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The *SAUR* gene family: the plant's toolbox for adaptation of growth and development

Running title: *SAUR* genes ensure dynamic growth adaptation

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Keywords: acid growth, auxin, brassinosteroids, cell elongation, growth adaptation, PIFs PP2C.D, Small Auxin Upregulated RNA, SAUR.

1

2 **Highlight:**

3 We discuss the importance of *SAUR* genes for plant growth adaptation, focussing on their
4 molecular functions and the various mechanisms for regulation of *SAUR* activity.

5

6

7 **Abstract**

8 The family of Small Auxin Up-Regulated genes (SAURs) is a family of auxin-responsive genes
9 with about 60 to 140 members in most higher plant species. Despite the early discovery of
10 their auxin responsiveness, their function and mode of action remained unknown for a long
11 time. In recent years, the importance of *SAUR* genes for the regulation of dynamic and
12 adaptive growth, and the molecular mechanisms by which SAUR proteins act are increasingly
13 understood. SAURs play a central role in auxin-induced acid growth, but can also act
14 independently of auxin, tissue-specifically regulated by various other hormone pathways and
15 transcription factors. In this review, we summarize the recent advances in *SAUR* gene
16 characterization in *Arabidopsis* and other plant species. We particularly elaborate on their
17 capacity to fine tune growth in response to internal and external signals, and discuss the
18 breakthroughs in understanding the mode of action of the SAURs in relation to their complex
19 regulation.

20

21

22 **Introduction**

23 The first discovery of small transcripts that rapidly responded to auxin dates back to 1987 from
24 experiments with elongating soybean hypocotyls (McClure and Guilfoyle, 1987). In the years
25 thereafter, these small auxin upregulated RNAs (*SAURs*) were also identified in tobacco,
26 *Arabidopsis* and maize (Gil *et al.*, 1994; Knauss *et al.*, 2003; Newman *et al.*, 1993), all showing
27 a rapid induction after auxin treatment. Both the transcript and protein half-lives were found
28 to be very short (Knauss *et al.*, 2003; McClure and Guilfoyle, 1989; Newman *et al.*, 1993),
29 indicating that SAUR activity can be quickly reduced after removal of the auxin stimulus,
30 allowing very dynamic responses. Because the transcripts were identified in elongating
31 hypocotyls and induced by the growth-hormone auxin, which had been proposed to induce
32 cell elongation via acid growth (Rayle and Cleland, 1970; Rayle and Cleland, 1980), a link

33 between auxin, *SAUR* gene expression and cell elongation was apparent. However, genetic
34 evidence demonstrating the role of SAURs in auxin-induced cell elongation remained absent
35 for a long time.

36 It was the renewed interest in *SAUR* gene function in combination with a strong
37 increase in the availability of genetic and molecular tools and resources, which recently
38 allowed to link the SAURs to auxin-induced growth in correspondence with the acid growth
39 theory. First, different *SAURs* were found to induce cell elongation in Arabidopsis when
40 overexpressed (Chae *et al.*, 2012; Li *et al.*, 2015; Spartz *et al.*, 2012; Stamm and Kumar, 2013),
41 and secondly, Spartz *et al.* (2014) made a major contribution to the field by showing that
42 SAURs can interact with PP2C.D phosphatases to inhibit their activity. This inhibition prevents
43 membrane H⁺-ATPases from being dephosphorylated, which increases their activity and
44 induces cell wall acidification. Thus, SAURs indeed induce plant growth by regulating cell wall
45 acidification. In addition to induction by auxin, *SAURs* can be regulated by a plethora of other
46 upstream factors, thereby regulating growth dynamically in response to internal as well as
47 environmental cues (e.g. Favero *et al.*, 2017; Hu *et al.*, 2018; Kodaira *et al.*, 2011; Oh *et al.*,
48 2014; van Mourik *et al.*, 2017). Because *SAUR* overexpression is sufficient to induce growth
49 (Fendrych *et al.*, 2016; Spartz *et al.*, 2017), other upstream factors may regulate SAUR-
50 mediated growth independent of the auxin pathway. SAURs have thus been unveiled as
51 growth-factors that are essential for both normal plant development as well as adaptation to
52 environmental conditions. In the last few years, *SAUR* studies from species other than
53 Arabidopsis have also been emerging, broadening our view on the importance of *SAURs* in the
54 plant kingdom.

55 Here, we review the recent advances in *SAUR* gene characterization in Arabidopsis as
56 well as in other plant species, and discuss their conservation and divergence in the plant
57 kingdom. We will summarize the novel insights into the molecular function of the SAURs, and
58 in particular elaborate on the different mechanisms of upstream and downstream regulation
59 of *SAUR* activity, which allow the plant to fine-tune growth in a tissue-specific manner under
60 different environmental conditions.

61

62 ***SAUR* gene evolution in the plant kingdom**

63 The *SAURs* form a plant-specific gene family, with the most basic members described in the
64 moss *Physcomitrella patens*, which contains 18 *SAUR* genes (Rensing *et al.*, 2008). Notably,

65 the Aux/IAA-ARF-mediated auxin signalling is also present from the moss lineages to the
66 higher plants (Lau *et al.*, 2009), suggesting that *SAUR* genes have been important for the
67 output of the auxin response from the beginning of land plant evolution onwards. Thanks to
68 recent advances in genome sequencing, *SAUR* families could be described in a large number
69 of species. Besides Arabidopsis, which contains 79 *SAUR* genes (Ren and Gray, 2015), most
70 higher plant species contain between 60 and 140 *SAUR* genes in their genomes, which are
71 often arranged in clusters (Chen *et al.*, 2014; Hu *et al.*, 2018; Jain *et al.*, 2006; Li *et al.*, 2017;
72 Wang *et al.*, 2010; Wu *et al.*, 2012). This high level of tandem and segmental duplications is
73 remarkable, but may to some extent be explained by the small size of the SAURs, permitting
74 duplication of the complete gene without loss of essential regions.

