*Journal of Experimental Botany, Vol. 54, No. 392, pp. 2589–2591, November 2003*
DOI: 10.1093/jxb/erg282

Journal of Experimental Botany

**GENE NOTE**

# *StCDPK1* is expressed in potato stolon tips and is induced by high sucrose concentration

Marcela Raíces[1], Rita M. Ulloa[1], Gustavo C. MacIntosh[2], Martín Crespi[3] and María T. Téllez-Iñón[1,*]

[1] Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, CONICET, FCEyN, Universidad de Buenos Aires. Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina
[2] Delaware Biotechnology Institute, 15 Innovation Way Newark, DE 19711, USA
[3] Institut des Sciences Végétales, CNRS, 1 Avenue de la Terrasse, F-91, 198 Gif sur Yvette, France

Received 7 May 2003; Accepted 21 July 2003

**Abstract**

*StCDPK1* encodes a calcium-dependent protein kinase (CDPK) from *Solanum tuberosum*, which is transiently induced upon tuberization in swelling stolons. *In situ* hybridization determined that StCDPK1 mRNA is localized in the apical dome of tuberizing stolon tips, close to the region where sucrose was reported to accumulate. The expression of *StCDPK1*, and other tuber-specific genes was enhanced when *in vitro*-cultured potato plants were transferred to high sucrose or high sorbitol containing media. Glucose, fructose or a mixture of both showed no effect on CDPK expression. Okadaic acid blocked sucrose-inducible gene expression, suggesting that phosphatases from the PP1/PP2A family could also participate in the regulation of *StCDPK1* and other tuberization-related genes.

Key words: Gene expression, okadaic acid, potato stolons, StCDPK1, sucrose.

Sugars are not only important energy sources and structural components, they are also central regulatory molecules controlling metabolism, the cell cycle, development, and gene expression (Sheen *et al*., 1999). It was suggested that sugars could act as morphogens providing positional information to the cell cycle machinery and different developmental programmes (Rolland *et al*., 2002).

Potato tuberization is an interesting system in which to study the sucrose regulation of gene expression. This process involves a switch in assimilate phloem unloading in the subapical region of the developing stolon. Sucrose is the most abundant sugar in swelling stolons, while glucose and fructose concentration remain lower and show a similar pattern at all developmental stages (Viola *et al*., 2001). *In vitro* tuber formation is dependent on sucrose concentration and microtuber production can be obtained without any addition of other growth regulators in the culture medium (Garner and Blake, 1989). Sucrose seems to be a specific signal since neither glucose nor fructose is as effective as sucrose in meeting the sugar requirement for tuber development (Ewing, 1987).

High sucrose concentrations induce the transcription of several genes involved in tuber storage metabolism (Salanoubat and

