

Terms

Important terms in molecular biology

- anti-parallel
Used to describe the opposite orientation of the two strands of a DNA double helix. The 5' end of one strand aligns with the 3' end of the other strand.
- complementary base pairing
Two DNA strands are said to complement one another if standard Watson-Crick base pairing can occur (AT and GC) when they are lined up in an antiparallel fashion.
- denaturation
The breaking of H-bonds that results in the separation of two strands of DNA. Separation of two strands of a DNA double helix or the severe disruption without breaking the covalent bonds of its chains.
- & melting
The breaking of H-bonds that results in the separation of two strands of DNA. The difference between melting and denaturation is that melting refers to small portions of DNA while denaturation refers to the whole strand. Enzymes typically melt DNA while boiling and harsh chemical treatments denatures it.

Important terms

- annealing. Refers to base-pairing between two nucleic acid strands.
- reannealing. When a single stranded nucleic acid re-establishes base pairs that it previously had, prior to melting, it is said to reanneal. renaturation is the equivalent term used when the molecule was denatured.
- hybridization. When two nucleic acid molecules are allowed to anneal to one another. Usually used to refer to the instance when two non-identical molecules are allowed to pair up. Typically one is a probe and one is a naturally occurring molecule.
- direction that enzymes synthesize nucleic acids
5' to 3'. If you don't understand this perfectly make me explain it to you. If you do not, you will probably fail the class.
- direction that chemists synthesize nucleic acids
3' to 5'. Its a chemistry thing.
-

Things you are expected to Know

- ▶ What is a plasmid?
- ▶ What are restriction enzymes?
- ▶ Transcription -> Translation->protein
- ▶ Read the first 4 chapters of your book.

Transcription prokaryotic

Ch 6 and 7

Transcription and Gene Expression

All gene expression involves the process called transcription.

Transcription is the method by which cells synthesize RNA molecules.

The process of transcription is very similar to the process of DNA synthesis. The primary differences between the two are that transcription:

- 1) uses ribonucleotides
- 2) uses uracil instead of the nitrogenous base thymine
- 3) produces a single stranded copy of the DNA coding strand (of course, the RNA may form intramolecular H-bonds)
- 4) only the subset of the genome that we refer to as genes are transcribed.

