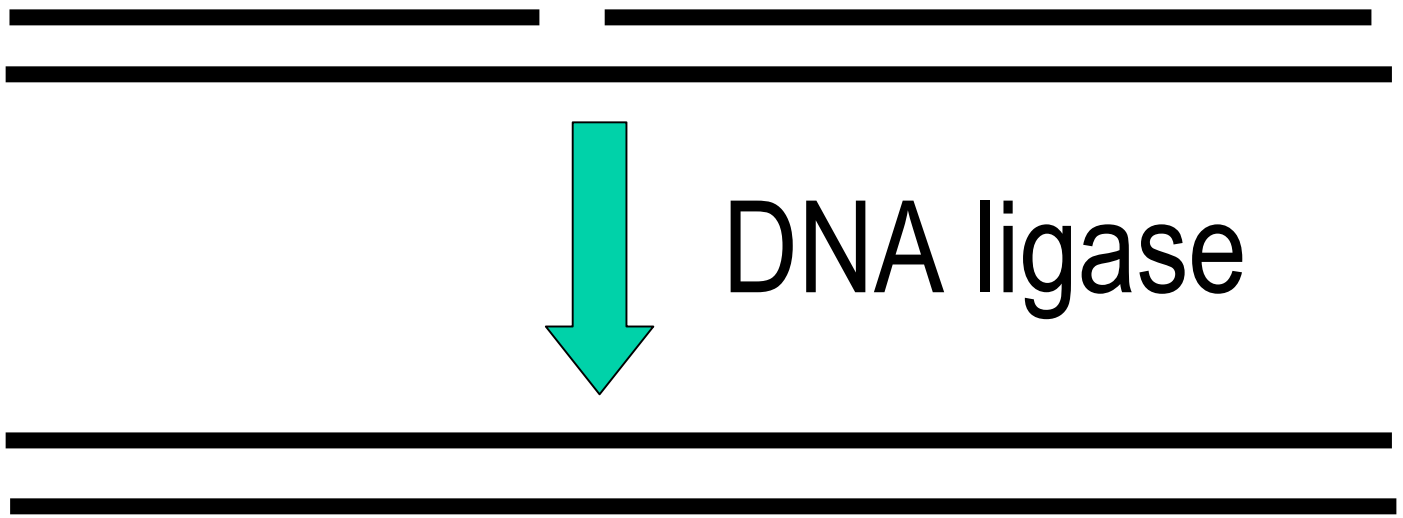
Also has 3'->5' proofreading activity

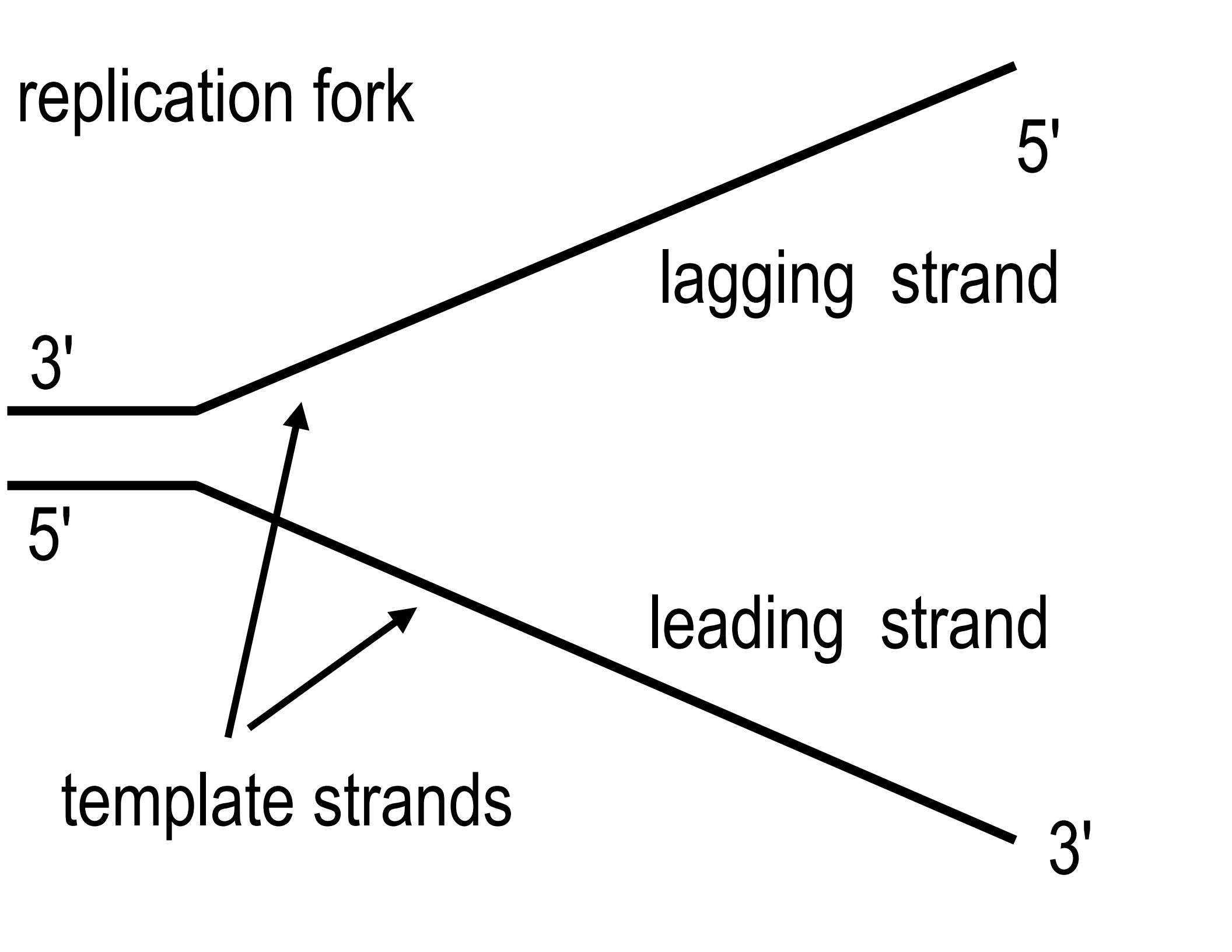
DNA polymerase I

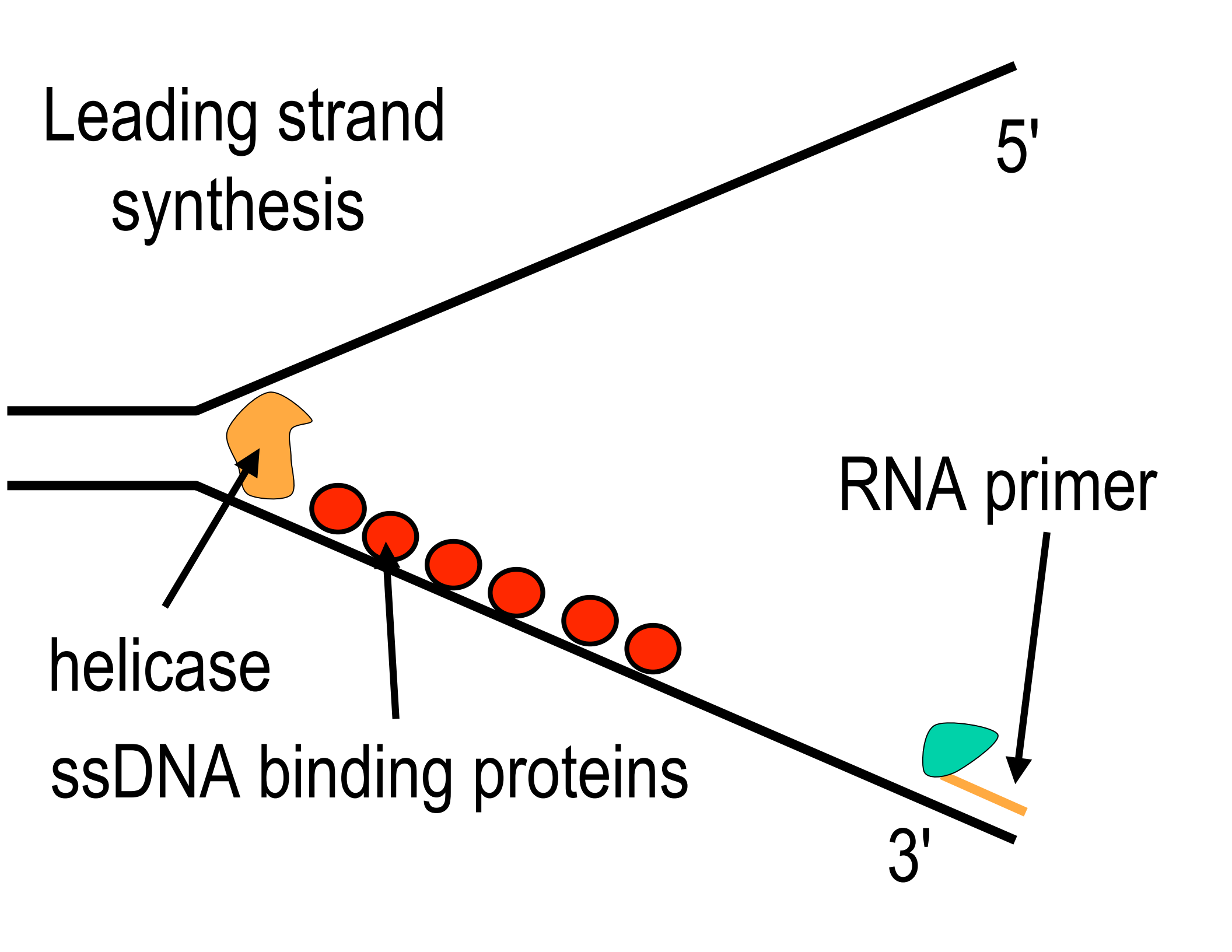
Synthesizes DNA from a DNA template and also removes RNA primers from the “Okazaki fragments”.

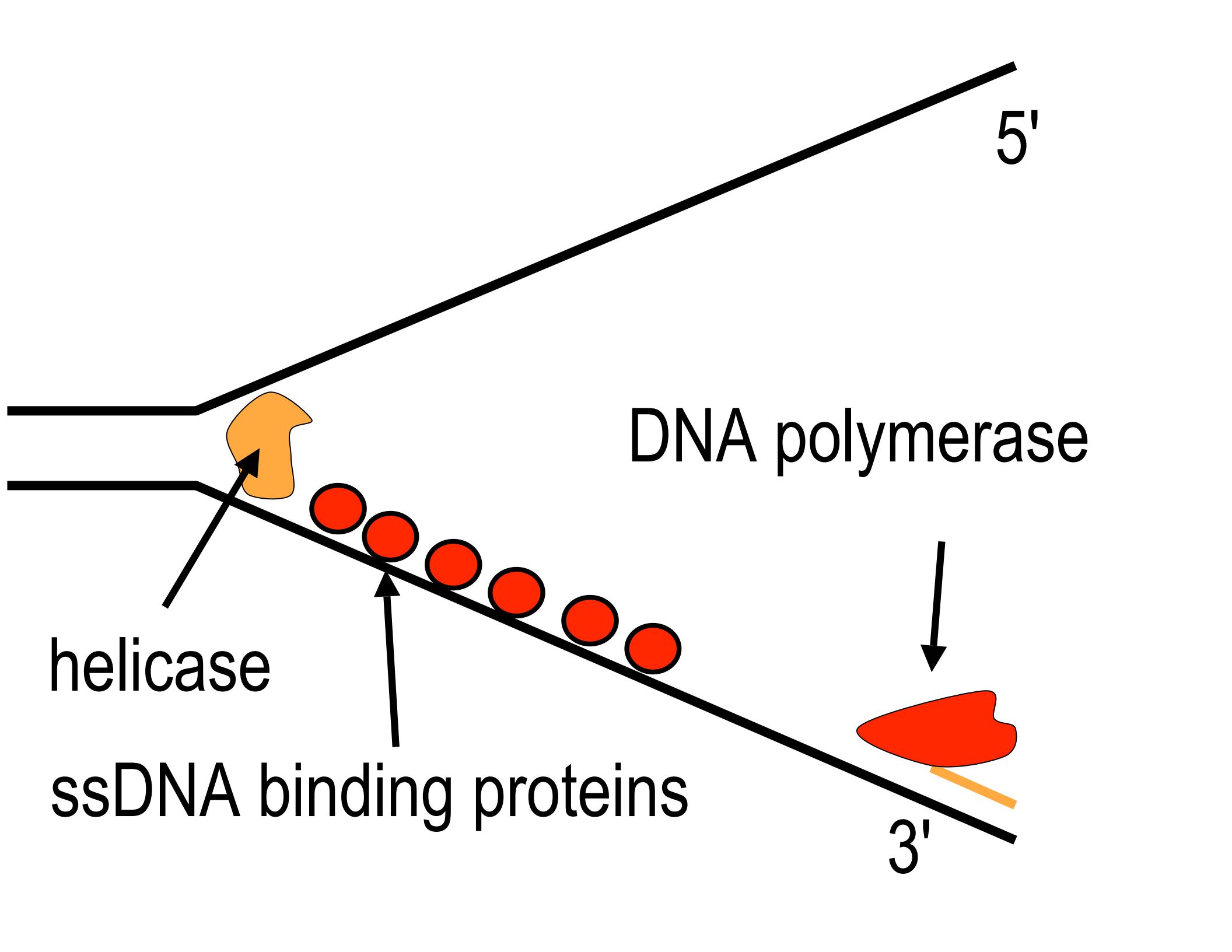
DNA ligase

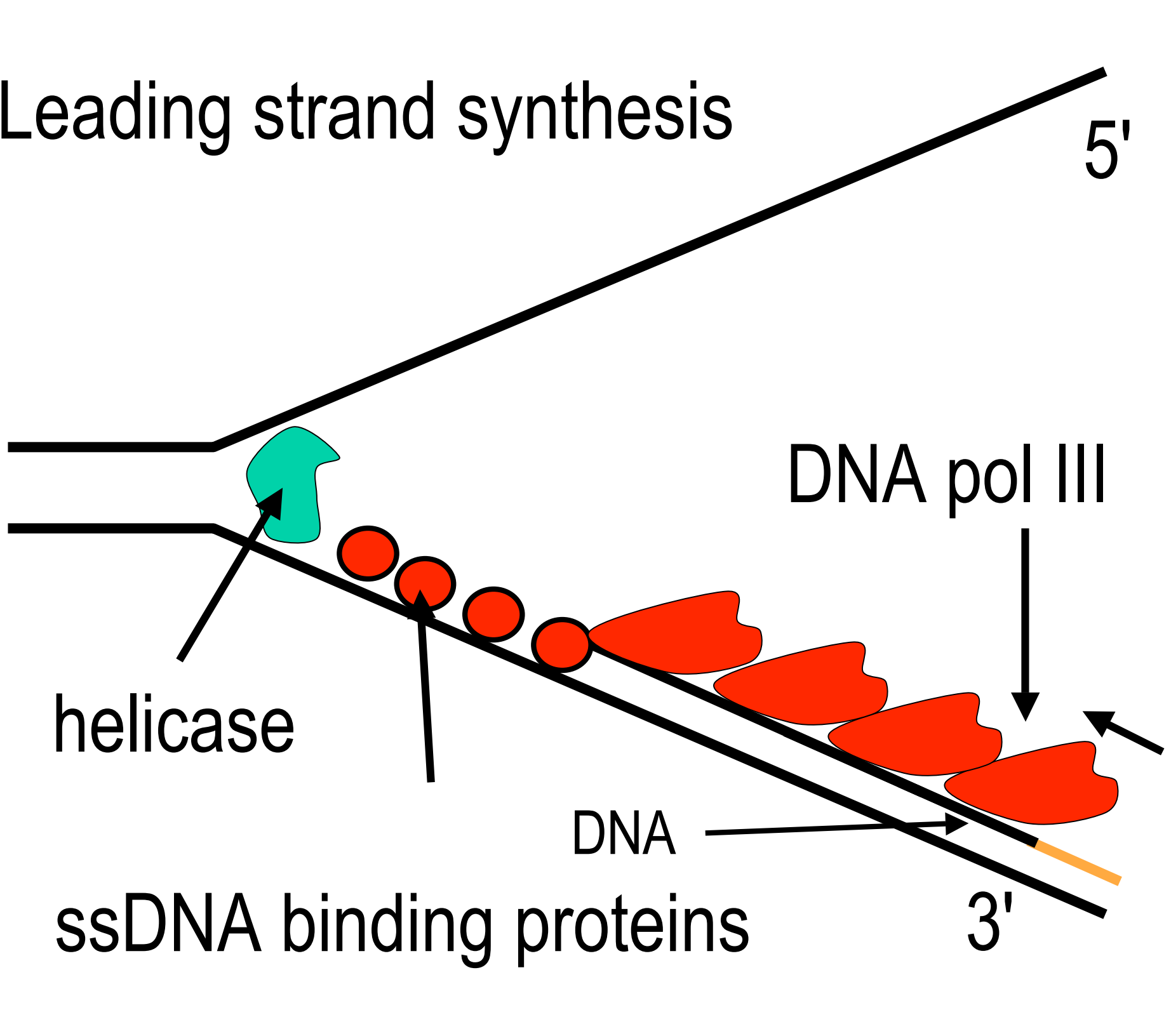
Joins DNA strands together by forming phosphodiester bonds




replication fork
5'
lagging strand
3'
5'
leading strand
template strands
3'


