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Clinical Diagnosis and Cancer Probe: A History of Unity and Mass Migration

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ABSTRACT

Cancer Diagnostic in the Making of PCR

In 1964 the mobility of Spanish biochemists from Europe to the United States resulted in a simple method to estimate the molecular weight of proteins (SDS-Page). Five years before, the idea that modifications of DNA-histones binding stopped RNA synthesis came at the hand of Vincent G. Allfrey in 1959. The discovery showed crucial and its interaction with the notable Spanish research program in US provided a new direction for the design of cancer diagnostic probes. In 1985, Manuel Perucho unified this view with the relevance of the polymerase that synthesizes DNA and RNA, in a method to detect single point mutations in oncogenic genes. It is expected to expose here the history of this useful approach who sought to use histone modifications in cancer clinical practice.

Keywords: Diagnosis - Genetics - Bioanalysis - Care

Introduction

According to Karl Marx, revolutions are the locomotive of history[1]. Scientists throughout history have appreciated this view in which a number of gradual changes are invariably connected with the sudden conception of a novelty. The most spectacular recent example is the polymerase chain reaction (PCR), an emergent scientific fact[2] beyond the domain of study of the Spanish investigative enterprise. Nonetheless, the progress in identifying human cancer genes has been greatly accelerated by molecular genetic methodologies developed by the Spanish biochemists in US[3]. The Spanish group demonstrated its major role as it was the first to characterize the molecular weight of proteins with a method (SDS-PAGE) which has its place in the early historiography of biology[4]. In the context of genetic engineering some narratives have been offered by specialized scientific journals where the author's own research orientation highlights some aspects of electrophoretic instrumentation[5], this is also the case of the Spanish pioneers who developed the technique of polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS)[6,7]. This paper searches to bring into historical attention how crossing the boundary from prokaryote to eukaryote resulted in the top results in cancer diagnostics obtained by a Spanish scientist at the New York University (NYU) School of Medicine (Stony Brook). This scientist was trained by the Spanish group involved in the discovery of the SDS-PAGE technique. He was part of a "mass migration" of biomedical researchers who responded to a call for relevance by obtaining crucial experimental results with eukaryotic organisms inspired by successes obtained with prokaryotes in the two preceding decades[8]. In spite of the relative neglect of this area of research, this is a good example of using historical data to understand the materiality of knowledge production[9]. The anthropological approach is then a focus of this study[10].

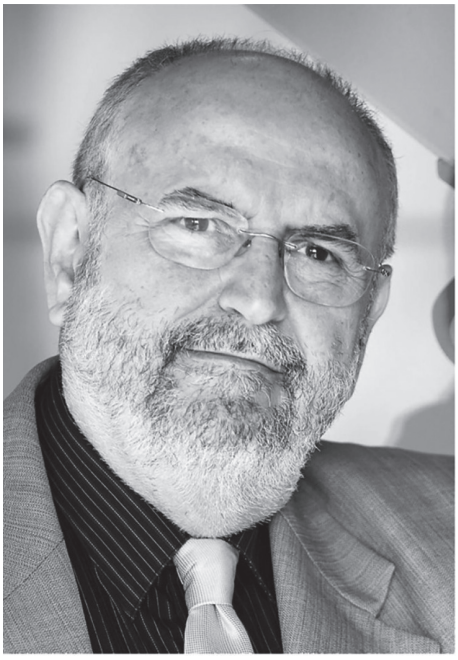

Fig. 1. Manuel Perucho, is an example of a scientist who crossed fields from phage biology into the biochemistry of RNA and then to the DNA world, especially concerned with oncogenic functions.

