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Encoding specificity in Ca^{2+} signalling systems

Martin R. McAinsh and Alistair M. Hetherington

Ca^{2+} acts as a second messenger in many of the diverse range of signal-transduction pathways of plants. This raises fundamental questions regarding the mechanism(s) by which these pathways can be specific and how Ca^{2+}-based signalling systems can be used to produce the graded physiological responses that are typical of many extracellular stimuli. Recent studies of stimulus–response coupling have begun to uncover some of the answers to these questions.

The Ca^{2+} cation is now firmly established as an intracellular second messenger that couples a wide range of extracellular stimuli to characteristic responses in plant cells. Since initial reports of a stimulus-induced increase in the concentration of cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyt}$) in higher plants[1], there has been a massive increase in the number of signalling systems known to use $[Ca^{2+}]_{cyt}$ as an intracellular second messenger[2]. However, the very widespread occurrence of this second messenger has prompted researchers to ask how Ca^{2+}-based signalling systems can be specific. The importance of this question is well illustrated in stomatal guard cells. To achieve the optimum stomatal aperture under a specific set of environmental conditions, guard cells integrate signals from a range of often conflicting extracellular stimuli, many of which use Ca^{2+} as a second messenger. For example, a potential problem arises when the guard cell encounters stimuli such as the plant hormones abscisic acid (ABA) (Ref. 1) and auxin[3]. Both of these employ Ca^{2+} in their signal-transduction pathways, but individually the two hormones have opposing effects on stomatal aperture. An additional problem is how plants produce graded responses using Ca^{2+}-based signalling systems. Most plant cells do not produce an 'all or nothing' response to extracellular stimuli – instead, the magnitude of the response is usually directly related to the strength of the stimulus. This article highlights some of the recent advances that help explain the specificity of Ca^{2+}-based signalling systems.

Physiological address

Specificity in Ca^{2+} signalling systems is initially controlled by whether or not a cell is competent to respond to a given stimulus. This is dependent on the cell expressing genes encoding the range of signalling components comprising the 'signalling cassette'[4] that is required for the transduction of a particular signal. In turn, the pattern of genes expressed will be dictated by the local environment of the cell and on the battery of environmental stimuli to which the cell has been exposed during development. We have termed this the 'physiological address' of the cell[5]. For example, it is unlikely that cells that do not express the genes encoding the elicitor-activated, Ca^{2+}-permeable ion channel would be able to respond to the oligopeptide elicitor derived from *Phytophthora sojae*[6]. Further support for the concept of the physiological address comes from investigations of ABA signalling in guard cells. It has been proposed that the growth history of the plant has a marked influence on whether guard cells employ a Ca^{2+}-dependent signalling pathway to transduce the ABA signal[7]. Therefore, these data suggest that specificity is dictated initially by the physiological address of the cell.

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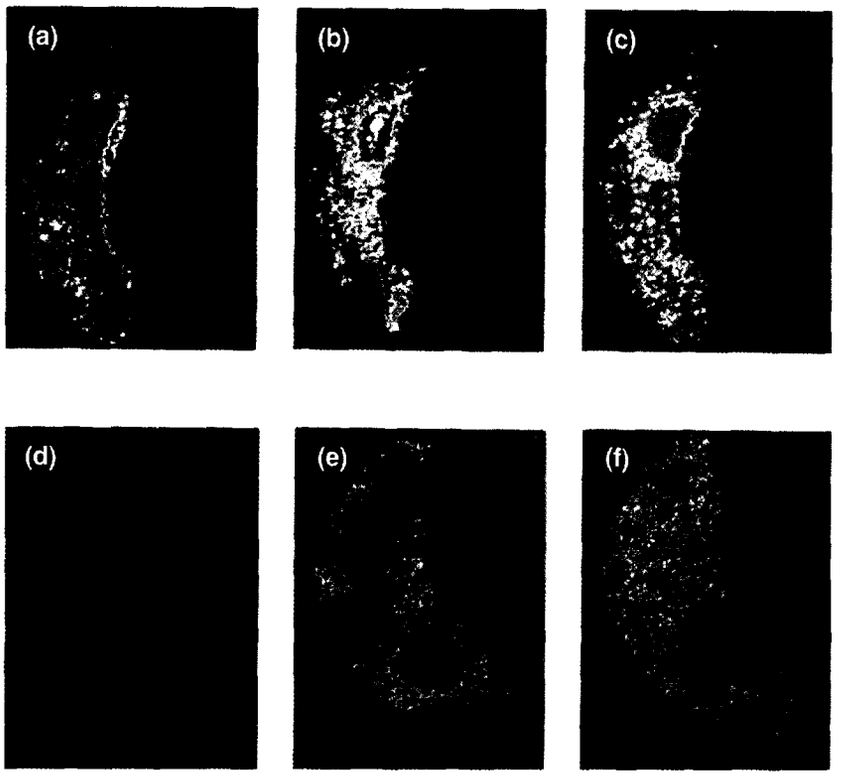