Leading strand
synthesis
5'
RNA primer
helicase
ssDNA binding proteins
3'


5'
DNA polymerase
helicase
ssDNA binding proteins
3'

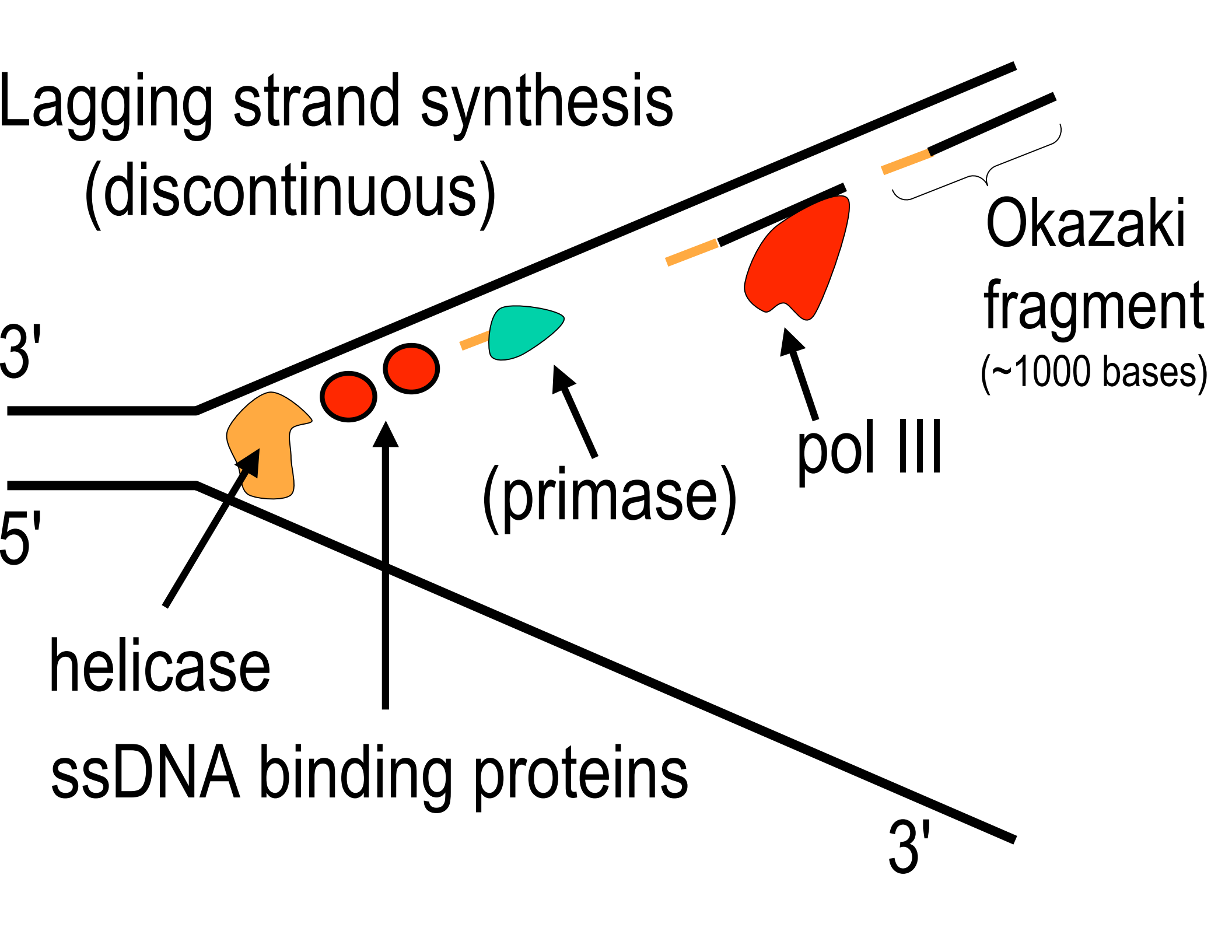

Leading strand synthesis
5'
DNA pol III
helicase
DNA
ssDNA binding proteins
3'

Proofreading

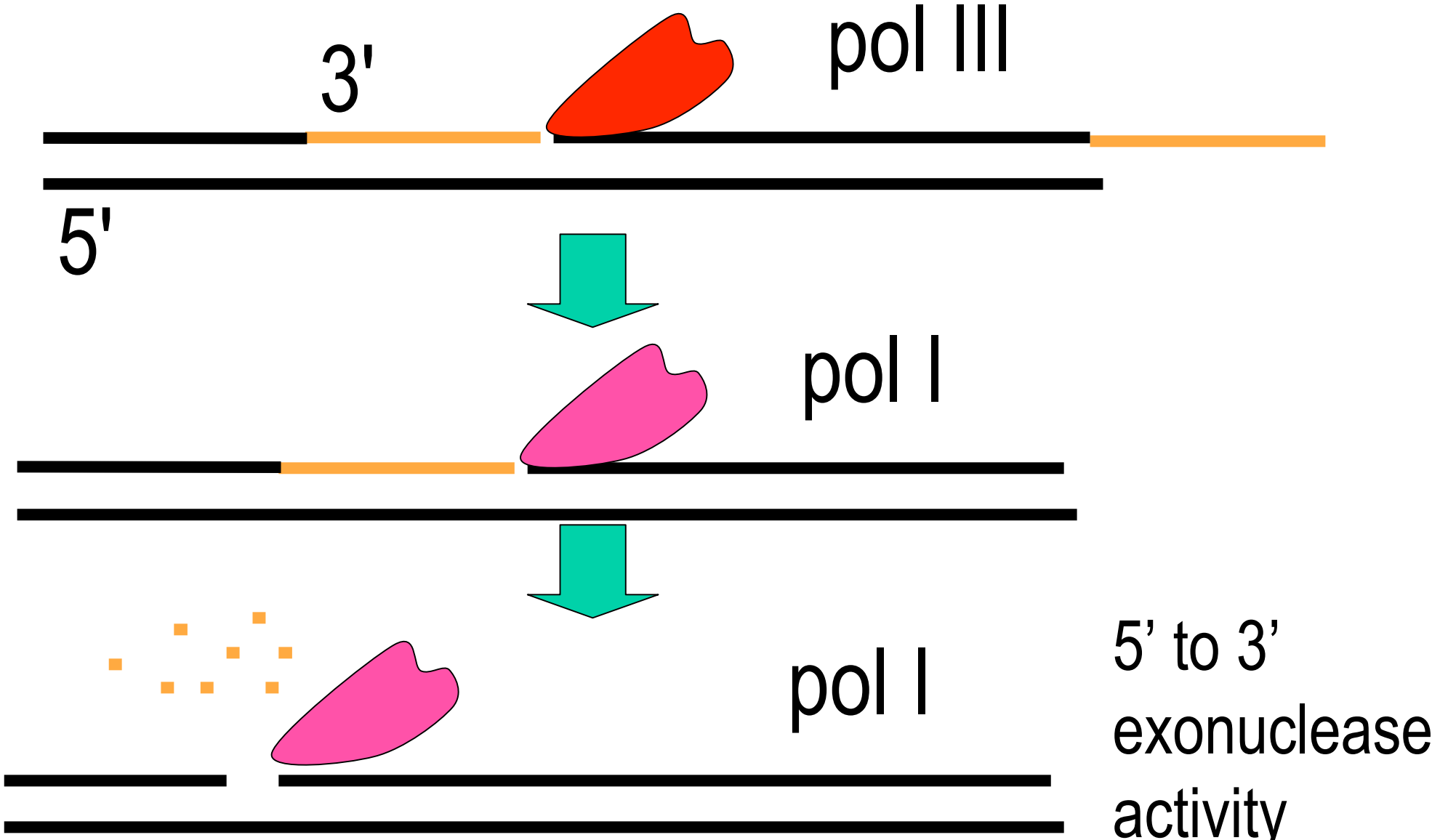
Pol III removes misincorporated bases using 3' to 5' exonuclease activity

This decreases the error rate to about 10^{-10} per base pair inserted

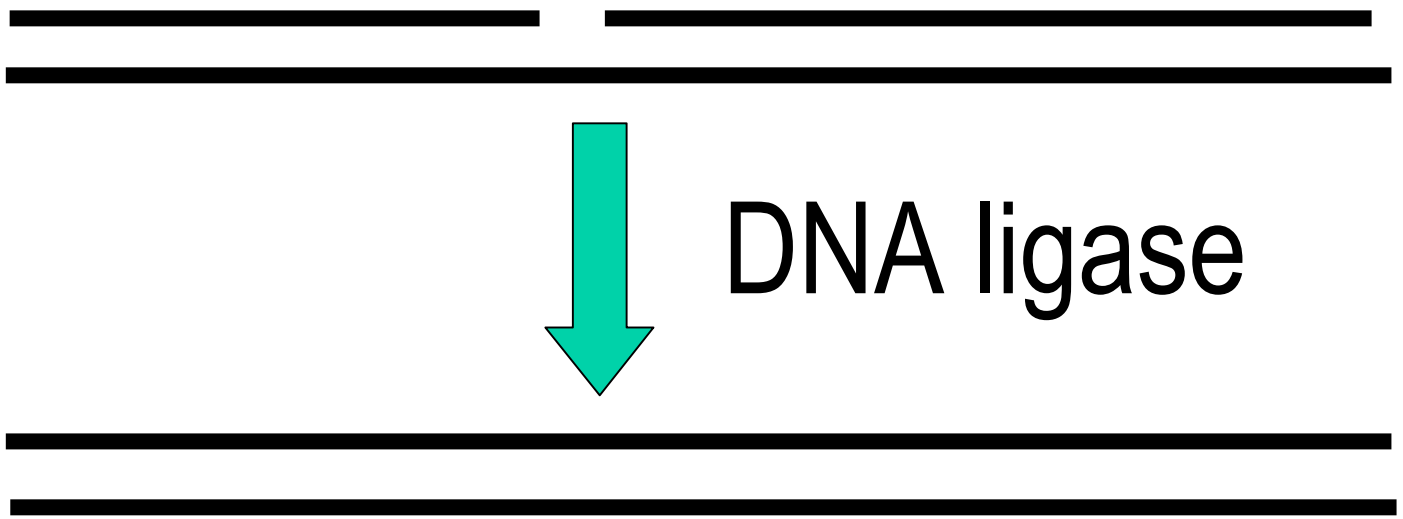
Diagram of DNA proofreading removed due to copyright restrictions.
See Figure 7-20 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.


Lagging strand synthesis
(discontinuous)
Okazaki
fragment
(~1000 bases)
3'
5'
(primase)
pol III
helicase
ssDNA binding proteins
3'

Primer removal



Ligation



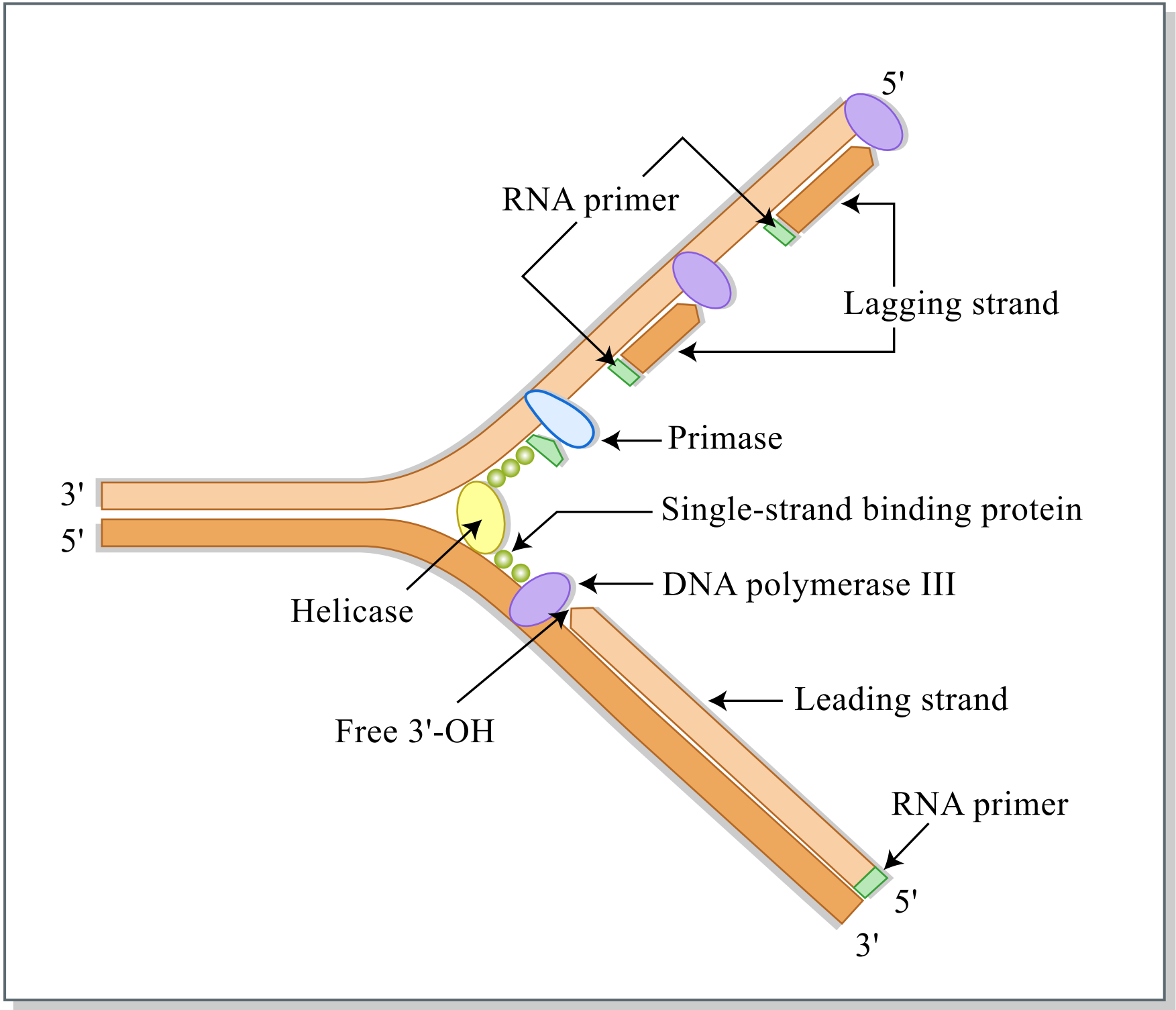
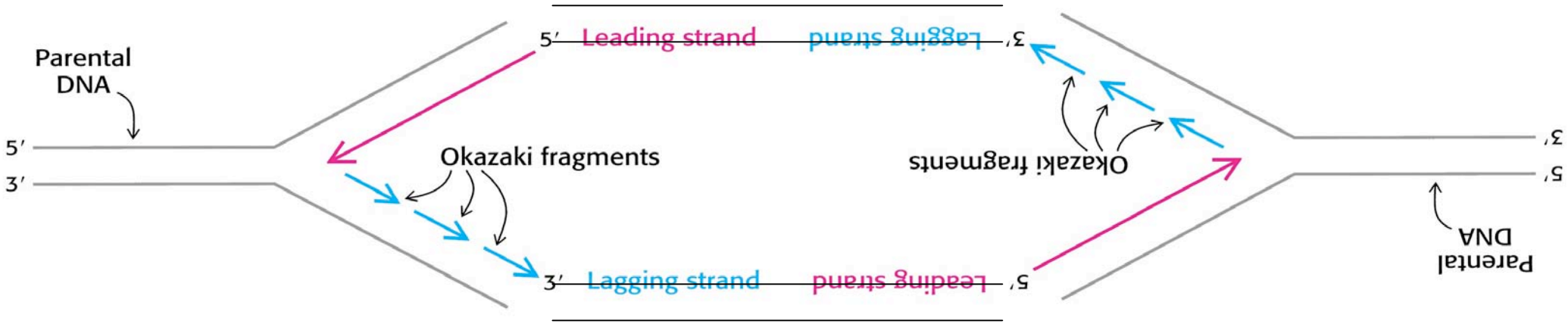


Figure by MIT OCW.

