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1     **Complex cellular and molecular events determining fruit size**

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12     **Key words:** organ growth, fruit development, molecular regulation, crop engineering

13

14

15 **Abstract**

16 The understanding of plant organ size determination represents an important challenge  
17 especially because of the significant role of plants as food and renewable energy sources and  
18 the increasing need for plant-derived products. Most of the knowledge on the regulation of  
19 organ growth and the molecular network controlling cell division and cell expansion, the main  
20 drivers of growth, is derived from arabidopsis. The increasing use of crops, such as tomato, for  
21 research, is now bringing essential information on the mechanisms underlying size control in  
22 agronomical important organs. This review describes our current knowledge, still very scarce,  
23 of the cellular and molecular mechanisms governing tomato fruit size and proposes future  
24 research to better understand the regulation of growth in this important crop.

25

26 **Tomato fruit, an excellent model to study growth and development ~~Fruit-size regulation~~**

27 In flowering plants, fruits are crucial organs since they protect the seeds during their  
28 development and allow their dispersal after maturation. Fruits also form an essential part of  
29 human diet and largely contribute to human health by providing a large variety of compounds  
30 including fibres, vitamins or phenolic compounds. Among the fruits produced worldwide,  
31 tomato is one of the most consumed. Tomato (*Solanum lycopersicum*) fruit is a low caloric  
32 source with high nutritional qualities since it contains lycopene, ascorbic acid, flavonoids and  
33 potassium. In addition to its specific biochemical properties and nutrient importance, with a  
34 short life cycle, high seed production, tomato has become a broadly used model for research on  
35 fleshy fruit physiology and development, a niche that cannot be filled by the model plant  
36 arabidopsis (*Arabidopsis thaliana*) producing a dry fruit, the silique. Indeed, while the silique  
37 grows after fertilization with little tissue differentiation, until it reaches its final length and then  
38 enters a senescence program, the growth of the tomato fleshy fruit is accompanied by important  
39 tissue differentiation, followed by the entry in a complex biochemical program, the ripening,  
40 which makes the fruit attractive and ready to disperse seeds [1]. The tremendous genetic  
41 diversity present in both wild and cultivated tomatoes, for a large number of which genome  
42 sequences are available, provides an extensive reservoir of resources available for genetic  
43 studies and trait discovery [2]. Especially, the domestication of tomato, which triggered the  
44 modifications of a wide range of morphological and physiological characters compared to its  
45 ancestral parents, resulted in a huge diversity in fruit weight and shape [3], thus providing key  
46 experimental systems to study growth determination. The diversity in fruit weight is very well

47 exemplified when comparing the wild *Solanum pimpinellifolium* producing fruits weighing  
48 around 1 g with the domesticated *Solanum lycopersicum* var. *lycopersicum* bearing fruits of  
49 more than 1 kg. Despite this huge diversity in fruit size obtained through domestication, the  
50 underlying cellular changes and genetic networks are still poorly known. Most of the knowledge  
51 on the molecular networks regulating growth is derived from studying the arabidopsis leaf, a  
52 model that was very valuable to define the actors in this process [4]. However, since the wiring  
53 of the networks might vary between different species, the translatability can sometimes be  
54 difficult. With the rapid advances in deep sequencing, in quantitative genetics and gene editing  
55 technologies, it is now possible to directly identify the components and connections of  
56 regulatory networks and potentially modify them in crops for breeding. These technologies are  
57 easily applicable to tomato, making of this plant an excellent model to dissect the genetic  
58 networks determining fruit growth. In this review, we present the current knowledge of the  
59 cellular and molecular mechanisms that govern tomato fruit size, and discuss future outlook on  
60 research to understand organ size determination.

61

## 62 **Tomato fruit growth: the pericarp, site of a complex set of cellular events determining** 63 **final fruit size**

64 After a period of vegetative growth, the transformation of the shoot apical meristem (SAM)  
65 into an Inflorescence Meristem (IM) marks the onset of the reproductive phase. In several  
66 plants, including tomato, the vegetative growth continues concomitantly to the reproductive  
67 phase through the activation, at the basis of the last initiated leaf, of a lateral meristem, also  
68 named sympodial meristem, thus defining the sympodial growth character [5]. As for the  
69 vegetative axis, the inflorescence shows a sympodial development in most of the cultivated  
70 tomatoes. The IM differentiates into a Floral Meristem (FM) to give rise to one flower and a  
71 new IM arises from the FM. This type of development gives the zigzag pattern to the tomato  
72 inflorescence as illustrated in **Figure 1A**. Once the FM differentiates, the development of the  
73 flower primordium will determine the organisation of the flower composed of four types of  
74 organs: the sepals, the petals, the stamens and the carpel. Wild tomato flowers are generally  
75 composed of 5 sepals, 5 petals, 5 stamens fused in cone and the pistil resulting from 2 fused  
76 carpels, is formed by the ovary, the style and the stigma [6], while cultivated tomatoes can  
77 contain up to 10 fused carpels such as in the Giant Heirloom tomato variety [7]. The tomato  
78 fruit differentiates after fertilisation from a pre-existing structure after fertilisation, the ovary,  
79 and is composed by the pericarp, the locular tissue surrounding the seeds, the placenta, the

80 columella and the septum corresponding to the fused edge of the carpels (**Figure 1B**). The  
81 pericarp, corresponding to the fleshy part of the fruit differentiates from the ovary wall. During  
82 and after flower development, two cellular events, cell division and cell expansion, occurring  
83 in different cell types, at different developmental times and different rates will determine the  
84 final fruit size. **Figure 2** illustrates in details the different steps of tomato fruit development,  
85 with a particular insight for the pericarp.

86 Tomato flower development can be divided into 20 stages and carpel formation initiates at stage  
87 4 [6]. Between stage 11 and maturity, the ovary displays intensive growth: the volume of the  
88 ovary wall increases by 2-fold and its thickness by 25% [8]. This growth is mostly supported  
89 by anticlinal cell divisions, increasing the number of cells in a given cell layer. Only one cell  
90 layer originating from a periclinal cell division is added to the ovary wall after stage 11 to reach  
91 around 9 cell layers at maturity. At stage 18, the ovary reaches its mature stage and growth is  
92 then arrested. Flower development ends at the anthesis stage which corresponds to the  
93 pollination and the fertilization of the ovary, leading to fruit set and triggering the onset of fruit  
94 development. At anthesis, the cells composing the ovary wall of the mature ovary, *i.e.* the future  
95 pericarp, have a homogeneous size with a square shape [8,9]. The pericarp is made up of  
96 different tissues, the exocarp, the mesocarp and the endocarp, corresponding to distinct cell  
97 layers which behave differently at the cellular level (**Figure 1C**) [10]. At anthesis, the exocarp  
98 corresponds to the three outer layers (E1, E2, E3), the endocarp to the two inner layers (I2, I1)  
99 and between the two, the four central layers form the mesocarp (M) [8]. Interestingly, the  
100 histological analysis of 20 tomato lines showing a wide diversity in final fruit weight, has shown  
101 that the pericarp characteristics at anthesis are mostly conserved regarding thickness, cell area  
102 and number of cell layers [11], showing that the size of the pericarp and consequently that of  
103 the fruit, is mainly determined after anthesis.

