Human insulin production by genetic engineering

By
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- Structure of the insulin
- Insulin gene
- Insulin processing in the body
- Disease
- Treatment
- Recombinant Insulin and process
- Host cells
- Vectors
- Bioreactors

INSULIN

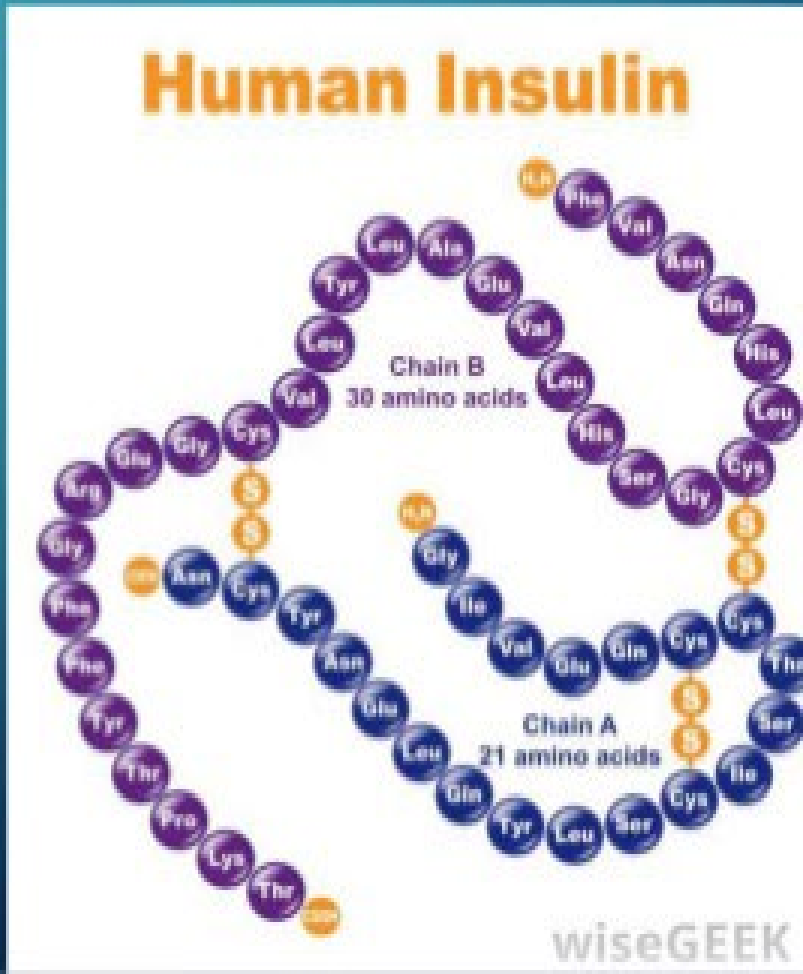
- Is a polypeptide hormone produced by the **β cells** of the islets of Langerhans in the pancreas
- Its main **function is enabling** the cells to **take up** glucose (providing it with energy it needs).
- It is required for normal **glucose homeostasis**.

Structure of Human Insulin

- Chemically Human insulin is small, simple protein composed of 51 amino acids sequences and has a molecular weight of 5808 Da.
- Insulin hormone is a dimer of a A- chain and a B-chain which are linked together by a disulphide bond.
- Fredrick Sanger et al (1954) gave the first complete description of insulin. Insulin consists of two polypeptide chain,
  o Chain A- 21 amino acids long
  o Chain B-30 amino acids long
  o Both chains are joined together by disulphide bond between two cysteine residue

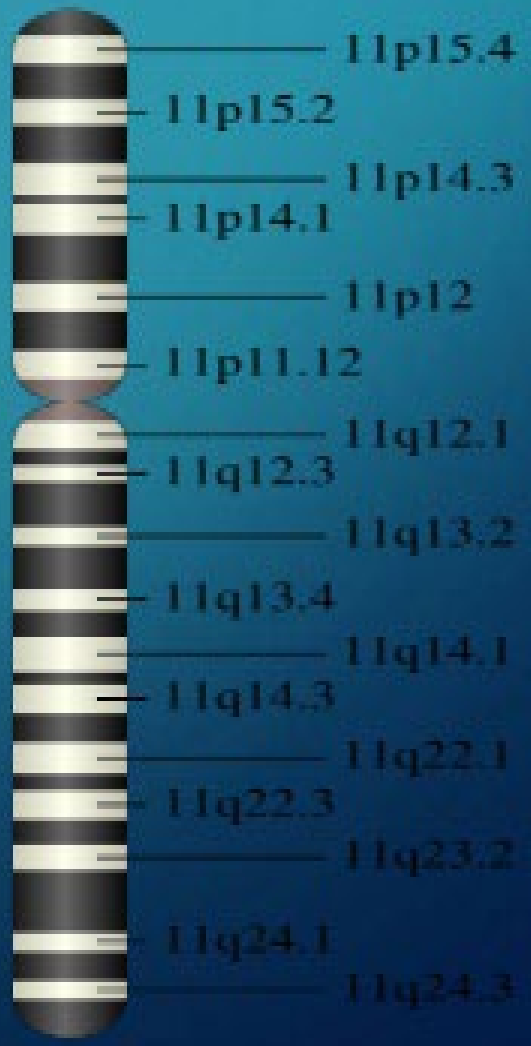
The Structure Of Insulin

- **Insulin** is composed of two peptide chains referred to as the **α chain** and **β chain**.
- **α** and **βchains** are linked together by two **disulfide bonds**, and an additional disulfide is formed within the **α chain**.
- **α chain** consists of **21 amino acids** and the **β chain of 30 amino acids**.