75 *SAUR* genes are generally intronless, with open reading frames predicted to encode
76 proteins of a size between 7 and 20 kDa (about 60 to 180 amino acids) (Chen *et al.*, 2014; Jain
77 *et al.*, 2006; Wang *et al.*, 2010; Wu *et al.*, 2012). These proteins have a conserved core of
78 approximately 60 residues, whereas the homology at the N-termini and C-termini is rather low
79 (Jain *et al.*, 2006; Park *et al.*, 2007; Ren and Gray, 2015). Within this core region, Wu *et al.*
80 (2012) identified four highly conserved motifs, present in the vast majority of the *SAUR*
81 proteins. The presence of these highly conserved motifs suggests that the *SAUR* proteins all
82 share a conserved basic function (see below). However, their variable N- and C-termini also
83 hint at distinct roles. For example, intracellular localization has been found to be different for
84 the *SAUR* proteins (e.g. Ma *et al.*, 2017; Markakis *et al.*, 2013; Park *et al.*, 2007; Qiu *et al.*,
85 2013; Spartz *et al.*, 2012), and may thus be encoded by the less conserved N- or C-terminus.
86 In addition, histidine-rich regions in the N- and C-termini of some Arabidopsis, sorghum,
87 tomato and potato *SAUR*s were suggested to allow metal-binding (Wu *et al.*, 2012), and some
88 maize, Arabidopsis (*SAUR70*) and soybean *SAUR*s have been shown to bind calmodulin via
89 their N-terminus (Popescu *et al.*, 2007; Yang and Poovaiah, 2000), while many more are
90 expected to have this capacity (Ren and Gray, 2015). The presence of the divergent N- and C-
91 termini thus suggest functional divergence amongst the *SAUR* proteins.

92 Kodaira *et al.* (2011) published a phylogenetic tree of the Arabidopsis *SAUR*s, in which
93 three distinct *SAUR* clades could be recognized (indicated as clades I to III). To discuss the
94 conservation and divergence of the *SAUR*s in a broader perspective and evaluate the position
95 of the Arabidopsis clades, we used the protein sequences from Arabidopsis, *Physcomitrella*,
96 potato, tomato, rice and sorghum *SAUR*s to construct a phylogenetic tree of the *SAUR* family.

97 Based on this analysis, the plant SAUR family can be divided into three subfamilies, which all
98 contain both monocot and eudicot sequences (see Figure 1 for an overview and Supplemental
99 Figure S1 for the complete tree). However, all *Physcomitrella* SAURs group together in one
100 clade of subfamily A (green in Figure 1), which consists of two *Physcomitrella* subclades that
101 are sister to a third subclade containing SAURs from sorghum, rice, potato and Arabidopsis.
102 These ancestral SAURs have sequences that are quite divergent from the other SAURs (see
103 Supplementary data File 1). The other two subfamilies, B and C, have only evolved after the
104 divergence of the mosses. These subfamilies contain clades that are lineage-specific for either
105 higher plants, monocots, eudicots, Arabidopsis or *Solanum*. This reveals that a considerable
106 number of recent gene duplication events have taken place throughout the evolutionary
107 history of the SAUR family, and that the duplicates have often been retained. This retention
108 may be explained by the advantage that a higher number of SAUR genes offers the plant. The
109 increasing complexity of higher land plants and their capability of colonizing different habitats
110 probably also raised a higher demand for growth adaptation in response to environmental
111 factors such as herbivory, shade and drought. The retention of duplicated SAUR clusters in
112 many different plant lineages suggests that SAUR copies are in general beneficial for the
113 plant's fitness, probably enhancing the plant's options to regulate growth.

114 Interestingly, proteins classified into clades I and II by Kodaira et al. were recovered in
115 two clades of subfamily C, most distantly related from the ancestral SAURs, while clade III
116 SAURs are dispersed over many clades. The Arabidopsis SAUR63-clade, placed into clade II by
117 Kodaira et al., can be found back as a separate clade ('clade IV') in our analysis. The SAURs
118 from clades I and II appear to possess functions distinct from those of clade III SAURs, as many
119 are responsive to abscisic acid (Kodaira *et al.*, 2011) and regulate cell elongation in seedlings
120 (Sun *et al.*, 2016) (see next section). This brings forward the intriguing possibility that these
121 functions have evolved more recently and are particularly important for the growth of higher
122 plants.

123

124 **SAUR function and mode of action**

125

126 *Cell elongation and growth*

127 The long period between the discovery of auxin-upregulated RNAs and their functional
128 characterization can be ascribed to the fact that single SAUR knock-outs rarely give a mutant

129 phenotype due to redundancy. In addition, distinct overexpression phenotypes could often
130 only be observed after stabilization of the protein through fusion with for example GFP (Chae
131 *et al.*, 2012; Spartz *et al.*, 2012). The first functional data therefore originated from
132 overexpression of fusion proteins or simultaneous downregulation of a group of paralogous
133 genes using amiRNA silencing. The majority of these studies showed that overexpression of
134 *SAUR* genes can induce cell elongation in Arabidopsis (Bemer *et al.*, 2017b; Chae *et al.*, 2012;
135 Franklin *et al.*, 2011; Kong *et al.*, 2013; Spartz *et al.*, 2012; Stamm and Kumar, 2013; van Mourik
136 *et al.*, 2017). Recently, Sun *et al.* (2016) used a comprehensive approach to show that light-
137 regulated seedling growth in Arabidopsis is controlled by a group of 32 redundantly acting
138 *SAURs*. These so-called *lirSAURs* (light-induced in cotyledons and/or repressed in hypocotyls)
139 are responsible for auxin-induced hypocotyl elongation in the dark and/or for the expansion
140 of cotyledons upon exposure to light. Phytochrome Interacting Factors (PIFs) are important
141 for this regulation in both tissues, but surprisingly, their breakdown upon exposure to light
142 reduces *SAUR* expression in the hypocotyls, while inducing it in the cotyledons (Sun *et al.*,
143 2016). The mechanisms behind this opposite effect remain to be resolved, but different co-
144 factors probably play a role (Sun *et al.*, 2016).

145 Although the function of the *SAURs* has thus far been mainly studied in Arabidopsis
146 seedlings, there is increasing evidence that their cell-elongating function goes far beyond the
147 juvenile stage, regulating growth in many different tissues. In addition to expression data,
148 which show plant-wide *SAUR* gene activity in various species (Hu *et al.*, 2018; Jain *et al.*, 2006;
149 van Mourik *et al.*, 2017; Wu *et al.*, 2012; Xie *et al.*, 2015), overexpression studies revealed that
150 *SAUR* activity can induce growth in leaves, stems and floral organs (Chae *et al.*, 2012; Spartz
151 *et al.*, 2012; van Mourik *et al.*, 2017). Interestingly, the specific expression of a *SAUR50*-like
152 gene from sunflower on the east side of the stem correlates with the diurnal bending of the
153 apex towards the sun (Atamian *et al.*, 2016), and there is evidence that the Arabidopsis
154 *SAUR10* gene, which is upregulated in shaded conditions, affects the degree of branch bending
155 (Bemer *et al.*, 2017b). This indicates that *SAURs* can also regulate light responses in the adult
156 phase in different plant species. In conclusion, the majority of *SAUR* genes probably play a role
157 in the induction of growth via cell elongation.

