Chapter 7: Developmental pathways altered in nematode feeding sites

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Abstract.-

Sedentary plant endoparasitic (root-knot and cyst) nematodes induce the formation of their feeding sites by directing the transdifferentiation of normal plant root cells into nematode feeding cells, namely giant cells and syncytia. In the last years, transcriptomic analyses combined with molecular cell biology have revealed dramatic and specific changes in gene expression in syncytia and giant cells. Among the genes whose expression is modified to establish feeding sites are those involved in hormone-regulated developmental pathways in the roots, particularly those related to auxins and cytokinins. The high concentrations of auxins and cytokinins in galls and syncytia have been described in detail by the use of reporter genes driven by specific promoters as “sensors” of both phytohormones, such as *DR5*, *ARR5* or *TCS*. Moreover, several molecular evidences link the formation of nematode feeding sites to developmental

processes as maintenance of the root apical meristem, lateral root initiation or vascular tissue development, in which the two hormones are involved. The mechanisms that nematodes use to interfere with plant developmental pathways are unclear, but some seem to involve nematode secreted molecules, such as the *CLE*-like and the *CEP* peptides. Only in a few cases, plant hormone transduction and developmental circuits hijacked by nematodes to induce and maintain feeding sites have been studied in detail. Analysis combining hormone genetic sensors, mutants and comparative transcriptomics, lead to the identification of relevant plant regulators that are exploited for NFS differentiation. We present the current knowledge connecting the hormonal-controlled developmental processes of the root with the development of the NFS, which seem to be different for giant cells and syncytia. For instance, *LBD16* and *WRKY23*, two key transcription factors in the signal transduction leading to lateral root formation mediated by auxins, play distinctive roles during gall/GC and syncytia formation, respectively. We also highlight the evidences linking gall and giant cell ontogeny to the pericycle and discuss the transfer cell-like identity of feeding cells.

Introduction.-

Plant endoparasitic nematodes induce the formation of sophisticated feeding structures inside the root that operate as physiological sinks to supply nutrients to the nematode (Perry and Moens, 2011). Among the most damaging groups of plant parasitic nematodes are the root-knot nematodes (RKNs; *Meloidogyne* spp.) and the cyst nematodes (CNs; *Heterodera* spp. and *Globodera* spp.), representing major threats to agriculture (Bird et al., 2009; Moens et al., 2009). One of the most remarkable changes regarding cell morphogenesis directly induced by nematode effectors (See Chapters 11, 12 and 13) is the formation of giant cells (GCs) by RKNs and syncytia by CNs. They are cells specifically induced by nematodes to sustain their feeding and their obligated development inside the plant. Together, both nematode groups are able to infect almost all species of agricultural crops, as RKNs show a polyphagous behaviour

(Moens et al., 2009), suggesting that the nematodes interfere with biological processes shared by most plant species in order to develop their feeding sites. One possibility is that they may “hijack”, at least partially, fundamental mechanisms of plant development, necessary for the survival and appropriate plant performance, as described for molecular transducers common to lateral root (LR) and gall formation (Cabrera et al., 2014a). Understanding NFS formation based on a deep knowledge of the developmental processes occurring in a non-infected root was proposed before the blast of the *omics* (Scheres et al., 1997). In the age of transcriptomics, when hundreds of genes have been identified as differentially expressed during the process of plant-nematode interaction, it becomes a prerequisite to connect those molecular evidences to the signalling cascades mediating developmental processes in a non-infected root. Several evidences show that plant parasitic nematodes develop their feeding sites through modulation or interloping of those developmental mechanisms present in the plant. This chapter summarizes the advances in this topic. The molecular mechanisms used by microorganisms to interfere with plant processes are surprisingly subtle but can effectively modify predefined plant developmental patterns. A recent example is the phytoplasma virulence protein SAP54 that promotes the degradation of flowering regulatory proteins, generating a short circuit in a developmental process that transforms flowers into leaves, helping attractiveness to leafhopper vectors for phytoplasma reproduction and propagation (MacLean et al., 2014). Similarly, effector molecules secreted by the nematodes seem to interfere with developmental pathways, although still there is a lack of clear evidence about the particular transduction cascades modified or perturbed by nematode effectors (this Chapter and Chapters 11 and 12; Lee et al., 2011).