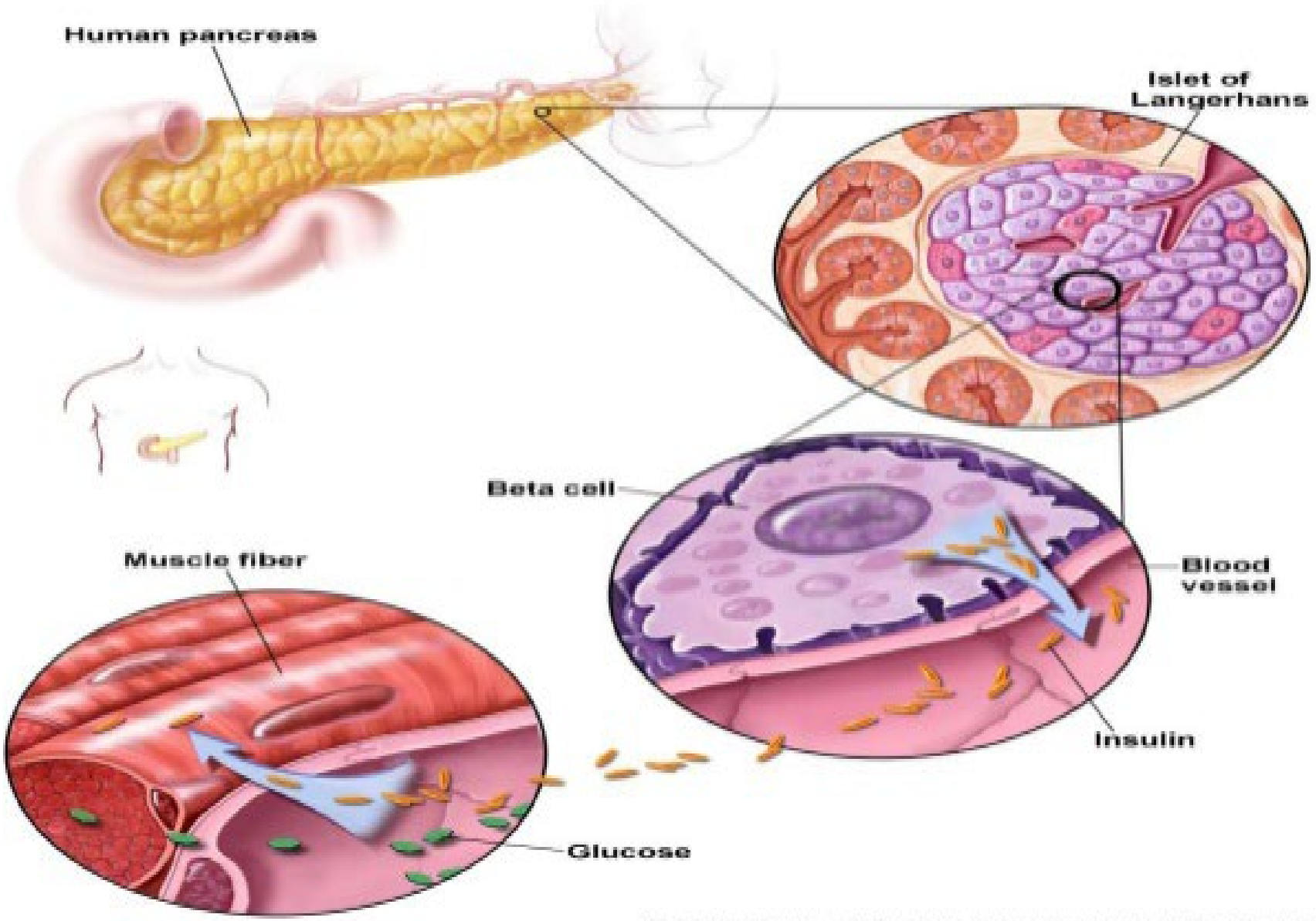
Which Chromosome Insulin Gene Is Found On?

- **INS: Insulin gene** is found on chromosome (11).
- It is found on **the short arm of chromosome 11** (p arm).
- It is located **region 1 , band 5, sub-band 5.**