DNA SYNTHESIS HAPPEN BIDIRECTIONALLY, FROM INITIATION SITE

“REPLICATION BUBBLE”



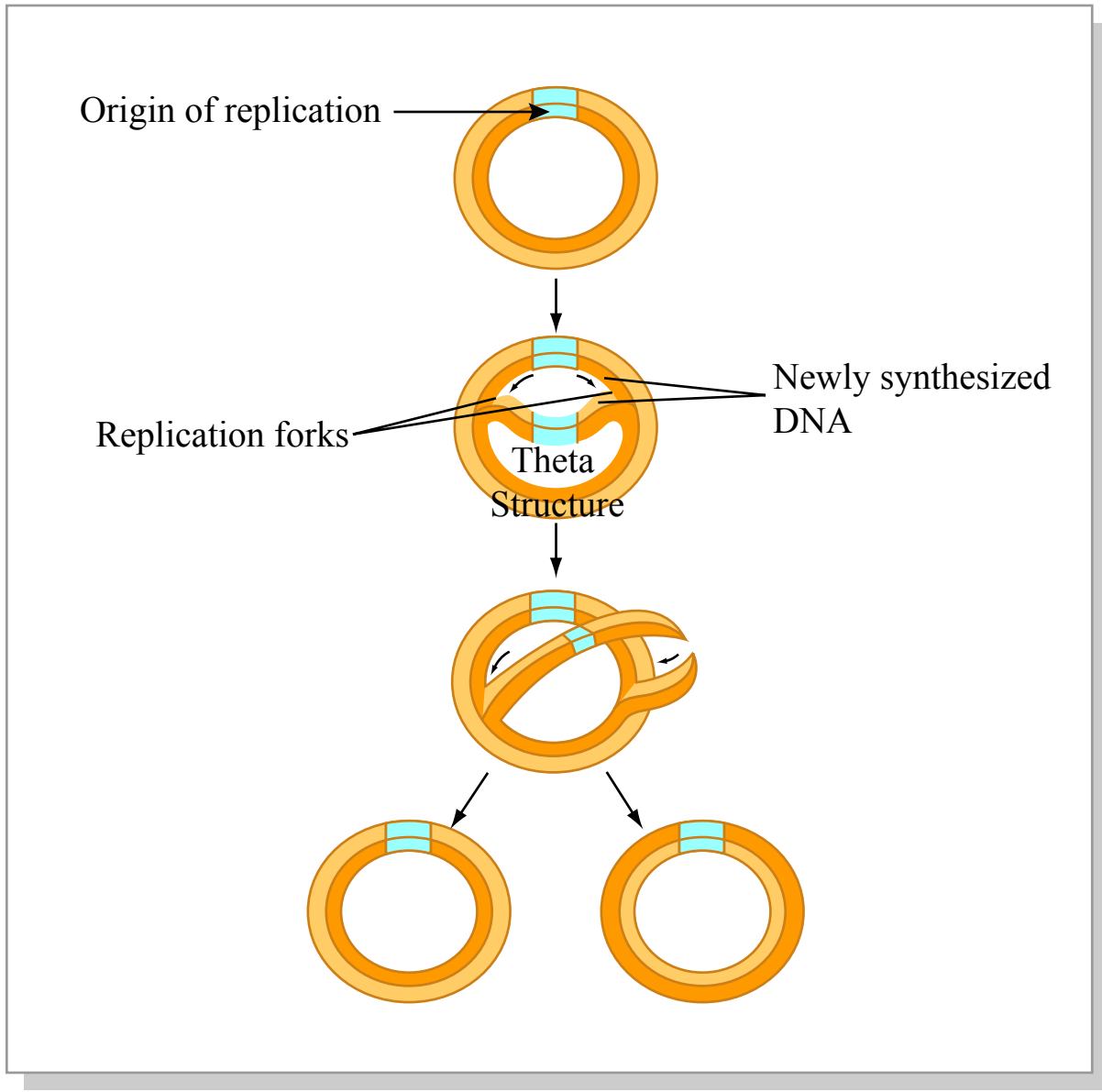
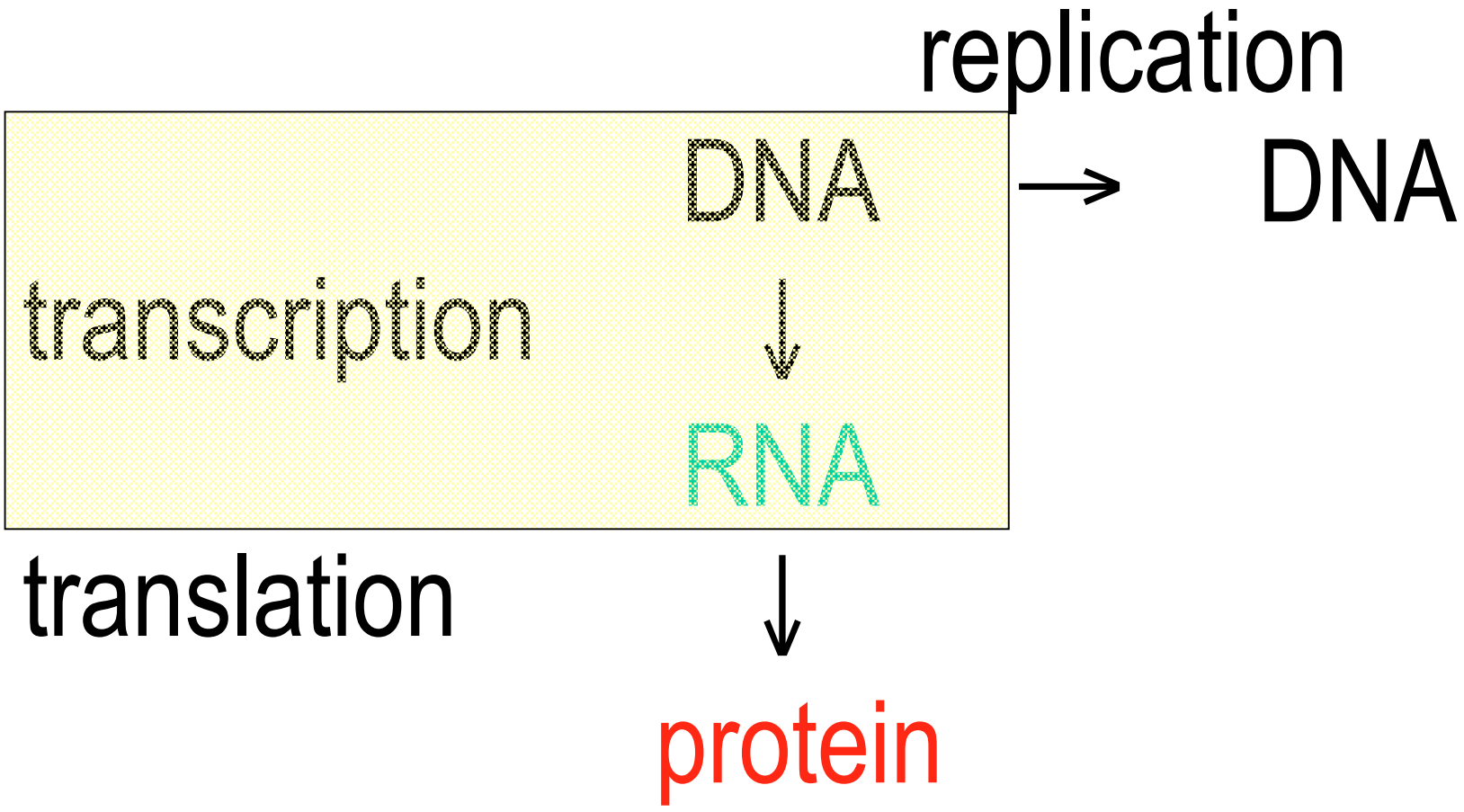


Figure by MIT OCW.

Flow of information



Regulatory pathways in prokaryotes

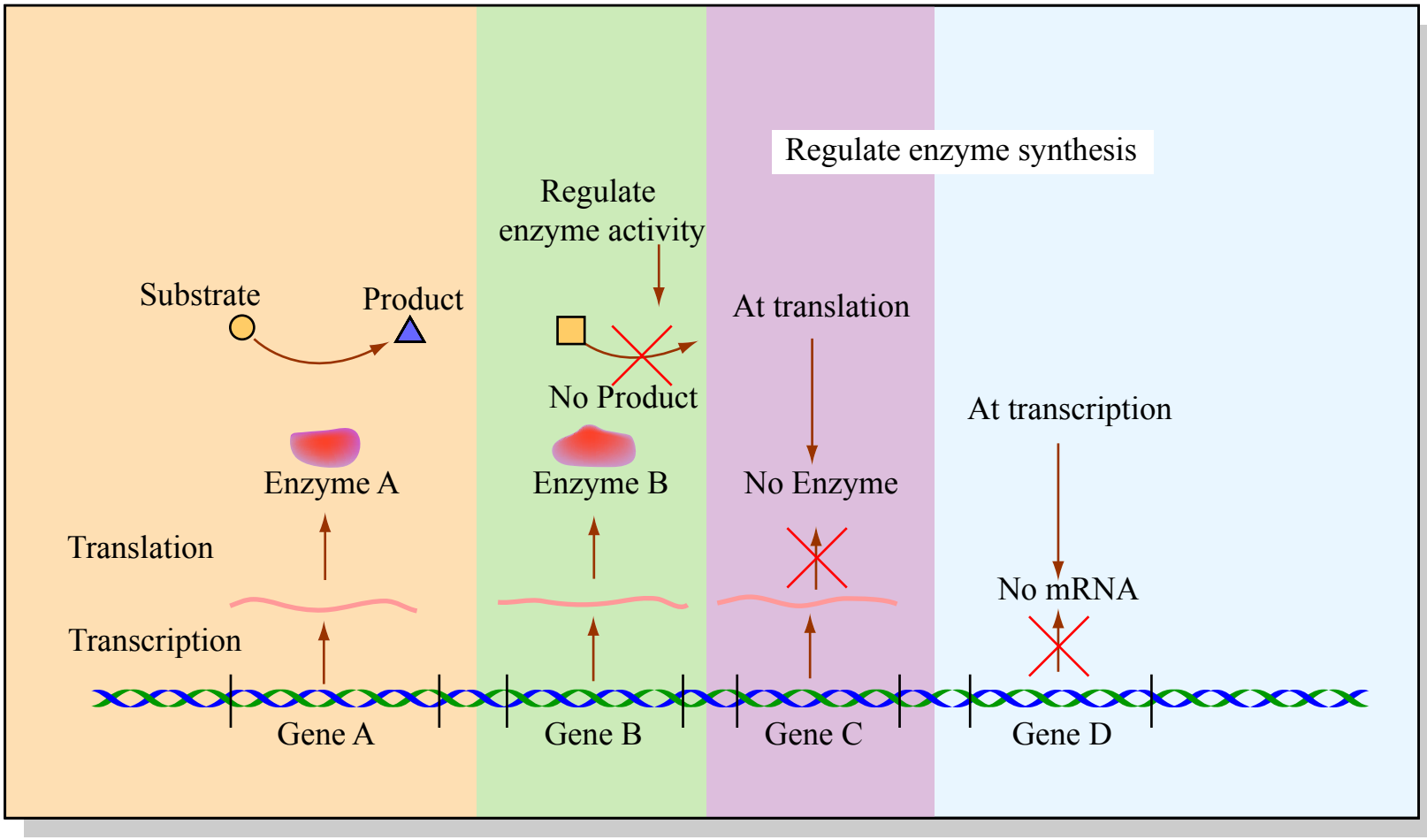
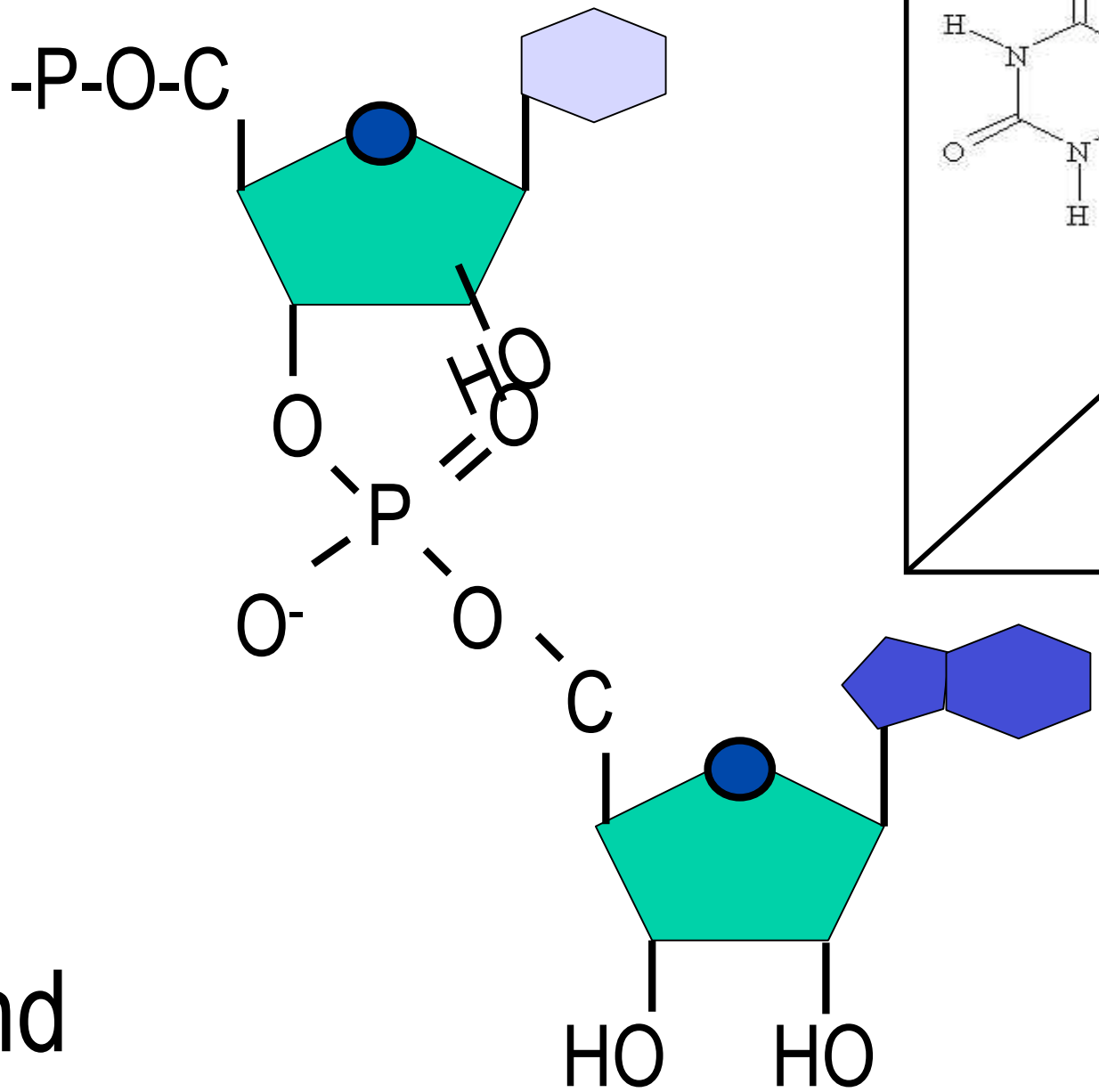


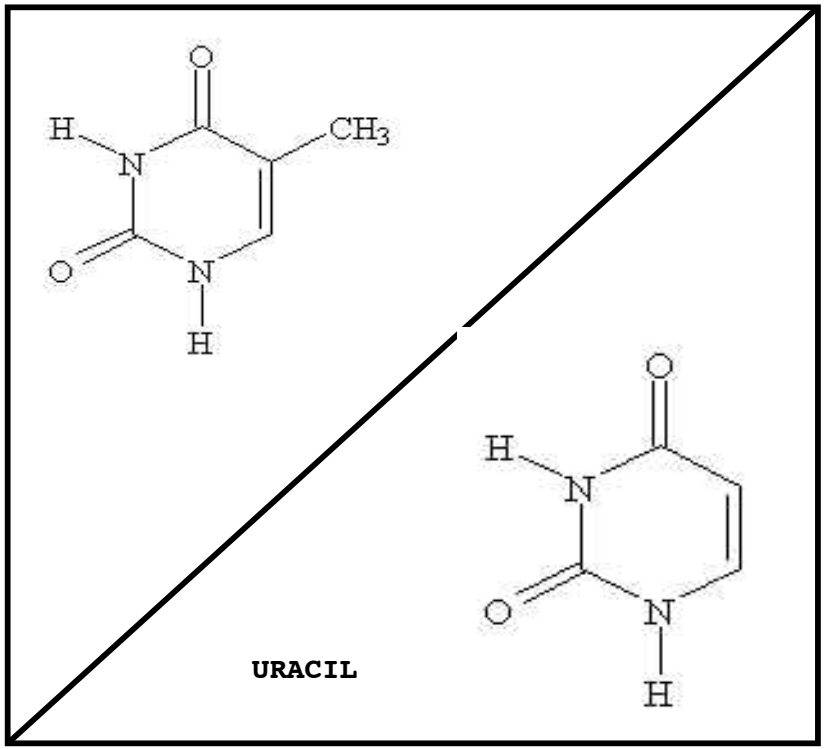
Figure by MIT OCW.