158 Auxin-induced cell elongation has been hypothesized to occur according to the acid
159 growth theory, based on the observation that a low pH induces cell wall loosening (Rayle and
160 Cleland, 1970) and that H⁺ excretion takes place in response to auxin application (Rayle and

161 Cleland, 1980). Recently, the mechanism by which acid growth occurs via auxin and SAURs
162 was step-by-step elucidated. First, Chen et al. (2010) showed that auxin induces
163 phosphorylation of the plasma membrane H⁺-ATPase AHA1 *in vitro*. Plasma membrane H⁺-
164 ATPases, of which *AHA1* and *AHA2* have the highest expression (Ren and Gray, 2015), require
165 phosphorylation of the C-terminal Thr-947 residue and subsequent binding of a 14-3-3 protein
166 for activation (Fuglsang *et al.*, 1999). Takahashi et al. (2012) then demonstrated that auxin
167 treatment increases the phosphorylation levels and 14-3-3 binding *in planta*, without changing
168 the amount of H⁺-ATPases. The localization of SAUR19-clade proteins to the plasma
169 membrane prompted Spartz et al. (2014) to investigate whether SAURs could regulate the H⁺-
170 ATPases, thereby discovering the link between auxin and cell membrane acidification, and
171 achieving a major break-through in the understanding of SAUR function. In their study, Spartz
172 et al. showed that SAUR proteins can interact with protein phosphatases of the PP2C.D family
173 to inhibit their function. This prevents dephosphorylation of the H⁺-ATPases, resulting in
174 increased H⁺-ATPase activity and induced membrane acidification (Figure 2A). Cell growth is
175 subsequently probably achieved by activation of cell-wall expansins due to the low apoplastic
176 pH, as well as increase of osmotic water flow due to plasma membrane hyperpolarization
177 (Spartz *et al.*, 2017). Arabidopsis SAURs from different clades were tested for their ability to
178 reduce PP2C.D activity *in vitro*, and they all exhibited this capacity (Spartz *et al.*, 2014; Sun *et*
179 *al.*, 2016), suggesting that repression of PP2C.D activity is the general mechanism by which
180 SAURs induce cell elongation. The Arabidopsis PP2C.D subfamily consists of nine members, of
181 which three (D2, D5 and D6) are located to the plasma membrane. In a recent paper, Ren et
182 al. (2018) showed that the three plasma-membrane localized PP2C.D members are the
183 primary regulators of AHA activity *in planta*, although small contributions of the other
184 PP2C.Ds, of which some can interact *in vitro* with SAUR19 as well, cannot be excluded. The
185 phenotype of the *d2d5d6* triple mutant is similar to that of *SAUR* overexpression lines, with
186 increased cell elongation in seedlings, leaves, stem and floral organs (Ren *et al.*, 2018),
187 suggesting that the SAUR-induced cell elongation is regulated via interaction with these
188 PP2C.Ds throughout the plant.

189

190 *SAUR function in other processes*

191 Interestingly, the overexpression lines of some *SAURs* were reported to display phenotypes
192 other than increased cell elongation, indicating that *SAUR* family genes may perform

193 additional functions. Some of these functions can probably be related to their interaction with
194 PP2C.Ds, while the mechanisms underlying other observed phenotypes may rely on different
195 factors. In this section, we shortly discuss the involvement of SAURs in other processes based
196 on the different phenotypes that have been reported.

197 An early senescence phenotype has been observed in overexpression lines of *SAUR10*,
198 *SAUR36* and the rice gene *OsSAUR39* (Bemer *et al.*, 2017b; Hou *et al.*, 2013; Kant *et al.*, 2009),
199 while *saur36* knock-out mutants exhibited a delayed leaf senescence phenotype (Hou *et al.*,
200 2013). Thus, SAURs appear to induce senescence, a function that may be regulated by
201 interaction with a PP2C.D phosphatase, as Xiao *et al.* (2015) identified the PP2C.D phosphatase
202 SENESCENCE-SUPPRESSED PROTEIN PHOSPHATASE (SSPP) as an important negative regulator
203 of leaf senescence. SSPP (PP2C.D7 according to TAIR, but designated PP2C.D1 in Ren *et al.*
204 2018), which is mainly cytosolic localized, dephosphorylates the senescence-inducing
205 receptor-like kinase AtSARK, localized at the plasma membrane (Figure 2B) (Xiao *et al.*, 2015).
206 SAURs may thus interact with SSPP in the cytosol, thereby repressing its activity and activating
207 AtSARK and leaf senescence.

208 Several other studies reported *SAUR* overexpression phenotypes not related to cell
209 elongation. In particular the few studies that published about nuclear-localized SAURs report
210 overexpression phenotypes different from cell elongation. Overexpression of *SAUR32*, the
211 first characterized Arabidopsis *SAUR* gene, leads to reduced hypocotyl growth and abolished
212 apical hook formation in the dark. The gene does not respond to auxin or light (Park *et al.*,
213 2007; Sun *et al.*, 2016) and is localized to the nucleus, suggesting that it does not interact with
214 the plasma membrane PP2C.Ds. Overexpression of *SAUR76*, which is predominantly nuclear
215 localized, does not promote cell elongation either, but affects the meristematic activity of the
216 tissues, with less cells in the leaves and more cells in the roots (Markakis *et al.*, 2013). Both
217 genes thus appear to have a function in the nucleus that may be unrelated to interaction with
218 PP2C.Ds, or involves nuclear-localized PP2C.(D)s. Interestingly, Ma *et al.* (2017) reported that
219 the cassava MeSAUR1 protein, also localized to the nucleus, can bind and regulate the
220 promoter of the ADP glucose pyrophosphorylase subunit *MeAGPs1a*, and would thus act as a
221 transcription factor. MeSAUR1 contains a specific N-terminus conserved in a clade of monocot
222 and eudicot SAURs, among which the Arabidopsis *SAUR10* and *SAUR50* proteins (Figure 1). It
223 is not very likely that this N-terminus provides DNA-binding activity however, as both *SAUR10*
224 and *SAUR50* exhibit canonical cell-elongation phenotypes upon overexpression. A more

225 thorough *in vivo* analysis of MeSAUR1 and other SAURs in the future is required to determine
226 whether some SAURs can act as transcription factors and to unveil the role of SAURs in the
227 nucleus.

228 *SAUR* overexpression can also have an effect on auxin levels, polar auxin transport
229 and/or expression of auxin pathway genes (Chae *et al.*, 2012; Kant *et al.*, 2009; Kong *et al.*,
230 2013; Ren and Gray, 2015; Spartz *et al.*, 2012; Xu *et al.*, 2017). Overexpression of growth-
231 inducing *SAURs* (*SAUR19*, *SAUR41*, *SAUR63*) results in increased auxin transport, while
232 overexpression of growth-inhibiting *SAURs* (*OsSAUR39*, *OsSAUR45*) has a repressive effect
233 (Kant *et al.*, 2009; Xu *et al.*, 2017). These effects on the auxin pathway can be indirect, because
234 the increase in H⁺-ATPase activity probably leads to an increased plasma membrane potential,
235 expected to induce auxin transport (Ren and Gray, 2015). However, since polar auxin transport
236 is regulated via phosphorylation of the PIN auxin efflux carriers via PP2C.A phosphatases
237 (Ballesteros *et al.*, 2012), one could also speculate that some SAURs might interact with other
238 PP2C phosphatases, thereby acting directly on polar auxin transport. Another plausible
239 explanation for the effect on polar auxin transport is the putative calmodulin-binding capacity
240 of many SAURs, because polar auxin transport depends on calcium signalling (Vanneste and
241 Friml, 2013; Ren and Gray, 2015).