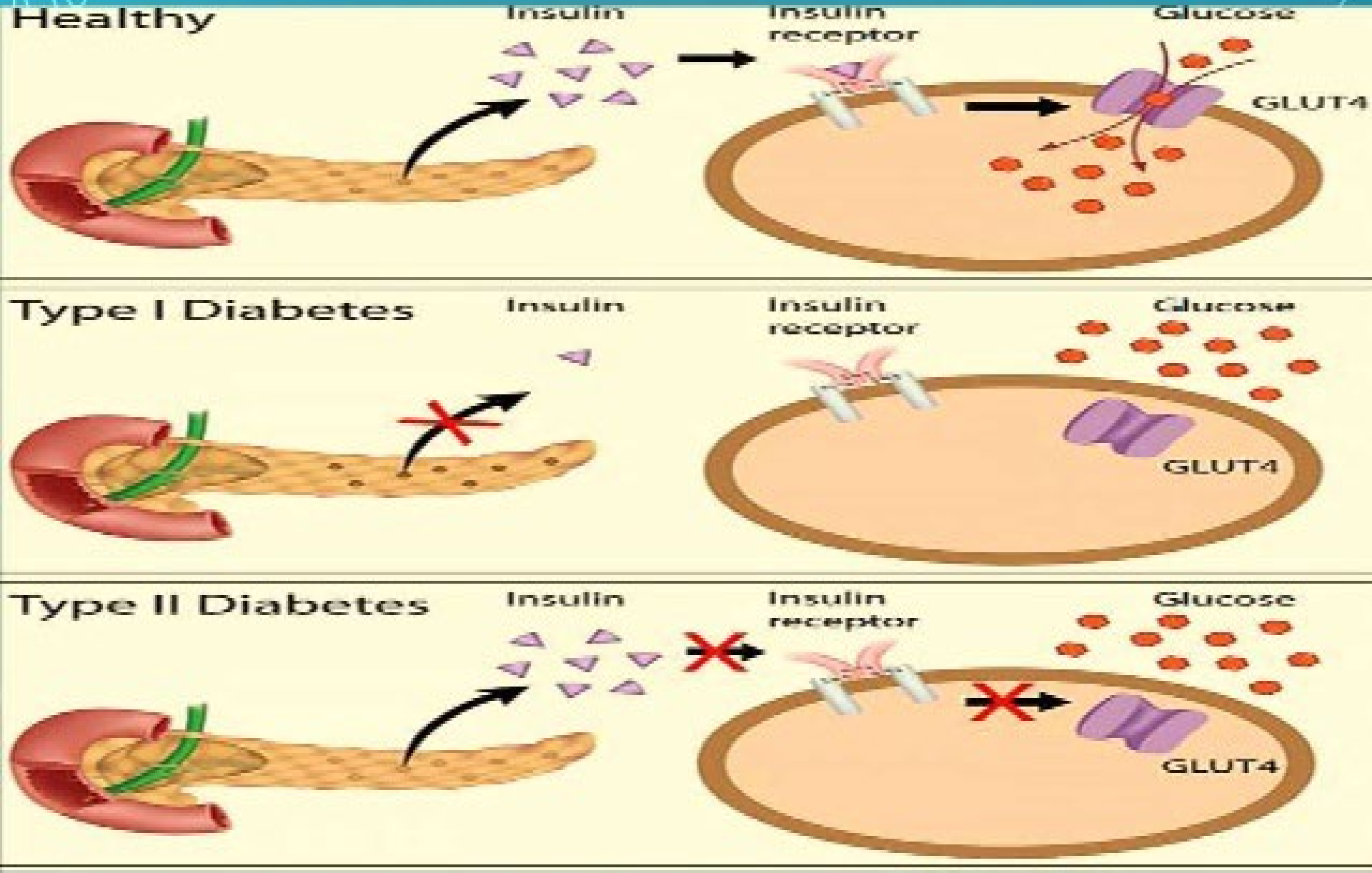


Insulin produced inside pancreas:

- At first Pancreatic β-cells synthesize pre-pro-insulin, which is a 109 amino acids long polypeptide
- Among 109 amino acids, 23 amino acids are signal molecules which allows the pre-pro-insulin to pass through cell membrane.
- Entering inside cell, it become 86 amino acids long pro-insulin. It is still inactive.
- Some Proteolytic enzymes cut and expose the active site of pro insulin converting it into active form of insuin of 51 amino acids long.

