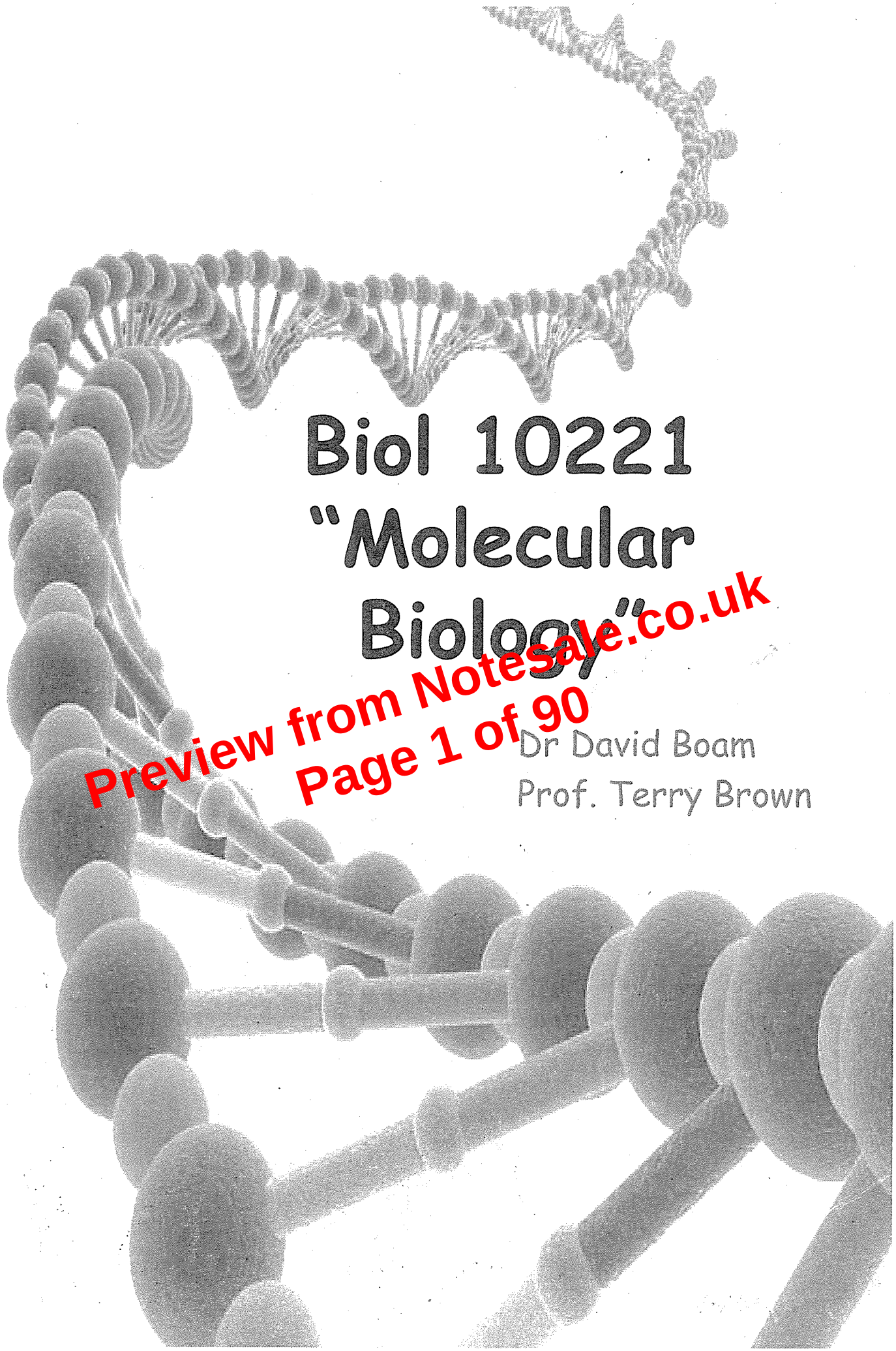

Biol 10221 "Molecular Biology"

