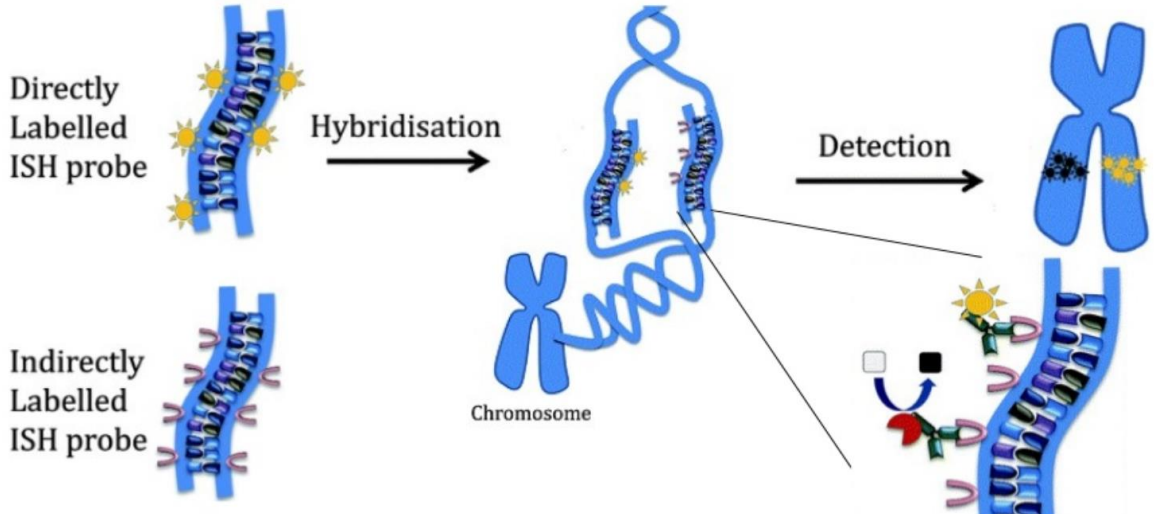
2.7. **Molecular mapping – ISH, FISH (brief idea)**

In 1953, James Watson and Francis Crick described the extensive network of hydrogen bonds that hold together the two antiparallel strands in the DNA double helix (Watson & Crick, 1953). Today, even schoolchildren know that adenine on one DNA strand binds to thymine on the complementary DNA strand, and that cytosine likewise binds to guanine. Because of the many hydrogen bonds formed between these bases, the double helix is a remarkably stable structure. Moreover, if the hydrogen bonds that hold the helix together are broken with heat or chemicals, the helix is able to re-form when conditions become more favourable. This ability of the DNA helix to re-form, or renature, provides the basis for molecular hybridization.

In molecular hybridization, **a labelled DNA or RNA sequence is used as a probe** to identify or quantify the naturally occurring counterpart of the sequence in a biological sample. In the 1960s, researchers Joseph Gall and Mary Lou Pardue realized that molecular hybridization could be used to **identify the position of DNA sequences in situ** (i.e., in their natural positions within a chromosome). In fact, in 1969, the two scientists published a landmark paper demonstrating that radioactive copies of a ribosomal DNA sequence could be used to detect complementary DNA sequences in the nucleus of a frog egg. Since those original observations, many refinements have increased the versatility and sensitivity of the procedure to the extent that in situ hybridization is now considered an essential tool in cytogenetics.

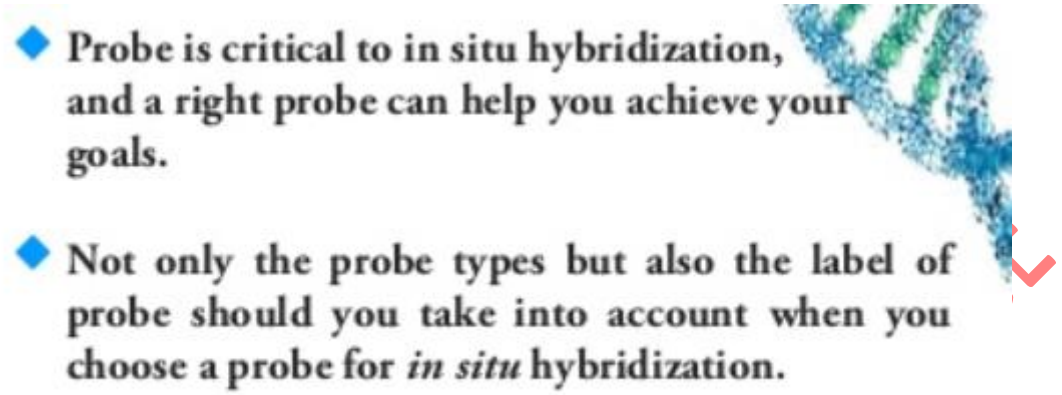
In Situ Hybridization (ISH)

