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# Induction of a homeodomain–leucine zipper gene by auxin is inhibited by cytokinin in *Arabidopsis* roots

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**Abstract**

Homeobox genes are essential regulators of the development of plants as well as other organisms. We chose eight putative *Arabidopsis* homeobox genes not previously characterized and examined their expression in response to treatment with auxin/cytokinin. One of them, *ATHB53*, was further studied because it was auxin-inducible and its induction was inhibited by cytokinin. Its full-length cDNA was cloned and found to encode a protein of the HD-Zip superfamily. Whole-mount in situ hybridization and RT-PCR showed that it was expressed in the root meristem, and auxin treatment increased its expression, especially in a region from 0.3 to 0.6 mm from the root tip. These results suggest that ATHB53 plays a regulatory role in auxin/cytokinin signaling during root development.


*Keywords:* Homeobox gene; HD-Zip; Auxin; Cytokinin; Root meristem

Homeobox genes are master regulators of various aspects of development in organisms ranging from plants to humans. Since the discovery of the *KNOTTED1* gene of maize, the first plant homeobox gene [1], a number of additional plant homeobox genes have been isolated. Mutation of *shootmeristemless* (*STM*) of *Arabidopsis* results in defects in shoot meristem formation and maintenance, clearly demonstrating that this homeobox gene is essential for meristem function [2,3]. Members of the homeodomain-Zipper (HD-Zip) superfamily contain a leucine zipper domain C-terminal to their homeodomain [4]. This protein family has so far been found only in plants and seems to have functions specific to plant development. Many members of the group such as ATHB1 and ATHB5 form homodimers or heterodimers [5,6]. Another member of the superfamily, *Athb-8*, is expressed in provascular cells during revascularization, suggesting that it controls vascular development [7,8].

The phytohormone, auxin, affects diverse aspects of plant development and responses to environmental changes. Auxin is transported via the phloem or by the polar transport system [9,10], and proteins

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controlling the influx and efflux of auxin have been identified and are essential to diverse auxin responses. Observations on auxin-dependent reporter gene expression and the effects of auxin transport inhibitors have revealed that the root apical meristem is a region of high auxin concentration [11], and a strong correlation was found between the location of the quiescent center and auxin concentration [12]. There is also evidence that some auxin responses are inhibited by cytokinin [13,14]. For example, auxin-stimulated responses are inhibited by cytokinin treatment in wild-type tomatoes, and the effect of inhibition is mimicked by the effects of the *dgt* (*diageotropica*) mutation.

Recent studies of transgenic plants expressing homeobox and hormone biosynthetic genes have pointed to links between plant hormones and homeobox genes [15]. Thus, the phenotype of plants overexpressing the isopentyl transferase (*ipt*) gene, a cytokinin-producing gene, which includes alterations of leaf shape and ectopic shoot meristem formation on leaves, is similar to those overexpressing *kn1* [16]. Moreover, increased cytokinin levels were observed in *kn1*-overexpressing leaves, implying that KN1 induces cytokinin production [17]. Transgenics overexpressing *HAT2*, a HD-Zip superfamily gene, have long hypocotyls, epinastic cotyledons, long petioles, and small leaves, similar to the phenotype of auxin-overproducing plants [18]. In addition, auxin induces several homeobox genes such as *prha* and *Athb-8* [7,19]. Links between phytohormones and homeobox genes have also been found in the case of ABA; thus *ATHB7*, *ATHB6*, and *Athb-12*, members of the HD-Zip superfamily, all turn out to be induced by ABA [20–22].

In this work, we studied eight previously uncharacterized putative homeobox genes (*HB-1* to *HB-8*) in *Arabidopsis*. We analyzed the response of these genes to auxin/cytokinin treatments and concentrated on *HB-8* since it proved to be regulated by both auxin and cytokinin. Cloning of the full-length cDNA showed it to encode a member of the HD-Zip superfamily. Its expression increased in root meristems in response to auxin treatment, and this response was inhibited by cytokinin.

