Correspondence

Pathogen-induced expression of plant ATP:citrate lyase[1]

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Hypersensitive response (HR) in plants is generally characterized by a rapid and localized cell death, cell wall strengthening and synthesis of phytoalexins and PR proteins [1]. In order to understand the molecular and cellular defense mechanisms better during HR that results from the interaction between plant pathogen and its non-host, we inoculated hot pepper (*Capsicum annuum* cv. Pukang) leaves with soybean pustule pathogen, *Xanthomonas campestris* pv. *glycines*8ra. A pool of genes induced or repressed by infection of the pathogen has been isolated by using a differential display-polymerase chain reaction (DD-PCR) technique (Yi et al., unpublished data). Approximately one half of the isolated genes had no sequence similarity in existing databases and the major portion of identified DNA fragments consisted of enzymes involved in primary or secondary metabolic pathways. One of the fragments was found to have significant sequence homology with ATP:citrate lyase (ACL) known from diverse organisms including alga, fungi, and mammals.

ACL is known to catalyze the following reaction: citrate+CoA+ATP → oxaloacetate+ADP+Pi+acetyl-CoA. In the cytosol of animals, oleaginous yeast, and fungi, acetyl-CoA produced by the action of ACL is the major precursor for fatty acid and sterol biosynthesis [2]. However, in plants, the biosynthetic origin of cytosolic acetyl-CoA that is needed for biosynthesis of phytochemicals is not established. In sweet potato, ACL activity has been observed to increase 10-fold in root tissues response to infection by the fungal pathogen, *Ceratocystis fimbriata*, and to correspond to the accumulation of phytoalexin ipomeamarone [3], but the molecular characterization of a plant ACL has not yet been reported.

In order to isolate a full-length cDNA clone encoding ACL, a cDNA library was constructed from hot pepper leaves inoculated with *X. campestris* pv. *glycines*8ra. After screening of the cDNA library by using a cDNA fragment of ACL produced by DD-PCR as a probe, a cDNA clone with an open reading frame containing 608 amino acids was finally isolated and designated as *Ca*-ACL1 (GenBank accession number: AF290958). The deduced amino acid of *Ca*-ACL1 gene has significant sequence homology with the C-terminal part of known animal ACLs. Calculated molecular mass of hot pepper ACL polypeptide was 66 kDa, which is only half of the animal ACLs. The ACL catalytic center, GHAGA, and the histidine residue that is autophosphorylated by ATP during catalysis are highly conserved in *Ca*-ACL1, but none of the three additional phosphorylation sites (Tyr446, Ser450, Ser454) that are involved in the regulation of rat ACL activity exists [2].

To investigate the expression kinetics of the *Ca*-ACL1 transcripts during inoculation with *X. campestris* pv. *glycines*8ra, total RNA was isolated from hot pepper leaves 0, 4, 8, and 12 h after inoculation, and Northern blot analysis was performed by using ^{32}P-labeled *Ca*-ACL1 as a probe. HR cell death on pepper leaves appeared at approximately 15 h post-infiltration, whereas no visible symptom was detected from the leaves infiltrated with buffer (0.9% NaCl). Pathogenesis-related protein 4 (PR4) probe was included as a positive marker of pathogen inoculation. Approximately 2 kb mRNA corresponding for *Ca*-ACL1 was significantly induced 4–8 h after inoculation of *X. campestris* pv. *glycines*8ra, but not in buffer-infiltrated tissues. In the duplicated blots, PR4 transcript was only expressed in leaf tissues inoculated with the pathogen (Fig. 1A).