Is a technique that allows for precise localization of **a specific segment of nucleic acid within a histologic section**. The underlying basis of ISH is that nucleic acids, if preserved adequately within a histologic specimen, can be detected through the application of a complementary strand of nucleic acid to which a reporter molecule is attached.

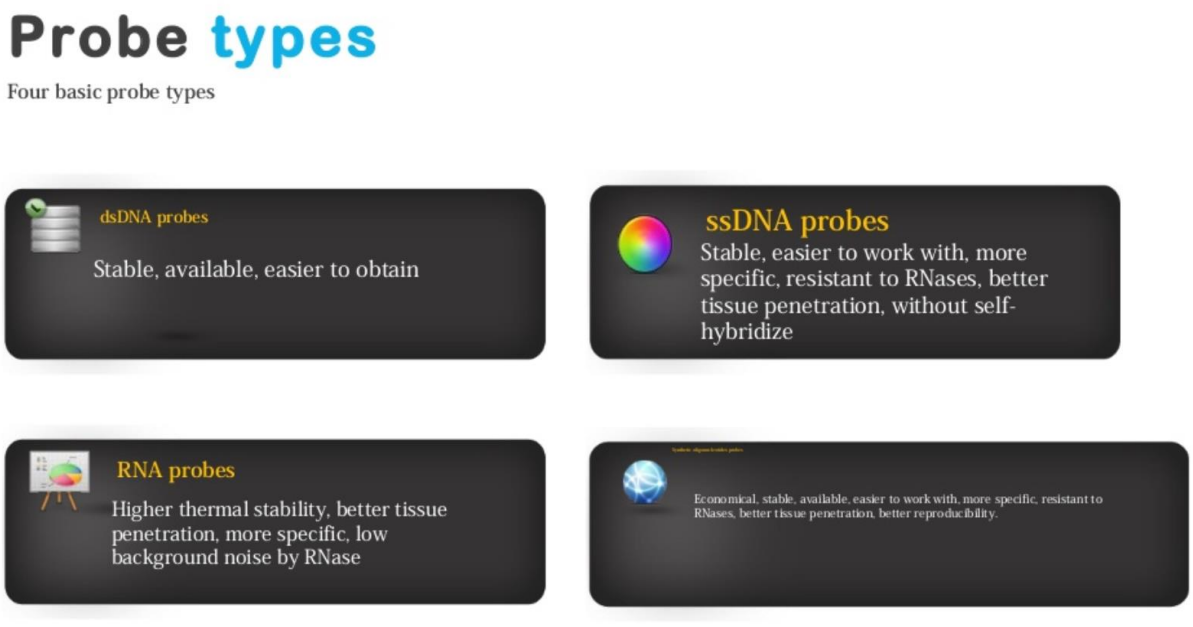


ISH works by using a tagging cDNA or probe that will hybridize with the target DNA sequence

The principle behind in situ hybridization is the specific annealing of a labelled nucleic acid probe to its complementary sequences in fixed tissue or cells, followed by visualization of the hybridization with radioactive decay, fluorescent signals, or enzyme histochemistry.



probe. (Science: **molecular biology**) general term for a piece of dNA or rNA corresponding to a gene or sequence of interest, that has been labelled either radioactively or with some other detectable **molecule**, such as biotin, digoxygenin or fluorescein.



Labeling Probe

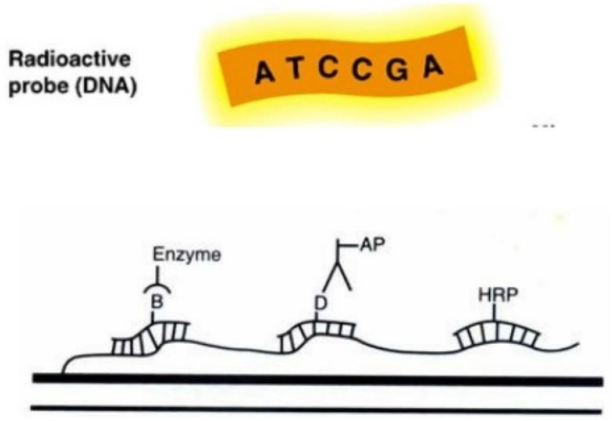
Labeling techniques

Radioactive isotopes

- ^{32}P
- ^{35}S
- ^{3}H

Non-radioactive labels

- biotin
- digoxigenin
- fluorescent dye (FISH)