The history of the methods for diagnostic mutation detection shows the relevance of a simple technique to detect point mutations in viral RNA genomes[11], that uses the RNase (RNA-degrading enzyme) in what is considered a historical moment for mutation de-

tection. Antisense riboprobes (a segment of labelled fragment of RNA used to detect mRNA or DNA targets) are hybridized with the total RNA of patients with possible mutations. Mismatches in the resulting RNA:RNA hybrids are cleaved with the enzyme RNase A and the products are examined electrophoretically. Antisense RNA is simply an RNA strand with a base sequence that is exactly complementary to a particular messenger RNA (the "sense" RNA). But as science does not come easily in Spain[12], there is almost no indication that will lead us to a particular connection between PCR invention by Kary B. Mullis[13] and the RNase A mismatch method obtained by Perucho[14]. The point is obvious that the inclusion of Perucho's contribution[15] in Mullis's monograph on his Nobel prize innovation[16], makes sense of its high value in the context of that revolution in molecular biology. This raises fascinating questions as to why and how a scientist might choose and manage such a 'diagnostic' task, and that because as J. Bangham says, "scholars in the history of biology are increasingly interested in the fact that although 'care' is often overlooked, research could not happen without it"[17].

As a question in history of cancer, the reliable detection of cancer is important to think about how medicine absorbs frustration or failure and how it addresses the success and actually think about what we mean when we use these words[18]. The central place of recombinant DNA technology in the historiography of biotechnology makes PCR a replacement of its conventional version, and an important issue regarding the Spanish participation in cancer diagnostic. As Doogab Yi underlines, "the prevalent historiography of commercialization often takes 'the Mertonian norm of open science' somewhat literally in its analysis of the impact of profit seeking on academic culture"[19]. The Spanish cancer research in the US with its recalcitrant insistence upon the finding of a vaccination for cancer, missed the development efforts on the 1985 cancer diagnostics method associated with PCR and available from Perucho's Lab. As way of consequence in 1991 Roche acquired the rights to the PCR from Cetus, a Californian biotech company[20], and from then on investments in diagnostic research went into tests for its own system.

The plan for the article is as follows. Section 2 sets out some background on Viñuela and Salas phage phi29 model system as a platform to build the Spanish molecular biology school. Section 3 describes how here two different traditions of research come together. We claim that it was a good idea to act in unity of purpose for the good of diagnostic procedures in hospital work. Section 4 turns to the trajectory of Vincent G Allfrey, and illustrates how the biochemical properties of the isolated nucleus have been at the core of both the thought-style and consequences of histone modifications research. This is an important history for a scientific audience, given how the Spanish collaborators of that process may be relevant to developments involving technical advancements and given structural similarities in the pursuit of ras oncogenes. Next (Section 5) this article considers three results obtained by Perucho in Germany as new paths in Allfrey's style, conducive to achieving a new technique for detecting point mutations. In Section 6 we

might see how a tactful approach unifying epigenetic and genetic principles inspired reversibility as a sign of success of this research. In the final part, Section 7, it is suggested that 'point mutations' at the root of cancer are of peculiar historical interest when considered through the inhibition of RNA synthesis by histones biochemical proof.

1. The phage phi29 in the context of a "mass migration"

Originally described by Shapiro, Viñuela and Maizel in 1967[21], the electrophoresis technique in polyacrylamide gels (PAGE) by using SDS detergent can separate and characterize the molecular weight of proteins; this is one of the best cited works in the scientific literature[22]. Eladio Viñuela developed this method in the Severo Ochoa Laboratory at the New York University, as he asked him if he could develop a project on his own. The project consisted of characterizing the proteins induced in E. coli after infection with phage MS2. Ochoa agreed, and Viñuela embarked on this project, for which he developed the SDS-PAGE technique for the separation of proteins according to their molecular weight[23]. Nowadays, this most popular electrophoretic technique uses SDS (sodium dodecyl sulphate), because numerous of its molecules are absorbed on each protein molecule forcing them to behave just like nucleic acids[24]. In these macromolecules, the charge-to-mass ratio becomes nearly constant above ca. 400 bp in length. This limiting mobility of the SDS complex has been found to be a linear, decreasing function of the logarithm of the molecular weight of proteins[25]

Fig. 2. Eladio Viñuela and Margarita Salas worked for three years (1963-66) with Ochoa, and built a school of molecular biologists at their return in the home country.

