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ATHK1 位于过氧化氢下游并通过调节钙通道介导保卫细胞 ABA 信号转导

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摘要 植物通过不断进化增强对环境中水分缺乏的抵抗能力. 脱落酸在植物干旱和渗透胁迫反应中起到重要的作用. 组氨酸激酶也被认为是作为感受子和调节子对水分亏缺作出反应. 本研究结果显示, 组氨酸激酶 1 介入了拟南芥脱落酸诱导的气孔信号转导, 该酶此前被认为是一种渗透调节因子. *ATHK1* 基因缺失突变体不能表现保卫细胞中正常的脱落酸反应, 包括气孔关闭、过氧化氢产生以及钙内流. 膜片钳及激光共聚焦结果显示, ATHK1 在脱落酸诱导的气孔关闭过程中可能位于过氧化氢下游, 并通过调节钙通道和保卫细胞钙震荡来起作用.

关键词
组氨酸激酶 1
过氧化氢
脱落酸
钙通道
保卫细胞

. , , . , [1]. , (abscisic acid, ABA) [2]. ABA , , (hydrogen peroxide, H_2O_2) [3~7]. (*Arabidopsis thaliana* histidine protein kinases, AtHKs) , 1(AtHK1) [8~11]. Urao [8,12] *ATHK1* , , , . , *ATHK1* mRNAs , . , . , [8,12,13]. , ATHK1 , . , ATHK1 [14]. ATHK1 ABA , ATHK1 ABA . , (H_2O_2) ( ) ABA [5,6,15]. , ATHK1 , , ATHK1 ABA/H_2O_2 .

1 材料和方法

( ) . ABA, H_2O_2,

英文版见: Lü D, Wang W, Miao C. ATHK1 acts downstream of hydrogen peroxide to mediate ABA signaling through regulation of calcium channel activity in *Arabidopsis* guard cells. Chin Sci Bull, 2012, 57, doi: 10.1007/s11434-012-5207-y

FURA3-AM, H_2DCF-DA, DPI Sigma . HPLC , . (DMSO) . ( ) . , (1 cm 0.5 cm), (MES-KCl, 50 mmol L^{-1} KCl, 10 mmol L^{-1} MES) . ABA , 0.3 mmol m^{-2} s^{-1} 2 h, , (Nikon TE100, 400 ×). ABA MES-KCl , 1 h . , 5 g, . , . ( ) . , , 16 h/8 h(/). 4~6 , [15] . (Narishige,). EPC-9 (Heka Instrument,) , PULSEFIT 8.7. 100 mmol L^{-1} $BaCl_2$, 0.1 mmol L^{-1} DTT, 4 mmol L^{-1} EGTA, 10 mmol L^{-1} HEPES-Tris, pH 7.1; 10 mmol L^{-1} $BaCl_2$, 0.1 mmol L^{-1} DTT, 10 mmol L^{-1} Mes-Tris, pH 5.6. ABA, H_2O_2 . ABA Ca^{2+} , 1 mmol L^{-1} NADPH, *D*- 510 490 mmol kg^{-1}. ( ) . H_2DCFDA *FURA3-AM* (DMSO) , 50 5 mmol L^{-1}. , , 10 μmol L^{-1} (*FURA3-AM*) 50 μmol L^{-1} (H_2DCFDA), 25 , 1 h (*FURA3-AM*) 30 min (H_2DCFDA). , . , 485 nm, 515~530 nm.

2 实验结果

T-DNA *athk1* WS Col-0, T-DNA . T-DNA , .

2.1 野生型与突变体在失水方面存在差异

, , ( ). , , 60 min 2 2 10% ((d)). , . , , ((c)). , . ATHK1 ABA ?

2.2 ATHK1 参与拟南芥 ABA 和 H_2O_2 诱导的气孔关闭过程

ABA . 2 . H_2O_2 ABA , H_2O_2 , ATHK1 . ABA 0~100 μmol L^{-1} (1(a)), H_2O_2 0~500 μmol L^{-1} (1(b)). ABA H_2O_2 . 2 ABA , 2 (1(a)); H_2O_2 ABA (1(b)). 10 μmol L^{-1} ABA 100 μmol L^{-1} H_2O_2 , (1(a) (b)), . ATHK1 ABA H_2O_2 . ?