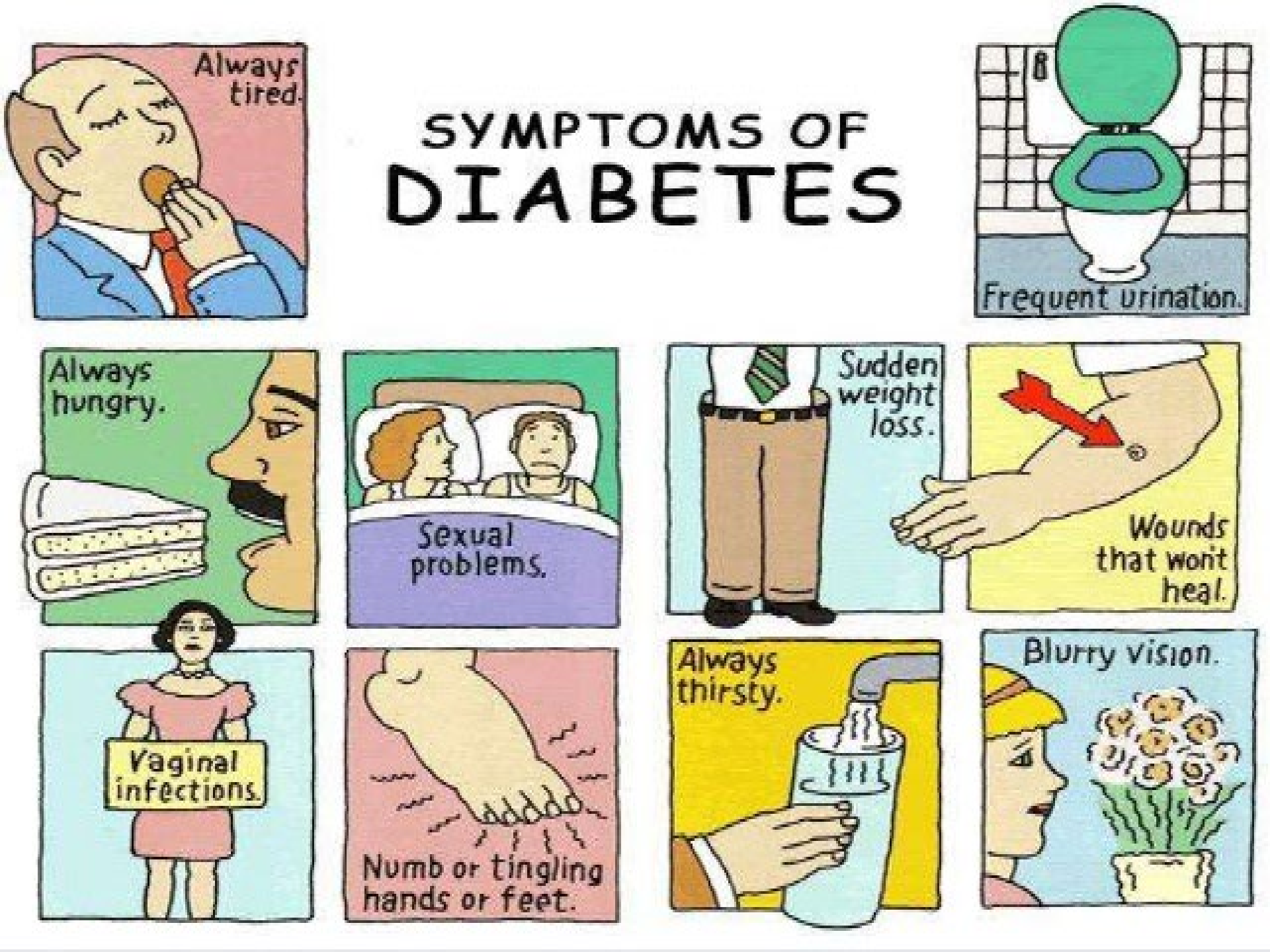

Human pancreas
Islet of Langerhans
Beta cell
Blood vessel
Muscle fiber
Insulin
Glucose
© 2001 Terese Winslow (assisted by Lydia Kibiuk)

Failure To Produce Or Use Insulin



Importance Of Insulin

- Without insulin, the **blood glucose builds up** in the blood and the cells are starved of their energy source.
- Some of the symptoms that may occur include **fatigue, constant infections,** blurred eye sight, numbness, **tingling in the hands** or legs, increased thirst, and slowed healing of bruises or cuts.
- The cells will begin to use fat, the energy source stored for **emergencies.**
- When this happens for too long a time **the body produces ketones**, chemicals produced by the liver.
- **Ketones** can poison and kill cells if they build up in the body over an extended period of time. This can lead to serious **illness and coma**.


SYMPTOMS OF
DIABETES
Always tired.
Frequent urination.
Always hungry.
Sexual problems.
Sudden weight loss.
Wounds that won't heal.
Vaginal infections.
Numb or tingling hands or feet.
Always thirsty.
Blurry vision.

TREATMENT

- Banting and Best invented the insulin hormone in 1921.
- Several individuals died since their glucose level increased due to disorder of insulin production .
- **Treatment *via* insulin injection** extracted from animal pancreas.

