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Jean Brachet and his School

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Birth of a Department

When in 1938 zoologist Paul Brien and physiologist Raymond Jeener offered Jean Brachet the Chair of Animal Morphology at the Université libre de Bruxelles (ULB), they were bringing into the Science Faculty a man who was soon to become one of its most eminent scientists and professors.

Not yet 30, Jean Brachet had already published some thirty papers, testimony to his intense and original activity in many laboratories: the Laboratory of Human Anatomy and Embryology of the Faculty of Medicine, the Zoological Stations of Naples, Sète, and Roscoff, the Biochemical Laboratory of the University of Cambridge, the Biology Department of Princeton University, and the Woods Hole Marine Biology Station. He had immediately sensed the importance of a new field of study which J. Needham had named «Chemical Embryology», a discipline focusing on the molecular causes of ontogeny. J. Brachet thus joined the young family of «chemical embryologists» whose members, besides Needham, included Chambers, Rapkine, Ephrussi, Wurmser, Runnström, and a few others.

During this initial stage of his career, J. Brachet studied variations in respiratory, protein, and carbohydrate metabolisms in the course of amphibian egg development. He notably demonstrated in 1937, in an article published with H.S. Shapiro, that the dorsal lip of the blastopore (Spemann's organizer) exhibits a more active respiratory metabolism than the corresponding ventral region.

He also contributed to ending a long-standing controversy over the chemical nature of the neural inducer by proving that basic dyes are as effective «evocators» as the acidic substances which German authors believed responsible for the induction produced by dead tissue. He further demonstrated, contrary to then-classical assumptions, that the germinal vesicle is neither the center of the growing oocyte's respiratory metabolism nor the site where cellular hydrolases accumulate.

But what science history will remember above all is the true, remote origin of Molecular Biology, although this discipline is generally said to have arisen around 1950, under the impetus of the «phage group» led by Max Delbruck and Salvatore Luria. At the young age of 19, Jean Brachet made an important discovery. Acting on a suggestion made by his mentor Albert Dalcq, he had used Feulgen's and Rossenbeck's newly developed staining method to observe the behavior of thymonucleic acid in the oocytes of various animal species. In those days, it was generally accepted that this low-molecular-weight substance was present only in animal cells, where it was believed to exert either a buffering or a viscostatic effect. Despite beliefs to the contrary, Brachet not only proved thymonucleic acid to be a permanent constituent of oocyte chromosomes, but also showed that in sea urchin fertilized eggs, its quantity increases in proportion to the number of cells. In perfect agreement with T.H. Morgan's chromosomal theory of heredity, his experiments thus suggested a genetic role for this substance, at a time when the «proteinic nature of genes» was a widely accepted theory.

Furthermore, seeking to confirm his cytochemical results by means of biochemical assays, J. Brachet reached the conclusion—a heresy in those days—that the sea urchin egg contains large amounts of «phytonucleic acid», a nucleic acid then considered exclusively vegetal and whose prototype was the zymonucleic acid of yeast. Today its name is RNA.

This, then, is the young iconoclastic biologist who chose to teach experimental embryology and cytology to future zoologists and who,

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in 1938, set up his laboratory in two rooms available in the buildings of the Science Faculty, built a few years earlier on the new Solbosch campus.

In 1940, applying Feulgen's staining technique to Amphibian oocytes, he described some thymonucleic-acid-rich (i.e., DNA-rich) granules in the nucleoli, thus discovering what we now call the nucleolar organizers. The same year, he published an article that would convince the most skeptical of his colleagues—including his friends at the Anatomy Institute—of the presence of RNA in animal cells. To achieve this goal, he applied UNNA's methyl green-pyronine simultaneous double-staining technique to sections of sea urchin oocytes pre-treated or not with ribonuclease. The latter enzyme had to be extracted and crystalized in the laboratory. As ribonuclease erased the pyronine stain from the cytoplasm and nucleoli, it became obvious that these two cell territories were rich in RNA.

This cytochemical technique, soon widely used by many embryologists, histologists, and cytologists, became universally known as the Unna-Brachet method. But it was Brachet himself who, applying it to a great variety of animal tissues, made a discovery that would determine the direction of his own research and the work of his many students for years to come: the perfect correlation between a cell's RNA content and its ability to synthesize proteins. Thus, the foundation of what was to become the central dogma of molecular biology (DNA makes DNA makes RNA makes proteins) was laid in Brussels in 1940. Here again, he applied a rule which he was always to follow and which he strove to pass on to his students, often with excellent results: to convince, always combine the biochemical and morphological approaches! Chemical pentose assays, in 1941, were thus used to confirm the hypothesis that RNA plays an essential role in protein synthesis. T. Caspersson in Stockholm independently reached the same conclusion by cytophotometric analysis of UV absorption by cellular nucleic acids.

The artificial limits between embryology, cytology, and biochemistry were thus fading in the light of a growing interest in the fundamental mechanisms of life, an interest which Jean Brachet would apply, throughout his life, to many different subjects, including cell physiology and differentiation, cancer, ageing and parasitology, yet never forsaking his first passion—the egg and the young embryo.

A first consequence of this interactive principle is his molecular interpretation of Dalcq and Pasteels' «morphogenetic potential» concept, and notably of the morphogenetic gradients that J. Mulnard mentions in this issue. Brachet believed that these result from two interacting RNA gradients, one a preexisting animal-vegetal gradient, and a second, cephalo-caudal gradient appearing during gastrulation.

In the thirties, Raymond Jeener, who held the Chair of Animal Physiology, had specialized in neurobiology. He had implanted a makeshift laboratory and his colony of cats in a cluster of small houses which the University had purchased near the Jean Massart Experimental Garden. The lab was not far from the beautiful Rouge-Cloître, at the edge of the Forêt de Soignes. Aware that he and Jean Brachet shared a more biochemically oriented (today we would say a more molecular) approach to biological research than did most of their colleagues, he suggested that J. Brachet should join him there, so as to pool their scientific equipment. This grouping of the Laboratories of Animal Physiology and Animal Morphology, shortly before the invasion of Belgium by the Nazi troops, was to give birth years later to the ULB's Department of Molecular Biology.