2.3 ATHK1 缺失不能阻止保卫细胞中 ABA 诱导的 H_2O_2 产生

ABA H_2O_2 [6]. , H_2DCF H_2O_2 .

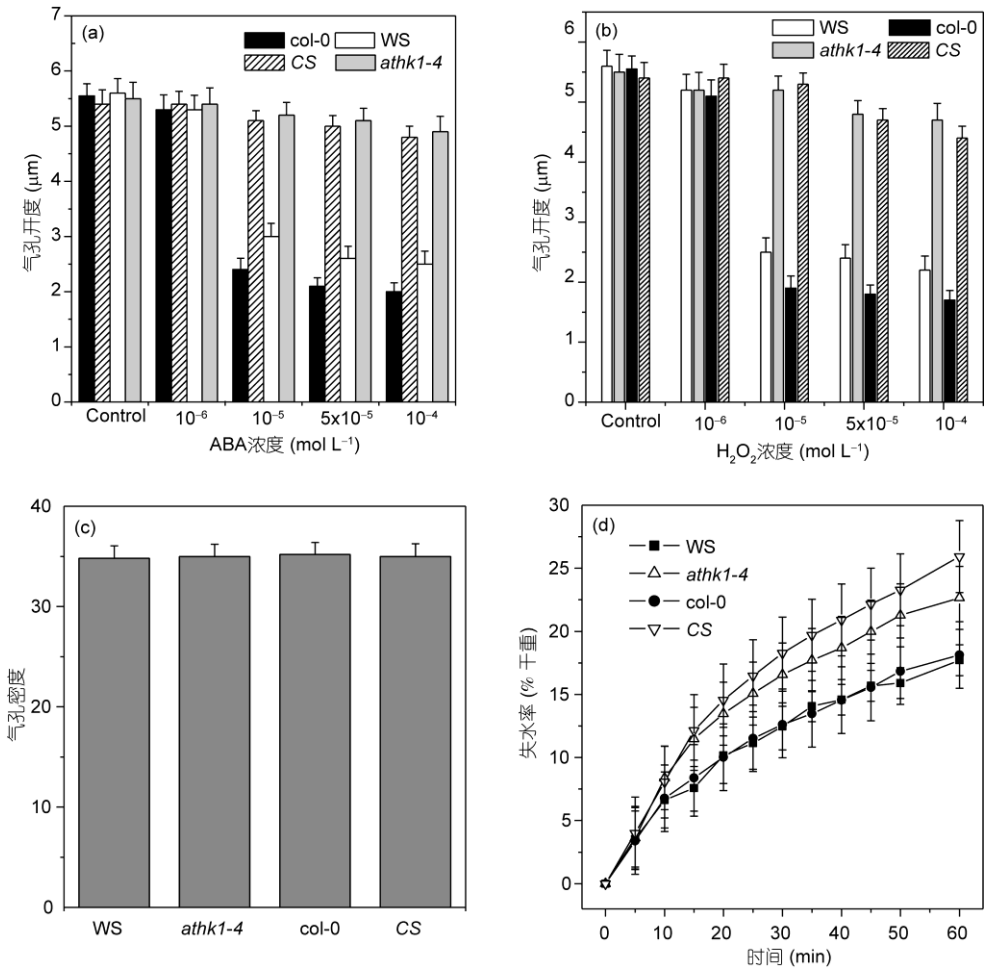

图 1　ATHK1 参与了 ABA 和 H_2O_2 诱导的气孔关闭过程

(a) ABA (WS & col-0) (*athk1-4* & *CS*) (*n* 250). (b) H_2O_2 (WS & col-0) (*athk1-4* & *CS*) (*n* 250). (c) (WS & col-0) (*athk1-4* & *CS*) . 5 , 100× 1 , . (d) (*athk1-4* & *CS*) (WS & col-0) (*n*=5)

ABA 10 min , .