Dr David Boam
Prof. Terry Brown

BIOL10221 Molecular Biology

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Course Outline

- DNA
  - DNA Structure
  - DNA replication
  - DNA repair
- Mutation and mutagenesis
- The genome and gene expression
  - Biology of RNA
  - Transcription
  - Translation
- Recombinant DNA technology

1

Online resources

- Its all on Blackboard
- Course resources
  - Learning modules Lecture notes, other resources, Assessments
- Discussion board
- Grades
- Announcements
- Assessment
  - Exam (85%)
  - Peerwise (5%)
  - Online test (10%)
    - Revisi… % of unit assessment
    - Further study - 4% of unit assessment

2

Online assessment

		type	% contribution to unit mark	open	Closed
1S	DNA structure and replication	lecture-based	1.25%	17th Oct	31st Oct
2S	Molecular basis of mutation	lecture-based	1.25%	31st Oct	14th Nov
3S	Mutation and repair	Further study	2%	31st Oct	21st Nov
4S	Transcriptional regulation	lecture-based	1.25%	14th Nov	28th Nov
5S	RNA biology and translational regulation	lecture-based	1.25%	28th Nov	12th Dec
6S	RNA biology and translational regulation	Further study	2%	28th Nov	19th Dec
7S	Basic genetic engineering	lecture-based	1%	11th Dec	23rd Dec

3

The amount of G + C nucleotides in an organism's DNA is called its GC content

Human DNA has a GC content of 40.3%

Plasmodium falciparum 19.0%

Streptomyces griseolus 72.4%

} unusual content

19

Content of G = Content of C.

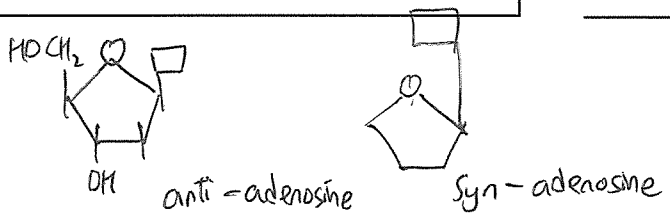
Most species has 40% - 60% GC content

Other important features of the double helix

- The two strands are antiparallel [opposite direction]
- There is a major and a minor groove
- The helix can exist in different forms

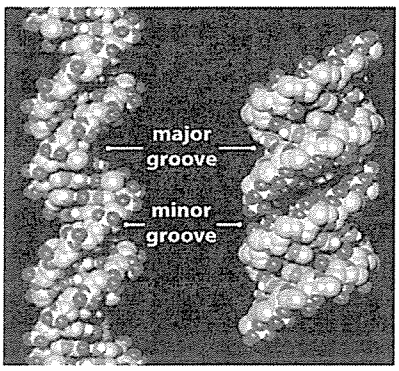
20

Major / Minor Groove allow reading of sequences of nucleotide bases without unwinding the DNA double helix.



anti-adenosine

syn-adenosine



A-DNA B-DNA

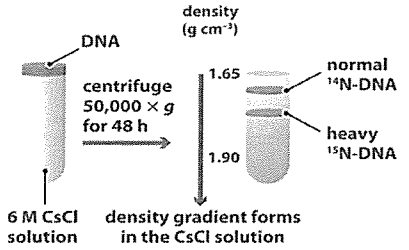
21

A- DNA more stretched out

nitrogen source

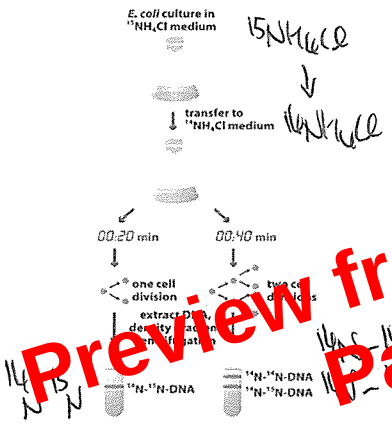
The culture medium contained $^{15}NH_4Cl$ - heavy ammonium chloride
- All of the bacteria's DNA became labelled with ^{15}N

^{15}N-DNA can be distinguished from ^{14}N-DNA by density gradient centrifugation



52

Density gradient centrifugation
- 6M CsCl solution
- 50,000 g @ 48 hours
- density [g cm⁻³]