The first priority of the two professors was to prove the involvement of RNA in protein synthesis. Their fellow countryman (and future Nobel prizewinner) Albert Claude, then Research Associate at the Rockefeller Institute in New York, had just developed a method of separating cell constituents by ultracentrifugation. J. Brachet and R. Jeener used this method to demonstrate that microsomes, which A. Claude had isolated from chick embryos, are a universal constituent of eukaryotic cells, containing lipids and «pentosenucleoprotide granules». To the latter, they attributed an essential role in protein synthesis, an hypothesis supported by the finding that a small proportion of the red-blood-cell hemoglobin, and a small proportion of the pancreatic insulin, were found bound to the granules extracted from these two respective sources.

I should mention here the contribution of a young chemistry graduate who joined the laboratory in 1941. Hubert Chantrenne had become, after Jean-Marie Wiame, J. Brachet's second graduate student, entrusted with the then-fastidious task of assaying the enzymes present in the granules. Together, they performed the first extraction and characterization of such granules from developing amphibian and chicken eggs. This work was unfortunately to be interrupted by the permanent closing of the University laboratories, imposed by the German authorities at the end of July, 1942. After a short stay at the «Institut des Fermentations» where J.-M. Wiame was teaching, the group broke up. J. Brachet was imprisoned as a hostage from December of 1942 to March, 1943. After that, he did some research on alcoholic fermentation and on viruses, notably at the Université de Liège, and set to work on his first book, *Embryologie Chimique* (Chemical Embryology), published in 1944, after the Liberation, republished in 1945 and translated into English by L.G. Barth in 1950. This original book, a synthesis of all the biochemical aspects of embryology, had a considerable impact on many generations of scientists and elicited many vocations, notably that of François Jacob *(La Statue Intérieure,* 1987).

With Liberation, the study of RNA-containing granules could be resumed. There was no lack of arguments in favor of their being the site of protein synthesis, but the impossibility of obtaining labeled amino acids prevented them from producing the final proof. This came, therefore, from several American laboratories between 1950 and 1955. Most people would have felt bitter about this. Brachet, evoking this exalting period a year before his death, wrote: «la lecture de ces travaux me rendit aussi heureux que si j'en avais été l'auteur» (to read those papers made me as happy as if I had been their author) (J. Brachet, 1987).

Those post-war years were decisive for the future of biology in Brussels. Intent above all on developing a research center of international stature, J. Brachet carried out scientific missions in Great Britain and the United States in 1945 and 1946. He was a Visiting Professor at the «Institut Pasteur» in Paris in 1946 and at the University of Pennsylvania (Philadelphia) in 1947. The same year, he spent some time at the Woods Hole Marine Biology station. The Rouge-Cloître laboratory was growing, two notable additions being students Maurice Errera and René Thomas, who a few years later were to become, with J.-M. Wiame and H. Chantrenne, the heads of new laboratories whose creation was obtained by J. Brachet. Brachet had convinced the academic authorities of the need to create new courses in the Science Faculty: biochemistry, biophysics, and genetics. He taught these courses himself until the corresponding chairs were opened.

During this period of great effervescence between the Liberation and 1950, J. Brachet published 46 articles, more than half of which

were devoted to problems in developmental biology. He studied the distribution of several enzymes, notably ribonuclease and alkaline phosphatase, in oocytes and embryos, investigated the synthesis of ATP in the dorsal and ventral halves of the neurula (in collaboration with H. Chantrenne), and examined the mode of action of the organizer, the morphological and biochemical effects of heat shock on morphogenesis, and, of course, the role of nucleic acids in regeneration, parthenogenetic development, and the development of lethal hybrids. He also drew special attention with a talk on nucleic acid metabolism during embryonic development at the Cold Spring Harbor Symposium in June of 1947. With Raymond Jeener, he examined the physico-chemical properties of thymonucleohistones, producing a paper that appeared in the first volume of the journal *Biochimica et Biophysica Acta*, in 1947. The paper was reprinted in the journal's hundredth volume, in 1989.

The next decade was the golden age of molecular biology, a field to which the Rouge-Cloître group was to make a preponderant contribution. The group's reputation ensured useful subsidies and above all attracted a growing number of young Belgian and foreign research scientists.

In keeping with the options of its founders, the group neglected no experimental approach. This resulted in the progressive specialization of the various members and in the emergence of units that later became the divisions of the Department of Molecular Biology. M. Errera's interest focused on the effects of UV and ionizing radiations on biological macromolecules. Later, he was to develop the laboratory of Biophysics and Radiobiology. R. Thomas studied the physico-chemical properties of DNA, notably discovering the hyperchromic (denaturing) effect of temperature. He then engaged in the study of the genetics of prokaryotes and was later to become the head of the Genetics Laboratory. H. Chantrenne, who would continue to collaborate with Jean Brachet, as we shall see, was to focus on the peroxidasic metabolism of yeast and on the basic aspects of protein synthesis. He later became the director of the Biological Chemistry Laboratory. As for R. Jeener, he was to discover the ribonucleic nature of the genome of the tobacco mosaic virus. After another decade or so of research on plant viruses and bacteriophages, he was to devote himself to immunology, a field which he continues to study, 19 years after becoming Professor emeritus, in «his» Laboratory of Animal Physiology, now directed by his brilliant successor, Jacques Urbain.

On the trail of the messenger

But in 1950, this structuring into autonomous divisions still belonged to the remote future. All of the group members shared the same passion, only the approaches and biological materials were as varied as possible. Among the latter, the egg and the developing embryo, to which Jean Brachet retained a special attachment, continued to occupy a place of honor. Students in zoology, admittedly few in number in those days but fascinated by Jean Brachet's teachings, often asked him to direct their master's thesis, sometimes followed by a Ph.D. One of them, Maurice Steinert, demonstrated the reality of the RNA gradients by quantitative assays in the various parts of the egg. He also showed, for the first time, that RNA synthesis could first be measured during gastrulation by means of a spectrophotometric method that was sufficiently sensitive to allow measurements on a single amphibian egg. In 1954, with his Ph.D. degree in his pocket, he chose to continue his career in the Belgian Congo, now Zaire. There, he devoted himself to the cellular

