HY5 Inhibits the Post-transcriptional Imprint of SAUR1/2/3/4 in Thermomorphogenesis

Authors: Feng Yu, Liu Zhouli, Wang Xin

Date: 2025-03-19T00:00:00+00:00

Abstract

The SAUR (SMALL AUXIN UP RNA) gene family represents an important family of downstream genes that promote hypocotyl elongation; however, the molecular mechanisms underlying SAUR regulation in high temperature-induced hypocotyl elongation remain largely elusive. Thermomorphogenesis is defined as a series of morphological changes that occur in higher plants within a moderately high temperature range between optimal and stressful high temperatures. Of these, high temperature-induced hypocotyl elongation is the most extensively studied. This study utilized Arabidopsis thaliana Col wild type, hy5 mutants, 35S::HY5-HA/Col-0 overexpression plants, and tobacco as experimental materials, and employed NPA treatment experiments, quantitative RT-PCR, chromatin immunoprecipitation, and dual luciferase reporter assays to investigate the molecular mechanism underlying high temperature regulation of SAUR1/2/3/4. The results revealed that: (1) In the thermomorphogenesis signaling transduction pathway, auxin acts downstream of HY5 (ELONGATED HYPOCOTYL 5). (2) HY5 represses transcription of SAUR1/2/3/4 at both 20 °C and 29 °C. (3) HY5 binds to E-box-containing regions in the promoters of SAUR1/2/3/4 under both normal and high temperatures, and this binding is inhibited by high temperature. (4) The regulation of SAUR1/2/3/4 by HY5 is dependent on auxin. Collectively, these findings indicate that high temperature regulates transcription of these four genes by modulating the binding affinity of HY5 to SAUR1/2/3/4 promoter chromatin, and that this regulatory process requires auxin. This study provides novel insights into the molecular mechanism by which high temperature regulates downstream genes involved in hypocotyl elongation.

Full Text

Preamble

DOI: 10.11931/guihaia.gxzw202410006
Title: HY5 Inhibits the Transcription of SAUR1/2/3/4 in Thermomorphogenesis

Authors: FENG Yu[1,2,3], LIU ZhouLi[1,2,3], WANG Xin[1,2,3]*
Affiliations:
[1] College of Life Science and Engineering, Shenyang University, Shenyang 110044, China
[2] Northeast Geological S&T Innovation Center of China Geological Survey, Shenyang 110000, China
[3] Key Laboratory of Black Soil Evolution and Ecological Effect, Ministry of Natural Resources, Shenyang 110000, China

Abstract: The SAUR (SMALL AUXIN UP RNA) gene family represents important downstream genes that promote hypocotyl elongation. However, the molecular mechanisms governing SAUR regulation during high temperature-induced hypocotyl elongation remain largely unclear. Thermomorphogenesis is defined as a series of morphological changes that occur in higher plants within a mild high temperature range between the optimal temperature and stressful high temperature. Among these changes, high temperature-induced hypocotyl elongation is the most intensively studied. Using Arabidopsis Col wild type, hy5 mutants, 35S::HY5-HA/Col-0 overexpression plants, and tobacco as materials, this study employed NPA treatment experiments, quantitative RT-PCR, chromatin immunoprecipitation, and dual luciferase reporter assays to explore the molecular mechanisms of high temperature regulation of SAUR1/2/3/4. The results demonstrated: (1) Auxin acts downstream of HY5 (ELONGATED HYPOCOTYL 5) in the thermomorphogenesis signaling pathway. (2) HY5 inhibits the transcription of SAUR1/2/3/4 at both 20°C and 29°C. (3) HY5 binds to E-box-containing regions of SAUR1/2/3/4 promoter chromatin, and these bindings are inhibited by high temperature. (4) The regulation of SAUR1/2/3/4 by HY5 still requires auxin. In summary, high temperature regulates the transcription of these four genes by affecting the binding strength of HY5 to SAUR1/2/3/4 promoter chromatin, and this regulatory process requires auxin. This study provides new insights into the molecular mechanisms by which high temperature regulates downstream genes involved in hypocotyl elongation.

Keywords: thermomorphogenesis, high temperature-induced hypocotyl elongation, HY5, SAURs, transcription regulation

CLC Number: Q943
Document Code: A

Funding: This work was supported by the Liaoning Provincial "Xingliao Talents Program" (XLYC2203070), the Liaoning Provincial Natural Science Foundation-General Project (2024-MSLH-506), the Northeast Geological S&T

Innovation Center Foundation Project (QCJJ2022-44), and the Liaoning Provincial Doctoral Research Startup Fund Project (201601216).