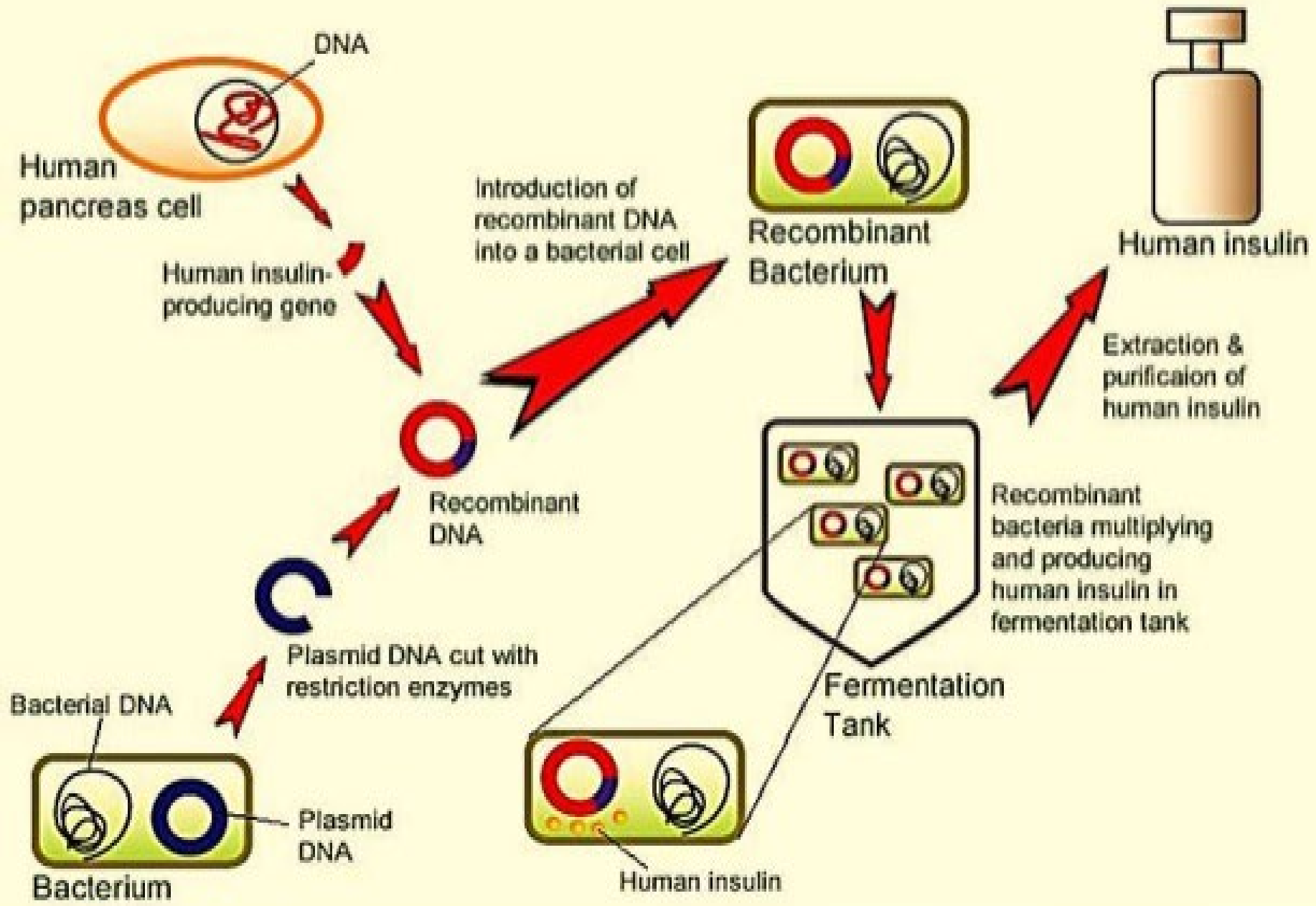
Human insulin production

- **Insulin** is a hormone produced by β-cells of islets of Langerhans of pancreas. It was discovered by sir Edward Sharpey Schafer (1916) while studying Islets of Langerhans.
- Pancreas is a mixed gland situated transversely across the upper abdomen behind stomach and spleen.
- Insulin is a peptide hormone produced by pancreas and is a central regulator of carbohydrates and fat metabolism in the body.

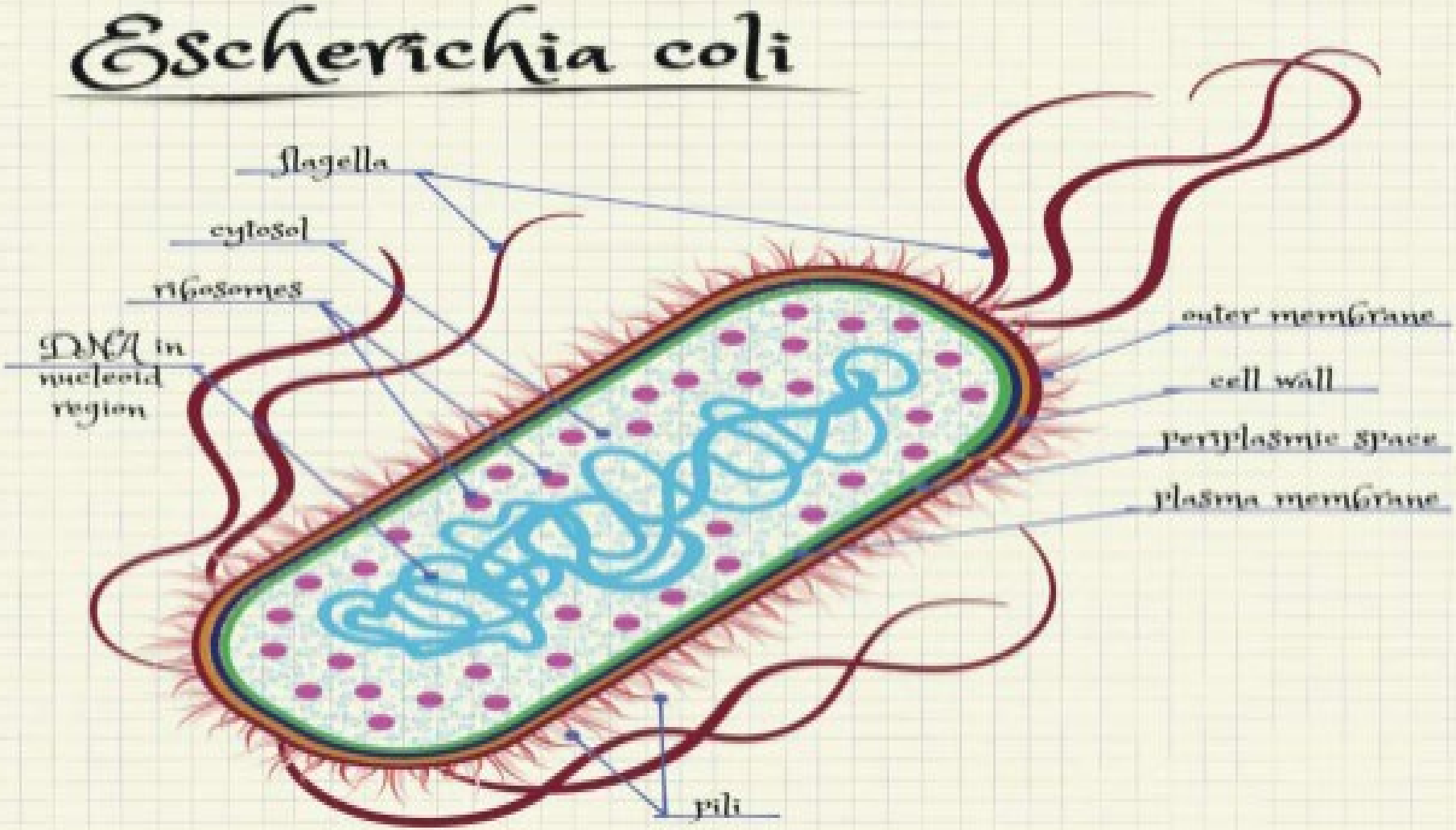
THE BREAKTHROUGH

- **Recombinant human insulin** :a form of insulin (trade name Humulin) made from recombinant DNA which is human insulin.

