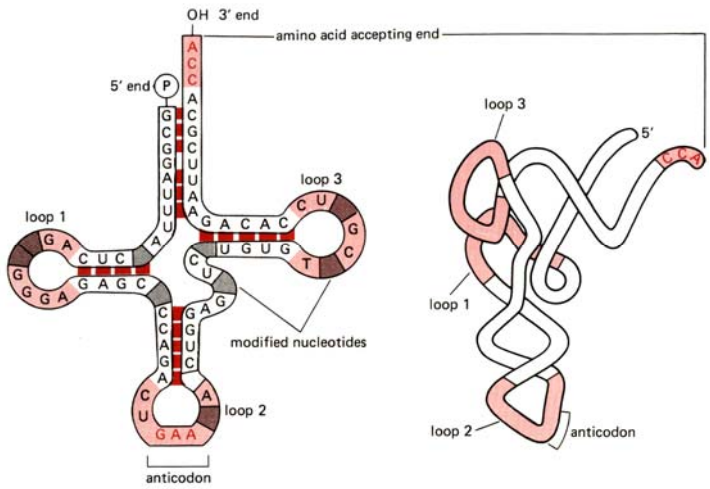
Reading for lecture 3

1. **Translation**
2. **Evolution by Natural Selection**
3. **Modern Genetics Techniques**

- Alberts et al, Chapter 5 (& end of ch. 4)
- Voet and Voet, Chapter 27



(Rasmol, 3D structure)

Translation

Transcription produces mRNA up to 1 million bases long. How is the genetic code translated into proteins?

[Transfer RNA] (2)

Adaptor – binds amino acid at 3' end (CCA is always conserved in all tRNAs), reads codon at anticodon.

Contains several modified nucleotides

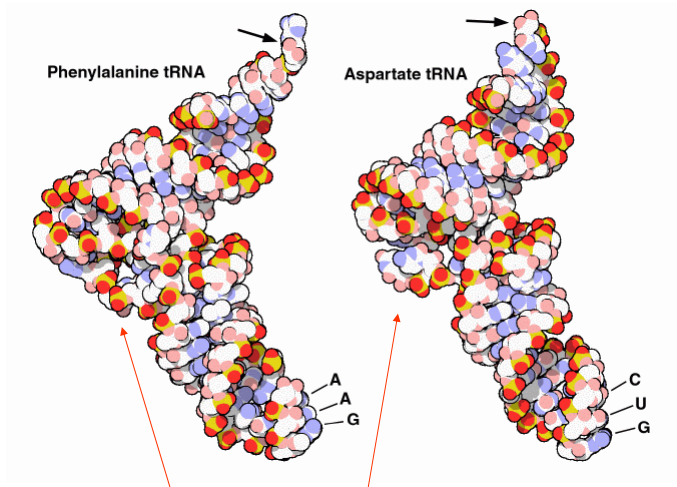
Structure includes base-paired helical regions and non-helical loops.

64 codons, 20 amino acids. How many tRNAs?

22-31 tRNAs. This is because many tRNAs can tolerate mismatch in the 3rd base in the codon. –

> *ie) lots of amino acids are specified by first 2 bases of codon – remember genetic code.*

About ¼ of the bases are conserved.

