

Part I Notes

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Preface

Hello there!

Part I

**Structure and Function of
Macromolecules**

Chapter 1

Paper I: Structure and Function of Macromolecules

Chapter 2

Physical Methods

Course aims:

- This course of 10 lectures introduces the principles and applications of biophysical tools that give information about the structure, dynamics and interactions of biological molecules, primarily in vitro but also in vivo.
- The techniques we describe mainly involve irradiating the specimen with electromagnetic radiation. They include: scattering (e.g. SAXS, SANS, DLS); refraction related phenomena (e.g. SPR); X-ray diffraction/crystallography; absorption and emission spectra (e.g. UV/visible, CD, fluorescence); magnetic resonance (NMR & MRI); microscopy and experiments on single molecules (single pair FRET, optical ‘tweezers’ etc.). Other important biophysical methods, such as analytical ultracentrifugation, calorimetry and mass spectrometry, will be covered in other lecture courses.
- In general, physical methods can have relatively difficult associated concepts. You should realise that this course is an introduction to the methodology. By the end of the course you should understand something about the physical basis of the various methods discussed, how they are applied, and what information they can provide. The goal is that you will be able to get the gist of a paper in a scientific publication that uses one or more of these methods. This means that you should know the implications of some of the jargon used; e.g. “data collected to 2.8Å resolution” in an X-ray diffraction paper.

Sources of help:

- All the Powerpoint lectures will be available on Weblearn and there is also a supplement there with useful Web-sites and links to relevant scientific papers (accessible through Oxford University on-line e-Journals).

- Basic textbooks, e.g. Alberts et al., cover some aspects of biophysical methods. However, some more specific books that describe biophysical techniques are recommended; e.g.:
 - Campbell ID, Biophysical techniques. OUP, 2012.
 - Branden and Tooze, Introduction to Protein Structure. Freeman (2nd edn.), 1998.
 - Hammes G, Spectroscopy for the biological sciences. Wiley, 2005.
 - Sheehan D, Physical Biochemistry. Wiley (2nd edn) 2009.
 - Van Holde, Johnson & Ho Principles of Physical Biochemistry, Prentice Hall (2nd edn), 2005.

2.1 Electromagnetic Radiation

2.1.1 Interaction of EM Radiation with Matter

- scattering
 - reflection and refraction can be explained by scattering
 - scattering from ordered arrays gives interpretable diffraction patterns
- Transitions between molecular energy levels
 - absorption and emission
- light-induced chemical events

2.1.2 Scattering

Incident electromagnetic radiation causes a particle to oscillate. The oscillations give rise to secondary radiation, which can give information about the concentration, shape, and motion of the scattering particle. The 3 main classes of scattering experiment are shown in Fig. 2.1

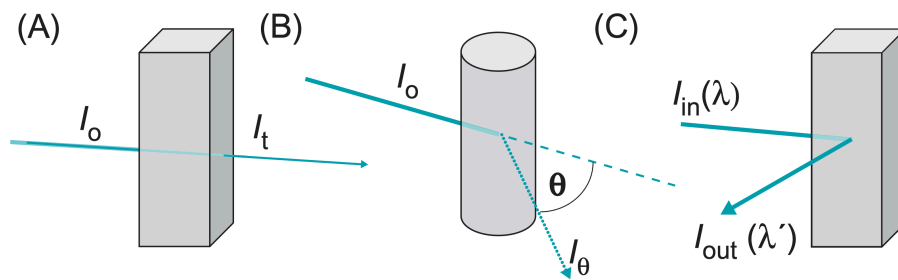


Figure 2.1: Some classes of scattering experiment, which measures (A) the reduction in transmitted intensity (turbidity); (B) the angular dependence of the scattering radiation (I as a function of θ); (C) changes in λ (or ν), measured at a fixed angle (usually 90°) to the incident beam

Part II

Energetics and Metabolic Processes

Chapter 3

An Introduction to Paper II: Energetics and Metabolic Processes

The aims of this course are:

Note that each of the courses in Part I contain material that is relevant to each and every other course; it is crucial that you interrelate this material as appropriate. Of particular relevance to this course is material on structural biology, proteins, and the control of prokaryotic gene expression.