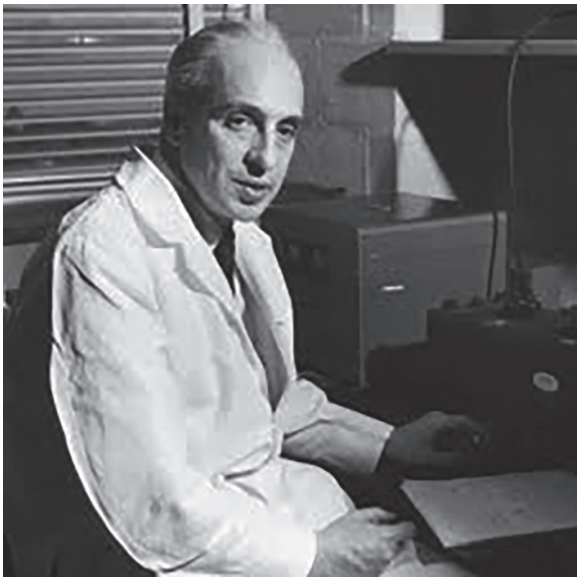

Fig. 3. Severo Ochoa threw himself into the genetic code race on the basis of his experience with polymer synthesis.

A valuable point of connection to this literature is the model system, the phage phi29, with which Viñuela and Salas worked in the Severo Ochoa's laboratory at the New York University Department of Biochemistry[26]. It was both the easiest model for the study of viral structure, and it was the "in-house" system that Salas was working on for several years. Otherwise, in the light of the use of electron microscopy for the structural study of viral molecular biology, the process of scientific migration between the US and Spain was a gradual, a non-revolutionary development. For the use of these techniques, Viñuela designed a strategy that led to cover the issue in a plan that was included at the launch of the new Center for Molecular Biology at the Autonomous University of Madrid. Viñuela and Salas chose a simple model system, the phage phi29, and with a titanic effort built up an impressive school of molecular biologists in Spain[27]. Working under the supervision of José Salas, Perucho defended his Ph.D. Thesis and went to Germany for a two-year postdoctoral training at the Max-Planck-Institut für Molekulare Genetik in Berlin, to aid in the isolation of eukaryotic genes and in studies on chromatin organization. Shortly after, in 1981, he moved to Cold Spring Harbor Laboratory (US), and joined the faculty of the New York State University (Stony Brook) in 1982. Between 1993 and 2007, he held posts at the California Institute of Biological Research and the Burnham Institute for Medical Research (La Jolla). He latter assumed the direction of the Institute of Predictive and Personalized Medicine of Cancer (IMPPC) in 2007.

Manuel Perucho was trained as a member of Eladio Viñuela's school at the Department of Molecular Biology in the Spanish Research Council (CSIC), between 1971 and 1977. Viñuela and Margarita Salas brought to Spain the teachings of Delbrück's school of microbiology, Delbrück's phage school, together with their background in biochemistry acquired at Ochoa's NYU lab between 1963 and 1966. The very first approach to the phenomena of point mutation was given by Delbrück during Spring 1944, at the Vanderbilt University School of Medicine, in a lecture series titled "Problems of Modern Biology in Relation to Atomic Physics". In his eighth lecture, "Radiation Effects", Delbrück turned to the inactivation of viruses by x-rays; he suggested that the virus particles are 'killed', and that the killing is a single hit effect. He noted that in some cases it was possible that "ionization did not 'kill' the gene, but altered it so that it now reproduces in this altered structure. Such 'point mutations' should also be inducible in viruses"[28].