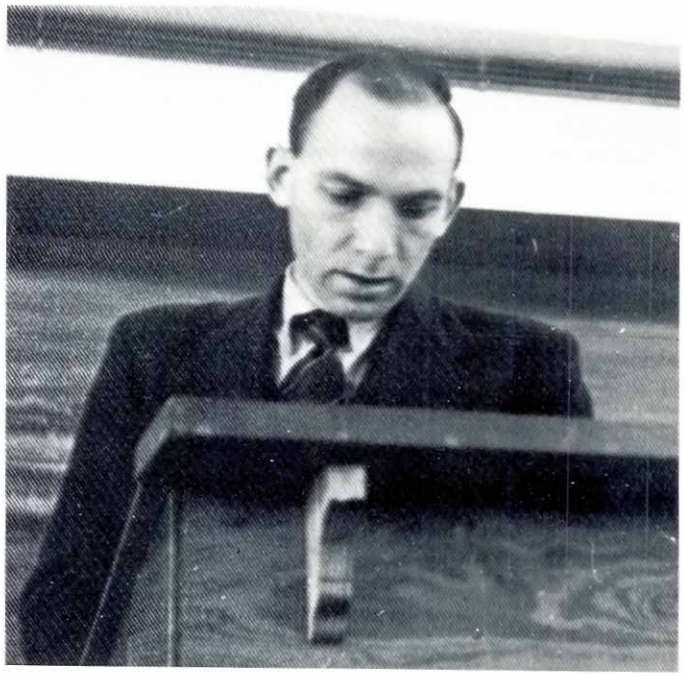

Fig. 1. Professor Jean Brachet teaching in 1940. *(Archives de l'Université libre de Bruxelles).*

biology and then to the molecular biology of trypanosomes, a field which he developed after his return to Belgium in 1964 within Jean Brachet's laboratory itself. Today, research in this area continues under the enthusiastic direction of Etienne Pays.

During this same period, Adrienne Ficq used autoradiography to simultaneously determine the amount of radioactive precursors incorporated and the site of this incorporation in biological materials. This enabled her to demonstrate, in 1955, the perfect coincidence between the basophilic character of cells and the rate of incorporation of radioactive amino acids into proteins. Autoradiography, an ideal link between the biochemical and morphological approaches, which in Jean Brachet's mind were inseparable, thus took its place among the arsenal of techniques that the successive generations of molecular embryologists working in the laboratory were to favor.

Results published by A. Ficq in 1955 and by J. Brachet and A. Ficq in 1956 were among the first obtained in this manner. The former showed that the nucleus of the starfish oocyte, or actually its nucleolus, is the site of RNA synthesis. The latter produced irrefutable proof that newt oocyte RNA is synthesized at the site of the lampbrush chromosome loops. This permanently established that the nucleus is where cytoplasmic RNA is synthesized, a fact suggested by Raymond Jeener, on the basis of biochemical experiments, as early as 1949! The role of the nucleolus, however, remained unclear, despite successful efforts to isolate this organelle from starfish oocytes, and despite the biochemical assays carried out by Elyane Baltus, a young graduate who arrived in 1950 and was to remain in the lab for her entire career. Happily, her efforts were crowned with success when in 1960, in collaboration with the American, Walter S. Vincent, she contributed to demonstrating that the nucleolus is the site of ribosome assembly.

It was during the fifties as well that Jean Brachet imagined and applied two particularly ingenious and very fertile strategies. He wanted to solve a double mystery: the role of the nucleus, clearly the site of RNA synthesis, and the role of RNA itself in biological processes.

Taking advantage of the fact that amoebae, onion roots, and oocytes absorb a great many acidic proteins, his idea was to treat them with purified ribonuclease. From 1954 to 1959, alone or in association with an increasing number of co-workers, he published 20 original papers describing the effects of enzymatic digestion of RNA on mitosis, cell metabolism, enzyme activities, embryo development, and even on the multiplication of the influenza virus! The general conclusion was that growth, morphogenesis, and protein synthesis are inhibited, while the pyroninophilia of the cytoplasm and nucleoli disappears. Furthermore, a partial reversion of this effect was obtained by adding exogenous RNA to the treated material. The effect exhibited some degree of specificity: onion RNA, for instance, was more effective on onion roots than yeast RNA. There is nothing surprising about these results today, but they were decisive at the time because they established, as Jean Brachet wrote, that «RNA is concerned with protein synthesis in the living cell as well as in simpler systems used by most biochemists» (J. Brachet, 1958).

As Jean Brachet was happy to repeat in many articles, the study of the biological functions of the nucleus owes much to the first cytology lecture he received from Pol Gerard in 1927. During that lecture, Brachet learned with surprise that a cell deprived of its nucleus can live for several days. Twenty-three years later, he published the first of a series of paper (25 from 1950 to 1960) devoted to the results of his merotomy experiments on *Amoeba proteus* and the unicellular alga, *Acetabularia mediterranea*, carried out with Hubert Chantrenne. He was notably able to show, by a cytochemical and biochemical approach, that RNA content and protein synthesis decrease over time in the anucleate halves of an amoeba, while they remain constant in the nucleate halves. These results were compatible with the notion that cytoplasmic RNA is indeed derived from nuclear RNA, which implies that it is synthesized in the nucleus and capable of crossing the nuclear membrane.

Some young graduates of those times, like Renée Tencer who arrived in 1954, still remember with nostalgia the days when the whole laboratory was mobilized, cutting amoebae in half to supply sufficient material for biochemical experiments.

Acetabularia, a larger organism, was more suitable in this respect, but its major advantage for embryologist Jean Brachet was the fact that it underwent a morphogenetic phase in the course of its biological cycle. Without dividing, the zygote develops into a long, tubular cell with, at its base, a rhizoid that holds the nucleus. At the apex of this siphon, an umbrella is formed, which will receive the many haploid nuclei resulting from the reductional division and equational divisions of the primary nucleus. Brachet had been acutely interested in and intrigued by a paradox resulting from Hämmerling's experiments in 1934: although the umbrella's structure is genetically determined by the nucleus, it develops normally and prematurely at the apex of an anucleate, thus necessarily gene-free, fragment. Biochemical experiments conducted with Hubert Chantrenne enabled him to demonstrate that anucleate fragments are perfectly capable of synthesizing proteins, including specific enzymes, several weeks after enucleation. As this strongly suggested a morphogenetic role for the RNAs accumulated at the apex of the alga, Brachet concluded that «specific DNA molecules (or parts of molecules), corresponding to each gene, would act as a template for RNA synthesis; there would be as many specific RNA molecules as there were genes. Finally, each specific RNA molecule would act as a template for a specific protein». This sentence was pronounced (and written) in April of 1959 at the «Sixth Weizmann Memorial Lecture» (J. Brachet, 1960: The biological role of nucleic acids). A few months later, François Jacob and Jacques Monod proposed the messenger RNA concept. Jean Brachet confided to me, one day, that if he had behaved like many colleagues worldwide, demanding that all of his co-workers, numerous by then, devote all their energy to the precise characterization of morphogenetic RNAs, he might, perhaps, have been cited as the «discoverer» of messenger RNA. But we know that Jean Brachet favored diverse approaches and, above all, had the utmost respect for each individual's freedom of endeavor.