In order to modify the molecular pathways used by the plants to develop their basic functional structures, it is reasonable to presume that the nematode interferes with the up-stream hormonal control of these particular transduction cascades. In this

respect, early experiments already pointed to the importance of auxins and cytokinins in the development of nematode feeding sites (NFS) induced within the root, as increased concentrations of both phytohormones or their precursors were detected in galls induced by RKNs (Balasubramaniam and Rangaswami, 1962; Krupasagar and Barker, 1969). More recently, mass spectrometric analysis confirmed the presence of auxins and cytokinins in the secretions of *H. schachtii* and *M. incognita* (De Meutter et al., 2003; 2005), which suggests a role for both hormones during NFS development. Moreover, the use of “hormone-sensor systems” based on reporter genes evidences the activation of both auxin and cytokinin signalling pathways in the formation/maintenance of GCs, galls and syncytia. Early experiments showing the activation of the auxin-responsive promoter *GH3* in the galls formed by RKNs in white clover (Hutangura et al., 1999) have been further confirmed with the use of the synthetic auxin responsive promoter *DR5*. It showed a clear activation in the GCs, galls and syncytia induced by plant parasitic nematodes in *Arabidopsis* either with GUS or GFP at early (Karczmarek et al., 2004; Grunewald et al., 2009) and late NFS developmental stages (Absmanner et al., 2013; Cabrera et al., 2014a). The activation of the *DR5* based sensor occurs very early during GCs formation, as brief incubation with the GUS substrate in conditions where diffusion is minimized shows a strong signal specifically within the GCs (Fig. 1A). However, the signal was also present in the surrounding vascular cells after longer incubation times, suggesting that, not surprisingly, auxins are also present in the vascular cell layers surrounding the GCs (Fig. 1B-C; Cabrera et al., 2014a). Similarly, the activation of cytokinin regulated genes has been demonstrated by using the responsive promoter *ARR5*, induced during early stages of the RKNs establishment (Lohar et al., 2004), and the synthetic cytokinin-responsive promoter *TCS*, that is induced in syncytia formed by *H. schachtii* in *Arabidopsis* but not in galls (Absmanner et al., 2013). Thereby, the overexpression in *Lotus japonica* of CKX, the enzyme that catalyzes the degradation of cytokinins, resulted in a reduction in the infection by RKNs (Lohar et al., 2004).

The auxin–cytokinin cross-talk is considered as the main hormonal control system regulating the developmental processes occurring in the roots, such as, the root apical meristem (RAM) maintenance, lateral root (LR) emergence or vascular tissue development (Dello Ioio et al., 2007; Bielach et al., 2012; Bishopp et al., 2011a). There are several molecular evidences showing that both hormones act antagonistically, i.e., auxin induces cell division in the meristems, while cytokinin stimulates the differentiation of these cells (reviewed in Moubayidin et al., 2009; Bishopp et al., 2011b; Bielach et al., 2012; Saini et al., 2013).

We present in this chapter the state of the art regarding our understanding of how nematodes interfere with hormone-regulated developmental pathways in the roots to establish their feeding sites, particularly those related to auxins and cytokinins. New data regarding morphometric parameters after GCs reconstruction are also discussed in relation to their acquisition of transfer cell-like nature.

Nematode peptide hormones as interceptors of plant development to form feeding sites.-

One of the major evidences demonstrating that nematodes actively interfere with the programmed development of the roots came from the discovery of a protein, Hg-SYV46, secreted by the CN *H. glycine* that contains a structural motif of the *CLAVATA3/ESR-related (CLE)* family in *Arabidopsis* (Wang et al., 2001; Wang et al., 2005). *CLV3-like* peptide hormones have been shown to have multiple functions in many aspects of plant development and morphogenesis (Leasure and He, 2012). The similarities between *CLV3* and the nematode peptide *Hg-SYV46* are not merely structural. The overexpression of *Hg-SYV46* in *clv3* mutant plants rescues their phenotype, while *Hg-SYV46* overexpression in wild type plants results in strong down-regulation of *WUSCHEL (WUS)*, similarly to that occurring in the plants overexpressing *CLV3* (Wang et al., 2005). In *Arabidopsis*, membrane receptor kinases and several of