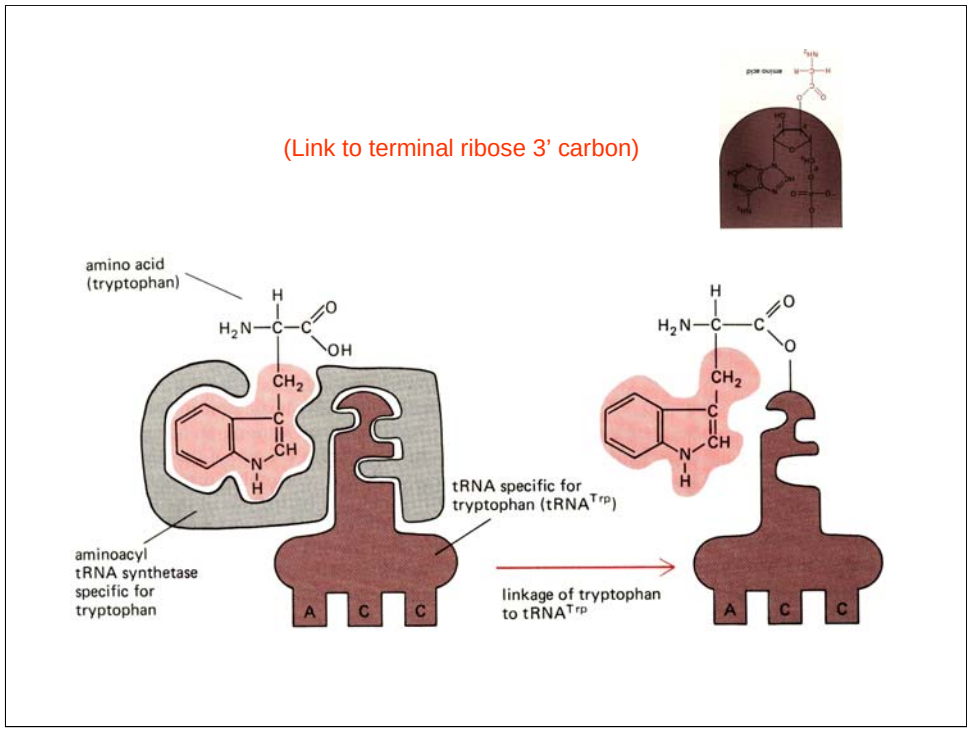


Notice different shapes here

[tRNA, space-filling] (3)

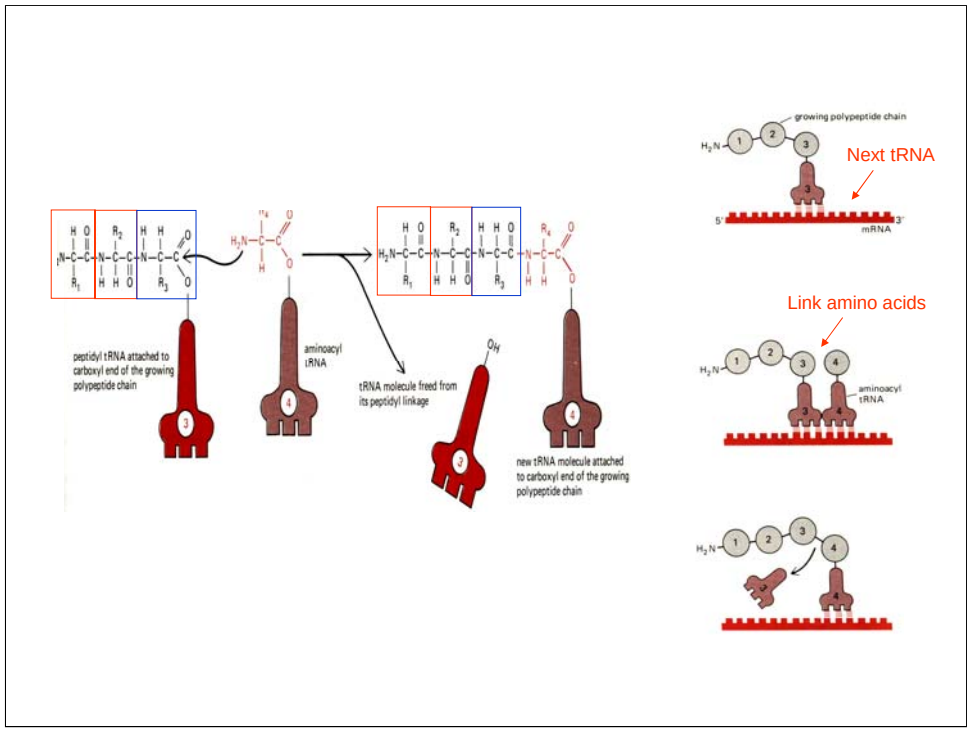
Different shapes allow recognition by machine that puts on the a.a.

Molecular recognition by 3-D shape



[Amino-acyl tRNA] (4)

The bond is unstable to addition of the "next" amino acid. "Head-growth" polymerization – energy for next monomer resides with the polymer.