His exceptional qualities as an enlightened guide, a determined researcher, and a great humanist, are probably the reason why, in addition to his own students, some 77 Russian, American, Chinese, Indian, Italian, French, English, Australian, Canadian, Japanese, Polish, German, Dutch, Yugoslavian, Finnish, and Chilean researchers enthusiastically visited his lab in the course of those ten years. This effervescence resulted in 240 published scientific papers, 103 of which bear the «Boss's» name. Furthermore, due to his authority in the fields of cytology, embryology, and molecular biology, he was asked to write three books. The first appeared in 1957 (*Biochemical Cytology*, Academic Press), the other two in 1960 (*The Biochemistry of Development*, Pergamon Press and *The Biological Role of Nucleic Acids*, Elsevier). He was also called upon to edit, in collaboration with Alfred Mirsky, the series *The Cell*, published in 6 volumes by Academic Press from 1959 to 1964. This amply shows that there were other research subjects besides those briefly mentioned above. One concerned the role of thiol groups in morphogenesis and therefore deserves a word in an article dealing with the history of embryology. As can be seen on several pages of his book, *Chemical Embryology*, this problem had intrigued him for many years. A first paper published in 1951 with Louis Rapkine showed that in amphibians, the inhibition of thiol groups is accompanied by inhibition of neurulation. After Rapkine's death, Brachet did not resume this work until 1958, first alone, then with several co-workers, including Jacqueline Quertier, Maud Decroly, Sylvie Limbosch and Viviane Pohl. They proceeded to study the effects of a reducing agent, mercaptoethanol, and of its oxidized counterpart, dithiodiglycol, on various biological systems: the development of amphibian and chicken embryos, umbrella formation in *Acetabularia*, regeneration of the tail in tadpoles and of the head in planarians. All these results led to the same conclusion: that oxidation of -SH groups to -S-S- groups plays an essential role in morphogenetic movements; but for lack of a means of determining the exact target or targets of regulatory oxidoreductions, this research was stopped in 1964. Jean Brachet would doubtless be interested in the work of Anne Van Langendonckt, a young researcher in his laboratory, who is currently completing a thesis on the presence and morphogenetic role of thioredoxins in *Acetabularia mediterranea*. This will probably also be the last of a long series of Brussels-based studies on this alga, promoted to the rank of «embryo *honoris causa*».

From Chemical Embryology to Molecular Embryology

We have now reached the dawn of the sixties. Molecular biology was developing at a vertiginous pace and embryology had amply

Fig. 2. The «Rouge-Cloître group» in the early fifties. *First row: Professors Raymond Jeener, Jean Brachet and Hubert Chantrenne. Second row: Professors Maurice Errera and René Thomas.*

contributed to this development. The European Molecular Biology Organisation (EMBO) was created in 1963, Jean Brachet being one of its founders. Paradoxically, as he himself stressed, embryology was making only slow progress at the time (J. Brachet, 1983), so the flow was reversed and embryology became molecular. This phenomenon was naturally felt first and foremost in the Laboratory of Animal Morphology, from which the Radiobiology, Biological Chemistry, and Genetics units had separated as distinct entities. The «Rouge-Cloître Group» had remained associated with J.-M. Wiame's Microbiology Laboratory, still located at the «Institut des Fermentations». These are the entities that proceeded to form a Research Department, officially recognized among the new structures created by the University in the wake of the 1968 protest movement. Thanks to the impetus produced by Euratom, when in 1963 it acknowledged the group as one of the five European centers worthy of its subsidies, the University decided to purchase a new campus site in Rhode-Saint-Genèse, a little town situated 15 km from Brussels, and to build modern laboratories there. The researchers moved there in 1965. The «Rouge-Cloître Group» thus became the «Rhode Group».

Students and foreign visitors continued to flow in, and with them the number of research subjects multiplied in both cytology and molecular embryology. I shall mention work in the latter field only, but I must briefly return to *Acetabularia*, which had generated an unexpected problem. It was a surprise indeed to discover that anucleate fragments, in addition to being a site of protein synthesis, were also the site of a net synthesis of RNA and...DNA! As early as 1959, Brachet had advanced the hypothesis that this difference in behavior between *Amoeba* and *Acetabularia* fragments was due to the presence of chloroplasts in the latter. In 1963, Baltus and Brachet detected DNA in chloroplasts; thanks to this «exotic» material, they thus provided final proof of the existence of chloroplast DNA by discrediting the «nuclear contamination» argument. A year later, André Goffeau (now Professor of Genetics at the Université Catholique de Louvain) and Jean Brachet showed that isolated chloroplasts from anucleate *Acetabularia* fragments are able to synthesize proteins from their own DNA. Finally, D. Shepard, an American post-doc, proved that chloroplasts can multiply. Subsequently, this discovery of the partial autonomy of cellular plastids was widely confirmed by a great many laboratories. It was then necessary to demonstrate that morphogenetic RNAs are not derived from chloroplasts. This goal was achieved by studying, in nucleate and anucleate fragments, the effects on morphogenesis and macromolecule synthesis of a wide variety of inhibitors, chosen for their ability to specifically affect chloroplast functions (ethidium bromide, rifampicin, chloramphenicol) and/or nuclear functions (ribonuclease, 5-fluoro-2' deoxyuridine, hydroxyurea, actinomycin, cordicepin, puromycin). These very detailed studies were undertaken by Françoise de Vitry from 1960 to 1965, and were continued thereafter by many of Jean Brachet's co-workers. One of these was Thérèse Vanden Driessche, who joined the laboratory as a teaching assistant in 1964. Trained as a botanist, she soon became interested in the circadian variations in *Acetabularia* photosynthesis, notably demonstrating the nuclear control of circadian rhythms. She later developed a chronobiology unit while continuing her studies on the molecular mechanisms of morphogenesis in *Acetabularia*. It was she who directed the above-mentioned work on thioredoxins.

