DNA REPAIR

Photoreactivation

Cleavage of the cyclobutane ring of pyrimidine dimers by DNA photolyases restores the original DNA structure. Photolyases have chromophores, which absorb blue light to provide energy for the reaction.

Alkyltransferase

An inducible protein specifically removes an alkyl group from the *O*6 position of guanine and transfers it to itself, causing inactivation of the protein.

Excision repair

In nucleotide excision repair, an endonuclease makes nicks on either side of the lesion, which is then removed to leave a gap. This gap is filled by a DNA polymerase, and DNA ligase makes the final phosphodiester bond. In base excision repair, the lesion is removed by a specific DNA glycosylase. The resulting AP site is cleaved and expanded to a gap by an AP endonuclease plus exonuclease. Thereafter, the process is like nucleotide excision repair.

Mismatch repair

Replication errors which escape proofreading have a mismatch in the daughter strand. Hemimethylation of the DNA after replication allows the daughter strand to be distinguished from the parental strand. The mismatched base is emoved from the daughter strand by an excision repair mechanism.

Hereditary repair defects

Mutations in excision repair genes or a translesion DNA polymerase cause different forms of xeroderma pigment of sum, a sun-sensitive cancer-prone disorder. Excision repair is also defective in Cockayne syndrome.

Gene mapping

One of the main objectives in genetic analysis is the determination of the **position of genes on the chromosome**. In isolation, this may seem a rather arcane occupation, but knowledge of the organization of the chromosome does play a major role in understanding **gene function** and has contributed extensively to the advances.

In bacteria, the classical methods of **gene mapping** depend on the production of **recombinants** by gene transfer using **conjugation, transformation and transduction.** These methods have now been supplemented, although not entirely supplanted, by methods based on in vitro gene technology. Nevertheless, a basic understanding of these methods is valuable for an appreciation of the development of our **knowledge of bacterial genetics**.

Conjugational analysis

In an earlier Lecture it was shown that **integration of the F plasmid** into the *E. coli* chromosome produces an **Hfr strain** which is capable of transferring a copy of the chromosome to a suitable recipient. Transfer of the whole chromosome would take about **100 min**. For this reason, the E. coli genetic map (Figure 5) is calibrated from 0 to 100 min, with each gene being assigned a **position** that corresponds to the time at which it is transferred from an arbitrary origin at the threonine locus (**thr, 0 min**) with transfer proceeding in a **clockwise direction**. The actual time at which transfer of a specific gene occurs and the direction of transfer will depend on the **Hfr** strain used, since the **F plasmid** can be integrated at different points and/or in a different orientation.

However, it is quite rare for the complete chromosome to be transferred. The mating pairs will tend to become separated at randomly distributed times. The longer it takes for transfer of a gene, the more chance there is that the mating pair will have separated before that gene is transferred. There will therefore be a gradient of transfer corresponding to the position of the genes with respect to the point at which transfer starts. This provided a convenient way of determining the relative position of genes on the E. coli chromosome (Figure 1).

If a prototrophic **Hfr strain** is mated with a multiply auxotrophic recipient (e.g. **thr leu trp his arg**), the number of recipients that have received each of the markers can be determined by **plating aliquots of the mixture on a minimal medium** supplemented with four **out of the five amino acids**. For example, the number of thr recombinants is measured using a medium that contains leucine, tryptophan, histidine and arginine, but not threonine. It is of course necessary to prevent growth of the prototrophic donor, for example by using a streptomycin-resistant recipient and including streptomycin in the medium.

Streptomycin in this instance is used as a counter selecting agent. On this medium, the donor will be unable to grow (because of the streptomycin) and the parental recipient will not grow (because of the absence of threonine). The only cells that can grow will be the recombinant recipients that have received the thr gene.

