Perspectives

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Molecular Genetics Under an Embryologist's Microscope: JEAN BRACHET, 1909–1988

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JEAN BRACHET, son of the well known Belgian embryologist ALBERT BRACHET, was himself fundamentally an embryologist by training. He remained so his entire life. But he was an unusual embryologist of the day; from the beginning he was convinced that development, and other biological phenomena, would eventually be understood in chemical terms.

BRACHET's entire career illustrated this conviction. His major books and monographs began with *Embryologie Chimique* (1944 and in English translation 1950), continued with *Le Rôle des Acides Nucléiques dans la Vie de la Cellule et de l'Embryon* (1952), *Biochemical Cytology* (1957), *The Biological Role of Ribonucleic Acids* (1960), *Introduction à l'Embryologie Moléculaire* (1974), and *Introduction to Molecular Embryology* (1974), and culminated with his monumental *Molecular Cytology* (1985). These are not the titles of a vitalist.

His fundamental biological interest was embryonic development. He focused this interest experimentally on the role of nucleic acids in protein synthesis, and the partitioning of morphogenetic determination between nucleus and cytoplasm. He thereby approached development "from the bottom up." From today's perspective, 30 years after the messenger-RNA hypothesis and 50 years after his germinal paper (BRACHET 1942), JEAN BRACHET was "the father of RNA."

Let us try to imagine the dark ages before the Golden Age of Molecular Biology. BRACHET (1987) tried to help us: ". . . I had of course read and reread the few pages that books on biochemistry dedicated to nucleic acids. They all stated that there exist two types (which was true), thymus nucleic acid in animals and zymonucleic acid in plants. The first one (DNA) has a mysterious sugar, deoxyribose, and the second one (RNA) a classical pentose, ribose . . . A rash and false generalization had led to the conclusion that DNA was an 'animal' nucleic acid and RNA was 'vegetal' nucleic acid. Early observations had led me to a quite different view. But could a young student, 22 years old, be iconoclastic to the point of breaking the dogma, accepted by all, that plant and animal nucleic acids are distinct? Before doing so, I had the wisdom to consult Joseph Needham, the 'pope' of chemical embryology. My hypothesis appeared so daring to him that he turned to his 'boss,' the Nobel Laureate Sir F. G. Hopkins. The wise advice of 'Hoppy' was: 'Tell that young man that he should not believe everything he sees written in books; they are full of errors. Let him do the experiments to check his hypothesis.'

"Which I did, at the marine station in Roscoff in Brittany, my exams passed! I took with me an apparatus to measure pentoses . . . in straw . . . which prompted ironic remarks from my French friends, Ephrussi, Lwoff, and Monod. Experiments rapidly showed I was on the right track: sea urchin eggs did contain large amounts of pentoses, mostly in the form of RNA.

"Back in Brussels, I rushed into the office of my master, Albert Dalcq, to tell him my nice tale. He listened to me placidly and told me that he would believe the presence of plant nucleic acid in sea urchin eggs only when I could show it under the microscope."

Some of the initial confusion about the nucleic acids persists in BRACHET's early papers. But in 1942 the fog lifts. In a long paper in French, BRACHET provides us with: (1) a method that permits clear distinction between DNA and RNA on histological sections; (2) by application of this method to a wide variety of tissues and organisms, proof that both RNA and DNA are constituents of animal cells, yeasts, and protists; (3) a clear description of the localization of RNA and

DNA within the cell; and (4) two clear suggestions of the respective roles of the two nucleic acids, thymonucleic acid (DNA) as the genetic material and pentosenucleic acid (RNA) as an essential actor in protein synthesis.