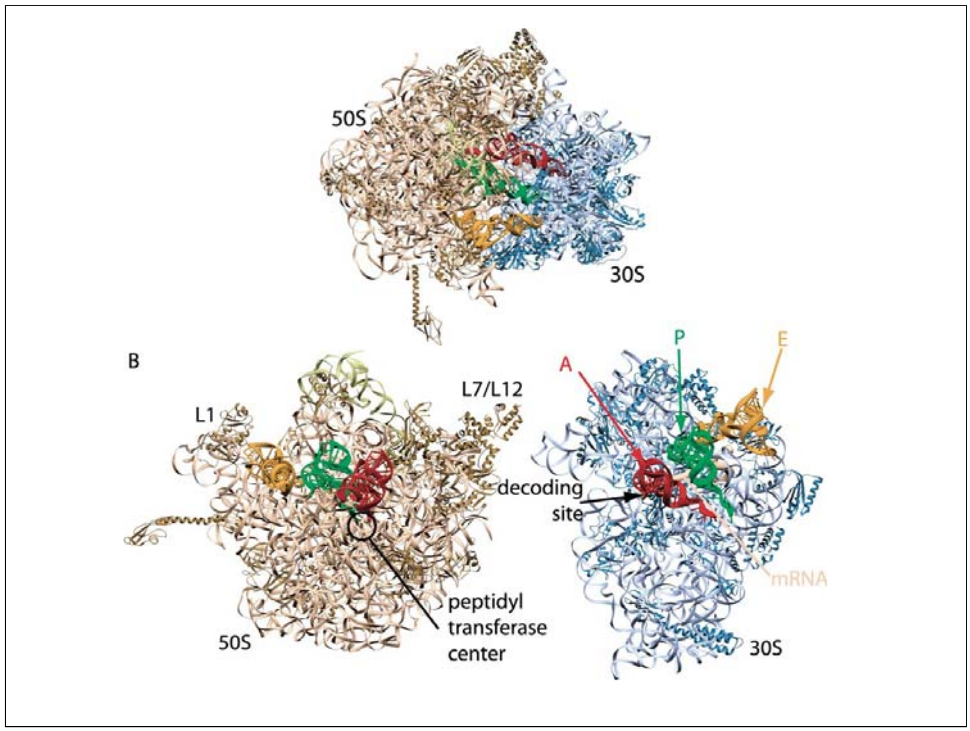


[“Polypeptide” synthesis] (5)

Codon/anticodon decides which tRNA is next

tRNA brings the appropriate a.a.

Directions: 5’ > 3’ on RNA, “N” > “C” terminals in amino acid.



As usual, the process is controlled tightly by a molecular machine: **The Ribosome**.

Binds and holds components in place.

Avoids bases being skipped.

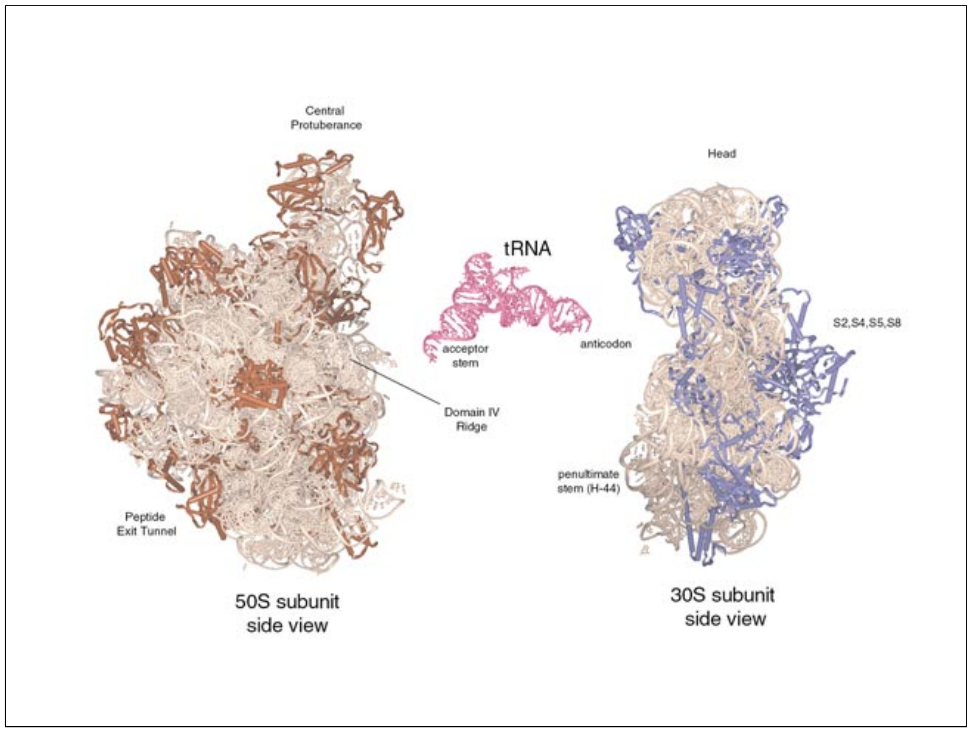
Catalyses all reactions.