To inform you of the diverse metabolic capabilities possessed by animal, bacterial and plant cells; To introduce you to the principles that underpin the diversity of metabolic processes that occur in different cell types; To introduce you to the types of control that regulate metabolic transformations. By the end of this course you should understand: Energy transduction by mitochondrial, bacterial and thylakoid membranes. The principles of, and the evidence for, the chemiosmotic theory; References: There are several Molecular Biology/Biochemistry textbooks that provide adequate factual material. None (other than perhaps 'The Genetic Switch') are good at providing a 'feel' for the strategies and approaches that lead to new discoveries and for consolidating these discoveries into biological understanding. Remember that knowing the facts is not enough; more important is knowing how we get to know the facts'.

Molecular Biology of the Cell (Alberts, Bray, Lewis, Raff, Roberts and Watson, 2008, Garland); now superseded by Essential Cell Biology-same authors (1998)

Chapter 4

Bacterial Warfare

4.1 Bacterial Cell Envelope

4.1.1 The outer membrane of Gram-negative bacteria

- not a phospholipid bilayer
 - phospholipid only in the inner leaflet
 - outer leaflet is composed of glycolipids, principally lipopolysaccharide (LPS)
- Most proteins are:
 - lipoproteins
 - * contain lipid moieties attached to an N-terminal cys
 - * these lipid moieties embed lipoproteins in the inner leaflet of the OM (not transmembrane proteins)
 - β -barrel proteins
 - * constitute almost all of the integral, transmembrane proteins of the outer membrane
 - * β -sheets that are wrapped into cylinders
 - * almost all comprise an even number of β -strands (VDAC is an exception)
 - * a.k.a. OMPs
 - * OmpF, OmpC
 - allow passive diffusion of small molecules such as mono- and disaccharides and amino acids
 - 16 TM strands
 - exist as trimers
 - very abundant; ~250000 copies per cell
 - * LamB (18 TM strands) and PhoE (16 TM strands)
 - also exist as trimers

- function in the diffusion of specific small molecules, maltose or maltodextrins and anions such as PO_4^{3-}
- * OmpA
 - another abundant OMP
 - monomeric
 - exist in two different conformations: a minor form with an unknown number of transmembrane strands can function as a porin, but the major, nonporin form has only 8 TM strands, and the periplasmic domain of this form performs a largely structural role
- * An additional class of OMPs with 20-24 TM strands are present at much lower levels, and they function as gated channels in the high affinity transport of large ligands such as Fe-chelates or vitamins such as VB-12
- Few enzymes
 - Phospholipase (PldA)
 - Protease (OmpT)
 - LPS-modifying enzyme (PagP)

4.1.1.1 LPS

LPS plays a critical role in the barrier function of the OM. It is a glucosamine disaccharide with 7 or 8 acyl chains, a polysaccharide core, and an extended polysaccharide chain that is called the O-antigen.

The acyl chains are largely saturated, and this facilitates tight packing

The nonfluid continuum formed by the LPS molecules is a very effective barrier for hydrophobic molecules

4.1.2 The peptidoglycan cell wall

Peptidoglycan is made up of repeating units of the disaccharide N-acetyl glucosamine-N-acetyl muramic acid (NAG-NAM), which are cross-linked by pentapeptide side chains

The OM is stapled to the underlying peptidoglycan by a lipoprotein called Lpp. Lpp is the most abundant protein in *E. coli*, more than 500,000 molecules per cell.

4.1.3 The inner membrane

The IM is a phospholipid bilayer. In *E. coli* the principal phospholipids are phosphatidyl ethanolamine and phosphatidyl glycerol (lesser amounts of phosphatidylserine and cardiolipin)

4.1.4 The Gram-positive cell envelope

Compared to Gram-negatives, Gram-positives lack the outer membrane, and have a thicker cell wall composed of several layers of peptidoglycan many times thicker than is found in *E. coli*.