H_2O_2 (2), , ABA . 10 10 , DPI H_2O_2 . min , 350 pA , , *AtHK1* ABA H_2O_2 70 pA ; , ATHK1 ABA H_2O_2 . NADPH DPI , 170 pA (3(a) (c)).

2.4　ATHK1 调节 ABA 和 H_2O_2 诱导的保卫细胞钙通道电流变化

, ABA (3(b) (c)), , 100 pA.

Pei [4] , H_2O_2 H_2O_2 , ABA . , , 350 pA (ABA H_2O_2 3(a) (d)), 100 pA , ATHK1 ? , (3(b) (d)).

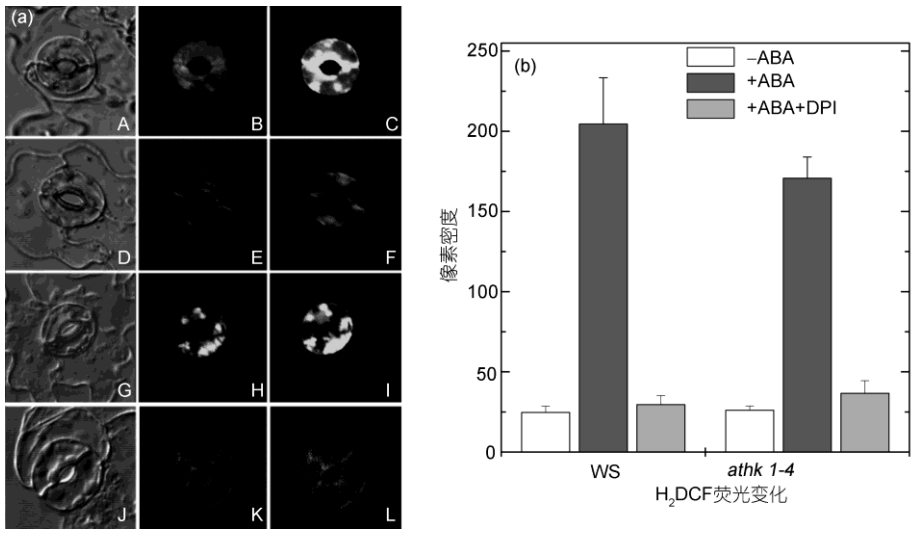

图 2 H_2DCF 荧光显示 ABA 处理前后 10 min 内 WS 野生型和 *athk1* 突变体保卫细胞中 H_2O_2 的产生情况

(a) (LSCM) ; A~C, WS+ABA; D~F, WS+ABA+DPI; G~I, *athk1*+ABA; J~L, *athk1*+ABA+ DPI. (b) H_2DCF (n=10). ABA 10 μmol L^{-1}, DPI 50 μmol L^{-1}