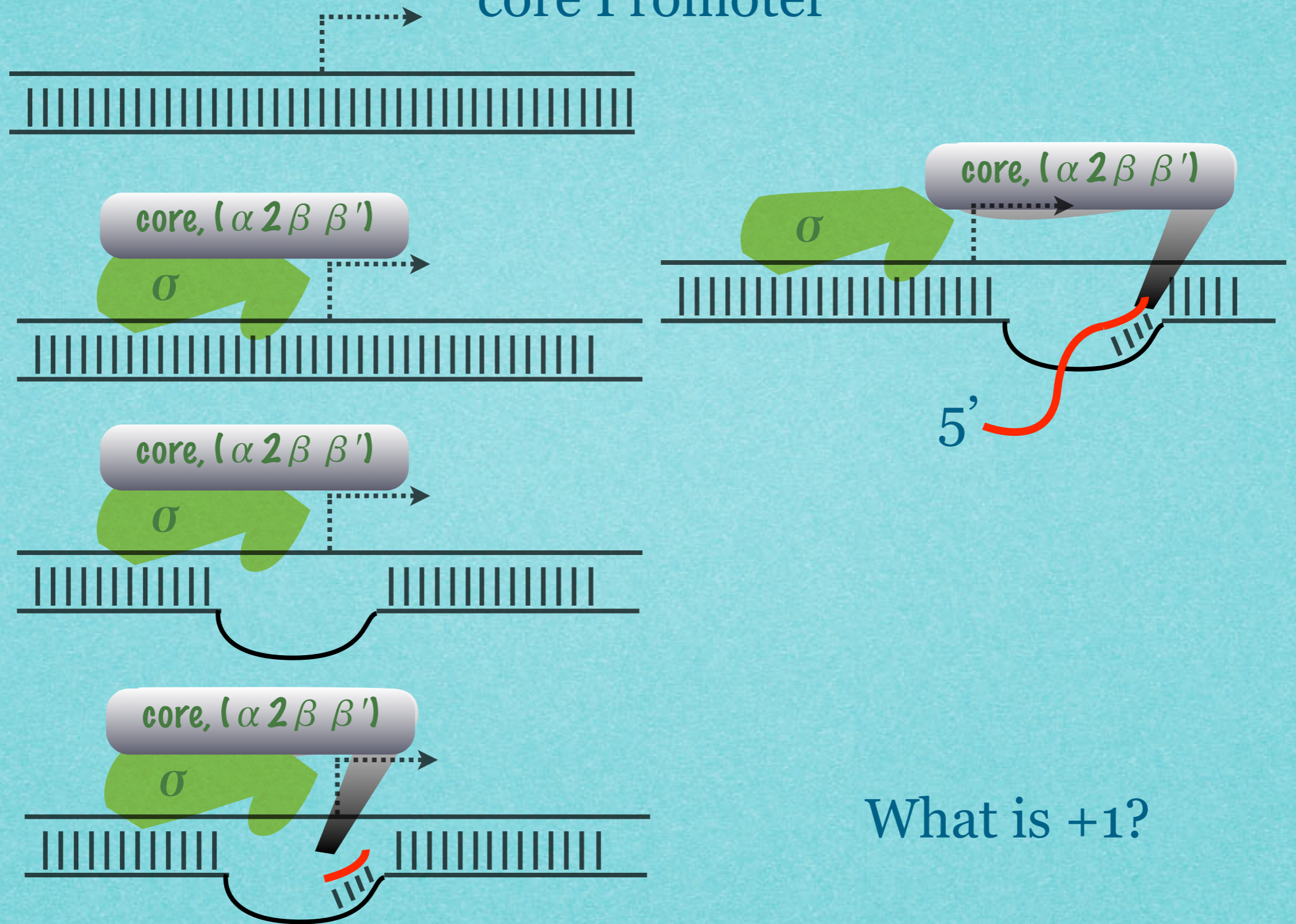
RNA synthesis rules

1. Precursors for synthesis are four ribonucleotide 5'-triphosphates, ATP, GTP, CTP and UTP (generically referred to as rNTP's)
2. During synthesis the 3' hydroxyl of nascent RNA chain reacts with the 5'-triphosphate of precursor rNTP.
3. Each new base to be added is 'chosen' based on its ability to base-pair with the template DNA strand. Watson and Crick base-pairing rules apply.
4. One strand of the DNA serves as template.
5. RNA chain is synthesized in the 5'->3' direction.
6. RNA is synthesized by DNA-dependent RNA polymerases (usually referred to simply as RNA polymerases).
7. RNA polymerases can initiate synthesis. **NO PRIMER IS NEEDED.**
8. In prokaryotes, the 5' most nucleotide keeps its triphosphate.
Therefore, the 5' end of RNA molecule has 3 phosphate groups.

Bacterial polymerase

- ▶ Bacteria have a single RNA polymerase that transcribes all genes which consists of 4 subunits (α_2 , β , β'). This is called the core polymerase.
- ▶ σ factor is a 5th subunit. Core+ σ =holoenzyme.
- ▶ σ can recognize promoters.