Prokaryotic transcription

- Transcribed regions
- RNA polymerase
- Promoters
- Terminators
- Sigma factor

5' end





3’ end

ssDNA

Transcription

Diagram of RNA transcription removed due to copyright restrictions.
See Figure 7-29a in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

DNA dependent RNA Polymerase (RNAP) recognizes promoter sequence and initiates transcription

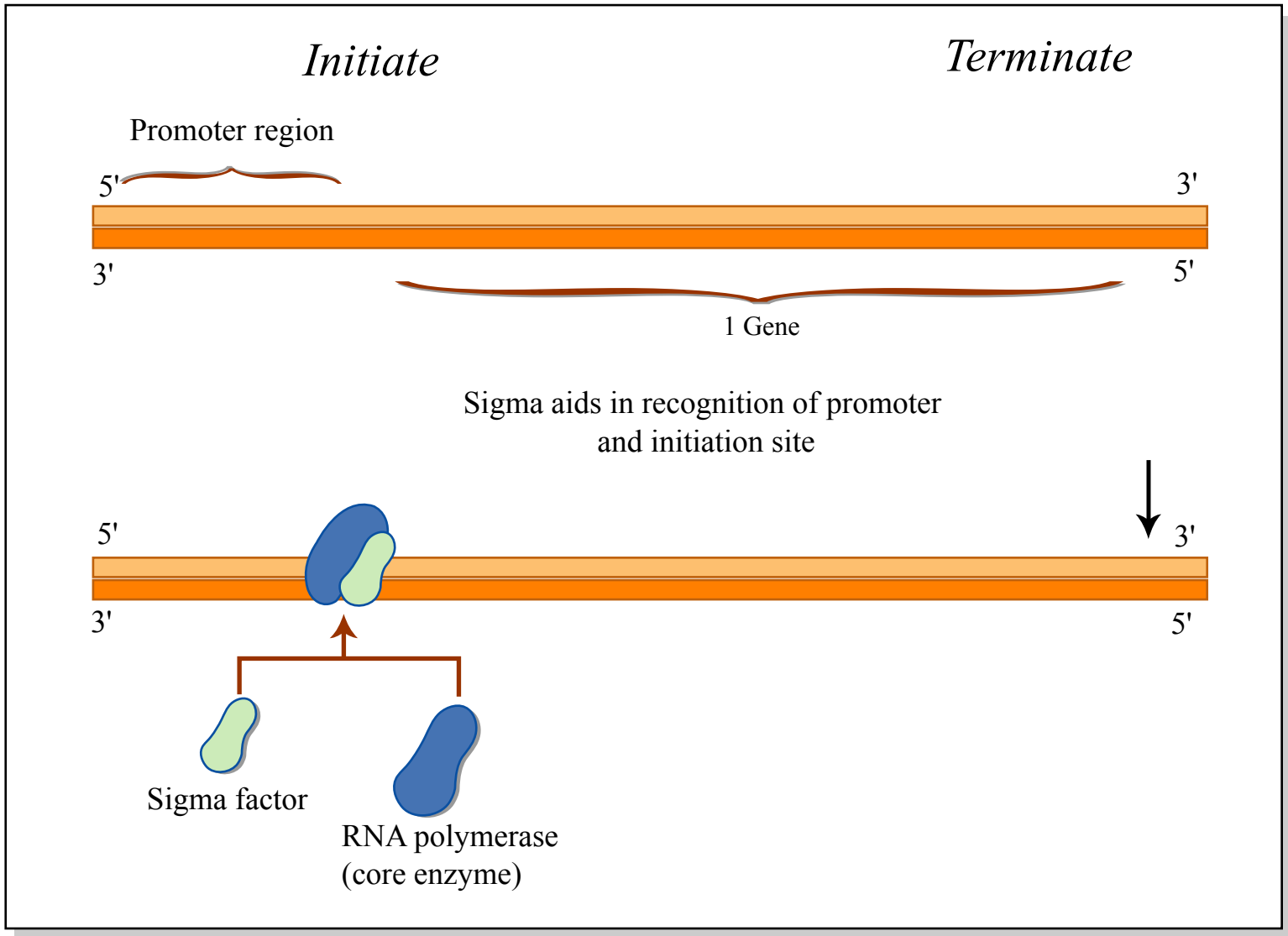


Figure by MIT OCW.

Synthesis of the mRNA transcript (5'→3') complementary to one of the twp strands – the template strand – sigma dissociates

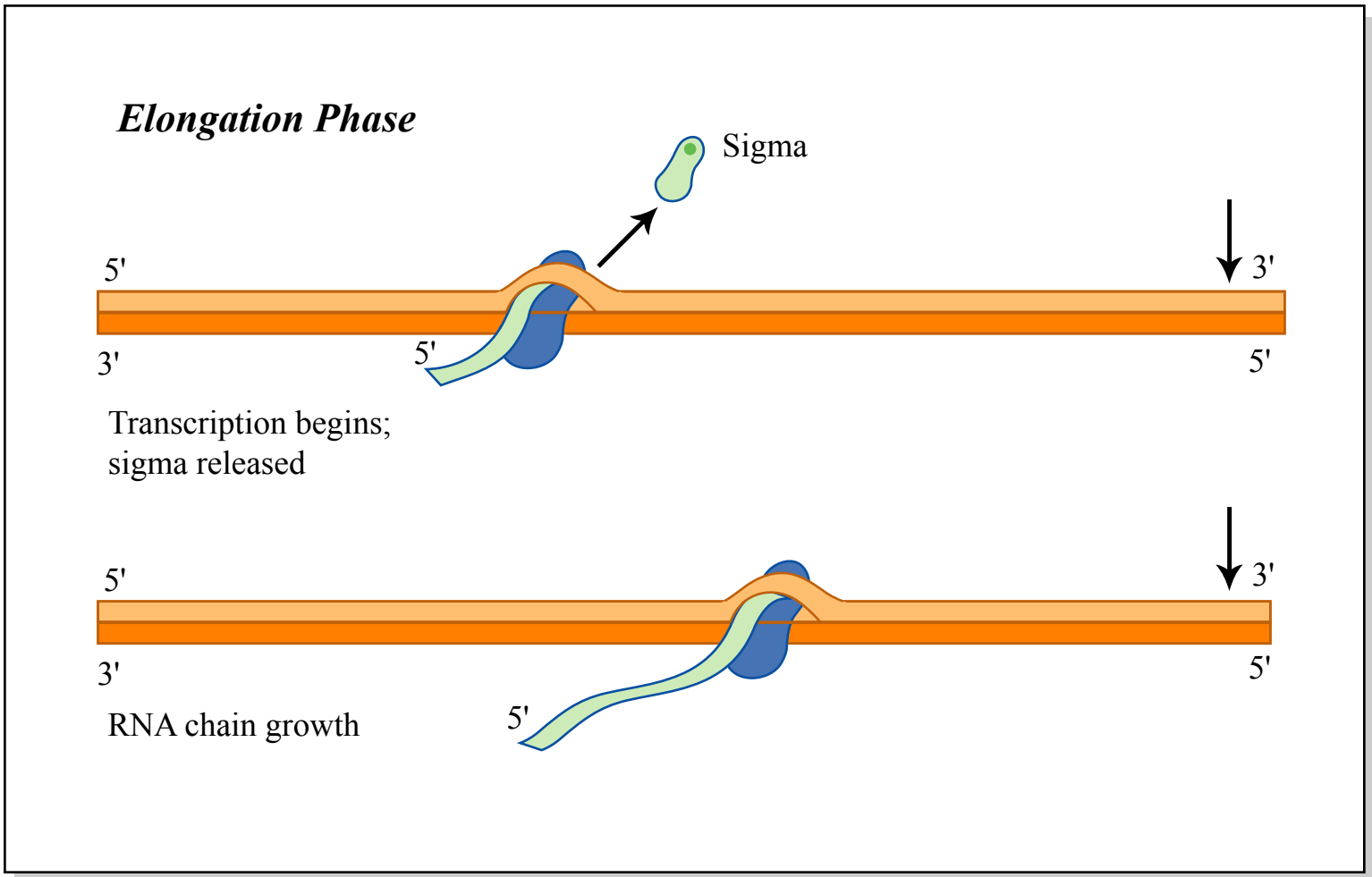


Figure by MIT OCW.

Elongation phase continues until the RNAP reaches a terminator and dissociates

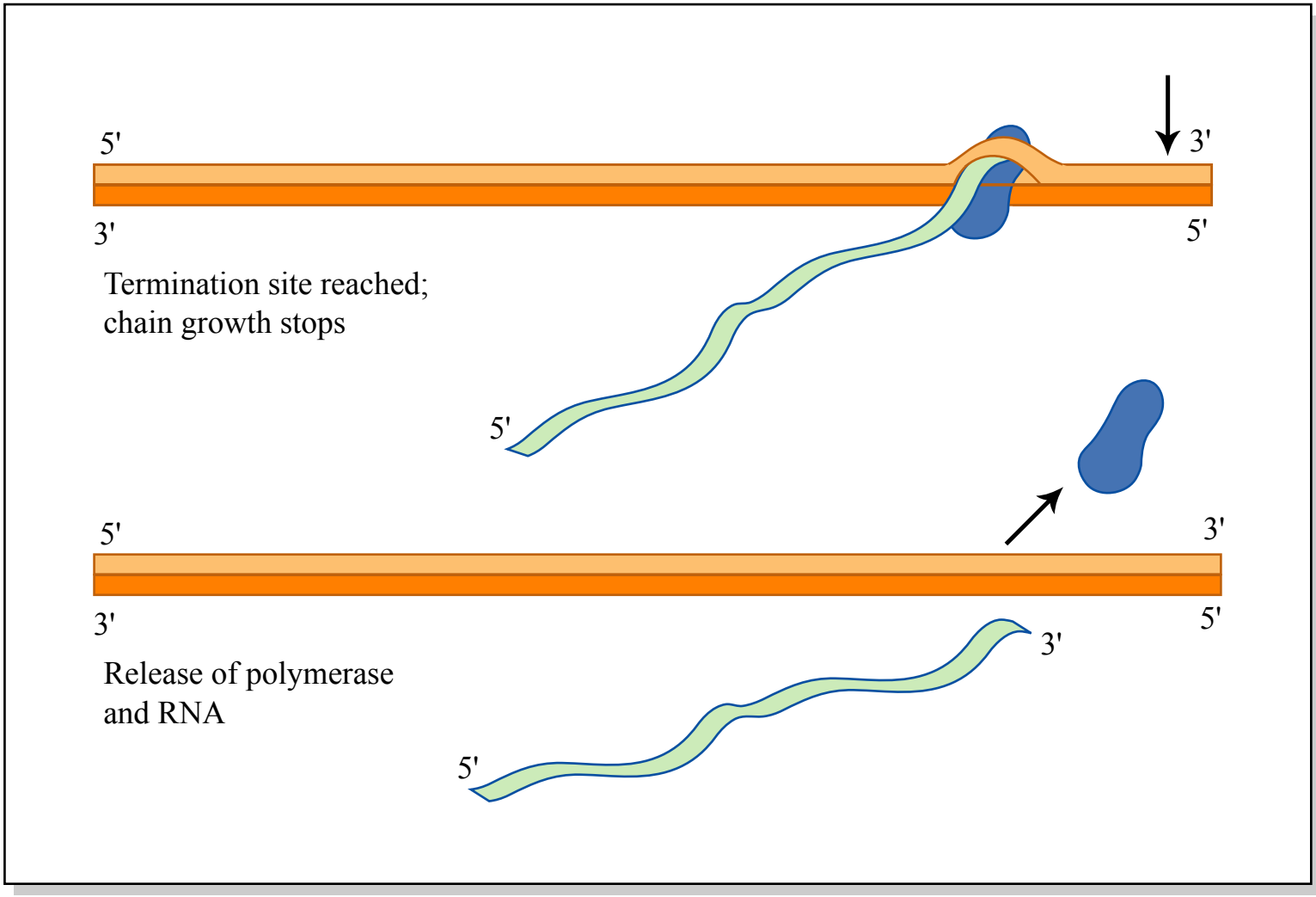


Figure by MIT OCW.

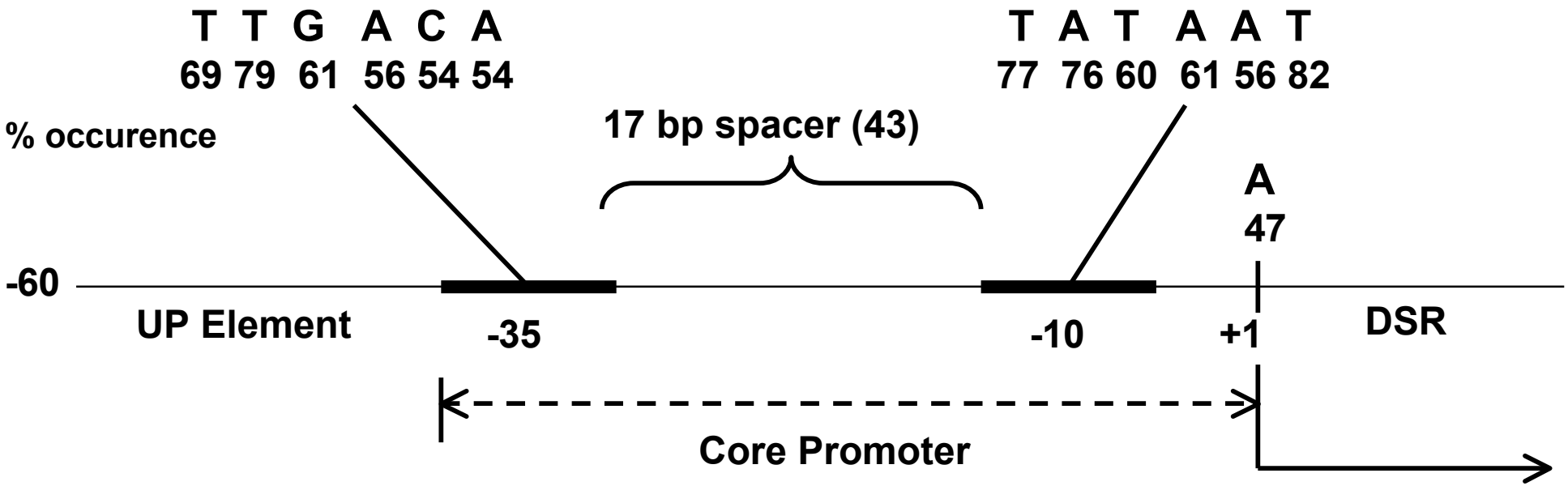
Initiation of transcription begins with promoter binding by RNAP holoenzyme

holoenzyme = RNAP core + Sigma

Diagram of RNA polymerase and transcription removed due to copyright restrictions.

Architecture of a vegetative (σ^{70}) promoter

-core promoter recognized by sigma factor



Alternative sigma factors bind to core RNA pol and direct it to different promoters.

E. coli RNA pol holoenzyme is $\alpha_2\beta\beta'\sigma$

Sigma 70 is used for 'normal' promoters

Sigma 32 is used for heat-shock promoters

Sigma 54 is used for N limitation promoters

Gene	**Sigma factor**	**-35**	**Spacing**	**-10**
rpoD	σ70	TTGACA	16-18 bp	TATAAT
rpoH	σ32	CCCTTGAA	13-15 bp	CCCGATNT
rpoN	σ54	CTGGNA	6 bp	TTGCA

What dictates the transcriptional activity of a gene?

Promoter strength

How similar are the promoter core elements (-10,-35, and their spacing) to the consensus?

- in general the more similar they are, the more active the promoter will be to initiate transcription
- however, some positions are more important than others

TTGACA ---17bp---TATAAT---A Consensus

TCGACA---17bp---TATTAT---A Strong promoter

TCAGTT---19bp---GATAAC---A Weaker promoter

Non-core sequences can affect promoter strength

1. Extended –10 sequences

   some promoters have longer –10 elements

2. UP elements

   other promoters have AT rich sequences just upstream of the –35 that elevate transcription rate

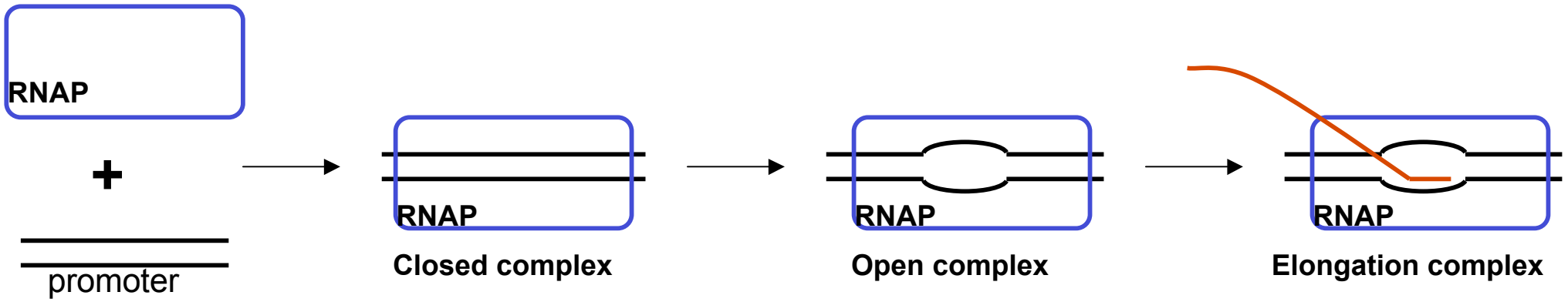
3. Downstream elements

   sequences immediately downstream of the start site can affect the overall efficiency of transcription initiation

Initiation complexes through elongation

Diagram removed due to copyright restrictions.