242 These examples show that SAUR function is not restricted to the promotion of cell
243 elongation. Other observed functions, such as senescence, are probably also regulated via the
244 interaction with PP2C.Ds, while other functions may depend on other mechanisms and be
245 more clade-specific. The presence of specific N- or C-termini could enable calmodulin-binding,
246 metal binding (Wu *et al.*, 2012), interaction with ethylene receptors (*SAUR76-78*, (Li *et al.*,
247 2015)), or even DNA-binding capacity. The clade-specific presence of conserved N- or C-
248 termini suggests that different sub-clades can have distinct functions. Interestingly, the
249 Arabidopsis SAURs that can induce cell elongation and were reported by Sun *et al.* (2016) to
250 be regulated during seedling morphogenesis, practically all fall into clades I and II defined by
251 Kodaira *et al.* (2011), while most clade III SAURs are either not expressed in the
252 hypocotyl/cotyledon, or do not exhibit differential expression upon transfer to the light
253 (except for *SAUR41*, *SAUR49* and *SAUR52*) (Sun *et al.* 2016). This could mean that the ability
254 to induce cell elongation, probably linked to plasma-membrane localization, is recorded in the
255 protein sequence. Likewise, the ability to perform functions other than cell elongation may
256 also depend on specific protein motifs. The future elucidation of protein motifs responsible

257 for localization and protein-protein interactions will give more insight into the possible
258 presence of clade-specific functions.

259

260 In conclusion, the main function of SAUR proteins is the plant-wide induction of cell
261 elongation, by repression of PP2C.D activity, in accordance with the acid growth theory. The
262 growth-inducing function appears to be executed by plasma membrane localized SAURs
263 interacting with PP2C.D2, D5 and D6. Furthermore, some SAURs probably perform roles in
264 other processes than cell elongation, such as leaf senescence or cell division. In agreement
265 with this, a number of SAURs (including MeSAUR1, OsSAUR39, OsSAUR45, SAUR32, SAUR36,
266 SAUR40, SAUR41, SAUR55 and SAUR71) do not localize to the plasma membrane, but to the
267 cytosol or nucleus (Kant *et al.*, 2009; Kong *et al.*, 2013; Narsai *et al.*, 2011; Park *et al.*, 2007;
268 Qiu *et al.*, 2013; Xu *et al.*, 2017). These SAURs can possibly interact with other PP2C.Ds, which
269 are localized to other cell compartments (Ren *et al.*, 2018) (see Figure 2), or even with PP2Cs
270 from other classes. Interestingly, only few rice and sorghum sequences group together with
271 the clade I and II Arabidopsis proteins, while the majority of the monocot sequences are
272 closest to the clade III Arabidopsis proteins, of which the function appears less restricted to
273 cell elongation. This may imply that the abundance of SAUR-proteins involved in cell
274 elongation has evolved in the eudicots, while the majority of the monocot SAURs displays
275 other functions.

276

277 **Regulation of the different SAURs is highly diverse**

278 In contrast to their general role in cell elongation, the regulation of different SAUR genes is
279 highly diverse (see Figure 3 for a graphical summary). In recent years, reports from Arabidopsis
280 as well as other species have unveiled that SAURs show tissue-specific expression patterns
281 and can be regulated by a plethora of upstream factors. Although many SAURs can be induced
282 by auxin (~70% in Arabidopsis (van Mourik *et al.*, 2017)), there is also a group of SAURs, named
283 class II SAURs by Van Mourik *et al.* (2017), which is not responsive to auxin. At least one of
284 these SAURs however (SAUR8), can induce cell elongation when overexpressed (van Mourik
285 *et al.*, 2017), indicating that class II SAURs can promote growth by repressing PP2C.D activity
286 in response to stimuli other than auxin

287 Factors that can up- or downregulate SAUR expression have been identified in
288 different species. Characterization of the SAUR family in tomato (Wu *et al.*, 2012), cotton (Li

289 *et al.*, 2017), poplar (Hu *et al.*, 2018), citrus (Xie *et al.*, 2015), watermelon (Zhang *et al.*, 2017),
290 maize (Chen *et al.*, 2014) and Arabidopsis (e.g. van Mourik *et al.*, 2017) all revealed that the
291 different *SAUR* genes exhibit specific expression patterns throughout plant development.
292 Moreover, the expression of different sets of *SAUR* genes can be positively or negatively
293 regulated by many different hormones, including auxin (summarized in Ren and Gray, 2015;
294 van Mourik *et al.*, 2017), cytokinin (van Mourik *et al.*, 2017), gibberellic acid (GA) (Bai *et al.*,
295 2012; Stamm and Kumar, 2013), brassinosteroids (e.g. Oh *et al.*, 2014; van Mourik *et al.*, 2017;
296 Wiesel *et al.*, 2015), ethylene (only *SAUR76-78* (Li *et al.*, 2015)), ABA (Kodaira *et al.*, 2011;
297 Nemhauser *et al.*, 2006), jasmonate (JA) (Nemhauser *et al.*, 2006) as well as by different light
298 conditions (e.g. OuYang *et al.*, 2015; Roig-Villanova *et al.*, 2007; Sun *et al.*, 2016; van Mourik
299 *et al.*, 2017), cold (Hu *et al.*, 2018; Wu *et al.*, 2012), drought (Guo *et al.*, 2018; Wu *et al.*, 2012),
300 high temperature (Franklin *et al.*, 2011), and high salt conditions (Guo *et al.*, 2018; Wu *et al.*,
301 2012) in different plant species. In general, *SAUR* genes are upregulated in response to
302 hormones and conditions that are known to induce growth, such as auxin, brassinosteroids,
303 gibberellin and decreased R:FR ratios, but downregulated in response to ABA, JA, and stress
304 conditions, such as drought, cold and high salt. This stress-induced down-regulation of growth
305 is probably compensating the plant's investment in resistance mechanisms. GUS reporter
306 analysis revealed that the response of *SAURs* to environmental and hormonal stimuli occurs
307 mainly in the tissue where they are already expressed (Markakis *et al.*, 2013; van Mourik *et al.*
308 *et al.*, 2017). This suggests that the tissue-specific expression of *SAUR* genes is determined by
309 upstream transcription factors (TFs) that may be mainly developmentally regulated, while the
310 amplitude of their expression in these tissues depends on their response to various
311 environmental and hormonal stimuli. Plants thus contain an extensive toolbox to regulate
312 growth dynamically in different tissues in accordance with environmental conditions.