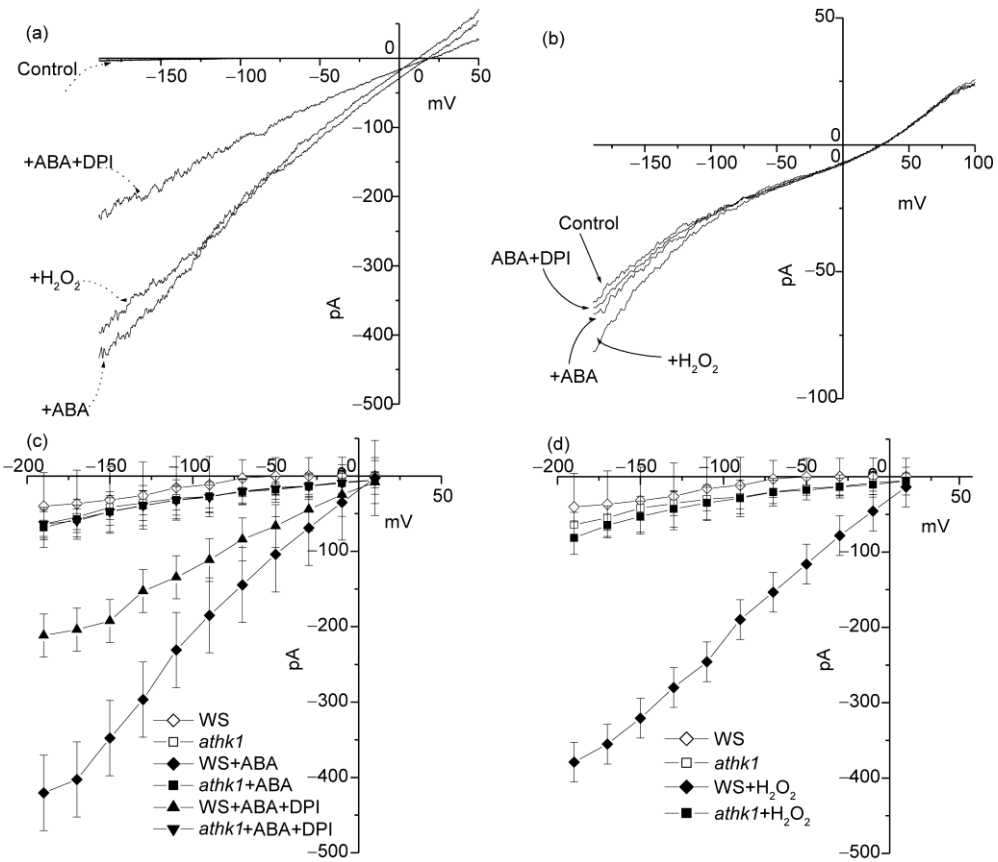

图 3 保卫细胞质膜上的钙通道受 ABA 和 H_2O_2 的影响在 WS 野生型和 *athk1-4* 中存在差异

(a) ABA DPI . (b) ABA DPI .
(c) , ABA DPI (n=10). (d) , H_2O_2 (n=10). ABA 10 μmol L^{-1}, H_2O_2 100 μmol L^{-1}, DPI 50 μmol L^{-1}

2.5 ATHK1 影响 ABA 和 H_2O_2 诱导的保卫细胞内钙浓度变化

ABA H_2O_2 , .

Ca^{2+} , ABA H_2O_2 Ca^{2+} . ATHK1 Ca^{2+} Ca^{2+} , ABA H_2O_2 . , Ca^{2+} . *FURA3-AM* .

, ABA *FURA3* , (4), 3 . DPI ABA , *FURA3* , .

2.6 ATHK1 调控保卫细胞内钙的浓度波动节律

. ATHK1 ?

time-course . , , ABA , (5(a)), (5(b)). , ( ) , (5(c)). , ABA , .

3 讨论

, ATHK1 ABA H_2O_2 . ABA , *ATHK1* ABA H_2O_2 ; , ABA , *athk1* (1). H_2O_2 [4,6], *athk1* ABA H_2O_2 (2), H_2O_2 , ATHK1 H_2O_2 . , ATHK1