104 The successful fruit set is characterized by the growth resumption inside the ovary due to cell  
105 division activity [8,12]. After fruit set, tomato fruit growth is classically described as the  
106 succession of two phases, a phase of cell division followed by a phase of cell expansion.  
107 However, this view is very simplistic, since detailed quantification of cell division and cell  
108 expansion within the pericarp has shown that these two processes co-exist and overlap very  
109 early during fruit growth (**Figure 2**) [8,10,13]. In addition, cell layer-specific patterns of cell  
110 division and cell expansion have been described, highlighting the complex coordination of these  
111 two processes during fruit growth. For example, the different parts of the pericarp behave  
112 differently with the highest mitotic index found in the exocarp. Moreover, within the exocarp,

113 the three cell layers do not show similar division plane orientations. The outermost layer, E1,  
114 increases the number of cells through anticlinal divisions thus driving the increase in fruit  
115 perimeter and thus volume. The cells from the two other exocarp layers (E2 and E3), generate  
116 the new mesocarp cells layers (M') through periclinal divisions. To a lesser extent, the endocarp  
117 layer I2 takes part in the formation of few new mesocarp layers [8]. Only few divisions,  
118 periclinal or oblique, occur in the mesocarp. Depending on the genotype, cell division in the  
119 pericarp extends until 5 to 25 Days Post Anthesis (DPA), respectively in the wild tomato  
120 *Solanum pimpinellifolium* and in a large cultivated tomato variety Levovil [13,14]. In the small  
121 cherry tomato wva106 variety, this period of 9 days after pollination results in an increase of  
122 cell number by 30-fold in E1 and 19-fold in E2 [8]. Depending on the genotype, the number of  
123 cell layers in the pericarp ranges from 9 to 26 cell layers [10,11,13].

124 Concomitantly to the intensive cell division period, the mesocarp cells (M) expand slightly  
125 before anthesis and then show a high rate of expansion directly after anthesis until 20 DPA,  
126 resulting in an increase of the initial volume by 1550-fold in the wva106 variety [8]. After the  
127 division phase, cells of the other layers enter progressively in expansion leading for example to  
128 an increase in volume of 12-fold for cells in the E1 and 400-fold in the I1. Contrary to the  
129 original mesocarp cells, the cells from the new mesocarp layer (M') start to expand directly  
130 after their formation and continue until 36 DPA to reach an increase of 1350-fold in volume in  
131 the wva106 variety. As for the cell division, the orientation of cell expansion is important for  
132 pericarp growth. Indeed, a detailed phenotypic characterization of fruit cellular parameters in  
133 12 mutants presenting different fruit weight and tissue morphology has revealed that anisotropic  
134 cell expansion, expansion along the abaxial-adaxial axis, is an important parameter for pericarp  
135 thickness control [9].

136 The intense cell expansion period during fruit growth is characterized by the occurrence of the  
137 endoreduplication process [15]. Endoreduplication is the result of a modified cell cycle, named  
138 endocycle, during which DNA synthesis occurs independently from mitosis [16,17].  
139 Endoreduplication which leads to an increase in ploidy level, takes place already before anthesis  
140 since few nuclei of an 8C DNA content have been observed [8]. During fruit development, the  
141 iteration of endocycles leads to increased DNA content from 4C until 512C in some tomato  
142 cultivars [11]. In the pericarp, ploidy levels and cell areas are positively correlated [18].  
143 Bourdon et al. (2012) showed that endoreduplication acts as a morphogenetic factor involved  
144 in cell size control according to the 'karyoplasmic ratio theory': it contributes to maintain  
145 homeostasis of cytoplasmic components in a highly structured cellular system, where multiple

146 physiological functions are integrated to support cell growth during fruit development [19]. The  
147 contribution of endoreduplication in the determination of cell size is supported by a dynamic  
148 model of tomato fruit development that includes cell division [20].

149 After this intense growth period, the number of cells is multiplied by 15, the cell volume by  
150 170 and the pericarp volume by 2600-fold in the wva106 variety [8]. The pericarp is thus made  
151 of a heterogeneous population of cells with small cells on the outside and larger cells in the  
152 middle of the tissue [18]. This variation in final cell size might result from the different  
153 contribution of each cell layer to the fruit growth. Indeed, external layers could support the  
154 increase in volume through periclinal divisions and the inner layers might contribute to the  
155 increase in volume of the fruit mostly through expansion [8]. The occurrence in a coordinated  
156 manner of these two events triggers the transformation of a 1-2 mm ovary into a 2-10 cm fruit  
157 in diameter.

158 Despite the existence of a large diversity in tomato fruit phenotypes, fruit pericarp growth can  
159 be described as the succession of overlapping and interconnected cellular events with different  
160 onsets, with different rates and duration in function of the cell layers: anticlinal, periclinal and  
161 oblique cell divisions, and isotropic and anisotropic cell expansion (**Figure 2**). As a  
162 consequence, the final fruit size can only be achieved through a strict spatial and temporal  
163 control and coordination of these events. The analysis of mutants, transgenic lines or tomato  
164 genotypes with altered fruit size and shape led to the identification of genes involved in some  
165 of these processes. In the following section, we describe genes that are involved in each process,  
166 and present how altering the different phases of fruit development starting from ovary  
167 development until the onset of ripening, can affect the final size of the fruit (**Figure 3**). We also  
168 point the still missing information that would allow building a genetic network for fruit growth  
169 determination.

170

## 171 **Final fruit size: a highly regulated process from the ovary development to fruit ripening**

### 172 *Increasing carpel/locule number by altering meristem size*

173 Since the fruit is derived from the pre-existing ovary after fertilization, one can expect that an  
174 alteration in ovary size might affect final fruit size. A first mean to produce larger fruits by  
175 modifying the size of the ovary is well exemplified in the tomato beefsteak variety. In this  
176 variety, the number of locules, the cavities derived from fused carpels harboring the seeds,

177 reaches up to 10, thus leading to a fruit that weighs approximating 1 kg, while wild small  
178 tomatoes or small-fruited cultivars only contain two locules [21]. This increase in locule  
179 number, corresponding to carpel number, is determined as early as floral meristem development  
180 and organisation start [22,23]. Two natural mutations, *lc* (*locule number*) and *fas* (*fasciated*),  
181 mainly control the number of locules in tomato. *Lc* and *fas* are affected in the arabidopsis  
182 orthologous genes *WUSCHEL* (*WUS*) and *CLAVATA3* (*CLV3*) respectively, which are involved  
183 in meristem organisation (**Figure 3A**). In arabidopsis, *WUS* encodes a transcription factor  
184 involved in the maintenance of stem cell identity within the shoot apical meristem [24]. An  
185 overexpression of *AtWUS* produces flowers with supernumerary organs lacking the most central  
186 organs [25]. In tomato, 2 single-nucleotide polymorphisms (SNP) in the downstream region of  
187 the putative orthologous gene, *SlWUS*, are responsible for the increase in locule number in the  
188 *lc* mutant [26]. The occurrence of these SNPs are hypothesized to suppress the binding of the  
189 transcriptional repressor AGAMOUS that negatively regulates *WUS* such as in arabidopsis  
190 [27]. As a result, the expression of *SlWUS* is increased in floral buds of *lc* which in turn may  
191 allow the maintenance of a larger stem cell population resulting in increased locule numbers  
192 [28,29]. As a second important locus for locule number determination in tomato, the *fas* locus  
193 harbours a modification in the promoter of *CLAVATA3*, *SlCLV3* [23]. In arabidopsis, *CLV3*  
194 encodes a secreted glycopeptide involved in the restriction of meristem size through the  
195 activation of the receptor kinase CLV1 [30]. The loss-of-function mutant *clv3* produces  
196 enlarged SAM and FM with supernumerary flowers [31]. In arabidopsis the production of  
197 double mutants demonstrated that a *WUS/CLV3* negative feedback loop determines the  
198 organisation and the number of flower organs [32]. Interestingly, *SlCLV3* shows similar pattern  
199 of expression as *SlWUS* [28], and *lc* and *fas* loci have synergistic effects on locule number and  
200 thus fruit size when combined. The presence of the *fas* locus alone increases inflorescence  
201 branching in addition to locule number [23]. The downregulation of *SlCLV3* through RNAi  
202 approach shows similar phenotypes, but also has deleterious effects such as the development of  
203 ovaries within the initial ovary [28]. In addition, *SlCLV3* is down-regulated in a *fas* background  
204 showing that the *fas* mutation is a partial loss-of-function of *SlCLV3* [28]. Interestingly, Chu et  
205 al (2019) showed a positive trend between locule number and FM size resulting from the effects  
206 of *lc* and *fas*. Two additional mutants, *fasciated and branched* (*fab*) and *fasciated inflorescence*  
207 (*fin*), also display enlarged meristems, fasciated flowers with more floral organs and produce  
208 larger fruits as a consequence of additional carpels [23]. The genes underlying these phenotypes  
209 in the *fab* and *fin* mutants correspond to *CLV1* and an arabinosyltransferase, respectively  
210 (**Figure 3A**). In *fab*, a missense mutation was found in *CLV1* and in *fin*, missense and deletion