313 The idea that tissue-specific *SAUR* gene expression is regulated by upstream
314 developmentally regulated TFs is supported by large-scale ChIP-seq data, revealing frequent
315 binding events of key developmental regulators such as LEAFY (LFY), APETALA 1 (AP1),
316 APETALA 2 (AP2) SEPALLATA 3 (SEP3) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1
317 (SOC1) (van Mourik *et al.*, 2017). Induced activity of the TCP (TEOSINTE BRANCHED
318 1/CYCLOIDEA/PROLIFERATING CELL FACTOR 1) TFs TCP4 and TCP20 can also rapidly
319 upregulate a set of *SAUR* genes (Challa *et al.*, 2016; Danisman *et al.*, 2012), but in the case of
320 TCP4, this occurs probably indirectly via the ARF-BZR pathway (discussed below) by direct

321 induction of the auxin biosynthesis gene *YUCCA5* (Challa *et al.*, 2016). Only the binding and
322 regulation of the MADS-domain TF FRUITFULL (FUL) to the *SAUR10* locus, involved in the
323 repression of its stem-specific expression (Bemer *et al.*, 2017b), and the direct repression of
324 *SAUR19*-clade genes by the AHL transcription factor SUPPRESSOR OF PHYTOCHROME B4-#3
325 (SOB3) in hypocotyls (Favero *et al.*, 2016) has been characterized in more detail so far. The
326 factors involved in the regulation of tissue-specific *SAUR* expression thus largely await further
327 investigation.

328

329 *Regulation by ARF-BZR-PIF*

330 The mechanisms controlling auxin, brassinosteroid, gibberellic acid (GA) and light-regulated
331 *SAUR* expression have been largely elucidated in recent years. Oh *et al.* (2014) showed that
332 ARF6, BZR1 and PHYTOCHROME INTERACTING FACTOR 4 (PIF4) can physically interact with
333 each other in hypocotyls and have largely overlapping target gene sets, including a large
334 number of *SAUR* genes. This points to a major role for an ARF-BZR-PIF complex in the
335 regulation of *SAUR* gene expression. In line with this, *SAUR* genes can be synergistically
336 upregulated by combined addition of auxin and brassinosteroids, (Bemer *et al.*, 2017b; van
337 Mourik *et al.*, 2017; Walcher and Nemhauser, 2012), are abundantly present in target lists of
338 ARF5, ARF7, ARF8 and ARF19 (Nagpal *et al.*, 2005; Okushima *et al.*, 2005; Schlereth *et al.*, 2010)
339 and downstream of different PIFs (Sun *et al.*, 2016; van Mourik *et al.*, 2017). Several other
340 studies have provided additional evidence for the role of a ARF-BZR-PIF complex in the *SAUR*-
341 induced growth response. Sun *et al.* (2016) showed direct binding of PIFs to the *lirSAURs*,
342 which induces their expression in dark-grown hypocotyls; Miyazaki *et al.* (2016) reported that
343 the hypocotyl elongation phenotype of *LOV KELCH PROTEIN 2 (LKP2)* overexpression is
344 accompanied by *SAUR* gene upregulation and depends on both auxin and PIFs; and Favero *et al.*
345 (2017) found that both brassinolide and auxin treatment enhanced transcript accumulation
346 of *SAUR19* subfamily genes in hypocotyls and that blocking polar auxin transport could
347 attenuate the growth responses of SOB3 mutants to exogenous brassinolide. Moreover,
348 family-wide *in silico* analysis of the regulatory regions of the Arabidopsis *SAUR* genes revealed
349 that inverted repeats of two AuxRE elements, bound by ARFs (Boer *et al.*, 2014), are enriched
350 in auxin-induced Class I SAURs, in combination with BZR and PIF5 binding motifs (van Mourik
351 *et al.*, 2017).

352 GA also plays a role in the ARF-BZR-PIF signalling module, as the growth-inhibiting
353 DELLA proteins interact with BZR1 and with ARF6 (Bai *et al.*, 2012; Bemer *et al.*, 2017a; Oh *et*
354 *al.*, 2014), thereby preventing their binding to the DNA. In the presence of GA, DELLAs are
355 degraded and the ARF-BZR-PIF complex can induce *SAUR* expression. DELLAs can also interact
356 with PIFs, thus controlling the activity of the inducing complex even more (De Bruyne *et al.*,
357 2014). In line with this, GA induced hypocotyl elongation requires both BZR1 and PIFs (Bai *et*
358 *al.*, 2012). These data indicate that at least in Arabidopsis, there is a distinct group of *SAURs*
359 that can be induced by auxin, brassinosteroids, gibberellin and light through ARF-BZR-PIF
360 complexes (van Mourik *et al.*, 2017). The light response of *SAURs* is regulated via the PIFs,
361 which are degraded by the phytochromes in the active Pfr state when the ratio of red to far-
362 red light is high (Castillon *et al.*, 2007). In low light conditions or at low red:far-red ratios (in
363 the shade), PIFs are active and induce *SAUR* expression. *SAURs* thus both regulate growth
364 downstream of photomorphogenesis and contribute to the shade avoidance response (Ren
365 and Gray, 2015; Sun *et al.*, 2016; van Mourik *et al.*, 2017). Also warm-temperature-induced
366 *SAUR* upregulation in Arabidopsis is mediated by the PIFs (Franklin *et al.*, 2011). Research in
367 other species have linked brassinosteroid and light signalling to *SAUR* gene expression as well.
368 Mutations in the *Medicago* brassinosteroid receptor MtBRI1 affected the expression of a set
369 of *SAUR* genes (Cheng *et al.*, 2017), a potato *SAUR* gene was identified as a marker for
370 induction of the brassinosteroid pathway (Wiesel *et al.*, 2015), *SAURs* were identified
371 downstream of PIFs in rice (Kudo *et al.*, 2017) and light treatments in Norway spruce also
372 induced *SAUR* gene expression (OuYang *et al.*, 2015). In conclusion, the cell elongation
373 capacity and light response of seedlings appears to a large extent regulated by the ARF-BZR-
374 PIF complex.

