

Letters

The origins of the quiescent centre concept

Introduction

To learn how new concepts are conceived and developed is a way to understand scientific progress, appreciate important achievements, and develop one's own skill at evolving new concepts. In plant developmental biology, the concept of quiescent centre (QC) is widely taken as an example of a stem cell population which, in this particular case, gives rise to most root tissues. Here we draw attention to the question of how the concept of the QC was conceived. It is appropriate to do so at this time because, as we show here, the concept was conceived 60 years ago. However, in marking this Jubilee event we do not consider either the subsequent history of the concept or the work done to more closely define the properties of the QC but, rather, to address how the QC was discovered, both conceptually and experimentally, and the evolution of its terminology.

Preliminaries

In 1953, during the course of analysing the organization and function of the root apices, Frederick Albert Lionel Clowes (born 10 September 1921; Fig. 1), at the School of Botany (now Department of Plant Sciences), University of Oxford, proposed the term 'cytogenenerative centre' to denote 'the region of an apical meristem from which all future cells are derived' (Clowes, 1953, p. 48). This term had been suggested to him by Mr Harold K. Pusey (Fig. 2), a lecturer in embryology at the Department of Zoology and Comparative Anatomy at the same University. The 1953 paper of Clowes reported results of his experiments on *Fagus sylvatica* and *Vicia faba*, in which small oblique and wedge-shaped excisions were made at the tip of the primary root, at the most distal level of the root body, near the boundary with the root cap. The results of these experiments were striking and showed that: the root which grew on following the excision was normal at the undamaged meristem side; the nonexcised meristem portion contributed to the regeneration of the excised portion; the regenerated part of the root had abnormal patterning and 'remained so for a time considered sufficiently long for the complete replacement of all the derivatives of the initials' (Clowes, 1953, p. 57). The main conclusion from these experiments was that the root tissues originated from a promeristem which was considered to be 'cytogenenerative centre', also called 'cytogenetic centre', 'ontogenetic centre' (Clowes, 1950), and 'constructional centre' (Clowes, 1954a). Frederick Albert Lionel Clowes indicated that the cytogenenerative centre was a property of

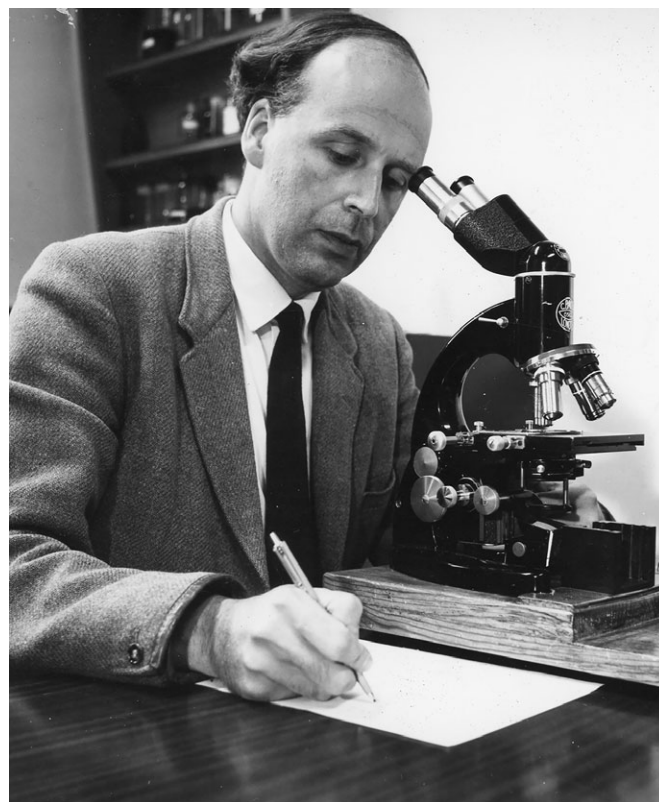


Fig. 1 Frederick Albert Lionel Clowes. Photograph from c. 1965.