Threading through these layers of peptidoglycan are long anionic polymers, called teichoic acids which are composed largely of glycerol phosphate, glucosyl phosphate, or ribitol phosphate repeats. Teichoic acids are divided into wall TA (WTA) and lipoteichoic acid (LTA). WTA are covalently attached to peptidoglycan; lipoteichoic acids are anchored to the head groups of membrane lipids. TA account for over 60% of the mass of the G+ cell wall.

The surfaces of G+ are decorated with a variety of proteins, some of which are analogous to proteins found in the periplasm of G- bacteria.

Part III

Genetics and Molecular Biology

Chapter 5

An Introduction to Paper III: Genetics and Molecular Biology

This is an intro to paper III

Chapter 6

Molecular Biology and Genetics

Chapter 7

Evolution of Genes and Genomes

7.1 The Origins of Life (Abiogenesis)

Abiogenesis is the process by which life has arisen from non-living matter, such as simple organic compounds.

The transition from non-living to living entities was not a single event, but an evolutionary process of increasing complexity that involved:

- molecular self-replication (RNA introns and Q phage)
- self-assembly (no enzymes)
- autocatalysis (RNA enzymes and self-splicing RNA)
- emergence of cell membranes

Assumption: all life forms descended from a single ancestral life form or progenote. Evidence:

- many essential building blocks of life could have assembled spontaneously from chemicals present on primitive Earth (prebiotic synthesis)
- paleobiologists show that the first fossil cells (microfossils) evolved from the prebiotically synthesised building blocks of life about 3-4 mya, when Earth was about 1-1.5 by
- based on analyses of meteorites, some components of RNA may have formed in space and arrived on Earth
- Molecular Fossils are structures (e.g. the placement of introns within a gene) or functions (e.g. the use of a particular metabolic pathway or the composition of a membrane or the information processing systems) that are shared by such diverse organisms that they must have been present in the first cells, as well as in earlier pre-cellular forms of life.

- the rate at which genome sequences are being obtained means rapid process is being made in understanding evolution of modern organisms

7.2 The Miller-Urey Experiments (1952)

- A chemical experiment that simulated the conditions thought to be present on the early earth and tested the chemical origin of life under those conditions (water, methane, ammonia, hydrogen, spark)
- well over 20 different amino acids produced; more than the 20 that naturally occur
- recent evidence suggests that Earth's original atmosphere might have had a composition different from the gas used in Miller experiment, but prebiotic experiments continue to produce racemic mixtures of simple to complex compounds under varying conditions

Amino acids

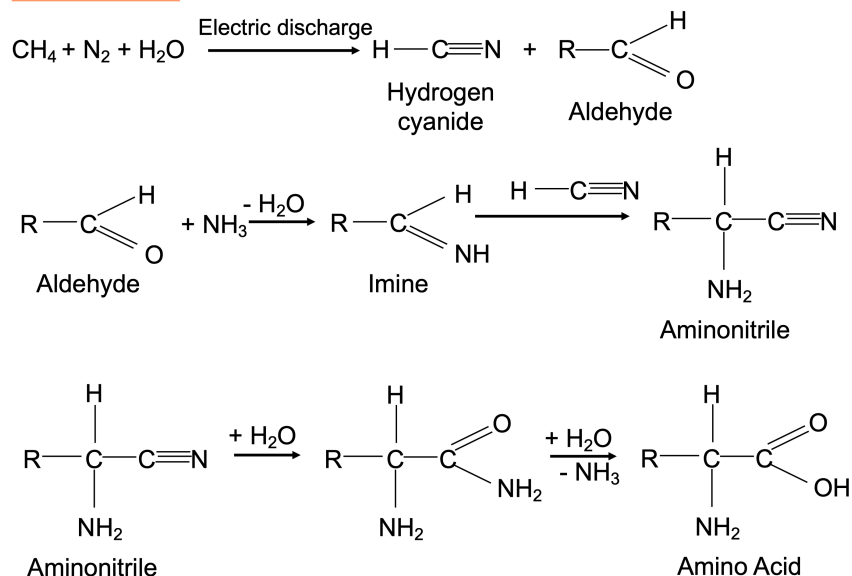


Figure 7.1: Prebiotic synthesis of amino acid

- These pathways are highly speculative
- These reactions could only have taken place in a reducing or non-reducing atmosphere but not in the modern strongly oxidising atmosphere
- Amino acids and other building blocks of life are regularly found buried deep in meteorites from elsewhere in the solar system