In the 1970s, as a response to the absence of medical progress an intellectual and political crisis in molecular biology had a major impact on the trajectory of the researchers in this area. So for example, until 1977, gene transfer methods were only successful when low complexity DNA was used. Subsequent improvements made transfer possible using total DNA from vertebrates and mammals, what also opened the way to the construction of viral strains with medical relevance. As a crucial answer to the call for medical relevance, Manuel Perucho was part of a "mass migration" of biomedical

researchers who have studied simple organisms like bacteria and bacterial viruses to eukaryotic organisms, such as animal viruses and human cells[29,30]. His discovery of the RNase A mismatch cleavage method is a potential topic where the participation of historians is required by scientists, because of the "obstacles"[31] raised in the face of its realization as a practical utility.

2. At the cross of two different research traditions

In 1978, Perucho left Spain for a two years' postdoctoral period in the Max Planck Institute of Molecular Genetics in Berlin. There, he researched H5 the main histone synthesized by the peripheral red blood cells and discovered that tissue-specific histone H5 transcript is polyadelynated[32]; polyadenylation is a mechanism for modifying mRNA function in eukaryotes and their viruses[33]. And histones, a suppressor of chromosomal RNA synthesis, are found in eukaryotic cell nuclei. From a historian perspective, the importance of this technical event outstands the early hope of the use of antisense RNA for potential therapeutic applications (through the modification of oligonucleotides).

Now studying eukaryotic organisms, the molecular biologist had found with histone H5 the unexpected hybridizations of two experimental systems: those of gene transfer and oncogenes. He had worked for a while with Michael Wigler on the ras gene, an oncogene, in a major development of genetic approach to cancer research; on Allfrey's work on the synthesis of H5; and on some of the diagnostics technology. It is worthwhile to note that histone H5 gene is unlinked to other histone genes and is found only once per genome. Thus, when Perucho succeeded in 1985 in "the characterization of mutations ... achieved with the RNase A mismatch cleavage method"[34], the definition of the exact single-base change in genes as a result of mutation was proved to be an important goal in his genetic research. As NCI's Section Chief (1983-88 Developmental Oncology) Mariano Barbacid said that the "method provides useful information regarding the levels of expression of ras oncogenes in ... tumors", while PCR "makes possible the routine use of oligonucleotide probes to identify ras oncogenes in clinical laboratories"[35]. Perucho's mutation detection by the RNAase A mismatch cleavage method, was fast and able to identify most base substitutions. It provided a means to detect point mutations in clinical specimens that was more sensitive than Northern analysis (a prior to PCR quantitation specific nucleic acid sequences from eukaryotic cells technique) and was highly specific for a single mutation or a small set of mutations[36]. Potentially beneficial to a collaborative research culture, by 1991 Kary Mullis gave as its own inventor an introduction to PCR in a course that took place in Spain[37]. This course followed some of the gene transfer and oncogene pioneers (Mike Wigler, Angel Pellicer, Jim Feramisco and Frank McCormick) previous venue to Spain in 1990, also invited by Perucho to give a course. Mullis' later

detour from science proved decisive for the scarce development possibilities available to the genetic characterization by RNase A mismatch method.

The inhibition of RNA synthesis by histones biochemical proof was a particular contribution by Allfrey and Mirsky, following their seminal study on the biochemical properties of the isolated nucleus[38]. The findings introduced the possibility that subtler mechanisms may exist which permit both inhibition and reactivation of RNA production at different loci along the chromosome. And when models for formation of mammalian RNA-containing sarcoma virus involving the transduction of oncogenic information were considered, a link was established between the detection of single base substitutions in eukaryotic genes (a method available from 1985[39]) and the findings from Allfrey and Mirsky (in 1958) that low molecular weight compounds are able to substitute for DNA in facilitating amino acid incorporation.

The isolated cell nuclei have a special interest because they ultimately bear on the function and mode of action of the gene. It is known that the role of polynucleotides fall within the realm of Allfrey and Mirsky interesting work on cell nucleus[40]. These authors demonstrated that the biosynthesis of nuclear proteins, as much as ATP and nucleotides formation, can be inhibited by means of deoxyribonuclease through depolymerizing desoxyribonucleic acid found at the nucleus. The activity of those systems therefore appeared to require the presence of a polyacid matrix with a relative lack of specificity. In fact, a specific protein enzyme can promote the syntheses of many if not all nucleic acids, as was shown by Ochoa[41]. The enzyme discovered by Ochoa and Grunberg-Manago is a polynucleotide phosphorylase (PNPase), which they described for the first time in 1955[42].

