[Plant Signaling & Behavior 2:6, 475-476, November/December 2007]; ©2007 Landes Bioscience

Article Addendum

Self-Incompatibility Involved in the Level of Acetylcholine and cAMP

Takafumi Tezuka[1,4,*]
Isamu Akita[2]
Natsuko Yoshino[3]

Divisions of [1]Life Sciences, Graduate School of Science; [2]Informatics for Natural Sciences, Graduate School of Human Informatics; [3]Technical Cooperation; Graduate School of Information Science; Nagoya University; Nagoya, Japan

[4]School of Health and Human Life; Nagoya Bunri University; Inazawa, Japan

*Correspondence to: Takafumi Tezuka; School of Health and Human Life; Nagoya Bunri University; Inazawa 492-8520 Japan; Fax: +81.52.835.8422; Email: shubunjp@yahoo.co.jp

Original manuscript submitted: 05/24/07
Manuscript accepted: 05/24/07

Previously published online as a *Plant Signaling & Behavior* E-publication: http://www.landesbioscience.com/journals/psb/article/4483

KEY WORDS

pollen tubes, self-incompatibility, *Lilium longiflorum*, cAMP, acetylcholie, AChE, ChAT

Addendum to:

Regulation of Self-Incompatibility by Acetylcholine and cAMP in Lilium Logiflorum

Tezuka T, Akita I, Yoshino N, Suzuki Y

J Plant Physiol; 2007; 164:878–85
PMID: 16882455
DOI: 10.1016/j.jplph.2006.05.013

ABSTRACT

Elongation of pollen tubes in pistils after self-pollination of *Lilium longiflorum* cv. Hinomoto exhibiting strong gametophytic self-incompatibility was promoted by cAMP and also promoted by some metabolic modulators, namely, activators (forskolin and cholera toxin) of adenylate cyclase and inhibitors (3-isobutyl-1-methylxanthine and pertussis) of cyclic nucleotide phosphodiesterase. Moreover, the elongation was promoted by acetylcholine (ACh) and other choline derivatives, such as acetylthiocholine, L-α-phosphatidylcholine and chlorocholinechloride [CCC; (2-chloroethyl) trimethyl ammonium chloride]. A potent inhibitor (neostigmine) of acetylcholinesterase (AChE) as well as acetylcholine also promoted the elongation. cAMP enhanced choline acetyltransferase (ChAT) activity and suppressed AChE activity in the pistils, suggesting that the results are closely correlated with self-incompatibility in *L. longiflorum*. In short, it came to light that cAMP modulates ChAT (acetylcholine-forming enzyme) and AChE (acetylchoine-decomposing enzyme) activities to enhance the level of ACh in the pistils of *L. logiflorum* after self-incompatible pollination. These results indicate that the self-incompatibility on self-pollination is caused by low levels of ACh and/or cAMP.

Cyclic AMP (cAMP) is an essential signaling molecule in both prokaryotes and eukaryotes.[1] The existence of cAMP in higher plants was questioned by some reviewers[2-4] in the mid 1970's, so that many workers were discouraged from studying roles in plant biology. However, its presence was confirmed by mass spectrometry[5] and infrared spectrometry[6] in the early 1980's and increasing evidence[7-12] now suggests that cAMP makes important contributions in plant cells, as in animals.