## Materials and methods

*Plant growth and phytohormone treatment*. Seeds of *Arabidopsis thaliana* ecotype Columbia were surface-sterilized in 20% hypochlorite containing 0.01% Triton X-100 for 5 min, washed in sterile D.W., and plated on solidified (0.8% agar) MS medium (Sigma, USA). Plants were grown in a growth room at 22 °C with a 16 h photoperiod. Ten-day-old seedlings were treated with 50 μM IAA for various times, as well as with various concentrations of IAA for 12 h, or of kinetin for 24 h. Twenty micromolar ABA, 20 μM 1-aminocyclopropane-1-carboxylate (ACC), 20 μM $GA_3$, and 20 μM epibrassinolide (BL) were also used. In order to examine the *ATHB53* induction in flowers, 6-week-old plants were treated with 50 μM IAA, or 50 μM kinetin for 30 min, and the hormone treatment was repeated 5 times. In addition, flowers were excised and incubated in the above hormone solutions for 4 h.

*Cloning a full-length ATHB53 cDNA*. A full-length cDNA for *ATHB53* was cloned with a CapFishing Kit (Seegene, Korea) according to the manufacturer's protocol. Full-length first strand cDNA was synthesized with oligo(dT)-annealing control primer (ACP), and PCR was performed with a 5′-RACE primer and a gene-specific primer. Primer sequences were as follows: 5′-RACE primer, 5′-GTCTACCGGCATTCGCTTCAT-3′; *ATHB53* gene-specific primer, 5′-CGTTGTCGTGGTGGTTTTTGAG-3′.

*Whole-mount in situ hybridization*. A DNA fragment of 0.4 kb containing the 3′ UTR of the *ATHB53* gene was used to make digoxigenin-labeled antisense/sense RNA probes. Tissue was prepared and whole-mount in situ hybridization was performed as described by Wilkinson [23].

*Semi-quantitative RT-PCR*. Total RNAs from *Arabidopsis* were used for reverse transcription with M-MLV Reverse Transcriptase (Promega, USA) while Ex *Taq* polymerase (Takara, Japan) was used in the subsequent PCR. As a quantitative control, we used the *Actin2* gene of *Arabidopsis* (*Actin2* forward primer, 5′-GAAAAG ATCTGGCATCACACTTTCTA-3′; *Actin2* reverse primer, 5′-ACAT ACATAGCGGGAGAGTTAAAGGT-3′). Specific primers for the *SAUR* gene and the *NIA1* gene were as follows: *SAUR* forward primer, 5′-CGTCATTACAGTGAAACGGCAGA-3′; *SAUR* reverse primer, 5′-AACCATAGTCAAAGAGCGCGTGA-3′; and *NIA1* forward primer, 5′-GGTGGACTCATGTCTCAACACTTAGA-3′; *NIA1* reverse primer, 5′- CTTCTAGCTCTTCCCTCACAAGAATG-3′. Each reaction was performed at least three times.

## Results

### *Identification of a putative Arabidopsis homeobox gene regulated by auxin and cytokinin*

Hormones control plant development at several different levels. Since homeobox genes are essential for development, we reasoned that the expression of many homeobox genes should be controlled by phytohormones. From putative homeobox genes annotated on the TIGR and MIPS web sites, we chose eight uncharacterized genes at random and designated them tentatively, *HB-1* to *HB-8*. In each case their amino acid sequence, as deduced from the predicted cDNA sequence, did include a homeodomain. To clone a cDNA fragment of each gene, RT-PCR was performed with total RNA from *Arabidopsis* seedlings and primers were designed to cover the homeodomain of each gene (Table 1). PCR products were cloned and sequenced to confirm that they contained the expected DNA sequences. The presence of transcripts and of homeodomains identified them as homeobox genes.

To examine their control by phytohormones, 10-day-old *Arabidopsis* seedlings were treated with 50 μM indole-3-acetic acid (IAA), or 50 μM kinetin, or both, and the expression of each gene was examined by semi-quantitative RT-PCR using RNAs from these treatments. *HB-5* and *HB-8* increased in expression in response to IAA whereas the others did not. None of