As a matter of fact the combination of different fields (in this case, gene transfer and oncogene), at the level of research, takes the form of important factors of unification with a specific connection. These sorts of interactions between the research programs described in this contribution, those of Severo Ochoa[43] and Vincent G Allfrey, are at play to help understand the diagnostic probe invented at Manuel Perucho's laboratory to screen for mutant ras genes and detect single point mutations in mammalian genes. The analysis noticed the rare signs of acknowledgment about this technique, progressively diluted between PCR instrumentations.

3. Histone modification, epigenetic change and mutation

In the 1980s it became possible to combine genetic engineering and analysis with cell culture to study the effect of inserted genetic material or of mutations and deletions in genome[44]. By then, molecular oncologists considered the best studied epigenetic marks (ie, those inheritance patterns that do not depend on the naked nucleotide sequence) were DNA methylation and histone modifications; they were essential to the cell survival. Indeed, cancer model systems and instruments such as PCR emerged as

urgent topics for historical investigation by that time[45]. In this sense, to explain the origin and the significance of the modification of nuclear histones, it would be necessary to return to the itinerary of Allfrey as main protagonist of the interest brought to the protein synthesis in the cell nucleus in the 1950s[46].

In conjunction with Allfrey's discussions on nuclear ATP synthesis and its relation to protein synthesis, the question aroused as to demonstrate that one role of the basic proteins (histones) in the organization of the chromosome was to reduce or control chromosome function. Allfrey's hypothesis was put into question by Chiu and Hnilica[47]. They stated that, it was a general conclusion that histones were not involved in regulating the activity of specific genes. As it is possible that the enzymes that epigenetically modify DNA and histone are themselves targets of genetic disruption, the experiment was performed to measure pieces of evidence indicating mutations in these genes. To provide an insight into the biological consequences of histone composition changes, the pursuit of ways to engage in the search to detect the occurrence of point mutations in ras oncogenes demonstrated effective results. Allfrey was correct when he surmised that histones were involved in controlling the dynamics of information transfer from DNA to RNA[48]. As a fact he was an exponent of those who continued to consider the nucleus a site of protein synthesis throughout the 1950s[49]. Allfrey deemed the protein synthesis in the nucleus and in the cytoplasm of a different nature[50], and described for the first-time translation in the nucleus reporting a rapid incorporation of radioactive amino acids into nuclear proteins[51] and in 1964 he discovered histone acetylation in vivo. He extensively worked with Spanish collaborators like Miguel Beato and Adolfo Ruiz-Carrillo in important technical developments (e.g. by searching for a strategy to define the size of a precursor of mRNA molecules[52]). Ruiz-Carrillo was well known and highly respected in France, where he worked at the Institut Pasteur in the days when Josep Maria Sala i Trepat, a biochemist who left Spain to be involved in cancer immunology, carried out his researches at the enzymology laboratory of the CNRS in Gif-sur-Yvette. But in 1976, he moved to Germany where another Spaniard was established at the Institut für Physiologische Chemie in Marburg, Miguel Beato. And he would remain in this country until 1981[53], when he moved to Canada after his fellowship to take an academic position at Laval's University.

4. Mutation detection within a context of epigenetics

With his cultural background as a member of the research school where the SDS-PAGE electrophoretic technique was discovered in 1967, Perucho obtained the means to explore altered messenger RNA levels to detect single base substitutions[54]. The identification of the mRNA target was crucial, because the mRNA target is part of the Ras pathway. mRNA in the context of cancer, tend to produce ten-fold more protein, which in turn may play a role in oncogene activation. While Viñuela, in 1967, developed the SDS-PAGE method in the laboratory of Severo Ochoa at New York

University, Perucho and Winter, in 1985, marked a turning point with their technique for detecting point mutations, in a context of influences from Allfrey. By July 25, 1985, their successful method of RNase A cleavage of mismatching errors was reported by Ochoa to the US National Academy of Sciences.