211 mutations leading to an absence of transcripts were found in a predicted HYDROXYPROLINE  
212 O-ARABINOSYLTRANSFERASE (HPAT). In arabidopsis, *CLV1* encodes a receptor kinase  
213 which binds to *CLV3* as to restrict *WUS* expression [33]. The rescue of arabinosyltransferase  
214 mutants by an arabinosylated *CLV3* showed that *CLV3* must be fully arabinosylated to perform  
215 its function [23]. In arabidopsis, the significance of arabinose modifications is less clear since  
216 null mutants for *HPAT* genes do not have a *clv* phenotype [34]. The *fab* and *fin* mutations have  
217 additive effect and thus act in the determination of meristem size through the WUS/CLV  
218 pathway. The loss of function of the EXCESSIVE NUMBER OF FLORAL ORGANS (SIENO)  
219 gene results in an increase of the FM size leading to the production of larger multilocular fruits,  
220 a phenotype that is much more pronounced in a *lc* mutation background [33]. SIENO encodes  
221 a transcription factor belonging to the superfamily APETALA2/ETHYLENE RESPONSIVE  
222 FACTOR (AP2/ERF), which is supposed to regulate directly *SIWUS* (Figure 3A) [33]. During  
223 domestication, a 85pb deletion in the SIENO promoter was selected leading to a reduction of  
224 its expression, and thus bigger fruits [33]. Among the actors putatively involved in locule  
225 number determination are the following genes: *INHIBITOR OF MERISTEM ACTIVITY*  
226 (*SIIMA*) and *KNUCKLES* (*SIKNU*) encoding respectively, a MIn zinc-Finger (MIF) and a  
227 transcription factor belonging to the C2H2 zinc-finger protein family (**Figure 3A**) [35,36]. The  
228 loss-of-function of *SIIMA* and *SIKNU* enlarges fruit size through an increase in carpel number,  
229 while the overexpression leads to the opposite effect, i.e. a decrease in fruit size. Together with  
230 TOPLESS, these two proteins form a transcriptional complex that recruits Histone deacetylase  
231 19, as to regulate negatively the expression of *SIWUS*, and thus impair stem cell activity within  
232 the floral meristem [35].

233 In tomato, the control of meristem size by the WUS-CLV pathway is thus essential for fruit size  
234 determination through the regulation of locule number (**Figure 3A**). Interestingly, this trait has  
235 been selected during domestication to produce large fruit-bearing plants by modulating the  
236 WUS-CLV signalling module mainly through mutations in cis-regulatory elements [37,38].  
237 Further research is now needed to identify the complete set of transcriptional regulators  
238 responsible for the modulation of this genetic network.

239 *Promoting cell division in the ovary and the fruit*

#### 240 Cell division control during ovary development

241 During flower development, cell division is the main driver for growth inside the ovary. The  
242 spatial modification of the rate or duration of cell division in the ovary will thus influence its  
243 final size and consequently final fruit size. Three QTLs, namely *fs8.1*, *sun* and *ovate*, controlling

244 fruit elongation within the cultivated tomato germplasm, are involved in the regulation of cell  
245 number along different growth axes of the ovary with *fs8.1* being the only one to ultimately  
246 increase fruit weight (**Figure 3B**) [21,39].

247 The *fs8.1* locus is present in processing tomatoes, referred to as square tomatoes [40]. In plants  
248 harbouring the *fs8.1* locus, the fruit shape index, corresponding to the ratio between the  
249 longitudinal and equatorial diameter, is different from plants harbouring the WT allele and leads  
250 to more elongated and heavier fruits [39]. This effect of *fs8.1* originates from the elongation of  
251 the ovary through the increase in cell number in the proximal–distal direction without any  
252 change in the medio-lateral direction (**Figure 3B**). In the abaxial-adaxial direction, an increased  
253 number of cell layers was also found in the fruit possibly leading to thicker pericarp. While cell  
254 size was not altered in the ovary, cells were smaller in the mature fruit pericarp of the *fs8.1*  
255 fruits. So far, the identity of the gene underlying the *fs8.1* locus remains unknown [39].

256 Two other major QTLs control fruit elongation: these are *sun* and *ovate* that do not lead to an  
257 increased fruit weight, on the contrary to *fs8.1*, [41,42]. *Ovate* confers to fruits a pear shape by  
258 increasing cell number in the proximo-distal direction and decreasing cell number in the  
259 mediolateral direction in the ovary, thus leading to increased proximal end of the fruit (**Figure**  
260 **3B**) [43]. In *sun*, elongated fruits are formed which contain more cells along the proximo-distal  
261 direction within the pericarp and the columella, while less cells are produced in the medio-  
262 lateral direction in the columella and the septum (**Figure 3 B**) [41]. As for *ovate* and *fs8.1*, the  
263 changes in *sun* occur during ovary development, but fruit elongation is mainly promoted shortly  
264 after anthesis. The genes underlying *ovate* and *sun* have been identified and correspond  
265 respectively to a member of the OVATE family protein (OFP) proposed to regulate cytoskeleton  
266 organisation [44,45] and to a member of the IQ67-domain (IQD) protein family that is involved  
267 in Ca<sup>2+</sup> signal transduction and cellular trafficking [46,47]. In *ovate*, the mutation results in a  
268 premature stop codon [44,45] while in *sun* the phenotype is caused by an interchromosomal  
269 duplication leading to increased expression of *SISUN* [48]. However, the mode of action of  
270 these two genes in the control of cell division remains totally elusive and further research should  
271 provide insight into the relation between cellular trafficking and cell proliferation.

272 In conclusion, *sun*, *ovate* and *fs8.1* control different mechanisms regulating ovary and fruit  
273 elongation acting on different spatial and temporal features of cell division (**Figure3B**). The  
274 synergistic interaction between these 3 loci suggest that these three genes are involved in  
275 distinct pathways which may converge at a common node for the regulation of proximo-distal  
276 organ patterning (**Figure 3B**) [42,49]. These pathways may involve hormone regulation

277 [42,49], but this still remains to be deciphered. Recently, it was shown that an auxin application  
278 during ovary development leads to elongated pear-shaped fruits resulting from cellular changes  
279 similar to the effects of *ovate* [49]. However how these three genes involved in fruit shape  
280 determination exert their roles in the control of cell division patterning still requires more  
281 investigations to be understood.