the WUSCHEL-RELATED HOMEBOX (WOX) transcription factors participate in *CLE* signalling (Leasure and He, 2012). While *CLV3* acts in the shoot apical meristem repressing the expression of *WUS* and regulating the SAM stem cell number through a negative feedback loop (Schoof et al., 2000), *CLE40*, another *CLV3*-like peptide, acts in the RAM regulating the expression of *WOX5* (Stahl et al., 2009) through the receptor-like kinase CRINKLY4 (ACR4). Overexpression of *CLE40* alters the expression of *WOX5* and promotes the differentiation of distal columella stem cells to columella cells (Stahl et al., 2009). Since the description of *Hg-SYV46*, more *CLE*-like genes have been described in *H. glycines* (Wang et al., 2010), *H. schachtii* (Patel et al., 2008; Wang et al., 2011) and *G. rostochiensis* (Lu et al., 2009; Guo et al., 2011; see chapters 11 and 12 for detailed explanation). Functional analysis *via* targeting these nematode *CLE*-like genes in plants by RNAi (Bakhetia et al., 2007; Patel et al., 2008) or by the infection of *Arabidopsis* *CLE* receptor mutants such as *clv1*, *clv2* and *rpk2* showed a decrease in the infection rate and in the size of syncytia. These constitute experimental evidences that confirm the role of *CLE*-like peptides in syncytia development (Replogle et al., 2011; 2013). All these data together suggest a role of the nematode peptide hormones in the development of the syncytia in *Arabidopsis*, possibly interfering with the aforementioned plant developmental pathways.

The *16D10* gene from *M. incognita* encoding a secretory peptide with a *CLE*-like sequence also showed functional characteristics of a component of a *CLE*-related pathway. *In vivo* expression of *16D10* dsRNA in *Arabidopsis* resulted in an increase in the resistance against RKN (Huang et al., 2006). Additionally, the overexpression of *16D10*, that directly interacts with *SCARECROW*-like transcription factors (*SCR*; Huang et al., 2006), does not rescue the *clv3* phenotype but stimulates root growth in *Arabidopsis* and tobacco, i. e., calli were formed in tips cut for subculturing (Huang et al., 2006). These results demonstrate *16D10* being an effector that substantially alters plant development. *SCR*, expressed specifically in the endodermis and cortex/endodermis initial cells of the root (Di Laurenzio et al., 1996), is a key regulator

of radial patterning in the *Arabidopsis* root (Levesque et al., 2006) and is also directly activated by *SHORT-ROOT* (*SHR*; Levesque et al., 2006), therefore regulating root meristem identity and root development. Strikingly, transcripts from both *SCR* and *SHR* were down-regulated in isolated GCs at 3 dpi (Barcala et al., 2010); however, transcriptomes specific of GCs are not available in earlier time points when developmental switches concerning cell development are probably crucial. Thus, the *16D10* gene constitutes another example of how nematodes could interfere with hormonal controlled developmental pathways of the root to generate their feeding sites.

The putative role of *CLE*-like nematode peptides might not be merely related to the meristems development. Yet, another group of *CLE* peptides as *TDIF*, a peptide hormone derived from *CLE41/44* (Ito et al., 2006) that induces the expression of *WOX4* in cambium cells (Hirakawa et al., 2010), participates in the vascular development by promoting the division of cambium cells preventing their differentiation into xylem. Changes in auxin levels, as those happening in nematode feeding cells, are necessary as the trigger signal for vascular development mediated by the *TDIF/WOX4* pathway (Scarpella et al., 2006; Wenzel et al., 2007; Donner et al., 2009). This agrees with the high degree of similarity found between the transcriptomes of 3 dpi GCs and suspension cells treated with brassinolide/boric acid that are differentiating into tracheids (Kubo et al., 2005; Barcala et al., 2010). These findings strongly point to pro-vascular cells as putative precursors of the GCs, and are in agreement to initial data based on histological observations that proposed metaxylem, protoxylem or xylem parenchyma cells as the initial cells that develop into GCs (Christie, 1936; Dropkin and Nelson, 1960; Bird, 1961; Niebel et al., 1993; Williamson and Hussey, 1996; Bird and Koltai, 2000). In addition, proliferating tracheids and phloem elements have been described around GCs and syncytia (Hoth et al., 2008; Absmanner et al., 2013; Bartlem et al., 2013). The development of these vascular elements is governed by the balance between auxins and cytokinins, as those vascular cells from the galls that

