PLS 622, Plant Physiology I: Friday, September 22, 2006

Vegetative development: Trichome, root hair, and stomatal Development:

Objectives:

- To examine the development of three specialized epidermal cell types, trichomes, stomata, and root hairs.
- To explore our knowledge of how the molecular control of trichome spacing is thought to occur.
- To construct an overview of the roles of the similar molecular players in the various developmental processes mentioned above.

Epidermal cell fate: Hitherto we have discussed cellular differentiation in the context of the maturation, from a ground meristem, of a contiguous array of cells comprising a particular tissue type. Assemblages of epidermal cells provide an interesting contrast in that there are two types of highly specialized cells present throughout the epidermis that develop in isolation, surrounded by pavement cells. These are the guard cells (making up the stomata), and the trichomes/root hairs. Two fundamental questions can be asked 1) how are the cells destined to differentiate into guard cells or trichomes/root hairs selected? 2) How is the spatial patterning of stomata and trichome/root hair placement in the pavement controlled?

Trichome Development:

Trichomes are present on the aerial portions of almost every terrestrial plant. Although some plants have but a single type of trichome, trichomes exist in several different forms, often on the same plant. Tomato has six different types of trichome. Trichomes are thought to perform a variety of functions, from plant defense (physical obstruction to marauding insects, to sites of synthesis and display of complex chemical inhibitors and repellents) to creation of a boundary layer of moister air to reduce transpirational loss of water. The nucleus of the cell resides in the base of the trichome and a thin strand of cytoplasm is continuous into the cell extension which is highly vacuolate. The cell wall of the mature trichome is thick and covered with numerous papillae the function of which is unknown. The most impressive of trichomes is that of the cotton ovule. The single cells of the ovule are capable of supporting a rate of cell extension (trichome elongation) in excess of 2 mm a day, leading to a final single cell length of 30-35 mm!!!

Trichomes mature basipetally. They expand laterally as the cell extension elongates so that the girth as well as the length of a mature trichome is much greater than that of the undifferentiated trichoblast. Additionally, a rosette of normal atrichoblast epidermal cells form a group of support cells at the base of the trichome stalk. Mature trichomes also increase their DNA content per cell above the usual diploid content by a process known as endoreduplication (synthesis of additional copies of the genome without subsequent cell division). This may be due to the fact that the trichome, although comprised of a single cell or a few large cells, expands considerably via the cell extension and will therefore require

additional copies of genes for house-keeping enzymes if the cell is to remain efficient. It seems logical that the cellular volume and the surface area serviceable by a single nucleus is limited and strictly correlated with the C-value (number of haploid genomes) present in a nucleus.

Trichome spacing.

So, how is trichome spacing determined? There are two models. The first suggests that precursor cells produce inhibitory signals that prevent other, neighboring cells, from going down the same developmental pathway. The second suggests that, once a precursor cell is induced to become a trichome it undergoes several divisions that separate it from other trichomes by a group of cells all derived from the same precursor daughter cell. In fact the first hypothesis appears to most closely describe control of trichome patterning. Let us see how the second hypothesis was debunked.

The second hypothesis has been refuted by cell lineage experiments using a transposon interrupted beta-glucuronidase (GUS) gene. With the transposon in place the gene does not make a functional enzyme and cells expressing the interrupted GUS gene stay clear in the presence of substrate. In cells that have had a transposition event occur, the excision of the transposon leads to the production of functional GUS from the now transposon-free gene and cells in which this occurs can turn the substrate blue. The transposition event can be induced in some cells prior to their mitoses in the expanding epidermis and thereafter, all daughter cells produced from the affected cell will be able to generate a blue color from the substrate. It was determined that the boundary of the cells developing from such transposition-positive cells passed through trichome producing and non-producing cells randomly, debunking the second hypothesis (Fig. 1).

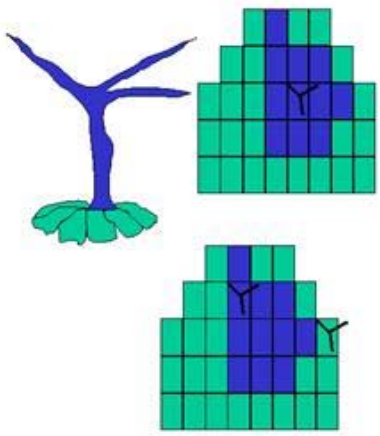


Figure 1: Trichome cell lineage experiments using a reporter gene.

Another way in which trichome spacing may be maintained is that the precursor cell, undergoes asymetric division with the different sized cells resulting in different developmental fates. Although this does occur in some plants (e.g. many monocots) it is not a universal method of determining trichome spacing. Additionally, epidermal cells of

arabidopsis can be artificially induced to form trichomes without the requirement of undergoing cell division.