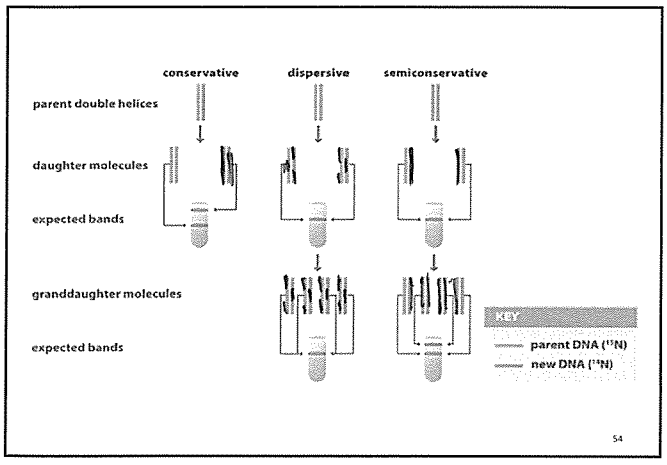


53

15-15

15-14 15-14

14/14



54

Variations of Semi conservative theme

1) Displacement replication

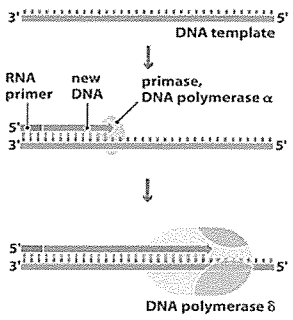
D-loop – point of beginning of replication

↳ a region of 500bp where DNA is disrupted by a RNA molecule. First strand [inner one] is synthesized while outer strand is displaced.

After 1st strand replication is complete, 2nd strand is copied afterwards.

Rapid synthesis of circular DNA mechanism → rolling circle replication

In eukaryotes the process is slightly different
- The RNA primer is first extended by DNA pol alpha
- DNA pol alpha adds about 20 nucleotides
- then DNA pol delta makes the rest of the new strand



70

(8-12 bp)

20 nucleotides

DNA pol α synthesis RNA primer

DNA pol δ & continue synthesising rest of the strand.

RNA primase closely attached to DNA pol α

DNA polymerase α – 4 subunit
- 1) of them make RNA
- 1) of them make DNA

↳ carry out synthesising of DNA for about first 20 nucleotides

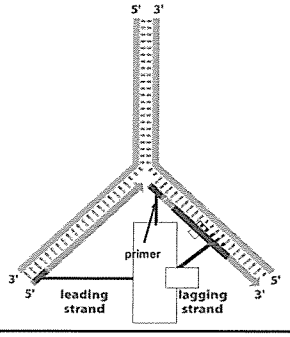
LECTURE 6

DNA REPLICATION III

71

How is the lagging strand copied?

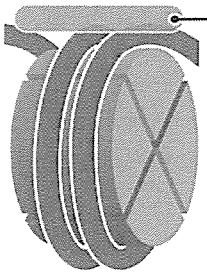
DNA synthesis is always in the 5′→3′direction
- the lagging strand must therefore be copied from a primer placed at the replication fork.



72

Histone H1 is the linker histone
- it attaches outside the nucleosome
- nucleosome + DNA + linker histone = chromatosome

a chromatosome



linker histone (H1)

H1 is a group of proteins, NOT an individual protein.

109

nucleosome + DNA + linker histone = chromatosome

H1 → H1 a-e, $H1^0$, H1t and H5

Beads on a string reduces the length of a DNA molecule by one-sixth
- there must be higher levels of packaging

The next level is the 30 nm chromatin fibre
- this reduces the overall length of the DNA by another one-seventh
- the 4.9 cm molecule in chromosome 8 would now be about 1.2 mm in length

side view

end view

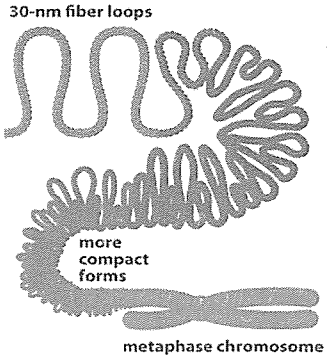
110

30nm chromatin fibre highest level of arrangement in non-dividing cell

↳ 30nm in diameter

↳ formed by packing individual nucleosomes

The higher levels of packaging are not understood
- the highest level - the metaphase chromosome - is only found in dividing cells