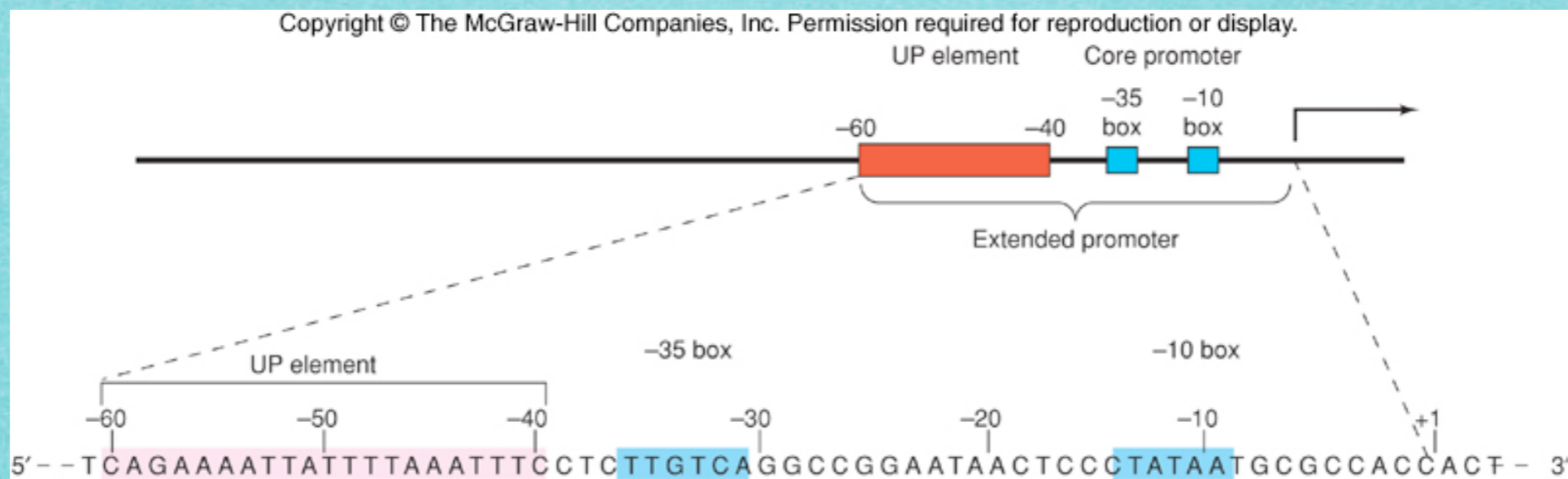
core Promoter



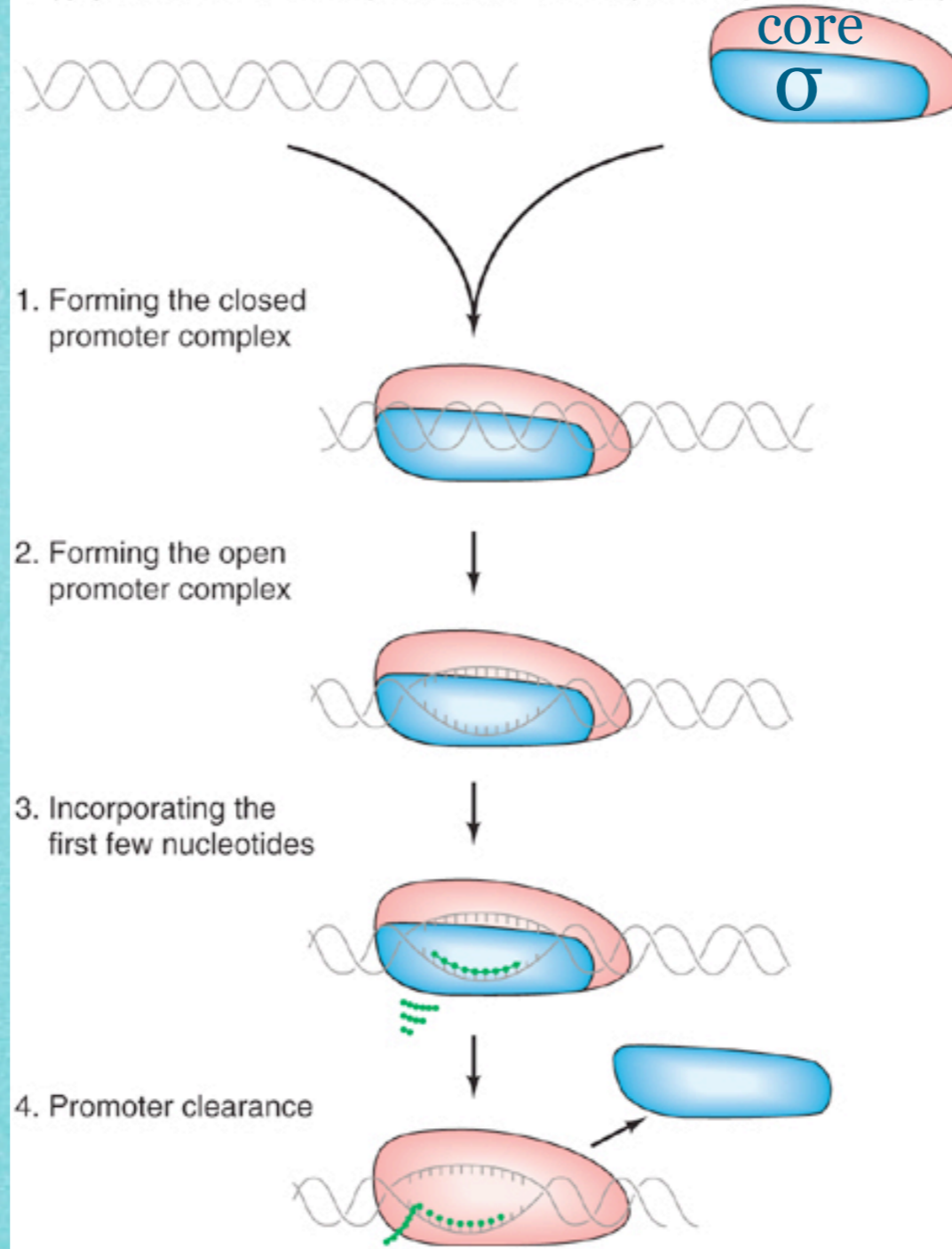
What is +1?

