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Plant cytoplasm. Contribution of French cytologists

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Introduction

Were one to recall the extent to which progress in biology depended on physical sciences, the history of the study of plant cells would provide a particularly obvious example. More than 300 years ago, the use of the first microscope led Robert Hooke, while observing a fragment of cork, to coin the word 'cell', the immense fortune of which we all know. Since that time (1665), the improvement in microscopes was slow and our knowledge of cellular structures progressed slowly also. A first leap forward was due to the work of Abbe and Zeiss. In the beginning of the 20th century their work led to the 'classic' concept of the structure of cells, plant or animal.

As concerns plant cytoplasm, the bulk of facts, whether accepted or disputed, was put forth in a fairly exhaustive manner in a number of French publications, and in a Treatise of Plant Cytology by A Guillermond, P Mangenot and L Plantefol (Le François, eds, 1933) and in the volume by P Dangeard (Lechevalier ed, 1947).

Thus, several researchers in Paris, Bordeaux, Lyon etc were seeking and ready to take advantage of the technical developments in electron microscopy and to apply them with some adaptations to the investigation of plant cells, which were often more difficult to fix and prepare than animal cells. Starting in the 1950s, the rapid improvement of these techniques suddenly opened an unexpected field of investigation which was widely explored in France.

New facts were not long to appear. Within some 15 years, all chapters of the so-called classic cytology were more or less profoundly perturbed and revised. French participation was important. Several examples, which do not pretend to be exhaustive, will illustrate the leading role played by French researchers in acquiring knowledge of plant cytoplasm.

The Golgi apparatus of plant cells

By the end of the 1950s, more than 50 years of often bitter debate had divided French researchers into those who believed in the existence of a Golgi apparatus in plant cells and those who denied it. In 1957, thanks to the hospitality of Pierre Paul Grassé who had created an electron microscopy laboratory at the Laboratoire d'Évolution des Êtres Organisés in Paris and who had already described 'dictyosomes' in animal cells [38], a morphological identical system was described for the first time in root cells from *Allium cepa* [4]. The same year, Chardar and Rouiller [20] in France, Perner [49] in Germany and soon after Lance [40] in Paris, observed the same configuration in various spermaphytes. The debate was closed. The Golgi apparatus, seen in 1961 in algae [3, 37] and with difficulty in fungi, could then be considered as a general constituent of eukaryotic cytoplasm in plant and animal. The behaviour and function of these structures was only elucidated later, but seemed *a priori* to be analogous in both kingdoms. The discovery added one further argument to the concept of the fundamental unity of eukaryotic cells.

The endoplasmic reticulum

Often observed in animal cells before 1957, it was recognized in plant cells the same year also in the electron microscopy department of the Laboratoire d'Evolution des Etres Organisés [13]. Since it was found many times in spermaphytes and is generally considered as a system common to all eukaryotes, notably gymnosperms [17], bryophytes [39], fungi [53] and algae [3,37]. Investigations in France on cyanophyceae [41] and on bacteria demonstrated the absence of endoplasmic reticulum in prokaryotes.

These observations, along with other results, showed the nuclear envelope as a differentiation of the endoplasmic reticulum, hence a cytoplasmic structure [5,10].

Mitochondria and plastids

A source of contention between the schools of A Guillermond and of P Dangeard was clarified by the Parisian cytologists in 1958 (see [41]). Despite structural analogies and cytochemical similarities between meristem mitochondria and proplastids, it was showed that these organelles were not identical and corresponded to two independent lineages as anticipated by A Guillermond. In fact, the least differentiated proplastids are distinct from meristem mitochondria by their greater size and by the appearance of their cristae. During differentiation, mitochondrial cristae increase in length and number as the metabolic activity of the cell itself increases [5, 14].

In chloroplasts, the considerable growth of thylakoids and their partial association in grana, already observed in spermatophytes, was often studied in France as well as abroad (see [43]). Giraud [37] and his disciples in Paris examined particularly the granular localisation of superimposed pigments in thylakoids of the rhodophyceae.

The plasmalemma

The plasmalemma was the object of intensive research in Parisian laboratories and the first suggestion of its participation in the process of endocytosis by invagination dates back to 1958 [29]. Exocytosis was also extensively investigated. One of the most remarkable examples concerns exocytosis of polysaccharide-containing vesicles during pecto-cellulose wall formation. Vian and Roland [55] followed the evolution of Golgi vesicles during their transport to the plasma membrane, allowing their fusion and the subsequent liberation of their contents into the extracellular space. Up to then, exocytosis had been studied in animal cells only. Besides adding wall precursors, exocytosis constitutes a means for compensating losses of plasmalemma fragments which occur during endocytosis. Integrity of the plasmalemma is therefore restored.

Extracellular secretions

Two examples can be mentioned which were studied at the Faculté des Sciences in Lyon and a third at the Département de Microscopie Electronique in Bordeaux.

An important work concerns hydatodes, a widespread system among vascular plants. Hydatodes secrete water which contains dilute substances. Perrin [50] showed that such excretions required the activity of special cells and involved cooperation between the endoplasmic reticulum and a highly active plasmalemma. The latter undoubtedly plays a role in intercellular exchanges notably as 'plasmalemmasomes'.

Another study, also in Lyon, concerned the stigmatic secretions of *Forsythia intermedia* [29]. Polysaccharide secretions were shown to be essentially 'granulocrine' involving vesicles fusing with the plasmalemma as is the case with wall secretions. Secretion of liposoluble substances were not observable in the electron microscope. Rather they probably traversed the plasmalemma as isolated molecules or associated to transporters.

Nectary cells were also studied in Bordeaux [30, 31]. The secretory vesicles accumulate in the cytoplasm before exocytosis. This is accompanied by a surprising modification of the endoplasmic reticulum called 'coat of mail' [31].