111

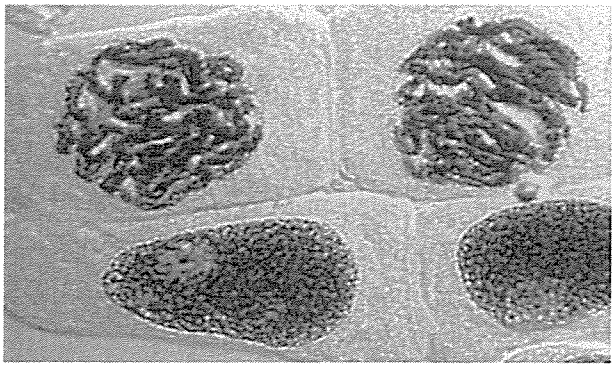
↳ most compact version of DNA

DNA wrapping around histone – 1/3 , twice – 1/6

nucleosome → 30nm chromatin fibre – 1/7

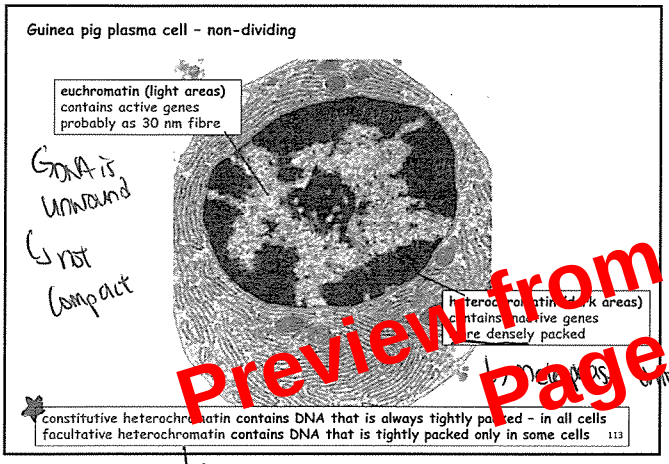
30nm chromatin fiber → euchromatin 1/750

Dividing bluebell cells



112

Guinea pig plasma cell – non-dividing



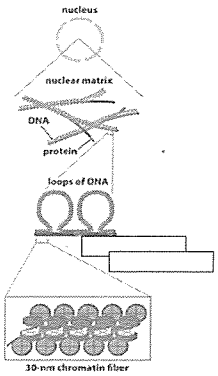
DNA is unwound → not compact

constitutive heterochromatin contains DNA that is always tightly packed – in all cells
facultative heterochromatin contains DNA that is tightly packed only in some cells 113

→ contains in differential cell

* Diff. forms of DNA allow different types of genes to be accessed at diff cells *

In euchromatin, DNA is in the form of the 30 nm fibre
- attached to the nuclear matrix so the DNA does not get tangled up

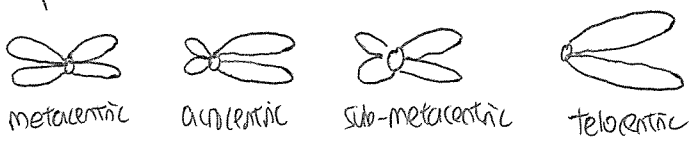


114

Nuclear matrix → complex network of protein & RNA fibils that permeate the entire nucleus.