A crucial aspect of the procedure is that the target nucleic acid be preserved in situ and be accessible for hybridization to the probe Unlike the hybridization of nucleic acids in solution to target sequences on membrane filters, the target in this case is crosslinked and embedded m a complex matrix that hinders access of the probe and decreases stability of the hybrids There are a number of different techniques in in situ hybridization, each dependent on the application of the technique, the type of probe and target nucleic acid to be used, and the type of visualization system available to display the hybridization signals For example, the probe could be double-stranded DNA, cDNA, single-stranded DNA oligonucleotides, or RNA; the target could be metaphase chromosomes, interphase cells, chromatin fibres, or tissue sections, the hybridization could be displayed by radioactive decay signals or by fluorescent dyes.

The Steps involved in-situ hybridization:

1. Direct or indirect labeling of probe.
2. Blocking DNA fragmentation
3. Preparation of slide.
4. Denaturation of Probe and blocking DNA in a hybridization mixture.
5. Addition of the probe and the blocking DNA with the hybridization mixture.
6. Chromosome DNA denaturation.
7. Hybridization of blocking DNA and probe in the target sequence of the chromosome.
8. Detection of the probe in the chromosome of one parent.
9. Chromosome DNA molecule of the second parent related to the unlabeled blocking DNA.
10. Visualization of hybridization signals in a fluorescence microscope. Unlabelled chromosomes are visualized with a counter-stain (blue).

***In situ* Hybridization (ISH) is a method that allows to localize and detect nucleic acid sequences within structurally intact cells or morphologically preserved tissues sections.**

Fluorescence *in situ* hybridization (FISH) is a kind of ISH which uses fluorescent probes binding parts of the chromosome to show a high degree of sequence complementarity.

The problems associated with radioactive probes (health hazard), nonradioactive in situ hybridization 1s now the preferred method. In situ hybridization, now free of radioactivity, it involved the stable labelling of the probe nucleic acid (with no theoretical shelf-half-life), by fluorescent- or enzyme-immunochemistry for hybrid molecule detection, and fluorescent or light microscopy. In situ hybridization is became a safer and faster technique with higher resolution. fluorescent labels quickly replaced radioactive labels in hybridization probes because of their greater safety, stability, and ease of detection (Rudkin & Stollar, 1977). In fact, most current in situ hybridization is done using FISH procedures (Trask, 2002; Speicher & Carter, 2005). Detecting a DNA sequence can be compared to looking for a needle in a haystack, with the needle being the DNA sequence of interest and the haystack being a set of chromosomes. This search is made much easier if the investigator has a powerful "magnet"—in this case, **a fluorescent copy of the DNA sequence of interest**. Hybridization occurs when the "magnet" meets the

"needle"; this requires both a probe and a target. In the **Figure -1**, The probe sequence, often a piece of cloned DNA, is shown in red. The target DNA—chromosomes on a glass slide—is shown in blue (in the right column). Hydrogen bonds that join the two strands of the DNA helix are represented by black lines.

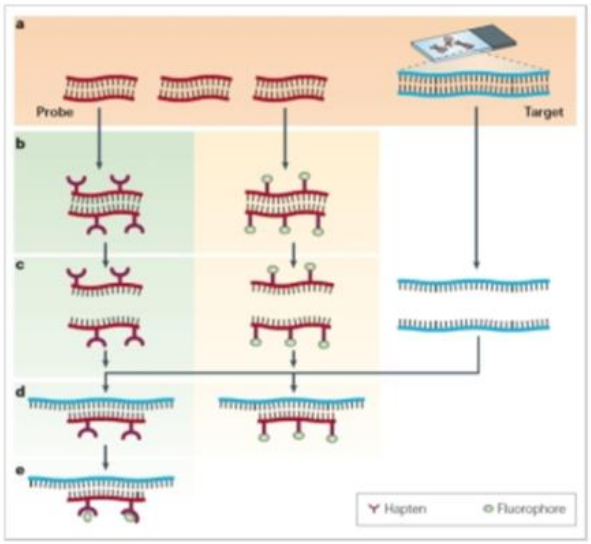
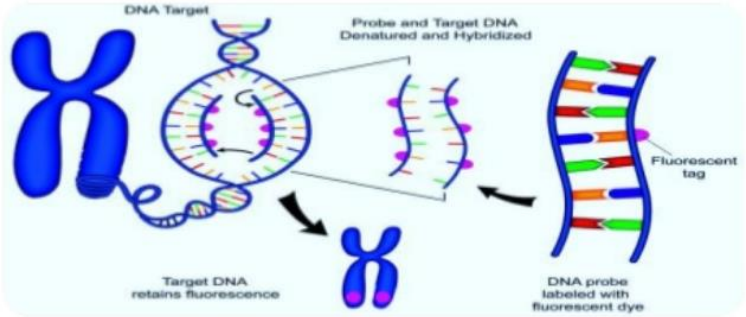