First Author: FENG Yu (1997-), Master' s student, 主要从事植物抗逆分子机制的研究, (E-mail) 738167787@qq.com.
Corresponding Author: WANG Xin, Ph.D., Associate Professor, 主要从事植物抗逆分子生物学的研究, (E-mail) xwang166@126.com.

Introduction

As global warming continues to intensify, high temperature will increasingly threaten plant survival, reproduction, and crop production worldwide (Cox et al., 2020; Challinor et al., 2014). When temperatures rise slightly above the optimal range, plants can cool themselves through morphological changes. This temperature range is referred to as mild high temperature (Zhu et al., 2022). However, when temperatures exceed a certain threshold, plants can no longer overcome the negative effects of high temperature through their own adjustments, leading to growth stagnation or even death. This temperature range is termed stressful high temperature (Zhu et al., 2022).

The model plant Arabidopsis thaliana has an optimal temperature of 20–22°C, a mild high temperature range of 23–29°C, and stressful high temperatures above 30°C. The morphological changes occurring within the mild high temperature range are collectively known as thermomorphogenesis (Quint et al., 2016; Quint et al., 2023). Among these morphological changes, hypocotyl elongation has become a focal point for researchers due to its experimental accessibility.

In the thermomorphogenesis signaling pathway, the transcription factor HY5 and auxin play crucial roles (Gray et al., 1998; Delker et al., 2014; Gangappa & Kumar, 2017; Lee et al., 2021). HY5 acts as a negative regulator of thermomorphogenesis, inhibiting mild high temperature-induced hypocotyl elongation (Delker et al., 2014). Conversely, auxin functions as a positive regulator, promoting high temperature-induced hypocotyl elongation (Gray et al., 1998; Kim et al., 2020; Bianchimano et al., 2023). At optimal temperature, HY5 binds to the promoter region of the auxin biosynthesis gene YUCCA8 (YUC8) and inhibits its transcription, thereby maintaining low auxin levels (Gangappa & Kumar, 2017). Under mild high temperature, HY5 is inhibited through multiple mechanisms. One such mechanism involves decreased binding strength of HY5 to the YUC8 promoter region, reducing its ability to repress YUC8 transcription (Gangappa & Kumar, 2017) and consequently increasing auxin synthesis. Notably, the promotion of YUC8 expression peaks 4 hours after transfer to mild high temperature and significantly decreases by 8 or 24 hours (Sun et al., 2012; Huai et al., 2018). This suggests that increased auxin sensitivity may play a major role in hypocotyl elongation during the initial hours after high temperature stress (Pucciariello et al., 2018). Furthermore, HY5 and the thermomorphogenesis positive regulator PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) bind to

the same region of the YUC8 promoter (Gangappa & Kumar, 2017). However, the binding strength of PIF4 to the YUC8 promoter is enhanced by high temperature, suggesting potential competition between these two transcription factors for YUC8 promoter binding (Gangappa & Kumar, 2017).

AUXIN RESPONSE FACTORS (ARFs) are transcription factors in the auxin signaling pathway. Arabidopsis contains 23 ARFs, with transcriptional activators including ARF5, ARF6, ARF7, ARF8, and ARF19 (Roosjen et al., 2018). These ARFs constitutively bind to the promoters of many auxin-responsive genes and promote their expression. In the absence of auxin, AUXIN/INDOLE-3-ACETIC ACID INDUCIBLE (AUX/IAA) proteins interact with these ARFs and remove them from target gene promoters, thereby inhibiting auxin-responsive gene expression (Ito et al., 2016). When auxin levels increase, auxin is recognized by its receptor TRANSPORT INHIBITOR RESPONSE 1 (TIR1)/AUXIN SIGNALING F BOX PROTEIN (AFB). The receptor-auxin complex promotes the binding of AUX/IAA to the Skp1-Cullin-F-box (SCF) TIR1/AFB E3 ubiquitin ligase complex, leading to ubiquitination and degradation of AUX/IAA proteins (Maraschin et al., 2009). This releases ARF transcription factors, which then activate auxin-responsive gene transcription. Since the SAUR gene family comprises auxin-activated genes, their expression is likely promoted by these five ARF transcription factors. SAURs play important roles in promoting plant growth and cell elongation (Ren & Gray, 2015). Their molecular mechanisms primarily involve two pathways: first, by acidifying the cell wall to increase its extensibility, thereby promoting Arabidopsis hypocotyl cell expansion and resulting in hypocotyl elongation (Spartz et al., 2014; Wong et al., 2021); second, by binding to ribosomal protein RPL12 and SAUR62/75 to enhance ribosome assembly and translation, thereby promoting pollen tube growth (He et al., 2018). In summary, plants in thermomorphogenesis increase auxin content by inhibiting HY5 to relieve the repression of auxin synthesis.

