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Abstract

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

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

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

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

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

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

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

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

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

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

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

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

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

Increasing carpel/locule number by altering meristem size

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

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

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

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

Promoting cell division in the ovary and the fruit

Cell division control during ovary development

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

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

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

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

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

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

Cell division control during both the ovary and the fruit development

The process of domestication in tomato has resulted in the selection of plants presenting a large diversity in fruit shape, but also in larger fruit size. About thirty QTLs related to fruit size/weight have been identified in tomato [50]. The *fw2.2* locus (for fruit weight QTL of chromosome 2, number 2) is responsible for up to 30% of the fruit weight variation [51]. The gene underlying *fw2.2* locus, *SlFW2.2,* encodes a protein containing a PLAC8 (Placenta-specific gene 8 protein) motif predicted to be important for the membrane localisation of the protein and belongs to the multigene *CELL NUMBER REGULATOR* (*CNR*) family [52]. Two different alleles, a "large fruit allele", present in modern tomatoes, and a "small fruit allele" inherited from wild tomato ancestors, differ mainly from polymorphisms in the upstream regulatory region of the gene and lead to spatial and temporal differences of expression [53]. The "large fruit" allele is expressed earlier during fruit development, while the "small fruit" allele is expressed later and maintained longer [54]. In plants harbouring the "large fruit" allele, the ovary is larger, mainly due to an increase in the number of cells without any change in cell size showing that SlFW2.2 is involved in regulation of cell number (**Figure 3B**) [53]. In addition, at early stage of fruit development, the mitotic index is increased without any change in cell size recorded in the placenta and the pericarp and is negatively correlated with the expression of *SlFW2.2* [54,55]. The increase in cell division not followed by a modification of the thickness of the pericarp might indicate that SlFW2.2 act as a negative regulator of anticlinal cell divisions (**Figure 3C**). In several plants species, orthologues of *SlFW2.2* are also involved in the regulation of the reproductive organ size such as in maize (*Zea mays*), where the overexpression of *ZmCNR1* leads to the formation of small organs [52]. Despite many studies on *FW2.2*, the mechanism of action by which such a membrane protein can negatively regulate cell number and thus fruit size, as well as the exact changes occurring at the cellular level (cell division rate, cell division duration...) are not yet understood.

Cell division control during fruit development

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

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

Effect of altering the cell cycle machinery

The correct control of cell number during flower or fruit development is thus a key component for final fruit size control. One way to study the impact of cell division on fruit growth is to target directly genes regulating the cell cycle. The progression throughout the successive phases of the mitotic cycle is controlled by heterodimeric protein complexes made of a catalytic subunit referred to as CYCLIN-DEPENDENT KINASE (CDK), and a regulatory subunit CYCLIN (CYC). The CDK-CYC complex is highly regulated at post-transcriptional level by proteolysis, phosphorylation or binding of regulatory proteins [60]. However, strongly altering the expression of cell cycle regulators can promote cell division rate, duration or pattern but also alter cell expansion, often not leading to an increase in fruit size, thus showing the interconnection existing between these two processes [61,62]. For example, gain and loss-of function of *SlCCS52A* (CELL CYCLE SWITCH 52A), encoding a protein part of the complex ANAPHASE PROMOTING COMPLEX/CYCLOSOME^{CCS52A} (APC/C^{CCS52A}) targeting CYCLINs for destruction through proteolysis, lead to similar fruit phenotype namely a

reduction of size but differing at cellular level. Indeed, downregulation of *SlCCS52A* impaired cell expansion without affecting cell division showing the involvement only after the cell proliferation phase. The kinetics study of the gain-of-function lines fruit growth revealed an extent of cell expansion in late stages of fruit development and a reduction of anticlinal division supposed to promote the increase in volume of the fruit [62]. This extend of cell expansion was accompanied by an increased ploidy level supporting the proposed role of endoreduplication in promoting growth.