Fig. 1. Increases in cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyt}$) in guard cells of open stomata of *Commelina communis* in response to 100 nM abscisic acid (a–c) and 1 mM external Ca^{2+} (d–f). The $[Ca^{2+}]_{cyt}$ was monitored using the fluorescent Ca^{2+} indicators fura-2 and indo-1 microinjected into the cytosol of individual guard cells. The distribution of 'resting' $[Ca^{2+}]_{cyt}$ (a, d) and stimulus-induced increases in $[Ca^{2+}]_{cyt}$ approximately 15 s (b, e) and 2 min (c, f) following the addition of the stimulus are indicated by colour (blue indicates low $[Ca^{2+}]_{cyt}$; red indicates high $[Ca^{2+}]_{cyt}$). The images show the heterogeneous nature of the stimulus-induced increases in $[Ca^{2+}]_{cyt}$. These data suggest that plant cells have the capacity to encode specificity in the Ca^{2+} signal in the form of localized increases in $[Ca^{2+}]_{cyt}$. *Reproduced, with permission, from Refs 2 and 10.*

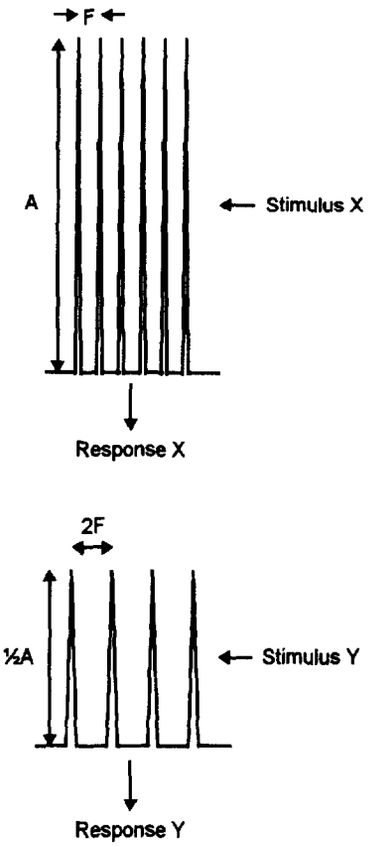

Fig. 2. Encoding signalling information in the amplitude, A (also termed 'analogue'-encoded information), and in the frequency, F (also termed 'digital'-encoded information), of Ca^{2+} spikes and oscillations in cytosolic free Ca^{2+}. For example, stimulus X may induce one pattern of oscillations, with amplitude A and frequency F, giving response X; a second stimulus, Y, may induce a completely different pattern of oscillations, with amplitude 0.5A and frequency 2F, to give a different response, Y.

The Ca^{2+} signature – a stimulus-specific Ca^{2+} signal

Given that the cell is competent to respond to a range of stimuli through the generation of a Ca^{2+} signal (i.e. a stimulus-induced increase in $[Ca^{2+}]_{cyt}$), how then does it differentiate between different Ca^{2+}-mobilizing stimuli? The answer may lie in the ability of cells to generate increases in $[Ca^{2+}]_{cyt}$ that are unique, in terms of their spatio-temporal characteristics, in response to an individual stimulus. We have referred to such stimulus-specific Ca^{2+} signals as 'Ca^{2+} signatures'[8]. Figure 1 shows a series of digital ratio images of stomatal guard cells with Ca^{2+} indicators loaded into the cytosol. This type of analysis provides maps of the concentration of Ca^{2+} inside the cell, and these images clearly show the heterogeneous nature of stimulus-induced increases in $[Ca^{2+}]_{cyt}$, with both hot-spots and Ca^{2+}-quiescent regions. This suggests that plant cells certainly have the capacity for specificity in the form of localized increases in $[Ca^{2+}]_{cyt}$. In animal cells, such increases are known to play an important role in defining signal specificity. For example, in AtT20 cells (a mouse pituitary cell line), it has been shown that elevations in nuclear Ca^{2+} control Ca^{2+}-activated gene expression via the cyclic AMP response element, while increases in $[Ca^{2+}]_{cyt}$ regulate gene expression through the serum response element[9].