roots with broad columellas (i.e. of roots having an 'open' type of apical meristem, as was the case in the roots of the two species studied). He then wished to know whether the same property applied to thinner roots with narrow columellas (i.e. roots with a 'closed' type of meristem) (Clowes, 1954a). Using roots of *Zea mays* and *Triticum vulgare*, this indeed turned out to be the case, and he was then able to state that 'the cytogenenerative centre is conceived as the part of the apical meristem from which all future tissues are derived' (Clowes, 1954a, p. 115). We may infer that Clowes regarded the cytogenenerative centre as a universal feature of roots because he used the term also with respect to the structure and function of the root meristem of conifers (Clowes, 1954b, p. 525). Notice that Clowes considers this centre to be concerned not only with cell production but also to be a source of root tissues. In this sense, the term 'cytogenenerative centre' supersedes the term 'ontogenetic centre', which he had used in relation to root apex construction of *F. sylvatica* (Clowes, 1950, see p. 253). In fact, this newer elaboration of 1954 was in keeping with developments in the science of plant morphology for, by using the term 'cytogenenerative centre', Clowes was now introducing into the English literature an equivalent of the German term 'Urmeristem' (primal meristem), which denotes a zone within which all potentialities for root



Fig. 2 Harold K. Pusey. Photograph from c. 1950.

development are preserved, an idea tracing back to J. W. von Goethe via Karl von Goebel and Hermann von Guttenberg, the latter also making contributions to the understanding of root development.

Date of birth of the QC

Of the cytogenenerative centre in *Zea*, Clowes writes that it 'consists of a promeristem whose constituent initials lie outside the surface of the cone frustum. The cells at the pole of the periblem–dermatogen complex enclosed within the initials, *play little or no part in the production of new cells in mature roots where the constructional pattern is preserved. They very rarely show mitotic figures or other evidence of division*, but they may play a cytogenenerative role when the architectural pattern is upset after wounding or in altering the size of the root' (Clowes, 1954a, p. 114, emphasis added; see Fig. 3a). Note that the term 'quiescent centre' has not yet been used, only 'cytogenenerative centre'. However, from Clowes's (1954a) paper it is clear that the 'cytogenenerative centre' and the zone which rarely showed mitotic figures, that he would later call 'quiescent centre', refer to the same group of cells. Note also that the term 'cytogenenerative centre', as used in his 1953 paper, refers only to its function in tissue regeneration following wounding and is not yet referring to the mitotic quiescence of this group of cells in undisturbed roots. Nevertheless, without any preliminaries, the very first words of a subsequent paper by Clowes (1956a), received by the *New Phytologist* on 30 December 1954, are as follows: 'In previous work on grass root apices (Clowes, 1954a) it was suggested that there is *a quiescent centre in the meristems where the cells divide rarely or never*' (emphasis added). He is thus underlining the mitotic, or proliferative, quiescence of this centre. Therefore, since this aspect is considered in the 1954a paper, and the term 'quiescent centre' is used here for the first time in his 1956 article (prepared in December 1954), we can consider that 1954 was the year of birth of the QC concept. Additionally, we found in the experimental notebooks of Clowes that the first experiments of feeding roots with ^{32}P -phosphate and $8\text{-}^{14}\text{C}$ -

adenine followed by application of autoradiographic film to the tissue sections, were performed in May and July 1954, respectively. These experiments showed for the first time that this area of the root tip had a low rate of DNA synthesis.

The nature of quiescence

Clowes' work with autoradiography was sufficient to confirm the presence of proliferatively quiescent cells within the root apex, a supposition which he had formed years earlier during his analysis of periclinal divisions in the root apex of *F. sylvatica* (Clowes, 1950). In this work, Clowes hints that there must be a way in which cell growth and division were organized in the location where initial cells of columella and cortex meet, so that these cells, with apparently different planes of cell division, could grow conformably together without 'recourse to "gliding growth" to overcome the tensions set up' (Clowes, 1950, p. 262). If gliding growth was not permitted, then, although not specifically stated here, the solution would be for the cells at this location to grow either not at all or slowly and 'differences in the rates of division and elongation of the different tissues can sufficiently account for the form of the root' (p. 262). Thus, as early as 1950, at the start of his work on root apices, Clowes recognized the geometrical necessity for a quiescent zone accompanied by different rates of cell division which would allow the growth of neighbouring groups of cells within and around the promeristem to proceed harmoniously. A clear statement about this was made in 1968: 'It [the QC] results from the geometrical necessity for a region of no growth between the initial cells of the cap and the dividing cells of the stele and cortex' (Clowes, 1969, p. 6). The autoradiographic work of 1954 confirmed what Clowes had suspected in 1950 to be a zone of limited growth in this same location.