The result is illustrated by Figure 6. In this instance, the **HfrH** donor has been used, from which the genes are transferred in a **clockwise direction** starting

very close to the thr locus. There is a linear relationship between the logarithm of the number of recombinants and the map position of the genes concerned. If it is assumed that the position of the trp gene is not known, determining the number of Trp recombinants will allow the gene to be mapped as shown in Figure 2. An alternative method for more accurate mapping of genes that are transferred relatively early in mating involves deliberately separating the mating pairs (**by violent agitation**) in samples of the mixture at different times after the start of mating (**interrupted mating**). Recombinants that have received a specific gene start to appear at a certain time after the start of mating (the time of entry), which is a measure of the distance of that gene from the origin of transfer.

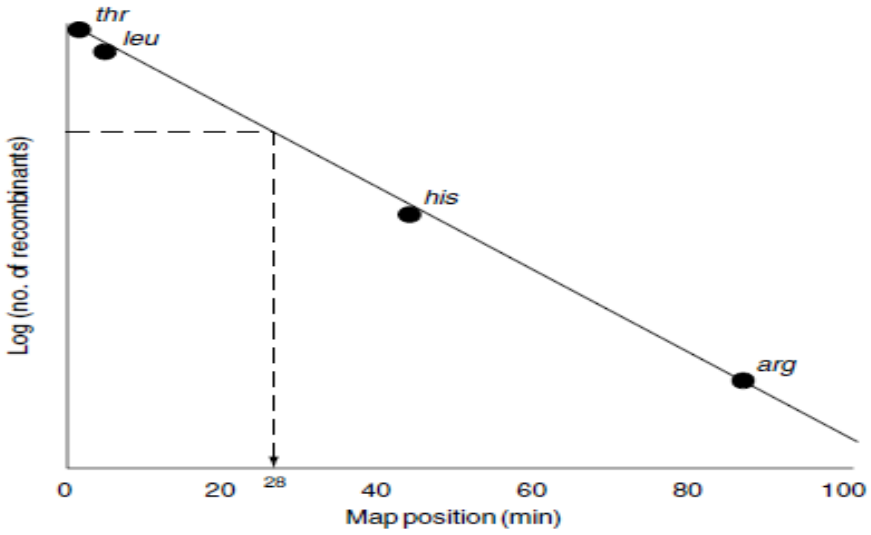

Figure 6 .Gene mapping using the gradient of transfer by conjugation. Determination of the position of the trp gene

Molecular techniques for gene mapping

Restriction Mapping

The **molecules of DNA composing** the genomes of living organisms are too long to be **analyzed directly**. However, they can be cleaved into relatively small fragments in a reproducible manner. For this **purpose**, about 400 different **restriction endonucleases** have been derived from various bacteria. Restriction endonucleases (**restriction enzymes**) cleave DNA at defined sites. Such enzymes **protect bacteria** from invading foreign DNA, which is cut into small pieces. A given enzyme typically **recognizes a specific sequence** of **4–8 (usually 6)**

nucleotides, called a restriction site, where it **cleaves the DNA**. The sizes of the DNA fragments produced depend on the distribution of the restriction sites

A. DNA cleavage by restriction nucleases

The recognition site of a common restriction enzyme, ***Eco*RI**, derived from the bacterium *Escherichia coli* restriction enzyme I, is **5′- GAATTC-3′** (**1**). The **enzyme cleaves double stranded DNA asymmetrically** to produce fragments with single-stranded ends (**2**). On one fragment, the single-stranded **3′-5′ end** has four nucleotides **(3′-TTAA**) **overhanging**, and on the other fragment the **5′-3′ overhang** is **AATT- 3′.** This common asymmetric cleavage pattern is called **palindromic** because it reads the same in opposite directions. Some restriction enzymes have a symmetric recognition site (**3**) and produce **blunted ends** (**4**), such as ***Hae*III (5′-CGCG-3′).** The ends of fragments with single-stranded overhangs can be easily connected in different ways, within molecules by **cyclization** or between molecules to form linear **concatemers.** This ligation requires the enzyme ligase Fig 5.