While SAURs promote plant growth and cell elongation (Ren & Gray, 2015), the molecular mechanisms by which high temperature regulates SAURs during high temperature-induced hypocotyl elongation remain poorly understood. Using Arabidopsis and tobacco as experimental materials, this study primarily employs molecular biological approaches to address the following questions: (1) the upstream-downstream relationship between HY5 and auxin in the thermomorphogenesis signaling pathway; (2) whether HY5 regulates the transcription of SAUR1/2/3/4; (3) whether HY5 can bind to SAUR1/2/3/4 promoter chromatin regions and the specific binding locations; and (4) whether HY5 can regulate SAUR1/2/3/4 transcription independently of auxin. This research focuses on how high temperature regulates SAURs in thermomorphogenesis, enriches the downstream target genes of HY5, and further deepens our understanding of the molecular mechanisms by which high temperature regulates cell elongation genes.

Materials and Methods

1.1 Experimental Materials

In this study, we used the model plant Arabidopsis thaliana as the research material. The Columbia-0 (Col-0) ecotype served as the wild type, the hy5 mutant was used for genetic analysis, and the 35S::HY5-HA/Col-0 transgenic plants were employed for chromatin immunoprecipitation (ChIP) experiments. All three plant materials were obtained from the Key Laboratory of Photobiology, Chinese Academy of Sciences.

1.2 Plant Culture Conditions

Seed sterilization and seedling culture were performed according to our previously published protocol (Wang et al., 2015). For quantitative real-time PCR (qRT-PCR) and chromatin immunoprecipitation (ChIP) experiments, seedlings were cultured under continuous white light at 20°C for 6 days, then transferred to either 20°C or 29°C for an additional 6 hours. After cultivation, whole seedlings were harvested, immediately frozen in liquid nitrogen, and stored at -80°C until use. For phenotypic experiments, seedlings were cultured under continuous white light at either 20°C or 29°C for 8 days. The light intensity was 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

1.3 Hypocotyl Photography and Data Processing

Seedlings of different genotypes were cultured on the same plate, with three independent replicates. After photography, hypocotyl lengths were measured using Image-J software (http://rsb.info.nih.gov/ij).

1.4 RNA Extraction from Arabidopsis

Total plant RNA was extracted using the Tiangen Polysaccharide and Polyphenol Plant Total RNA Extraction Kit (DP432) according to the manufacturer' s instructions.

1.5 Reverse Transcription of RNA

For reverse transcription, we used the Tiangen FastKing cDNA First Strand Synthesis Kit (KR116) to synthesize cDNA from the extracted RNA.

1.6 Quantitative Real-time PCR (qRT-PCR) Experiments

We performed qRT-PCR using Tiangen SYBR Premix Kit with UBQ1 as the internal reference gene. PCR primer sequences are listed in Table 1 .

Table 1 qRT-PCR primer sequences

Primer	Sequence (5 $→3)|*Primer*|*Sequence*(5→$3)		
UBQ1-F	TTCCTTGATGATGCTTGCATC	SAUR2-R	GGCTTTACGCAACAAGGCTT
UBQ1-R	TTGACAGCTCTTGGGTTCAAG	SAUR3-F	ACTTCAGGGCTTGTTCCGAA
SAUR1-F	TGATCGGACTCTCTCAGCTA	SAUR3-R	GGTATCGTCAAGCCACCCAT
SAUR1-R	GTACCCGACGTAGCCAAGAG	SAUR4-F	TGTCGCGTGTGATCAACTCT
SAUR2-F	GGAGAAGACGAGATGGAGACG	SAUR4-R	TCTCCGACATAAACCGCAACA

1.7 Chromatin Immunoprecipitation (ChIP) Experiments

ChIP experiments were performed according to previously described methods (Jing et al., 2013). HA antibody or IgG serum was used for protein-DNA complex precipitation. DNA quantification was performed by qRT-PCR (primers listed in Table 2).