Recent evidence also suggests a role for temporal heterogeneities – differences in the kinetics of stimulus-induced increases in $[Ca^{2+}]_{cyt}$ – in the generation of the Ca^{2+} signature in plants. Oscillations[10,11] and waves[12] in $[Ca^{2+}]_{cyt}$ have both been reported in plant cells. In guard cells, it has been shown that the pattern of oscillations induced by external Ca^{2+} correlates directly with the concentration of the stimulus and the magnitude of the final response[10]. In addition, the process of cold acclimation is associated with a change in the cold-shock Ca^{2+} signature[13]. Taken together, these data demonstrate a potential mechanism for producing the important graded response described previously via modifications to the kinetics of the Ca^{2+} signature.

Work in animal cells has shown clearly that differences in the kinetics of the increase in $[Ca^{2+}]_{cyt}$ are important for encoding specificity in the Ca^{2+} signal. Stimulus-induced increases in $[Ca^{2+}]_{cyt}$ can occur in the form of spikes, waves, oscillations and plateaus, all of which have the potential to encode signalling information. It was recently demonstrated that the amplitude and duration of Ca^{2+} signals differentially control the activation of transcriptional regulators[14]. Furthermore, work in pancreatic acinar cells[15] has shown that agonist-induced Ca^{2+} spikes in the micromolar range are necessary for the induction of exocytosis, whereas Ca^{2+} spikes in the submicromolar range are associated with the activation of luminal and basal ion channels.

Perhaps the most intensively studied aspect of Ca^{2+} signalling in terms of encoding