[the Ribosome] (6)

Largest X-ray crystal structure to date.

mRNA and tRNA sites are in the middle

Mostly RNA. “rRNA”

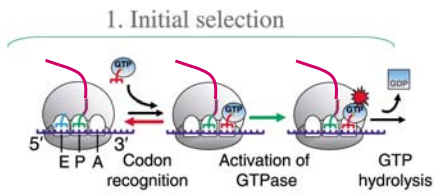


[Subunits showing rRNA vs protein] (7)

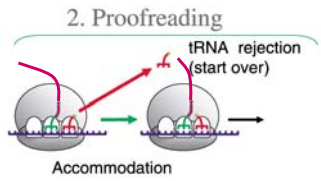
Unusual in that “rRNA” is the catalyst, not protein. Proteins are accessories.

rRNAs are highly conserved in all living things – very early in evolution?

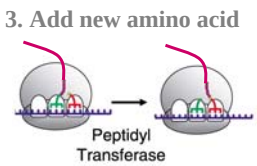
Also an exit tunnel for polypeptide



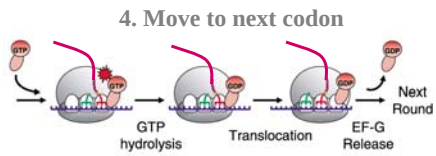
Select correct tRNA by complimentary base pairing



Option to reject tRNA if recognition is poor



Catalysed polymerisation



Back to the beginning

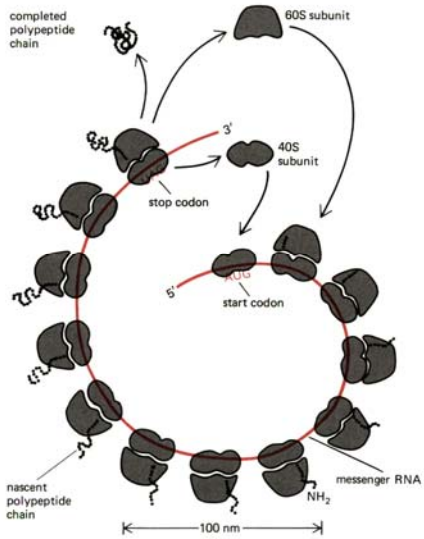
V. Ramakrishnan, V. (2002). Ribosome Structure and the Mechanism of Translation. *Cell* 108:557-572

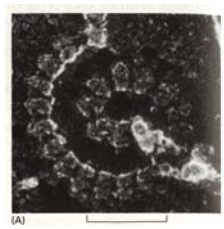
[Ribosome translocation] (8)

Even this is a simplification of all the steps involved

Termination is when stop codon binds release factor (Protein ?)

NB: Textbooks (my editions ~1989) out of date. This is common in this field. See for example review article referred to in the slide.





[poly-ribosome] (9)

In prokaryotes, translation begins on un-finished mRNA transcript

The mechanistic details of how the ribosome works are beginning to emerge

helped enormously by the structure

Classical biochemsitry, genetics, biology.

