S. S. College, Jehanabad

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Subject: Zoology

Topic: Histochemical localization of DNA by Fuelgen reaction

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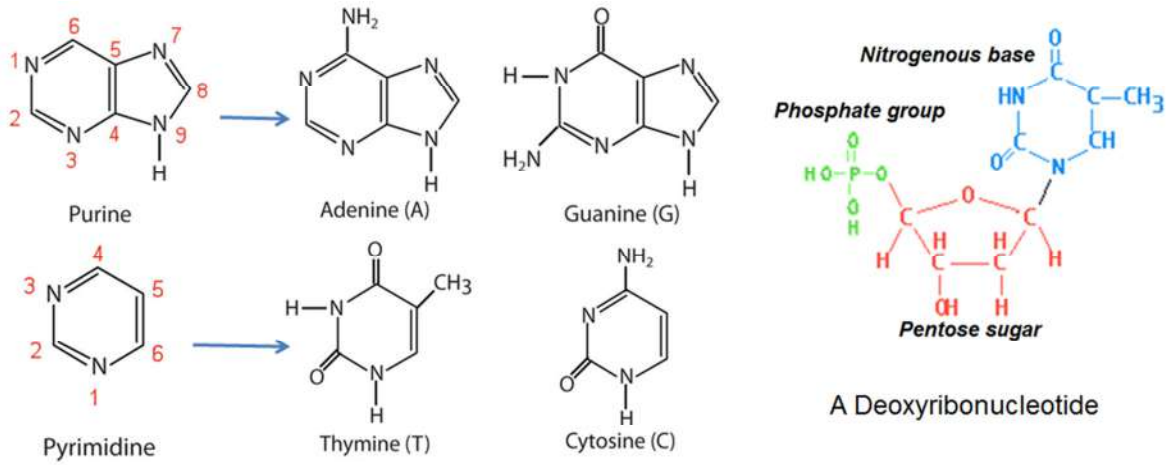
HISTOCHEMICAL LOCALIZATION OF DNA BY FEULGEN REACTION

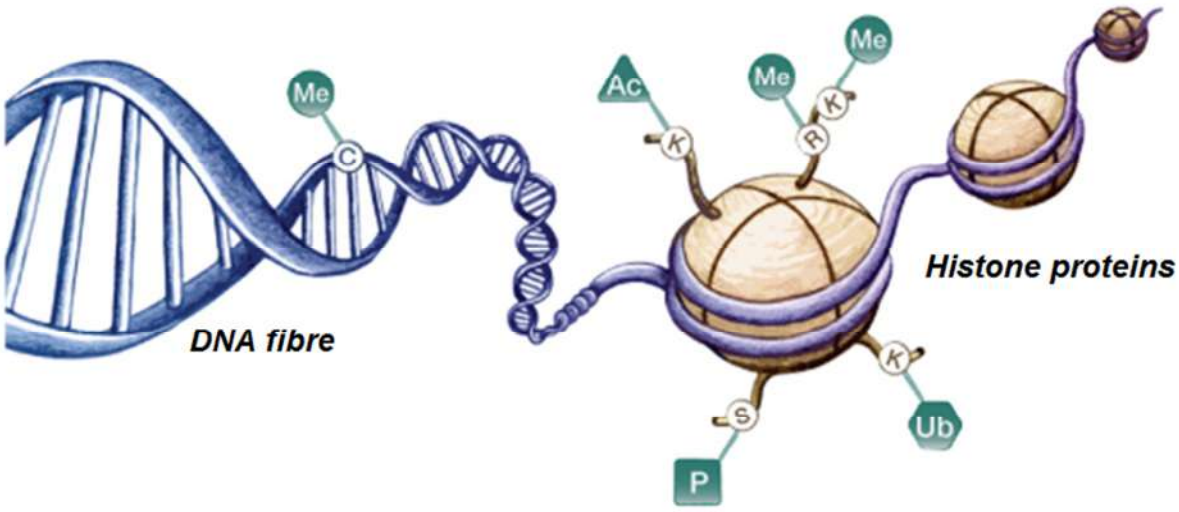
Deoxyribonucleic acid, more commonly known as DNA, is a complex molecule that contains all of the information necessary to build and maintain an organism. All living things contain DNA either in their subcellular compartment or in cytoplasm as in prokaryotes. All multicellular organisms possess the full set of DNA in the nucleus required for normal functioning of cells. It is not only essential for normal physiological function of cells, but it also serves as the primary unit of heredity in organisms of all types by allowing a transmission of genetic information from one generation to next and ensure a certain level of continuity between the generations, while still allowing for slight changes that contribute to the diversity of life.