Table 1
Primers for cloning putative *Arabidopsis* homeobox genes

| Genes | Loci | Primers used for RT-PCR |
|---|---|---|
| HB-1 | At1g28420 | 5′-CTCGTCTTCCTCTATTGCCACATTT-3′ |
| | | 5′-GGATCAAACAGAAAGGTGAAGAGGA-3′ |
| HB-2 | At2g18550 | 5′-CAATGTAATCTGGCGAGTAGAGCAA-3′ |
| | | 5′-AAAAGGAAGAGCAAGAGTGTTGTGG-3′ |
| HB-3 | At2g33880 | 5′-AACAAATCTGAGTCTGTGGCTGTTG-3′ |
| | | 5′-TCGGTGATGCTAACGTCTTCTACTG-3′ |
| HB-4 | At4g35550 | 5′-TTATTTCCAGAAGAACCTCCACCAT-3′ |
| | | 5′-GATGGAATGGGATAATCAGCTACAA-3′ |
| HB-5 | At4g36740 | 5′-AGACGAAATTGGACTGTTGCTTGAG-3′ |
| | | 5′-TGAACTACACGGTGGATGATCAGAA-3′ |
| HB-6 | At5g02030 | 5′-CCACGACCCACATTACAAAACTCAT-3′ |
| | | 5′-ATCTCTCGATTCCCACTTCCACTTT-3′ |
| HB-7 | At5g46880 | 5′-AGCTTTCTTGAAGCATCAGCTCAAC-3′ |
| | | 5′-TCAGGACATGCCTAATGGATACTCA-3′ |
| HB-8 | At5g66700 | 5′-CTGTTGGTTGGCATTTCTTCAAGTC-3′ |
| | | 5′-GCTCTCAAGTGTACCCCTACACGAC-3′ |

the genes showed any change in expression in response to kinetin, whereas *NIA1* [24], a positive control, was induced under the same conditions. Intriguingly, simultaneous treatments with both hormones led to no induction of *HB-8*, indicating that cytokinin inhibited its induction by auxin. The expression of this gene was therefore further studied.

### *Isolation of the full-length ATHB53 cDNA*

The full-length cDNA of the *HB-8* gene was cloned with a CapFishing Kit (Seegene, Korea) (see Materials and methods). The ORF obtained contained 687 nucleotides and the deduced amino acid sequence consisted of 229 amino acids (Fig. 2A). The deduced amino acid sequence included not only a homeodomain (amino acid no. 68–127), but also a leucine zipper motif (amino acid no. 128–157) to the carboxyl side of the homeodomain (Figs. 2B and C). The homeodomain was very similar to the consensus of the homeodomain sequences found in a number of eukaryotes [25,26]. In addition, the leucine zipper motif was in a position identical to that of the other homeodomain–leucine zipper (HD-Zip) proteins. Since this protein was found to be a member of the HD-Zip superfamily, it would be proper to name it according to conventional designation for the superfamily. Besides, the name ATHB53 had already been given to this protein on the basis of sequence information (albeit not characterized) [26], so that we decided to refer to it as ATHB53, rather than HB-8. According to previous classifications of HD-Zip proteins [27,28], ATHB53 is an HD-Zip I protein. The presence of a homeodomain and a leucine zipper motif in ATHB53 suggests that it may be a DNA-binding protein and form dimers [5]. ATHB53 has the highest identity to HB-5: 83% and 66% in the homeodomain and leucine zipper motif, respectively (Fig. 2).

### *Effects of phytohormones on the expression of ATHB53*

To examine the hormonal induction of *ATHB53*, *Arabidopsis* seedlings were treated with various phytohormones and its expression was assayed by semi-quantitative RT-PCR. First, seedlings were treated with auxin and transcript levels were measured with time (Fig. 3A). As a positive control, we examined the expression of *SAUR* under the same conditions. Induction could be detected by 2 h and increased continuously up to 24 h. We then determined the concentration of IAA needed for induction (Fig. 3B). Treatment with various concentrations of IAA led to dose-dependent expression, with concentrations as low as 50 nM IAA able to induce *ATHB53*.

When we applied exogenous kinetin to *Arabidopsis* seedlings and examined the accumulation of *ATHB53* transcripts (Fig. 3C), their level remained low regardless of the kinetin concentration, as also seen in Fig. 1. Total RNAs were isolated from plants treated with 1-aminocyclopropane-1-carboxylic acid (ACC), abscisic acid (ABA), gibberellic acid (GA), and brassinosteroid (BR), and assayed for *ATHB53* transcripts by RT-PCR (Fig. 3D). None of them induced *ATHB53*. Evidently *ATHB53* is induced by the auxin signaling pathway and is not directly responsive to other phytohormones.