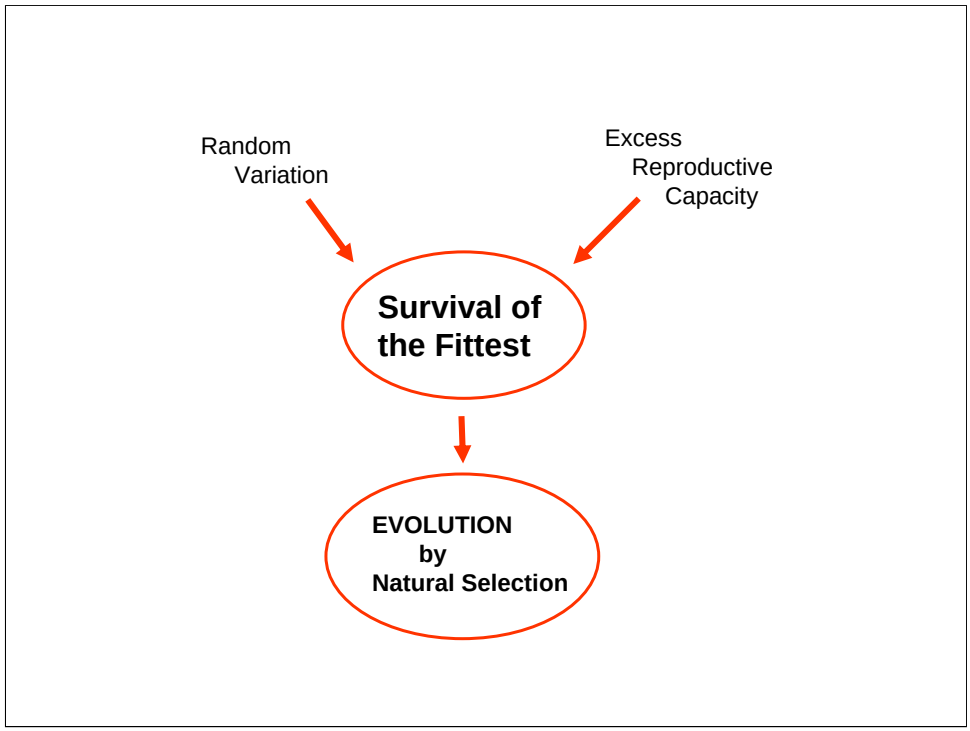
Mechanical experiments planned – biophysical techniques of later lectures

The internet is a good source of illustrations
and information on Biology and Biological Physics:

For example: Animations of DNA replication, transcription, translation at…

http://www.wehi.edu.au/education/wehi-tv/dna/

(show examples ?)



Interlude

1 GB (human genome) is very little information compared to what you'd need fully to specify a human. (Fit on a CD!)

The genetic information is more a "seed" than a "blueprint"

Processes that convert it into an organsim are highly specific and have evolved over a few billion years.

Themes…

Self-organization. Eg Base-pairing. RNA- and Protein Folding.

Networks / complexity. Large numbers of interacting components, "emergent" behaviour ?

(Developmental biology)

Very topical research area.

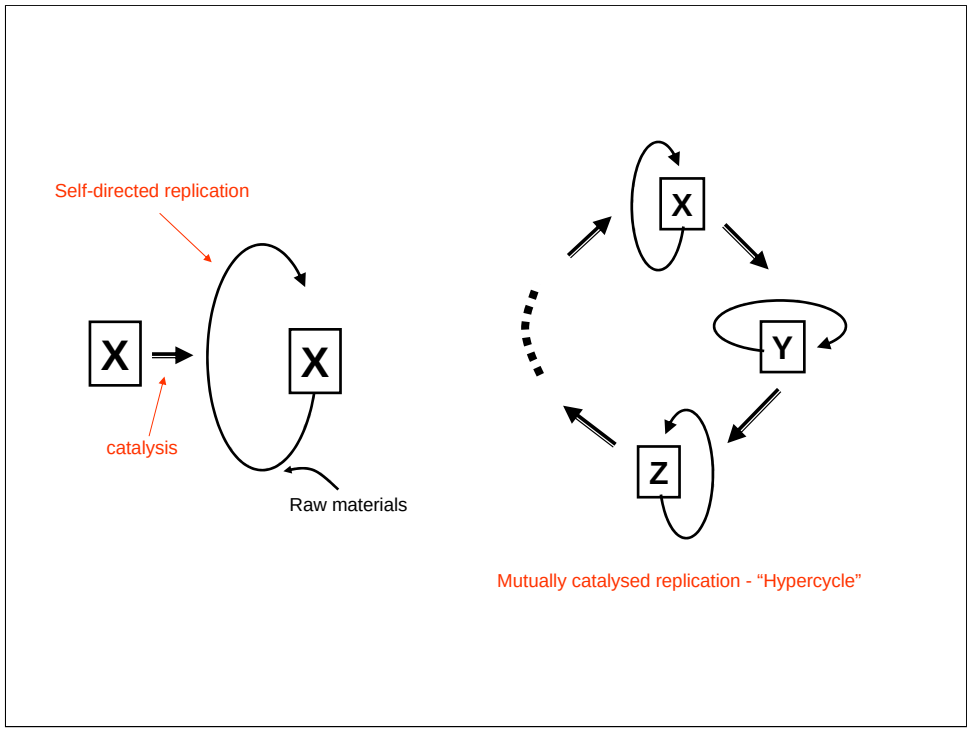
Evolution by Natural Selection

What does DNA really "do" ?