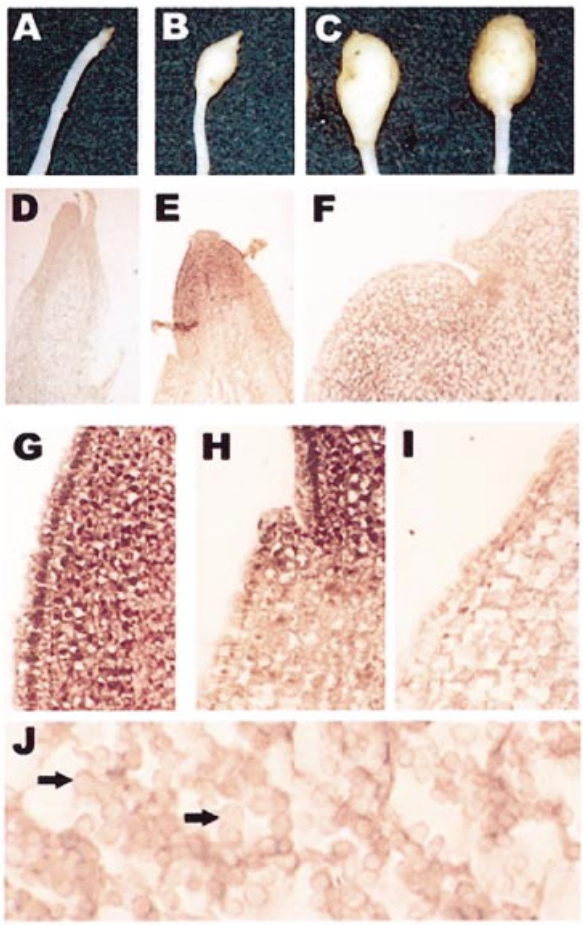


**Fig. 1.** Localization of StCDPK1 mRNA in potato tuberizing stolons from *Solanum tuberosum*, L. cv. Spunta plants cultivated in a greenhouse under a regime of 16/8 h light/dark at 25/20 °C. Semi-thin (7 μm) longitudinal sections (D–I) of early stolons (A) and induced stolons (B, C) were *in situ* hybridized with StCDPK1 DIG-labelled antisense mRNA probes. (D) Early stolons. (E) Shoot apical region of induced stolons expressing *StCDPK1*. (F) Late induced stolons. (G, H, I) Magnified images of sections equivalent to the one shown in (E). (J) Detailed image of (I), arrows indicate starch granules.

* To whom correspondence should be addressed. Fax: +54 11 4786 8578. E-mail: mtellez@dna.uba.ar

 

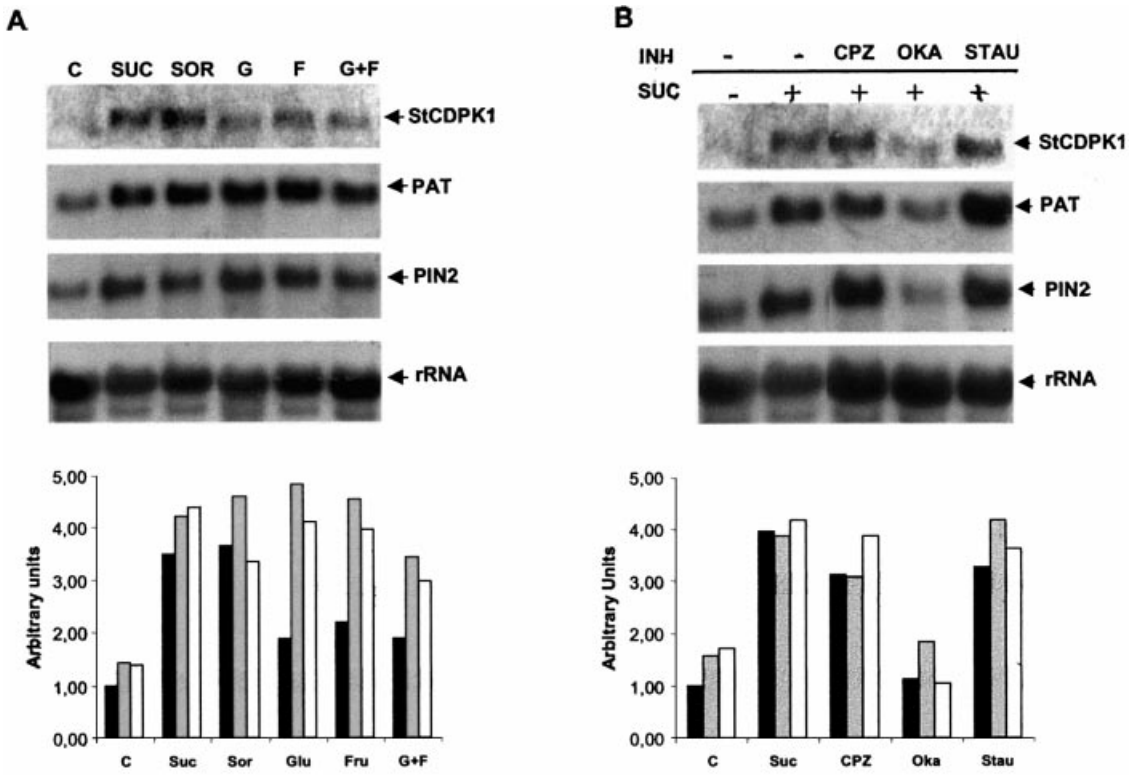


**Fig. 2.** For northern blot analysis, plants grown under a 16 h light photoperiod at 21 °C for 30 d, were transferred for 16 h under continuous light to: (A) liquid medium containing 2% (58 mM) sucrose (C), 8% (230 mM) sucrose (SUC) or equal mM concentrations of sorbitol (SOR) glucose (G), fructose (F) or a mixture of both (G+F); (B) liquid medium containing 2% sucrose (–), 8% sucrose (+) or 8% sucrose with the addition of inhibitors (INH): 0.5 mM chlorpromazine (CPZ), 100 nM okadaic acid (OKA) or 1 μM staurosporine (STAU). Total RNA (15 μg per lane) was hybridized with StCDPK1, patatin (PAT) or *Pin2* probes. Equal RNA loading was checked with a 17S rRNA probe. Relative quantification of StCDPK1 (black bars), patatin (grey bars) or *Pin2* (white bars) mRNAs is shown.