Karp, 1999, Molecular Cell Biology, Wiley and Sons



The transcription cycle

- can be viewed as a cycle

1. Initiation

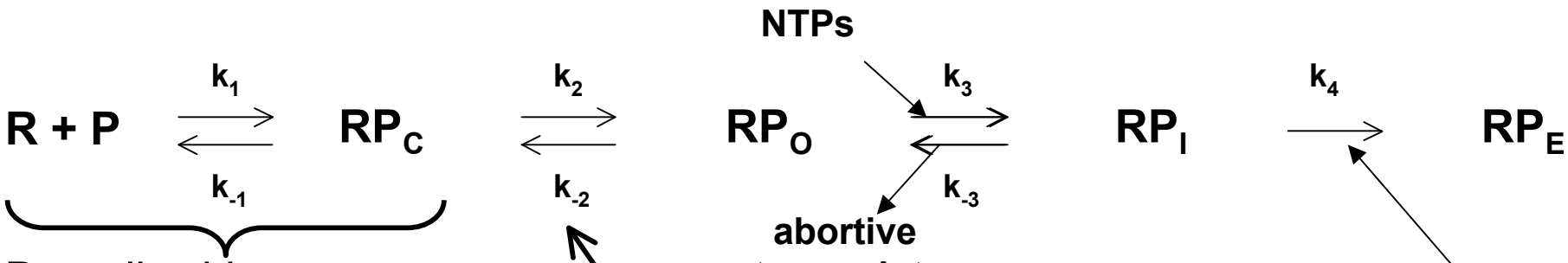
2. Elongation

3. Termination

Diagram removed due to copyright restrictions.

Mooney and Landik, 1999. Cell 98: 687

Mechanism of transcriptional initiation



Described by a equilibrium constant called K_I

$K_I = RP_C/(R + P)$

R – RNAP
P – Promoter
RPC – closed complex
RPO – open complex
RPI – initiation complex
RPE- elongation complex

The rate of open complex formation is often called k_{II}

This transition is called "promoter clearance"- described by k_{IV}

Transcription termination

Diagram of transcription termination removed due to copyright restrictions.
See Figure 7-32 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Some differences between eukaryotic & prokaryotic transcription.

Eukaryotic mRNAs are usually spliced,capped and tailed, in the nucleus.

Eukaryotes do NOT have classical operons.

RNA polymerase structure/function differ

Initiation complexes differ Sigma factor vs. TBP

Prokaryotic genes very very rarely have introns

DRUGS THAT INHIBIT TRANSCRIPTION &/or DNA REPLICATION

ANTIBIOTIC	TARGET; MODE OF ACTION
Actinomycin D	**Transcription**; inhibits DNA-dependent RNA synthesis by binding DNA
Adriamycin·HCl	**DNA replication & Transcription**; Inhibits DNA and RNA synthesis by binding DNA
Aphidicolin	**DNA replication** ; Inhibits alpha-type polymerase (eukaryotic and viral)
Bleomycin·sulfate	**DNA replication** ; reacts with DNA and causes chain break
Chromomycin A_3	**Transcription** ; Inhibitor of DNA-dependent RNA-synthesis
Mithramycin A	**Transcription ;** inhibits RNA synthesis by complexing with DNA
Mitomycin C	**DNA replication** ; Anti-tumor antibiotic. Binds covalently to DNA
Nalidixic acid	**DNA replication**; Inhibitor of bacterial DNA gyrase (a topisomerase inhibitor)
Netropsin·	**DNA replication**; Peptide antibiotic. Binds to AT-rich regions in the minor groove of DNA
Novobiocin	**DNA replication;** Inhibitor of bacterial DNA gyrase
Rifampicin	**Transcription ;** Inhibitor of DNA-dependent RNA-polymeras
Novobiocin·	**DNA replication**; Causes DNA methylation and DNA strand breaks

Translation

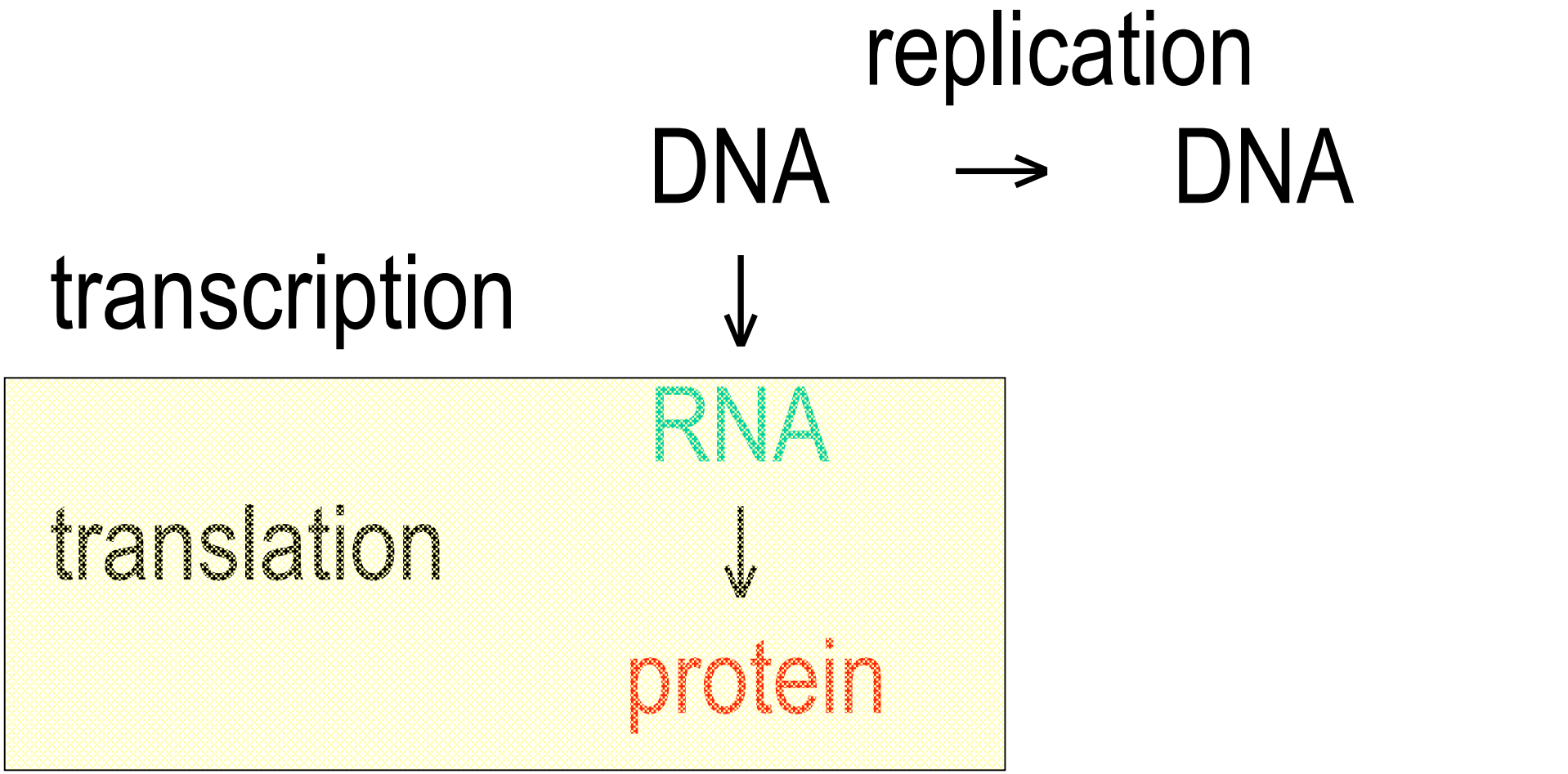
Coupled transcription/translation **Compartmentalization/transcript processing**

Diagram of transcription and translation in prokaryotes vs. eukaryotes removed due to coypyright restrictions.

Coupled transcription/translation

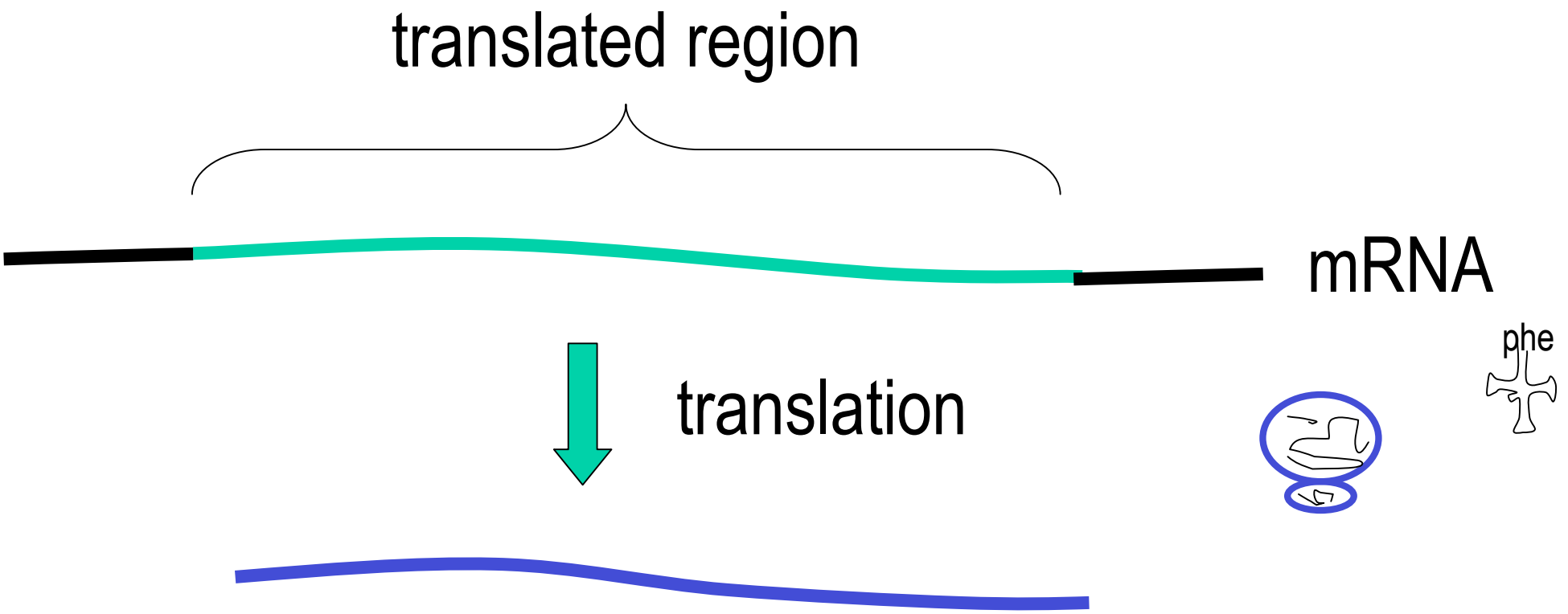
Microscopic photographs of transcription and translation removed due to copyright restrictions.

Flow of information



Overview of prokaryotic translation

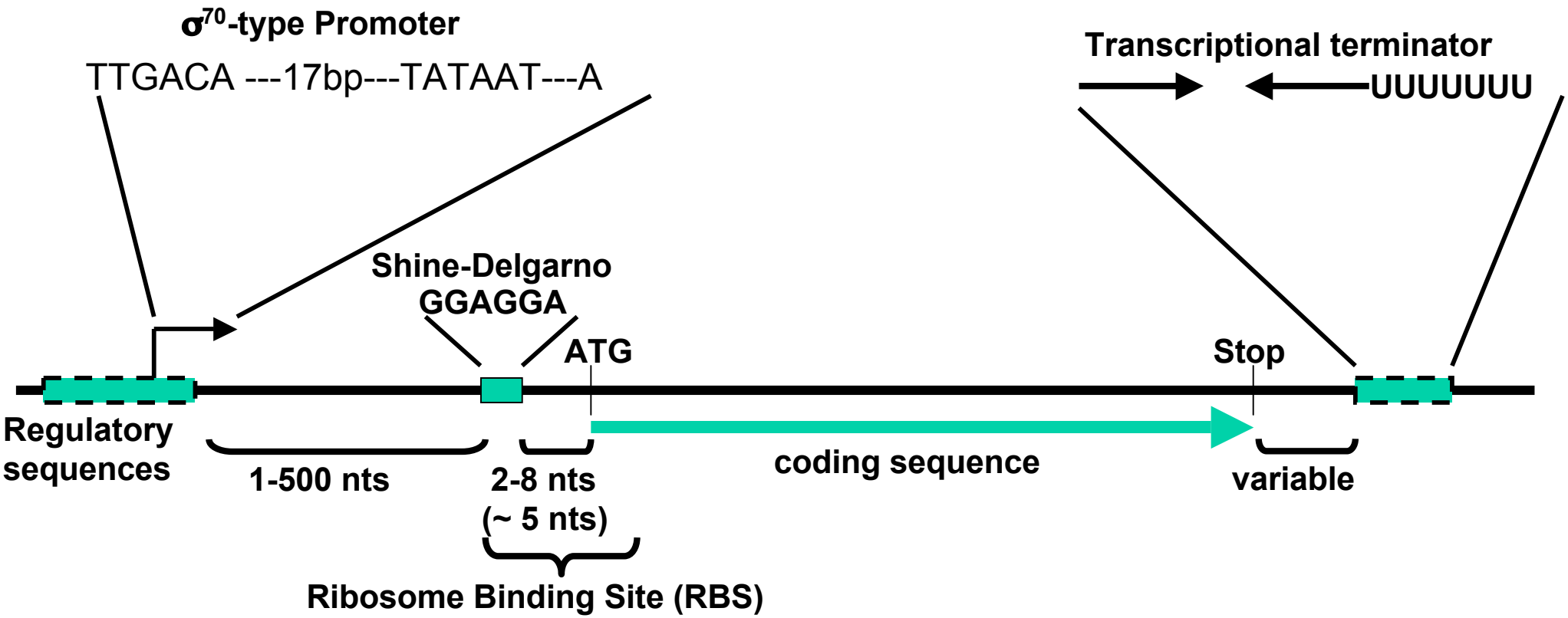
Protein synthesis from an mRNA template.