The deciphering of the geometrical pattern of cellular branching within the root meristem of *F. sylvatica* was facilitated by Otto Schüëpp's analytical work in this direction (Schüëpp, 1926). In the paper of 1950 Clowes also briefly suggests a theory for the regulation of growth of the promeristem. He states that the cells '... have the properties of initials by virtue of their positions within the promeristem ...', and also that 'the changes which they bring about by their own activity cause ... changes in the shape of the promeristem ...'. Clowes also indicates: 'These changes are probably not automatic, but probably result from external factors, such as nutrition' (1950, p. 266). Clowes also hints that the regulation of divisions is due to the way in which cell walls are able or unable to expand owing to the presence of neighbouring groups of cells: 'The cessation of longitudinal divisions in the initials of the outer periblem is considered to be related causally ... to the lateral expansion of the columella ...' (p. 263). The latter view was expanded upon and took precedence over the nutritional theory, as detailed in his account of the QC in 1975 (Clowes, 1975a): 'The view that I find most attractive is that the QC is maintained by the pressure exerted by the growth of the surrounding cells. The critical fact here is that the QCs of different kinds of roots are of different shape and their shape fits into the pattern of the files of dividing cells' (p. 16). Quiescence was thought to be due to a restraint imposed on cell growth by pressure from neighbouring cells.

Stem cell function

Importantly, Clowes suggests that ‘... the cells which lie over the surface of the QC can usefully be regarded as the initials of the root. By the division and growth of these initials and their derivatives the QC is carried forward throughout the life of the root ...’ (Clowes, 1956a, p. 29). In essence this is a hypothesis that stem cells are present in the root. But only in 1977 did Peter Barlow suggest that the QC functions as a stem-cell population, operating in plant roots. He proposed (Barlow, 1997) that the main body of the QC is constituted of structural initial cells, and that its peripheral cells constitute the functional initial cells. Although the staining properties of both the ‘ontogenetic centre’ (Clowes, 1950) and its equivalent, the ‘cytogenenerative centre’ (Clowes, 1956a), were found to be sufficiently distinct to indicate their low level of cellular activity, it was by analysing the size of nucleoli and the degree of incorporation of radioactive precursors of DNA into roots of *Z. mays*, that Clowes was able to conclude that ‘There is a region in the apical meristems of *Zea* roots where the cells are distinguished from the surrounding cells by their smaller nucleoli, lower RNA content of the cytoplasm, and by not synthesizing DNA from externally supplied phosphate or adenine. This region corresponds to the QC previously postulated on anatomical grounds and it may be concluded that its cells rarely or never divide and grow ...’ (Clowes, 1956a, p. 33). Furthermore, Clowes proposed how the ‘cytogenenerative centre’ is organized and what are the locations of the initial cells (Fig. 3a). In 1956 he also uses the drawing of exactly the same root to show how theoretically the QC should be organized (Fig. 3b). Finally, in this same work (Clowes, 1956a), he published an autoradiograph preparation of median sections of *Zea* roots which shows an image of the QC that has become a classic (Fig. 3c). By 1959, autoradiographic evidence for the presence of a QC in roots of four species, *Z. mays*, *Pisum sativum*, *V. faba*, and *Sinapsis alba*, had been assembled (Clowes, 1959a); a similar image for *Allium ascalonicum* had appeared in 1956 (Clowes, 1956b). Unpublished evidence also shows that Clowes delineated, by autoradiography, a QC in roots of *Pinus sylvestris*.

Conclusion concerning the significance of the QC

In the work in which autoradiography was used to demonstrate ‘quiescence’, Clowes (1956a) cites the seminal work of Howard & Pelc (1953) who formulated the concept of cell cycle (see Dubrovsky & Ivanov, 2003), acknowledging them also for their discussion of his own experiments. This is an important insight because it demonstrates that Clowes understood the QC from the point of view of cell population dynamics, as having generally low rates of DNA synthesis and cell proliferation as compared with cells in the main body of the root meristem. This represents a fundamental step forward for developmental plant biology. In his own research, Clowes developed these ideas in three main directions: functional analysis of the cell cycle and cell population dynamics within root apical meristem both outside