#### 282 Cell division control during both the ovary and the fruit development

283 The process of domestication in tomato has resulted in the selection of plants presenting a large  
284 diversity in fruit shape, but also in larger fruit size. About thirty QTLs related to fruit  
285 size/weight have been identified in tomato [50]. The *fw2.2* locus (for fruit weight QTL of  
286 chromosome 2, number 2) is responsible for up to 30% of the fruit weight variation [51]. The  
287 gene underlying *fw2.2* locus, *SIFW2.2*, encodes a protein containing a PLAC8 (Placenta-  
288 specific gene 8 protein) motif predicted to be important for the membrane localisation of the  
289 protein and belongs to the multigene *CELL NUMBER REGULATOR (CNR)* family [52]. Two  
290 different alleles, a “large fruit allele”, present in modern tomatoes, and a “small fruit allele”  
291 inherited from wild tomato ancestors, differ mainly from polymorphisms in the upstream  
292 regulatory region of the gene and lead to spatial and temporal differences of expression [53].  
293 The “large fruit” allele is expressed earlier during fruit development, while the “small fruit”  
294 allele is expressed later and maintained longer [54]. In plants harbouring the “large fruit” allele,  
295 the ovary is larger, mainly due to an increase in the number of cells without any change in cell  
296 size showing that *SIFW2.2* is involved in regulation of cell number (**Figure 3B**) [53]. In  
297 addition, at early stage of fruit development, the mitotic index is increased without any change  
298 in cell size recorded in the placenta and the pericarp and is negatively correlated with the  
299 expression of *SIFW2.2* [54,55]. The increase in cell division not followed by a modification of  
300 the thickness of the pericarp might indicate that *SIFW2.2* act as a negative regulator of anticlinal  
301 cell divisions (**Figure 3C**). In several plants species, orthologues of *SIFW2.2* are also involved  
302 in the regulation of the reproductive organ size such as in maize (*Zea mays*), where the  
303 overexpression of *ZmCNR1* leads to the formation of small organs [52]. Despite many studies  
304 on *FW2.2*, the mechanism of action by which such a membrane protein can negatively regulate  
305 cell number and thus fruit size, as well as the exact changes occurring at the cellular level (cell  
306 division rate, cell division duration...) are not yet understood.

#### 307 Cell division control during fruit development

308 Starting with the same pool of cells inside the ovary, a modification of the cell division rate or  
309 duration after anthesis can also affect the final size of the fruit. Among the QTLs related to fruit

310 weight/size, *fw3.2* is the second major locus controlling tomato fruit mass [56]. The study of  
311 nearly isogenic lines that differ for the allele at *fw3.2*, has revealed that the increase in fruit size  
312 appears during fruit development. At mature stage, cytological analysis showed an increase in  
313 the number of cell layers within the pericarp, leading to larger fruit whereas cell size remains  
314 unchanged (**Figure 3C**) [56]. The increase in cell layer number and the delayed fruit ripening  
315 suggest an extension of the cell division period. The gene underlying *fw3.2* was identified as  
316 being an orthologue of *AtKLUH/CYP78A5* shown to control organ size in arabidopsis and  
317 encoding a CYTOCHROME P450 [56,57]. In plants carrying the large fruit allele of *fw3.2*, a  
318 mutation in the upstream region was proposed to lead to an increase in *SIKLUH* gene  
319 expression. However, recently, the pan-genome establishment after long read sequencing of  
320 100 diverse tomato lines, revealed that the increased expression of *SIKLUH* is caused by a  
321 tandem duplication of the gene at the *fw3.2* locus [58]. This gene dosage effect at *fw3.2* was  
322 confirmed by the use of CRISPR-Cas9 genome editing targeting one to several copies of  
323 *SIKLUH* [58]. Several orthologues of *SIKLUH* may also regulate fruit mass since in chile pepper  
324 a *fw3.2* QTL associated with *KLUH* has been found as in tomato [56]. In maize leaves, the  
325 orthologue of *SIKLUH*, *ZmPLA1*, triggers an extended phase of cell division allowing a higher  
326 biomass production and an improved seed yield when overexpressed [59]. Although *KLUH*  
327 seems to control cell division in different plant species, its mechanism of action, postulated to  
328 act through the production of a still unknown mobile signal, remains to be elucidated [57].

329

### 330 Effect of altering the cell cycle machinery

331 The correct control of cell number during flower or fruit development is thus a key component  
332 for final fruit size control. One way to study the impact of cell division on fruit growth is to  
333 target directly genes regulating the cell cycle. The progression throughout the successive phases  
334 of the mitotic cycle is controlled by heterodimeric protein complexes made of a catalytic subunit  
335 referred to as CYCLIN-DEPENDENT KINASE (CDK), and a regulatory subunit CYCLIN  
336 (CYC). The CDK-CYC complex is highly regulated at post-transcriptional level by proteolysis,  
337 phosphorylation or binding of regulatory proteins [60]. However, strongly altering the  
338 expression of cell cycle regulators can promote cell division rate, duration or pattern but also  
339 alter cell expansion, often not leading to an increase in fruit size, thus showing the  
340 interconnection existing between these two processes [61,62]. For example, gain and loss-of  
341 function of *S/CCS52A* (CELL CYCLE SWITCH 52A), encoding a protein part of the complex  
342 ANAPHASE PROMOTING COMPLEX/CYCLOSOME<sup>CCS52A</sup> (APC/C<sup>CCS52A</sup>) targeting  
343 CYCLINs for destruction through proteolysis, lead to similar fruit phenotype namely a

344 reduction of size but differing at cellular level. Indeed, downregulation of *SICCS52A* impaired  
345 cell expansion without affecting cell division showing the involvement only after the cell  
346 proliferation phase. The kinetics study of the gain-of-function lines fruit growth revealed an  
347 extent of cell expansion in late stages of fruit development and a reduction of anticlinal division  
348 supposed to promote the increase in volume of the fruit [62]. This extend of cell expansion was  
349 accompanied by an increased ploidy level supporting the proposed role of endoreduplication in  
350 promoting growth.

351 Unfortunately, of all genes described above that are involved in the control of cell division, no  
352 direct connection to the regulation of the cell cycle machinery has been described. Obtaining  
353 this information would help finding potential common targeted components of this machinery  
354 and thus further building the gene regulatory network determining tomato fruit size. In addition,  
355 too few mutants affecting the endocycle have been studied and for mutants affected in cell  
356 expansion, as described below, often, no information is available on cell ploidy. To demonstrate  
357 the role of endoreduplication in cell expansion control, the analysis of both parameters in  
358 mutants should be done systematically.

359

#### 360 *Altering cell expansion*

361 Cell expansion starts right after fruit set in the mesocarp cells, extends till ripening [8,10,11,13]  
362 and is responsible for a rapid and major increase in fruit size. Among the QTLs controlling fruit  
363 mass, *fw11.3* explained as much as 8% of fruit weight variation [50]. The *CELL SIZE*  
364 *REGULATOR (CSR)* gene underlies the *fw11.3* locus [63]. *SICSR* encodes a protein of unknown  
365 function with a low level of expression in the fruit, only after the cell division phase. Cytological  
366 analysis of near isogenic lines showed an increase in pericarp thickness resulting from an  
367 increase in mesocarp cell size without any change in the number of cell layers [63]. The  
368 expression of the mutated allele in the wild type background highlighted that the allele  
369 increasing fruit weight encodes a truncated protein acting as a dominant allele acting as a gain  
370 of function. The orthologous gene for *SICSR* in arabidopsis belongs to the FANTASTIC FOUR  
371 proteins (FAF) involved in the regulation of SAM size through a negative regulation of AtWUS  
372 [64]. *SICSR* and AtFAFs are likely to share the same biochemical function within the cell but  
373 in different tissues [63]. This function needs to be investigated in addition to the speculated  
374 involvement of *SICSR*, based on co-expression data, in the antagonistic action of auxin and  
375 cytokinin on cell enlargement [63].