The distribution of *ATHB53* mRNA in different plant organs of 5-week-old plants was monitored by semi-quantitative RT-PCR (Fig. 4). The mRNA was detected mainly in roots and late flowers containing

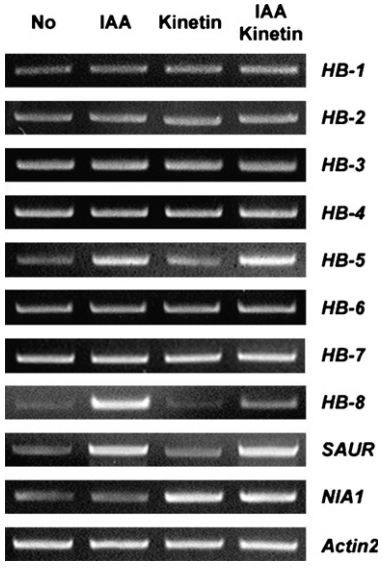


Fig. 1. Expression of eight putative homeobox genes in response to auxin/cytokinin treatment. RNAs were isolated from 10-day-old *Arabidopsis* seedlings treated with IAA or kinetin and used in semi-quantitative RT-PCR. The *SAUR* gene was used as positive control for auxin induction, *NIA1* for cytokinin induction, and *Actin2* as a loading control.

**A**

```
1        ACCAAAGTCATATCCTATTGCATCACTCTCCTTTAAACACAGTCAGAGAAAACAGCAACT

61       CTTGTCTGAAAAACTAGAGAGAGAAAATGGATCATGGTAGGTTAATGGATGATCAAATGA
                                    M  D  H  G  R  L  M  D  D  Q  M

121      TGCTAGGCTCTCAAGTGTACCCCTACACGACCCAACCCCAAAATTCACACTGCATCATCG
         M  L  G  S  Q  V  Y  P  Y  T  T  Q  P  Q  N  S  H  C  I  I

181      TTAACCAGATCGATGGAGGCGAAGAATCAAAACCGGTGAAGCGGAGGAGGAAGAGGAGGA
         V  N  Q  I  D  G  G  E  E  S  K  P  V  K  R  R  R  K  R  R

241      GTAAAGGTTCATCAGCCACCAACGAAGAAGACGTGGCGGAGATCGGAGGGATGTTGAGGA
         S  K  G  S  S  A  T  N  E  E  D  V  A  E  I  G  G  M  L  R

301      AGAGGAAGTTGACCGATGAACAAGTGAACATGCTCGAATATAGCTTCGGGAACGAGCATA
         K  R  K  L  T  D  E  Q  V  N  M  L  E  Y  S  F  G  N  E  H

361      AGCTGGAGTCAGGGAGGAAGGAGAAGATCGCCGGAGAGTTAGGTCTTGACCCGAGACAGG
         K  L  E  S  G  R  K  E  K  I  A  G  E  L  G  L  D  P  R  Q

421      TGGCTGTTTGGTTCCAGAACCGCCGTGCACGTTGGAAGAACAAGAAACTAGAGGAAGAGT
         V  A  V  W  F  Q  N  R  R  A  R  W  K  N  K  K  L  E  E  E

481      ACGCCAAACTCAAAAACCACCACGACAACGTCGTACTCGGCCAATGCCAACTCGAGTCTC
         Y  A  K  L  K  N  H  H  D  N  V  V  L  G  Q  C  Q  L  E  S

541      AGATATTGAAACTAACAGAACAATTGAGTGAAGCTCAAAGTGAAATTCGAAAACTGTCGG
         Q  I  L  K  L  T  E  Q  L  S  E  A  Q  S  E  I  R  K  L  S

601      AACGACTTGAAGAAATGCCAACCAACAGTTCAAGTTCATCGCTTTCTGTTGAAGCCAACA
         E  R  L  E  E  M  P  T  N  S  S  S  S  S  L  S  V  E  A  N

661      ATGCACCAACTGATTTCGAGCTTGCCCCGGAAACTAATTATAACATCCCGTTTTATATGT
         N  A  P  T  D  F  E  L  A  P  E  T  N  Y  N  I  P  F  Y  M

721      TAGATAATAATTATTTACAAAGCATGGAGTATTGGGATGGTTTGTATGTATAATCAGTTT
         L  D  N  N  Y  L  Q  S  M  E  Y  W  D  G  L  Y  V  *

781      ATCAACTAAATATCTCGTGCTTTATTGATGCAACTCATGTTATTATAAGTAATGTAGAAA
841      TAGGTGTTGGTGAAACGGCTGTAATTATGTGTTGTTTTCAGTATTTATTTTAATATAAAA
901      AAAAAAAAAAAAAAAAAAAA
```