Belliard, 1989; Müller-Röber *et al*., 1990; Visser *et al*., 1994). Previously, it was suggested that a calcium-dependent protein kinase activity (CDPK) could be involved in the events leading to tuber formation (MacIntosh *et al*., 1996). Furthermore, StCDPK1, an active CDPK that is differentially expressed in swelling stolons was isolated (Raíces *et al*., 2001). It was then interesting to study if *StCDPK1* could be a potential target of sucrose regulation.

The spatial distribution of StCDPK1 transcripts was analysed in thin stolons (Fig. 1A), induced stolons (Fig. 1B, C) and mature tubers, using *in situ* hybridization as described by Crespi *et al*. (1994). Purple staining, indicating a positive hybridization signal, was found in the shoot apical dome of swelling stolons (Fig. 1E) whereas no hybridization signal was observed in early stolons (Fig. 1D), larger induced stolons (Fig. 1F), mature tubers (data not shown) or in sections incubated with the sense StCDPK1 probe (negative control, data not shown). These results correlate with previous Northern analysis (Raíces *et al*., 2001) except for the larger induced stolons. The weak signal detected at this stage could suggest that the kinase transcripts are dispersed or that mRNA expression was already down-regulated, since StCDPK1 is completely absent in mature tubers.

The strong signal detected in induced stolons (Fig. 1E) was observed in the cells from the apical region (Fig. 1G, H) and faded towards the proximal portion of the developing tuber, which is adjacent to the attached stolon. A large amount of starch granules was observed in the parenchyma of the region that lacks *StCDPK1* expression (Fig. 1I), suggesting that StCDPK1 transcript levels decline in the differentiated storage tissue.

StCDPK1 localization, close to the site where sucrose concentration accumulates upon tuber differentiation, prompted the authors to analyse whether sucrose was involved in the induction of *StCDPK1* expression. *In vitro*-cultured adult plants were transferred to liquid media containing 2% sucrose (control), 8% sucrose or osmotically equal concentrations of sorbitol, glucose, fructose or glucose plus fructose for 16 h. Northern blots indicated that sucrose and sorbitol treatments induced a 3.5-fold accumulation of StCDPK1 transcripts (Fig. 2A) that correlated with a 2.5–4-fold increase in CDPK activity (data not shown). Although a slight increase in transcript accumulation could be observed with glucose, fructose or a combination of both, none mimicked the induction obtained with sucrose or sorbitol (Fig. 2A). When the tuber-specific genes Patatin and *Pin2,* which are known to be up-regulated by sugars (Roitsch, 1999), were analysed, sucrose, sorbitol and all the monosaccharides tested were able to increase mRNA accumulation (Fig. 2A).

The fact that *StCDPK1* expression is specifically up-regulated by sucrose, but not by hexoses, could be of physiological relevance since sucrose is the most abundant sugar in swelling stolons tips and acts as a specific signal during *in vitro* tuber induction (Ewing, 1987). The effect of sorbitol on *StCDPK1* expression suggests that

osmotic stress could be a component of the sugar signal. It is possible that osmotic-stress activation of sucrose-phosphate synthase, which leads to an increase in sucrose concentration (Toroser and Huber, 1997; Geigenberger *et al*., 1999), could be involved.