DNA is a long molecule, a polymer chain of ~ 100 ro several thousand deoxyribonucleotides sometimes also called as bases. There are four different types of deoxyribonucleotides in DNA which are known as deoxyadenosine mono-phophate (dAMP), deoxyguanosine mono-phosphate (dGMP), deoxycytosine mono-phosphate (dCMP), and deoxythymidine mono-phosphate (dTMP) made of purine such as A (adenine) and G (guanine), and pyrimidine such as C (cytosine) and T (thymidine) nitrogenous base respectively esterified covalently attached with deoxyrobose sugar (a pentose sugar) and esterified with a phosphate group. Purine forms hydrogen bonds with their corresponding pyrimidine, for example A to T and G to C.



Examples of bases present in the DNA molecule

The four ribonucleotides are necessary to make a code for a cell that describes entire function of cell life, known as genetic codes. DNA is generally formed of 2 polynucleotide chains twisted around each other in a double helix. Double stranded DNA is simply two chains of single-stranded DNA positioned so their 'bases' can interact with each other with hydrogen bonding which is, though, weak but it adds up to enough strength to hold the strands together. In addition to this hydrogen bonding, strands are also held together by an interaction called "base stacking". Importantly, the two strands align each other in opposite direction, hence the structure is said to be "anti-parallel". The DNA is present in the nucleus as part of chromosomal structure which carries the genetic information from generation to generation. However, mitochondria as well as chloroplasts are also known to possess DNA. It usually occurs as nucleoproteins or a complex of DNA with a basic protein such as histone or protamine. The histone proteins complexes with DNA and results in a compact structure called chromatin fiber, whereas protamine is highly basic proteins mostly found in sperm chromatin.



Packaging of DNA with histone proteins to form a structure known as chromatin fiber (Source: Promega Connection)

The DNA in a cell or tissue slides are commonly identified and localized by carrying Feulgen reaction, acridine orange, methylene blue and methyl green – pyronin Y methods. Acridine orange is an organic cationic compound that serves as a nucleic acid-selective fluorescent dye which enters cell by diffusion and interact with DNA by intercalation and RNA via electrostatic attraction, and is generally used in microbiological study. Methylene blue is a cationic dye which is attracted to negatively charged DNA and is generally used in determining the bacterial morphology. Methyl green – pyronin Y staining methods is performed for the identification and localization both DNA and RNA by utilizing its differential staining property.

Feulgen Reaction

It is a relatively new method for demonstrating DNA in a tissue sections. It is actually a sensitive means of detecting aldehydes of deoxyribose sugars that is exposed after the removal of bases from the DNA molecule, which makes it the ideal method for detecting the presence of DNA. The sugar part that remains in the DNA backbone reacts as an aldehyde ultimately forming a visible color. Therefore, this method can be said to be divided in to two main parts:

- The first part of the procedure is the hydrolysis phase that involves the use of 5N HCl 40°C min. This step is aimed at separately selecting two purine bases (adenine and guanine) that are removed from the DNA molecule.
- The second step is the staining phase. The reagent used is preferred because it is highly selective for DNA rather than RNA.

Here, RNA does not react because of the presence of hydroxyl on carbon 2 of ribose, which prevents HCl from hydrolyzing sugar.

Principles

Basic fuchsin is a magenta coloured dye which is rendered colourless when treated with hydrochloric acid (HCl) and sodium bisulfate. This bleached dye may react with aldehydes to

give a new dye with stronger colour magenta. Such aldehydes may be produced when cellular DNA is slowly hydrolysed on treatment with HC1 at 40° to 60°C, if the section is placed in the bleached dye, the aldehydes liberated from the DNA will react with the dye and exhibit the magenta colour. Since both DNA and RNA are basophilic (take up same type of stains) the Feulgen reaction allows a distinction to be made as to which basophil material is DNA.

Fixation and Tissue Processing

Tissue sections of usually 5µm thickness are fixed in 10% neutral buffered formalin fixative solution and processed for paraffin infiltration and embedding. The concentration of formaldehyde higher than 10% is avoided. In a less practiced method, the tissue is fixed using a saturated aqueous solution of mercuric chloride with 5% glacial acetic acid. The mercurial deposits were then removed from the tissue by treating with iodine (to convert into mercuric iodide which is soluble in alcohol) and washed in alcohol.

Other fixatives such as Carnoy's, formol saline and Zenker's fluid are also used, however, acid fixative is never used for the histochemical identification and localization of nucleic acids i.e. DNA & RNA. This staining method is also carried out in freeze formalin fixed tissue.