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

Altering cell expansion

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

In tomato, the up-regulation of several transcription factors belonging to the GROWTH REGULATING FACTOR (GRF) family led to pleiotropic effects including shorter cotyledons, large flowers and higher plants [65]. In these plants expressing higher levels of *SlGRF1* to *-5*, fruit size and weight are increased resulting from an increased size of the epidermal cells [65]. The *SlGRF* genes are thought to regulate growth by different means since opposite phenotypes on cell size are observed in the cotyledons and fruits of the SlGRF1 to -5 mutants. These differences are also observed in arabidopsis leaves with AtGRF1 and -2 controlling cell size whereas AtGRF5 regulates cell proliferation [66,67]. Further studies would be needed to specify the function of each individual *SlGRF* in the control of these cellular processes and to identify the targets of these transcription factors.

The overexpression lines for the putative transcription factor, FRUIT SANT/MYB-LIKE1 (SlFSM1) harbour smaller fruits [68] characterized by a thinner pericarp resulting from a decreased cell expansion. In these plants, cell expansion in leaves and hypocotyl is also impaired showing that SlFSM1 acts as a suppressor of cell expansion in various organs. The closest orthologues of *SlFSM1*, the *Antirrhinum* RADIALIS (AmRAD) and arabidopsis RAD-like 2 (AtRL2) are involved in the asymmetries and radially symmetric flowers, respectively [69,70]. Looking for the protein partners of SlFSM1 allowed the identification of FRUIT SANT/MYB BINDING PROTEIN 1 (SlFSB1) and SlMYB1 was found to interact with SlFSB1 [68]. Based on this interaction study using the tomato proteins and the AtRAD model network in arabidopsis, a binding competition of SlFSB1 by SlFSM1 and SlMYB1 was proposed as the mechanism involved in the regulation of differential cell expansion during fruit development [68,70]. It will be necessary to verify this model by altering the expression of these different players in single and higher order mutants and study the effect on fruit growth.

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

Hormone and fruit growth regulation

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 pathenocarpic 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

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

Gibberellins and cell expansion

Many studies have demonstrated the important role of GA for fruit growth regulation using mutants or exogenous treatments [81,82]. As for auxin, a treatment of unpollinated ovaries with gibberellins (GA) leads to the formation of parthenocarpic fruit [83]. Auxin and GA pathways are interconnected as GA treatment induces an increase in auxin content and, in turn, auxin induces GA biosynthesis [84]. Nevertheless, GA seems to be mainly involved in cell expansion process in the fruit. PACLOBUTRAZOL RESISTANCES 2 (PRE2), belonging to the bHlH transcription factor family, is induced by GA and mediates plant response to GA [85]. *SlPRE2* overexpression lines exhibit a slight increase in fruit diameter whereas loss-of-function lines show a decrease in fruit size [86]. In RNAi lines for *SlPRE2*, the mesocarp cell size is reduced leading to a thinner pericarp. SlPRE2 seems to show similar function as AtPRE1 since both proteins are involved in cell elongation through GA modulating response [86–88]. The downregulation of another transcription factor involved in GA pathway, *SlGRAS2*, leads to a reduction of pericarp cell size and thus smaller fruit [89]. *SlGRAS2* is expressed from ovary wall at anthesis to 10 DPA fruit. In the RNAi lines targeting *SlGRAS2*, the ovary wall is thinner but no change in the final number of cell layers in the pericarp was observed showing the involvement in SlGRAS2 in cell expansion regulation. In these lines, both GA biosynthesis and signal transduction pathways are inhibited.

The increased expression of the transcription factor CYCLIN DOF FACTOR 4 (SlCDF4) under the control of PHOSPHOENOLPYRUVATE CARBOXYLASE promoter (pPPC2) used for a fruit specific expression with highest expression during the cell expansion phase [90], leads to the production of larger fruits through an increase in both cell layers and cell sizes [91]. In these plants, the hormone content is modified with higher GA and lower auxins levels. SlCDF4 may play a dual role on auxin and gibberellin synthesis thus regulating both cell division and expansion. The increase in fruit size and in GA content had already been observed with the overexpression of another member of the family, *SlCDF3* [92] although its effect at cellular level was not described. As for auxin, GA plays an important role all along fruit development for cell enlargement control, but many of the genes involved in GA signal transduction still need to be discovered. In addition, even if a crosstalk between auxin and gibberellins seems to exist, its regulation to maintain the balance between cell division and cell expansion is not properly understood.