differentiate into phloem elements respond to auxins, but not to cytokinins, before differentiation (Absmanner et al., 2013). In contrast, the phloem around syncytia responded to both auxins and cytokinins (Absmanner et al., 2013). An example of the interference of the RKNs with the process of the vascularization in the galls is that overexpression of the enzyme chorismate mutase, secreted by *M. javanica*, inhibits the final differentiation of root vascular cells (Doyle and Lambert, 2003), a phenotype that can be rescued by adding IAA. This suggests that nematode-secreted chorismate mutase acts by reducing IAA levels. Interestingly, both CNs and RKNs encode in their genome proteins homologues to plant chorismate mutases (Bekal et al., 2003; Jones et al., 2003; Huang et al., 2005; Vanholme et al., 2009; Chronis et al., 2014). Therefore, the secretion of chorismate mutases by PPNs might be a way to interfere with the auxin/cytokinin balance within the vascular cylinder in order to redirect the differentiation of vascular elements to NFCs.

Auxin gradients, generated by the PIN proteins, have been shown to be a common signal for the formation of different new organs in the plant (Benková et al., 2003; Vanneste and Friml, 2009). In this context, tomato plants treated with the polar auxin transport inhibitor NPA showed a reduction in the establishment of CNs and abnormal syncytia development (Goverse et al., 2000). Grunewald et al. (2009) studied the differential expression of *PIN* coding genes during early syncytia development in *Arabidopsis* confirming opposite regulation for different members of the family. A model was proposed in which PIN1 mediates the influx of auxin to the initial syncytia cells and PIN3 and PIN4 distribute the accumulated auxin laterally, allowing the expansion of the NFS (Grunewald et al., 2009). Thereby, CN infection rates and syncytia development are affected in *pin3* mutant plants (Grunewald et al., 2009). Together with PIN1, another auxin influx carrier, LAX3, that is expressed in the syncytium and in cells to be incorporated into the syncytium together with LAX1 (Lee et al., 2011), allow the syncytia growth by increasing the auxin levels in the neighbouring cells. Strikingly, an

effector protein from *H. schachtii* (*Hs19C07*) can interact with LAX3 (Lee et al., 2011). Additionally, *AUX1*, a closely related *AUX/LAX* family member, was up-regulated in syncytia developed by *H. schachtii* and in galls induced by *M. incognita* in *Arabidopsis* (Mazarei et al., 2003). Both the *aux1/lax3* double mutant and the *aux1/lax1/lax2/lax3* quadruple mutant showed significant decrease in the number of female nematodes at both 14 and 30 dpi (Lee et al., 2011), suggesting that the *LAX3-Hs19C07* interaction could alter auxin levels to promote syncytia establishment. The analysis of *Arabidopsis* mutant lines *pin1/ttg-1* and *pin2* after *H. schachtii* infection, resulting in the reduction of nematode and syncytia development, confirmed the role of auxin transporters during the plant-nematode interaction (Goverse et al., 2000). However, most of the existing data are based on cyst nematodes and further analysis should be made in galls induced by RKNs to know the putative role of these proteins in this process.

Novel classes of peptide hormones as CEP (C-terminally Encoded Peptide) are emerging as regulators of the developmental process leading to gall formation. *CEP* genes have been identified in *M. incognita* and *M. hapla* genomes (Goverse and Bird, 2011; Bobay et al., 2013) but not in the false RKN *Nacobbus aberrans* (Eves-van den Akker, et al., 2014), neither in cyst nematodes (*H. glycines* and *Globodera rostochiensis*), migratory nematodes (*Radopholus similis* and *Pratylenchus coffeae*) nor the free-living nematode (*Caenorhabditis elegans*; Bobay et al., 2013). In particular, *MhCEP11* from *M. hapla* shows a significant sequence homology (Bobay et al., 2013) with the *Arabidopsis*-encoded CEP1 that is expressed in LR primordia. Overexpression of *AtCEP1* with a constitutive promoter resulted in a reduced number of cells in the RAM (Ohshima et al., 2008). In *Medicago truncatula*, the constitutive overexpression of *MtCEP1* altered root development in several ways, by inhibition of lateral root formation, enhancement of nodulation and cortical, epidermal and pericycle cell divisions (Imin et al., 2013). Future functional studies involving these molecules will elucidate their role as putative regulators of the gall and/or GC formation.

