

Garapin et al. 1978

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We will discuss the topics, methodology, and historical importance of this paper by Garapin, Roskam, Kourilsky, LePennec, Perrin, Gerlinger, Cochet and Chambon (1978) about Intron/Exon Structure [*Electron Microscopy and Restriction Enzyme Mapping Reveal Additional Intervening Sequences in the Chicken Ovalbumin Split Gene*].

This paper came along at a time in history when the genome was thought to be a complete 1:1 copy of the proteins and products of cellular machinery for life. Until this point little to nothing was known about post translational modifications of

proteins, let alone splice variants.

Using the following pages and links as well as some personal interpretation, we will enlighten you about the processes used and discoveries made through this research paper.

Biography

Pierre Chambon born and raised in Mulhouse, France on the 7th of February, 1931. He is now an Honorary professor at the College de France, as well as a honorary professor, at the faculté de médecine at Strasbourg University. He is well known for founding and directing the [IGBMC](#) (Institute for Genetics and Cellular and Molecular Biology) from 1994-2002. This institute is one of the leaders in biochemical research that focuses on High order eukaryotic genomes with sub-focus on control and expression of these genes, protein products and produced biological effects; with the hope that this can apply to the human model. Chambon also was the founder and former director of the Institute Clinique de la Souris (ICS/MCI) in 2002. This Institute functions to facilitate translational and functional genomics research, with close association to INSERM, CNRS and the University of Strasbourg. The ICS is known for its high capacity of generating mutant mice, a large variety of phenotypic analysis of animals and the analysis of therapeutic models using mice.



An Expert in the field of genetics, his extensive work in endocrinology has been focused on the structure and function of nuclear receptors. Chambon has made discoveries that have shaped the world of genetics and endocrinology. He work is so influential that he was won many awards, including: Louisa Gross Horwitz from Columbia University [1999], The March of Dimes Prize in developmental Biology [2003], The Albert Lasker for Basic Medical Research [2004] & the Gairdner Foundation International Award [2010]. Chambon received these awards because ...

Knowledge of the era

Before the time of this paper little was known about the nature of introns and exons. The genetic sequence was assumed to be copied essentially verbatim with some knowledge of short intervening sequences beginning to surface. The splicing system and modifications were either unknown or poorly understood at the time depending on the system. The polymerase chain reaction had not yet been developed (It was developed in 1983 by Kary Mullis) and therefore any DNA replication that occurred at the time was done through molecular cloning by *e. coli* bacteria (as outlined below in the methods). This process was very labour intensive when compared to PCR but had the advantage that each vector only takes up one piece of DNA. Molecular cloning is therefore extensively used in Genomics applications where target specificity is crucial in constructing a genome "scaffold". This molecularly cloned scaffold is then commonly used as a framework to assemble the products of high throughput sequencing.

It is also important to note that at the time the genetic code for the translation of proteins was not yet known and sequencing technology was far behind what it is now. In modern times the gene would be sequenced and the amino acid sequence analyzed to compare sections and determine the areas which were not present in the final code. The technology at the time necessitated a more indirect approach which would be considered by many to be more elegant in design.

At the time of publication, this paper offered concrete and well documented visual evidence of the existence of spliced out structures in a gene. This evidence had not existed until this paper, making it a landmark achievement in the field of molecular biology.

MOLECULAR CLONING

Since the process of PCR (polymerase chain reaction) that is more commonly used this day wasn't invented until 1983,

a similar but different method had to be used to replicate DNA en mass. This method is known as Molecular cloning. The process created in 1972 involves a variety of steps:

1. The DNA from the organism of interest is obtained
2. It is treated with a variety (or just one kind) of restriction enzymes, that slice the DNA into smaller fragments.
3. At the same time a vector DNA is also cut with the same restriction enzyme (eg. Commonly in e.coli host organisms the restriction enzyme EcoRI is used.)
4. Then both host and the DNA of interest are mixed together, and by using a enzyme such as DNA ligase both strands with combine to form recombinant DNA.
5. This recombinant DNA is then transferred into a host organism genome, by various means. In the case of this paper, it was achieved by means of a viral phage, transferring the recombinant DNA into hosts such as e.coli.
6. Host cells that have accepted and contain the recombinant DNA are selected for using a selectable marker such as a gene that gives resistance to a particular antibiotic. By introducing the selected antibiotic into the bacterial colonies, only cell containing recombinant DNA will survive and replicate.
7. Lastly, the clones are screened for and the DNA can be obtained.

In the paper by Chambon et. al. they cloned the λ gtWES. λ c DNA (the chicken ovalbumin DNA) by digesting it with EcoRI, isolating the separate cuts by sucrose gradient sedimentation., and replicating them in a e.coli test strain.

Electron Microscopy

Major Components of this paper

Restriction enzyme mapping

We studied this a lot in 2250. This process involves the digestion of a fragment of DNA with enzymes which cut the DNA strand in a semi-predictable way. These predictable enzymes are then used in groups and their combined effects are analyzed and compared to make a map of the whole fragment with the locations of each enzymes site(s) indicated.