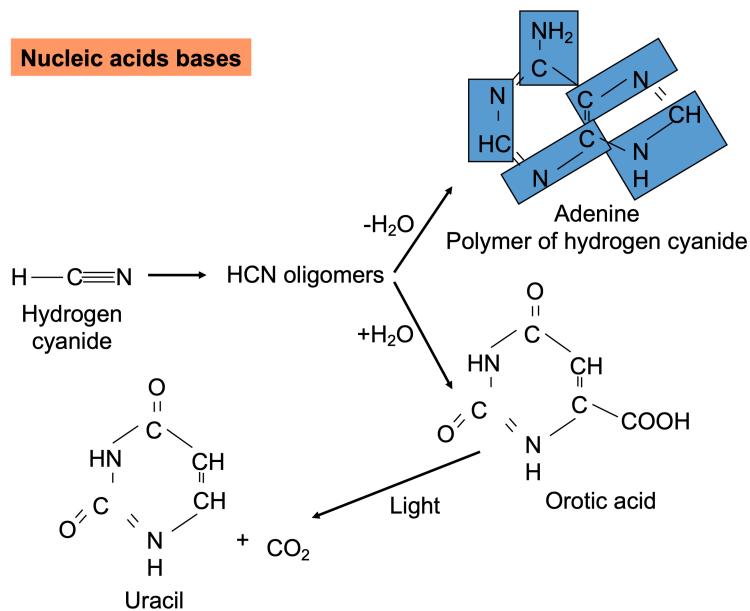


Figure 7.2: Prebiotic synthesis of bases

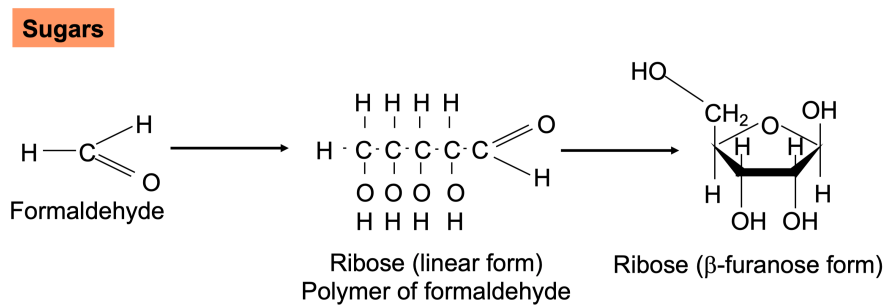


Figure 7.3: Prebiotic synthesis of sugars

7.3 RNA World Hypothesis

Why RNA not DNA?

- enzymatic activity
 - No enzymatic activity has ever been attributed to DNA
 - RNA has a 2'OH and has the ability to fold into complex 3d structures (e.g. tRNA, rRNA) essential to create an active site for catalysis
 - the 2'OH of ribose plays a direct catalytic role in many ribozymes
 - very short RNAs can be catalytic i.e. 52 nucleotides of RNA from a plant virus has catalytic activity (essential if this ability evolved from the primordial soup)
- Although all cells have DNA genomes, DNA precursors (deoxyribonucleotides) are always synthesised from RNA precursors (ribonucleotides) using **ribonucleoside diphosphate reductase**
- reverse transcriptase could have copied RNA into DNA

7.4 Self-splicing intron of *Tetrahymena*

Chapter 8

Molecular Biology Toolbox

8.1 Recombinant DNA

Techniques that allow the **isolation, manipulation, amplification, and expression** of identifiable sequences of DNA.

8.2 RE- and ligation-dependent methods

Restriction enzymes serve as bacterial immune response against phages and foreign DNA. They recognise specific DNA sequences and cleave the DNA in a Mg^{2+} -dependent manner, acting as molecular “scissors”. Type II RE is commonly used in lab.

Bacteria protect their own DNA from cleavage by methylating A.