Auxins, lateral root formation and feeding sites.-

CEPs are one of the most recently identified molecules that relate a root developmental process, LR formation, to gall development (Imin et al., 2013), but it is not the only connecting link. The aforementioned local increase of auxin levels favoured by PIN proteins is needed as well during the formation of LRs (Benkova et al., 2003). There are several pieces of evidence that point to similarities and molecular connections between the processes of LR and NFS development. Among them, it was early shown that the auxin-insensitive tomato mutant *diaegotropica*, *dgt* (Richardson and Price, 1982), which lacks LRs, was resistant to *M. incognita* and developed smaller syncytia upon cyst nematode infection (Goverse et al., 2000). In tomato and *M. truncatula*, two transcription factors, *KNOX* and *PHAN*, are induced in both GCs and LR meristems (Bird and Koltai, 2000; Koltai et al., 2001). In *Arabidopsis* the down-regulation of the *KNOX* transcription factor *KNAT6* yields an increment in the number of LRs (Dean et al., 2004), in agreement to the suggested antagonistic action between auxin and *KNOX* transcription factors in organogenesis (reviewed in Scofield and Murray, 2006). Moreover, Barthels et al. (1997) used a promoter-tagging strategy to identify specific regulatory regions differentially activated in NFS as compared to uninfected roots. Surprisingly, among the 103 promoter tag lines that displayed a distinct activation response to nematode infection, 39 also exhibited induction at LR initiation sites. This has been further confirmed by *in silico* analysis of transcriptomes from galls and GCs in *Arabidopsis* that showed an enrichment of characteristic genes from LR initial cells in the transcriptome of 3 dpi GCs and galls (Cabrera et al., 2014a). LRs originate from divisions in the xylem pole pericycle (XPP) cells following an auxin-mediated signalling pathway. Two XPP marker lines, J0121 and J0192, showed strong and distinct GFP expression in the galls formed by *M. javanica* (Cabrera et al., 2014a; Fig.2A-B). Strikingly, in both lines GFP expression was mostly observed at both sides of the vascular cylinder and progressed inwards during gall development, which

partially differs with the expression pattern found during LR formation (Laplaze et al., 2005; Cabrera et al., 2014a). In addition, the regular anticlinal and periclinal divisions observed during LR formation (Lavenus et al., 2013), were substituted by abnormal division planes in the cells proliferating inside the galls (Cabrera et al., 2014a). The expression pattern of *LBD16*, the gene whose promoter drives GFP expression in the J0192 enhancer trap line, mimics that of J0192 line (Fig. 2C). *LBD16* expression is detected in galls from *M. javanica* and *M. arenaria* from 1 dpi up to 11–15 dpi and was regulated by auxins, as shown by its inhibition by PEO-IAA, an antagonist of IAA, similarly to what happens in LR primordia (Cabrera et al., 2014a; Okushima et al., 2007; Lee et al., 2009). Although its expression at early stages appeared to correlate with the presence of auxins in the same cell types, at later stages the mere presence of auxins in the gall was not sufficient to activate *LBD16* expression (Cabrera et al., 2014a). These results may indicate the necessity for a threshold level of auxins in the gall to allow *LBD16* expression, which would mimic the scenario that occurs during the first divisions of LR development. However, the absence of signal in the syncytia formed in the *LBD16::GUS* line, where the “auxin sensor” DR5 was also activated (Karczmarek et al., 2004), suggests that other signals apart from auxins could be contributing to the early activation of *LBD16* in galls and GCs. In this respect, secretions from *M. incognita* juveniles were also able to induce *LBD16* expression in *Arabidopsis* leaf protoplasts, suggesting an activation of the *LBD16* promoter by nematode secretions, in an autonomous manner. Furthermore, a reduction in gall formation of at least 20% was observed in *LBD16* loss of function lines as compared to wild type controls (Cabrera et al., 2014a). Interestingly, the expression pattern of the marker line *ProCycB1;1:CycB1;1(NT)-GUS*, active only during the G2/M transition, mimicked that of J0192 in XPP cells during early nematode establishment, and in most cells inside the vascular cylinder of the gall as the infection progressed. These results suggest that the founder cells contained in the XPP that divide to form a new LR (Péret et al., 2009) also divide during early gall formation (Cabrera et al., 2014a). *LBD16* loss

of function lines showed abnormal GC development, pointing to a role of the pericycle during this process. The importance of XPP-specific genes during infection in *Arabidopsis* was further demonstrated by genetic ablation using a *J0121>>DTA* line that showed a dramatic reduction in the infection and in the size of the GCs as compared to a control *J0121>>GFP* line (Cabrera et al., 2014a).