Sigma recognizes specific sequences

Figure 6.7



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Promoters & the idea of consensus sequences

The idea of a consensus sequence is a very important one.

(a) Strong *E. coli* promoters

tyr tRNA	TCTCAACGTAACAC	TTTACAGCGGCG	CGTCATTTGAT	TATGAT	GC	GCCCC	GCTTCCCGATAAGGG
rrn D1	GATCAAAAAAATAC	TTGTGCAAAAAA	TTGGGATCCC	TATAAT	GCGCCTCC	GTTGAGACGACAACG	
rrn X1	ATGCATTTTTCCGC	TTGTCTTCCTGA	GCCGACTCCC	TATAAT	GCGCCTCC	ATCGACACGGCGGAT	
rrn (DXE) ₂	CCTGAAATTCAGGG	TTGACTCTGAAA	GAGGAAAGCG	TAATATAC	GCCAC	CTCGCGACAGTGAGC	
rrn E1	CTGCAATTTTTCTA	TTGCGGCCTGCG	GAGAACTCCC	TATAAT	GCGCCTCC	ATCGACACGGCGGAT	
rrn A1	TTTTAAATTTCTC	TTGTCAAGGCCGG	AATAACTCCC	TATAAT	GCGCCACC	ACTGACACGGAAACAA	
rrn A2	GCAAAAAATAAATGC	TTGACTCTGTAG	CGGGAAGGCG	TATTATGC	ACACC	CCGCGCCGCTGAGAA	
λ P _R	TAACACCGTGCGTG	TTGACTATTTTA	CCTCTGGCGGTG	AATAATGG	TTGCATGTACTA	AAGGAGGT	
λ P _L	TATCTCTGGCGGTG	TTGACATAAATA	CCACTGGCGGTG	AATACTGA	GACATCAGCAGGAC	GCAC	
T7 A3	GTGAAACAAAACGG	TTGACAACATGA	AGTAAACACGGT	ACGATGT	ACCACATGAAACGAC	AGTGA	
T7 A1	TATCAAAAAGAGTA	TTGACTTAAAGT	CTAACCTATAGG	AATACTTA	CAGCCATCGAGAGGG	ACACG	
T7 A2	ACGAAAAACAGGTA	TTGACAACATGA	AGTAACATGCAGT	AAGATAC	AAATCGCTAGGTA	AACTAG	
fd VIII	GATACAAATCTCCG	TTGTACTTTGTT	TCGCGCTTGGT	TATAATCG	CTGGGG	GTCAAAGATGAGTG	
		-35		-10		+1	

