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Studying Gene Expression and Function

PRESENTATION

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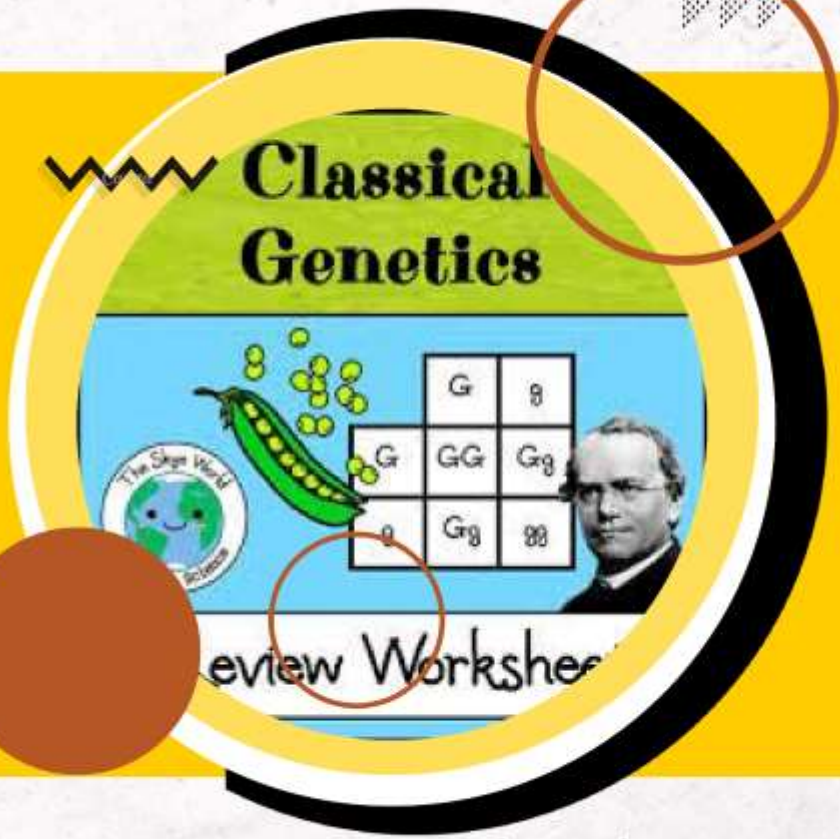
One direct way to discover what it does then is to look at what happens to an organism when that gene is not present

Mutations interrupt the cell's procedures because mutations hold the key to the informational function of genes

With the present time, the discovery of gene function regularly begins with DNA ancestry with hidden gene initiatives. Identifying cellular pathways that have been disrupted or compromised by such mutations regularly affects a window on the biological function of the gene.

In this phase we describe several unique methods for determining the characteristic of a gene, whether one starts with a DNA sequence or from an organism with a phenotype.
Where we begin with the classical approach to the study of genes and genetic characteristics, then this research begins with genetic screens that separate samples of interest, and then proceeds towards identifying the gene or genes responsible for the phenotype.

The Classical Approach Begins with Random Mutagenesis



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The method of chemical or radiological mutagenesis is called insertional mutagenesis, as it relies on the fact that randomly inserted exogenous DNA can produce mutations if a specific part in a gene or its regulatory sequence is cut off. The inserted DNA of known sequence serves as a molecular marker that assists in subsequent identification and in the cloning of the disruptor.