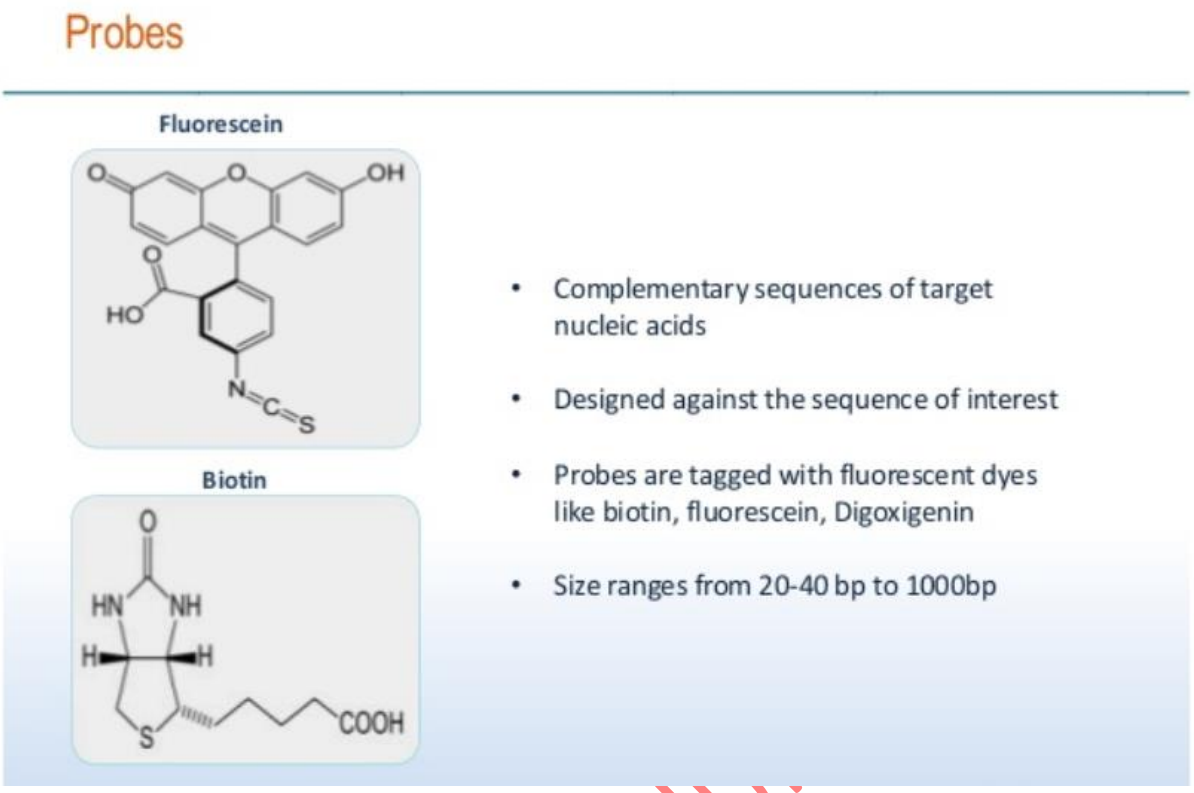
Figure 1: Principles of fluorescence *in situ* hybridization (FISH).