Table 2 ChIP-qPCR primer sequences

Primer	Sequence (5 $→3)|*Primer*|*Sequence*(5→$3)		
SAUR1-F1	GGGTCAAGAGGTCACCAAGA	SAUR3-F1	TGTGTCTTAAACGCGATATCTTTT
SAUR1-R1	ACTTCATTATGTTCTAGAAAG	SAUR3-R1	AGCAACAAACCTTCACCCCT
SAUR1-F2	AGCCACCTTTGAGAGAAAGA	SAUR3-F2	GGAGAAGACGAGATGGAGACG
SAUR1-R2	TGGGAACAAAAGCATCTGCA	SAUR3-R2	AGAATCTACCAACTGTATTAGTGCG
SAUR1-F3	AGCCACCTTTGAGAGAAAGA	SAUR3-F3	GCAAATAGCTATAGATCTATGGGGA
SAUR1-R3	TTGTTTGTGGGGTGAGGCAT	SAUR3-R3	TGTTTGTTAAATTTTCAGCTCCATAGA
SAUR1-F4	AGGACAAGGCATAAGCAGCA	SAUR3-F4	TGAGCTAAGCCATGTGAGCC
SAUR1-R4	TGCAAATGAGTAAGAAGCGA	SAUR3-R4	AAACAGTACGATGCGATATTTTGA
SAUR2-F1	GGAACCACAAACCTCTTCTTC	SAUR4-F1	TGGTTGAAAAATCCATCTTACAAAGA
SAUR2-R1	TCCAAACATAAATAAGAAGCA	SAUR4-R1	TGATGTAAGTCAGAGCTGGGT
SAUR2-F2	AGACAACGAGGGGAAGAAGA	SAUR4-F2	AGGATGGTGGATCACATGCA
SAUR2-R2	AGCTAAGCCATGTGAGCCAG	SAUR4-R2	TGATAAAATTGTGCAGTACTGTTTCA
SAUR2-F3	TCAGCTCCATAGATAGTTATTGAT	SAUR4-F3	CAATAAATAATGGAAATGAAACACGTGAC
SAUR2-R3	AGCCACAGTCACAATCAGCT	SAUR4-R3	TTAATCAGTTGAGTTAATATCACTGCT
SAUR2-F4	AAAATCATGCACTAATTAAGAATT	SAUR4-F4	TCGCCACCATTTTCAAGAGCCA
SAUR2-R4	AGAATCTACCAACTGTATTAGTGCG	SAUR4-R4	AGTTTGCAGAGTTTCACATGGT

1.8 Vector Construction

The promoter regions of SAUR1, SAUR2, SAUR3, and SAUR4 (2.0 kb DNA fragments upstream of the start codon ATG) were amplified from wild-type seedling genomic DNA using pfu DNA polymerase. The coding region of HY5 was amplified from wild-type seedling cDNA using pfu DNA polymerase (primers listed in Table 1). After sequence verification, the amplified fragments were inserted into the pEASY-Blunt vector (TransGen) to generate pEASY-SAUR1p, pEASY-SAUR2p, pEASY-SAUR3p, pEASY-SAUR4p, and pEASY-HY5.

The E-box-containing regions in the HY5-binding sites of SAUR1/2/3/4 promoters were mutated using the TaKaRa MutanBEST kit to obtain pEASY-SAUR1p(mut), pEASY-SAUR2p(mut), pEASY-SAUR3p(mut), and pEASY-SAUR4p(mut).

The HY5 coding region from pEASY-HY5 (digested with EcoRI and XhoI) was inserted into the pGreenII 62-SK vector (digested with EcoRI and XhoI) to generate 35S:HY5 (used as the effector in experiments). Promoter fragments from pEASY-SAUR1p, pEASY-SAUR2p, pEASY-SAUR3p, and pEASY-SAUR4p and their mutant versions were obtained by XhoI and BamHI digestion and inserted into the pGreenII 0800-LUC vector (digested with XhoI and BamHI) to generate pSAUR1:LUC, pSAUR2:LUC, pSAUR3:LUC, and pSAUR4:LUC and their mutant versions (used as reporters in experiments).

1.9 Dual Luciferase Reporter Gene Assay

Transient promoter activation assays were performed using the dual luciferase reporter gene detection method (Ji et al., 2021).

1.10 Data Analysis

One-way ANOVA was performed using IBM SPSS Statistics, and Duncan' s multiple comparison test was used to analyze differences between groups. * indicates significant difference (P<0.05), ** indicates highly significant difference (P<0.01). Different lowercase letters also indicate significant differences.

Table 3 DNA fragment cloning primer sequences

Primer	Sequence (5 $→3)|*Primer*|*Sequence*(5→$3)		
HY5-F	GAATTCATGCAGGAACAAGCTCAC	pSAUR2-R	GGATCCATCTATCTTTCAAATGAAC
HY5-R	CTCGAGCTAGACTCGTAAATCTG	pSAUR3-F	CTCGAGTGGAACAAAGGATGGT
pSAUR1-F	CTCGAGCTCCATTATGTGAATAG	pSAUR4-R	GGATCCGTCTTCTTATTTATGTTTG

Primer	Sequence (5 $→3)|*Primer*|*Sequence*(5→$3)		
pSAUR1-R	GGATCCCTTTGAGGACTTAATTC	pSAUR4-F	CTCGAGCATTGAGGAACGCATTA
pSAUR2-F	CTCGAGGGAACCACAAAGCATTC	pSAUR4-R	GGATCCCTTTAAAAGCAAGAAG