Equipment

- Slides
- 22mm square coverslips
- Whatman #4 filter paper
- Ceramic staining rack
- Staining dish
- Coplin staining jar
- Forceps
- Latex globes

Reagents

- Saturated formalin solution (40% formaldehyde solution)
- Glacial Acetic Acid – Corrosive; store at room temperature.
- Basic fuchsin dye
- Light green SF
- Anhydrous Potassium metabisulfite ($K_2S_2O_5$)
- Sulfurous acid (H_2SO_3)
- Conc. Hydrochloric acid (HCl)
- Absolute alcohol (100% ethanol) – Flammable
- Activated charcoal
- Double distilled water
- Xylenes – Flammable; store room temp in flammable cabinet.
- DPX as a mounting medium

Solution

I. Schiff's reagent solution

- It is prepared by dissolving 5gm of basic fuchsin in 900ml of boiling distilled water in a container and cooled to 50°C.
- 100ml of 1N HCl is slowly added in the solution, again cooled to about 25°C.
- 10gm of potassium metabisulfite ($K_2S_2O_5$) is lowly added, shaken for 3 minutes and incubated for 24 hours in the dark at room temperature.
- Finally, 5gm of fine activated charcoal is added and shaken well for 3 minutes.

2. 1N Hydrochloric acid solution

- It is prepared by mixing slowly 8.3ml of conc. HCl in a container containing 80ml of distilled water.
- Then, the volume is adjusted to 100ml by adding suitable amount of distilled water.

3. Light green SF yellow stain solution

- It is prepared by mixing 0.2gm Light green SF yellowish 100.0ml double distilled water and 0.2ml glacial acid is added to this solution.

4. Alcohol 50 %

- Reagent alcohol ~50 ml
- Deionized water ~50 ml

5. Alcohol 70 %

- Reagent alcohol ~70 ml
- Deionized water ~30 ml

6. Alcohol 80 %

- Reagent alcohol ~80 ml
- Deionized water ~20 ml

7. Alcohol 95 %

- Reagent alcohol ~95 ml
- Deionized water ~ 5 ml

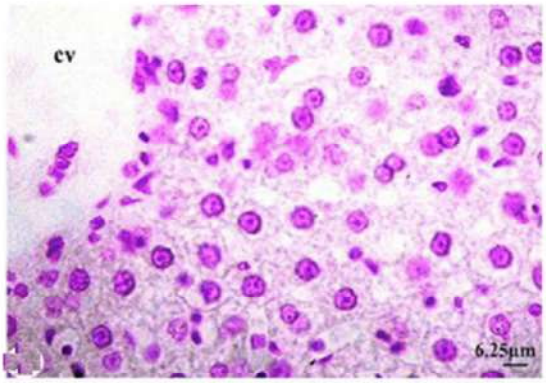
Procedure

1. Deparaffinize the tissue section thoroughly in trhee changes of xylene, 3 minutes each.

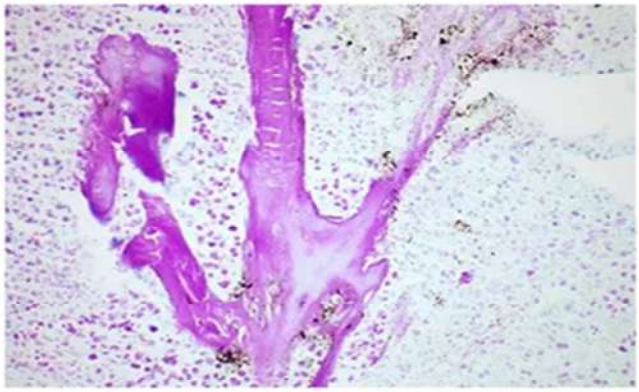
2. Hydrate the tissue section through two changes each of 100% and 95% ethyl alcohols, 10 dips each and wash well with distilled water.
3. Hydrolyze the sections in hydrochloric acid working solution at 60°C for 10 minute.
4. Place slides directly in Schiff's reagent solution for 45 minutes.
5. Wash in running tap water for 5 minutes and rinse in distilled water.
6. Counterstain in Light Green SF yellowish stain for 1 minute.
7. Wash in distilled water.
8. Dehydrate in two changes each of graded alcohol from 70%, 95% and 100% ethyl alcohol.
9. Clear in xylene with three to four changes, 10 dips each.
10. Mount with DPX.

Result

Deoxyribonucleic acid (DNA) in the tissue section appears to be red – purple and nuclei also appear to be red – purple in colour.



Section in the liver stained with Feulgen' reaction.
Comunicata Scientiae 3(3): 162-180, 2012



Small cell lung carcinoma stained with Feulgen' reaction.
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