[Natural Selection] (10)

"Fitness" means the ability to reproduce. (accurately enough that copies can also reproduce –Grandchildren are the acid test!)

nb: Evolution is not necessarily progressive…



Natural selection applies even at the level of molecules.

Origins of life : self-replicating RNA molecules.

Preferential catalysis of **their own** replication, or hypercycle.

[RNA world] (11)

But it is incredibly powerful. given enough time – all of life.

“Selfish Gene” – we are (merely) machines for replicating our DNA!

..whatever its chemical nature, a gene must be extremely small and composed of few atoms. Otherwise the very large number of genes required to generate an organism would not fit in the cell nucleus.

On the other hand, because it is so small, a gene would be expected to undergo significant changes as a result of spontaneous reactions induced by random thermal collisions with solvent molecules.

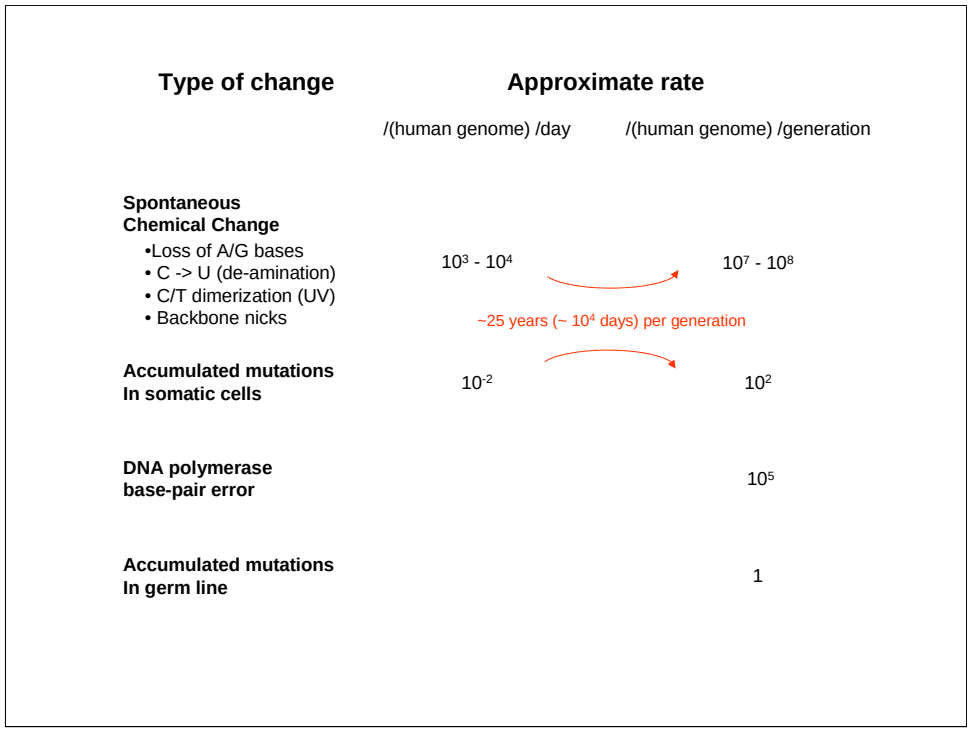
This poses a serious dilemma, since genetic data imply that genes are composed of remarkably stable substance in which spontaneous changes occur rarely.

Erwin Schroedinger, 1945

Mutation

[Schroedinger's dilemma] (12)

Inherited DNA changes are called "mutations"



[Mutation and error Rates] (13)

Mutation rates are 5 orders of magnitude lower than error rates

One reason is that most mutations do not survive.

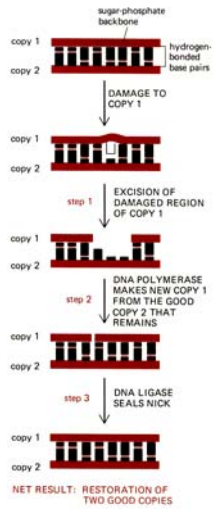
Everything is just right, random changes will mess it up

Cancer

Also, error correction…

Properties of DNA that help error correction…

- Base-paired double helix : backup copy *in situ*
- (Also a spare double-helical copy in diploid organsims)
- Bases are chosen such that naturally occurring chemical changes result in unnatural bases, that can be recognised by repair machinery
- 5' -> 3' polymerisation direction: energy for adding bases comes with new bases



[error correction] (14)

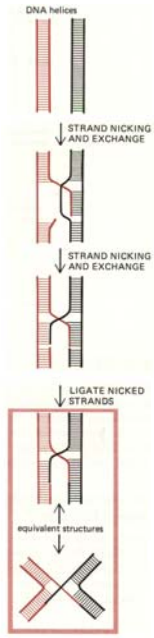
Yeasts have ~50 genes dedicated to. DNA repair

Bacteria have special DNA repair sytems that are activated when DNA damage is detected.