How did BRACHET reach this clarification? In his words (1987), "After several not very encouraging attempts, I found in 1939 a simple cytochemical technique for detecting RNA in cells. To my great joy, I found RNA, like DNA, to be a universal constituent of cells–bacterial, vegetal, and animal. The intracellular localization of these two types of nucleic acid is, however, quite different: whereas DNA is found in chromatin and chromosomes, RNA accumulates in cytoplasm and nucleoli. In addition, whereas the amount of DNA per nucleus remains constant in a particular species (allowing for its doubling when cells prepare for division), the amount of RNA varies considerably from one tissue to another; I saw a completely unexpected correlation between the quantity of RNA in a cell and its capacity to synthesize proteins. This led me to another iconoclastic proposition: proteins are not synthesized by proteolytic enzymes operating backwards, as was generally thought, but by an unknown mechanism implicating RNA. The same conclusion was arrived at simultaneously (1941) by T. Caspersson in Stockholm, who was using a completely different technique for the cytochemical detection of nucleic acids."

What was that "simple cytochemical technique"? At the time, RNAse was one of the few enzymes that had been purified and crystallized, by KUNITZ. A fortunate property of RNAse was its heat stability, in contrast to most other enzymes. Thus, by heating to 100°, a crude "ferment" could be converted into an RNAse preparation presumed to be uncontaminated by other activities. "This specificity, together with the ease with which one isolates ferment in its active state thanks to its thermostability, suggested a simple way to detect pentosenucleic acid on slides: it would suffice to stain two cuts of the same organ with the UNNA mixture, one of them previously treated with the ferment" (BRACHET 1942).

The UNNA stain, a mixture of the red pigment pyronin and methyl green, was a happy choice: staining by pyronin is sensitive to RNAse, while that by methyl green coincides with that by the Feulgen reagent and is directed toward DNA. This permitted BRACHET to classify structures as containing RNA alone (red, sensitive to boiled ferment), DNA alone (green, insensitive to boiled ferment), or both (blue, converted to green by boiled ferment). The paper carefully validated the relationship between pyronin staining and chemically measured pentoses.

How did BRACHET convert his chemical determination into functional cellular information? Applying his simple method to a number of tissues and organisms, from yeasts to protists to vertebrates, he described the following pattern: the cytoplasm contains granules, then called "microsomes" (CLAUDE), whose basophilicity is due to RNA by the above criteria. Microsomes were later shown to be fragments of endoplasmic reticulum carrying ribosomes. The nuclear sap is not stained, but chromosomes are commonly stained blue, becoming pure green after RNAse treatment. These fundamental principles of the cellular deployment of the two nucleic acids were pursued in more cytological detail in this study. The RNA content of untreated chromosomes was quantitated as about 10% of the amount of DNA. In the giant chromosomes of Chironomus, BRACHET clearly distinguished euchromatin (bands staining green) from heterochromatin (which contains RNA in addition). The nucleoli were shown to contain RNA by BRACHET's criteria.

The central points distilled by BRACHET in his 1942 paper were that DNA is clearly localized in the chromosomes, and more specifically in the bands; and that the abundance of RNA in the cytoplasm is strongly correlated with the rate of protein synthesis. All the elements were in place for the so-called "central dogma" that DNA makes RNA which makes protein. What was lacking, 20 years before the messenger-RNA hypothesis, was a clear recognition of what is now known as genetic information, stored in one macromolecule and transferred to another. [The very word "information" had not moved from common parlance into the technical language of biologists. Indeed, a paper was sent to *Nature* as a joke pointing out that every biologist was using three combinatorial words–transformation, transduction, and induction–but nobody was using the fourth combination–information–which could neatly replace the other three (EPHRUSSI *et al.* 1953).]

It was clear to BRACHET that the relationship between DNA, RNA and protein was not a simple chemical transformation of one into another. Rather, his cytological analysis made it apparent that DNA played a role in the synthesis of RNA which, once transferred to the cytoplasm, would play a role in the synthesis of proteins.