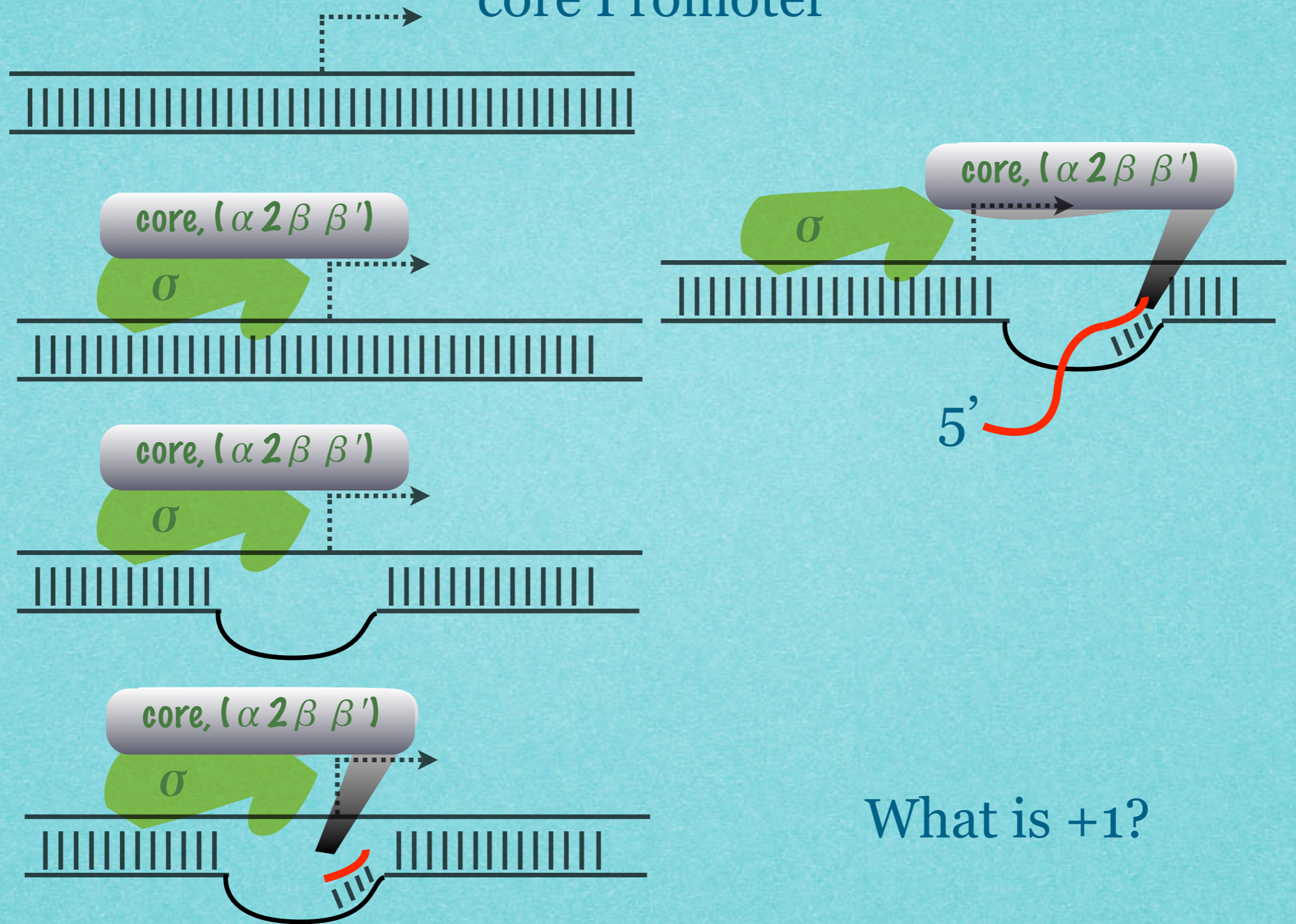
(b) Consensus sequences of σ^{70} promoters



(c) *Lac* promoter sequence



core Promoter



What is +1?