The induction of *LBD16* during gall formation not only connects this process with LR formation but with the generation of calli (Cabrera et al., 2014a; Demeulenaere and Beeckman, 2014). *LBD* transcription factors and pericycle cells have been shown to be essential for the generation of calli from different organs through ectopic activation of a LR developmental program (Sugimoto et al., 2010). The ectopic expression of *LBDs* triggers spontaneous callus formation but its suppression inhibits the process (Fan et al., 2012); whether or not gall development is somehow related to this process of callus formation through the activation of a LR initiation-like program remains to be elucidated. *In silico* data comparison supported this hypothesis, as those genes co-regulated with *LBD16* in different transcriptomes were integrated in signalling cascades mediated by auxins during LR and callus formation, as a particular feature of early developing RKN feeding sites (3dpi) distinct to CNs. In contrast, cytokinin-induced genes were enriched in syncytia, whose transcriptomes hold a high similarity with the transcriptome of shoot regeneration from callus, modulated by cytokinins (Cabrera et al., 2014b, c). In agreement with these analyses, subtle changes in the balance between cytokinin and auxin levels could be mediating the appearance of chloroplast-like structures inside 7dpi GCs induced by *M. graminicola* in rice, as showed by confocal microscopy (Kyndt et al., 2013; Ji et al., 2013) and in syncytia (Szakasits et al., 2009). Transcriptomic studies performed in isolated GCs and syncytia in the last recent years reflected the predominance of genes regulated by phytohormones among the differentially expressed genes as compared to non-infected tissues (reviewed in Escobar et al., 2011; Cabrera et al., 2014b, c). In this way, a direct *in silico* comparison

of the transcriptomes of isolated GCs and syncytia at early developmental stages in *Arabidopsis* and the transcriptomes from seedlings treated with exogenous phytohormones contributed to increase the vast number of genes regulated by hormones that are also induced or repressed in NFCs (Cabrera et al., 2014b, c). Clear differences between the hormone-related transcriptional balances of the two NFC types were found. While the percentage of auxin-induced genes stands out (26%) in GCs, in syncytia there are 21% of cytokinin-induced genes. On the contrary, in GCs there are more cytokinin-repressed genes as compared to those up-regulated; but in syncytia the number of cytokinin up-regulated genes is higher than the number of down-regulated ones. Interestingly, the number of genes repressed by auxins or cytokinins in GCs or syncytia was high, suggesting that gene repression driven by these hormones may be also crucial for the development of the NFS (Cabrera et al., 2014b, c).

WRKY23 is another transcription factor regulated by auxins and induced by both RKN and CN. Loss of function lines showed an increased resistance to CNs (Grunewald et al., 2009). *WRKY23* acts downstream of the signalling cascade (*SLR/IAA14*)-(auxin responsive factors) (*ARF7/ARF19*) during NFs and LR formation (Grunewald et al., 2008; Grunewald et al., 2012) and it is needed for LR development through the stimulation of local flavonols biosynthesis (Grunewald et al., 2012). However, *WRKY23* expression is activated in NFS by an independent auxin pathway, suggesting the existence of other nematode-dependent signals in regulating *WRKY23* expression. This could be also the case for *LBD16* expression that is activated by nematode secretions as previously mentioned (Cabrera et al., 2014a). Other members of the *LBDs* transcription factors family as *LBD41*, are activated in both *M. incognita* and *H. schachtii* feeding sites (Fuller et al., 2007), in agreement with transcriptomic analysis of GCs in *Arabidopsis* (Barcala et al., 2010).