Attachment between loops of 30nm chromatin fibre & nuclear matrix is made by matrix-associated regions (MARs) or scaffold attachment regions (SARs)

metaphase chromosome → contains 2 daughter chromosomes as it is seen after "S" phase

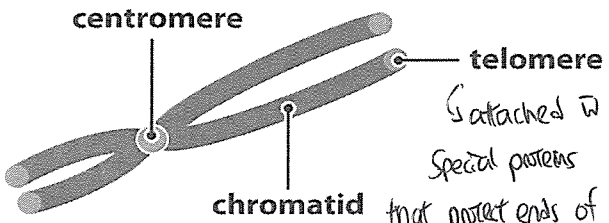


meta ≈ centre
sub-meta ≈ a little off centre
acro ≈ towards one end
telo ≈ very close to one end

The metaphase chromosome is the highest level of packaging

The centromere hold the daughter chromosomes together
- contains special histones - CENP-A instead of H3

The telomeres protect the ends
- from exonuclease attack
- from being mistaken for chromosomes breaks and joined together



115

to hold chromatids together

↳ much stickier, allows centromere

↳ attached to special patterns that protect ends of chromosome

linker histone - H1

centromere — CENP-A

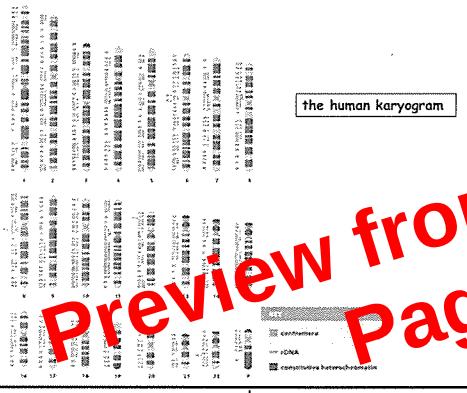
centromeric DNA is largely made up of repeat sequences

repeats are 171 bp in length ⇒ alphoid DNA

CENP-A form an outer shell at which the kinetochores are constructed.

↳ attachment points for microtubules

Metaphase chromosomes can be stained to give a banding pattern
the complete set is the called the karyogram



116

functions: allow genetic disease of newborn to be determined before birth.

staining techniques to produce chromosome banding patterns

Technique	Procedure	Banding Pattern
G banding	Mild proteolysis followed by staining with Giemsa	

Y chromosome contains mainly heterochromatin

Staining Technique to produce chromosome banding patterns → G, R, Q, C } banding

LECTURE 9

CAUSES OF MUTATIONS

Molecular basis of mutation

Lecture 9 Causes of mutations

Lecture 10 Repair of mutations

Lecture 11 Effects of mutations on genes

Lecture 12 Effects of mutations on organisms

117

Aa × aa

aa

Aa

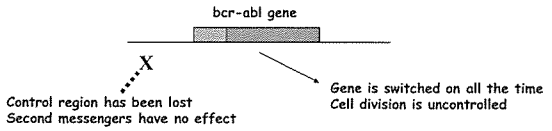
0.5 + aa

0.5 Aa or aa

AA or Aa

0.5

The Philadelphia chromosome is a common cause of leukaemia



↳ control region shifted to other chromosome

178

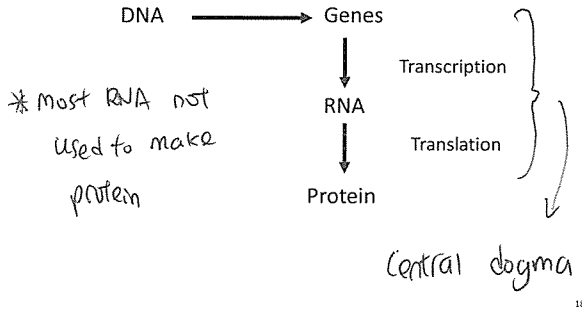
Lecture 13 Genes, polymerases and promoters

Key concepts
- Genomes
- Sense and antisense
- RNA polymerase
- Prokaryotic and eukaryotic gene structure

Keywords
- Sense and antisense
- Coding/non-coding
- template
- RNA polymerase
- Promoter
- Intron
- Exon
- UTR
- Spacer
- Leader
- Cistron

179

Transcriptional regulation



* most RNA not used to make protein

Central dogma

180

Genes summary

- All genes contain a regulatory element - promoter/enhancer
  - direction
  - position
  - spacial/temporal/inducibility
- prokaryotic genes differ in organisation and function from eukaryotic
  - cistrons vs introns/exons
- intervening DNA accounts for the difference in genome sizes

190

Lecture 14 RNA polymerases

Aims

- To show that there are differences in complexity and organisation between the prokaryotic and eukaryotic transcriptional apparatus
- RNA polymerases in prokaryotes and eukaryotes
- RNA polymerase-associated general factors