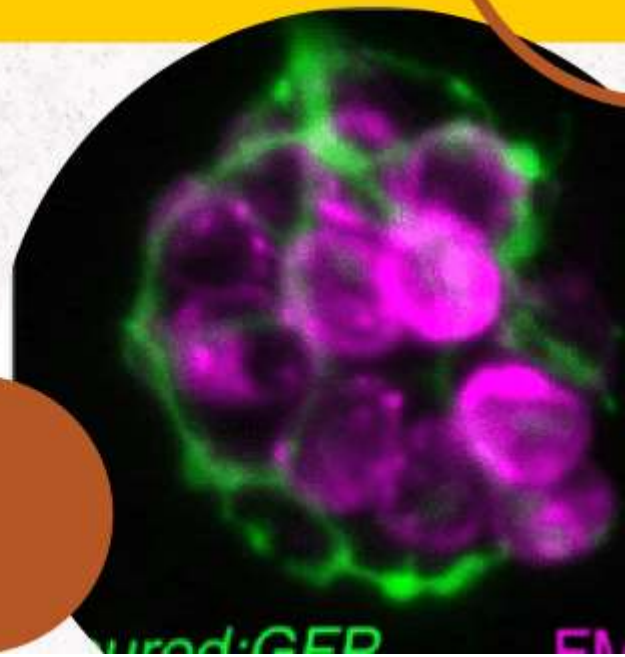
-As in the fruit fly, where the use of the transmissible P element to disrupt genes led to a revolution in the appearance of the genetic trait in it.
-Transposable elements have also been used to create mutations in bacteria and yeast, and retroviruses (which clone themselves into the host's genome) have been used to disrupt genes in zebrafish and mice.
-The mutation in the unpaired gene encoding a regulatory protein causes the development of leaf buds instead of flowers, as in flowering bulbs of Arabidopsis.

Unlike previous organisms, humans do not reproduce rapidly and are not treated with mutations. Any human being with a serious defect in a basic process (such as DNA replication) will die long before birth.

-But how do we study human genes?
That is through **two ways**:
-**First**, because genes and their functions have been conserved throughout evolution, studying less complex model organisms reveals important information about genes and similar processes in humans.
-**Second**, the corresponding human genes can then be studied in cultured human cells. Analyzes of the phenotypes of affected individuals as well as their culture cells have provided many unique insights into important human cell functions.

Genetic Screens Identify Mutants Deficient in Cellular Processes

-Once a set of mutations has been produced in a model organism such as yeast or flies, thousands of individuals must be screened to find the altered phenotype.
-Given that obtaining a mutation in the gene of interest depends on the possibility of its disruption or mutation in another way during random mutations, the greater the size of the genome, the less likely it is that any particular gene will be mutated. Therefore, the more complex the organism, the more mutations must be examined.
-The phenotype examined can be simple or complex.



For example, a screen was designed specifically to search for genes involved in visual processing in zebrafish, where one mutation was discovered in this screen called lakritz, which is missing 80% of the retinal ganglion cells that help transmit visual signals from the eye to the brain. Since the cellular organization of the zebrafish retina is similar to that of vertebrates, its study provides insights into visual processing in humans.

The gene in the protein product can be inactivated by increasing or decreasing the temperature as it works at a medium temperature As in temperature sensitive bacterial or yeast mutants, these mutants are subsequently **used to** :

-identify and characterize the corresponding DNA replication proteins.

-It also led to the identification of several proteins involved in the regulation of the cell cycle .

-and in moving proteins through the secretory pathway in yeast.