There are at least 10 known genes that affect trichome development, of which we shall discuss 7. There are three core protein types in trichoblasts (cells destined to become trichomes) and three in atrichoblasts. Two of these core protein types are the same in the two types of cells. The trichoblast stimulating complex is formed from GL1:GL3:TTG1 (GL1 = GLABRA1; GL3 = GLABRA3 and; TTG1 = TRANSPARENT TESTA GLABRA1). The atrichoblast complex is formed from TRY:GL3:TTG1 (TRY = TRIPTYCHON). It is the competition between TRY and GL1 to interact with the other proteins in the complex that decides trichoblast fate. The *GLABRA1* (*GL1*) gene (glabrous = "free from hair") which, when defective (*gl1*), results in the loss of trichomes on most surfaces, and the TRY gene, are both MYB transcription factors (The **MYB** moniker is from the first protein of this type identified, an oncogene from the Avian **My**elo**b**lastosis virus, a DNA binding protein). The GLABRA3 (GL3) protein and ENHANCER OF GL3 (EGL3) protein both encode basic Helix-Loop-Helix (bHLH) proteins that interact as homo (GL3:GL3 or EGL3:EGL3) or hetero-dimers (GL3:EGL3) and act as scaffolding to which TTG1 and GL1/TRY proteins bind. Neither the *gl3* nor the *egl3* mutants have striking phenotypes but the double mutant is totally without trichomes. The TRANSPARENT TESTA-GLABRA1 protein is a member of both the trichoblast simulating and trichoblast inhibiting complex. Mutants in *ttg1*, as its name suggests, is a pleiotropic mutant that results, among other things, in the loss of trichomes from most surfaces of the plant, akin to *gl1* mutations. An additional player that influence this competition is CAPRICE (CPC), also a MYB transcription factor (a DNA binding protein). CPC negatively regulates trichome initiation by binding to GL1, making it unavailable to participate in the trichome initiating complex. Both the trichoblast stimulating and the trichoblast inhibiting complex bind to the promoter region of the *GLABRA2* (*GL2*) gene (encoding a homeobox protein). In trichomes, *GL2* is up-regulated by the trichoblast stimulating complex (GL1:GL3:TTG1) and down-regulated by the trichoblast inhibiting complex (TRY:GL3:TTG1).

The model in figure 2 attempts to synthesize what we know about the control over trichome initiation and spacing in arabidopsis. Initially, all cells of the leaf have an equal opportunity to become trichoblastic. However, early during development, one of the cells at the margin of the distal portion of the leaf primordia acquires the trichoblastic fate through a fortuitous stimulation of GL2. From now on, trichoblast acquisition is dependent on how close the nearest trichoblast is to an undifferentiated cell. The complexes (stimulating or inhibiting) require two bHLH proteins to associate (GL3:GL3; EGL3:EGL3 or; GL3:EGL3) and they hold TTG1 (a WD-repeat protein [W = tryptophan; D = aspartic acid] sometimes called WD40-repeat proteins because the conserved W and D of the motif is within a region of approximately 40 amino acids) and either GL1 (a MYB protein) or by TRY (another MYB transcription factor) in association. If the trimeric protein complex GL1:GL3:TTG1 is formed, the cell is stimulated to adopt the trichome fate (Fig. 2). If the trimeric protein complex TRY:GL3:TTG1 is formed, the cell is steered into the atrichoblast fate. The CAPRICE protein CPC is a negative regulator of trichome initiation in arabiodopsis due to its propensity to sequester GL1 protein away from the initiation

complex, providing more GL3 and TTG1 subunits for interaction in a trichome-limiting complex with TRY (Fig. 2).

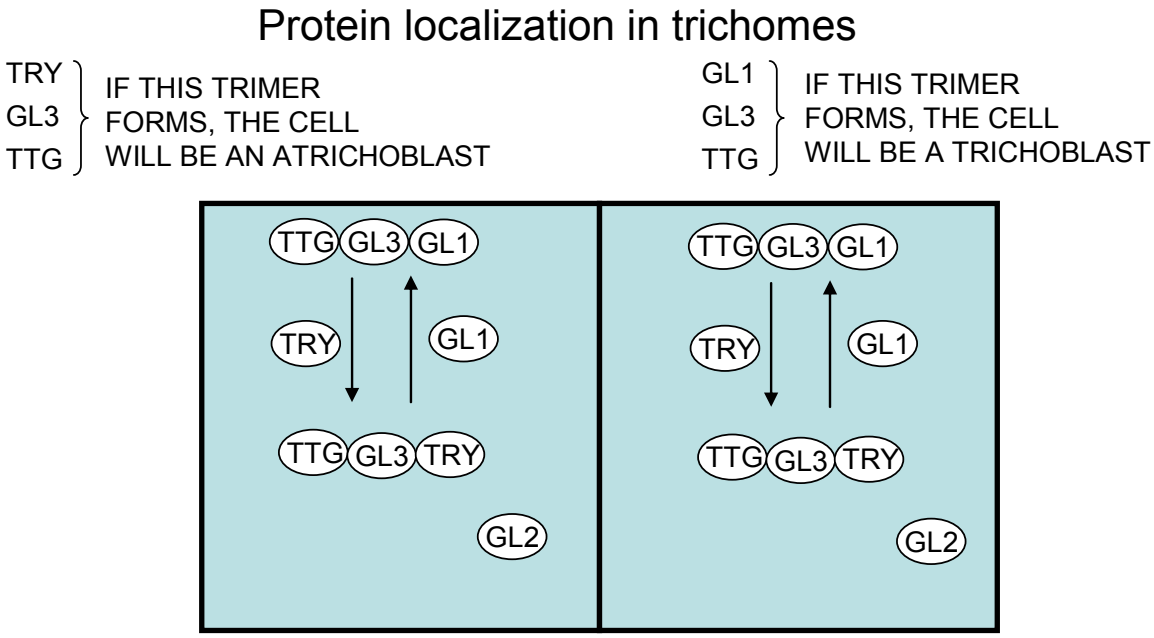
The timing of trichome development:

The timing of trichome development varies among plants. In arabidopsis leaves, although the first trichome does not commence differentiation, at the distal leaf tip, until the leaf primordium has expanded to circa 100μm, the trichomes are the first cells to terminally differentiate. On cotton ovules, the trichomes (which ultimately become single-celled cotton fibers) do not commence elongation until after all other epidermal cells have ceased to divide.

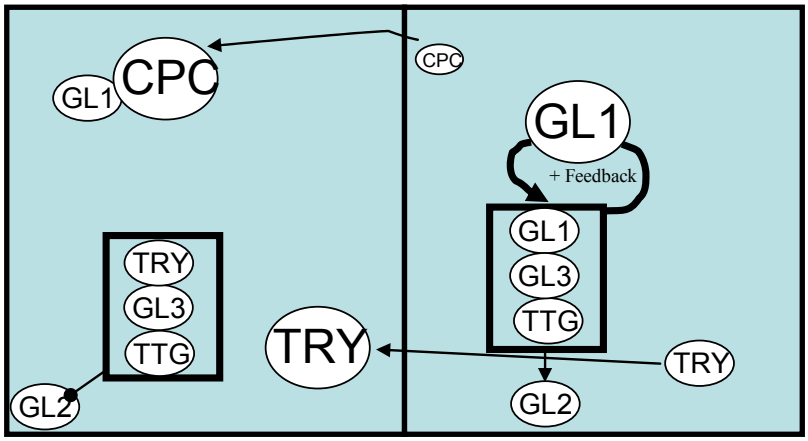
Fusing the nuclear targeted maize R gene that promotes trichome formation, to a steroid receptor from mice permitted the inducible expression of the R gene in the trichome-less *transparent testa glabra1* (*ttg1*) mutant. With no steroid application, the R gene remained cytosolic and the mutant remained trichome-less. Upon application of the steriod, the R gene product traversed the nucleous and the mutant reverted to a trichomed phenotype. When application of steroid was delayed, the more distal, older, sections of expanding leaves failed to revert, signifying that the trichome precursor cells are determined early in leaf expansion.

Additionally, the *REDUCED TRICHOME NUMBER* (*RTN*) gene may be responsible for determining the duration a particular cell is competent to make trichomes. This gene has been shown to be an allelic variant between Landsberg erecta and Columbia strains of arabidopsis. The Ler strain typically has fewer trichomes than the Col strain and this was demonstrated to be due to a shorter period of trichome initiation during the development of a Ler leaf. Hence, RTN may control the expression of either TTG1 or GL1 or control the period during which epidermal cells are receptive to TTG1 or GL1.

There is also some evidence that TTG1 and/or GL1 may be involved in determining the spacing of trichomes. Weak mutant alleles of both TTG1 and GL1 have been identified that do not completely suppress trichome development. In these plants, trichomes appear in clusters. Additionally, heterozygous TTG/ttg plants that ectopically overexpress GL1 often develop numerous clusters of trichomes. This suggests that a stoichiometric balance between TTG1 and GL1 must be maintained to prevent trichome clustering. An additional gene, TRY, may act downstream of TTG1 and GL1. Mutations in this gene increases the numbers of trichomes that occur in clusters.



FIRST, ALL CELLS HAVE AN EQUAL CHANCE OF PRODUCING A TRICHOME (BECOMING AN EPIDERMAL CELL CALLED A 'TRICHOBLAST'.



NEXT, ONE CELL ACQUIRES MORE OF THE GL1/GL3/TTG COMPLEX AND COMMITS TO TRICHOBLAST FATE. THIS UPREGULATES GL1, POSITIVELY ENFORCING TRICHOBLAST FATE. ADDITIONALLY, TRY AND CPC MIGRATE INTO ADJOINING CELLS VIA PLASMODESMATA. THIS INHIBITS TRICHOME DEVELOPMENT IN THESE CELLS MAKING THEM 'ATRICHOBLASTS'.

Figure 2: A Model of trichome initiation/suppression.

References used in the preparation of these notes.

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