SDS-PAGE (1967)	**RNAse A cleavage (1985)**
Viñuela developed SDS-PAGE for the separation of proteins according to their molecular weight. Nowadays, this electrophoresis method is the most popular.	Perucho obtained the means to explore levels of messenger RNA alteration to account for the existence of simple base substitutions at the point of a cancer patient's diagnosis.

Fig. 4. Answering to the call for medical relevance, molecular biologists migrated from studying simple organisms (bacillus virus *phi29)* to eukaryotic systems with the focus being in the mutation detection techniques for diagnostic use.

Over the few years Perucho spent in Berlin (1978-79) he worked together with Adolfo Ruiz-Carrillo, who had collaborated on several issues with VG Allfrey. Above all, Allfrey and Ruiz-Carrillo formulated the premise that a block in DNA synthesis might slow the utilization of newly synthesized histone molecules for chromatin assembly. The unresolved problem of overcoming an energy blockage in protein synthesis pushed them to accept previous results putting into question prokaryotic-based views on mRNA[55]. And as there is a tight coupling between histone and DNA synthesis in a variety of eukaryotic cells, the hypothesis had been suggested that histones were an obstacle to RNA polymerase. The identification of that connection contributed to understand how epigenetic deregulation occurs in cancer. The historical fact is that the problem of overcoming an energy blockage in protein synthesis was oriented towards the possibility that cancer can arise as a result of DNA instability without direct evidence of mutation[56].

The exception is histone H5, which continues to be synthesized after the synthesis of the other histones had virtually ceased. Histone H5 tissue specificity and its continued synthesis after the cessation of synthesis of DNA and of the other histones raised particularly interesting questions about the control of its expression. And this was the main result of joint research by Perucho and Ruiz-Carrillo, the purification of H5 messenger RNA[57]. In 79, one year after he arrived at the Max Planck Institute for Molecular Genetics in Berlin, Perucho assumed that histone H5 may be considered as an H1 variant, as it has many homologies in its amino acid sequences. So to study histone H5 mRNA, he proposed a method to prepare un-degraded polysomes on a large scale and in high yield[58].

In line with the vision of the recombinant technology that helped to decipher the intricacies of eukaryotic gene structure, Ruiz-Carrillo used Perucho's results in Germany (H5 mRNA activity determination by translation, the fact that H5 mRNA is polyadenylated and a method to purify H5 mRNA) to generate an exact DNA complement

(cDNA) of the H5 messenger RNA (H5 mRNA), aimed at characterizing the structure of the H5 gene. Otherwise it could be say (as Fleck might have put it[59]) that thanks to this Allfrey's style performed by an expert from his team (Ruiz-Carrillo), Perucho was able to set a system of experiments to establish a method used to detect cancer.

5. A competent collaborator from Cold Harbor Spring to Stony Brook

Fig.5. Edward Winter, a professor of biochemistry at the State University of New York (Stony Brook)

When Edward Winter joined Manuel Perucho's laboratory at the State University of New York, Stony Brook, in 1985, Winter had certainly been in contact with the H5 messenger RNA purification technique, which played for him the role of a research promoting conceptual tool. Perucho main focus was the phenomenon of activation of the oncogenic potential or how the mutant ras allele shifts from the normal to the amplified state[60]. The diagnostic query carried out a search for knowledge needed to localize a mismatch. Perucho selected Winter on the base of his RNA extraction method[61], that had a focus on the diagnostic detection of single point mutations in mammalian genes, in a particular strain of human lung tumor cells, Calu-1, and in colon tumor cell lines. Winter's probe technique was designed to allow hospital technicians to screen for mutant ras genes.