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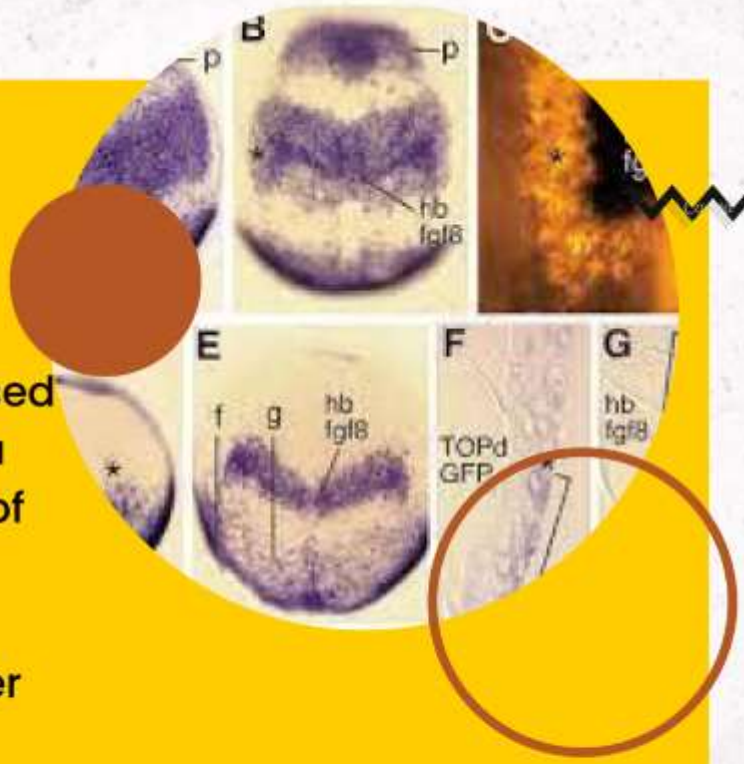
Reporter Genes Reveal When and Where a Gene Is Expressed

Evidence of gene function is obtained by examining where and when a gene is expressed by replacing part of the gene encoding with a reporter gene. The fluorescence and activity of its protein product are then monitored.

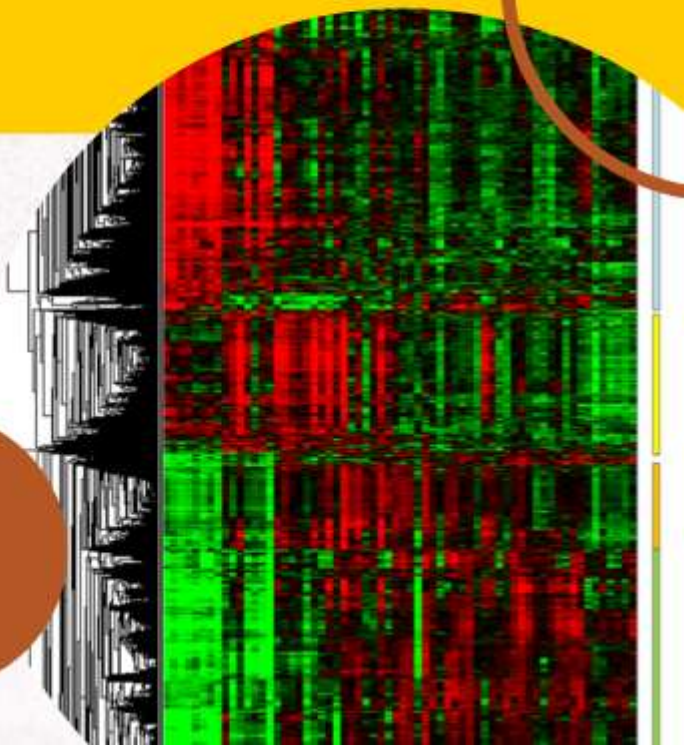
The level, timing and cell specificity of reporter protein production reflects the action of regulatory sequences belonging to the original gene



Microarrays Monitor the Expression of Thousands of Genes at Once

DNA microarrays, developed in the 1990s, revolutionized the way gene expression is now analyzed by allowing the RNA products of thousands of genes to be monitored simultaneously.

Denseer arrays may contain tens of thousands of these fragments in an area smaller than a postage stamp, allowing for thousands of hybridization reactions in which some microarrays are created from large fragments of DNA that were **generated by PCR** and then spotted on slides by a robot.



- First, mRNA is extracted from the cells under study and converted into cDNA, then the microarray is incubated and allowed to cross, then the matrix is washed to remove the cDNA. The positions of the array are then matched to the specific gene detected at that location.

The fluorescent DNA from the experimental samples is mixed with a reference sample of cDNA.