The place of experimental and molecular embryology was nevertheless preponderant in the lab in those early sixties. It remained so until Jean Brachet became Professor emeritus in October, 1977. As we have seen, he was to devote much energy to organizing and managing a new institute but in 1968, in order «to do bench work in the lab», he accepted a position as Research Director at the Laboratorio internazionale di Genetica e Biofisica (LIGB) and, after the crisis in 1968, at the Laboratorio di Embriologia molecolare (LEM) in Naples. In a short autobiography, he wrote that the work he did there «deserved publication, but was not of prime importance» (J. Brachet (1988): «Autobiographical Sketch» in *Life Science Reviews, vol. 1.* Chronobiology. Souvenir of the Brachet Institute of Cell and Molecular Biology. Ed. by M. Glory, Changanacherry, India; pp 21-25). Naples, however, is where, between 1963 and 1965, with A. Ficq, E. Baltus, R. Tencer, and J. Quertier from his lab and Arsène Burny of the Biological Chemistry Laboratory, he discovered the presence of maternal «masked» messenger RNA in sea urchin eggs. His idea had been to work on anucleate fragments of virgin eggs obtained by centrifugation in a sucrose gradient, a method he had seen used by E.B. Harvey, in 1936, while he was in the United States. Parthenogenetic activation of this material was found to trigger massive, puromycin-sensitive stimulation of protein synthesis, similar to that described in 1960 by Brachet's friend Alberto Monroy, in whole fertilized eggs. This discovery was the starting point of many studies on the post-transcriptional control of gene expression. Shortly before his death, Jean Brachet resumed work on this subject with the help of a technician, the aim being to study the relative stability of mRNAs in nucleate and anucleate fragments. Sadly, he was unable to complete this project.

Jean Brachet worked three months a year in his lab in Naples to continue his research on the eggs of marine organisms (essentially sea urchins, ascidians, and *Chaetopterus*). He was sometimes accompanied, at the beginning, especially, by one or two young co-workers, possibly post-docs. The bulk of the embryological research was done in Rhode-Saint-Genèse, however, on the most appropriate material for the problem at hand: amphibian eggs *(Pleurodeles, Axolotl, Xenopus), Ilyanassa* eggs, chick embryos, and from 1972 on, the preimplantation mouse embryo.

To ensure better complementarity of morphological and molecular data, a first electron microscope was purchased for the laboratory in 1960 and a second in 1965, when the group settled in Rhode-Saint-Genèse. To put this new tool to the best possible use, J. Brachet called upon an electron microscopy expert who had been his student in the forties. Paulette Van Gansen had completed her masters' thesis under R. Jeener, her doctoral thesis under P. Brien, and had continued her research in the laboratory of A. Claude, who had returned to Belgium to direct the Institut de Cancérologie Jules Bordet. Her first work at the «Rouge-Cloître» was an ultrastructural study of *Acetabularia*, continued by Monique Boloukhère, her first graduate student.

Oogenesis, oocyte maturation, oocyte activation, zygote cleavage, and morphogenesis were thus studied in all of their aspects by dozens of scientists, some of whom already held or were to hold an academic position in the University or a permanent position with the Fonds National de la Recherche Scientifique (FNRS): A. Ficq, R. Tencer, P. Van Gansen, M. Boloukhère, and C. Thomas, for the University, and E. Baltus, J. Quertier, M. Decroly, M. Geuskens, and H. Alexandre for the FNRS. The results were recorded from 1960 to 1977 in 220 original articles and 30 review papers written by the «Boss», out of 460 publications emanating from the laboratory during this period under Jean Brachet's management. It would obviously be impossible and beside the point to mention each of

Fig. 3. The campus of Rhode-Saint-Genèse.

these contributions. I shall limit my account to the research themes and principal conclusions that had a major impact on our understanding of developmental biology.

After the work of E. Baltus and W.S. Vincent on the molecular nature of nucleoli isolated from starfish oocytes, two lab members began to investigate nucleic acid and protein metabolism during oogenesis. These were M. Geuskens, who arrived in 1959 and used starfish, and A. Ficq, focusing on amphibians. In 1965, M. Geuskens joined the electron microscopy unit headed by P. Van Gansen. He notably studied various ultrastructural aspects of cell differentiation and malignant transformation during frequent stays at the Institut de Recherches Scientifiques sur le Cancer in Villejuif, France. A. Ficq, on the other hand, continued her research on oogenesis until becoming Professor emeritus in 1984. Her achievements include the cytochemical and autoradiographic discovery, in 1968, that the DNA of *Xenopus* nucleolar organizers undergoes considerable amplification during the pachytene stage of meiosis, forming a cap-like structure that is clearly visible in the nucleus. It is copies of this rDNA that, once released from the cap, will later form, at the diplotene stage, the 1500 nucleoli of the germinal vesicle. This complex nucleologenesis was clearly understood thanks to the outstanding autoradiographic and ultrastructural observations of P. Van Gansen, published in 1972 and 1974. Christian Thomas made yet another discovery: he showed that ribosomal genes, despite their amplification and unlike the 5S RNA genes, are not transcribed in previtellogenic oocytes. This young scientist, who joined the lab in 1965, was considered by all of his colleagues and Jean Brachet himself to be one of the best molecular embryologists of his generation. He did a post-doc in Donald D. Brown's laboratory in Baltimore, the aim being to locate fibroin genes in cells of the silkworm's silk gland. He then resumed his studies on the control of transcription during oogenesis. There was no doubt he was destined for a brilliant career. Despite the near impossibility of hiring new researchers in the academic framework, he was appointed First Assistant to Professor Brachet in 1980. Sadly, he was to die three years later of a dour illness which he faced with immense courage, never abandoning the practice of his profession.

A chapter of tome XIV of the *Traité de Zoologie*, published by Pierre-Paul Grassé, was devoted to oogenesis in amphibians. P. Van Gansen was entrusted with the task of writing it. This brilliant synthesis was her last contribution to embryology, as she was to switch her focus to cell ageing, then to Schistosome-induced hepatic fibrosis.