376 In tomato, the up-regulation of several transcription factors belonging to the GROWTH  
377 REGULATING FACTOR (GRF) family led to pleiotropic effects including shorter cotyledons,  
378 large flowers and higher plants [65]. In these plants expressing higher levels of *SIGRF1* to -5,  
379 fruit size and weight are increased resulting from an increased size of the epidermal cells [65].  
380 The *SIGRF* genes are thought to regulate growth by different means since opposite phenotypes  
381 on cell size are observed in the cotyledons and fruits of the *SIGRF1* to -5 mutants. These  
382 differences are also observed in arabidopsis leaves with *AtGRF1* and -2 controlling cell size  
383 whereas *AtGRF5* regulates cell proliferation [66,67]. Further studies would be needed to  
384 specify the function of each individual *SIGRF* in the control of these cellular processes and to  
385 identify the targets of these transcription factors.

386 The overexpression lines for the putative transcription factor, FRUIT SANT/MYB-LIKE1  
387 (*SIFSM1*) harbour smaller fruits [68] characterized by a thinner pericarp resulting from a  
388 decreased cell expansion. In these plants, cell expansion in leaves and hypocotyl is also  
389 impaired showing that *SIFSM1* acts as a suppressor of cell expansion in various organs. The  
390 closest orthologues of *SIFSM1*, the *Antirrhinum* *RADIALIS* (*AmRAD*) and arabidopsis *RAD-*  
391 *like 2* (*AtRL2*) are involved in the asymmetries and radially symmetric flowers, respectively  
392 [69,70]. Looking for the protein partners of *SIFSM1* allowed the identification of FRUIT  
393 SANT/MYB BINDING PROTEIN 1 (*SIFSB1*) and *SIMYB1* was found to interact with *SIFSB1*  
394 [68]. Based on this interaction study using the tomato proteins and the *AtRAD* model network  
395 in arabidopsis, a binding competition of *SIFSB1* by *SIFSM1* and *SIMYB1* was proposed as the  
396 mechanism involved in the regulation of differential cell expansion during fruit development  
397 [68,70]. It will be necessary to verify this model by altering the expression of these different  
398 players in single and higher order mutants and study the effect on fruit growth.

399 In some cases, the alteration in pericarp thickness can be uncoupled from fruit size. For instance  
400 the loss-of-function of the GUANYLATE-BINDING PROTEIN1 (*SlGBP1*), induces a  
401 decrease in pericarp thickness through a decrease in cell size; however, the final fruit size  
402 remains unchanged [71]. In these plants, the difference in pericarp thickness only appears after  
403 20 DPA, and is accompanied by an early stop of cell expansion and the re-entry in division state  
404 of the cells indicating that *SlGBP1* is involved in the maintenance of the differentiation program  
405 in pericarp cells through a yet unknown mechanism.

406

407 *Hormone and fruit growth regulation*

408 Auxin and cell division

409 The modification of genes involved in hormonal regulation can impact on fruit size since  
410 important changes in hormone contents occur during fruit growth [72]. In pre-anthesis ovary in  
411 arabidopsis, the AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) factors repress the auxin  
412 signal by sequestering the AUXIN RESPONSE FACTOR (ARF), transcription factors which  
413 regulate the expression of auxin response genes. Hence the ovary remains in a phase of  
414 dormancy, in which cell division activities are inhibited [73]. Fertilization triggers an increase  
415 in auxin content leading to the destruction of the Aux/IAA and the release of the ARFs which  
416 then become available to transcribe their target genes and consequently trigger the resumption  
417 of the cell division process [74]. In tomato, treatment of unpollinated ovary by auxin can mimic  
418 the fecundation and leads to the development of parthenocarpic fruit [75]. The increase in auxin  
419 concentration inside the ovary following pollination is an important trigger for the growth of  
420 the ovary [72]. In tomato, the ARF family consists in 22 proteins [76]. *SlARF9* expression is  
421 triggered by pollination and reaches the highest expression at 6 DPA, a pattern similar to auxin  
422 accumulation in the fruit [72]. The loss- and gain-of-function of *SlARF9* result in larger and  
423 smaller fruits respectively [77]. Both at early and mature fruit stages, the *SlARF9* RNAi lines  
424 show a decrease in cell sizes and more cell layers in the pericarp. In contrast, fruits from the  
425 *pTPRP-SlARF9* line present an early increase in cell size and a decrease in cell layer number at  
426 later stages. Thus, *SlARF9* seems to act as a negative regulator to fine-tune cell division during  
427 fruit growth [77]. Surprisingly the decrease in *SlARF5* expression, using an amiRNA, leads to  
428 similar small fruit phenotype as the *SlARF9* overexpression [78]. No obvious difference on  
429 fruit morphology was observed in early dividing fruit of *amiSlARF5* lines but at later stages,  
430 the pericarp contained less cell layers, due to a shorter period of cell division, and larger cells  
431 compared to wild type plants. *SlARF5* could thus be a positive regulator of cell division [78].  
432 Auxin can also modulate the regulation of auxin responsive genes through the action of  
433 repressor proteins such as *SlIAA17* [79]. *SlIAA17* is highly expressed in the fruit at 10 DPA  
434 when cell expansion starts and its expression declines gradually up to the breaker stage. This  
435 peak of expression corresponds to one of the bimodal peak of auxin occurring at 10 and 30 DPA  
436 [12]. The study of *SlIAA17* by RNAi approach has shown that the decrease in expression of this  
437 gene leads to the production of larger fruits through an increase of the pericarp cellular size  
438 without modification in the number of cell layers [79]. *SlIAA17* interacts with several ARF  
439 proteins including *SlARF5* [80]. Interestingly, the decrease of *SlIAA17* and *SlARF5* expression  
440 leading to opposite phenotype could corroborate with a repressor function of *SlIAA17* on  
441 *SlARF5*. The role of auxin in regulating fruit growth seems complex and depending on the  
442 developmental stage of the fruit, different responsive proteins are involved. Additional research

443 is thus needed to understand the role of these auxin responsive genes and their possible  
444 connections during the course of fruit development.

#### 445 Gibberellins and cell expansion

446 Many studies have demonstrated the important role of GA for fruit growth regulation using  
447 mutants or exogenous treatments [81,82]. As for auxin, a treatment of unpollinated ovaries with  
448 gibberellins (GA) leads to the formation of parthenocarpic fruit [83]. Auxin and GA pathways  
449 are interconnected as GA treatment induces an increase in auxin content and, in turn, auxin  
450 induces GA biosynthesis [84]. Nevertheless, GA seems to be mainly involved in cell expansion  
451 process in the fruit. PACLOBUTRAZOL RESISTANCES 2 (PRE2), belonging to the bHLH  
452 transcription factor family, is induced by GA and mediates plant response to GA [85]. *SIPRE2*  
453 overexpression lines exhibit a slight increase in fruit diameter whereas loss-of-function lines  
454 show a decrease in fruit size [86]. In RNAi lines for *SIPRE2*, the mesocarp cell size is reduced  
455 leading to a thinner pericarp. *SIPRE2* seems to show similar function as AtPRE1 since both  
456 proteins are involved in cell elongation through GA modulating response [86–88]. The  
457 downregulation of another transcription factor involved in GA pathway, *SIGRAS2*, leads to a  
458 reduction of pericarp cell size and thus smaller fruit [89]. *SIGRAS2* is expressed from ovary  
459 wall at anthesis to 10 DPA fruit. In the RNAi lines targeting *SIGRAS2*, the ovary wall is thinner  
460 but no change in the final number of cell layers in the pericarp was observed showing the  
461 involvement in *SIGRAS2* in cell expansion regulation. In these lines, both GA biosynthesis and  
462 signal transduction pathways are inhibited.