Keywords

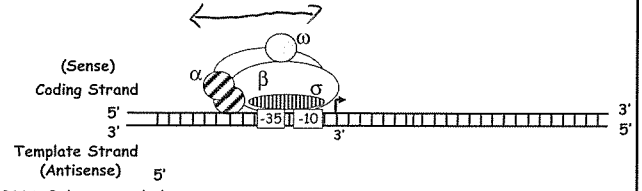
- RNA polymerase
- Recruitment
- Multi-protein complex
- Sigma
- TBP
- TFIIA - J
- tRNA promoter
- rRNA promoter
- mRNA promoter

191

after changing shape,
bp no.
change to ≥100 at
start of transcription
↓
70bp – 100bp

multi subunit
many polypeptides } each w distinct function

Transcription initiation by prokaryotic RNA polymerase



RNA Polymerase holoenzyme

One sigma (σ) - promoter recognition

Two alpha (α) - assembly, activation of transcription

Two beta (β) - catalysis, termination
β, β'

One omega (ω) assembly, folding; required for some

192

of the RNA polymerase genes

σ – only site that recognises DNA sequence

α – stimulates process of transcription

β | β' – reads DNA sequence, polymerase function

Lecture 14 Summary

- There are differences in complexity and organisation between the prokaryotic and eukaryotic transcriptional apparatus
- RNA Polymerases are macromolecular complexes
- prokaryotes have 1 polymerase, eukaryotes have 3
- eukaryotic polymerases
  - specialised, gene type-specific
  - use basal transcription factors

Useful websites

- http://www.mun.ca/biochem/courses/3107/Lectures/Topics/euk_transcription.html
- http://gator.uhd.edu/~uzmana/webwk.htm

199

Lecture 15 Gene Regulation

- **Aims**
- Describe a simple mechanism of gene regulation
- Demonstrate how intermolecular interactions play a key role in gene regulation
- Show how function of operons can be elucidated by study of mutations

- **Learning outcomes**
- By completion of this lecture you should be able to
- Define and describe the functional components of a typical operon
- Understand the mechanism of regulation of the lac operon by lactose and glucose
- Understand the concept of negative feedback regulation
- Understand the mechanism of catabolite repression
- Understand the following general concepts of molecular structure , intermolecular interactions and how they apply to mechanisms of regulation by operons

200

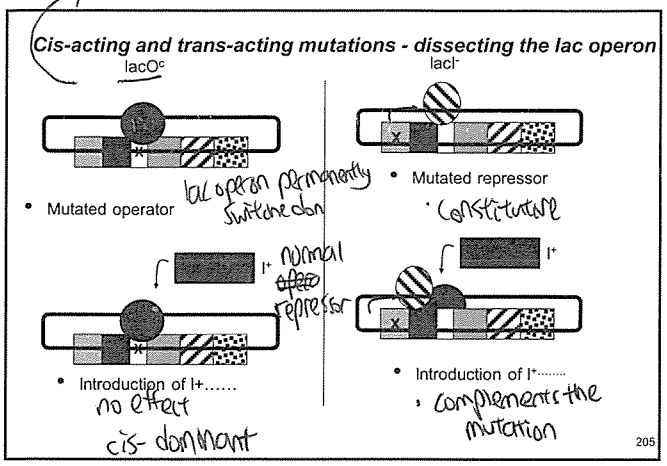
Keywords/concepts

- **Concepts**
- DNA-protein interactions
- protein-protein interactions
- protein-small molecule interactions
- conformational change
- inducers/co-repressors

- **Keywords**
- repressor
- inducer
- co-repressor
- operator
- promoter
- operon
- cistron