Results

2.1 Auxin Acts Downstream of HY5 in the Thermomorphogenesis Signaling Pathway

Both auxin and the transcription factor HY5 regulate mild high temperature-induced hypocotyl elongation (Delker et al., 2014), but their upstream-downstream relationship in the thermomorphogenesis signaling pathway remains unclear. In thermomorphogenesis, auxin synthesized in cotyledons is transported to the hypocotyl to promote its elongation. NPA is an auxin transport inhibitor that blocks polar auxin transport from cotyledons to hypocotyl. Consistent with previous studies (Gray et al., 1998), NPA inhibited hypocotyl elongation at both 20°C and 29°C (Figure 1 [Figure 1: see original paper]). Notably, NPA treatment strongly inhibited hypocotyl elongation in HY5 under both temperature conditions (Figure 1). This indicates that auxin acts downstream of HY5 in the thermomorphogenesis signaling pathway.

Figure 1 The genetic relationship between HY5 and auxin in the thermomorphogenetic signaling pathway. A, C, E: Standard phenotypes, scale bars = 2 mm; B, D, F: Quantitative statistics, error bars represent the standard error of 20 seedlings. Plant culture conditions: continuous white light at 20°C or 29°C for 8 days. Different letters indicate significant differences between samples ($P<0.05$), the same below.

2.2 HY5 Regulates the Expression of SAUR1/2/3/4

We examined whether HY5 regulates the transcription of SAUR1/2/3/4. Consistent with another concurrent study from our laboratory, mild high temperature promoted the transcription of SAUR1/2/3/4 in Col seedlings (Figure 2 [Figure 2: see original paper]). At both 20°C and 29°C, the transcript abundance of SAUR1/2/3/4 was significantly higher in hy5 mutants than in Col (Figure 2), demonstrating that the transcription factor HY5 inhibits the transcription of SAUR1/2/3/4 under both temperature conditions.

Figure 2 Effects of HY5 on the expression of SAUR1/2/3/4. A: Transcript abundance of SAUR1 in Col and hy5; B: Transcript abundance of SAUR2 in Col and hy5; C: Transcript abundance of SAUR3 in Col and hy5; D: Transcript abundance of SAUR4 in Col and hy5. Culture conditions for Col-0 and hy5:

6 days of continuous light culture at 20°C, then transferred to 20°C or 29°C for 6 hours before sampling. Error bars represent the standard error of three biological replicates.

2.3 Transcription Factor HY5 Binds to SAUR1/2/3/4 Promoter Chromatin

We investigated whether HY5 directly inhibits the transcription of SAUR1/2/3/4. Previous studies have shown that HY5 binds to E-box (CANNTG) cis-acting elements in target gene promoters (Leivar & Quail, 2011). We found numerous E-box cis-acting elements distributed throughout the promoter regions of SAUR1/2/3/4 (Figure 3 [Figure 3: see original paper]A). ChIP results showed enrichment in fragment 3 and 4 of the SAUR1 promoter (Figure 3B), fragment 1 and 2 of the SAUR2 promoter (Figure 3C), fragment 4 of the SAUR3 promoter (Figure 3D), and fragment 3 and 4 of the SAUR4 promoter (Figure 3E). Moreover, enrichment at 29°C was significantly lower than at 20°C (Figure 3B-E). These results demonstrate that HY5 binds to specific E-box-containing chromatin regions in the SAUR1/2/3/4 promoters, and these bindings are inhibited by mild high temperature.

Figure 3 The relationship between HY5 and SAUR1/2/3/4 promoter region chromatin. A: Distribution of E-box elements in SAUR1/2/3/4 promoters and primers used in qRT-PCR (P1, P2, P3, and P4); B: HY5 binding to the promoter region of SAUR1; C: HY5 binding to the promoter region of SAUR2; D: HY5 binding to the promoter region of SAUR3; E: HY5 binding to the promoter region of SAUR4. Culture conditions for Col-0: 6 days of continuous light culture at 20°C, then transferred to 20°C or 29°C for 6 hours before sampling. SAUR1/2/3/4 are gene coding sequences amplified using corresponding qRT-PCR primers. Error bars represent the standard error of three biological replicates (*P<0.05, **P<0.01), the same below.

2.4 HY5 Inhibits SAUR1/2/3/4 Expression in Dual Luciferase Reporter Assays

We further examined whether HY5 regulates SAUR1/2/3/4 transcription using dual luciferase reporter assays. In tobacco leaves co-expressing ProSAUR1p:LUC and Pro35S:HY5, LUC expression was significantly lower than in leaves co-expressing ProSAUR1p:LUC and empty vector (Figure 4 [Figure 4: see original paper]C). This phenomenon was also observed for the other three SAUR genes (Figure 4D-F), further confirming that HY5 inhibits the transcription of SAUR1/2/3/4. Additionally, we mutated the E-box elements in the HY5-binding regions of SAUR1/2/3/4 promoters and examined whether HY5 could still regulate reporter genes under the control of these mutated promoters. In tobacco leaves co-transformed with ProSAUR1p:LUC(mut) and Pro35S:HY5, LUC expression was similar to that in leaves co-expressing ProSAUR1p:LUC(mut) and empty vector (Figure 4C), with similar results observed for the other three SAUR genes (Figure 4D-F).