B. Examples of restriction enzymes

Restriction enzymes can be classified according to the type of ends they produce: (**1**) **5′ overhangs (e.g., *Eco*RI, see above),** (**2**) 3′ overhangs (e.g., ***Pst*I**), (**3**) **blunted ends** (e.g., ***Alu*I, *Hae*III** , ***Hpa*I**), or (**4**) **nonpalindromic ends** (***Mln*I**). Some have a bipartite recognition sequence with different numbers of nucleotides at the **single-stranded ends** (e.g., ***Bst*I**).

In ***Hind*III** it suffices that the **two middle nucleotides** are a **pyrimidine and a purine (GTPy- PuAC),** and it does not matter whether the former is **thymine (T)**

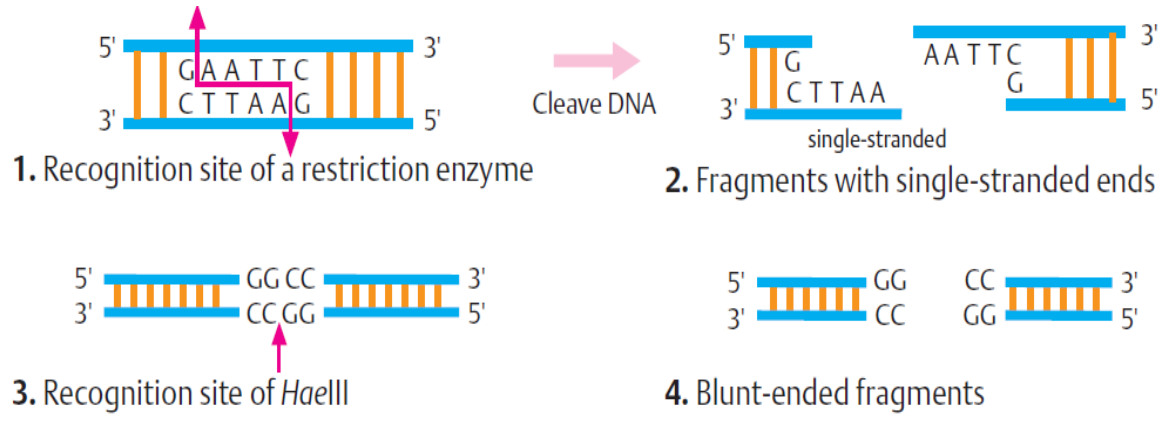

Fig 5.DNA cleavage by restriction nucleases

or cytosine (C) or whether the latter is **adenine (A) or guanine (G).** Such recognition sites occur frequently and produce many relatively small fragments. **Rare-cutters recognize** long sites of 10 and more nucleotides. Consequently they produce large fragments, which is useful for many purposes. Some enzymes have cutting sites with limited specificity Fig 6.