A second oogenesis-related problem was elucidated in the laboratory around this same period. Since the work of Hoff Jorgensen

and Zeuthen in 1952, it was known that virgin eggs of amphibians contain DNA well in excess of the nuclear diploid value. Baltus and Brachet showed in 1962 that 2/3 of this excess DNA is bound to the yolk platelets (the remaining third belonging to the many mitochondria). This discovery was received with skepticism. It was again necessary to convince both morphologists and biochemists. Jean Brachet and A. Ficq thus developed, in 1963, an original and very sensitive method for «staining» DNA, by binding radioactive actinomycin to oocyte sections and detecting it by autoradiography. Later applied at the ultrastructural level by Gilberte Steinert and P. Van Gansen (1971), the method finally convinced morphologists. The very detailed and equally convincing molecular analysis was performed by E. Baltus, F. Hanocq, J. Quertier, and a graduate student, Micheline Kirsch, working on her doctoral thesis. The latter, now a professor at the Vrije Universiteit te Brussel, supplied the final proof of the exogenous origin of this DNA associated with liver vitellogenin, a hypothesis formulated by Jean Brachet in 1969. It was at last proven that this DNA has no genetic role.

Efforts to determine the origin and fate of a third type of extranuclear DNA were to mark the beginning of a new research trend in the laboratory, one that was to result in 34 publications. As early as 1940, Jean Brachet had observed the presence of Feulgen-positive granules in spontaneously maturing frog oocytes. He confirmed this finding 25 years later with the oocytes of several anuran species, where maturation was induced *in vitro* by pituitary extracts, with a technique published by Dettlaf in 1964. Very detailed and precise cytochemical, ultrastructural, and autoradiographic analyses combined with *in situ* molecular hybridization later proved that these granules are produced by fusion of many nucleolar organizers that migrate, thereafter, to the cortex. This work was carried out in collaboration with F. Hanocq, P. Van Gansen, G. Steinert and C. Thomas.

As this research on oocyte DNA progressed, Jean Brachet focused with E. Baltus and J. Quertier on other aspects of maturation, notably demonstrating that its cytological manifestations are under post-transcriptional control. One of their doctoral students, Martine Wiblet, detected the appearance of a new histone kinase. The possibility that there could be a relationship between this enzyme and the maturation promoting factor (MPF) was thus considered, more than ten years before the fact was clearly established. The same researchers demonstrated the essential role of Ca^{++} ions in the cascade of molecular events leading to chromatin condensation and to rupture of the germinal vesicle, thus explaining the unexpected inducing effect of various chemicals applied to the membrane, such as organomercurials, lanthanum chloride, or propanolol, all of which are able to mimic the action of progesterone. Another noteworthy finding was the demonstration, in 1976, of the inducing action of MPF on small, incompetent oocytes. These examples are convincing testimony to the diversity of maturation-related problems tackled in the laboratory during those years.

Yet without a doubt, the subject to which the most time and the greatest number of papers were devoted was the regulation of gene activity during early embryo development. Here again, it all began with an unexpected discovery that was widely confirmed inside the laboratory and abroad.

As early as 1957, Jean Brachet was studying with Renée Tencer the synthesis of macromolecules in *Axolotl* gastrulae, using A. Ficq's autoradiographic method and the only penetrating precursor available, $^{14}CO_2$. Three years later, Nicolas Bieliavsky, a new graduate, and R. Tencer published work that initially involved monitoring the incorporation of tritiated uridine into the RNA of dissociated cells of early amphibian embryos. In this work, they showed that the precursor is incorporated into the DNA of cleaving eggs. The same observation was made on sea urchin eggs by A. Ficq in 1963 and on mice, to a lesser extent, by H. Alexandre in 1976. Tencer and Bieliavsky further proved that this newly revealed «salvage» pathway of deoxyribonucleotide synthesis protects eggs from the inhibiting effect of fluorodeoxyuridine (FUDR) on DNA synthesis. Indirect, complementary experiments using ribonucleotide reductase inhibitors (hydroxyurea, d-adenosine) enabled Jean Brachet, in 1967 and 1968, to establish that this enzyme is indispensable for replenishing the thymidine pool, rapidly depleted after fertilization. The ultrastructural effects of the two agents were studied by M. Geuskens. The enzymatic activity was characterized in the laboratory on amphibians (1975), and at the LEM, in Naples, on sea urchins (Benita de Petrocellis, 1976).

It was clear, moreover, that induction of this enzymatic activity was under post-transcriptional control: Jean Brachet, always acutely interested in the role of nucleic acids in morphogenesis, had shown with Herman Denis of the Université de Liège that unlike the morphogenetic movements of gastrulation and neurulation, egg cleavage relies on no *de novo* transcription but does require protein synthesis. Brachet then proposed his famous model involving a dorso-ventral gradient of mRNAs synthesized by the nuclei at the gastrula stage, activating the pre-existing animal-vegetal ribosome gradient. These absolutely fundamental conclusions, published in 1963 and 1964, were based on the use of newly available inhibitors of either transcription (actinomycin D) or translation (puromycin). They sparked countless new studies. Work in Brachet's lab alone gave rise, by 1975, to some 30 papers.

The extensive study of the morphological and biochemical effects of actinomycin D on the development of amphibians was continued by H. Denis who, upon his return to Liège in 1965, pursued a brilliant career, first in his alma mater, then at the Université de Paris VI, where he is currently a Professor. The hypothesis that mRNAs play a role in morphogenesis was verified in the case of echinoderms and birds, and further strengthened later in studies using specific inhibitors of the various forms of RNA polymerase (α-amanitin, the rifampicins) and new inhibitors of protein synthesis (cycloheximide, fusidic acid).