375

376 *Other factors involved in SAUR regulation*

377 The regulatory networks involved in the repression of *SAURs* upon stress conditions such as
378 cold, drought and increased salinity have been less well characterized. However, Kodaira *et*
379 *al.* (2011) showed that the cold- and high salt-inducible TFs ARABIDOPSIS ZINC-FINGER 1
380 (AZF1) and AZF2, which function in the ABA response pathway, can repress 15 *SAUR* genes.
381 Electrophoretic Mobility Shift Assays (EMSAs) also showed that both TFs can bind to the
382 upstream region of *SAUR20* and *SAUR63*, indicating that the regulation of the *SAURs* by
383 AZF1/2 occurs via direct binding. The repressive effect of JA is probably transduced via the

384 ARF-BZR-PIF complex, because JAZ proteins can interact with the DELLA proteins, thereby
385 inhibiting the interaction of the DELLA proteins with the PIFs. In the presence of JA, JAZ
386 proteins are degraded, resulting in increased DELLA-mediated inhibition of ARF-BZR-PIF (Yang
387 *et al.*, 2012). The fact that the *pifq* mutant is impaired in JA-induced growth inhibition (Yang
388 *et al.*, 2012), confirms this dependency of JA signalling upon the ARF-BZR-PIF complex.

389 *SAUR* transcript levels are also regulated in a circadian manner. The sunflower *SAUR50*-
390 like gene for example, is particularly highly expressed in the morning at the east-side of the
391 stem (Atamian *et al.*, 2016), while the circadian movement of waterlily flowers is under control
392 of auxin, associated with day-time dependent expression of 25 *SAUR* homologs in the petals
393 (Ke *et al.*, 2018). In *Arabidopsis* hypocotyls, *SAURs* are induced by PIFs (Oh *et al.*, 2014; Sun *et*
394 *al.*, 2016), which accumulate at dawn in short-day (SD) seedlings (Soy *et al.*, 2014). This
395 suggests that *SAUR* transcripts may also be most abundant around dawn, at least in SD
396 conditions, in agreement with the timing of maximum hypocotyl elongation (Soy *et al.*, 2014).
397 Indeed, *SAUR63* subfamily genes revealed to be diurnally expressed, with highest expression
398 in the early morning (Chae *et al.*, 2012). The clock genes PSEUDO-RESPONSE REGULATOR 5
399 (PRR5) and PRR7 are negative regulators of hypocotyl growth expressed in the course of the
400 day, and act as transcriptional repressors (Nakamichi *et al.*, 2010). Both factors can directly
401 bind to many *Arabidopsis SAUR* genes (van Mourik *et al.*, 2017), thereby probably repressing
402 their expression in the afternoon. Thus, the majority of the *SAUR* genes may be higher
403 expressed in the early morning and repressed in the afternoon through the upstream control
404 of clock genes. Family-wide temporal expression analyses are required however, to validate
405 this circadian expression pattern.

406 In addition to upstream regulation of *SAUR* gene transcription, post-transcriptional
407 and post-translational regulation of *SAUR* activity also contributes considerably to the *SAUR*-
408 mediated dynamic growth control. *SAUR* overexpression gives a much more severe phenotype
409 when fused to a tag such as GFP, which probably stabilizes the protein that has a very short
410 half-life (Chae *et al.*, 2012; Knauss *et al.*, 2003; Ren and Gray, 2015). Besides the rapid protein
411 decay, which has not been further investigated so far, several studies have shown that *SAUR*
412 transcript levels quickly drop after removal of the inducer (e.g. auxin) (Markakis *et al.*, 2013;
413 van Mourik *et al.*, 2017). This post-transcriptional regulation is at least in part regulated by a
414 ~40-nucleotide downstream (DST) element in the 3' untranslated region (UTR) of a number of
415 *SAUR* genes. This region was initially characterized in a few *SAURs* from soybean, mung bean

416 and Arabidopsis (McClure and Guilfoyle, 1989; Newman *et al.*, 1993), and confers mRNA
417 instability (Newman *et al.*, 1993). Sullivan and Green (1996) identified two functionally
418 important conserved regions within the DST element (ATAGAT and GTA) by mutational
419 analysis in tobacco. The DST element, more precisely defined as GGA(N)xATAGAT(N)xGTA, is
420 present in 30 of the 79 Arabidopsis *SAURs* (Ren and Gray, 2015). Overexpression of
421 Arabidopsis *SAURs* including the DST element resulted in much less severe phenotypes than
422 when the element was excluded (Hou *et al.*, 2013; van Mourik *et al.*, 2017). Putative DST
423 elements were also identified in *SAURs* from rice (Jain *et al.*, 2006) and tomato (Wu *et al.*,
424 2012). The DST element has been associated with circadian control of mRNA, because several
425 other transcripts with a DST sequence, which are upregulated in the *dst1* and *dst2* EMS
426 mutants, are regulated in a circadian manner (Pérez-Amador *et al.*, 2001). However, more
427 recently, also oxidative stress was found to induce transcript degradation via 3'UTR DST
428 sequences (Ravet *et al.*, 2012), suggesting that several upstream cues can induce DST-
429 mediated transcript degradation. Which upstream factors regulate *SAUR* mRNA decay
430 remains to be investigated. Identification of the loci causal for the *dst1* and *dst2* molecular
431 phenotypes would certainly contribute to the elucidation of DST-controlled *SAUR* mRNA
432 decay.

433

434 The data summarized in this section illustrate the complex regulation of the *SAUR* genes via
435 both developmental, environmental and clock-controlled pathways at the transcriptional and
436 post-transcriptional levels (see Figure 3). Despite the high level of complexity, many regulatory
437 modules appear to converge at the ARF-BZR-PIF complex, which integrates various upstream
438 cues. In addition, tissue-specific TFs and other upstream regulators also contribute
439 significantly to the dynamics of *SAUR* activity, and also determine the expression of the *SAURs*
440 that are not regulated via ARF-BZR-PIF.

441

442 **Concluding remarks**

443 Land plants need to constantly adapt their growth to the environmental circumstances in
444 accordance with their developmental stage. To achieve this, they evolved dynamic growth
445 factors that can rapidly induce growth in response to a wide range of internal and
446 environmental stimuli. These growth factors, the SAUR proteins, generally share a common
447 function in repression of PP2C.D phosphatases, but their genes exhibit a great regulatory