In order to determine fragment sizes the cut fragments are run out on an agarose gel next to a size ladder of fragments of known size. This allows for the size fragments from the digest to be measured and their profile compared to other digests. At the time of this experiment this would be done by digesting purified phage lambda with a restriction enzyme, likely Hae III and comparing the fragments from the known size digest to the unknown one.

Ovalbumin

Generally considered to be a storage protein, Ovalbumin is the main constituent of chicken egg whites. It is very readily available and is therefore quite often used in research applications. It's used most often as a molecular weight marker in proteomics studies.

Eco RI restriction enzyme

Eco RI is a 6 base restriction enzyme which is commonly used in many molecular biology applications. It's a common enzyme in molecular cloning studies as the plasmids designed for these studies incorporate multiple eco R1 sites into their design. Uses the restriction site

5' G^AAATTC 3'

3' CTTAA^G 5'

This restriction enzyme has the advantage of creating the "sticky end" we see that allows it to anneal to strands with a similar end. This is very important in molecular cloning and other hybridization reactions.

Hind III restriction enzyme

The Hind III restriction site is less commonly used then Eco R1 however it serves many of the same functions in its uses in research. It creates a sticky end much like that of ECO RI which is also used to anneal digested strands together

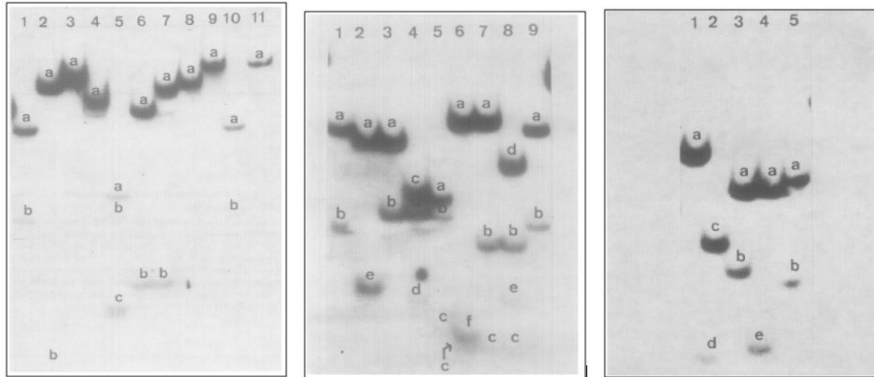
5' AAGCTT 3'

3' TTCGAA 5'

e. coli

Escherichia coli are gram negative rod shaped bacteria which are often called the workhorse of molecular biology. It's a very well studied organism in many respects and was among the first few organisms to have its genome sequenced. Lab grown strains of e. coli are used around the world in thousands of different research applications and it was (and still is) the go to organism for molecular cloning applications in the lab.

GEL MAPS-



When Chambon et al. started this experiment they already had a hunch about existing intervening sequences (what we now today call introns). And they set this experiment to validate that such sequences exist in the chicken ovalbumin gene. To do so they needed to construct a restriction map. The first step in which was to create gel maps. These are maps created by running gel electrophoresis on DNA samples treated with differing restriction enzymes. And comparing them to a "ladder" (restriction enzyme treated DNA of known band sizes). The restriction enzymes chambon et al used where HaeIII, Hinf I, Mbo II and Pst I. They used these enzymes separately as well and in different combinations and then ran them through gel electrophoresis. After which by comparing the various bands created in the ovalbumin DNA by the various restriction enzymes chambon et. al where able to create a restriction map.

Restriction Mapping Figure

Electron Micrography Images Analyzed

CONCLUSION

Just to sum things up, Chambon et. al used the gel maps to create and restriction map; in doing so they managed to find 3 intervening sequences in the 2.35 kb segment of the chicken ovalbumin gene. These sequences are 194±45 bp, 590±90 and 350±50 in length. They were visualized in different clones, using electron micrographs, and each of the four recombinant strands show intervening sequence motifs that fit the description of the lengths given above, thus showing conclusively that introns exist via experimental methods not just theoretical methods.

Modern knowledge

Things have changed greatly in molecular biology since this paper was published.

We now have access to the polymerase chain reaction, which allows much faster and more efficient amplification of desired DNA sequence. Molecular cloning is now a much less utilized technique which is only really used in a select few circumstances

The availability of sequencing technology has made restriction enzyme mapping a much less popular technique as well. The hours and hours of work which went into restriction mapping is now giving way to the ease and precision of sequencing and the following data analysis. This sequence data explosion has led to corresponding growth in bioinformatics, evolutionary biology, and molecular biology among many others.

The knowledge of introns and exons which has come forward between this publication and now is absolutely amazing. We have also raised many more questions about the nature of how a protein goes from mRNA to a finished product. There are over 300 known post-translational modifications to proteins and countless questions of how proteins fold and their interactions.