Note that as late as 1960, the only strong argument for the existence of an informational intermediate between DNA and protein was the cytological separation between nucleus and the RNA-rich microsomes. In interpreting the classic experiments of PARDEE, JACOB and MONOD (1959) and RILEY *et al.* (1960) on expression from a conjugationally transferred *lacZ* gene, RILEY and her colleagues wrote: "The assumption that the *z* gene acts directly as a template in the synthesis of β-galactosidase would of course perfectly account for the observations. This assumption appears

unlikely, however, in the face of a growing body of evidence suggesting that the seat of protein synthesis, in many types of cells including bacteria, is not the nucleus but rather certain cytoplasmic constituents (ribosomes). We are therefore left to consider the only other alternative, namely that the transfer of information involves functionally unstable intermediates, and to ask which cell constituents might be likely candidates for such a function." Soon afterward, as we know, this intermediate was identified as messenger RNA, and it was assumed always to be unstable.

Here again, BRACHET entered with an unorthodox observation. Continuing to focus on the dialog between nucleus and cytoplasm, a classic theme in embryology, he, a Professor of Animal Morphology, chose to work on *Amoeba proteus* and on the giant unicellular alga Acetabularia, because each could easily be cut into two parts, one with and one without a nucleus. When starved, the anucleate portion of *A. proteus* steadily decreased in RNA content, consistent with the notion that RNA is synthesized in the nucleus. However, protein synthesis continued for several days in the anucleate portion, indicating that at least some messenger RNAs are stable. With Acetabularia, a remarkable species-specific morphological marker is the "cap." HAMMERLING (1953) had shown previously that anucleates of Acetabularia could develop a cap and, by nuclear graft experiments, had shown that the morphology of the cap is determined by the nucleus. GOLDSTEIN and PLAUT (1955) and BRACHET (1955) resolved the apparent paradox by demonstrating that RNA is synthesized in the nucleus and chased into the cytoplasm. The messenger is not always unstable.

In persuing the issue of the life span of eukaryotic messenger RNAs, it was natural to focus on mammalian erythrocytes and their reticulocyte precursors as natural anucleates. BRACHET's disciple CHANTRENNE with his young colleagues BURNY and MARBAIX (1965) explicitly showed that a messenger RNA species could be purified from rabbit reticulocytes and injected into Xenopus oocytes to direct the synthesis of rabbit globin chains (in collaboration with GURDON). This tour de force, one of the crucial experiments in molecular biology, illustrated the breadth of biological material with which the school of BRACHET pursued the fundamental issue of the dialog between nucleus and cytoplasm.

As BRACHET remarks (1987), "It can be seen that as early as 1942 we already knew the fundamental principles of the 'central dogma of molecular biology.' However, during the war, my colleague R. Jeener and I tried to demonstrate biochemically the hypothesis of intervention of RNA in protein synthesis. The results of our experiments went the right way, but it was impossible for us to obtain definitive proof because we did not have the necessary tool, radioactive amino acids. Just after 1950, several American laboratories demonstrated that the integrity of RNA was indispensable for radioactive amino acids to be incorporated into nascent proteins. Reading these papers made me as happy as if they had been mine. However, no one understood at the time how an RNA molecule could direct the synthesis of a specific protein. The astounding developments of molecular biology in the 1960s gave clues to this enigma."

From a local viewpoint, BRACHET and his colleague JEENER created the Belgian school of molecular biology. The small group, initially including their very first students WIAME, CHANTRENNE and ERRERA, gradually grew into a powerful institute of molecular biology on a new site of the Université libre de Bruxelles in Rhode-St-Genese. This group exerted a strong influence on the remarkable group in Ghent (FIERS, SCHELL and VAN MONTAGU). Till his death BRACHET remained *le patron* to all his students, including myself. An impressive personality, he clearly affirmed his scientific and political opinions. But, as in his science, he was able to free himself from dogma. Like many European scientists who had resisted the Nazi oppression, he was not only leftist, but for some time a member of the Communist party. Soon after his only visit to the USSR, he abruptly resigned the party because he was urged to support LYSENKO.

BRACHET was a very generous boss, never subjugating gifted young colleagues. He typically gave students complete freedom, seasoned with an occasional sharp but useful warning. He remained extremely careful to list himself as author only on papers to which he had contributed with his own hands. Those are the hands with which the embryologist JEAN BRACHET, *le patron* to me, developed his youthful iconoclasm and became the father of RNA.

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