Down-regulation of plant V-type H^+-ATPase genes after light-induced inhibition of growth

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Received 18 December 1995; revised version received 21 March 1996

Abstract Cell extension growth in the mesocotyl tip of dark-grown *Zea mays* L. seedlings is dependent on vacuole enlargement and massive flux of ER and Golgi vesicles. Water flow into the expanding vacuole is driven by ion accumulation, which in turn is energized by the vacuolar H^+-ATPase (V-ATPase). The V-ATPase energizes the secondary ion transport into the expanding vacuole. As light exposure leads to a strong inhibition of extension growth, the effect of light on transcript levels for subunits A and c of the V-ATPase was analyzed. Partial homologous cDNAs for subunit A and two isoforms of subunit c were cloned by RT-PCR. In dark-grown seedlings transcript levels for both subunits were much higher in the growing mesocotyl tip than in the fully differentiated mesocotyl tissue. Only in the tip region did light exposure lead to a strong and coordinate down-regulation of both mRNAs whereas in the differentiated mesocotyl only a slight decrease was observed. The results indicate that expression of the 'housekeeping' V-type H^+-ATPase is strongly regulated in response to growth rate.

Key words: V-type H^+-ATPase; Subunits A and c; Extension growth; Etiolation; Light; Gene expression; *Zea mays* L.

1. Introduction

In higher plants the V-ATPase creates an electrochemical H^+-gradient across the tonoplast membrane [1,2]. It also acidifies the internal volume of Golgi vesicles [3]. Vacuolar acidification serves several important 'housekeeping' functions, but may also be involved in the salt stress response. Thus, salt exposure may affect transcript levels of V-ATPase genes [4-7]. However, expression of the V-ATPase is also under developmental control, as was recently demonstrated for *Mesembryanthemum crystallinum* [6,7], *Gossypium hirsutum* [8], and *Hordeum vulgare* [9]. In particular, expression of subunit c increases transiently during expansion of cotton petals [8], and in barley leaves the transcript level for subunit E was highest in the growing zone at the leaf base [9]. These observations suggest that expression of V-ATPase genes positively correlates with cellular growth. The aim of the present study was to test this hypothesis in a plant tissue, where growth can easily be manipulated without simultaneously inducing significant developmental changes. The mesocotyl of etiolated maize seedlings was chosen as a well defined experimental system. Here, elongation is the result of directional cell extension and is inhibited by light exposure [10-12]. In the etiolated seedling, cell division and elongation in the mesocotyl tip (5 mm) are dependent on intense traffic of ER and Golgi vesicles and vacuole expansion [13]. These processes are in turn dependent on luminal acidification by V-ATPase and, possibly, H^+-PP_iase, which resides on the same membrane [14]. We have studied the expression of V-ATPase genes coding for the catalytic subunit A (part of the peripheral V_1-complex) and the proton channel forming subunit c (part of the membrane-integral V_o-complex) [1]. Transcript levels of both subunits were analyzed in the growing mesocotyl tip (0–5 mm) and the mesocotyl base (5–70 mm) of 6-day-old etiolated *Zea mays* seedlings before and after light exposure. The results indicate that expression of V-ATPase genes is strongly correlated with extension growth.

2. Materials and methods

2.1. Plant material

Seeds of *Zea mays* L. cv. Lixis (Force Limagrain, Darmstadt, Germany) were germinated for 6 days at 23°C in the dark on moist filter paper. For light treatment 6-day-old dark-grown seedlings were exposed to light (300 μmol photons·m^{-2}·s^{-1}) and harvested after different time intervals. Control seedlings were grown in a growth chamber with a 16/8 h day/night cycle at 23°C. For RNA isolations the mesocotyls of etiolated seedlings were cut into two parts, the mesocotyl tip (0–5 mm) and the mesocotyl base (5–approx. 70 mm). For light-grown control seedlings the complete mesocotyl (length ca. 3 mm) was harvested.

2.2. Growth measurements

Extension growth of the mesocotyl was determined for three 5 mm zones starting from the mesocotyl tip using ink for demarcation. The length of mesocotyl zones after different growth periods was determined under a binocular microscope (resolution ±0.25 mm). Values are means of 20–40 replicates (± S.D.).