The precise definition of a mutation at a molecular level and the mismatch-driven reasoning, proved fruitful, as far as the reversibility of the malignant transformation was achieved with success[62]. These results were supported by the consideration of an original mutation event as the initial trigger of cancer and the concept that genetic instability (alterations in the form of amplification of mutant ras genes) may be a critical step in the development of a tumor. A unified approach for yielding diagnostic results that involved genetic and epigenetic changes. From Perucho's perspective, this series of two cancer articles specified as a criterion, diagnostic indicators of the presence of oncogenes such as c-K-ras.

Nevertheless, J. Feramisco, Perucho's collaborator, had achieved his investigations on total cellular RNA isolation in 1982[63]. His total high-quality RNA isolation was important, as the successful analysis of RNA depends greatly on the extent to which one can effectively protect it from degradation. In 1983 he had been part of Perucho's team when a third transforming human ras gene was discovered in neuroblastoma and was designated NRAS[64]. But in 1985, as the choice of phenomenon is relative to the scientist's interests, Perucho invited Winter. Winter was also, like him, a professor of biochemistry at the State University of New York at Stony Brook.

Both professors argued the reversible transformation of rat fibroblasts by mutant ras oncogenes, as a sign of success of their research[65]. As a sensitive molecular essay, performed by biochemists, the new method to detect mutations had been used to study human ras expression.

Fig. 6. James Feramisco, at Cold Spring Harbor Laboratory in New York

A surprising aspect of the RNase A mismatch cleavage method is that it may be possible to demonstrate its close relation with the first inklings of the chemical nature and potential synthetic capacities of the nucleus. To paraphrase VG Allfrey, neither the histone neither the ribonucleic acid contents of the nucleus are appreciably diminished by DNase (DNA degrading enzyme) treatment[66]. In certain cases, nevertheless, DNase treatment of the RNA is required to obtain meaningful PCR results. Otherwise, the soluble and readily extractable RNA of the nucleus was also successfully used to achieve the detection of point mutations in total genomic DNA. So the conception of the cleavage by RNases links point mutations to carcinogenesis on the basis of both RNA:RNA[67] and RNA:DNA[68] heteroduplexes.

6. Concluding remarks. Molecular-biology techniques in the diagnosis of monogenic diseases

If a technique that went down to history in 1967, SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), was elaborated under Ochoa's influence, the eighties saw the detection and characterization of oncogenic single point mutations by the RNase A mismatch cleavage method also communicated by Severo Ochoa to the National Academy of Sciences (NAS). The first one pointed to permit easy assessment of protein purity and reasonable measurement of relative molecular mass values of denaturated polypeptide chains, the second was originally developed to detect single base substitutions in transcribed genes as a diagnostic method for cancer. The paper's view is that the case permits the historian to acknowledge the disciplined and even-tempered mood preserved in one and the same person, persisting through consecutive generations of a collective, and producing a real methodology in both cases. At the basis of this diagnostic tool is the material world of Allfrey and Mirsky; both had determined the inhibition of RNA synthesis by histones biochemical proof. A strong thought-style which lies at the root of this cancer probe for point mutation, that made a biochemist from Ochoa's school develop it into a well-established structure. His emphasis on clinical care is what makes this knowledge of a peculiar historical interest, as biomedical research could not happen without it. This mysterious unity of

the right methods, problems and results enable the historian to understand the materiality of knowledge production.

The shifting meaning of gene manipulation in the search for medical relevance involved a mass migration of biochemists' research from prokaryotic systems to eukaryotic systems. Those addressing key questions in molecular biology centered on phage phi29 were able to contribute with a technical framework where to characterize the molecular weight of proteins (SDS-PAGE). This is roughly the framework from which the contributors of a new method that appeared to detect point mutations began. This important method in work on cancer used labeled antisense RNA transcripts, and is relevant for historians because it was carried out in full recognition of its clinical possibilities. The innovative method opened up new applications associated with PCR and enabled the solution of longstanding problems; since the definition of the exact single-base change in genes, as a result of mutation, is an important goal in genetic research.

Bibliography and notes

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