Thus, if the amount of RNA expressed in cells increases from a certain time in relation to the reference sample, the spot is red. If gene expression decreases, the spot is green.

-With such an internal reference, gene expression profiles can be accurately tabulated.

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DNA microarrays have been used to examine everything from the expression of a strawberry gene change to the expression of human cancer cells.

Half of these genes have no known function but comprehensive studies of gene expression also provide an additional layer of information useful for predicting gene function.

Using the method of **cluster analysis** where groups of coordinately regulated genes can be identified where the function of an unknown gene is described by its clustering with known genes that share its behavior where cluster analyzes were used to analyze gene expression profiles that underlie many interesting biological processes, including **wound healing in humans**.

- If the starting point is a protein, the time encoding it or its nucleotide sequence is determined, after that the genetic sequence can be changed in the laboratory to create a mutant copy, then this designer gene is transferred into the cell where it merges with the chromosome to become a permanent part of the cell.

-All descendants cells will then contain this mutagen gene, **for example**:
the fertilized egg can obtain multicellular organisms that contain this mutant gene (even humans can now transform in this way), but despite this, no remedial measures have been taken so far for fear of Other approaches to discovering gene function have also been discussed earlier, including searching for homologous genes in other organisms and determining when and where a gene is expressed.

All of these approaches can be used either to study single genes or for a large trial

Gene Targeting Makes It Possible to Produce Transgenic Mice That Are Missing Specific Genes

- If a part of the DNA carrying the mutated body is transferred inside a mouse cell, it is inserted into the chromosomes randomly.

but rarely, once out of a thousand times, one of the two copies is replaced from the normal time by homologous recombination. Any specific gene in a mouse cell can be altered or inactivated by direct gene replacement and in the special case that the gene of interest is inactivated, the resulting animal is called a 'knockout' mouse.

After a period of cell proliferation, rare colonies are isolated from cells in which a homologous recombination event most likely caused a gene substitution.

-**In the second step**, individual cells from the selected colony are taken into a micro-pipette and injected into an early mouse embryo. **In the first step**, a modified version of a gene is introduced into cultured embryonic stem cells. Only a few rare embryonic stem cells will have their corresponding normal genes replaced. Mice are bred with transgenes to produce both a male and a female.

The ability to prepare transgenic mice that lack a known normal gene was a major advance, and the technique is now used to dissect the functions of a large number of mammalian genes.

The most popular recombination systems are widely used to engineer gene variants in mice and plants

The target gene is replaced in embryonic stem cells by a fully-functional copy of the gene flanked by a pair of short DNA strands, called lox sites, that a chemical recombination protein recognizes as the transgenic mice that result are normal-looking. They are then mated with transgenic mice that express the gene. Similar recombination systems are used to generate conditional mutants in Drosophila.

Undifferentiated cells can form apical tissue which can then give rise to an entire new plant, including gametes. These can be exploited to generate transgenic plants from cells grown in culture. When a piece of plant tissue is grown in a sterile medium containing nutrients and appropriate growth regulators, many cells are stimulated to proliferate indefinitely in an unregulated manner, resulting in a mass of relatively undifferentiated cells called a callus. If nutrients and growth regulators are carefully manipulated, in many species, a whole new plant can be regenerated.

Just as mutant mice can be derived by genetic manipulation of embryonic stem cells in culture, so transgenic plants can be created from full-potent plant cells transfected with DNA in culture

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Summary

- Within the classical genetic method, random mutagenesis is coupled with screening to become aware of mutants which can be poor in a specific biological system. These mutants are then used to discover and take a look at the genes responsible for that manner. DNA engineering techniques may be used to mutate any gene and to re-insert it right into a cellular's chromosomes so that it turns into a everlasting a part of the genome.
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If the cell used for this gene switch is a fertilized egg or a totipotent plant cell in tradition, transgenic organisms can be produced that explicit the mutant gene and bypass it directly to their progeny.