Key components of translation

- Messenger RNA
- Transfer RNA
- ribosomes and rRNA

Simple structure of a prokaryotic gene



Shine-Dalgarno sequence

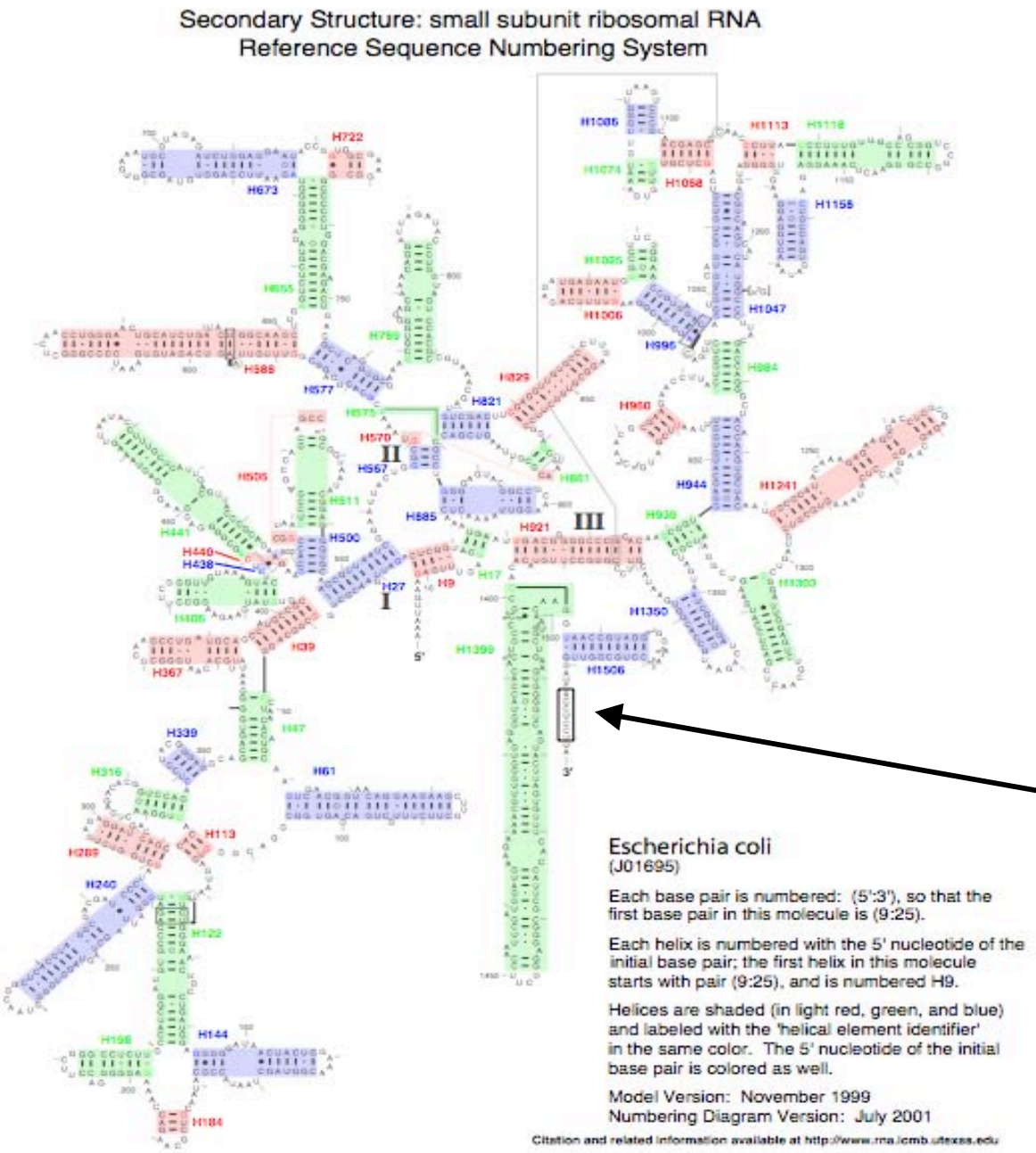
~AGGAGG, ribosome binding sequence, critical for ribosome binding

Start codons

AUG, GUG, or UUG

Stop codons (nonsense codons)

UAA, UGA, or UAG




```
5′mRNA AGGAGGU 3′
3′rRNA UCCUCCA 5′
```

Shine Delgarno seq.

Blue = Universal sites

THE GENETIC CODE

- Series of codons that determines the amino acid sequence of the encoded protein.
- Coding sequences have an average of about 300 codons.
- Except for the stop codon, each codon specifies a particular amino acid.

The genetic code

Table of the genetic code removed due to copyright restrictions.
See Table 7-5 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

The genetic code is degenerate.

more than one codon can code for the same amino acid

UUU → phenylalanine
UUC → phenylalanine

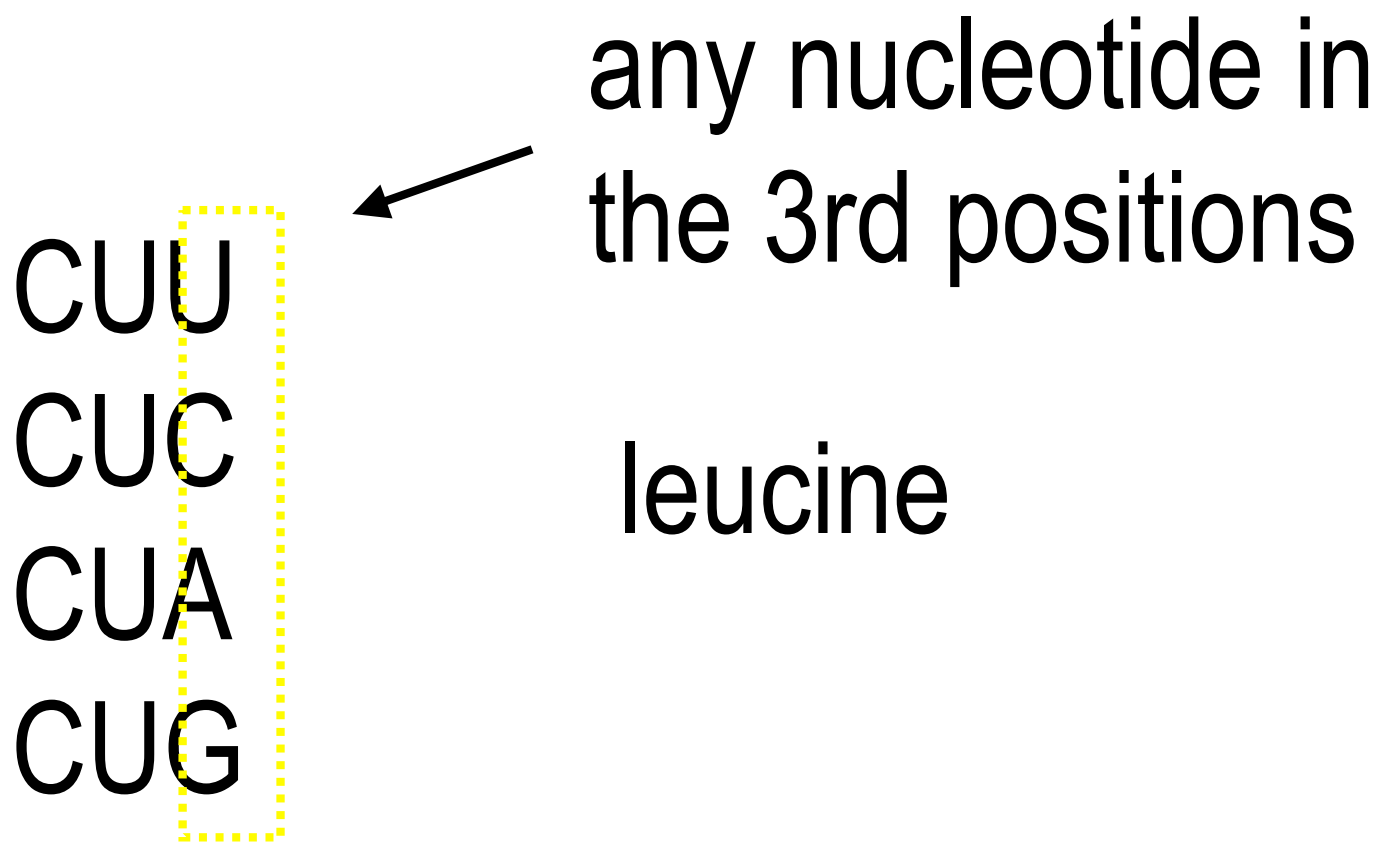
Synonyms

Different codons can code for the same amino acid

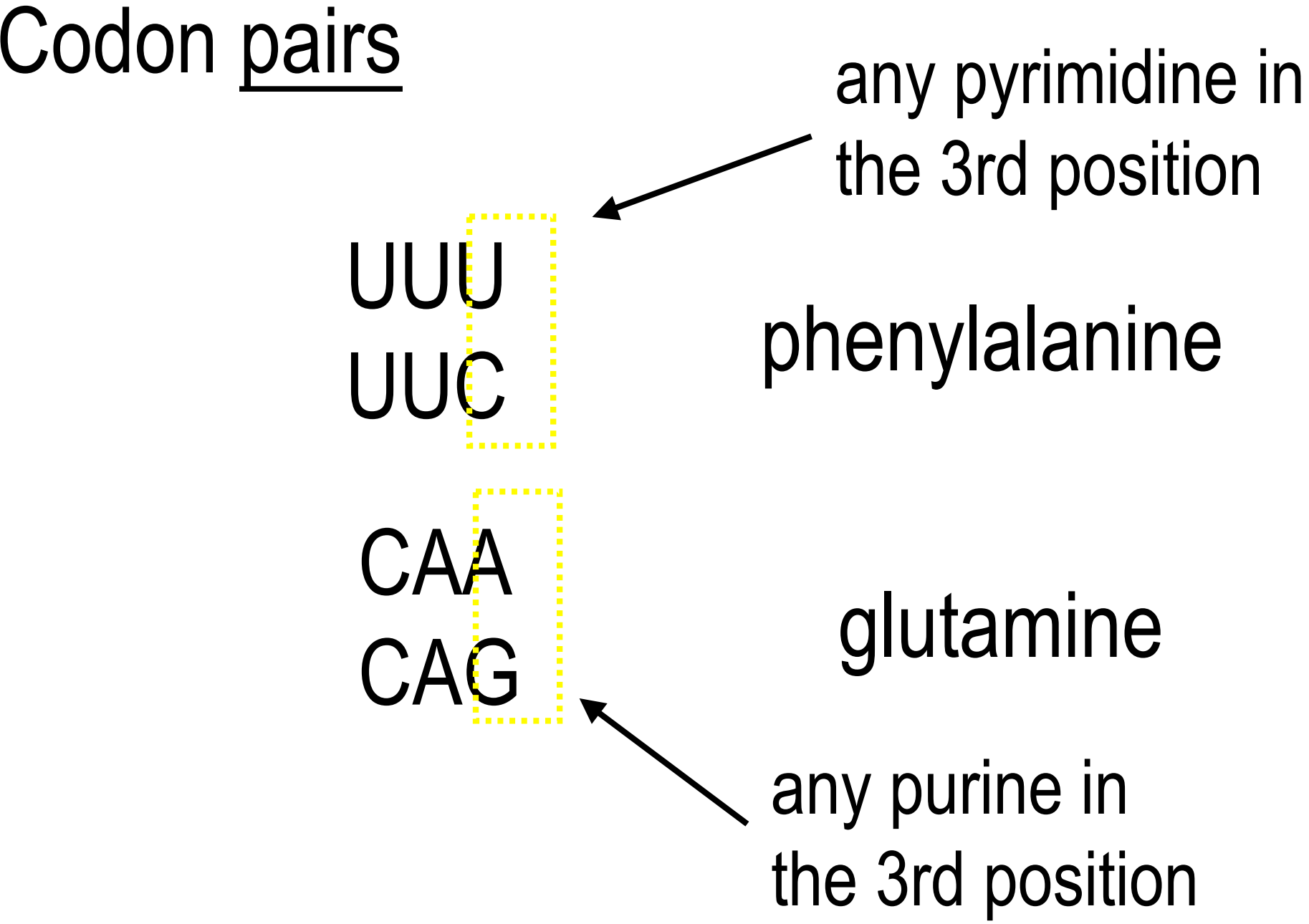
UUU → phenylalanine
UUC → phenylalanine

Not all synonyms are used with equal frequency. This is called "codon usage bias".

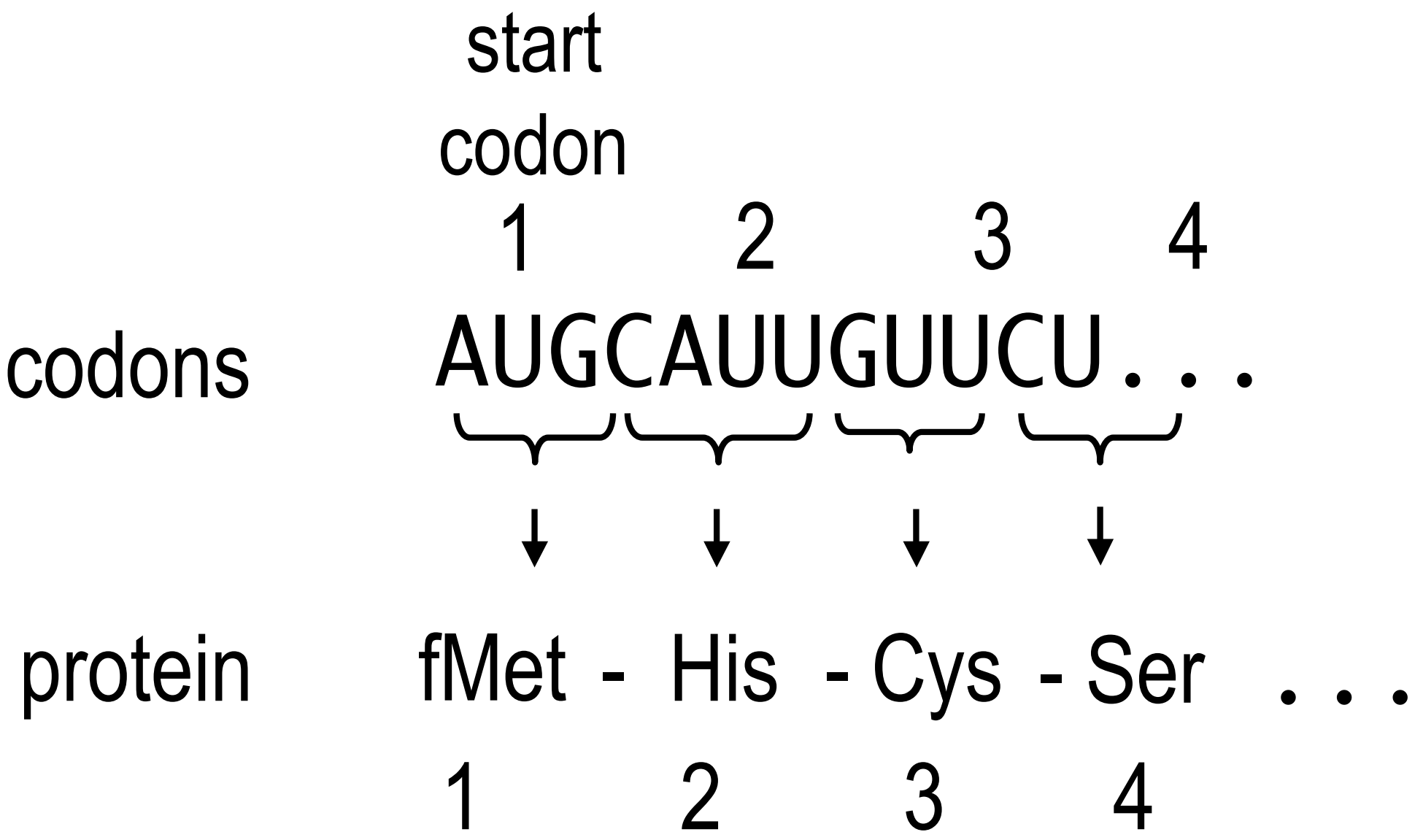
Codon families



Codon pairs


any pyrimidine in
the 3rd position
UUU
UUC
phenylalanine
CAA
CAG
glutamine
any purine in
the 3rd position

Codons consist of 3 bases



Reading frames

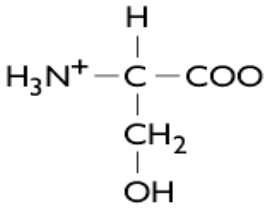
```
     TTC TCA TGT TTG ACA GCT
RF1  Phe Ser Cys Leu Thr Ala>
RF2   Ser His Val *** Gln Leu>
RF3     Leu Met Phe Asp Ser>
     AAG AGT ACA AAC TGT CGA
RF4 <Glu *** Thr Gln Cys Ser
RF5    <Glu His Lys Val Ala
RF6  <Arg Met Asn Ser Leu
```

	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	***	***	A
	Leu	Ser	***	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

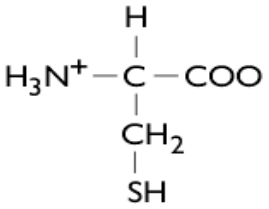
Structures of amino acids commonly found in proteins (20).