Concluding remarks and future outlook

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

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

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

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

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

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

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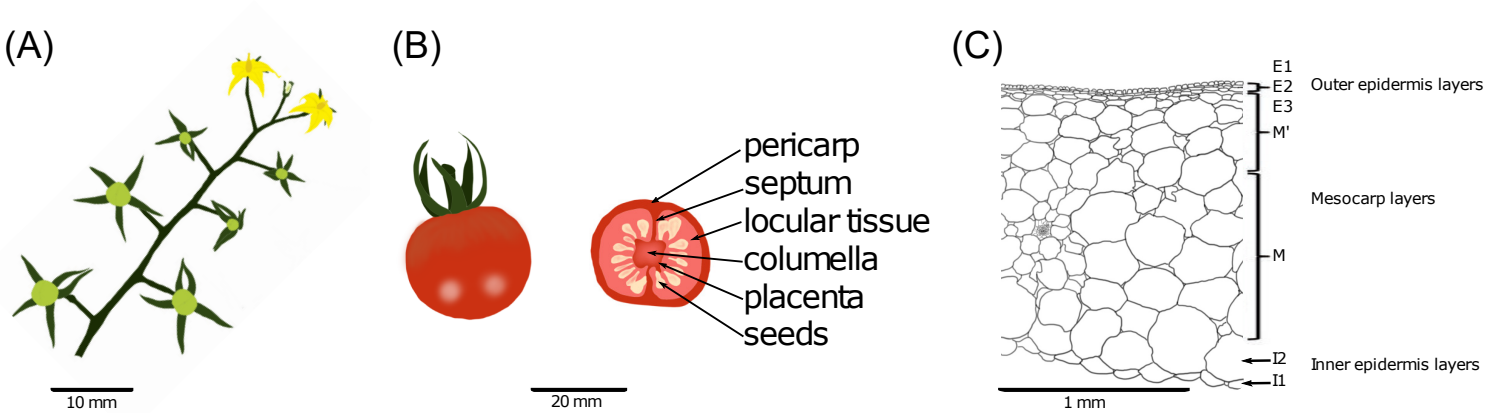
802 **Figure Legends**