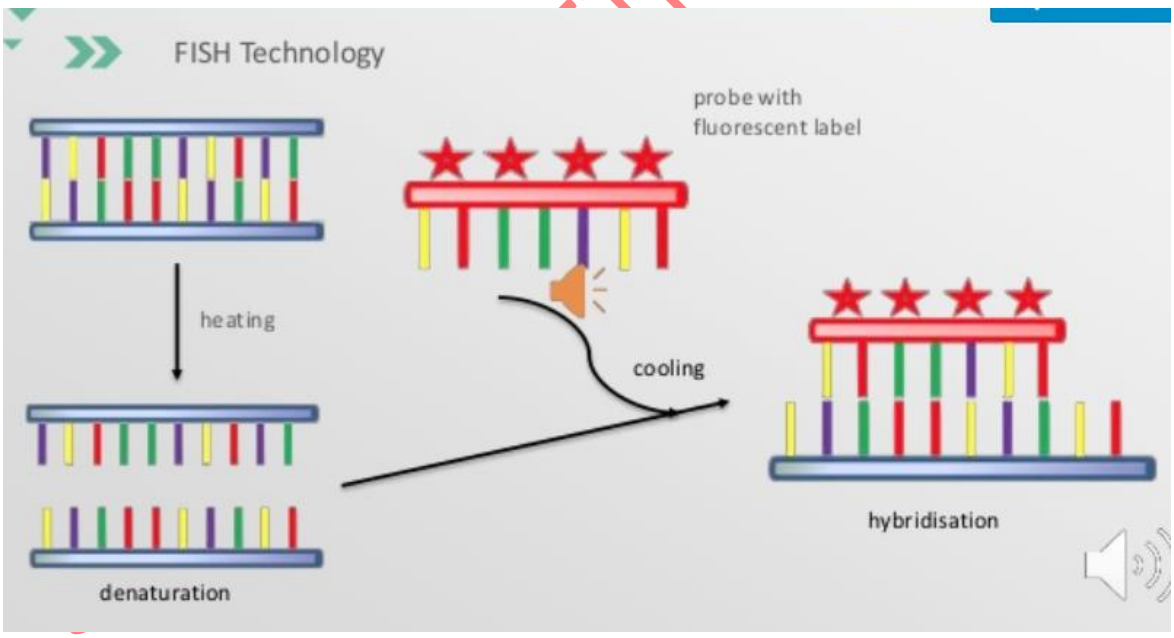
(a) The basic elements of FISH are a DNA probe and a target sequence. (b) Before hybridization, the DNA probe is labeled by various means, such as nick translation, random primed labeling, and PCR. Two labeling strategies are commonly used: indirect labeling (left panel) and direct labeling (right panel). For indirect labeling, probes are labeled with modified nucleotides that contain a hapten, whereas direct labeling uses nucleotides that have been directly modified to contain a fluorophore. (c) The labeled probe and the target DNA are denatured. (d) Combining the denatured probe and target allows the annealing of

FISH technique



Schematic representation of FISH technique. A DNA probe is tagged with a fluorescent marker. The probe and target DNA are denatured, and the probe is allowed to hybridize with the target. The fluorescent tag is then detected with a fluorescent microscope.





FISH has a major application for several diagnostic applications: identification of numerical and structural abnormalities, characterization of marker chromosomes, monitoring the effects of therapy, detection of minimal residual disease, tracking the origin of cells after bone marrow transplantation, identification of regions of deletion or amplification, detection of chromosome abnormalities in non-dividing or terminally

differentiated cells, determination of lineage involvement of clonal cells, the examination of the karyotypic pattern of non-dividing or interphase cells etc. Moreover it has many applications in research: identification of non-random chromosome rearrangements, identification of translocation molecular breakpoint, identification of commonly deleted regions, gene mapping, characterization of somatic cells hybrids, identification of amplified genes, study the mechanism of rearrangements. FISH is also used to compare the genomes of two biological species to deduce evolutionary relationships.