201

repressor can't bind to operator → constitutive mutation



↳ can't be rescued

↳ can be rescued

lacI⁻ – non-functional repressor or no repressor

constitutive – gene always switched on

I⁺ – normal repressor proteins

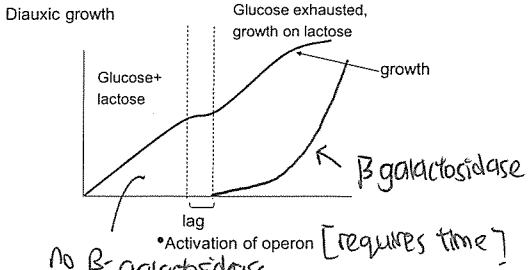
Lac operon - Summary

- Repressor binds operator – inhibits initiation
- Repressor activity regulated by lactose
- Mutations in regulatory elements
  - Cis acting
  - Cannot be complemented
- Mutations which alter activity of regulatory proteins
  - Trans-acting
  - May affect many genes
  - Can be complemented

206

lactose	glucose	β-galactosidase
×	×	×
✓	×	✓
✓	✓	× *
* switched on		

Catabolite repression



No β-galactosidase activity as there is glucose supply

glucose represses lac operon!

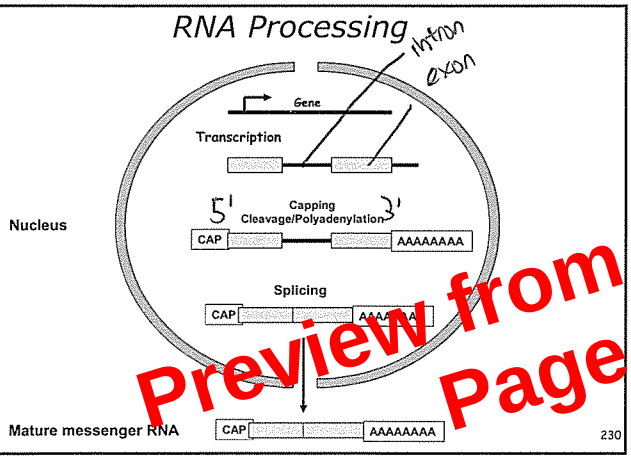
- several operons allowing use of alternate carbon sources are repressed by glucose and only became active when all glucose is used up.
- Preferential use of glucose
- catabolite repression

Lecture 18 RNA Processing

- Aim
  - Introduction to eukaryotic mRNA processing mechanisms
  - Importance for regulation and diversity
- Objectives
  - You should understand main processing reactions and how they are carried out
    - Capping
    - Polyadenylation
    - Splicing

229

RNA Processing

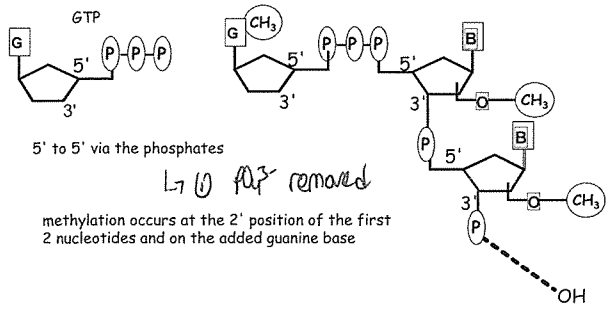


230

heterogenous nuclear mRNA (hnmRNA)
↳ premature mRNA transcript

capping is done after RNA pol transcribes the first few bases
↳ sends signal to RNA pol after capping is done

mRNA capping



5' to 5' via the phosphates
↳ Ø 8th removed

methylation occurs at the 2' position of the first 2 nucleotides and on the added guanine base

231

addition of Ø nucleotide – guanine (GTP)
methylation of first few nucleotides of mRNA at the 2' OH group
- linked to transcription initiation
- ↑ stability of mRNA
- required for efficient splicing
- nuclear export & translation initiation

Reading Frames

The sequence of codons from a *specific start codon to a specific stop codon* is a **reading frame**

Almost every protein chain initiates with AUG - codes for Methionine...but not all met codons are equal! (next lecture....)