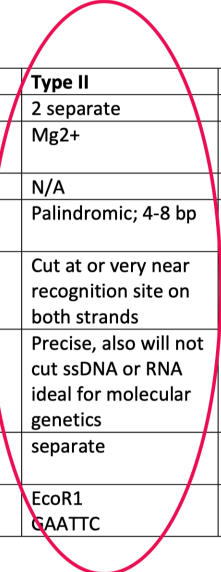
Bacteriophage T4 ligase is used to ligate GOI with the vector

Blunt end methods:

- removal of overhang: S1 nuclease
- (or) filling in an overhang: Klenow fragment + dNTPs + Mg^{2+}
- Blunt end ligation: DNA ligase

8.3 Other Applications of RE

Restriction fragment length polymorphism (RFLP) is a molecular method of genetic analysis that allows individuals to be identified based on unique patterns of restriction enzyme cutting in specific regions of DNA.



Characteristic	Type I	Type II	Type III
No. subunits	3 bifunctional	2 separate	2 bifunctional
Co-factors required	ATP, Mg ²⁺ and SAM	Mg ²⁺	ATP, Mg ²⁺ and SAM
ATP hydrolysis?	Yes	N/A	no
Recognition site	Bipartite, asymmetric,	Palindromic; 4-8 bp	Asymmetric, 5-7 bp
Cleavage site	Cut 24-26 bp downstream from recognition site	Cut at or very near recognition site on both strands	400-7000bp away from recognition site
Specificity	too imprecise to be useful in molecular genetics	Precise, also will not cut ssDNA or RNA ideal for molecular genetics	not precise enough for molecular genetics
Restriction and modification	mutually exclusive	separate	simultaneous
Eg.	EcoB TGAN ₈ TGCT)	EcoR1 GAATTC	Eco P15 CAGCAG

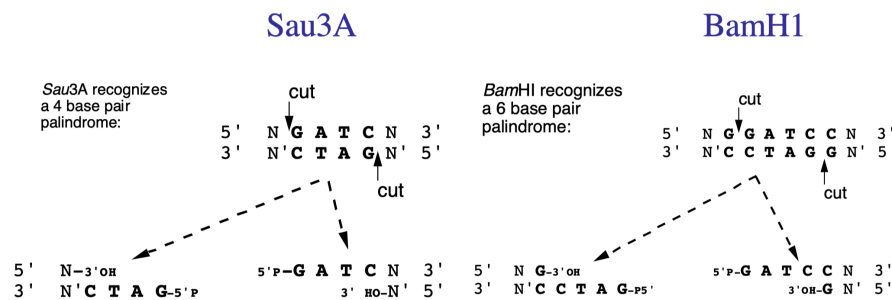
Figure 8.1: Types of restriction enzymes.

8.4 Alternatives to RE- and ligase-based cloning

Gateway cloning (via homologous recombination): the Gateway cloning system exploits the site-specific recombination system utilized by bacteriophage lambda to shuttle sequences between plasmids bearing flanking compatible recombination attachment (att) sites

TOPO cloning (uses topoisomerase I to hold vectors open and to promote ligation); exploits Taq polymerase's feature that adds non-specific A to the 3' end

Type II restriction enzymes: isoschizomers

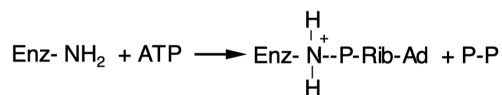


- There is a 4 base 5' overhang (sticky end)
- Sau3a and BamH1 produce compatible ends
- they are **isoschizomers**

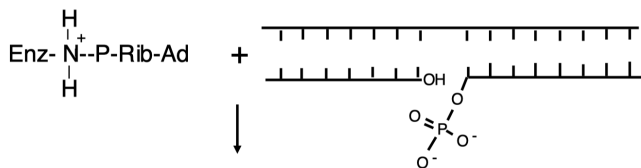
Figure 8.2: REs which produce compatible ends are called isoschizomers. In this case, both have the 4 base 5' overhang GATC