Intracellular secretions

These are inseparable from the history of vacuoles. They were studied particularly in laticifers and in pseudolaticifers at the Institut de Cytologie et de Biologie Cellulaire in Marseille-Luminy [32, 35, 44, 46–48]. Such secretions represent the most remarkable example of vesicle formation by autophagy. The central vacuole of the 'true' laticifers results from the sequestration of the major portion of the protoplasm. The protoplasm thus circumscribed is then destroyed by hydrolases. In the residual cytoplasm the plastids differentiate into giant amyloplasts which are partially extruded into the vacuole where they mix with polyphenolic secretions. They are then introduced into canal-like vesicles and are expulsed into the vacuole. The laticifers of *Euphorbia characias* harbor an exceptionally high level of peroxisomes [45].

In the case of pseudolaticifers of *Lactuca sativa*, interest was focused on the mechanisms of cell wall perforation which enables the protoplasm of several cells to communicate [33–35]. In particular, the plasmalemma of areas undergoing degradation form vesicles containing the remnants of cell walls. These vesicles migrate within the cytoplasm and empty into vacuoles in which their contents are lysed. Giordani [35] showed clearly that only the vacuoles contain the cellulases necessary to hydrolyze cellulose, in contrast to vesicles of the plasmalemma. Very recently, biochemistry of isolated elements has completed the conclusions based on cytochemistry [36].

The origin of vacuoles

This was also an important subject of studies in France. In the sixties, pictures obtained after permanganate fixation strongly suggested that vacuoles arose from dilatations of the endoplasmic reticulum [15, 51]. Numerous preparations soon demonstrated that in plant cells, only smooth membranes could give rise to dilatations analogous to young vacuoles [10, 11].

The origin of vacuoles was more related to the discovery of lysosomes in plant cells. First suspected from morphological observations alone [16], their existence was confirmed by the revelation of acid phosphatase activity in electron-dense bodies and in Golgi extensions [22, 52]. The dense bodies were related to primary lysosomes. However, the existence of sequestering double membranes and the destruction of imprisoned cytoplasm led to the conclusion that the 'autophagic vacuoles' were homologous to secondary lysosomes and transformed into ordinary vacuoles after hydrolysis of their contents [11, 22]. Plant lysosomes were studied furthermore at the Institut de Cytologie et de Biologie Cellulaire in Marseille-Luminy. After isolation by centrifugation, their biochemical characteristics and properties were found to be the same as those established by De Duve (1963) for animal lysosomes, with the exception of one enzyme (β-glucuronidase) [23]. Heterophagic phenomena implying digesting vacuoles were also described in root meristems [21, 24].

Other studies were more particularly oriented towards the origin of young vacuoles in meristems and towards the formation of the sequestering double membranes already mentioned. These studies led to the concept of a 'provacuolar apparatus' which derives from the extensions of the Golgi system [46, 48]. The provacuolar apparatus, and later the typical vacuoles, therefore, arise from the Golgi apparatus and not from the rough endoplasmic reticulum. Again, it appeared that the two systems differ greatly in their functions and their evolution [5, 12].

The ultrastructure of the vacuole membranes, the tonoplast, is very close to that of the plasmalemma. This is doubtless a necessary condition for anastomosis between plasma membrane and the vacuoles of Golgi vesicles, mentioned before *a propos* of the exocytosis of polysaccharide precursors of cell wall.

Intercellular communications or plasmodesmata

The structure of plasmodesmata was first clarified in 1957 and in 1960 at the Laboratoire de Botanique at the Ecole Normale Supérieure [6]. Plasmodesmata ensure the continuity of the endoplasmic reticulum, plasmalemma and hyaloplasms of neighboring cells. The pecto-cellulose wall which seems to separate cells does not in fact prevent the coenocytic configuration of tissues, different from what occurs in animal tissues where cells are rigourously separated. The organization of the plasmodesmata channels which cross

the pecto-cellulose wall also contradicts the notion that the walls are like prisons enclosing the protoplasm ('The Cell', R Hooke; [12]). Cell walls represent the base for an internal medium which, like that of animals, ensures circulation of necessary metabolites, *ie* the 'apoplastic' way [6, 12, 28, 54].

Conducting tissues

The differentiation of specialized cells from the phloem and the xylem was studied in detail and jointly with Anglo-Saxons and German researchers notably at the Ecole Normale Supérieure and at the Institut de Cytologie et de Biologie Cellulaire in Marseille-Luminy. Examples of such problems are the interpretation of 'P-proteins' [1, 2], differentiation of sieve cell walls [1, 18], evolution of the plasmalemma, tonoplast, and the 'mictoplasm' of sieve cells [8]. Numerous articles and notes were published which cannot be exhaustively summarized here. We are thus limited to a few citations [8, 9, 19, 25–27, 42].

From the above account, it can be seen that although French research in ultrastructural cytology was not unique, it played nonetheless an important role. The efficiency of the work accomplished relies perhaps on two facts which confer to it a certain degree of originality. First, the attachment of the French researchers to data from classical cytology kept them from going astray in their electron microscopic observations where the scale change might have given an impression, sometimes enrapturing, of penetrating into an unknown, groundless domain. Secondly, their investigative approach founded on the ontogenic basis of continuing evolution of living structures, allowed them to bring together aspects and images which, described separately, might have appeared estranged from each other, as for example extension of Golgi apparatus and vacuoles.

Today, elucidation of cytoplasmic structures in plants, that of their interrelationships, their evolution and their movements provide a concrete basis for researchers in cellular physiology. Progress achieved in biochemistry and molecular biology are now available to them. The physiologists are now in the fore but they seem to be aware of the necessity to consolidate their investigations with the help of electron microscopy and ultrastructrural cytochemistry.

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