3 stop codons UAA, UGA, and UAG

Reading frame 1	U U A U G A G C G C U A A A U Leu **Stop** Ala Leu Asn	mRNA polypeptide
Reading frame 2	U U A U G A G C G C U A A A U Tyr Glu Arg **Stop**	
Reading frame 3	U U A U G A G C G C U A A A U Met Ser Ala Lys	

238

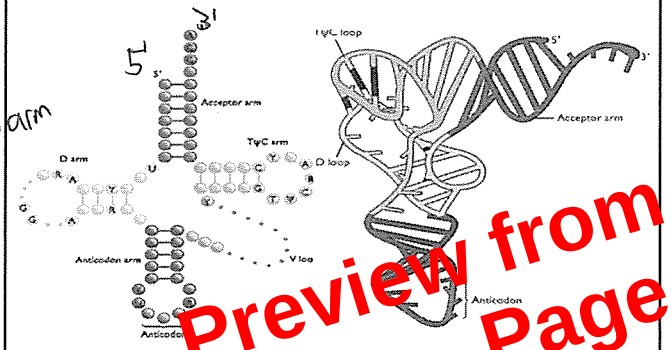
5' 3' acceptor arm

D arm — TψC arm

variable loop

Anticodon arm

Transfer RNA (tRNA) Structure and Function



D arm

clover leaf structure

239

→ interacts w codon

Anticodon - sequence of 3 bases which is complementary to mRNA

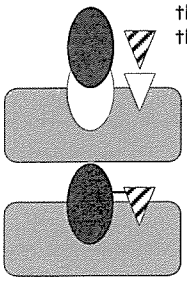
3' - [illegible] the appropriate amino acid

→ acceptor arm

transcription rate = 60 nucleotide /s

Transfer RNA (tRNA) Structure and Function

Function - to specifically link to a particular amino acid and to recognise a codon in mRNA - ensures amino acid-codon match



tRNA and amino acids paired by aminoacyl-tRNA synthetases

Each synthetase is specific for amino acid and tRNA - proofreading!

Some tRNAs recognise more than one codon; some amino acids have more than 1 tRNA

240

20 aminoacyl-tRNA synthetases

amino acid selected based on size, charge, polarity, etc

aminoacyl-tRNA synthetases catalyses formation of a covalent bond between 3' and amino acid by a process of esterification.

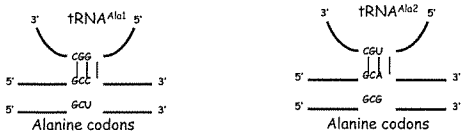
20 < tRNA < 61

Bond between phosphate group & ribose sugar in RNA - glycosidic bond

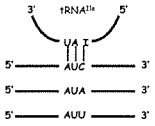
How does one tRNA recognise more than one codon?

Wobble

(A) G-U Base pairing



(B) Inosine base-pairs with A, C and U



Wobble - allows unconventional base pairing between third base in codon and first base in anticodon

241

A) 5 base w U instead of C

↳ due to flexibility of 1st & 3rd base in the codon.

↱ purine

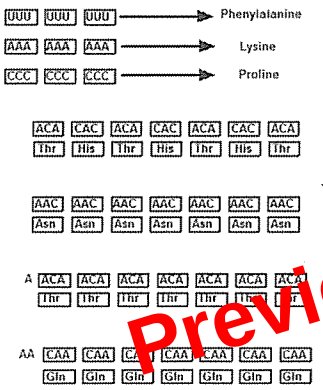
B) Inosine – similar in structure w guanine

↳ binds w A, C, U

no steric hinderance between 2 purines, allowing complementary base pairings to occur

How the genetic code was broken

Polynucleotides



1) Polynucleotides + bacterial extracts competent for translation

2) tRNA binding

By altering the open reading frame

242

Add specific polynucleotides into 20 test tubes each containing different radioactively labelled amino acids + other amino acids.

Find which precipitation of the radioactively labelled amino acids

61 / 64 codons code for amino acid

3 Stop codon

Lecture 20 protein synthesis

- **Aims**
- to introduce the basic mechanism of translation
- how the sequence of mRNA is used to code for a polypeptide chain
- stages in translation
- the role of cofactors and nucleotides in translation
- the role of the ribosome in translation

- **ILOs**
- Students should have a basic understanding and knowledge of
- Initiation elongation and termination phases of translation
- The role of the ribosome in catalysing translation and in translation start site selection
- The identity and roles of translation cofactors
- The roles of nucleotide triphosphates in driving translation

243

5'- IAU -3'

3'- UA -5'

AU