Action of bacteriophage T4 ligase

Reaction 1



Reaction 2



Reaction 3

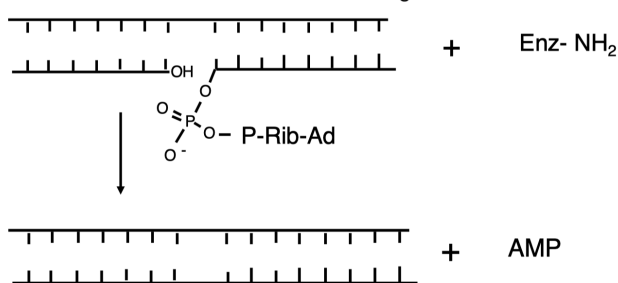
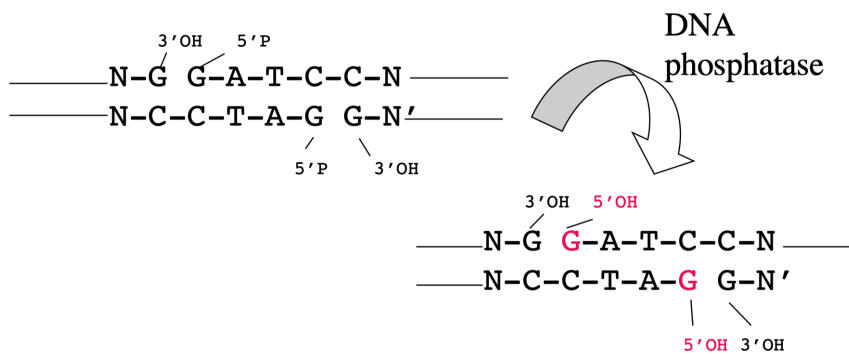


Figure 8.3: Action of T4 ligase.



Note that insert DNA will still have 5' phosphates so can be ligated into a phosphatased vector.