Hydrophilic amino acids

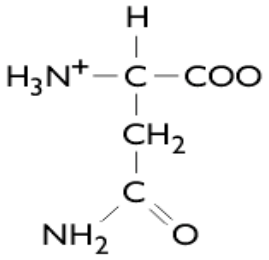
Polar



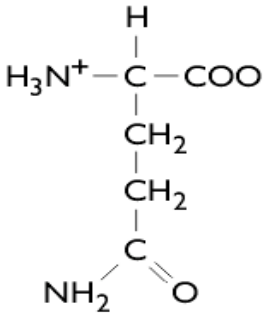
Serine (Ser) S



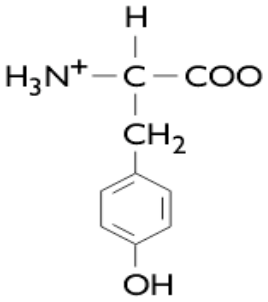
Cysteine (Cys) C



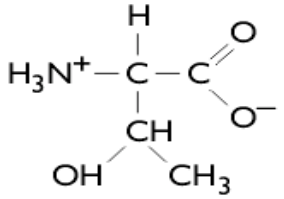
Asparagine (Asn) N



Glutamine (Gln) Q

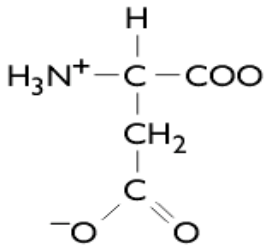


Tyrosine (Tyr) Y

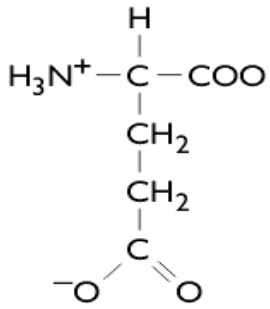


Threonine (Thr) T

Negatively charged

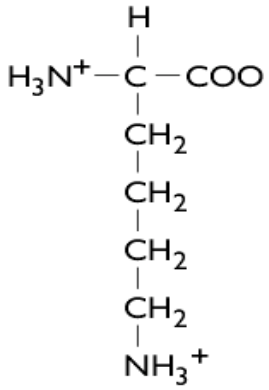


Aspartic acid (Asp) D

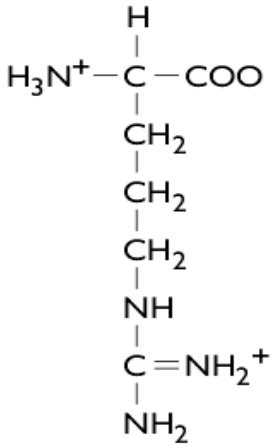


Glutamic acid (Glu) E

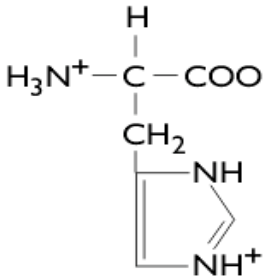
Positively charged



Lysine (Lys) K

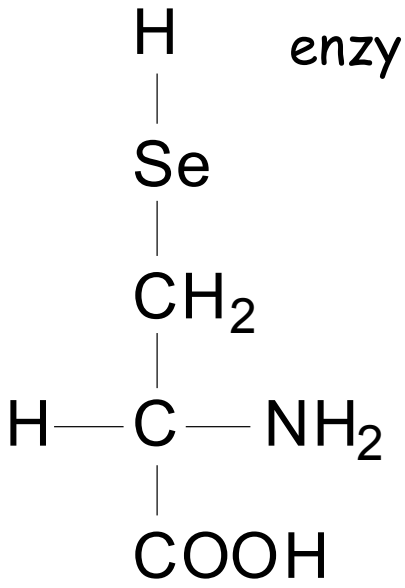


Arginine (Arg) R



Histidine (His) H

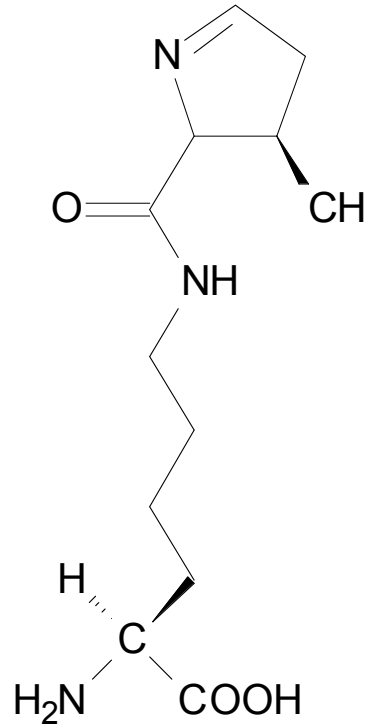
Selenocysteine – the 21st amino acid



Selenocysteine appears in a number of oxidoreductase enzymes e. g. formate dehydrogenase, glycine reductase.

UGA codon, normally nonsense !
(surrounding context allows to serve as 'sense' codon)

Pyrrolysine – the 22nd amino acid



UAG codon, normally nonsense !

Pyrrolysine is found in enzymes involved in methanogenesis in a few bacteria and archaebacteria.

Key components of translation

Messenger RNA

Transfer RNA

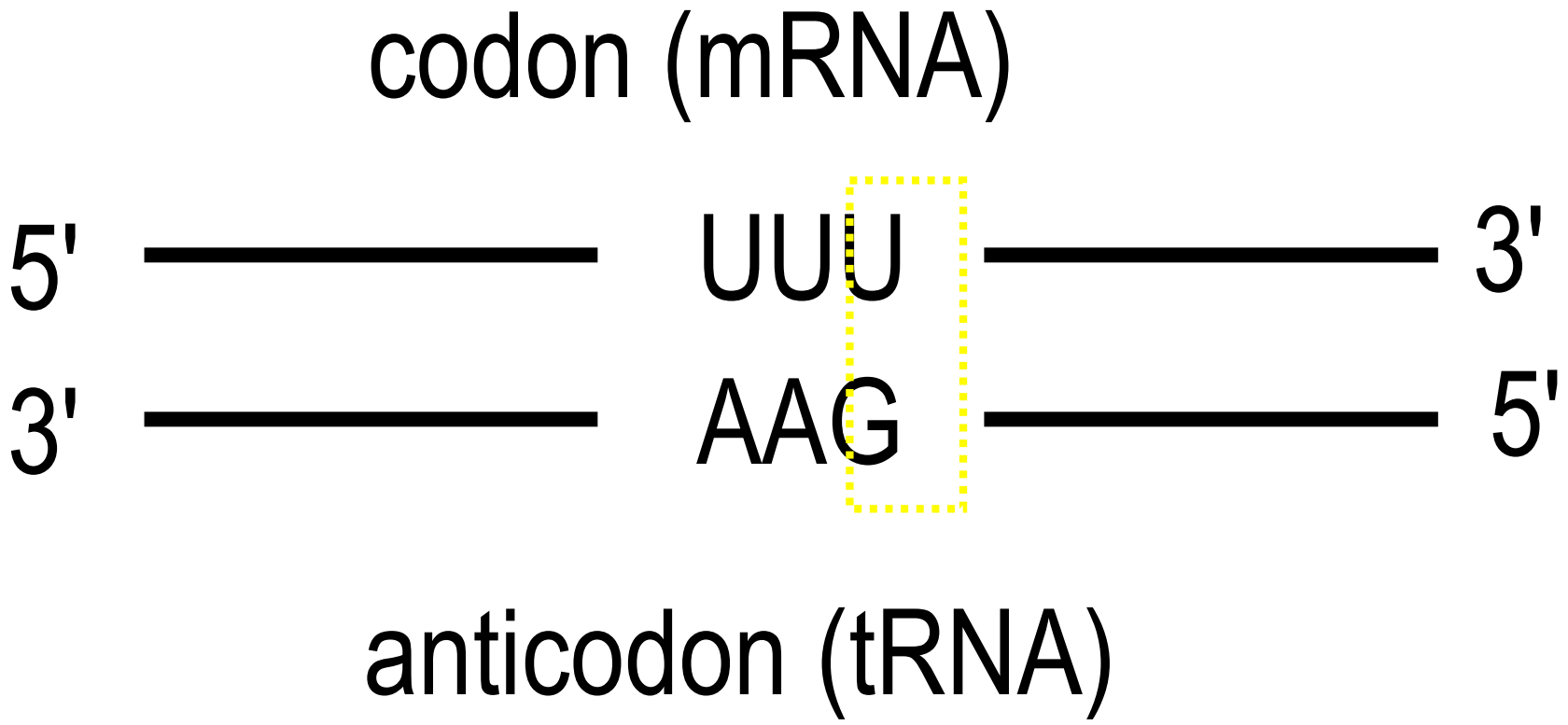
ribosomes and rRNA

Diagrams removed due to copyright restrictions.
See Figures 7-36 and 7-34 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

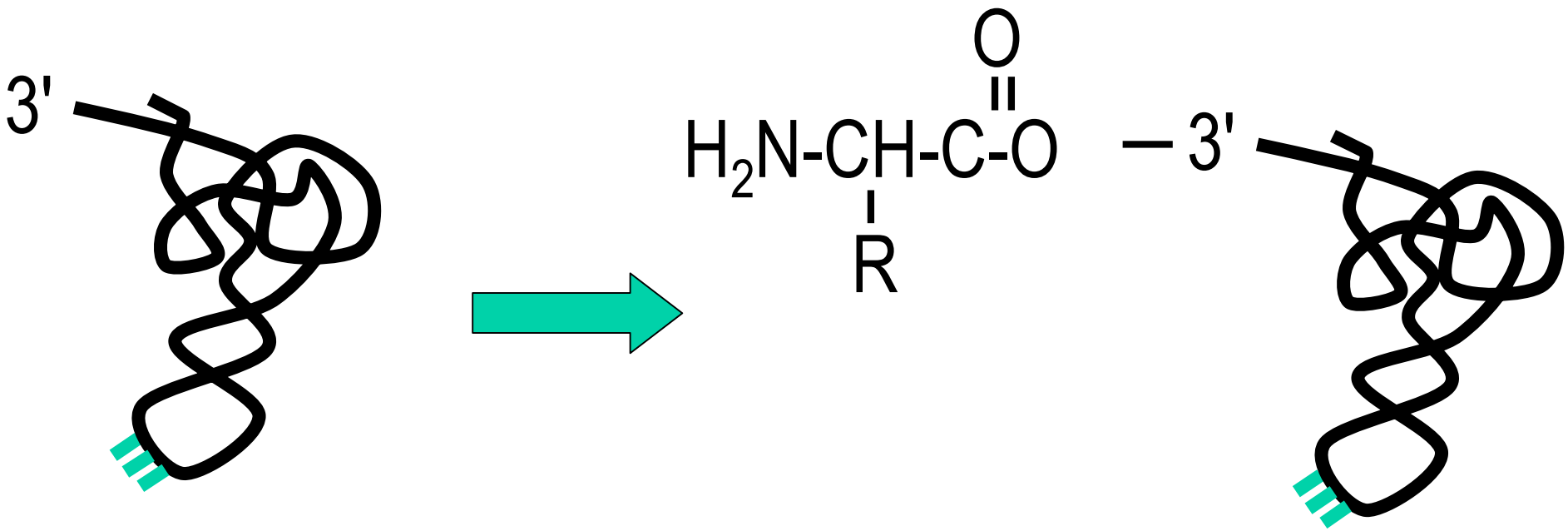
Wobble base pairing

UUU UUC	phenylalanine

U-G and G-U base pairs are allowed in the 3rd position of the codon.



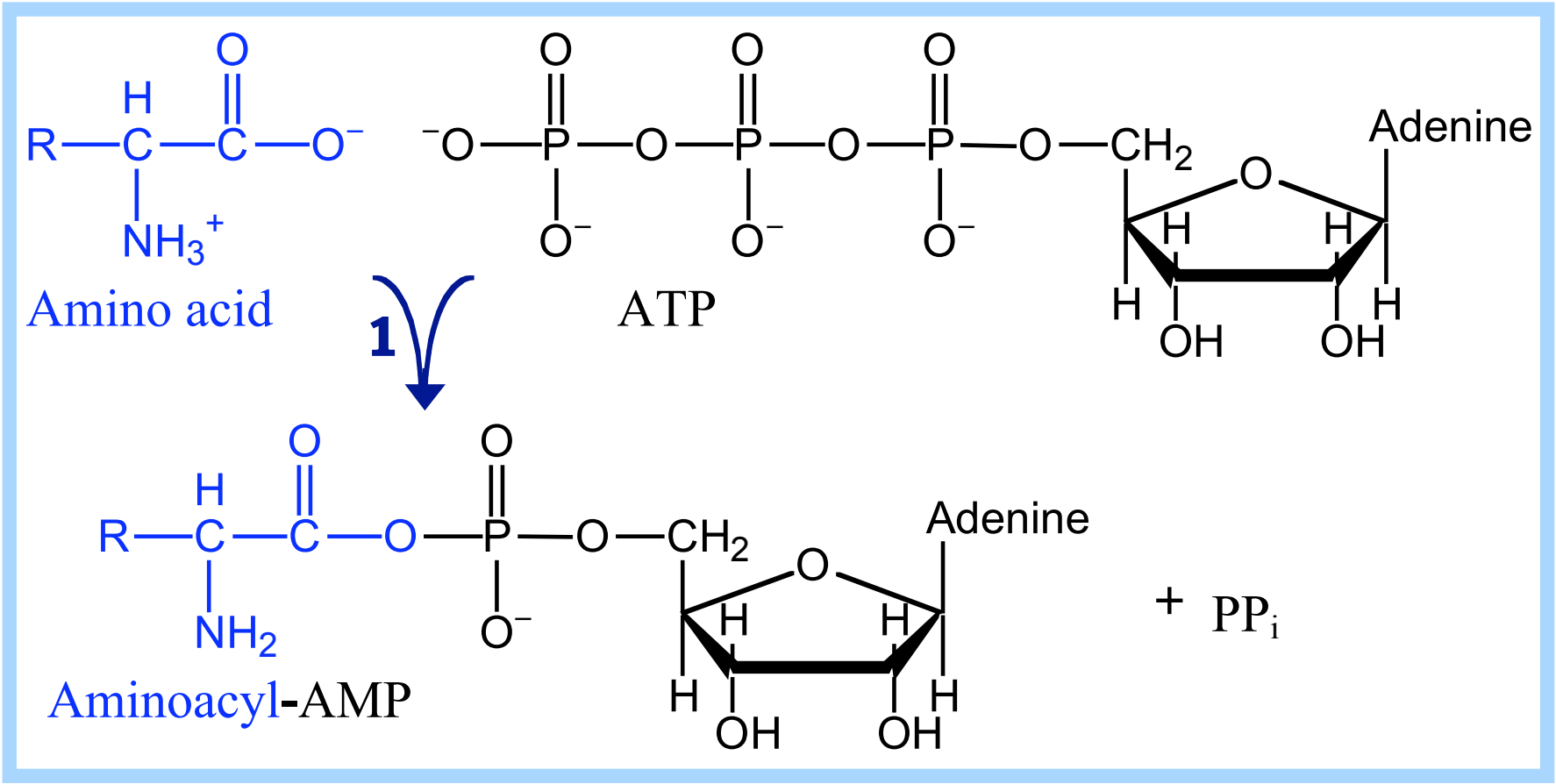
tRNA charging (adding amino acid)



tRNA
(uncharged)

aminoacyl-tRNA
(charged)

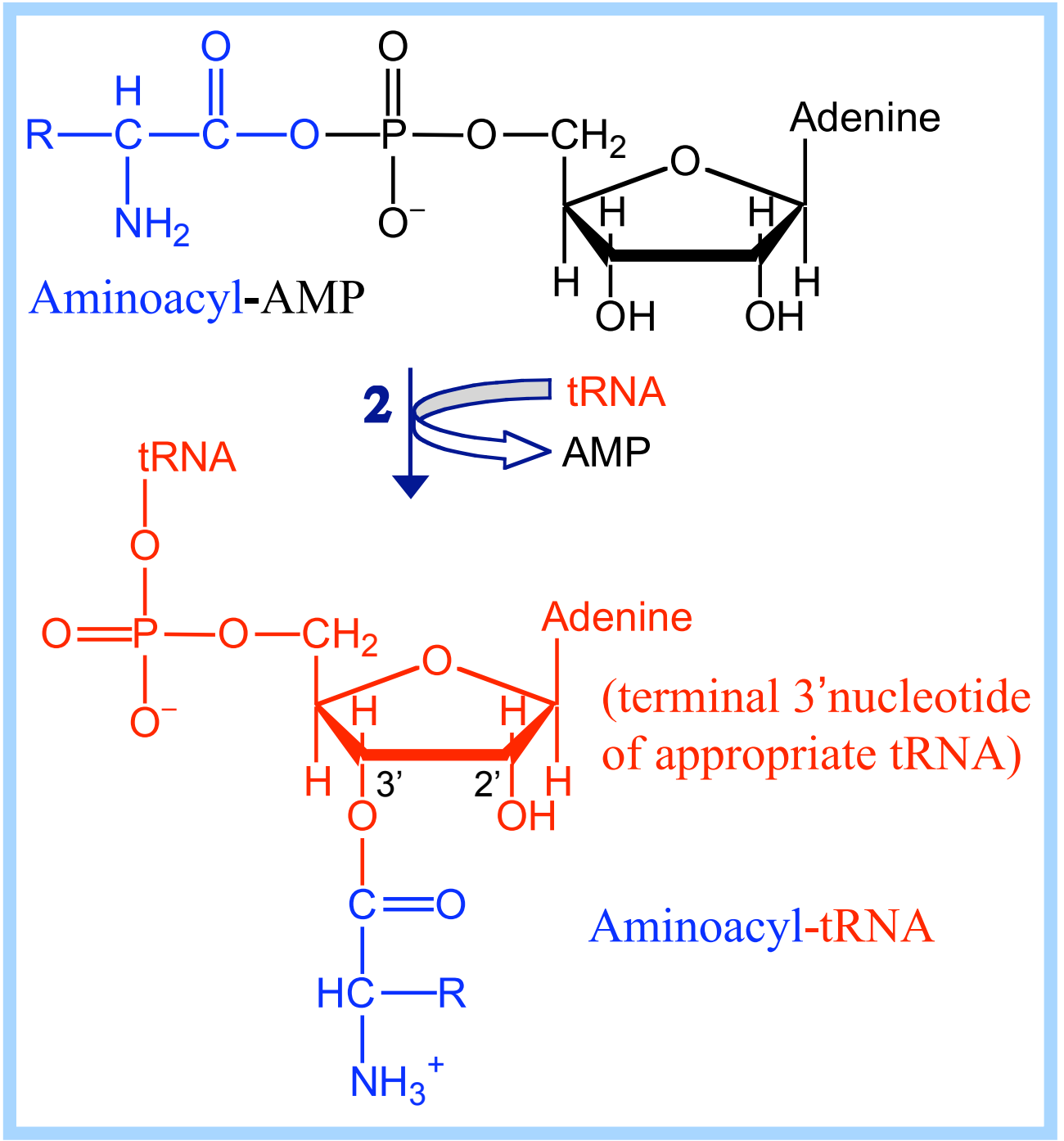
tRNA charging uses the energy of ATP



Aminoacyl-tRNA Synthetases catalyze linkage of the appropriate amino acid to each tRNA. The reaction occurs in two steps.