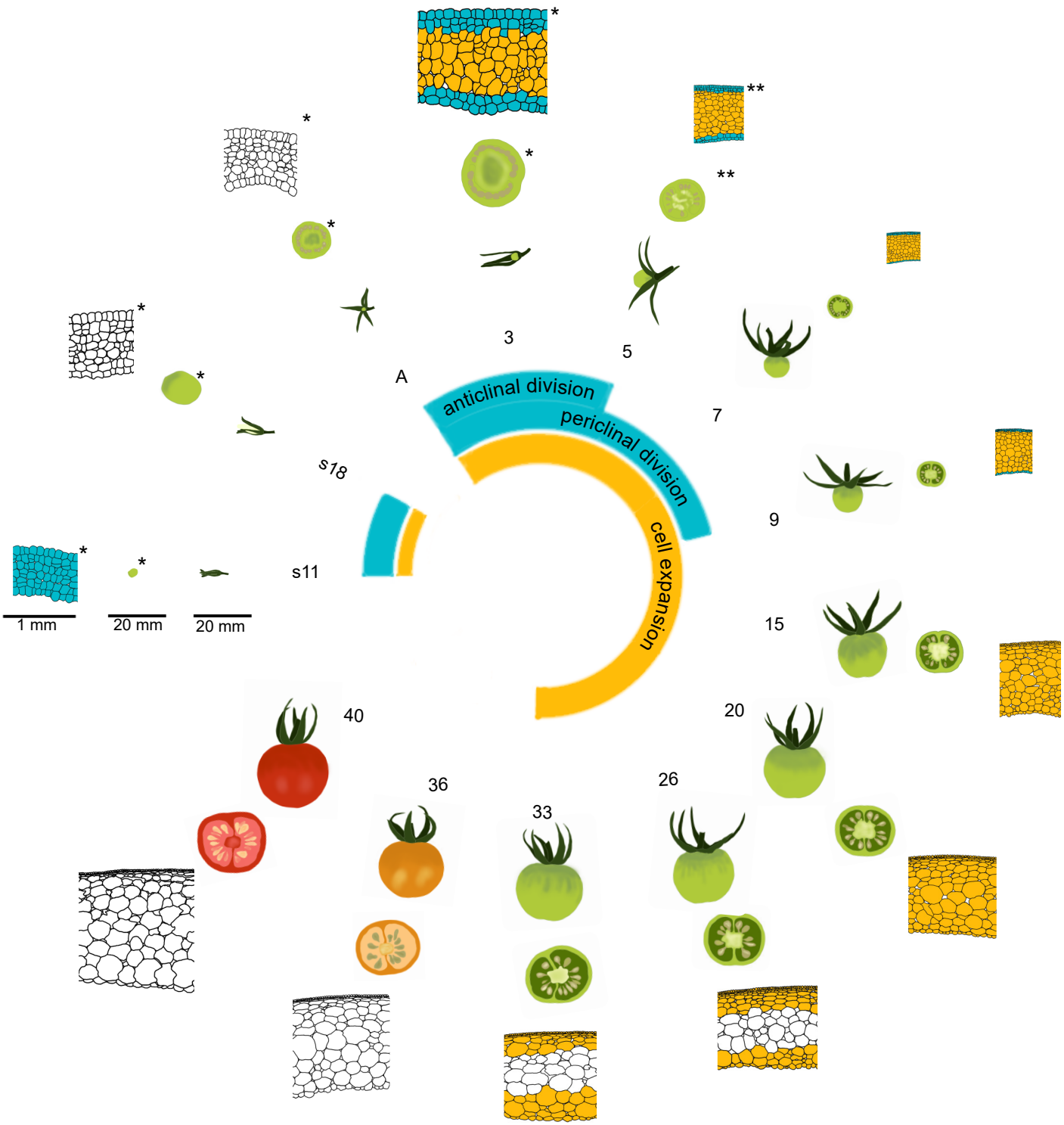
803 **Figure 1.** Inflorescence architecture, fruit tissues and pericarp cell layers. A: Inflorescence of
804 wva106 variety showing the zigzag inflorescence pattern. B: Tomato fruit at mature stage and
805 equatorial cross section. C: cellular drawings of a pericarp section at mature stage. The names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