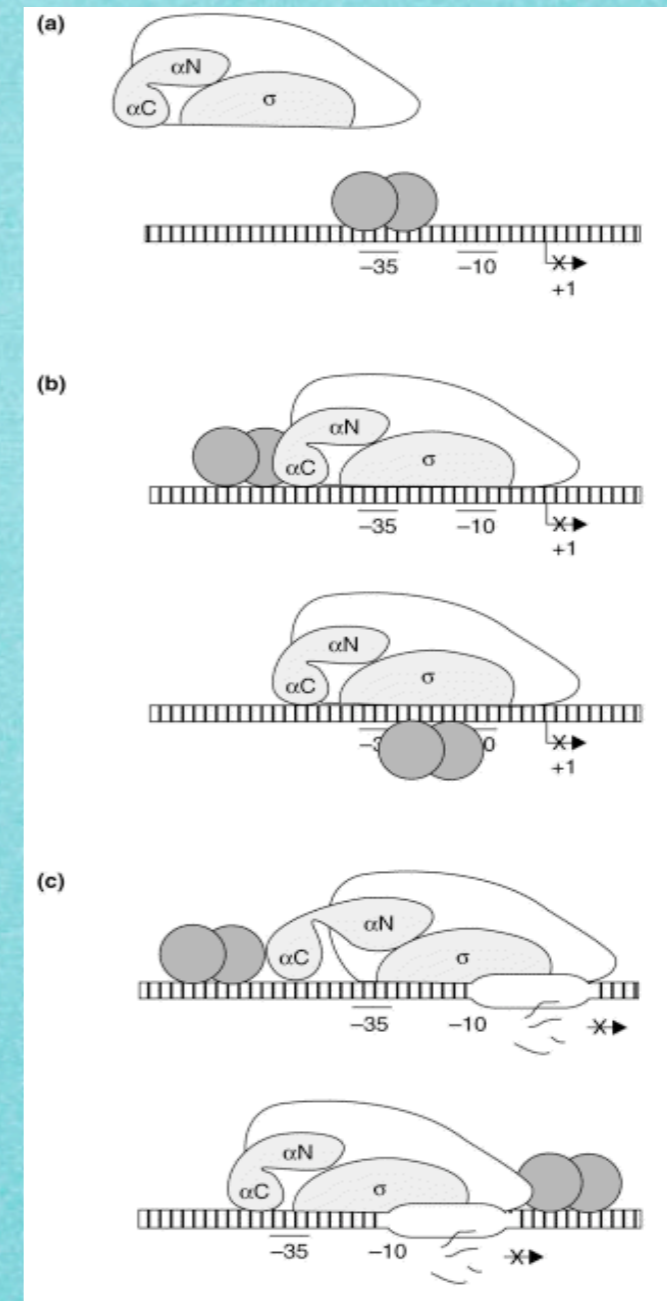
Termination

Regulation

- ▶ Can be positive
- ▶ Can be negative
- ▶ Usually both occur
- ▶ If it is important usually one sees that every step is regulated in a fashion that seems redundant to us.

Negative regulation

- ▶ A. Steric hinderance.
- ▶ B. Inhibition of transition from closed to open complex.
- ▶ C. Inhibition of promoter clearance. Open complex can form. Abortive transcripts appear.



Reference: Mechanisms of transcriptional repression

Rojó F CURRENT OPINION IN MICROBIOLOGY 4 (2): 145-151 APR 2001

Positive regulation

- ▶ Help stabilize the binding of polymerase to the core promoter.
- ▶ Enhance the rate of a step (e.g. melting, promoter clearance)

Transcription factors

- ▶ Anything except RNA polymerase that is required for transcription.
- ▶ Basal transcription factors are part of the pre-initiation complex
- ▶ Regulatory transcription factors control the rate of initiation. It is very common for them to bind as dimers.
- ▶ Multimerization can lead to the DNA being tied up in shapes.

Changing the shape of DNA can be used to regulate gene expression

Terms

- ▶ 5' to 3'
- ▶ Promoter
- ▶ core promoter
- ▶ Closed complex
- ▶ Open complex
(tighter)
- ▶ Promoter clearance
- ▶ Repressor
- ▶ Activator
- ▶