2.3. PCR-cloning of partial homologous cDNAs for subunits A and c of maize V-ATPase

Partial cDNAs for V-ATPase subunits A and c were amplified by RT-PCR from total RNA, which was isolated from coleoptile tips of 6-day-old dark-grown seedlings. Total RNA was purified by LiCl precipitation and used for first strand cDNA synthesis with M-MLV reverse transcriptase (Gibco BRL). Oligonucleotide primers were designed on the basis of conserved regions from subunit A of *Daucus carota* [15] and subunit c of *Avena sativa* [7,16]. Conditions for PCR [7] and nonradioactive probe labelling by PCR amplification [17] have been previously described. The amplified partial cDNAs were cloned blunt-ended into the *Eco*RV site of the pBluescript II SK vector (Stratagene). The cloned PCR fragments were sequenced with the DIG Taq DNA Sequencing Kit for Standard and Cycle Sequencing (Boehringer Mannheim).

2.4. RNA isolation and nonradioactive Northern blot

Total RNA was isolated by the method of Logemann et al. [18]. RNA samples were dissolved in formamide and stored at −80°C.

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The nucleotide sequences reported in this paper have been submitted to the GenBank/EMBL Data Bank with accession numbers X92373 (subunit A of V-ATPase), X92374 and X92375 (subunit c of V-ATPase).

PII S0014-5793(96)00336-5

Transcript levels for subunits A and c of V-ATPase were analyzed by nonradioactive Northern blot according to Löw and Rausch [17].

3. Results and discussion

3.1. Cloning of homologous partial cDNAs for subunits A and c of Zea mays V-ATPase by RT-PCR

Alignment of the partial cDNA sequence of subunit A from *Zea mays* (Fig. 1, upper) with the corresponding sequence of subunit A of *Daucus carota* (478–1113 bp) [15] reveals homologies at the DNA and protein level of 78% and 88%, respectively. Also, the putative active site, which was identified in the *Daucus carota* cDNA sequence (region B) [15], is present in the *Zea mays* sequence. This confirms the identity of the *Zea mays* sequence as a partial V-ATPase subunit A cDNA. For subunit c, two different partial cDNAs were amplified, which share 95% sequence identity at the DNA level (Fig. 1, lower); whether the different cDNAs represent isoforms, or, alternatively, result from Taq polymerase errors during PCR is not yet known. Sista et al. [19] have compared amino acid sequences of subunit c in different organisms and hypothesized on possible locations for the four membrane-spanning domains. Based on this comparison the partial subunit c cDNA sequences of *Zea mays* would include helix I, helix II, and part of helix III. For the expression analysis by Northern blot the cloned partial cDNAs were used as probes. As they derive from the highly conserved coding regions the detected transcript levels may always include more than one isoform.

3.2. Transcript levels for subunits a and c of V-ATPase in mesocotyl of light- and dark-grown Zea mays seedlings: evidence for light-induced coordinate down-regulation in the mesocotyl tip of etiolated seedlings

Message levels for both V-ATPase subunits were significantly higher in the mesocotyl tip of etiolated seedlings as compared to the base (Fig. 2). The difference appeared to be slightly more pronounced for subunit c than for subunit A. In the mesocotyl tip light exposure led to a strong decrease of both mRNAs (Fig. 2). The down-regulation of transcript levels in the mesocotyl tip after cessation of growth is first noticeable after about 3–6 h. After 8 h mRNA levels for both subunits were similar to those found in the mesocotyl base. To evaluate the possible contribution of age to the observed down-regulation, mRNA levels in 6.5-day-old etiolated seedlings were compared with those of 6-day-old seedlings exposed for an additional 12 h to light (Fig. 3). The result confirms that the down-regulation of mRNAs was unrelated to mesocotyl age.

The data presented support the hypothesis that expression of V-ATPase genes in the *Zea mays* mesocotyl is strongly linked to extension growth. While the mesocotyl tip zone (0–5 mm) of etiolated seedlings shows an extension growth of 86% during a 12-h growth period, this growth is reduced to 13% when seedlings are exposed to light (Fig. 4). It has been reported earlier that the growth rate declines rapidly after light exposure following a biphasic mode [11]. While a 50% reduction of growth rate occurs even within 2 h, a further reduction down to less than 10% is observed after 6–8 h [11]. The time course of transcript reductions for subunits A and c (Fig. 2) indicates that the decline of V-ATPase expression follows the light-induced cessation of growth with some delay.