This indicates that HY5 requires these mutated E-box elements to inhibit SAUR1/2/3/4 transcription.

Collectively, the results from Figures 2, 3, and 4 demonstrate that under normal temperature conditions, HY5 strongly binds to SAUR1/2/3/4 promoter chromatin, strongly inhibiting their transcription and resulting in low expression levels of these four genes. When mild high temperature occurs, HY5 binding to SAUR1/2/3/4 promoter chromatin weakens, reducing transcriptional inhibition and consequently increasing expression of these genes.

Figure 4 HY5 inhibited the transcription of SAUR1/2/3/4 in tobacco leaf transient expression experiments. A: Construction of HY5 overexpression vector; B: Construction of wild-type and mutant promoter reporter vectors for target genes; C-F: Relative LUC activity in various co-transformation combinations. LB T-DNA: left border; CaMV 35S promoter: cauliflower mosaic virus 35S promoter; Rluc: reference gene; luciferase: marker gene; T7 promoter: promoter; polyA: polyadenylate; HY5: target gene; RB T-DNA: right border; EV: empty vector.

2.5 The Auxin Signaling Pathway Is Still Required for HY5 Regulation of SAUR1/2/3/4 Expression

To investigate whether HY5 can regulate SAUR1/2/3/4 expression independently of the entire auxin signaling pathway, we examined the transcript levels of these four genes in seedlings from Figure 1. As shown in Figure 5 [Figure 5: see original paper], NPA inhibited the transcription of SAUR1/2/3/4 in Col-0 under both temperature conditions, confirming the effectiveness of our drug treatment. Notably, the transcript abundance of SAUR1/2/3/4 in hy5(NPA) was significantly lower than in hy5 and similar to that in Col-0(NPA), indicating that the auxin signaling pathway is involved in HY5-mediated regulation of SAUR1/2/3/4 transcription.

Figure 5 Effects of NPA treatment on the regulation of SAUR1/2/3/4 transcription by HY5. A: Effect of NPA treatment on SAUR1 expression; B: Effect of NPA treatment on SAUR2 expression; C: Effect of NPA treatment on SAUR3 expression; D: Effect of NPA treatment on SAUR4 expression. Plant culture conditions: continuous white light at 20°C for 6 days. NPA concentration: 10 $\mu mol \cdot L^{-1}$.

Discussion and Conclusion

As genes that promote hypocotyl elongation, SAUR1/2/3/4 expression must be tightly regulated. Therefore, plants require both transcription factors that promote and those that inhibit their expression. HY5 functions to inhibit SAUR1/2/3/4 expression. At 20°C, plants need to maintain hypocotyl elongation at a low level. This study demonstrates that at 20°C, HY5 strongly

inhibits SAUR1/2/3/4 expression, keeping hypocotyl elongation gene expression low and thereby meeting the plant' s requirements for hypocotyl growth. At 29°C, plants require vigorous but not excessive hypocotyl elongation, as over-elongation would make plants prone to lodging or breakage, causing severe damage or death. This study shows that at 29°C, HY5' s ability to inhibit SAUR1/2/3/4 transcription is strongly suppressed but not completely abolished, preventing excessive expression of hypocotyl elongation genes and avoiding over-elongation.

Plants require numerous cell elongation genes to support hypocotyl elongation growth, including during high temperature-induced hypocotyl elongation. However, as an important negative regulator of thermomorphogenesis, only two HY5 target genes involved in cell elongation—EXP8 and XTR7—have been identified (Gangappa & Kumar, 2017). This study discovered new HY5 cell elongation target genes, further improving our understanding of HY5-mediated regulation of thermomorphogenesis.