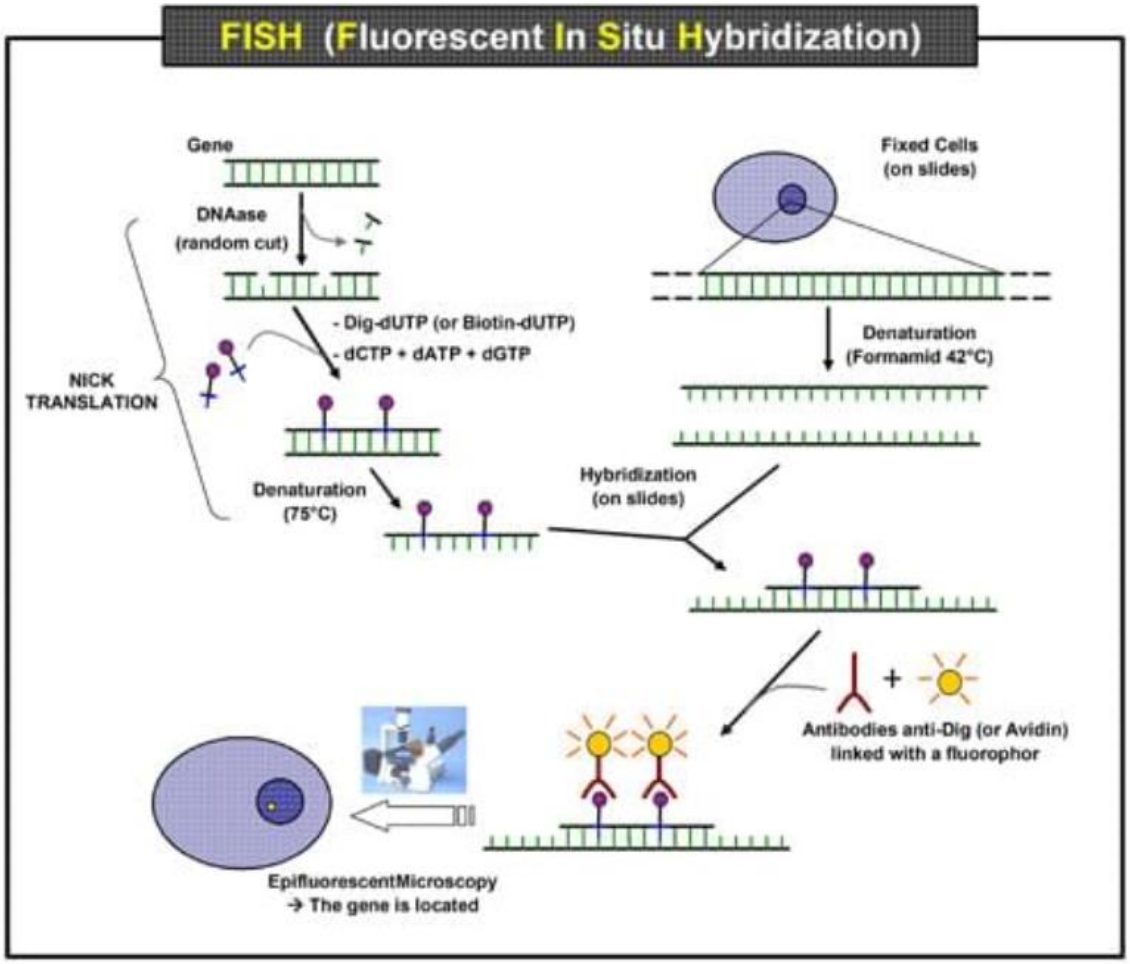


Fig. 1 Scheme of the principle of the FISH experiment to localize a gene in the nucleus.

7.1. **One Gene–one polypeptide concept**

The "**one gene, one enzyme**" hypothesis dates back to the early 1940s when researchers George Beadle and Edward Tatum showed "that genes act by regulating definite chemical events". They used an organism (the fungus *Neurospora*) with simple nutritional requirements, whose genetics could be analysed, X-irradiated it and selected for mutants with novel requirements (*Neurospora* could grow on a minimal medium composed of a sugar, salts, and the one vitamin, biotin and with x-rays radiation to attempt to produce *Neurospora* mutants that had lost the ability to grow on their minimal medium.). Their method, which also works for studies of behaviour and development, show that a mutation in a given gene affected one enzyme in a biosynthetic pathway. They concluded that genes specify the structure of enzymes, and **one gene codes for the structure of one enzyme.**

This was soon found to be oversimplified since many enzymes are made up of multiple polypeptide chains (or subunits), so the hypothesis was modified to "**one gene, one polypeptide**" hypothesis.

The contribution of the Beadle–Tatum experiment was twofold. It provided a methodology that connected biochemistry and genetics, and it revealed a possibly simple relationship between genes and biochemical characteristics (traits).

In reality, this too is oversimplified because some enzymes are composed of RNA as well as protein. Also, because of alternative splicing (which was discovered in the 1970s), multiple proteins can be encoded by the same gene. Another thing to keep in mind is not all proteins are enzymes, so there are plenty of genes that encode proteins that may not have enzymatic activity but are still important for cellular functions.