Lily (*Lilium longiflorum*) exhibits strong gametophytic self-incompatibility.[13,14] Thus, elongation of pollen tubes in the pistil after self-incompatible pollination in *L. longiflorum* cv. Hinomoto stops halfway, in contrast to the case after cross-compatible pollination (cross with cv. Georgia).[14] This self-incompatibility appears to be associated with the stress and self-incompatible pollination on stigmas of lilies results in activation and/or induction of enzymes such as NADH- and NADPH-dependent oxidases, xanthine oxidase, superoxide dismutase (SOD), catalase and ascorbate peroxidase in the pistils.[15] The activities of NADH- and NADPH-dependent oxidases (O_2^--forming enzymes), however, are known to be suppressed by cAMP[16] and increase in the level of cAMP in guinea pig neutrophils results in their decreased expression.[17] The level of O_2^- reactions with SOD is also decreased by cAMP.[18] In the case of the lily, inhibition of NADH- and NADPH-dependent oxidases by cAMP was found to be noncompetitive with NAD(P)H.[16] We hypothesized that decrease in active oxygen species such as O_2^- and suppression of stress enzyme activities in self-pollinated pistils of lily by cAMP might cause elongation of pollen tubes after self-pollination and this proved to be the case. Namely, elongation of pollen tubes after self-incompatible pollination in lily was promoted by exogenous cAMP at a concentration as low as 10 nM, a conceivable physiological level.[13] Moreover, similar elongation could be achieved with adenylate cyclase activators [forskolin(FK) and cholera toxin] and cAMP phosphodiesterase inhibitors [3-isobutyl-1-methylxanthine (IBMX) and pertussis toxin].[14,19] These phenomena led us to examine the involvement of endogenous cAMP in pistils after self-incompatible or cross-compatible pollination. As expected, the level of endogenous cAMP in pistils after self-pollination was approximately one half of that after cross-pollination. Furthermore, this was associated with a concomitant decrease in adenylate cyclase and increase in cAMP phosphodiesterase.[19]

Many researchers in the field of plant biology have been unsuccessful in attempts to estimate the quantity of cAMP and to detect activities of adenylate cyclase and cAMP phosphodiesterase. On major difficulty is the presence of proteases and we have

overcome this problem by using protease inhibitors, such as aprotinin and leupeptin.[19]

In 1947, acetylcholine (ACh) of higher plants was first reported in a nettle (*Urtica urens*) found in the Himalaya mountain range.[20] In 1983, its existence in plants was confirmed by mass spectrometry of preparations from *Vigna* seedlings.[21] In our preliminary studies, CCC (chlorocholinechloride), a plant growth retardant (specifically an anti-gibberellin), enhanced the elongation of the pollen tubes in pistils after self-incompatible pollination in lilies. This led us to investigate whether other choline derivatives cause similar effects and positive findings were obtained with ACh, acetylthiocholine and L-α-phosphatidylcholine.[22] Moreover, the elongation was also promoted by neostigmine, an inhibitor of acetylcholine esterase (AChE) activity. In line with these results, choline acetyltransferase (ChAT) demonstrated low and AChE high activity in pistils after self-incompatible pollination.

The positive influence of cAMP[14,19] and ACh[22] in pistils of *L. longiflorum* after self-incompatible pollination encouraged us to examine the involvement of these two molecules in regulation of pollen tube elongation of lily after self-incompatible and cross-compatible pollination. As a result, it was revealed that cAMP promotes ChAT and suppresses AChE activity in pistils after both self- and cross-pollination. In other words, the self-incompatibilty in pistils of *L. longiflorum* appears to be due to levels of ACh and/or cAMP below certain threshold values.

Hitherto, these substances have not been recognized to play important roles in the metabolic systems of higher plants. However, given their conservation through evolution, it is natural that such central metabolic substances make essential contributions, regardless of the organism. We have succeeded in establishing physiological functions of cAMP and ACh in pistils of lily[14,19,22] and this points to use of plant reproductive organs such as research materials. The exact responsibilities of the two molecules may depend on differences in tissues or organs of plants and further molecular biological studies in this area are clearly warranted. This issue is currently being investigated.