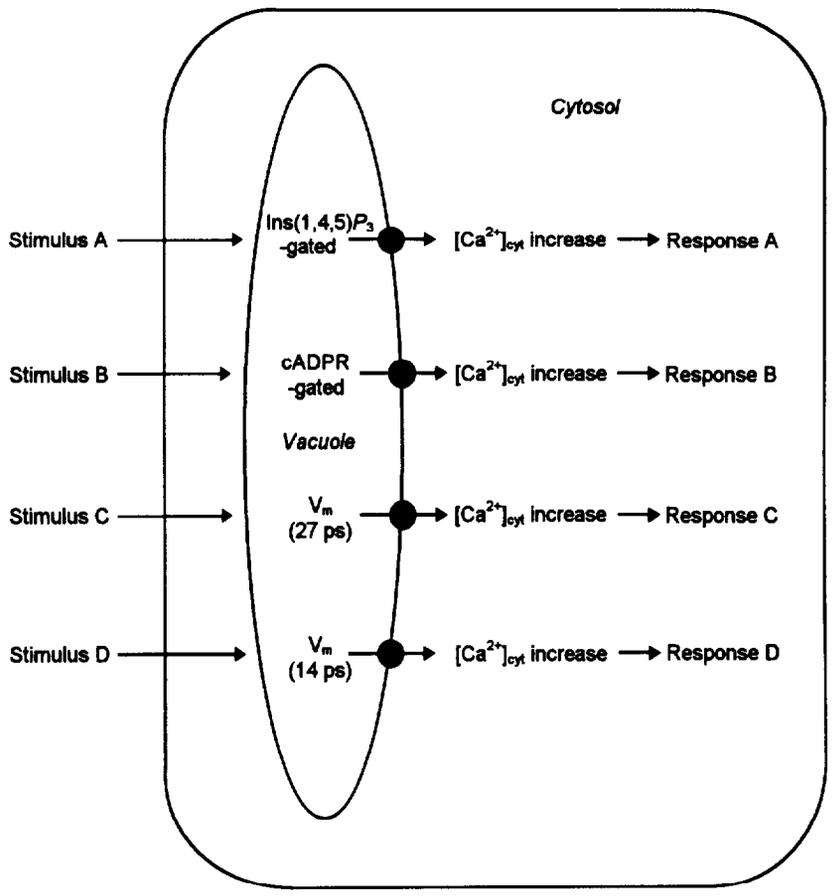

Fig. 3. A model illustrating the potential role of vacuolar Ca^{2+} channels in the generation of a stimulus-specific Ca^{2+} signature in stomatal guard cells. Different stimuli could induce an increase in cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyt}$) through the release of vacuolar Ca^{2+} via separate Ca^{2+}-mobilizing pathways. For example, stimuli A and B may cause Ca^{2+} release through channels gated by inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3] and cyclic ADP-ribose (cADPR), respectively, whereas stimuli C and D may cause Ca^{2+} release through two distinct, voltage-gated channels (V_m). Differences in the distribution, gating properties or sensitivity to regulatory factors (voltage, Ca^{2+} and pH) of these channels will all contribute to the spatial and temporal heterogeneities observed in stimulus-induced increases in $[Ca^{2+}]_{cyt}$. In turn, this will increase the amount of information encoded in the Ca^{2+} signature. For reasons of clarity, the contribution of 'cross-talk' between signalling cassettes (i.e. whether specific Ca^{2+} release channels and/or Ca^{2+}-mobilizing second messengers act as components of the signal transduction pathways of several different stimuli) is not included in this model.

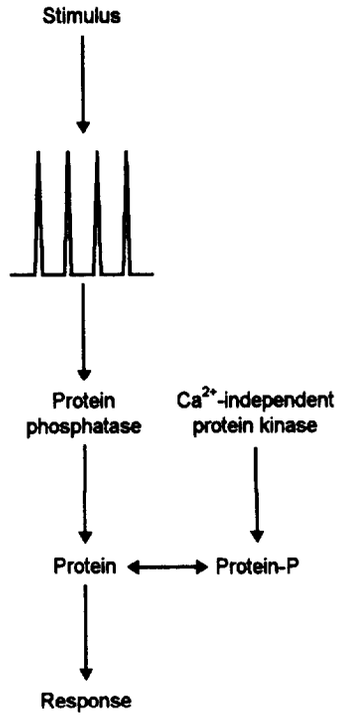

Fig. 4. A model for decoding the information encoded in spikes and oscillations in cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyt}$) based on phosphorylation–dephosphorylation events (e.g. the coupled action of a Ca^{2+}-activated phosphatase and a Ca^{2+}-independent kinase). Stimulus-induced Ca^{2+} spikes or oscillatory changes in $[Ca^{2+}]_{cyt}$ would modify the activity of a Ca^{2+}-dependent phosphatase while having no effect on the activity of a Ca^{2+}-independent kinase. Therefore, the level of the phosphorylated form of a target protein would go up and down against the constant background of kinase activity. During low-frequency Ca^{2+} spiking and oscillations in $[Ca^{2+}]_{cyt}$ with a long period, the protein will become highly phosphorylated in-between Ca^{2+} peaks; during spiking and oscillations with a higher frequency and a shorter period, much less phosphorylation will occur, resulting in the maintenance of a larger fraction of the dephosphorylated protein. The presence of phosphatases and kinases that exhibit different Ca^{2+} activation kinetics could allow differential decoding of stimulus-specific patterns of Ca^{2+} spikes and oscillations in $[Ca^{2+}]_{cyt}$ into a range of physiological responses that can occur downstream of phosphorylation events.

specificity in the Ca^{2+} signal has been the role of Ca^{2+} spikes and oscillations in $[Ca^{2+}]_{cyt}$ (Ref. 16). In animals, the mechanisms of generation and maintenance of such kinetics include both positive and negative feedback, often invoking the release of Ca^{2+} from intracellular stores through the action of additional second messengers such as inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3], and fluxes of Ca^{2+} across the plasma membrane or between intracellular stores[17–19]. Theoretical models also exist to account for how information might be encoded in the pattern of oscillations[20]. Information can be encoded in both the amplitude and frequency (also termed 'analogue' and 'digital'-encoded information, respectively) of the spikes and oscillations in $[Ca^{2+}]_{cyt}$ (Fig. 2) (Ref. 19).