The molecular mechanisms regulating translation of long-life informational RNAs stored during oogenesis were also the subject of several studies by M. Decroly and M. Goldfinger. Their contribution led to the hypothesis that a ribosomal dissociation factor could be one of the components limiting the rate of protein synthesis in oocytes. This very complex problem, however, required that the messengers themselves be investigated. This is certainly what made Jean Brachet encourage Gérard Marbaix to go to Oxford. There, in John Gurdon's excellent laboratory of molecular embryology, Marbaix was able to take part in experiments undertaken by Gurdon, involving microinjection of RNA into *Xenopus* oocytes. G. Marbaix was one of Hubert Chantrenne's students. In 1964, with A. Burny, he had been the first to isolate and characterize a eukaryotic messenger RNA, the rabbit globin messenger. In Oxford, he thus took part in Gurdon's famous 1971 experiment which showed that the rabbit hemoglobin messenger is immediately translated in the cytoplasm of the *Xenopus* oocyte. This microinjection method, used in J. Brachet's laboratory from then on, is what enabled G. Huez, another of H. Chantrenne's students, to understand the stabilizing

role of the polyadenylated sequence of mRNAs in oocytes. The problem was still far from being solved, however, and today, thanks to the spectacular progress of molecular genetics, G. Huez and his co-workers study AU-rich mRNA sequences and their role in translational control.

Among the biological systems that seemed adequate for studying the reciprocal regulation of nuclear and cytoplasmic activities, lethal interspecific hybrids are another case worth mentioning. Today they are out of fashion, but at the time, they had long held embryologists' attention. Since the work of the Swiss biologist Fritz Baltzer in 1910, for instance, it was known that in a cross between two taxonomically distant species, the incompatibility of the two gametes does not become manifest until gastrulation, a fact observed in echinoderms and amphibians alike. What particularly interested Jean Brachet in this system was the fact that development stops precisely at the stage where the information contained in the DNA is expressed. He had already demonstrated the system's complexity in 1944 by showing that a presumptive notochord fragment taken from a young hybrid gastrula was «revitalized» when grafted onto a normal gastrula. The mechanism of this «revitalization» remains obscure today, but the laboratory's contribution during the sixties increased our knowledge of lethal hybrid biochemistry. Combining, as usual, both cytochemical and biochemical techniques, R. Tencer, N. Bieliavsky, A. Ficq and J. Brachet showed, at the same time as Baltzer and his students, that neither DNA synthesis, RNA synthesis, nor protein synthesis stops when morphogenesis stops. The responsible factor appeared to be the nature of the proteins alone. Brachet concluded, in an homage to Professor Baltzer written in 1964, that «the problems posed by lethal hybrids can only be solved with the help of the methods and concepts of molecular biology» (J. Brachet, 1964, *Rev. Suisse Zool.*).

This is precisely what Herman Denis did during a stay a few years later at Jean Brachet's lab at the LIGB in Naples. He showed that RNA synthesized by an interspecific sea urchin hybrid blocked at the gastrula stage is preponderantly paternal, despite the preferential elimination of paternal chromosomes during egg cleavage. With elegant saturation and competition experiments using molecular hybridization on filters, he explained the paradox by the existence of two classes of genes that become active at the onset of gastrulation. The first and most abundant class of genes, destined to be expressed at all stages of development, are normally subject to negative control, which a foreign cytoplasm cannot exert. The second group of genes, which are functional only during the early developmental stages, fail, on the other hand, to be activated in the same foreign cytoplasm. These outstanding results already underline the full importance of the activation and repression of genes exerting specific control over the stages of morphogenesis. They were published in 1969 and 1970, in three papers signed H. Denis and J. Brachet that received much attention.

This sums up, imperfectly to be sure, the activities of the embryologists working in the Laboratory of Animal Morphology, rebaptised «Laboratory of Molecular Cytology and Embryology» in 1970. One should bear in mind that all this happened at a time when descriptive, comparative, and experimental embryology no longer seemed as attractive to young would-be researchers. Young scientists were drawn to other fields such as molecular genetics, sometimes even despising those «morphological sciences from another era». Jean Brachet was among those who believed in the complementarity of genetics and embryology. He foresaw the current success of developmental genetics. While encouraging all

Fig. 4. Professor Jean Brachet and Professor Hubert Chantrenne at Rhode-Saint-Genèse in 1987.

reductionistic approaches that might solve a problem concerning cell differentiation, he knew better than anyone, and often repeated, that the best way to understand embryo development is to work with the embryo itself. We can only rejoice, therefore, in the plethora of published gradients of regulatory-gene transcription products, while regretting the almost systematic absence of any reference to the «old» morphogenetic gradients.

On May 4th, 1977, Jean Brachet gave his last lecture before an auditorium of third-year Zoology students. All of his collaborators were there, listening with emotion. They were remembering the man who guided them step by step in their research, the exceptional pedagogue that he was. The lecture was devoted to nucleocytoplasmic interactions, a field he had explored for half a century. In one hour, he had made a densely informative, penetrating sketch of the whole subject. These same qualities are to be found in all of his books. Two books, published in 1974, concerned molecular embryology. One, entitled *Introduction to Molecular Embryology*, was aimed at the widest possible educated public, and its translation into Italian, Spanish, and Japanese is testimony to its success. The second, *Introduction à l'Embryologie moléculaire*,

published by Masson, was intended, rather, for students enrolled in a third university cycle.

In conformity with its rules, the ULB was thus depriving itself of one of its most outstanding Professors. The research leader remained, however, to the immense satisfaction of all members of his old lab.

Jean Brachet's succession

To fill the shoes of a man like Jean Brachet was by no means an easy task. There was certainly no lack of worthy candidates, each in charge of an independent research group within the large Laboratory of Molecular Cytology and Embryology. Their interests, however, had somewhat diverged. It was thus decided to create a Laboratory of Developmental Biology in addition to the existing lab. Its management was entrusted to Raphaël Kram, an MD and chemist who had created a division of normal and pathological cell physiology. Within this laboratory, Renée Tencer directed an embryology unit with which E. Baltus, J. Quertier and F. Hanocq were associated. With Claude Szpirer, she became co-director of the laboratory after R. Kram's sudden death in 1983. As co-directors of the Laboratory of Molecular Cytology and Embryology, where Brachet continued to work until his death, A. Ficq, P. Van Gansen, and M. Steinert were appointed. This laboratory included, in addition to the co-directors' research units, T. Vanden Driessche's chronobiology unit and, from 1984 on, H. Alexandre's mammalian embryology unit.

Jean Brachet thus continued his own research both in Naples (until 1982) and in Rhode-Saint-Genèse, while maintaining a close interest in the work of P. Van Gansen on cellular ageing, of H. Alexandre on the early developmental stages of mouse embryos, of R. Tencer on membrane dynamics in early amphibian embryos, and of E. Baltus, J. Quertier and F. Hanocq on oocyte maturation.