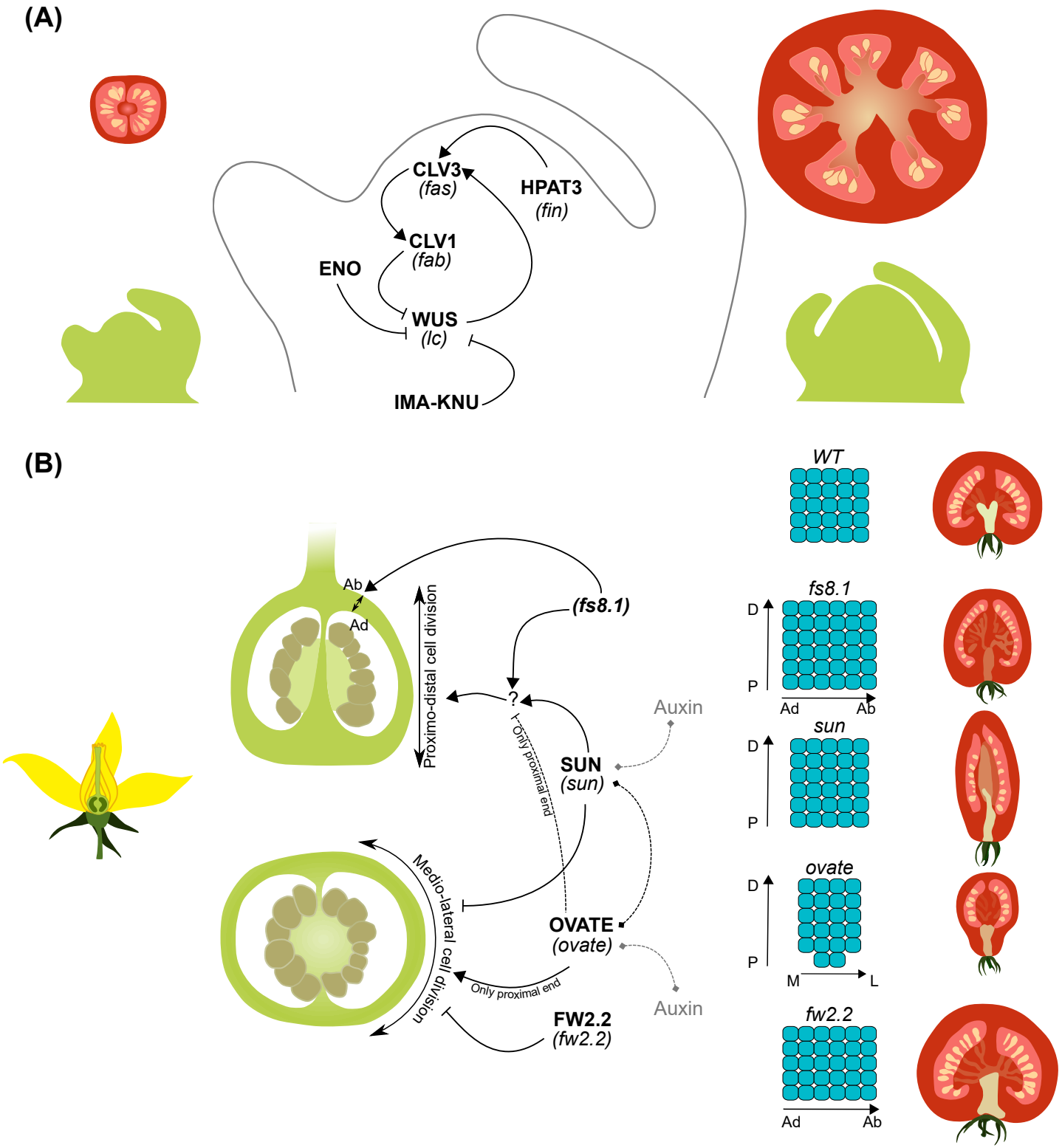
807 **Figure 2.** Different developmental stages of tomato fruit development (cultivar wva106) at
808 fruit, pericarp and cellular level. From inside to outside the circle, the different phases of
809 development (cell division phases, cell expansion phase and ripening) are shown as pictures of
810 ovaries 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,

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

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


(A)
10 mm
(B)
pericarp
septum
locular tissue
columella
placenta
seeds
20 mm
(C)
E1
E2
E3
M'
M
I2
I1
Outer epidermis layers
Mesocarp layers
Inner epidermis layers
1 mm


anticlinal division
periclinal division
cell expansion
A
3
5
7
9
15
20
26
33
36
40
s11
s18
1 mm
20 mm
20 mm


(A)
CLV3
(fas)
HPAT3
(fin)
CLV1
(fab)
ENO
WUS
(lc)
IMA-KNU
(B)
Ab
Ad
Proximo-distal cell division
(fs8.1)
?
Only proximal end
Auxin
SUN
(sun)
Medio-lateral cell division
OVATE
(ovate)
Only proximal end
Auxin
FW2.2
(fw2.2)
WT
fs8.1
D
P
Ad
Ab
sun
ovate
M
L
fw2.2
(C)
FW2.2
(fw2.2)
KLUH
(fw3.2)
ARF5
Auxin
ARF9
IAA17
CDF4
SIFSM1
GRAS2
PRE2
GBP1
GRF1-5
CSR
(fw11.3)
Gibberellins
less and/or smaller cells
WT
more and/or larger cells