DNA polymerization:

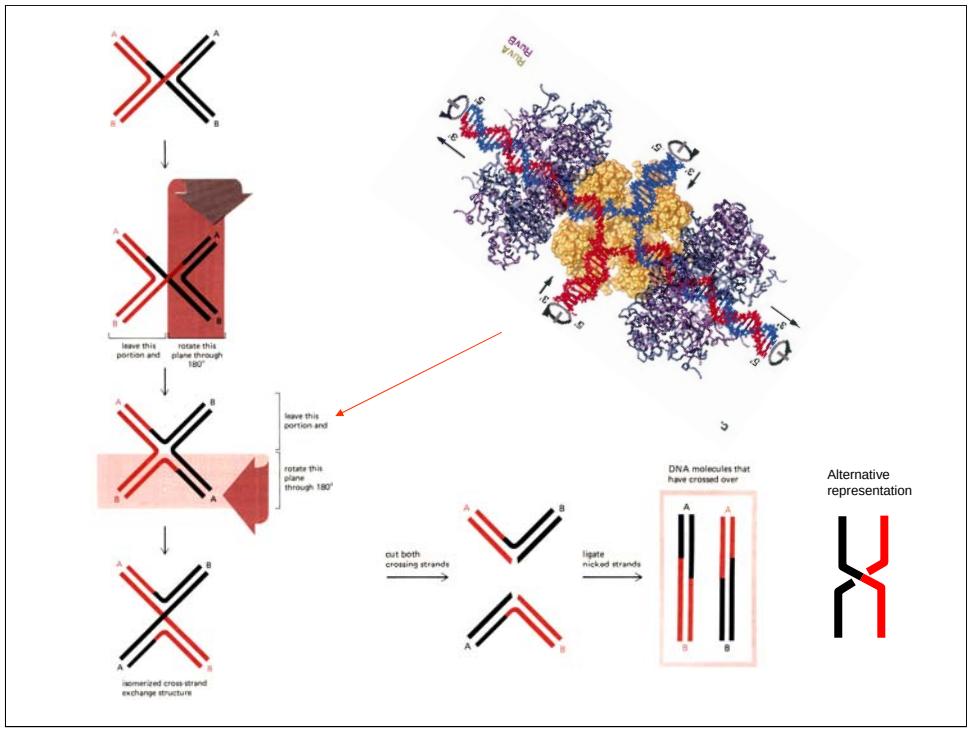
5' – 3', incoming NTP has energy. Therefore "proofreading exonucleases" can chop out any errors from chain, which can continue where it left off.

(Compare to "Head growth" in ribosome.)



Homologous recombination

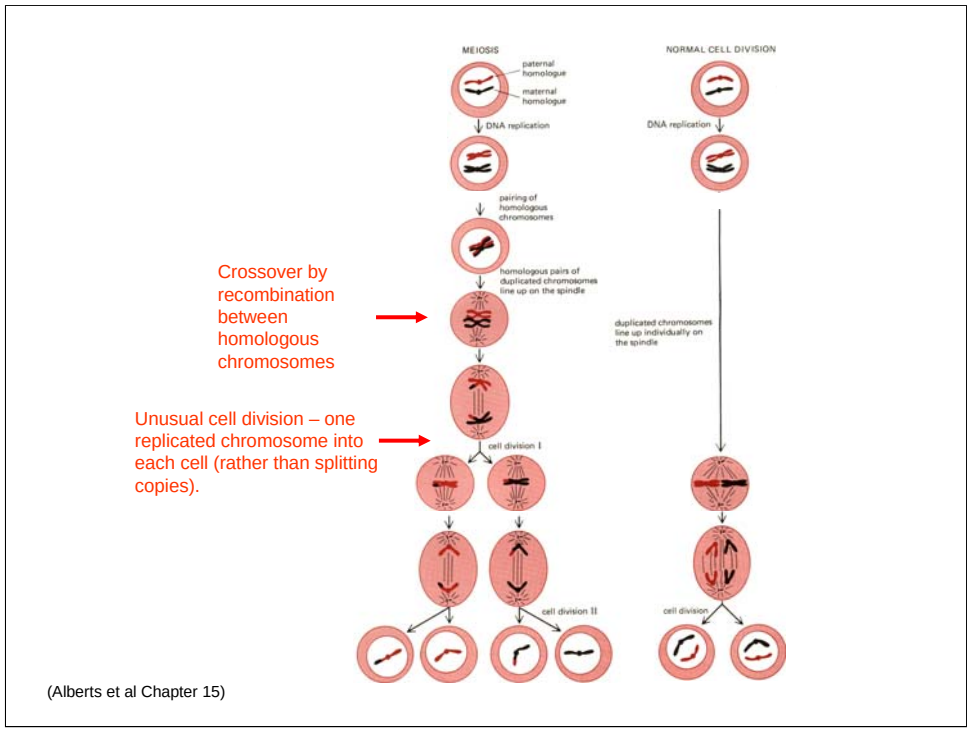
[strand exchange] (15)