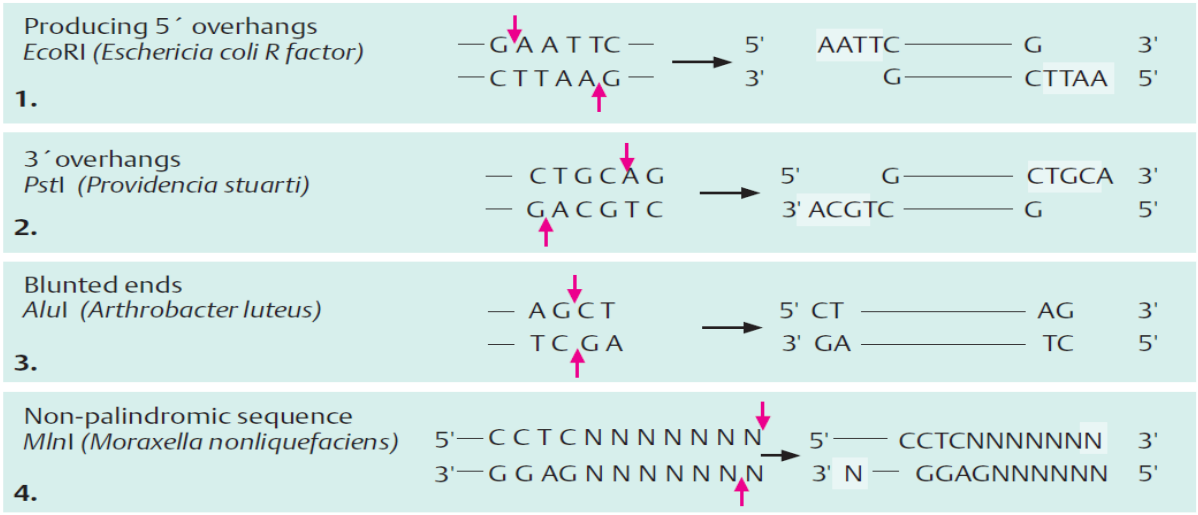

Fig 6.Examples of restriction enzymes

C. Determining of the location of restriction sites

Since the **fragment sizes reflect** the relative positions of the **cutting sites**, they can be used to characterize a DNA segment (**restriction map**). For example, if a **10-kb DNA segment cleaved** by two enzymes, A and B, results in three **fragments, of 2 kb, 3 kb, and 5 kb,** then the relative location of the cleavage sites can be determined by using **enzymes A and B alone** in further experiments. If enzyme A yields two fragments of **3 kb and 7 kb**, and enzyme **B** two fragments of 2 kb and 8 kb, then the two recognition sites of enzymes A **and B must** be located 5 kb apart. The recognition site for A must be 3 kb from the left end, and that for **B 2 kb from** the right end (red arrows). This establishes a restriction map to **characterize this fragment** Fig 7.

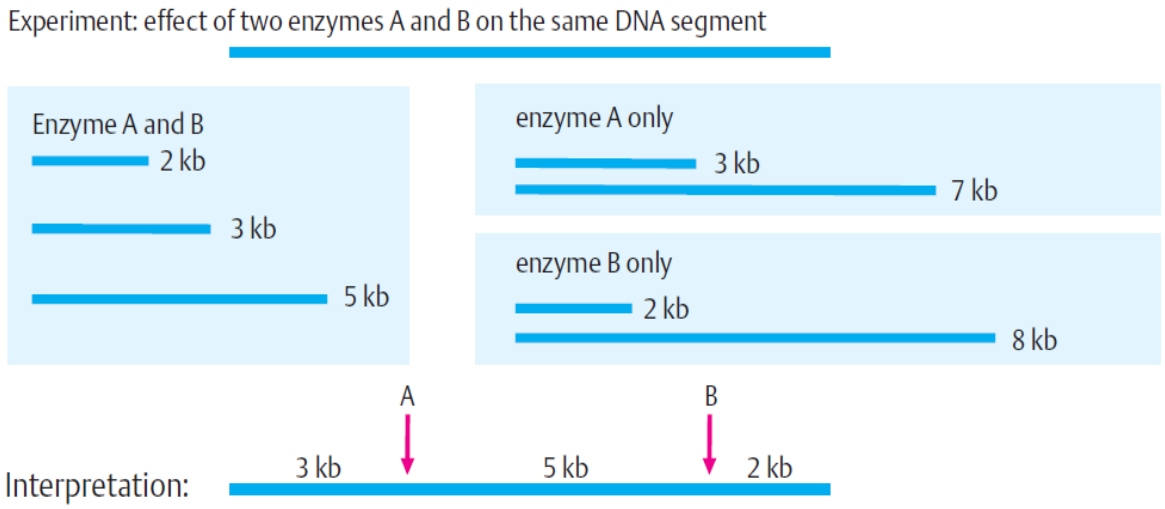

Fig 7.Determining the location of restriction sites

D. Restriction map

A **given DNA segment** can be characterized by the distribution pattern of restriction sites. In the example shown, a DNA segment is characterized by the distribution of the recognition sites for **enzymes E (*Eco*RI) and H (*Hind*III).** The individual sites are separated by intervals defined by the **size of the fragments** after digestion with the enzyme. A **restriction map** is a linear sequence of restriction sites at defined intervals along the DNA. **Restriction mapping** is of considerable importance in **medical genetics** and evolutionary researchFig 8.

Fig 8 .Restriction map