Human Insulin Production

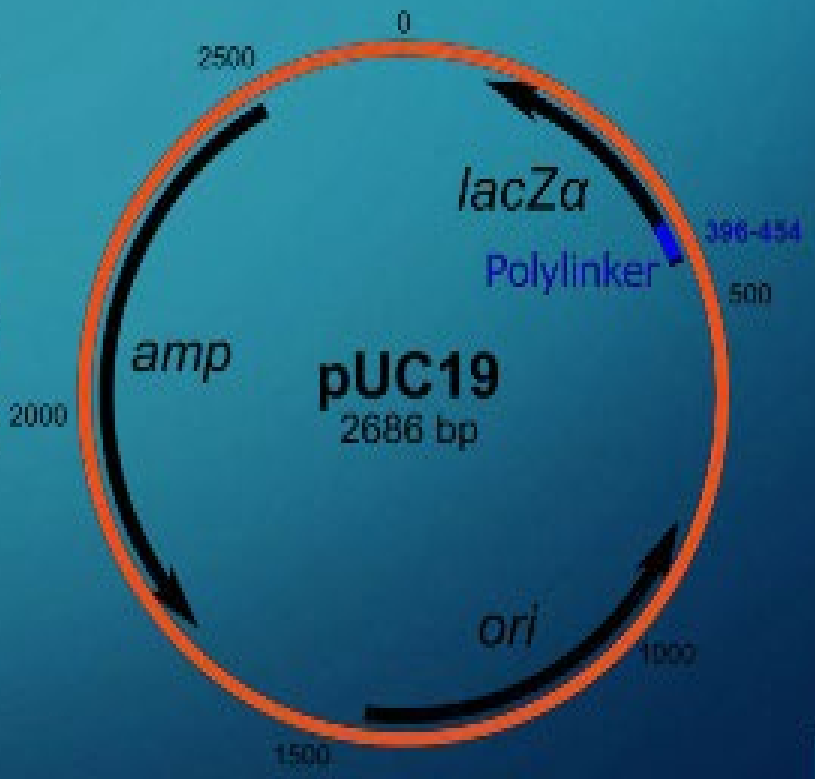


HOST CELL USED FOR INSULIN PRODUCTION



EXPRESSION VECTOR

- A circle of double-stranded DNA that is separate from the chromosomes, which is found in bacteria.



ADVANTAGES OF USING BACTERIAL PLASMID

- Small, easy to handle
- Straightforward selection strategies
- Useful for cloning small DNA fragments
- (< 10kbp)

PHASES INVOLVED IN INSULIN PRODUCTION

Upstream Process

Downstream Process

UPSTREAM PROCESS PHASE

Shaking bioreactor

Advantages:

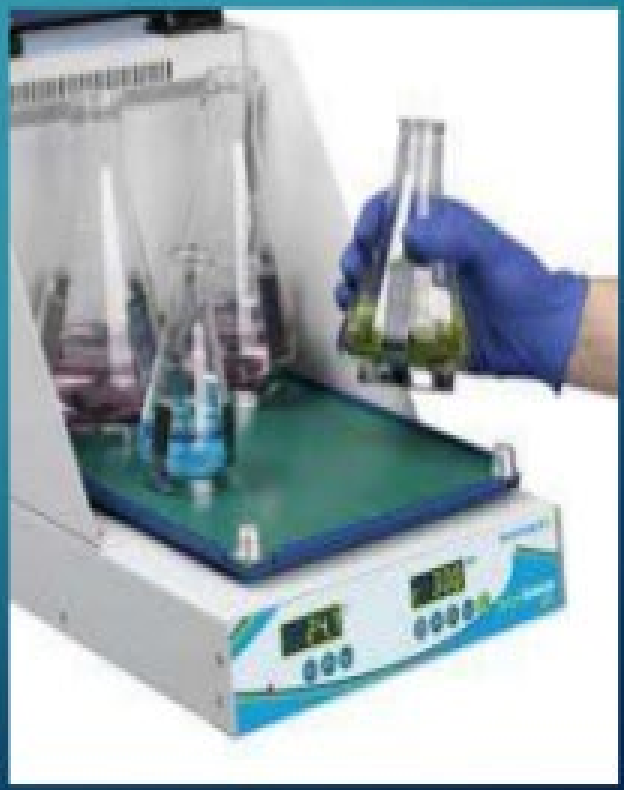
Easy ,Visible ,Cheap, Depyrogenation feasible

Disadvantage:

Poor aeration

Impeller jams

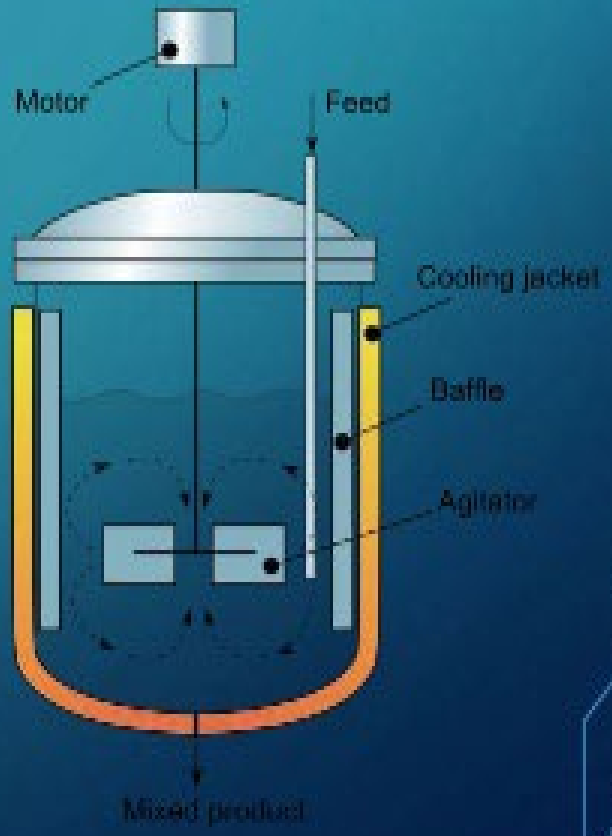
Requires cleaning siliconizing & sterilization
lHigh space requirements in incubator



UPSTREAM PROCESS PHASE

Stir tank bioreactor

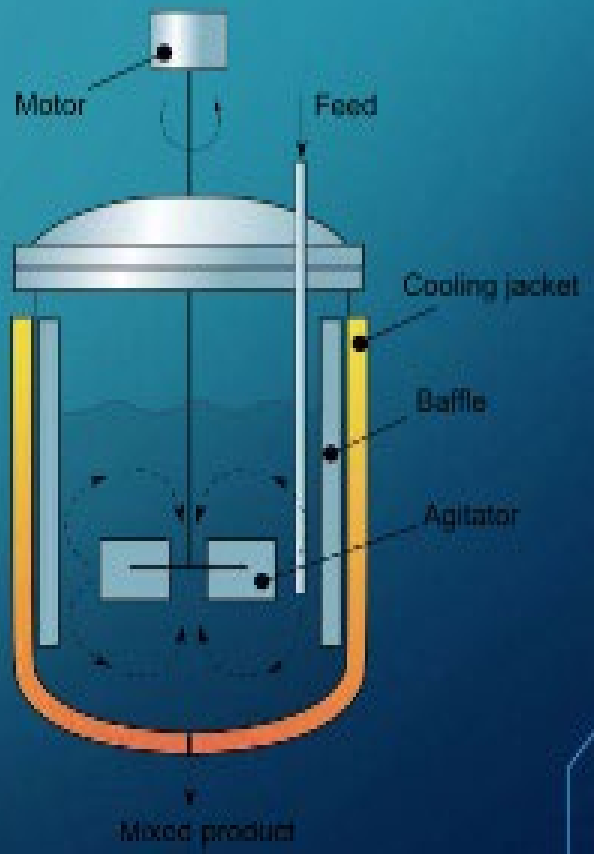
- **Continuous bioreactor processes** continually feed nutrients and medium into the bioreactor while also continually harvesting material from the bioreactor.



UPSTREAM PROCESS PHASE

Stir tank bioreactor

- Agitator is introduced to disperse the reactants thoroughly into the reaction mixture immediately as they enter the reactor.
- Product is continuously drawn out and that's why known for perfect mixing.
- Compositions at outlet and inside reactor are the same.



DOWNSTREAM PROCESS

- **Removal of insolubles** is the first step and involves the capture of the product as a solute in a particulate-free liquid. Typical operations to achieve this are filtration, centrifugation.

- **Product isolation** is the removal of those components whose properties vary considerably from that of the desired product. Solvent extraction, adsorption, ultrafiltration, and precipitation are some of the unit operations involved.

DOWNSTREAM PROCESS

- **Product purification** is done to separate those contaminants that resemble the product very closely in physical and chemical properties. operations include affinity, size exclusion, reversed phase chromatography, ion-exchange chromatography, crystallization and fractional precipitation.

- **Product polishing** describes the final processing steps which end with packaging of the product in a form that is stable, easily transportable and convenient.

Insulin produced by recombinant DNA technology

- The basic step in recombinant DNA technology is similar for insulin production also.
  - At first suitable vector (plasmid) is isolated from E. coli and then it is cut open by restriction endonuclease enzyme.
  - The gene of interest (ie. Insulin coding gene) is isolated from β-cell and inserted in opened plasmid.
  - Plasmid and gene of interest are recombined together by DNA ligase enzyme
  - This recombined plasmid is inserted into suitable host cell (ie E. coli) and now this recombined host cell starts producing insulin hormone.

contd.....