463 The increased expression of the transcription factor CYCLIN DOF FACTOR 4 (*SICDF4*) under  
464 the control of PHOSPHOENOLPYRUVATE CARBOXYLASE promoter (pPPC2) used for a  
465 fruit specific expression with highest expression during the cell expansion phase [90], leads to  
466 the production of larger fruits through an increase in both cell layers and cell sizes [91]. In these  
467 plants, the hormone content is modified with higher GA and lower auxins levels. *SICDF4* may  
468 play a dual role on auxin and gibberellin synthesis thus regulating both cell division and  
469 expansion. The increase in fruit size and in GA content had already been observed with the  
470 overexpression of another member of the family, *SICDF3* [92] although its effect at cellular  
471 level was not described. As for auxin, GA plays an important role all along fruit development  
472 for cell enlargement control, but many of the genes involved in GA signal transduction still  
473 need to be discovered. In addition, even if a crosstalk between auxin and gibberellins seems to  
474 exist, its regulation to maintain the balance between cell division and cell expansion is not  
475 properly understood.



476

## 477 **Concluding remarks and future outlook**

478 Tomato fruit is a complex 3-dimensional structure that relies on cell division and cell expansion  
479 to develop fully. These two processes occur in different cell types at different developmental  
480 stages and at different rates creating a high complexity of interconnected events, requiring a  
481 highly fine coordination. This complexity and fine coordination were already described for the  
482 planar arabidopsis leaf, often used as model to study growth mainly in 2-D, along two axes  
483 ‘base-to-tip’ and the ‘middle-to-margin’ [93] while in the silique, cell expansion is the main  
484 driver of growth after fertilisation [94]. In tomato, the large diversity in fruit shape results from  
485 growth patterns occurring along three axes -“proximo-distal”, “medio-lateral” and “abaxial-  
486 adaxial”- making of this organ an excellent model, although challenging, to study growth in 3-  
487 D. In tomato, final fruit size can be altered through changes in cell division, in its direction,  
488 duration or rate, but these changes can occur as early as in the ovary or in the developing fruit  
489 itself, in different zones of the fruits, and according to cells dividing in an anticlinal, periclinal,  
490 or oblique manner. Similarly, an alteration in cell expansion either anisotropic or isotropic can  
491 influence the final fruit size. Due to this large diversity of events that can influence final fruit  
492 size, yet not all described in detail, the changes that can affect positively fruit size are difficult  
493 to apprehend fully, since they can occur alone or in combination. Capturing the effects of the  
494 alteration of one or several events at a 3-D level will definitely provide essential information to  
495 better apprehend the high complexity of cellular events needed to form a fully grown and  
496 functional fruit but will require computational models to integrate multiple cellular parameters.  
497 So far only a few models have been developed to integrate some, but not all, cellular parameters  
498 possibly influencing final fruit size in tomato [20,95,96].

499

500 Through the study of mutants and the identification of genes underlying important QTLs, a  
501 number of fruit growth regulators influencing cell division or cell expansion have been  
502 identified. In many cases, these potential homologues of these genes/QTLs were also described  
503 in other plants species. For example, homologues of FW2.2 have been found in papaya (*Carica*  
504 *papaya*), peach (*Prunus persica*), grape vine (*Vitis vinifera*) [97] in which they are associated  
505 with fruit weight QTLs, and also in physalis (*Physalis floridana*) or rice (*Oryza sativa*) in which  
506 they regulate leaves, floral organs, berries, and seeds size, and plant height and seed size,  
507 respectively [97]. A homologue of SISUN may also underlie fruit shape variation in cucumber  
508 [98] and an orthologue of SIKLUH may regulate fruit weight in chile pepper [56]. In several of

509 these plants, functional studies are not always easy to carry to understand the mode of action of  
510 these genes and for the search of new growth regulators. The availability of genome sequences  
511 for multiple wild and cultivated tomato plants, the possibility to edit the genome of tomato and  
512 easily transform it make that information obtained from tomato on the mechanisms underlying  
513 size control could be used to search for genes controlling organ size in other crops and/ or for  
514 engineering favourable alleles. However, although several genes/QTLs involved in organ size  
515 determination have been identified, in numerous cases, their nature, exact effect on the cellular  
516 processes are often not described and the mode of action of these regulators remains poorly  
517 understood. It is regretful that of the almost 30 FW QTLs identified so far only for 3, the gene  
518 behind the QTL is known, even if the function of the related protein is often not understood.  
519 This lack of knowledge on the genetic regulation of several processes determining fruit growth  
520 does not allow to build a solid regulatory network that could be manipulated in order to modify  
521 fruit growth. The important nodes and their associated partners need to be discovered. The  
522 construction of this network can be achieved through the identification of proteins partners of  
523 the growth regulating proteins, their targets or regulatory elements. With additional information  
524 on the exact effect and mechanism of action for each regulator, rational combinations between  
525 mutants of genes enhancing for example cell division and cell expansion could be a good  
526 solution for modifying fruit size and thus probably fruit quality. These combinations could be  
527 achieved by crossing mutants or through the use of CRISPR-Cas9 genome editing technologies  
528 that allow the production of deletions, but also creating targeted insertions, exchanging amino  
529 acids and modulating gene expression for one or several targeted genes [99,100].

530 Tomato domestication led to the selection of favourable traits essentially aiming at improving  
531 yield through the increase of fruit size or fruit number. Polymorphism/mutations in several of  
532 the genes described in this review, such as *FAS*, *OVATE*, *SUN* or *FW2.2* contributed to this  
533 improvement. However, often, this selection was at the expense of other desirable traits such  
534 as nutritional features or stress tolerance. For example, modern commercial tomato varieties,  
535 which often produce numerous and/or large fruits, contain lower amounts of important flavour  
536 metabolites (sugars, acids, amino acids, volatiles...) than old varieties [101], suggesting a trade-  
537 off between quality and size/yield. This trade-off originates from a loss of genetic diversity in  
538 domesticated cultivars, which, probably randomly/indirectly, triggered the loss of favourable  
539 alleles or the co-selection of unfavourable alleles in the absence of positive selection [101–103].  
540 The analysis of the genome, transcriptome and metabolome in more than 400 tomato accessions  
541 has shown that changes in metabolite content through domestication might have been caused

542 by a linkage of genes nearby the selected alleles of genes associated with larger fruits [104]. In  
543 the actual context of climate change, a loss of crop yield due to the increasing occurrence of  
544 environmental stresses together with the actual consideration of consumers preferences toward  
545 fruits of better quality create a need to combine yield-related traits and tolerance-to-stress and/or  
546 quality-related traits. To achieve rapidly these combinations, molecular engineering using  
547 CRISPR-cas9 technology could be exploited for producing superior tomato varieties with  
548 multiple favourable traits by specifically targeting genes involved in fruit growth, fruit flavour  
549 and response to stress. Wild relatives or old varieties show a better adaptation to environmental  
550 constraints or can produce larger amounts of fruit quality related metabolites [101,103,105], de  
551 novo domestication could help combining these traits. In a wild tomato, Zsögön et al. (2018)  
552 have targeted six important loci, including OVATE, FW2.2 and CLV3, for key domestication  
553 traits through molecular engineering using CRISPR-cas9 technology to create loss of function  
554 alleles, and have succeeded in improving most of the targeted traits in these plants [106].  
555 However, as mentioned previously, this targeted molecular breeding will only be possible  
556 through a better knowledge of the genetic basis of the traits of interest including fruit size  
557 control.