Subunit A

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Z.m.  LKTIAIKSGDVYIPRGVSVPALDKDVLWEFQPTKLGVG  38
D.c.  -----KR----------------T------K-I-E-

Z.m.  DVITGGDLYATVFENTLMQHHVALPPGSMGKISYIAPA  76
D.c.  -LL----------S----------DA----T-V---

Z.m.  GQYNLQDTVLELEFQGIKKKFTMLQTWPVRSPRPVASK  114
D.c.  ---S-K----------V--Q----------T-------

Z.m.  LAADTPLLTGQRVLDALFPSVLGGTCCIPGAFGCGKTV  152
D.c.  -------------------------A----------

Z.m.  ISQALSKYSNSEAVVYVGCGERGNEMAEVLMDFPQLTM  190
D.c.  ----------DT-----------------------

Z.m.  QFADGRRESVMKRTTLVANTSN                  212
D.c.  TLP---E--------------
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Subunit c

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Z.m.  VPVVMAGVLGIYGLIIAVIISTGINPKAKPYYLFDGYA  38
A.s.  ------------------------------F------
O.s.  ------------------------------------
A.t.  ----------------------------S--------
G.h.  ----------------------------S--------
M.c.  ----------------------------S--------
C.m.  ----------------------------S--------
D.c.  ----------------------------S-------S
B.v.  ----------------------------S--------

Z.m.  HLSSGLACGLAGLPAGMAIGIVGDAGVRANAQQPKLFV  76
A.s.  ------------A-----------------------
O.s.  ------------A-----------------------
A.t.  ------------S-----------------------
G.h.  ------------S-----------------------
M.c.  ------------S-------------R---------
C.m.  ------------S-----------------------
D.c.  ------------S-----------------------
B.v.  ------------S-----------------------
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Fig. 1. Alignment of partial protein sequences of V-ATPase subunits A and c of *Zea mays* (Z.m., clones 70-3 and 16-2) with several corresponding sequences from other higher plants. Subunit A from *Daucus carota* (D.c.; [15]); subunit c from *Avena sativa* (A.s.; M73232), *Oryza sativa* (O.s.; U27098), *Arabidopsis thaliana* (A.t.; L44584), *Gossypium hirsutum* (G.s.; U13669), *Mesembryanthemum crystallinum*, *Clusia minor*, and *Daucus carota* (M.c., C.m., D.c.; [7]), and *Beta vulgaris* (B.v., Matthias Kirsch, unpublished). The partial cDNA clones from *Zea mays* were amplified by RT-PCR according to [7]. Nonconserved amino acids are indicated. Accession numbers are from GenBank/EMBL Data Bank.

However, whether this is due to a delayed down-regulation of transcription or is the result of high mRNA stability is not yet known.

Recently, Perera et al. [20] reported that in etiolated *Arabidopsis thaliana* seedlings the strong expression of V-ATPase subunit c was confined to a single isoform (AVA-P2). In the present study no isoform-specific probes were used; however, as under the stringency conditions employed all isoforms would have been co-detected, our results suggest that total expression of subunit c is massively reduced after light-induced cessation of growth.