Previous studies have shown that HY5 inhibits the transcription of the auxin synthesis gene YUC8 in thermomorphogenesis (Gangappa & Kumar, 2017). Therefore, HY5 can influence the auxin signaling pathway by regulating auxin synthesis, ultimately altering SAUR gene transcription. However, this signaling pathway involves multiple steps and requires a relatively long time to respond to high temperature environments. This study discovered that HY5 also directly regulates the transcription of SAUR1/2/3/4, representing a shorter signaling pathway that responds more quickly and may complement the previously identified long signaling pathway. Interestingly, our results indicate that this regulatory process still requires auxin, but this does not negate the direct regulation of SAUR1/2/3/4 by HY5. We propose that this regulatory process may involve modulation of auxin sensitivity. Combined with another concurrent study from our laboratory, we found that HY5 and PIF4 bind to the same regions of SAUR1/2/3/4 promoter chromatin, with high temperature inhibiting HY5 binding while promoting PIF4 binding. Moreover, HY5 inhibits while PIF4 promotes SAUR1/2/3/4 transcription. This suggests that enhanced binding of either HY5 or PIF4 may lead to reduced binding of the other, indicating potential competition between these two transcription factors for SAUR1/2/3/4 promoter binding. Previous research has shown that PIF4 and ARF6 interdependently promote the expression of hypocotyl elongation-related genes; weakening either one inhibits the other' s promoting effect, while enhancing either one strengthens the other's effect (Oh et al., 2014). Integrating this information, we propose the following molecular mechanism in plant thermomorphogenesis: at normal temperature, HY5 binding to SAUR1/2/3/4 promoter chromatin is enhanced while PIF4 binding is weakened, reducing PIF4' s ability to promote SAUR1/2/3/4 transcription and consequently decreasing ARF6' s ability to promote SAUR1/2/3/4 transcription, thereby reducing plant auxin sensitivity. At high temperature, HY5 binding to SAUR1/2/3/4 promoter chromatin is weakened while PIF4 binding is enhanced, increasing PIF4' s ability to promote SAUR1/2/3/4 transcription and consequently enhancing ARF6' s ability

to promote SAUR1/2/3/4 transcription, thereby increasing plant auxin sensitivity. Thus, HY5 can enhance the growth-promoting effect of auxin even when auxin levels have not increased.

The biological significance of this regulatory mode may be twofold. First, it accelerates plant response to high temperature. The pathway by which HY5 regulates SAURs through auxin synthesis involves multiple steps and thus requires more time. By increasing auxin sensitivity, plants can regulate SAUR1/2/3/4 transcription during the period before the auxin elevation signal is transmitted, thereby accelerating the response to high temperature. Second, it sustains the promoting effect of auxin on hypocotyl elongation. As mentioned in the introduction, the promoting effect of high temperature on auxin synthesis is relatively short-lived, and the subsequent promotion of hypocotyl elongation by high temperature may depend on increased auxin sensitivity (Huai et al., 2018; Sun et al., 2012; Pucciariello et al., 2014).

Ideally, this study would construct a hy5/saur1/saur2/saur3/saur4 quintuple mutant to observe whether the long hypocotyl phenotype of hy5 is suppressed, but this experiment is currently unfeasible for various reasons. Single mutants of saur1, saur2, and saur3 have not shown phenotypic differences from wild type at the morphological level, and the saur4 single mutant has not yet been isolated. We constructed a saur1/saur2/saur3 triple mutant, which also lacks a thermomorphogenesis phenotype, likely because SAURs constitute a large gene family with over 70 identified members and highly redundant functions. We also planned to use RNA interference technology to silence as many SAUR genes as possible, but could not find homologous sequences of the minimum length required for RNA interference even among these four SAUR1/2/3/4 genes. Overall, to our knowledge, current technology makes it difficult to reveal phenotypes upon SAUR gene deletion or downregulation, and no reports of such phenotypes have been retrieved. We subsequently constructed a hy5/saur1/saur2/saur3 quadruple mutant. As predicted, this quadruple mutant showed no difference from hy5 in thermomorphogenesis phenotype.

Plant signaling pathways form a complex network, often involving multiple pathways with intricate interconnections when regulating the same target. For example, in thermomorphogenesis, plants likely regulate SAURs through multiple interconnected pathways. These pathways have distinct characteristics and can complement each other, enabling plants to possess strong adaptability to high temperature. Based on this, we believe that other signaling pathways regulating SAURs likely exist and await further exploration. Overall, this study deepens our understanding of the molecular mechanisms underlying plant responses to high temperature and provides potential theoretical support for heat-resistance breeding in crops, which is receiving increasing attention.

References

BIANCHIMANO L, DE LUCA MB, BORNIEGO MB, et al., 2023. Temperature regulation of auxin-related gene expression and its implications for plant growth[J]. Journal of Experimental Botany, 74(22): 7015-7033.

CHALLINOR A, WATSON J, LOBELL D, et al., 2014. A meta-analysis of crop yield under climate change and adaptation[J]. Nature Climate Change, 4(3): 287-291.

COX DTC, MACLEAN IMD, GARDNER AS, et al., 2020. Global variation in diurnal asymmetry in temperature, cloud cover, specifc humidity and precipitation and its association with leaf area index[J]. Global Change Biology, 26(12): 7099-7111.

DELKER C, SONNTAG L, JAMES GV, et al., 2014. The DET1-COP1-HY5 pathway constitutes a multipurpose signaling module regulating plant photomorphogenesis and thermomorphogenesis[J]. Cell Reports, 9(6): 1-12.