558 In conclusion, the combination of in-depth understanding of gene regulatory networks, of their  
559 effects at cellular level when mutated with the use of genome editing represent a promising  
560 engineering strategy for future crop improvement and tomato represents an excellent model  
561 both for obtaining this knowledge and for direct application (see also outstanding questions).

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566

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## 802 **Figure Legends**

803 **Figure 1.** Inflorescence architecture, fruit tissues and pericarp cell layers. A: Inflorescence of  
804 wva106 variety showing the zigzag inflorescence pattern. B: Tomato fruit at mature stage and  
805 equatorial cross section. C: cellular drawings of a pericarp section at mature stage. The names  
806 of cell layers are according to Renaudin *et al.* (2017) [8].

807 **Figure 2.** Different developmental stages of tomato fruit development (cultivar wva106) at  
808 fruit, pericarp and cellular level. From inside to outside the circle, the different phases of  
809 development (cell division phases, cell expansion phase and ripening) are shown as pictures of  
810 ovaries and fruits (stage 11 to 40 DPA), fruit equatorial cross sections and cellular drawings of  
811 pericarp sections (s: stage, A: anthesis and number for DPA). Dividing cells are represented in  
812 blue and expanding cells in orange. The scale has been conserved along the development,

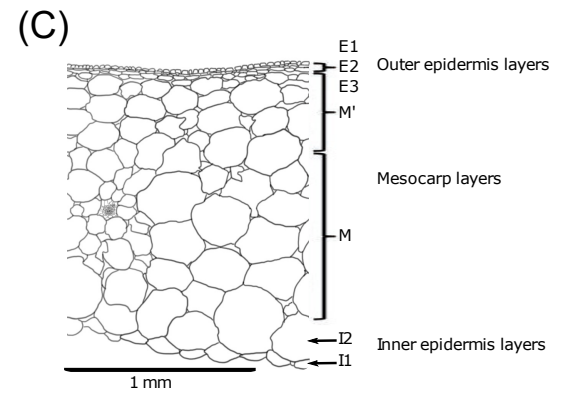
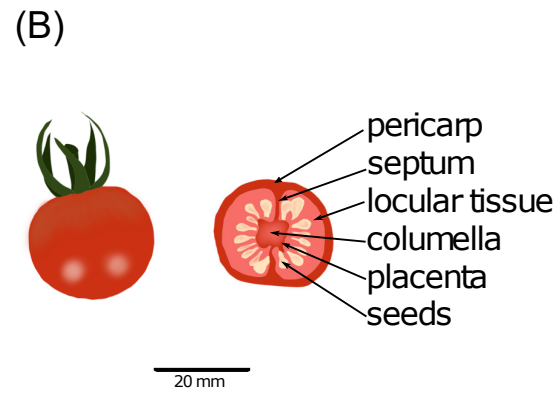
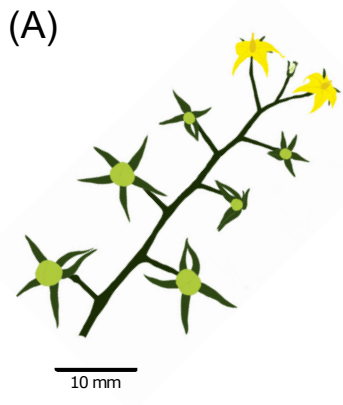


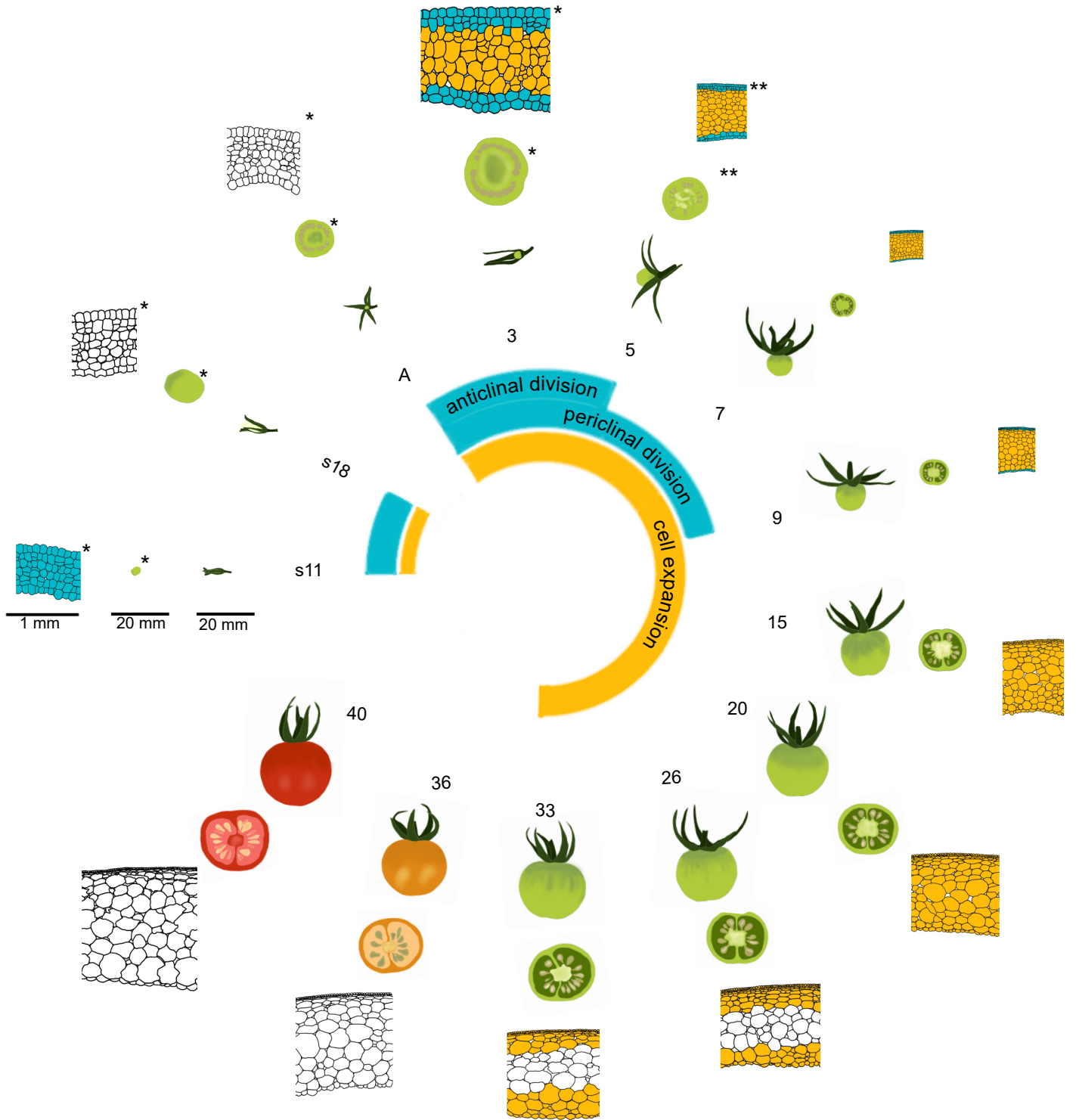
813 except for the stages marked with a \* and a \*\* for which a magnification of x10 and of x2.5  
814 has been done for optimal visualisation.