He conducted a detailed study of ions, the cytoskeleton, and macromolecule synthesis in the differentiation without cleavage of the *Chaetopterus* egg, in collaboration with A. Ficq and H. Alexandre. This was Brachet's second encounter with a fascinating biological system, discovered by Lillie in 1902: in 1938, he had studied some of its metabolic parameters. He then investigated the role of DNA replication cycles in morphogenetic movements, using specific inhibitors of polyamine synthesis and of DNA polymerase α. This work led to research, in Brussels and abroad, on the constitutive mechanisms of the biological clock that controls morphogenetic events, such as cavitation in the mouse egg.

His last personal work was devoted to certain cellular aspects of fertilization and cleavage in the sea urchin. He also wanted to continue his research on the relative stability of mRNAs in nucleate and anucleate fragments, but was unfortunately unable to complete this project. His last article, written with his devoted technician, Annette Pays, concerned the effect of monensin, a monovalent ion ionophore, on the eggs and embryos of sea urchins. The paper appeared a few weeks after his death in *Archives de Biologie*, the journal where, sixty years earlier, he had published his first work devoted to the behavior of thymonucleic acid during oogenesis.

Although the above account may lead one to doubt his word, Jean Brachet claimed that the main task to which he devoted most of his time during this period was that of writing a brand new version of his famous book, *Biochemical Cytology* (J. Brachet, 1988: «Autobiographical Sketch» in *Life Science Reviews, vol. 1.* Chronobiology. Souvenir of the Brachet Institute of Cell and Molecular Biology. Ed. by M. Glory, Changanacherry, India). This masterful synthesis, outstandingly updated, was published in two volumes by Academic Press in 1985 under the title *Molecular Cytology*. This was the achievement of a man who, alone and without any computer, had spent entire days among students and researchers, reading thousands of articles in the department library, always excited about developments in molecular, cellular, and developmental biology, an evolution to which he had never ceased to contribute. With the modesty of the great, he commented on this episode as follows: «I hope that it will be of some use to young biologists as a reference book». He was to have the satisfaction of seeing it hailed with enthusiasm worldwide. And needless to say, his book will be not only a reference but an inspiration to researchers young and old for many years to come.

At the request of the Springer publishing company, he also produced in 1986 a second edition of *Introduction to Molecular Embryology*. He asked me to help write it, thereby doing me a great honor. My contribution was humble, but my joy was immense.

An account, however imperfect, of Jean Brachet's exceptional career, is enough to show that the death of the «Boss» on August 10th, 1988 made orphans of us all. The man has left us, but his gigantic work remains, and with it a Research Department that is the pride of the Science Faculty and of the whole Université libre de Bruxelles.

There remains an embryology unit in each of the laboratories derived from the old Animal Morphology Laboratory. Research on oocyte maturation in amphibians was recently abandoned. E. Baltus retired in 1989, while J. Quertier and F. Hanocq joined the Molecular Parasitology unit of E. Pays. Their last contribution to embryology concerned the role of the *ras* oncogene in the regulation of *Xenopus* egg cleavage. Two excellent papers were published on the subject, one of which bears Jean Brachet's signature. As for R. Tencer, she had pursued interesting research on the role of cell membrane biogenesis in early morphogenetic processes in amphibians. In recent years, she has been joined in this endeavor by N. Bieliavsky, her working companion in the early sixties. In the laboratory of Molecular Cytology and Embryology, members of the mammalian embryology unit, working in close collaboration with Professor Jacques Mulnard (Human Anatomy and Embryology Laboratory, Faculty of Medicine), have been working for four years on the pleiotropic control exerted by MPF on the cytoplasmic manifestations of oocyte maturation in mice.

Renée Tencer's vast culture in embryology made Jean Brachet appoint her, in 1970, to teach experimental embryology to Zoology students. Her rigorously scientific frame of mind, her permanent concern for applying biochemical and morphological approaches to typically embryological problems (furrowing during cleavage, anisotropy, mesodermal induction for instance), have made her the future director of a new Embryology Laboratory that should come into being when P. Van Gansen becomes Professor emeritus in September of 1992. At the same time, H. Alexandre will leave the Department for the Université de Mons, where he will head an Embryology laboratory in the Faculty of Medicine.

It would be unfair, of course, to give all the credit to the two above-mentioned units for the body of research done by the Molecular Biology Department as a whole in the general field of developmental biology. We have already mentioned the major contribution of G. Huez, now co-director of the Laboratory of Biological Chemistry, to the problem of translation of maternal mRNAs in *Xenopus* oocytes and eggs, a work that has now been extended to mammalian ova.

I should also mention two people who used to work with R. Thomas, whose initial training was in molecular genetics, and who have been contributing for some fifteen years to the spectacular progress in the developmental genetics of *Drosophila:* Alain Ghysen, head of the Neurobiology Laboratory and Christine Dambly-Chaudière, who is soon to direct a Laboratory of Developmental Genetics—each animates a young and dynamic team and collaborates closely with foreign labs. Their respective contributions to this issue are perfect illustrations of the importance of their work in understanding the genetic determinants of cellular commitment and differentiation in the embryo. To end this list, I shall mention Claude Szpirer, who now co-directs the Laboratory of Developmental Biology with R. Tencer, and Josiane Szpirer, head of a unit in the Genetics Laboratory. These scientists, who specialized in the somatic genetics of mammals during a stay in Henry Harris's laboratory in Oxford, are pursuing research in one of today's «hottest» fields: the control of differentiation gene expression. The article by C. Houart, J. Szpirer, and C. Szpirer in the present issue is an illustration.

The wealth of the Molecular Biology Department resides, today as it did yesterday, in the diversity of problems tackled and of strategies used to solve them. As long as this remains true, Jean Brachet's inheritance will remain intact. From the very beginning of his career, he consciously favored the multiplication of research subjects, himself making a judicious choice of the biological material best suited for the problem he wanted to solve. Thus, the egg, his first focus, was joined by *Amoeba* and *Acetabularia.* Reticulocytes, *Escherichia coli,* and bacteriophages had their time of glory, and with them new problems, new techniques and new concepts were born. The powerful methods of molecular biology and genetics are now used in the service of developmental biology. The egg and the embryo seem to have been rediscovered by new generations of scientists. Jean Brachet foresaw this as well!

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