Figure 8.4: Preventing self-ligation

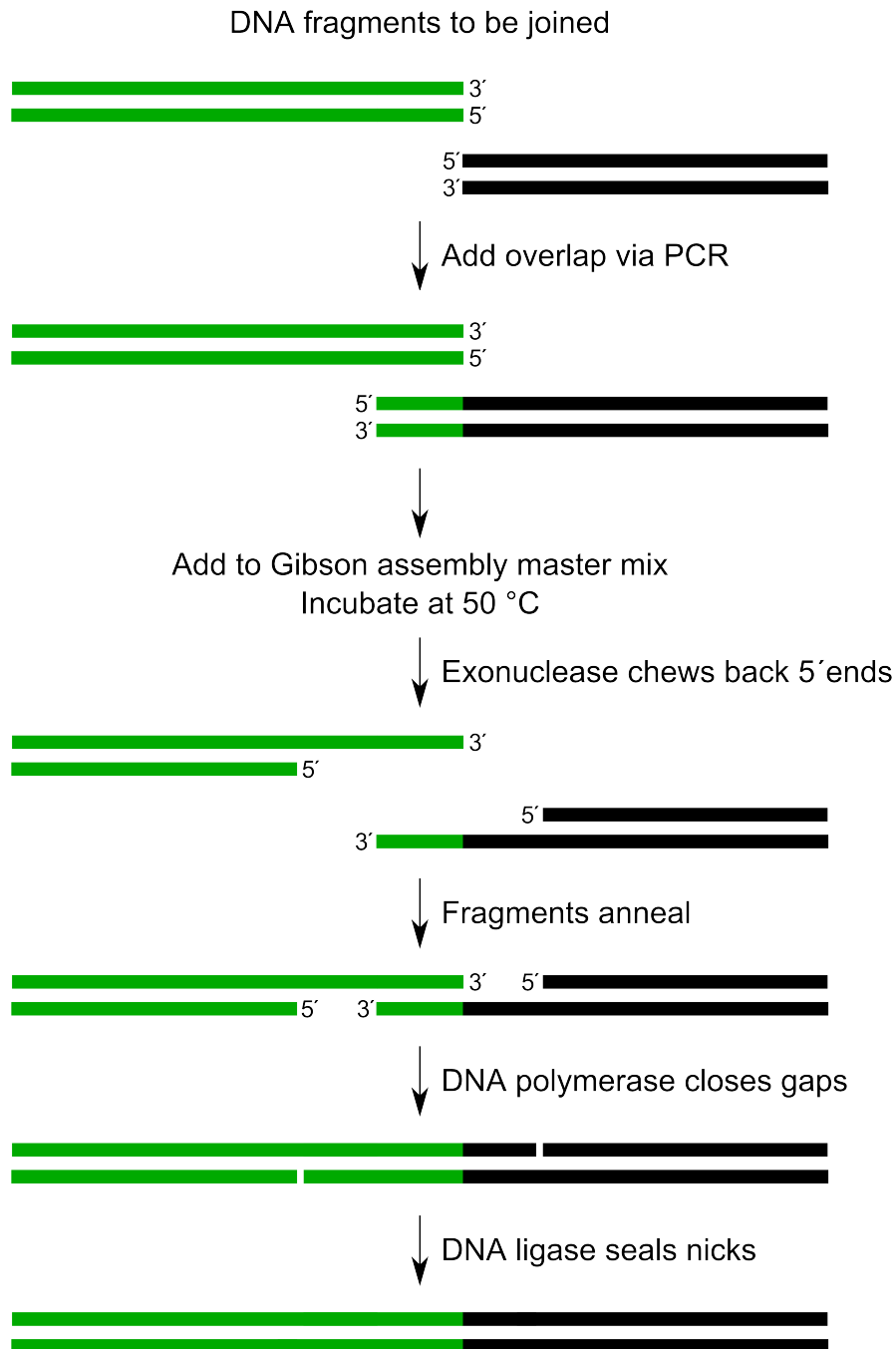


Figure 8.5: Gibson cloning. Gibson assembly is a molecular cloning method which allows for the joining of multiple DNA fragments in a single, isothermal reaction. The PCR products do not need to be digested. The method can simultaneously combine up to 15 DNA fragments based on sequence identity. It requires that the DNA fragments contain 20-40 base pair overlap with adjacent DNA fragments.

Chapter 9

The Mechanism of Protein Synthesis: The Ribosome

Overview

- charging tRNAs: maintaining accuracy
- the structure of the 70S ribosome and the 30S and 50S subunits
- the factors involved in the initiation, elongation and termination of translation
- maintaining the fidelity of translation (decoding)
- the peptidyl transferase reaction

9.1 Antibiotics inhibit translation

9.2 Aminoacyl-tRNA synthesis

9.3 tRNA structure

- With one exception (initiator tRNA) all aminoacyl-tRNAs interact with the ribosome and with elongation factors in the same way, so they must all have features in common:
 - cloverleaf-shaped secondary structure; stems with Watson-Crick base pairing and 3 loops (D loop, psi loop and the anticodon loop); modified nucleotides; CCA3' terminal group in the acceptor stem
 - L-shaped tertiary structure; 3D structure contains non-Watson-Crick base pairs and intercalated bases ('base stacking')

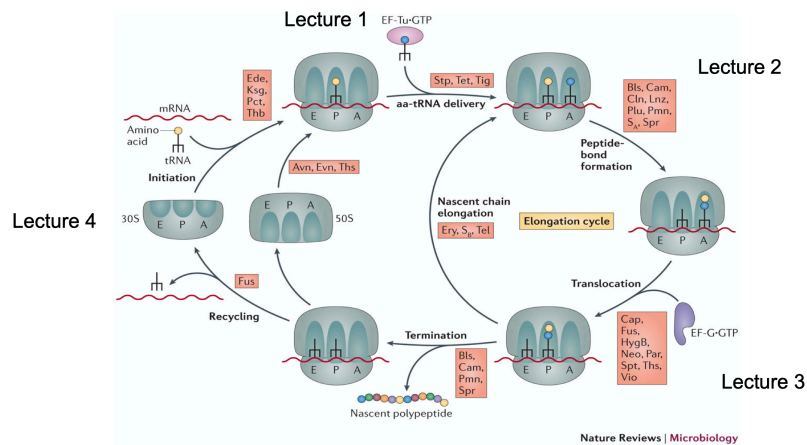


Figure 9.1: Antibiotics inhibit different steps of translation

- rRNAs and tRNAs are heavily stacked and show triple base interactions in which a base (usually A/G) forms an interaction in the minor groove of a Watson-Crick-base pair.
- conserved nucleotides maintain the shape but at the same time all tRNAs must be able to be distinguished for binding to different amino acids by the cognate amino acyl tRNA synthetases