Several components of Ca^{2+} signalling systems in plants have been identified that may constitute part of the mechanism(s) by which information is encoded in the Ca^{2+} signature. Two distinct voltage-gated Ca^{2+} release channels, which have the ability to perform almost exactly the same function *in vivo*, co-reside in the vacuolar membranes of guard cells[21]. More recent work has also reported the presence of Ins(1,4,5)P_3 and cyclic ADP-ribose-sensitive Ca^{2+} release pathways in the same vacuole in red beet[22]. These channels may form part of the signal transduction pathways of different stimuli. Differences in their distribution, gating properties or sensitivity to other factors such as voltage, Ca^{2+} and pH may all contribute to the spatial and temporal heterogeneities

observed in stimulus-induced increases in $[Ca^{2+}]_{cyt}$, and thus increase the amount of information encoded in the Ca^{2+} signature (Fig. 3). Although research into this area is at an early stage in plants, it is already apparent that the concept of the Ca^{2+} signature warrants further investigation.

Decoding Ca^{2+} signatures

Having generated the Ca^{2+} signal, the next requirement is to position the other elements of the signalling cassette in such a way that the Ca^{2+} signal can be relayed to the final effector responsible for the production of the response. For efficient transduction of the Ca^{2+} signal, the downstream elements in the signalling cassette need to be located close to the site of the increase in $[Ca^{2+}]_{cyt}$. This is particularly important when these increases are highly localized. As already discussed, increases in nuclear Ca^{2+} are important in the control of specificity. There is also evidence that indicates that Ca^{2+} concentrations immediately below the plasma membrane may be subject to rapid and dramatic changes. Use of a new Ca^{2+} indicator, FFP18, to monitor immediately submembrane, depolarization-induced changes in $[Ca^{2+}]_{cyt}$ has revealed that submembrane increases in $[Ca^{2+}]_{cyt}$ reach micromolar concentrations, while the mean $[Ca^{2+}]_{cyt}$ recorded using fura-2 rises only to a few hundred nanomolar[23]. These data suggest a mechanism for selectively activating Ca^{2+}-dependent processes located in the plasma membrane, and also warn of the potential dangers of conclusions based solely on indicators of global increases in $[Ca^{2+}]_{cyt}$. The importance of these highly localized increases in $[Ca^{2+}]_{cyt}$ in the control of stimulus specificity has recently been highlighted[24]. If highly localized increases in $[Ca^{2+}]_{cyt}$ also occur in plants, they could be very important in the control of plasma membrane ion channels that are known to be activated by Ca^{2+}. Several studies suggest that plant cells are capable of generating increases in $[Ca^{2+}]_{cyt}$ that are highly localized and that occur in the immediate region of the plasma membrane. For example, hypoosmotic shock induces transient elevations in $[Ca^{2+}]_{cyt}$ that are initiated at the apex of rhizoids of the marine alga *Fucus*[25]. Similar localized increases have also been reported in growing pollen tubes[26].

It is only possible to speculate about the signalling machinery downstream of Ca^{2+} that is responsible for decoding the information encoded in the Ca^{2+} signature. However, recent work has pointed to a role for calmodulin isoforms in the control of specificity[27,28]. In turn, differential expression of calmodulin genes could be strongly influenced by the physiological address of the cell. In animals, it has also been proposed that protein phosphorylation may provide a mechanism by which the signalling information encrypted in Ca^{2+} spikes and oscillations in $[Ca^{2+}]_{cyt}$ may be deciphered via the coupled action of a phosphatase and kinase[29]. A model involving a Ca^{2+}-activated phosphatase and a Ca^{2+}-independent kinase could be envisaged (Fig. 4). Ca^{2+}-dependent phosphorylation and dephosphorylation events have been implicated in the regulation of guard cell turgor[30,31], and therefore provide a plausible mechanism for decoding the signalling information encoded in $[Ca^{2+}]_{cyt}$ oscillations observed in this cell type. The presence of phosphatases and kinases with different Ca^{2+} activation kinetics could allow differential decoding of stimulus-specific patterns of oscillations in $[Ca^{2+}]_{cyt}$ into a range of physiological responses that can occur downstream of phosphorylation events.