In **step 1**, an O atom of the amino acid α-carboxyl attacks the P atom of the initial phosphate of ATP.

In **step 2**, the 2' or 3' OH of the terminal adenosine of tRNA attacks the amino acid carbonyl C atom.



Aminoacyl-tRNA Synthetase

Summary of the 2-step reaction:

1. **amino acid + ATP → aminoacyl-AMP + PP_i**

2. **aminoacyl-AMP + tRNA → aminoacyl-tRNA + AMP**

The 2-step reaction is **spontaneous** overall, because the concentration of **PP_i** is kept low by its hydrolysis, catalyzed by Pyrophosphatase.

There is a different **Aminoacyl-tRNA Synthetase** (**aaRS**) for each amino acid.

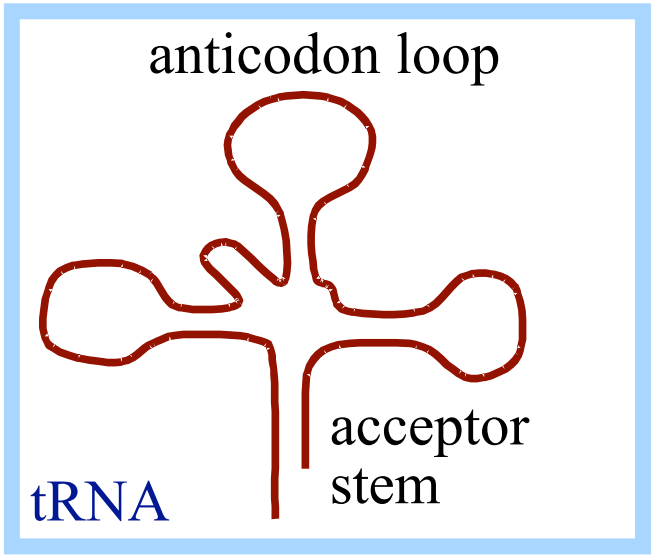
Each aaRS recognizes its particular amino acid and the tRNAs coding for that amino acid.

Accurate translation of the genetic code depends on attachment of each amino acid to an appropriate tRNA.

Domains of tRNA recognized by an aaRS are called **identity elements**.

Most identity elements are in the **acceptor stem** & **anticodon loop**.

Aminoacyl-tRNA Synthetases arose **early in evolution**. The earliest aaRSs probably recognized tRNAs only by their acceptor stems.



Key components of translation

Messenger RNA

Transfer RNA

ribosomes and rRNA

Structure of the *E. coli* Ribosome

Diagram of the structure of the *E. coli* ribosome removed due to copyright restrictions.

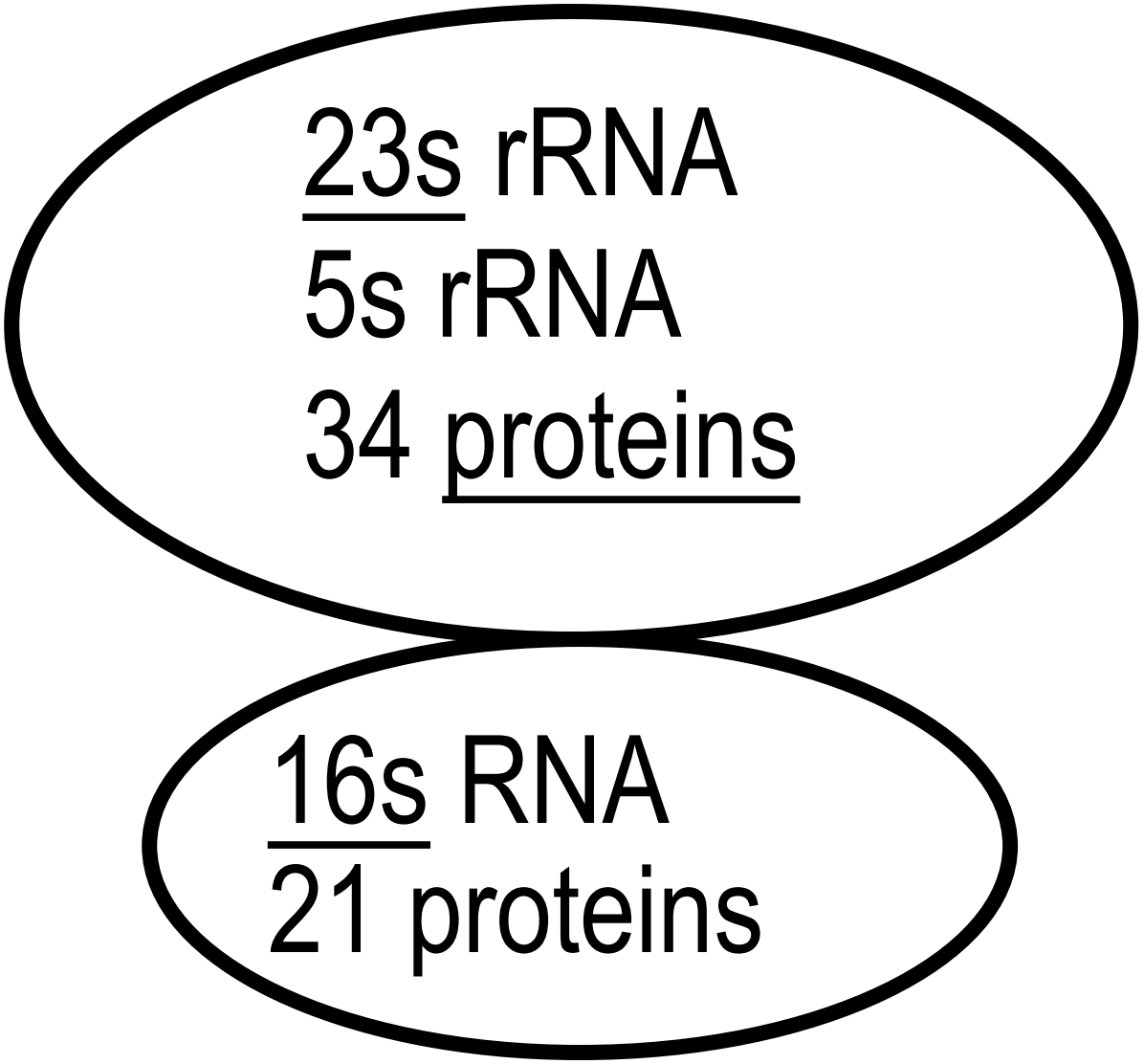
The cutaway view at right shows positions of tRNA (P, E sites) & mRNA (as orange beads).

Table of ribosome structure removed due to copyright restrictions.
See Table 7-6 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

DRUGS THAT INHIBIT TRANSLATION

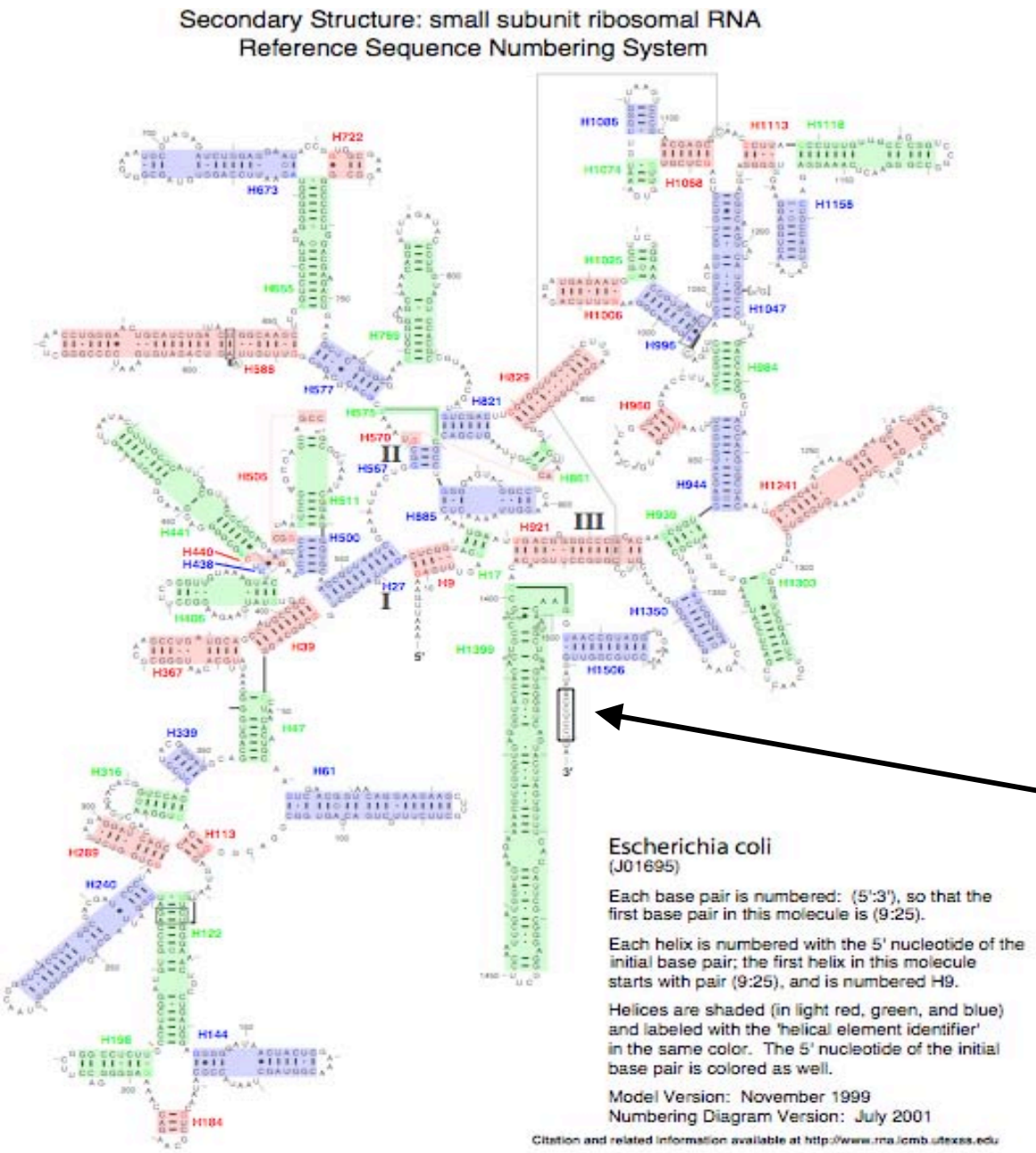
	Inhibitor	Comments
BACTERIAL	**Chloramphenicol**	inhibits prokaryotic peptidyl transferase
	Streptomycin	inhibits prokaryotic peptide chain initiation, also induces mRNA misreading
	Tetracycline	inhibits prokaryotic aminoacyl-tRNA binding to the ribosome small subunit
	Neomycin	similar in activity to streptomycin
	Erythromycin	inhibits prokaryotic translocation through the ribosome large subunit
	Fusidic acid	similar to erythromycin only by preventing EF-G from dissociating from large subunit
	Puromycin	resembles an aminoacyl-tRNA, interferes with peptide transfer resulting in premature termination in both prokaryotes and eukaryotes
EUKARYOTE	**Diptheria toxin**	catalyzes ADP-ribosylation of and inactivation of eEF-2
	Ricin	found in castor beans, catalyzes cleavage of the eukaryotic large subunit rRNA
	Cycloheximide	inhibits eukaryotic peptidyltransferase

Prokaryotic 70S ribosome



50s subunit

30s subunit




```
5′mRNA AGGAGGU 3′
3′rRNA UCCUCCA 5′
```

Shine Delgarno seq.

Blue = Universal sites

Courtesy of the Comparative RNA Web Site. Used with permission.

Diagram removed due to copyright restrictions.
See Figure 7-38 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

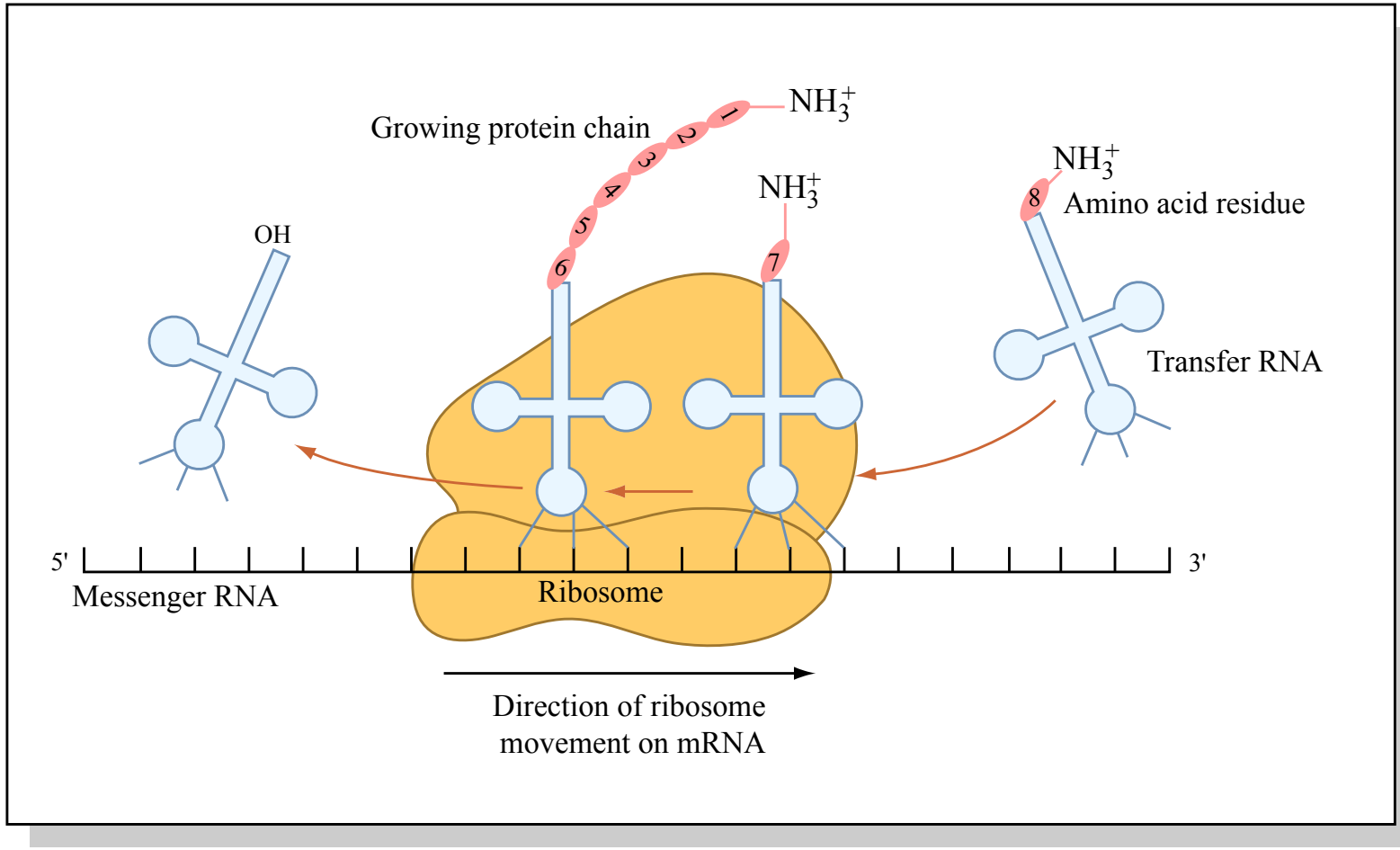


Figure by MIT OCW.

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See Figure 7-39 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

RIBOZYMES ARE CATALYTIC RNAS

EXAMPLES :

Rnase P - (cleaves t-RNA presursor -> tRNA

Self splicing introns in eukaryotes

Ribosomes !!!!

The RNA Moiety of Ribonuclease P Is the Catalytic Subunit of the Enzyme

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