References

1. Taiz L. Signal transduction in eukaryotes. In: Taiz, L, Zeiger, E. eds. Plant Physiology. 2nd ed. Sinauer Associates, Inc., 1998:379-407.
2. Alvarez R, Moore TC, Vandepeute J. Formation of adenosine 5'-phosphorofluoridate and the assay of adenyl cyclase in barley seeds. Plant Physiol 1974; 53:144-8.
3. Niles RM, Mount MS. Failure to detect cyclic 3',5'-adenosine monophosphate in healty and crown gall tumorous tissues of *Vicia faba*. Plant Physiol 1974; 54:372-3.
4. Bredemeijer GMM. Effect of bud-pollination and delayed self-pollination on the induction of a possible rejection peroxidase in styles of *Nicotiana alata*. Acta Bot Neerl 1976; 25:107-16.
5. Newton RP, Gibbs N, Moyse CD, Wiebers JL, Brown EG. Mass spectrometric identification of adenosine 3':5'-cyclic monophosphate isolated from a higher plant tissue. Phytochemistry 1980; 19:1909-11.
6. Janistyn B. Gas chromatographic, mass- and infrared-spectrometric identification of cyclic adenosine-3':5'-monophosphate (c-AMP) in maize seedlings (*Zea mays*). Z Naturforsch 1981; 36:193-6.
7. Ishioka N, Tanimoto S. Involvement of cyclic AMP in adventitious bud initiation of Torenia stem segments. Plant Cell Physiol 1990; 31:91-7.
8. Lusini P, Trabalzini L, Franch GG, Bovalini L, Martelli P. Adenylate cyclase in roots of *Ricinus communis*: Stimulation by GTP and Mn^{2+}. Phytochemistry 1991; 30:109-11.
9. Pacini B, Petrigliano A, Diffley P, Paffetti A, Brown EG, Martelli P, Trabalzini L, Bovalini L, Lusini P, Newton RP. Adenyl cyclase activity in roots of *Pisum sativum*. Phytochemistry 1993; 34:899-903.
10. Kurosaki F. Role of inward K^+ channel located at carrot plasma membrane in signal cross-talking of cAMP with Ca^{2+} cascade. FEBS Lett 1997; 408:115-9.
11. Legendre L, Dercker JP, Wrisez F, Correze C, Audran JC, Haye B, Lambert B. Evidence for the existence of cAMP in lily plant flower tissues. Phytochemistry 1997; 44:769-74.
12. Jin XC, Wu WH. Involvement of cyclic AMP in ABA- and Ca^{2+}-mediated signal transduction of stomatal regulation in *Vicia faba*. Plant Cell Physiol 1999; 40:127-33.
13. Hiratsuka S, Tezuka T, Yamamoto Y. Use of longitudinally bisected pistils of *Lilium longiflorum* for studies on self-incompatibility. Plant Cell Physiol 1983; 24:765-68.
14. Tezuka T, Hiratsuka S, Takahashi SY. Promotion of the growth of self-incompatible pollen tubes in lily by cAMP. Plant Cell Physiol 1993; 34:955-8.
15. Tezuka T, Tsuruhara A, Suzuki H, Takahashi SY. A connection between the self-incompatibility mechanism and the stress response in lily. Plant Cell Physiol 1997; 38:107-12.
16. Tsuruhara A, Suzuki H, Tezuka T. Inhibition of the activity of NAD(P)H-dependent oxidase in pistils of *Lilium longiflorum* by cAMP. Plant Cell Physiol 1999; 40:1093-8.
17. Takenawa T, Ishitoya J, Nagai Y. Inhibitory effect of prostaglandin E2, forskolin, and dibutyryl cAMP on arachidonic acid release and inositol phospholipid metabolism in guinea pig neutrophils. J Biol Chem 1986; 261:1092-8.
18. Gulyaeva NV, Obidin AB, Marinov BS. Modulation of superpxide dismutase by electron donors and acceptors. FEBS Lett 1987; 211:211-4.
19. Tsuruhar A, Tezuka T. Relationship between the self-incompatibility and cAMP level in *Lilium longiflorum*. Plant Cell Physiol 2001; 42:1234-8.
20. Emmelin N, Feldberg W. The mechanism of the sting of the common nettle (*Urtica urens*). J Physiol 1947; 106:440-5.
21. Hoshino T. Identification of acetylcholine as a natural constituent of *Vigna* seedlings. Plant Cell Physiol 1983; 24:829-34.
22. Tezuka T, Akita I, Yoshino N, Suzuki Y. Regulation of self-incompatibility by acetylcholine and cAMP in *Lilium longiflorum*. J Plant Physiol 2007; 164:878-85.