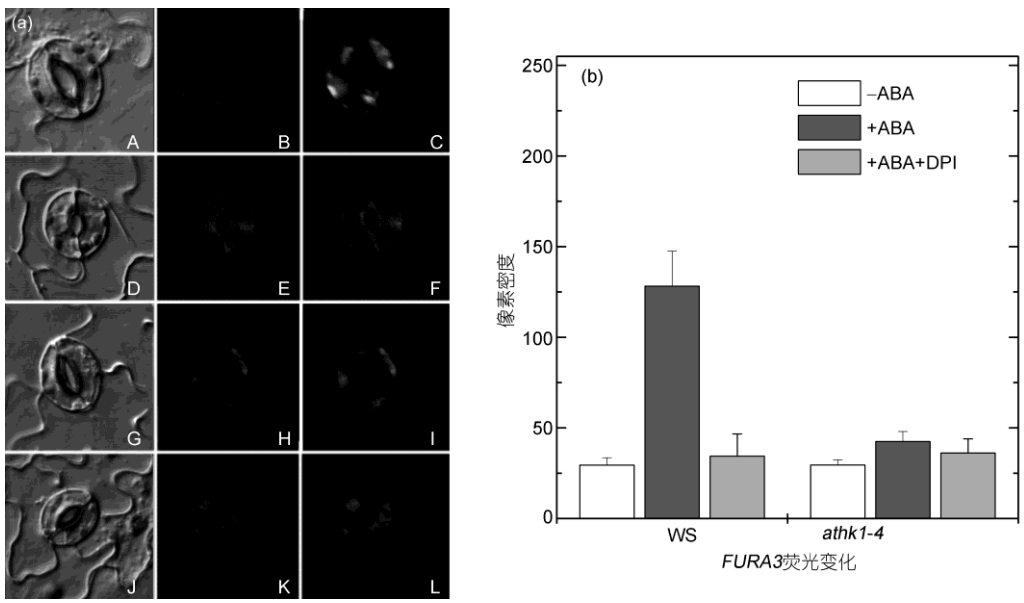

图 4 *FURA3* 荧光显示在 ABA 处理 10 min 后 WS 野生型和 *athk1* 突变体保卫细胞中钙的分布情况

(a) *FURA3* (*n*=10). A~C, WS+ABA; D~F, WS+ABA+DPI; G~I, *athk1*+ABA; J~L, *athk1*+ABA+DPI. (b) *FURA3* ABA 10 μmol L^{-1}, DPI 50 μmol L^{-1}