815 **Figure 3.** Molecular mechanisms determining tomato fruit growth. A. Genes influencing fruit  
816 locule number through the regulation of meristem size. B. Genes influencing fruit size and  
817 shape through the regulation of cell division patterns in the ovary. From the left to the right are  
818 represented a longitudinal section of a tomato flower, a longitudinal (top) and a transversal  
819 (bottom) section of an ovary with the different division patterns (proximo-distal, adaxial-  
820 abaxial and medio-lateral, the genes influencing these cell division patterns, the models  
821 explaining the changes in cell division pattern in mutants of these genes and the consequences  
822 on fruit shape and size of these mutations. C. Genes influencing fruit growth through the  
823 regulation of cell division and /or expansion in the pericarp and consequences on pericarp  
824 thickness. Abbreviations: ARF (AUXIN RESPONSE FACTOR), CDF (CYCLIN DOF  
825 FACTOR), CLV (CLAVATA), CSR (CELL SIZE REGULATOR), ENO (EXCESSIVE  
826 NUMBER OF FLORAL ORGANS), FSM (SANT/MYB-like), FW (FRUIT WEIGHT), GBP  
827 (GUANYLATE-BINDING PROTEIN), GRAS (GIBBERELIC ACID INSENSITIVE,  
828 REPRESSOR OF GAI, and SCARECROW), GRF (GROWTH REGULATING FACTOR),  
829 HPAT (HYDROXYPROLINE O-ARABINOSYLTRANSFERASE), IAA (INDOLE-3-  
830 ACETIC ACID), IMA (INHIBITOR OF MERISTEM ACTIVITY), KNU (KNUCKLES), PRE  
831 (PACLOBUTRAZOL RESISTANCES) and WUS (WUSCHEL). Ab: abaxial; Ad: Adaxial;  
832 M: medio; L: lateral; P: proximal; D: distal.

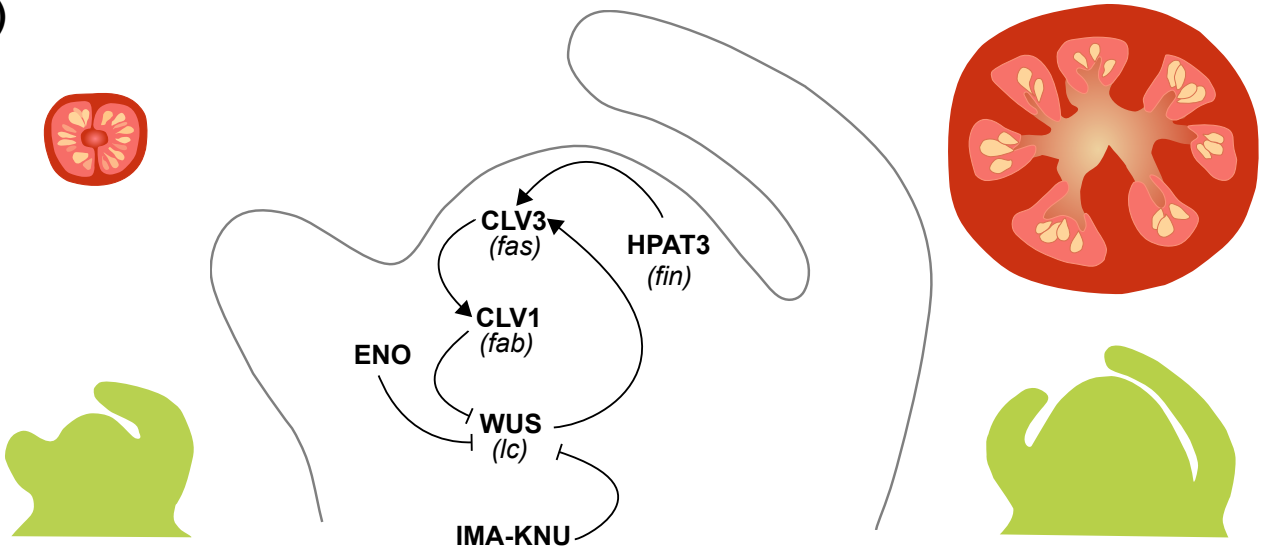
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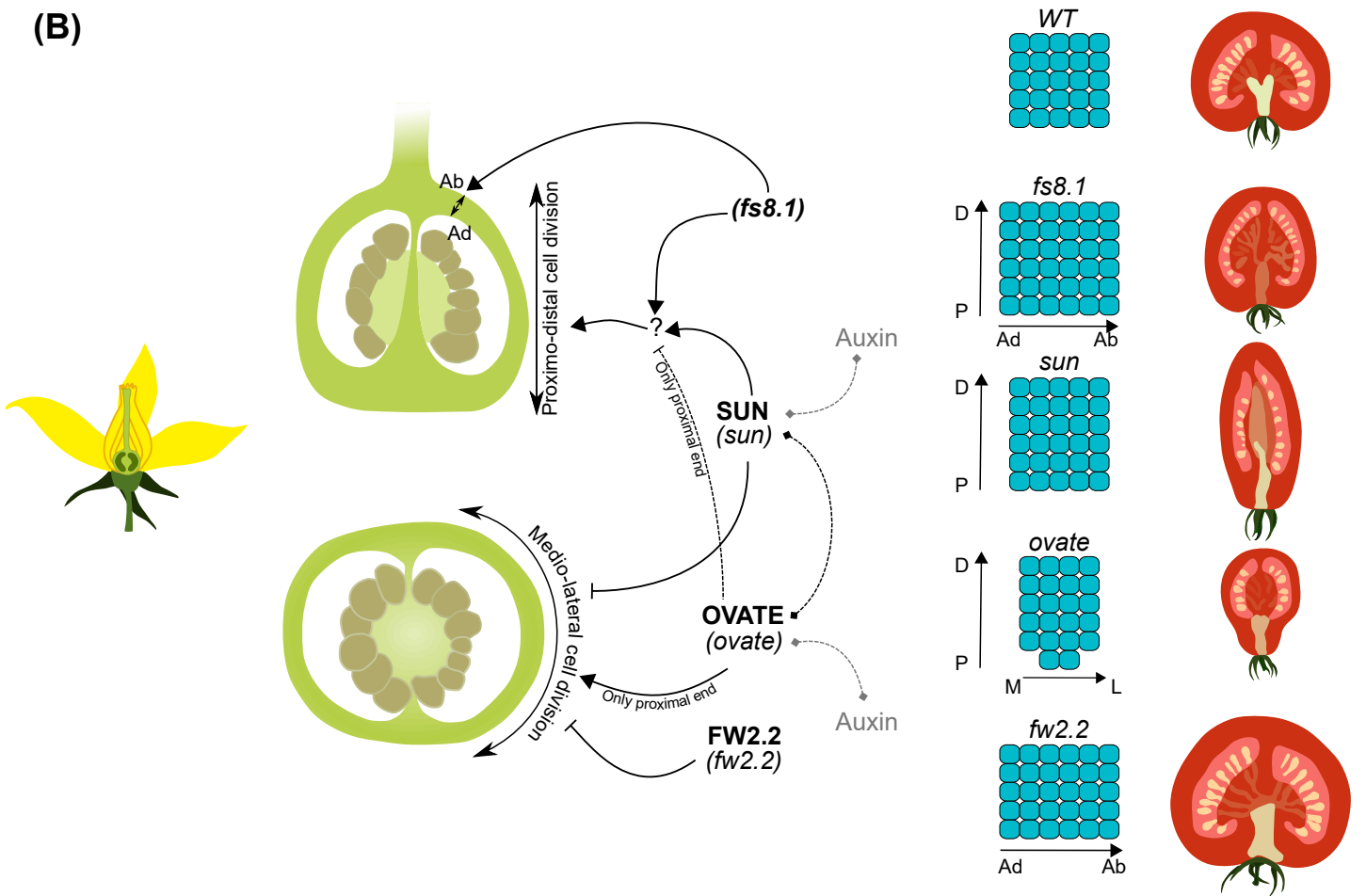




(A)



(B)



(C)