Acknowledgements: The work was supported by a grant of the

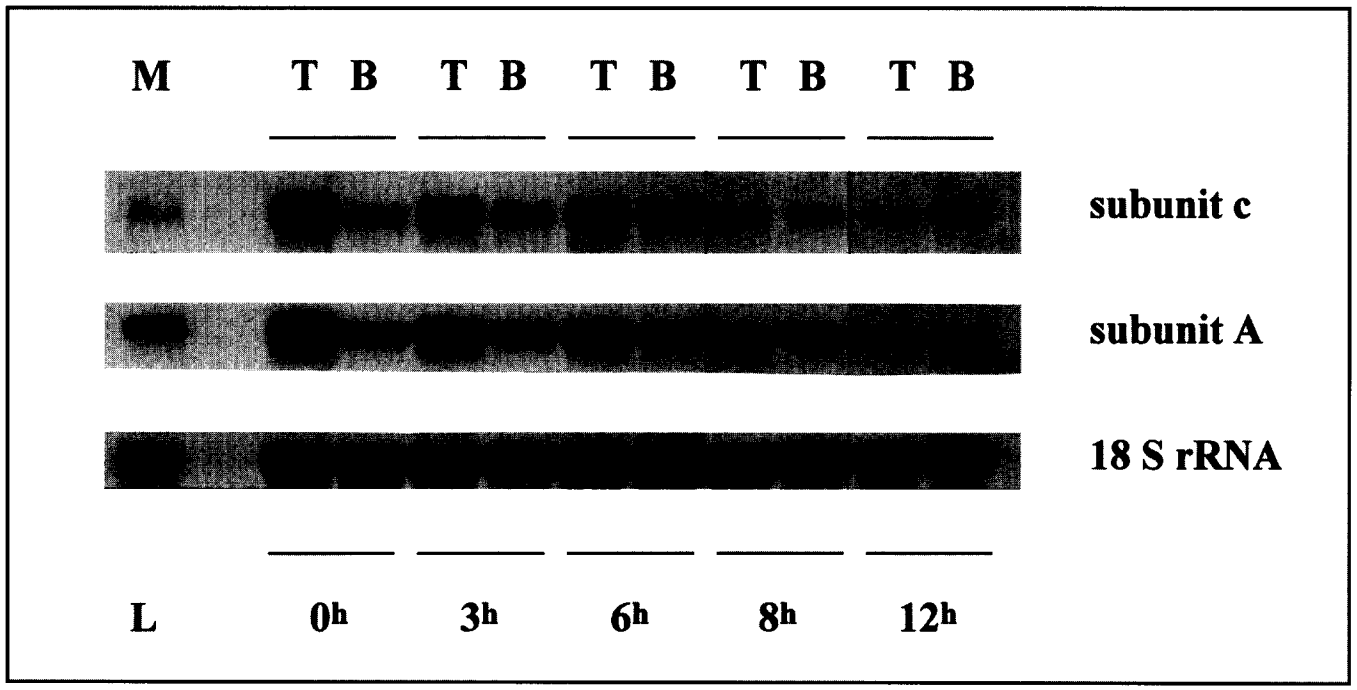

Fig. 2. Time course of mRNA decline for subunits c and A in 6-day-old etiolated seedlings after light exposure (0–12 h) as revealed by Northern blot. Left lane: Transcript levels in total mesocotyl (M) of seedlings grown at a day/night regime of 16/8 h. T, mesocotyl tip; B, mesocotyl base.

Deutsche Forschungsgemeinschaft to T.R. (SFB 199, TP B4). Stimulating discussions with Prof. Dr. U. Lüttge and PD Dr. R. Ratajczak are gratefully acknowledged.

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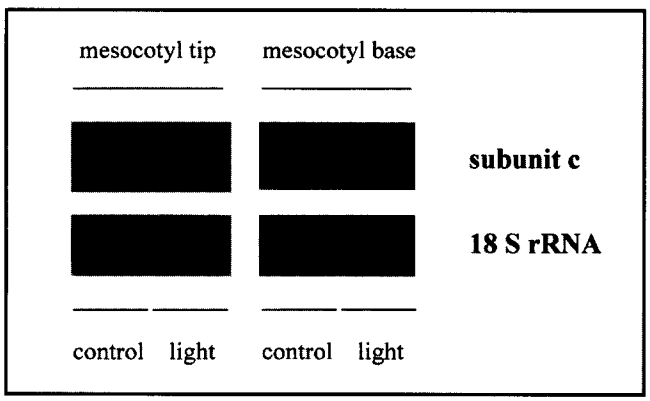

Fig. 3. Subunit c transcript levels in 6.5-day-old etiolated *Zea mays* seedlings (control) as compared to etiolated seedlings which after 6 days in the dark were exposed for an additional 12 h to continuous light (light).

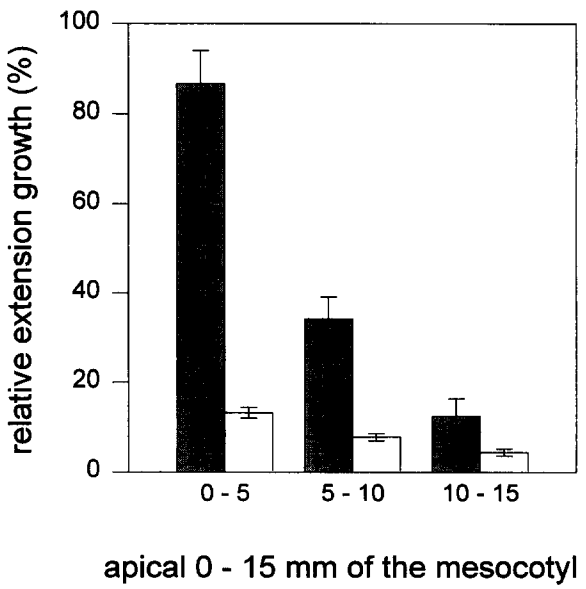

Fig. 4. Relative extension growth of different apical mesocotyl zones of 6-day-old etiolated seedlings during the following 12 h in light (empty bars) versus dark (shadowed bars). Growth is expressed as percentage increase of initial length (5 mm = 100%).

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