448 region diversity, allowing tissue-specific and stimuli-specific expression patterns. This provides
449 the plant with a great toolbox for growth adaptation. The high retention of *SAUR* genes after
450 duplication indicates that expansion of this toolbox delivers an evolutionary advantage. In
451 *Arabidopsis*, about ~70% of the *SAUR* genes is responsive to auxin and probably regulated by
452 the ARF-BZR-PIF complex. The majority of these *SAURs* regulate cell elongation, at least in the
453 seedling (Sun *et al.*, 2016), which is linked to interaction of their proteins with the plasma-
454 membrane localized PP2C.Ds (D2, D5 and D6) (Ren *et al.*, 2018). Plasma-membrane localized
455 *SAURs* are presumably the main determinants of cell elongation, at least in part regulated via
456 the ARF-BZR-PIF module. *SAURs* that are localized to the cytosol at the other hand, could
457 repress the cytosolic PP2C.D PPSL, thereby inducing senescence. Several *SAURs*, such as
458 *SAUR10* and *SAUR36* (Bemer *et al.*, 2017b; Hou *et al.*, 2013), can both induce cell elongation
459 and senescence, and are thus expected to localize both to the plasma membrane and the
460 cytosol. Interestingly, several *SAURs* exhibit nuclear localization (Narsai *et al.*, 2011; Park *et al.*,
461 *et al.*, 2007), and can possibly target the nuclear-localized PP2C.Ds: D1, D3 and D4 (Ren *et al.*,
462 2018). The nuclear-localized *SAUR32*, which has been characterized in detail (Park *et al.*,
463 2007), inhibits cell elongation and is not responsive to auxin, suggesting that nuclear-localized
464 *SAURs* may fulfill a function different from promoting cell elongation, possibly linked to
465 induction by other factors, such as cytokinin. *SAUR51*, expressed in meristematic cells, is also
466 non-responsive to auxin, but upregulated by cytokinin (van Mourik *et al.*, 2017). Future
467 experiments will have to elucidate whether the localization of *SAUR* proteins is indeed
468 predictive for their function, and whether this also correlates with their response to particular
469 stimuli. Other outstanding questions regarding *SAUR* regulation and molecular function (see
470 Table 1) will hopefully be solved in future studies as well.

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480 **Supplementary data**

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483 **Figure S1. Full version of the phylogenetic tree displayed in Figure 1.** The colours of the clade
484 correspond with the colours in Figure 1. The tree was generated in MEGA based on a hand-
485 adjusted Bio-Edit alignment (Supplementary data file 1)

486

487 **Data File S1. Alignment of all SAUR proteins used to generate the phylogenetic tree.**

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496

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Tables

Table 1. Outstanding questions

• Which protein motifs determine the intracellular localization of the SAUR proteins?
• Can SAURs also interact with other PP2C clades?
• Which protein motifs are required for the interaction with PP2C.Ds?
• Is the effect on senescence regulated via the interaction with PPSL?
• Are only plasma membrane localized SAURs involved in cell elongation?
• What is the biological function of the calmodulin binding SAURs?
• Does the predicted metal-binding capacity of some SAURs have a biological function?
• Which TFs are involved in tissue-specific <i>SAUR</i> expression?
• Which SAURs act redundantly in the different tissues?
• Which pathways are involved in the response of <i>SAURs</i> to abiotic stresses?
• Is the response to ARF-BZR-PIF linked to plasma membrane localization?
• How is the DST-mediated <i>SAUR</i> mRNA decay regulated?
• What is the reason for the short half-life of SAUR proteins?

Figures

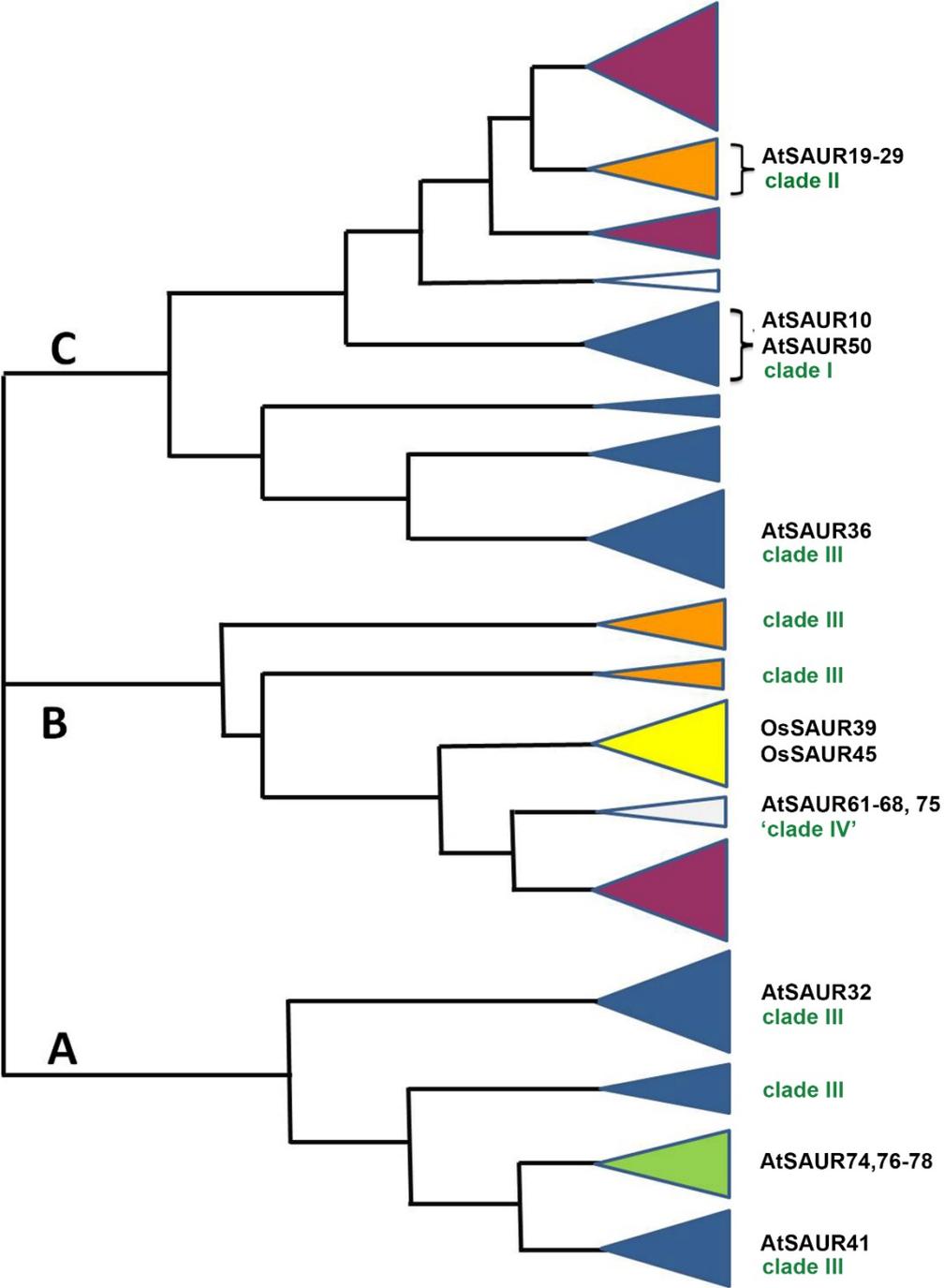


Figure 1. phylogenetic tree of the SAUR family. The unrooted tree was constructed from a hand-adjusted BioEdit alignment of all SAURs from Arabidopsis (www.Arabidopsis.org), Physcomitrella (Rensing *et al.*, 2008), potato (Wu *et al.*, 2012), tomato (Wu *et al.*, 2012), rice (Jain *et al.*, 2006) and sorghum (Wang *et al.*, 2010) (Supplemental data) using the maximum

likelihood method in the MEGA software (Hall, 2013). The colours of the triangles indicate the species represented in that clade. Green: all species (including *Physcomitrella*); Blue: eudicot and monocot; Yellow: monocot; Orange: eudicot; White: *Arabidopsis*; Purple: *Solanum*. In some cases, the separation of the clades is uncertain and supported by low bootstrap values (Supplemental Figure S1). Some characterized SAURs have been listed alongside the clades. The clade division from Kodaira et al. (2011) is indicated in dark green.

