Functional architecture of a late pollen promoter: pollen-specific transcription is developmentally regulated by multiple stage-specific and co-dependent activator elements

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Abstract

The tomato lat52 gene encodes an essential cysteine-rich protein preferentially transcribed in the vegetative cell during pollen maturation. Detailed analyses of the identity, organization and role of cis-regulatory elements in controlling the precise developmental and tissue-specific expression of lat52 during pollen development were performed. Analysis of a series of F₀ promoter deletion mutants stably introduced into tobacco demonstrated differential developmental activation of deletion mutants during pollen development. All major cis-regulatory elements required for pollen-specific transcription were located within the upstream region −492 to −52. This region was shown to comprise three independent activator domains A, B and C, each sufficient to activate the minimal CaMV 35S promoter in a pollen-specific manner. F₀ deletion and gain of function approaches were used to show that domain A and the previously defined motif PBII (sub-domain B1) were largely redundant in the presence of downstream sequences in mature pollen. Within domain B two novel pollen-specific sub-domains B2 and B3 were identified. Within domain C, the activity of the PBI motif (sub-domain C1) was shown to be strictly dependent upon a downstream 20 bp pollen-specific activator unit −72 to −52 (sub-domain C2), containing two novel co-dependent regulatory elements AGAAA and TCCACCATA. These results demonstrate that transcriptional activation of lat52 is controlled by a complex of pollen-specific cis-regulatory elements which cooperate to achieve maximum levels of gene expression throughout pollen maturation. Alternative models of the interaction of identified cis-regulatory elements with putative trans-acting factors within the lat52 promoter and their developmental utilization are presented.

Introduction

The pathway of pollen maturation provides an accessible and well characterised system in which to study the tissue-specific and developmental control of gene expression. At maturity the pollen grain contains about 20 000 unique transcripts of which 2000 are estimated to be specifically or preferentially expressed in pollen [25, 39, 40]. The similar expression patterns of a number of these ‘late pollen genes’ suggests a common mechanism of regulation since transcripts appear at pollen mitosis I and accumulate in the maturing pollen grain up to anthesis [30]. In order to further dissect the pathway of late pollen gene regulation it is important to functionally define the cis-regulatory elements responsible for coordinate and specific activation in the developing pollen grain. One of the most well understood late pollen genes in terms of its regulatory properties is the late anther tomato, lat52 gene [18], which shows significant homology to cDNAs isolated from maize [12], rice [42], sorghum [22], lilac [1] and olive [17]. The lat52 gene encodes an abundant cysteine-rich protein which is distantly related to Kunitz trypsin inhibitor family [19]. The lat52 homologue in olive encodes the major pollen allergen, OLE EL, which is localised to the endoplasmic reticulum [24] and accounts for ca. 1% of total dry mass of mature pollen [38]. An essential gametophytic role for
the LAT52 protein in pollen hydration and tube growth has been demonstrated by antisense-mediated down-regulation in tomato [20].

Previous studies of native lat52 mRNA expression in tomato demonstrated an essentially pollen-specific expression pattern with only low levels of transcripts detectable in the anther wall [37] and in petals [18]. During gametophyte development lat52 transcripts were first detectable in spores undergoing pollen mitosis I, leading to a substantial increase in mature pollen [18]. The specificity of transcriptional activation was further demonstrated since the lat52 5′-flanking region (−492 to +110) was shown to direct pollen-specific GUS activity in tomato, tobacco and Arabidopsis [35] and pollen-specific cell ablation when linked to the cytotoxic diphtheria toxin A chain [31]. Furthermore, the developmental accumulation of GUS activity in pollen of transgenic plants closely reflected the accumulation of the native lat52 transcript [33, 6].

Sequence analysis of the lat52 promoter, led to the identification of the reiterated PB core motif TGTG-GTT. (PBI, II and III) which is closely related to the rbcS-3A Box II cis-regulatory element [9, 16]. Analysis of lat52 5′ promoter deletion mutants in tomato led to the identification of two pollen-specific activator regions: −492 to −125 (containing PBI II and PBI I) and −124 to −72 (containing PBI). This analysis also defined the minimal proximal promoter sufficient to direct pollen-specific expression as the region −71 to +110. Further gain of function analyses demonstrated that both PBI II (−194 to −176) [36] and the 30 bp proximal promoter region (−84 to −55) [7] were sufficient to direct pollen-specific expression.

Here the results of experiments designed to further elucidate the precise sequences and organization of cis-regulatory elements required for the developmental and cell-specific regulation of the lat52 gene are described. Analyses of promoter deletion mutants demonstrated a differential utilization of regulatory domains during pollen development. Three independent pollen-specific activator domains and functionally redundant sequences were defined. Minimal sequences sufficient for pollen-specific expression were localised to a novel bipartite 20 bp sequence. The results are discussed in relation to working models of the interaction of regulatory modules involved in the control of gene expression during pollen development.

Materials and methods

Plasmid constructions

Construction of plasmids containing the lat52 promoter 5′ deletion mutants (−492 to +110, −100 to +110, −71 to +110 and −41 to +110) fused to the Escherichia coli β-glucuronidase gene uidA (gus) and the nopaline synthase 3′ region (nos3′) have been described previously [36]. The lat52-gus-nos3′ fusions were excised as SalI/EcoRI fragments and cloned into the binary vector pBIN19 [3]. lat52 promoter 5′ deletion mutants (−492, −225, −145, −100, −71, −41 and −17) fused to the luciferase coding region (luc) [21] and the CaMV 35S 3′ terminator region (CaMV3′) [27] were created by subcloning lat52 promoter regions from lat52-gus-nos3′ cassettes [36] as SalI/NcoI fragments into a promoterless luc-c3′ cassette in pBluescript KS+ (Stratagene). The lat52 promoter regions −194 to +110 and −176 to +110 were amplified from the template pNBL52-5 [2] using the polymerase chain reaction (PCR) in conjunction with specific 5′ oligonucleotides containing a KpnI site at the relevant 5′ border sequence of the lat52 promoter and a 3′ oligonucleotide complementary to the non-sense strand of luc. The generated PCR products were digested with KpnI/NcoI and cloned into the above luc-c3′ cassette. The lat52 promoter 5′ deletion +1 to +110 was created by subcloning the lat52 5′-utr-luc-c3′ cassette from pNBL52-27 [2] as a Xhol/HindIII fragment into pBluescript KS+. Plasmids used for the substitution mutational analysis of the region −100 to −42 of the lat52 promoter were created as follows. The 2 bp series of substitution mutants throughout PBI were introduced using PCR and degenerate oligonucleotides. The amplified region −100 to +110 containing the introduced 2 bp mutation was cloned as a KpnI/NcoI fragment into the luc-c3′ cassette. The block substitution mutants of the region −84 to −42 were created by amplifying the promoter region downstream of the desired mutation using PCR. This fragment was then cloned into a luc-c3′ cassette as a SalI/NcoI fragment. The upstream region of the desired mutation was then amplified using PCR and cloned into the SalI site.

Hybrid CaMV 35S-lat52 mutants were constructed by amplifying the duplicated CaMV 35S region (−418 to −90/−418 to −78) from the plasmid pRTL2LUC [29] using PCR. The duplicated CaMV 35S enhancer region was cloned directly upstream of the appropriate lat52 5′ promoter deletion mutant-luc-c3′ cas-