- Hakura et al (1977) chemically synthesize DNA sequence of insulin for two chains A and B and separately inserted into two PBR322 plasmid vector.
  - These gene are inserted by the side of β-galactosidase gene of the plasmid.
  - The recombinant plasmid were then separately transformed into E. coli host.The recombinant host produced pro-insulin chains ie. fused β-galactosidase-A chain and β-galactosidase-B-chain separately.
  - These pro-insulin chains A and B were separated from β-galactosidase by treatment with cyanogen bromide. The detachment of pro-insulin chains from β-galactosidase is possible because an extra codon form methionine was added at N-terminal of each gene for A and B-chain.
  - After detachment, A and B chains are joined invitro to reconstitute the naïve insulin by sulphonating the peptide chains with sodium disulphonate and sodium sulphite.
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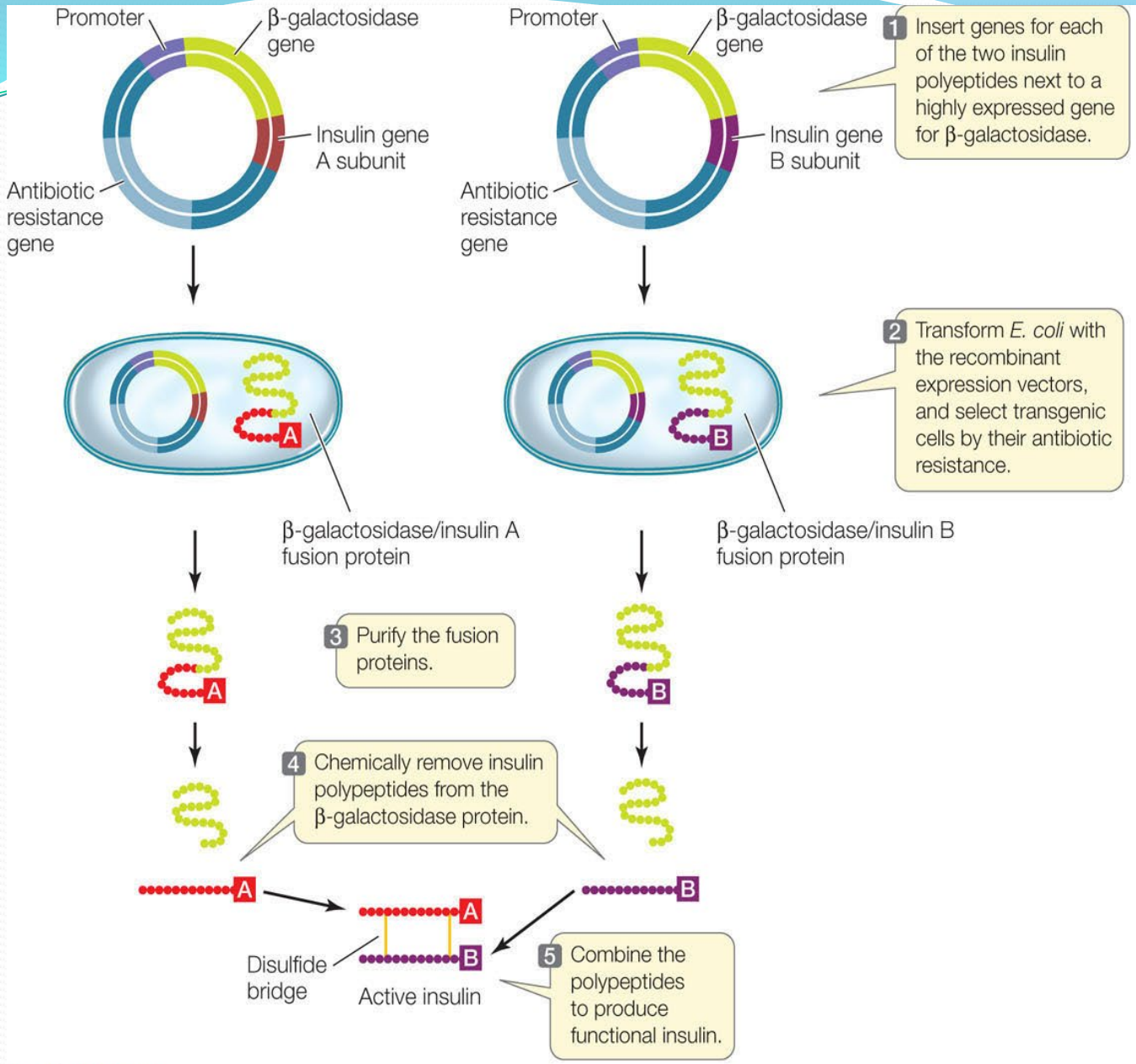

13.12: David McIntyre.

Production by recombinant DNA technology is designed by Gilbert

- In this method, m RNA for pre-pro-insulin is isolated from islets of Langerhans cell
- mRNA is reverse transcribed to form DNA and then it is inserted into PBR 322 plasmid in the middle of the gene for penicillinase.
- Then the recombinant plasmid is transformed into suitable host ie E. coli cell
- The host produced penicillinase + pre-pro insulin
- Insulin is later separated by trypsin treatment

Roles of insulin in body:

- Insulin causes cells in liver, skeletal muscles and fat tissue to take up glucose from the blood. In liver and skeletal muscle, glucose is stored as glycogen and in adipose tissue, it is stored as triglyceride.
- Insulin stops the use of fat as energy source by inhibiting the release of glucagon hormone.
- With the exception of the metabolic disorder such as Diabetes mellitus and metabolic syndrome, insulin maintain constant proportion of glucose in blood by removing excess glucose from the blood which otherwise would be toxic.
- When blood glucose levels fall below a certain level, body begins to use stored glycogen as energy source through glycogenolysis; which breaks down glycogen stored in liver and muscles into glucose, which is then utilized as energy source.

- Failing to control the level of insulin in body results in a disorder called diabetes mellitus. As a consequences Insulin is used medically to treat some forms of diabetes mellitus.
- Patients with type I diabetes depends on insulin shots. Most commonly insulin is injected subcutaneously for the patients because the hormone is no longer produced in their body. Type I diabetes is also known as Insulin dependent diabetes mellitus.
- Patients with type II diabetes are often resistant to insulin and because of such resistance many suffer from relative insulin deficiency. This is also known as Insulin independent diabetes. Some patients with type II diabetes may eventually require insulin shots if other medication fails to control blood glucose level. Over 40% of type II diabetes patients require insulin shots as part of their diabetes management plan.

- THANKU