ELSEVIER

Cell Calcium 38 (2005) 527–538

cell calcium

www.elsevier.com/locate/ceca

Proteinaceous and oligosaccharidic elicitors induce different calcium signatures in the nucleus of tobacco cells

David Lecourieux [a,1], Olivier Lamotte [a], Stéphane Bourque [a], David Wendehenne [a], Christian Mazars [b], Raoul Ranjeva [b], Alain Pugin [a,*]

[a] *UMR INRA 1088/CNRS 5184/Université de Bourgogne, Plante-Microbe-Environnement, 17 rue de Sully, BP 86510, 21065 Dijon cedex, France*
[b] *UMR CNRS-Université Paul Sabatier, Signaux et Messages Cellulaires chez les Végétaux, Pole de Biotechnologies Végétales, 24 chemin de Borde Rouge, BP 42617, Auzeville, 31326 Castanet-Tolosan, France*

Received 18 March 2005; received in revised form 18 May 2005; accepted 15 June 2005
Available online 28 September 2005

Abstract

We previously reported elevated cytosolic calcium levels in tobacco cells in response to elicitors [D. Lecourieux, C. Mazars, N. Pauly, R. Ranjeva, A. Pugin, Analysis and effects of cytosolic free calcium elevations in response to elicitors in *Nicotiana plumbaginifolia* cells, Plant Cell 14 (2002) 2627–2641]. These data suggested that in response to elicitors, Ca^{2+}, as a second messenger, was involved in both systemic acquired resistance (RSA) and/or hypersensitive response (HR) depending on calcium signature. Here, we used transformed tobacco cells with apoaequorin expressed in the nucleus to monitor changes in free nuclear calcium concentrations ($[Ca^{2+}]_{nuc}$) in response to elicitors. Two types of elicitors are compared: proteins leading to necrosis including four elicitins and harpin, and non-necrotic elicitors including flagellin (flg22) and two oligosaccharidic elicitors, namely the oligogalacturonides (OGs) and the β-1,3-glucan laminarin. Our data indicate that the proteinaceous elicitors induced a pronounced and sustainable $[Ca^{2+}]_{nuc}$ elevation, relative to the small effects of oligosaccharidic elicitors. This $[Ca^{2+}]_{nuc}$ elevation, which seems insufficient to induce cell death, is unlikely to result directly from the diffusion of calcium from the cytosol. The $[Ca^{2+}]_{nuc}$ rise depends on free cytosolic calcium, IP_3, and active oxygen species (AOS) but is independent of nitric oxide.

Keywords: Calcium; Elicitor; Cryptogein; Signal transduction; Nucleus; Protein kinases; Tobacco

1. Introduction

In the course of evolution, Ca^{2+} has emerged as one of the most versatile intracellular messengers carrying information to most processes that are important to cell life [1,2]. In recent years, most environmental challenges faced by plants including plant growth regulators, light, mechanical disturbances, abiotic stress, and pathogen elicitors have been shown to induce changes in cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) [3–7]. It has also been established that stimuli such as ABA and auxin, which elicit the closure and opening of stomata, respectively, induce increments in $[Ca^{2+}]_{cyt}$ [8–10]. There is now a firm recognition that changes in free calcium do not proceed in a stereotypical manner [3,6]. The nature, strength and duration of a stimulus are perceived differentially, and determine characteristic calcium changes, called calcium signatures, in cytosol and organelles [11–13]. Each signature is specified by its magnitude, shape, temporal and spatial parameters, which ultimately control the reversible binding of calcium to specific protein sensors before passing the decoded information onto targets. As a result, specific calcium signatures trigger altered protein phosphorylation, gene expression patterns [3,6] and the subsequent responses in plant cells [11,14,15,1]. Alternatively, Ca^{2+} is also proposed to act as a switch in the signalling process [16].

Both the vacuole and the endoplasmic reticulum (ER) are usually described as internal Ca^{2+} sources and/or buffering compartments for $[Ca^{2+}]_{cyt}$ regulation [17,18]. The mobilization of Ca^{2+} from these intracellular pools can

* Corresponding author. Tel.: +33 3 80 69 31 77; fax: +33 3 80 69 32 26.
E-mail address: pugin@dijon.inra.fr (A. Pugin).
[1] Present address: UMR CNRS 6161 Transport des Assimilats, Bâtiment de Botanique, 40 av. du recteur Pineau, F-86022 Poitiers cedex, France.

0143-4160/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ceca.2005.06.036

involve various second messengers as inositol tri-phosphate (IP_3), cyclic adenosine di-phosphate ribose (cADPR), nicotinic acid adenine dinucleotide phosphate (NAADP) or sphingosine-1-phosphate [13,19,20]. Changes in free calcium with physiological significance are also apparent in chloroplasts, mitochondria and nucleus [21–24]. The interplay between nuclear and cytosolic calcium in determining a global calcium signature and the elicitation of biological responses has been established [25,15]. The evidence for the importance of nuclear calcium in signalling processes is strengthened by the presence of calcium-dependent activities in the plant nucleus, including CDPK, calmodulin-binding protein and calcium-calmodulin regulated protein phosphatase [26–30]. It has been also shown that Ca^{2+}-ATPase and some components of the phosphoinositide signalling pathway localized to the plant nucleus [31,75]. Furthermore, it has been established that the plant nucleus is able to generate its own calcium changes even in an acellular environment [32,33].