**B Homeodomain**

```
                      helix1    loop   helix2  turn     helix3          Identity(%)
ATHB53   GMLRKRKLTDEQVNMLEYSFGNEHKLESGRKEKIAGELGLDPRQVAVWFQNRRARWKNKK     100%
HB-5     -LF--------------M---D------E--DRL-A----------------------R      83%
Athb-1   LPEK--R--T---HL--K--ET-N---PE--TQL-KK---Q----------------T-Q     63%
Oshox5   APEK--R--A---Q---R--EE-N---PE--TEL-RR--MA----------------T-Q     63%
VAHOX1   AEKK--R---N--QF--K---E-N---PE--VQL-K----Q---I-I----------T-Q     63%
Hfi22    ISEK--R-SV---KA--KN-EV-N---PE--V-L-Q----Q----------------T-Q     63%
ATHB6    LSEK--R-SIN--KA--KN-EL-N---PE--V-L-Q----Q----------------T-Q     62%
CPHB-5   LPEK--R--A---HL--K--EA-N---PE--AEL-KK---Q-----I----------T-Q     62%
ATHB5    AAEK--R-GV---KA--KN-EIDN---PE--V-L-Q----Q-----I----------T-Q     60%
```

**C Leucine zipper motif**

```
ATHB53   LEEEYAKLKNHHDNVVLGQCQLESQILKL                                  100%
HB-5     -----N----S-----VDK-R---EVIQ-                                   66%
Athb-1   --RD-DL--STY-QLLSNYDSIVMDND--                                   28%
Oshox5   --HDFDR--AAY-ALAADHHA--L-DNDR-                                  28%
VAHOX1   --KD-DE-R-RY-TLKSNYNN-LKEKED-                                   28%
Hfi22    --RD-GV--SNF-ALKHNYES-KHDNEA-                                   28%
ATHB6    --KD-GV--TQY-SLRHNFDS-RRDNES-                                   28%
CPHB-5   --RD-D---SSY-SLLSTYDSIRQEND--                                   31%
ATHB5    --RD-GV--SNF-ALKRNRDS-QRDNDS-                                   28%
```

Fig. 2. Full-length cDNA sequence and deduced amino acid sequence of *ATHB53*. (A) Full-length cDNA was cloned with a CapFishing Kit (see Materials and methods). The nucleotide sequence has been submitted to GenBank with Accession No. AY683477. The homeodomain is underlined, and the leucine and valine residues of the putative leucine-zipper motif are marked with bold letters and underlined. The asterisk indicates the termination codon. (B,C) Comparison of the homeodomain and leucine zipper motifs of ATHB53 with those of several HD-Zip family members; HB-5, Athb-1, -5, -6 (*A. thaliana*), Oshox5 (*Lycopersicon esculentum*), VAHOX1 (*L. esculentum*), Hfi22 (*Nicotiana tabacum*), and CPHB-5 (*Craterostigma plantagineum*). Dashes indicate identical amino acids. Conserved leucine residues in the leucine zipper motif are boxed.