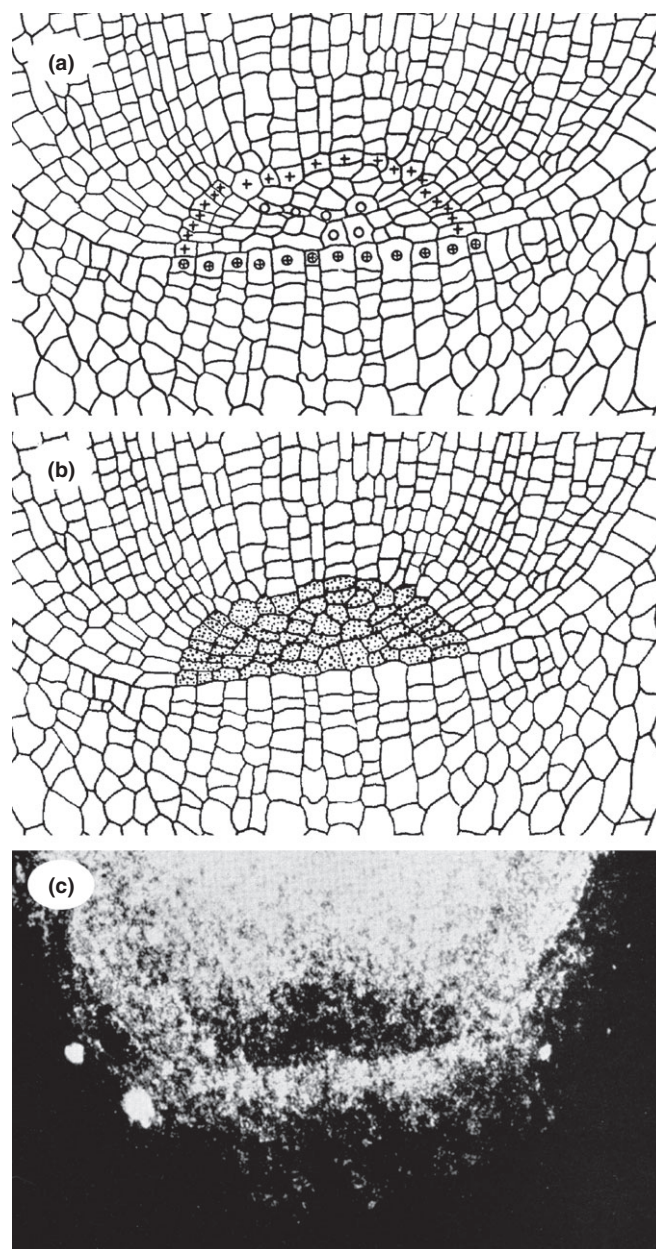


Fig. 3 Conceptual transition from ‘constructional centre’ to ‘quiescent centre’. All panels show longitudinal median sections of *Zea mays* root. (a) In his study of 1954a, Clowes, on the basis of an anatomical analysis of the promeristem (outlined), shows an image which includes both initial cells (+) and the cells that constitute a minimal constructional centre (o). (b) The same image as in (a) was used in the 1956a paper to outline a hypothetical quiescent centre where cells rarely divide. It is indicated in the text of this 1956a paper that the basicopic boundary of the quiescent centre can fluctuate and is not well defined. (c) An autoradiographic preparation of *Zea* root; section made after the live root was fed with $8\text{-}^{14}\text{C}$ -adenine. A pseudo-darkfield image shows white areas where silver grains are present in the autoradiographic film, and a dark area which corresponds to the quiescent centre, where no adenine incorporation into DNA had taken place. Figure panels reproduced with permission.

and inside the QC (Clowes, 1961, 1971, 1975b, 1982a,b; de la Torre & Clowes, 1974); the developmental origin of the QC during embryogenesis and lateral root formation (Clowes, 1958, 1978); and the role of the QC during regeneration of root tissue

(Clowes, 1959b, 1969, 1970a; Clowes & Stewart, 1967), particularly after acute or chronic ionizing radiation (Clowes, 1967). He also examined experimentally the role of nutrition in respect to the quiescence of the QC (Clowes, 1970b). The wide distribution of the QC within roots of the Angiosperms was also shown (Clowes, 1984). For a widely used model species, *Arabidopsis thaliana*, the structural and functional initials of the root are considered to be the QC and stem cells, respectively (reviewed in Bennett & Scheres, 2010). Important research areas developed by Frederick Albert Lionel Clowes expanded rapidly and have led to the current understanding of the cellular bases and genetic networks involved in root meristem maintenance and root growth. This would not have been possible without the formulation of the QC concept.

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