Processing tRNAs:

- RNAP III; processing of primary transcripts in nucleoplasm
- 5' end sequence is removed by RNase P; CCA is added to the 3' end; multiple internal bases are modified (methylated A, C, G, T=U); pseudouridine, dihydrouridine

9.4 Mischarging: the accuracy of matching amino acids to tRNAs

- mutations that lead to mis-charging (an amino acid being linked to the wrong tRNA) can be in the anticodon, the D loop or in the acceptor stem of the tRNA, suggesting that the synthetase inspects the whole conformation of the tRNA as well as details of base recognition
- the presence of modified nucleotides (with the potential for unusual interactions) suggests also that local details of shape are important for recognition
- the frequency of missense errors can be determined by the rate of incorpo-

9.4. MISCHARGING: THE ACCURACY OF MATCHING AMINO ACIDS TO TRNAS⁴¹

ration of amino acids into protein in which it is not encoded (e.g. cysteine in flagellin)

- frequency $\sim 10^{-4}$ as the overall rate of error of mis-incorporation
- therefore the two recognition steps in protein synthesis (matching amino acids to tRNAs and matching tRNAs to codons on the mRNA) must each have an error frequency no worse than 10^{-4}
- recognition of the amino acid by aa-tRNA synthetase must involve non-covalent interactions between amino acids and enzyme
- however, the difference between some amino acids is only a single methyl group (e.g. I/V; S/T; A/G). One methyl group contributes very little to the binding energy and would allow discrimination only to an error level of 10^{-2}
- the recognition system therefore need two steps of discrimination independently, involving two separate attempts by the enzyme to recognise the amino acids
- to make the error frequency multiplicative, these must be separated by an irreversible (energy-requiring) step (hydrolysis of ATP)
- there are two different ways of achieving these two separate attempts at recognition: 'pre-transfer editing' and 'post-transfer editing' (hydrolytic editing)

An example with the isoleucine tRNA synthetase:

- the first attempt at recognition is by the original choice of amino acid. The binding of Ile and formation of Ile-adenylate is only 10^2 better than binding of Val and formation of Val-adenylate
- the second step: the valyl-adenylate is hydrolysed by the enzyme much more rapidly than isoleucyl-adenylate — and does not proceed to tRNA reaction — so total discrimination is 10^4

A second example of hydrolytic editing occurs at a second active site on the synthetase and is illustrated by valyl-tRNA synthetase.

The initial recognition of valine does not discriminate well against threonine, and after the formation of valyl/threonyl adenylate and then the aa tRNA, threonyl-tRNA is hydrolysed 3000 times faster than valyl-tRNA at a separate site on the enzyme. Also, in the presence of tRNA^{Ile}, there is > 99% hydrolysis of the incorrectly activated valyl-tRNA^{Ile}

- This 'double sieve' sorts correct and incorrect charging.
- the first sieve invokes size and steric requirements.
- the second sieve invokes chemical characteristics.

9.5 The structure of the 70S ribosome

- both ribosomal units are about 60% RNA and 40% protein.
- The

Part IV

Cell Biology and the Integration of Function

Chapter 10

An Introduction to Paper IV: Cell Biology and the Integration of Function

Part V

General Paper

Chapter 11

An Introduction to Paper V: General Paper

Part VI

Data Analysis and Intepretation

Chapter 12

An Introduction to Paper VI: Data Analysis and Intepretation

Appendix A

Physical Principles

A.1 Oscillators and Simple Harmonic Motion

A simple harmonic oscillator has its frequency (ν) independent of amplitude (a)

$$m\ddot{x} = -kx$$