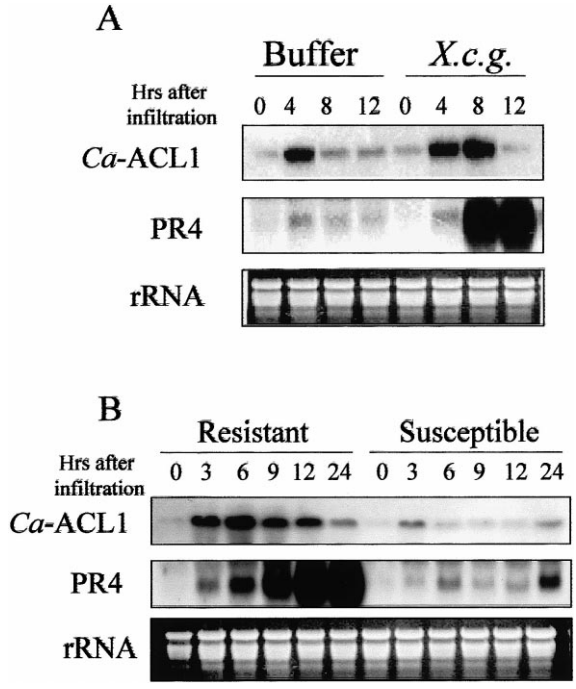

Fig. 1. Induction kinetics of *Ca*-ACL1 in hot pepper leaves after inoculation of bacterial pathogens. (A) 8-week-old hot pepper leaves were inoculated by syringe infiltration of buffer (0.9% NaCl) and *X. campestris* pv. *glycines*8ra (5×10^7 colony forming units (CFU)/ml), respectively. (B) The resistant *C. annuum* cv. ECW2DR (*BS2*) and susceptible *C. annuum* cv. ECW (*bs2*) cultivars were inoculated by syringe infiltration of *X. campestris* pv. *vesicatoria* race3 (avrBS2, 5×10^7 CFU/ml), respectively. The inoculated leaf tissues were harvested at indicated time points. An equal amount (20 μg) of total RNA from each sample was loaded in each lane. Ethidium bromide staining of the gel prior to transfer indicates equal loading of RNA.

To analyze whether induction of *Ca*-ACL1 mRNA level is specific to incompatible plant–microbe interaction, we inoculated two nearly isogenic pepper lines, *C. annuum* cv. ECW2DR (*BS2*) and *C. annuum* cv. ECW (*bs2*) with a natural pepper pathogen *X. campestris* pv. *vesicatoria* race3 (avrBS2). In Fig. 1B, significant accumulation of the *Ca*-ACL1 transcript was observed in the resistant (*C. annuum* cv. ECW2DR) but not in the susceptible (*C. annuum* cv. ECW) strain. In the identical blot, a higher level of PR4 mRNA was only detected in the resistant strain. In both cases, induced levels of *Ca*-

ACL1 transcript were detected within 4 h of pathogen infiltration that preceded the expression of PR4 mRNA and preferentially associated with the HR in pepper.

The HR triggered by incompatible plant–microbe interaction is often associated with systemic acquired resistance, leading to the activation of plant defense responses in the surrounding and distal uninfected parts of the plant [1]. Salicylic acid and hydrogen peroxide are generally considered as cellular signals for the activation of plant defense responses. To investigate whether *Ca*-ACL1 transcripts are systemically expressed, Northern blot analysis was carried out using total RNA samples isolated from healthy leaves, inoculated leaves, and the first upper leaves from the inoculated site. The induced level of *Ca*-ACL1 transcript was only detected in the inoculated leaves, indicating that *Ca*-ACL1 transcript is not systemically induced upon incompatible pathogen infection (data not shown). To identify cellular signaling molecules that induce the expression of *Ca*-ACL1, we treated hot pepper leaves with salicylic acid, H_2O_2, or wounding. The expression of *Ca*-ACL1 mRNA was only mildly affected by these treatments (data not shown).

In this paper, we describe a novel plant ACL cDNA, whose expression is up-regulated by pathogen infection. Our genetic evidence is consistent with the biochemical data on sweet potato and fungal pathogen interaction [3]. Many plants induce the synthesis of chemically heterogeneous phytoalexins family to protect themselves against pathogen attack at infection site. For example, the sesquiterpene ipomeamarone is produced from farnesyl diphosphate in sweet potato infected by *C. fimbriata*, and bicyclic diterpene casbene from geranylgeranyl diphosphate by caster bean (*Ricinus communis*) seedling is greatly stimulated following infection with *Rhizopus stolonifer* [3,4]. It has been reported that pyruvate is a more effective precursor for sesquiterpene biosynthesis than citrate, and a cytosolic pathway which utilizes the enzymes pyruvate decarboxylase, aldehyde dehydrogenase and acetyl-CoA synthetase, may play an important role in supply of acetyl-CoA for sesquiterpene biosynthesis [5]. However, there is now a need to further analyze whether ACL1 might be involved in the production of acetyl-CoA required for the formation of phytoalexins after pathogen infection in the hot pepper.

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[1] GenBank accession number of hot pepper ATP:citrate lyase is AF290958.

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