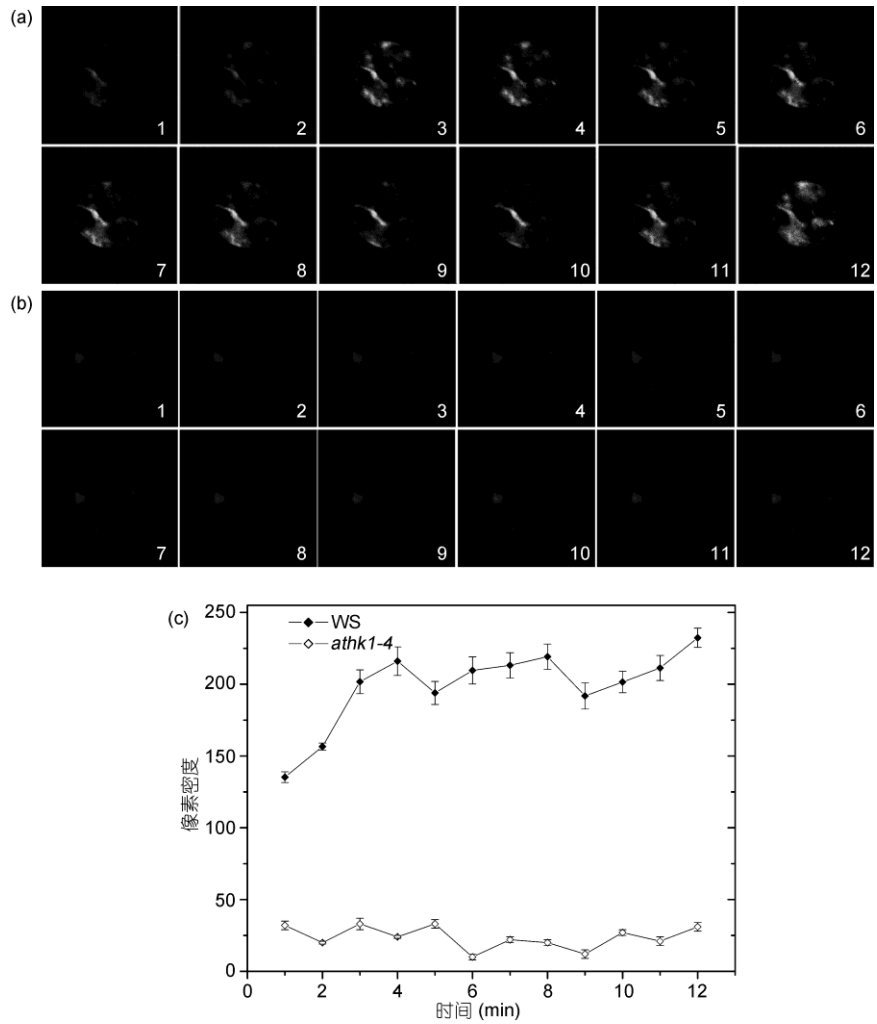

图 5 ***FURA3*** **荧光显示在 ABA 处理后 WS 野生型和** ***athk1*** **突变体保卫细胞内钙离子浓度的波动**

(a) . (b) *athk1* . (c) *FURA3* (n=5). ABA 10 μmol L^{-1}. ABA 1~12 min

H_2O_2 , ABA H_2O_2 , . ATHK1 ABA . : ATHK1, ATHK1 ? ATHK1 . H_2O_2 , ABA H_2O_2 , ABA , Ca^{2+} , , [4,5,15]. , AtHK1 , ABA H_2O_2 Ca^{2+} , [5,6,15]. , ABA H_2O_2 ATHK1 , H_2O_2 . ABA NADPH DPI . , WS , H_2O_2 ATHK1 ABA H_2O_2 (3). , (3). *athk1* ABA ABA, H_2O_2,

ATHK1 . 2),
H_2DCFDA *FURA3-AM* , ATHK1
H_2DCFDA ABA 10 min , .
10 , DPI
H_2O_2 (2). , ABA 10 min ,
FURA3 , ,
athk1 (4). , ATHK1 ATHK1, ABA, H_2O_2 Ca^{2+}
. . ATHK1 NADPH
, , , H_2O_2
ATHK1 , . , ATHK1 H_2O_2
, . 5 , . ATHK1
(Ca^{2+} ,
), (*athk1* , .

4 结论

参考文献

1 Schroeder J I, Allen G J, Hugouvieux V, et al. Guard cell signal transduction. Annu Rev Plant Physiol Plant Mol Biol, 2001, 52: 627–658
2 Wang P T, Song C P. Guard-cell signaling for hydrogen peroxide and abscisic acid. New Phytol, 2008, 178: 703–718
3 Kovtun Y, Chiu W L, Sheen J, et al. Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc Natl Acad Sci USA, 2000, 97: 2940–2945
4 Pei Z M, Murata Y, Benning G, et al. Calcium channels activated by hydrogen peroxide mediate abscisic acid signaling in guard cells. Nature, 2000, 406: 731–734
5 Zhang X, Miao Y C, An G Y, et al. K^+ channels inhibited by hydrogen peroxide mediate abscisic acid signaling in *Vicia* guard cells. Cell Res, 2001, 11: 195–202
6 Zhang X, Zhang L, Dong F C, et al. Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in *Vicia faba*. Plant Physiol, 2001, 126: 1438–1448
7 Murata Y, Pei Z M, Schroeder J I, et al. Abscisic acid activation of plasma membrane Ca^{2+} channels in guard cells requires cytosolic NAD(P)H and is differentially disrupted upstream and down-stream of reactive oxygen species production in *abi1-1* and *abi2-1* protein phosphatase 2C mutants. Plant Cell, 2001, 13: 2513–2523
8 Urao T, Miyataa S, Shinozakia K Y, et al. Possible His to Asp phosphorelay signaling in an *Arabidopsis* two-component system. FEBS Lett, 2000, 478: 227–232
9 Hwang I, Chen H C, Sheen J. Two-component signal transduction pathways in *Arabidopsis*. Plant Physiol, 2002, 129: 500–515
10 , , , . *ATHK1* . , 2004, 30: 553–560
11 Pareek A, Singh A, Kumar M, et al. Whole-genome analysis of *Oryza sativa* reveals similar architecture of two-component signaling machinery with *Arabidopsis*. Plant Physiol, 2006, 142: 380–397
12 Urao T, Yakubov B, Satoh R, et al. A transmembrane hybrid-type histidine kinase in *Arabidopsis* functions as an osmosensor. Plant Cell, 1999, 11: 1743–1754
13 Tran L P, Urao T, Qin F, et al. Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in *Arabidopsis*. Proc Natl Acad Sci USA, 2007, 104: 20623–20628
14 Wohlbach D J, Quirino B F, Sussmand M R. Analysis of the *Arabidopsis* histidine kinase ATHK1 reveals a connection between vegetative osmotic stress sensing and seed maturation. Plant Cell, 2008, 20: 1101–1117
15 Miao Y C, Lv D, Wang P C, et al. An *Arabidopsis* glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. Plant Cell, 2006, 18: 2749–2766