The key role of Ca^{2+} in the signalling pathways received particular attention in the area of plant defense against pathogens [34]. We have participated in the identification of the initial events implicated in defense reaction by challenging tobacco (*Nicotiana tabacum* var. Xanthi) cell cultures with elicitors, particularly cryptogein, a 10 kDa polypeptide which is secreted by the oomycete *Phytophthora cryptogea* [35]. Cryptogein binding to its putative plasma membrane receptor [36,37] provokes a large and sustained calcium influx [38] which triggers numerous downstream events including the activation of a plasma membrane (PM) NADPH oxidase, the production of active oxygen species (AOS), cytosol acidification and the PM depolarisation [39], MAPK activation [40], protein phosphorylation [41], microtubule depolymerization [42], nitric oxide (NO) production [43,44], anion channel activation [45] and the inhibition of glucose transporter(s) [46]. The calcium influx led to a biphasic $[Ca^{2+}]_{cyt}$ elevation involving both extracellular and intracellular Ca^{2+} pools [7]. NO appears to be a fundamental component of the pathway(s) leading to Ca^{2+} release from intracellular pools [44]. We have shown further that the long sustained $[Ca^{2+}]_{cyt}$ elevation could be responsible for the sustained MAPK activation, microtubule depolymerization, defense gene activation and cell death [7]. The comparison of necrotic elicitors (cryptogein and other elicitins) and non-necrotic elicitors (carbohydrate derivatives) highlighted the role of the $[Ca^{2+}]_{cyt}$ signature in hypersensitive response (HR).

Here, we have used tobacco cell suspensions expressing the chimeric construct nucleoplasmin/apoaequorin in the nucleus [25,15], in order to monitor changes in free nuclear calcium ($[Ca^{2+}]_{nuc}$), to investigate the possible participation of the nuclear compartment in determining specific cellular calcium signature and to address the physiological significance of calcium rise in the nucleus. We compared the effects of necrotic and non-necrotic proteinaceous elicitors to those of non-necrotic carbohydrate derivatives. We provide data showing that both necrotic elicitins and harpin and the non-necrotic flagellin induced comparable biphasic $[Ca^{2+}]_{nuc}$ elevations with higher amplitude and longer duration than the $[Ca^{2+}]_{nuc}$ increases observed with the non-necrotic carbohydrate elicitors. The cryptogein-induced $[Ca^{2+}]_{nuc}$ elevation is not related to NO production but apparently involves an IP_3-dependent mechanism and depends partly of AOS production.

2. Results

2.1. Comparison of $[Ca^{2+}]_{nuc}$ elevations in response to different elicitors and relationships with cell death

To investigate the contribution of the nucleus to cellular calcium signatures in response to elicitor challenge, we have stably transformed *N. tabacum* cv. Xanthi cells with a chimeric gene using a nucleoplasmin/apo-aequorin construct [47,25,15]. In these conditions and using gold-labeled secondary antibodies, van der Luit et al. [25] demonstrated that about 90% of aequorin localized in nuclei of tobacco cells. As shown on Fig. 1, the expressed fusion protein was immunodetected mainly in the nucleus. By contrast, no fluorescence was observed in the nucleus of control tobacco cells; the fluorescence observed on the surrounding of both control and transformed cells can be explained by a non-specific binding of the aequorin antibody on the cell wall.

The transformed cells were challenged with elicitors that induce cell death (elicitins and harpin) or do not induce cell death (OGs, laminarin and flg22) and the subsequent changes in nuclear calcium were monitored. A resting $[Ca^{2+}]_{nuc}$ of 20 ± 1.4 nM ($n = 5$) was measured in aequorin-transformed tobacco cells and a cryptogein treatment induced a biphasic $[Ca^{2+}]_{nuc}$ elevation (Fig. 2A). Within 3 min, a first and transient $[Ca^{2+}]_{nuc}$ peak occurred, reaching 250 ± 31 nM ($n = 5$) after 5 min. This peak was immediately followed by a second $[Ca^{2+}]_{nuc}$ elevation. The additional increase in $[Ca^{2+}]_{nuc}$ reached a maximal of 1.5 ± 0.3 μM ($n = 5$) at 20 min, and decreased slowly but remain higher than the basal level even after 2 h of treatment (Fig. 2A and D). Increasing cryptogein concentrations from 2.5 to 100 nM resulted in the subsequent elevation of the two calcium peaks. The first one always appeared 5 min after addition of cryptogein. The appearance of the second one was accelerated by increasing the cryptogein concentration: from 35 min with 10 nM cryptogein to 15 min with 100 nM cryptogein (Fig. 2C).

Our previous data [7] which described the changes in cytosolic calcium, and the experiments described above provide for a comparison of the kinetics of $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{nuc}$ elevations induced by cryptogein in tobacco cells (Fig. 2B). In response to cryptogein treatment, bimodal increase in free Ca^{2+} concentration was elicited in both cytosolic and nuclear compartments. However, both the magnitude and kinetics of these increases differed considerably across the two compartments (Fig. 2B). Specifically, a 90-s