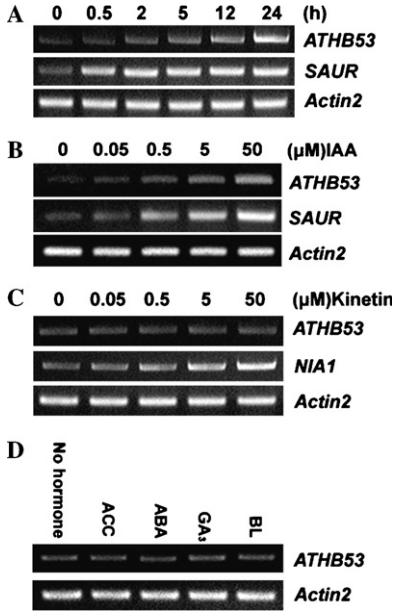


Fig. 3. Expression of *ATHB53* in response to hormones. (A) Time-dependent expression of *ATHB53* after IAA treatment. Ten-day-old seedlings were treated with 50 μM IAA for the indicated times. (B) Concentration-dependent expression of the *ATHB53* gene in response to various concentrations of IAA. Ten-day-old seedlings were treated with the indicated concentration of IAA for 12 h. The *SAUR* gene was used as positive control. (C) Concentration-dependent induction of *ATHB53* by kinetin. Ten-day-old seedlings were treated with the indicated concentration of kinetin for 24 h. *NIA1* served as positive control for cytokinin response. (D) Induction of *ATHB53* by various hormones. The concentrations of hormones used are described under Materials and methods.

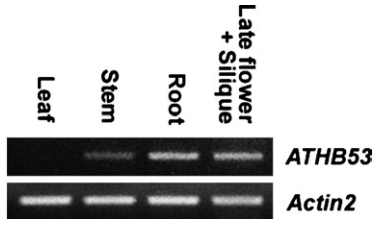


Fig. 4. Expression of *ATHB53* in various plant tissues. RNAs were isolated from tissues of 6-week-old *Arabidopsis* and used in semi-quantitative RT-PCR. The tissues were not treated with any hormone.

siliques, but very little in leaves and stems. We therefore examined the pattern of its expression in roots in response to auxin/cytokinin treatment in more detail.

### *ATHB53 expression in root*

Since *ATHB53* was highly expressed in roots, we thought it likely that the auxin-inducibility and inhibition by cytokinin observed previously (Fig. 1) would also occur in roots. We treated *Arabidopsis* seedlings with 50 μM IAA, or 50 μM kinetin, or both and examined the root tissues by whole-mount in situ

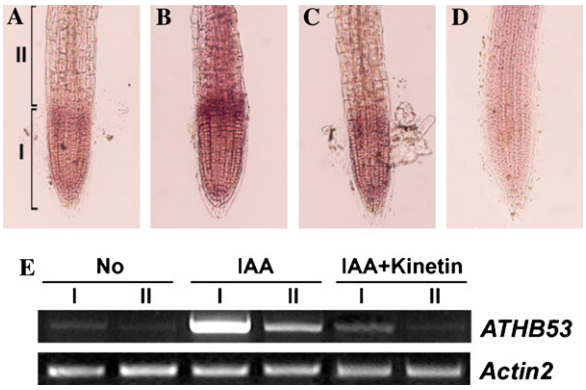


Fig. 5. *ATHB53* gene expression in root. Whole-mount in situ hybridization of *ATHB53* in control roots (A,D), roots treated with IAA (B), and those treated with IAA and kinetin simultaneously (C). Samples were hybridized with DIG-labeled antisense (A, B, and C) or sense (D) *ATHB53* riboprobes. (E) Expression of *ATHB53* in response to hormone treatment in regions of the root (segment I and II as in A), determined by RT-PCR.

hybridization. Without exogenous hormonal treatment, *ATHB53* was expressed at a low level in root meristem (Fig. 5A). Auxin treatment resulted in strong induction in both the meristem and elongation zone (Fig. 5B) with the highest expression in the root apical region approximately 0.3–0.6 mm from the root tip. When cytokinin and IAA were added at the same time, the level of *ATHB53* expression remained essentially at its basal level (Figs. 5A and C), thus replicating the inhibition of auxin-mediated induction by cytokinin in seedlings. To confirm the expression pattern obtained by whole-mount in situ hybridization, we isolated total RNA from segment I (from the root tip to approximately 0.6 mm from the tip) and segment II (the following 0.6 mm region) (see Fig. 5A), and performed RT-PCR (Fig. 5E). Upon treatment with auxin, region I had approximately three times more *ATHB53* transcripts than region II. When auxin and cytokinin were added together, the level of *ATHB53* transcripts resembled that of untreated samples. Overall, the results of RT-PCR analysis confirmed those from the whole-mount in situ hybridization. Taken together, these results indicate that ATHB53 plays a role in root development under the control of auxin and cytokinin.