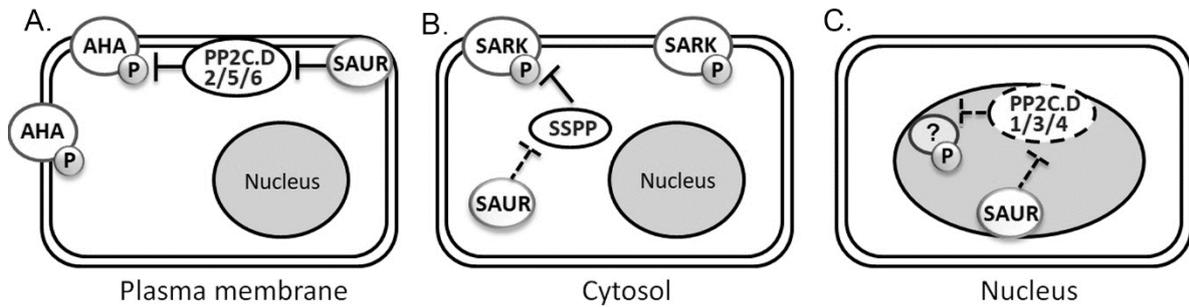


Figure 2. Schematic model of the putative molecular functions of SAURs in different cell compartments. A) In the plasma membrane, SAURs interact with PP2C.D2/5/6, thereby repressing dephosphorylation of the H⁺ATPases AHA1/2 and inducing cell elongation. **B)** In the cytosol, SAURs can probably interact with SSPP (PP2C.D1), thereby repressing dephosphorylation of AtSARK and inducing senescence. **C)** In the nucleus, the function of SAURs is still unclear, but they may interact with the nuclear localized PP2C.D1/3/4.

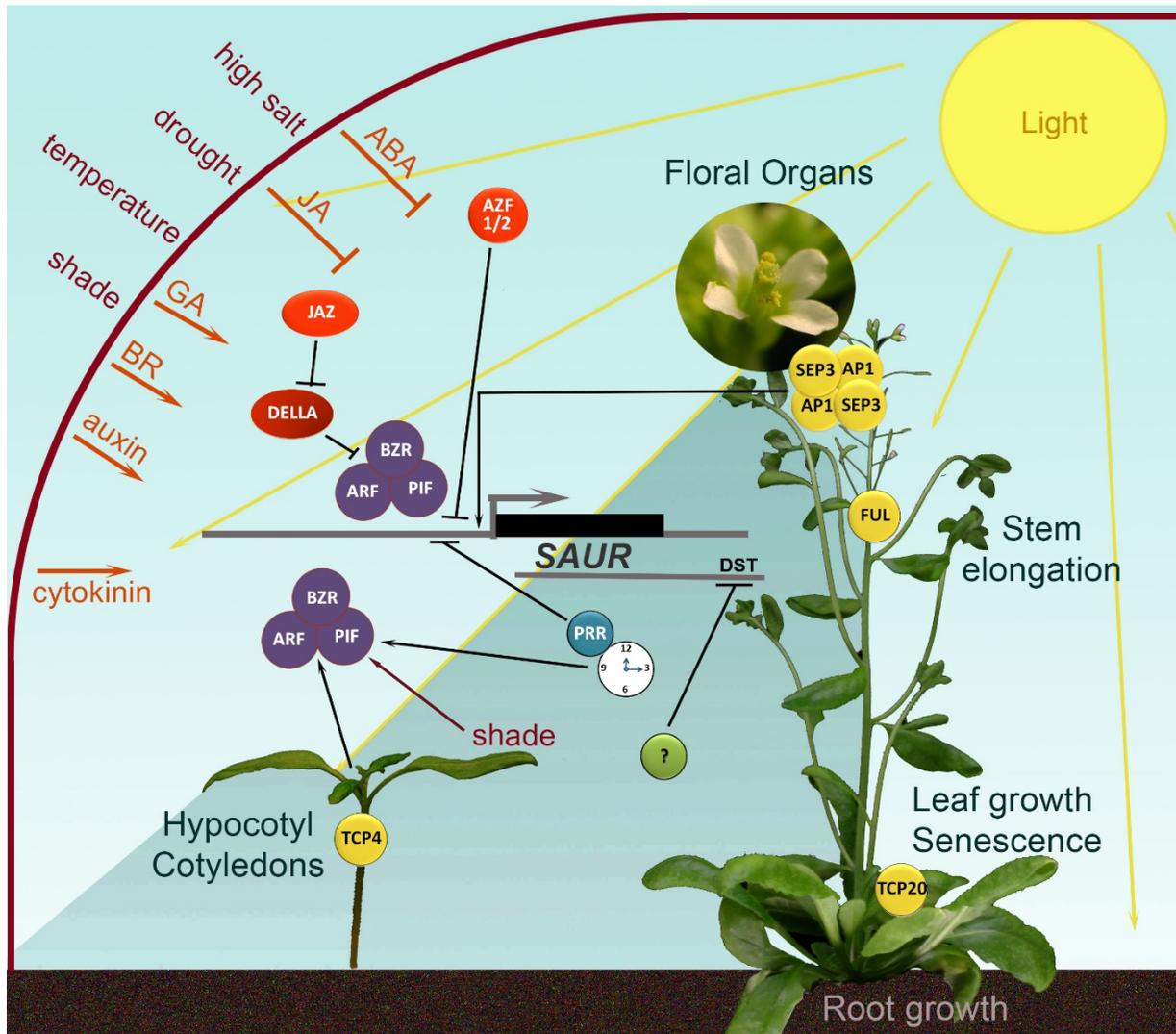


Figure 3. Regulation of SAUR genes by developmental, environmental and clock-controlled factors. The different tissues where SAURs play a role are indicated, as well as some upstream tissue-specific regulators (in yellow). Environmental signals (dark red) are transduced via hormones (orange). Most pathways converge at the level of the ARF-BZR-PIF complex (purple), while others directly act on the upstream region of SAUR genes or affect transcript stability. The black lines indicate direct or indirect activation or repression. The circadian regulation is indicated with a clock symbol.