Both kinase and phosphatase activities are induced at the onset of tuberization (MacIntosh *et al*., 1996) and have been implicated in the signal transduction pathways triggered by sugars (Smeekens, 2000). Kinase and phosphatase inhibitors were added to sucrose-treated plants to analyse their effect on sugar-inducible *StCDPK1* expression. The protein kinase inhibitor, staurosporine, and the calmodulin antagonist, chlorpromazine, had no effect on *StCDPK1* expression or any of the sugar-regulated genes analysed. By contrast, the addition of okadaic acid, a potent inhibitor of protein phosphatases from the PP1 and PP2A family, blocked the induction of *StCDPK1*, *Pin2* and patatin (Fig. 2B). These results suggest that, in the transduction of carbohydrate signals, protein dephosphorylation is required for the transcriptional activation of some tuberization-related genes. It can be proposed that StCDPK1 could be a key mediator in the signal transduction pathways triggered by sucrose during tuber development.

## Acknowledgements

We thank Dr Kikuta for the patatin clone and Dr Prat for the *Pin2* clone. We also thank Dr Maximiliano D'Angelo for critical reading of the manuscript. MTTI and RMU are members of CONICET and MR is fellow of UBA. This work was supported by grants from FONCyT, ICGEB (Trieste, Italy), CONICET, and UBA, and international co-operation CONICET-CNRS.

## References

**Crespi MD, Jurkevitch E, Poiret M, D'Aubenton-Carafa Y, Petrovics G, Kondorosi E, Kondorosi A.** 1994. *enod40*, a gene expressed during nodule organogenesis, codes for a non-translatable RNA involved in plant growth. *EMBO Journal* **13,** 5099–5112.

**Ewing EE.** 1987. The role of hormones in potato (*Solanum tuberosum* L.) tuberization. In: Davies PJ, ed. *Plant hormones and their role in plant growth and development*. Dordrecht: Kluwer Academic Publishers, 515–538.

**Garner N, Blake J.** 1989. The induction and development of potato microtubers *in vitro* on media free of growth-regulating substances. *Annals of Botany* **63,** 663–674.

**Geigenberger P, Reimholz R, Deiting U, Sonnewald U, Stitt M.** 1999. Decreased expression of sucrose-phosphate synthase strongly inhibits the water-stressed induced synthesis of sucrose in growing potato tubers. *The Plant Journal* **19,** 119–129.

**MacIntosh GC, Ulloa RM, Raíces M, Téllez-Iñón MT.** 1996. Changes in calcium-dependent protein kinase activity during *in vitro* tuberization in potato. *Plant Physiology* **112,** 1541–1550.

**Müller-Röber B, Kossmann J, Hannah CL, Willmitzer L, Sonnewald, U.** 1990. One of two different ADP-glucose pyrophosphorylase genes from potato responds strongly to elevated levels of sucrose. *Molecular and General Genetics* **224,** 134–146.

**Raíces M, Chico JM, Téllez-Iñón MT, Ulloa, RM.** 2001. Molecular characterization of StCDPK1, a calcium-dependent protein kinase from *Solanum tuberosum* that is induced at the onset of tuber development. *Plant Molecular Biology* **46,** 591–601.

**Roitsch T.** 1999. Source–sink regulation by sugar and stress. *Current Opinion in Plant Biology* **2,** 198–206.

**Rolland F, Moore B, Sheen J.** 2002. Sugar sensing and signaling in plants. *The Plant Cell* **14,** S185–205.

**Salanoubat M, Belliard G.** 1989. The steady-state level of potato sucrose synthase mRNA is dependent on wounding, anaerobiosis and sucrose concentration. *Gene* **84,** 181–185.

**Sheen J, Zhou L, Jang JC.** 1999. Sugars as signaling molecules. *Current Opinion in Plant Biology* **2,** 410–418.

**Smeekens S.** 2000. Sugar-induced signal transduction in plants. *Annual Reviews in Plant Physiology and Plant Molecular Biology* **51,** 49–81.

**Toroser D, Huber SC.** 1997. Protein phosphorylation as a mechanism for osmotic-stress activation of sucrose phosphate synthase in spinach leaves. *Plant Physiology* **114,** 947–955.

**Viola R, Roberts AG, Haupt S, Gazzani S, Hancock RD, Marmiroli N, Machray GC, Oparka KJ.** 2001. Tuberization in potato involves a switch from apoplastic to symplastic phloem unloading. *The Plant Cell* **13,** 385–398.

**Visser RGF, Vreugdenhil D, Hendriks T, Jacobsen EJ.** 1994. Gene expression and carbohydrate content during stolon to tuber transition in potatoes (*Solanum tuberosum*). *Physiologia Plantarum* **90,** 285–292.