### *ATHB53 expression in flowers*

Since *ATHB53* was highly expressed in flowers and siliques (Fig. 4), we decided to check if the auxin-inducibility and inhibition by cytokinin seen in seedlings and roots (Figs. 1 and 5) could also be observed in these organs. We therefore treated 6-week-old *Arabidopsis* plants with 50 μM IAA, or 50 μM kinetin, or both, and examined *ATHB53* expression in flowers and siliques by semi-quantitative RT-PCR. The *SAUR* gene,

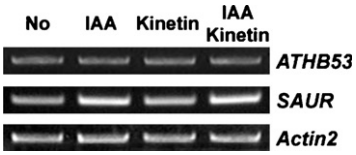


Fig. 6. *ATHB53* gene expression in flower. Six-week-old plants were treated with auxin and cytokinin as in Materials and methods. RNA was isolated from late flowers and siliques of plants treated with hormones. The *SAUR* gene was used as positive control.

a positive control, was induced by auxin (Fig. 6). However, no induction of *ATHB53* was found in auxin- or cytokinin-treated plants or in plants treated with both hormones although the transcripts themselves were detected. The *AtGH3a* gene was also induced under the same condition, as another positive control for auxin induction in flowers (data not shown). Even when flowers were excised and incubated in hormone solutions, no induction of *ATHB53* was observed. This indicates that in flowers, the *ATHB53* gene is not induced by auxin, and hence cytokinin-mediated inhibition cannot occur either.

## Discussion

Since the discovery of the HD-Zip superfamily of plants [29–31], many members of the family have been cloned and classified into four subfamilies [27,28]. Two of the putative homeobox genes studied in this work (*HB-5* and *HB-8*) were inducible by exogenous auxin. In addition, auxin induction of *HB-8* gene was abolished by cytokinin treatment (Fig. 1). It turned out that HB-8 had been previously named ATHB53 [26] although only on the basis of sequence information, and we decided to adhere to that name in this study as well as when submitting sequence data to GenBank.

Expression of *ATHB53* was induced by auxin, especially in roots (Figs. 4 and 5). When the full-length cDNA sequence was determined (Fig. 2A), a TGTCTC auxin response element (AuxRE) [32] was found 1233-bp upstream of the transcription start site although it is not necessarily responsible for the auxin-inducibility of *ATHB53*. *Athb-8*, *HAT2*, and *prha*, other proteins containing both homeobox and leucine zipper motifs, are also auxin-induced [7,18,19]. In situ hybridization of *Arabidopsis* roots revealed expression of *Athb-8* in the meristem and elongation zone. Expression was associated with procambia and lateral root primordia. *Prha* is also highly expressed in root tip and lateral root primordia although it is expressed in other tissues as well. Auxin treatment caused strong induction of *ATHB53* in root meristem (Fig. 5), with the highest expression in segment I, which may correspond to the boundary between meristem and elongation zone [33]. Although auxin induction has been observed in other HD-Zip genes, *ATHB53* is expressed in different regions of the root than those others. It remains to be seen whether the pathway by which auxin activates *ATHB53* is similar to that affecting other HD-Zip genes.

The auxin induction of *ATHB53* was inhibited by cytokinin treatment (Fig. 1). While both hormones serve as major integrators of developmental processes, they act synergistically in some instances and antagonistically in others. In tissue cultures, auxin and cytokinin promote shoot and root developments, depending on their ratios [34]. The expression of the auxin-inducible *SAUR* gene in soybean is unaffected by cytokinin alone, but auxin induction of the gene was decreased by 50% with cytokinin also present [35]. Similarly, ectopic expression of a *SAUR-GUS* reporter in an *axr3 Arabidopsis* background was abolished by cytokinin [36]. Coenen et al. [13], using the *dgt* (*diageotropica*) mutant of tomato, found that cytokinin inhibits a subset of auxin responses. Likewise, it inhibits the expression in roots of certain genes, including *ATHB53*, which are auxin-modulated. Further characterization of this gene at the physiological and molecular levels may provide insight into the basis of the interaction between these two hormones in root development.

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