Conclusion

These data indicate that increases in $[Ca^{2+}]_{cyt}$ in plants can be either localized or global, and show how specificity can be dictated by the local environment and the conjunction of the appropriate signal effectors. In addition, it is clear that the effective transmission of the signal is dependent on the appropriate downstream elements in the signalling cassette being positioned correctly. The subcellular location of these signalling elements in plants is currently being investigated.

Acknowledgements

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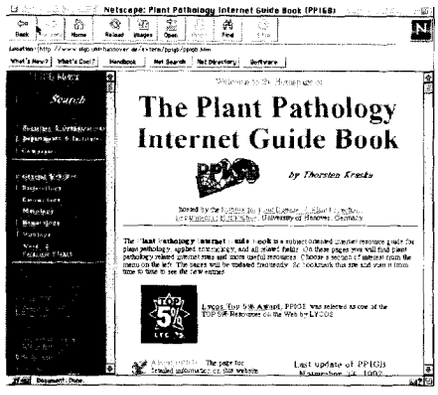
web review

The plant–fungus network: pathology online

Plant pathology is a vast research field, and pinning down key sites depends very much on your particular area of study. Perhaps because mushrooms are of interest to amateurs and specialists alike, those fungi that produce impressive fruiting bodies seem to be the most featured. A highly recommended place to begin is the **Mycology** site in **The World-Wide Web Virtual Library**, which is well organized and contains links to many informative sites (Box 1). Happily, this is true whether your interest is plant pathology or symbiosis. The linked sites are carefully selected, obviating the need to sift through dead-end URLs. There are essential connections to individual labs, institutions, publications and societies, with the best sites being mostly US-based. And if you need to regenerate the zeal for discovery, or to instil it in others, there is plenty of useful material. In **Fun Facts About Fungi**, you can watch as a morel fungus (Ascomyces) shoots its spores from the ascus, or learn that the fruiting body of the giant puffball (*Calvatia gigantea*) contains 7×10^{12} spores (which would take >221 000 years to count at one a second). It calls itself a 'kidpage', but don't be put off. **Fungi Images on the Net** is largely self-explanatory – there are >650 images, many of which are of exceptional quality.

The Plant Pathology Internet Guide Book, hosted by the **British Society for Plant Pathology (BSPP)** also has excellent links to resources, and the initial focus on plant pathology is helpful. For example, important research tools include access to culture collections and genetic stocks. The only disappointing feature is that the original, clear listing of linked sites on the **BSPP** site is not being updated regularly.



Box 1. Sites of interest to plant pathologists

The World-Wide Web Virtual Library: Mycology
http://www.keil.ukans.edu/~fungi/

Fun Facts About Fungi
http://www.herb.lsa.umich.edu/kidpage/factindx.htm

Fungi Images on the Net
http://www.pip.dknet.dk/%7Efvl/mushimage/imageframe.htm

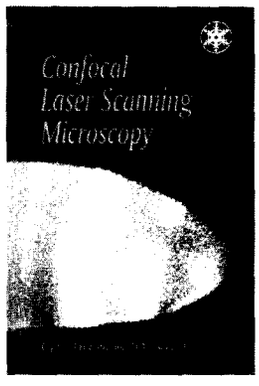
The Plant Pathology Internet Guide Book
http://www.ifgb.uni-hannover.de/extern/ppigb/ppigb.htm

British Society for Plant Pathology
http://www.bspp.org.uk/

book reviews

Lasers and plant cells

Confocal Laser Scanning Microscopy
by C.J.R. Sheppard and D.M. Shotton
Bios, 1997. £17.95 pbk (xii + 106 pages)
ISBN 1 872748 72 4



This book is one of a series of concise handbooks published in association with the Royal Microscopical Society and, in accordance with the fields of expertise of the two authors, deals with many aspects of confocal microscopy. As discussed, the confocal laser scanning microscope is used in the fluorescent mode for observing biological specimens. This is mostly because cell components and molecules can then be visualized after they have been tagged, directly or indirectly, with fluorescent dyes.

Plant cell biologists received the technique of confocal fluorescence microscopy with enthusiasm, because it could solve the problems,