GANGAPPA SN, KUMAR SV, 2017. DET1 and HY5 control PIF4-mediated thermosensory elongation growth through distinct mechanisms[J]. Cell Reports, 18(2): 344-351.

GRAY WM, ÖSTIN A, SANDBERG G, et al., 1998. High temperature promotes auxin-mediated hypocotyl elongation in Arabidopsis[J]. Proceedings of the National Academy of Sciences, 95(12): 7197-7202.

HE SL, HSIEH HL, JAUH GY, 2018. SMALL AUXIN UP RNA62/75 are required for the translation of transcripts essential for pollen tube growth[J]. Plant Physiology, 178(2): 626-640.

HUAI JL, ZHANG XY, LI JL, et al., 2018. SEUSS and PIF4 coordinately regulate light and temperature signaling pathways to control plant growth[J]. Molecular Plant, 11(7): 928-942.

ITO J, FUKAKI H, ONODA M, et al., 2016. Auxin-dependent compositional change in Mediator in ARF7- and ARF19- mediated transcription[J]. Proceedings of the National Academy of Sciences, 113(23): 6562-6567.

JI NN, WANG J, ZUO XX, et al., 2021, PpWRKY45 is involved in methyl jasmonate primed disease resistance by enhancing the expression of jasmonate acid biosynthetic and pathogenesis-related genes of peach fruit[J]. Postharvest Biology and Technology, 172(2): 111390.

JING Y, ZHANG D, WANG X, et al., 2013. Arabidopsis chromatin remodeling factor PICKLE interacts with transcription factor HY5 to regulate hypocotyl cell elongation[J]. The Plant Cell, 25(1): 242-256.

KIM S, HWANG G, KIM S, et al., 2020. The epidermis coordinates thermoresponsive growth through the phyB-PIF4-auxin pathway[J]. Nature Communications, 11(1): 1053.

LEE S, WANG W, HUQ E. 2021. Spatial regulation of thermomorphogenesis by HY5 and PIF4 in Arabidopsis[J]. Nature Communications, 12: 3656.

LEIVAR P, QUAIL PH, 2011. PIFs: pivotal components in a cellular signaling hub[J]. Trends in Plant Science, 16(1): 19-28.

MARASCHIN FS, MEMELINK J, OFFRINGA R, 2009. Auxin-induced, SCF(TIR1)- mediated poly-ubiquitination marks AUX/IAA proteins for degradation[J]. The Plant Journal, 59(1): 100-109.

OH E, ZHU JY, BAI MY, et al. 2014. Cell elongation is regulated through a central circuit of interacting transcription factors in the Arabidopsis hypocotyl[J]. Elife, 3(5): e03031.

PUCCIARIELLO O, LEGRIS M, COSTIGLIOLO RC, et al., 2018. Rewiring of auxin signaling under persistent shade[J]. Proceedings of the National Academy of Sciences, 115(21): 5612-5617.

QUINT M, DELKER C, FRANKLIN KA, et al., 2016. Molecular and genetic control of plant thermomorphogenesis[J]. Nature Plants, 2(1): 15190.

QUINT M, DELKER C, BALASUBRAMANIAN S, et al., 2023. 25 Years of thermomorphogenesis research: Milestones and perspectives[J]. Trends in Plant Science, 28(10): 1098-1100.

REN H, GRAY WM, 2015. SAUR proteins as effectors of hormonal and environmental signals in plant growth[J]. Molecular Plant, 8(8): 1153-1164.

ROOSJEN M, PAQUE S, WEIJERS D. 2018. Auxin Response Factors: output control in auxin biology[J]. Journal of Experimental Botany, 69(2): 179-188.

SPARTZ AK, REN H, PARK MY, et al., 2014. SAUR inhibition of PP2C-D phosphatases activates plasma membrane H+-ATPases to promote cell expansion in Arabidopsis[J]. The Plant Cell, 26(5): 2129-2142.

SUN JQ, QI LL, LI YN, et al., 2012. PIF4-mediated activation of YUCCA8 expression integrates temperature into the auxin pathway in regulating Arabidopsis hypocotyl growth[J]. Public Library of Science Genetics, 8(3): e1002594.

WONG JH, KLEJCHOVÁ M, SNIPES SA, et al., 2021. SAUR proteins and PP2C-D phosphatases regulate H+-ATPases and K+ channels to control stomatal movements[J]. Plant Physiology, 185(1): 256-273.

ZHU T, VAN ZM, DE SI, 2022. Wandering between hot and cold: temperature dose-dependent responses[J]. Trends in Plant Science, 27(11): 1124-1133.

Note: Figure translations are in progress. See original paper for figures.