[Recombination] (16)

“Holliday Junction”

As always, special machines perform this job – this example is RuvAB(C) complex



[Mitosis and Meiosis] (17)

Physical separation of chromosomes is done by molecular motors – later lectures

(actually many crossovers on each arm)

“Shuffles” genes – better way of introducing variation than (random) mutagenesis.

Mendelian gentics…

NB: Eukaryotes also have **RNA splicing** (Alberts p531) after transcription

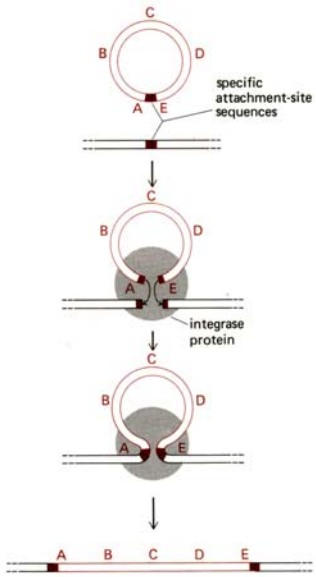
“introns”: Sequences in DNA but missing in mRNA and protein

“exons”: Sequences in DNA, mRNA and proteins

Makes genes **modular**

Can mix up protein domains without modifying DNA – eg to produce massive variation in the immune system.

Enhances the power of recombination to shuffle genes and parts of genes, by recombination in introns.

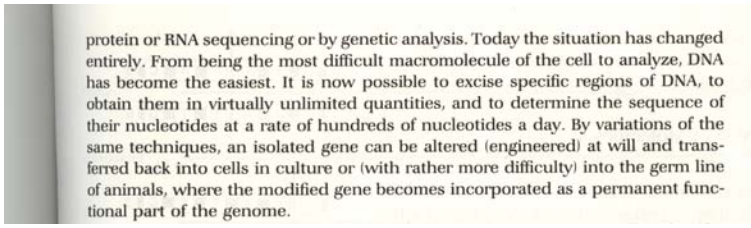


Modern Genetic Techniques (More detail in Lecture 11)

[site-specific recombination] (18)

Allows non-homologous DNA sequences to be inserted

Different mechanisms.



protein or RNA sequencing or by genetic analysis. Today the situation has changed entirely. From being the most difficult macromolecule of the cell to analyze, DNA has become the easiest. It is now possible to excise specific regions of DNA, to obtain them in virtually unlimited quantities, and to determine the sequence of their nucleotides at a rate of hundreds of nucleotides a day. By variations of the same techniques, an isolated gene can be altered (engineered) at will and transferred back into cells in culture or (with rather more difficulty) into the germ line of animals, where the modified gene becomes incorporated as a permanent functional part of the genome.

From Alberts et al, 2nd edition (1989)

[Quote from Alberts et al , ~1989 edition, page 180] (19)

Now we have the entire human genome and several others besides.

Entire bacterial genome can be sequenced in a day (>Mbp).

Transgenic crops and animals are common

Cloned mammals

“Bioinformatics” to handle the huge quantities of data

“Post genomics” – what do all the genes do? How do the proteins work? How are they regulated. Some emerging answers later in this course.

Use bacteria to produce inserted DNA or proteins in large quantities

Proteins for drug use (biotech bubble - only insulin so far)

Toolbox for whatever you can think of…

Eg. Turberfield group…