The expression patterns of crucial molecular components of the auxin signalling pathway, encoded by genes of the *ARFs* family, have been recently

addressed during syncytium development in *Arabidopsis* (Hewezi et al., 2014). At early infection stages, 2–3 days after *H. schachtii* infection, *ARF3*, 6, 10–12, 14, 15 and 20–22 were expressed inside the developing syncytium, while *ARF1*, 2, 4, 5, 9, 18 and 19 were active in both syncytial and neighbour cells. *ARF7* and 17 were mainly expressed at the edges of the syncytial and neighbouring cells and *ARF8* and 16 showed a weak response to *H. schachtii* infection (Hewezi et al., 2014). At 9–10 days after infection, *ARF1–3*, 7, 17 and 20–22 were expressed in fully developed syncytium, whereas the expression of the other *ARFs* was restricted to the syncytial cells around the nematode head (Hewezi et al., 2014). Although still under investigation, the differential expression patterns of the *ARF* genes seem to be essential for the correct development of the CNs feeding cells. All these data suggest a subtle and complex regulation of auxin-mediated pathways based on a tight temporal and spatial control of molecular components such as *ARFs* in CNs feeding sites. A lack of knowledge of the regulation of these genes after RKN infection is still faced.

Giant cells morphogenesis and transfer cells nature.-

In the previous sections, we described parallelisms between feeding site formation and developmental programs during the plant life cycle, such as LR formation. Although syncytia and GCs differ in their ontogeny and global transcriptional signatures, both develop cell wall ingrowths (CIs) to facilitate high rates of apoplastic/symplastic solute exchange. Both feeding site types also show similarities to the transfer cells (TC) that appear in different plant organs during plant development (reviewed in Offler et al., 2003)

In GCs the amplification of the plasma membrane surface area could be up to 20-fold (reviewed in Jones and Goto, 2011). Syncytia induced by *H. schachtii* are symplastically isolated at 10–15 dpi (Hofmann et al., 2007) and the CIs are smaller in male than in female-developed syncytia, suggesting that CI size control is based on the nutrient demand of the nematode (reviewed in Sobczak and Golinowski, 2008).

Recently, three-dimensional reconstruction and volume measurements of GCs in *Arabidopsis* (Cabrera et al., 2014e) brought some interesting findings that might explain, at least partially, their TCs characteristics. The abnormally large size of the GCs implies a reduction in their surface area to volume ratio (SA:V ratio; Cabrera et al., 2014e), what may compromise its functioning during nematode nourishing. Thus, the extensive formation of wall ingrowths lined with plasma membrane at certain developmental stages that defines them as TCs (Jones and Dropkin, 1976; Siddique et al., 2012) could be a response for a functional requirement to compensate the decrease of the S/V ratio as the GCs expand (reviewed in Rodiuc et al., 2014; Cabrera et al., 2014e). Moreover, size regulation affects cell function in multiple ways, e.g., not only nutrient and water movement can be changed by altered surface/volume ratio, but intercellular signalling might be influenced by changes in cell size and geometry. Recent studies in yeast revealed that transcription also changes specifically in response to cell dimensions (Wu et al., 2010; Sablowski and Dornelas, 2014).

Cell wall anatomy and composition in NFCs are quite similar to other TCs that develop in plants, and it is mainly composed of polysaccharides such as cellulose, hemicelluloses and pectin (reviewed in Rodiuc et al., 2014). Although regulatory signals for TC differentiation are not well known, the transcription factor *ZmMRP-1*, that has been described as a key component in the pathway leading to the formation of the TCs (Gomez et al., 2002), is also induced in *Arabidopsis* galls (Barrero et al., 2009). Auxins and ethylene are the major phytohormones described as regulators of TC differentiation from several cell types (Dibley et al., 2009; Thiel et al., 2008, 2012a,b; Zhou et al., 2010; Xiong et al., 2011). Common genes related to the auxin signalling cascades and transport from the *IAA/ARF/PIN* families play a role and/or show differential regulation in both TCs and NFCs (reviewed in Cabrera et al., 2014d; this chapter). Genes related with ethylene synthesis and signalling as those encoding membrane receptors, ACC oxidases or ACC synthase, are also to be induced in NFS

(Tucker, 2010; Cabrera et al., 2014d). Increased concentration of ethylene in tomato galls was described long ago (Glazer et al., 1983). Moreover, functional analysis performed with ethylene-related mutants reinforce the importance of this hormone during plant-nematode interaction as ethylene overproducing mutants *eto2* and *eto3* were more susceptible to CNs (Goverse et al., 2000; Wubben et al., 2001). Interestingly, ethylene overproduction in *eto2* mutants stimulated the formation of CIs or protuberances in syncytia along the vascular tissue, at late infection stages (Goverse et al., 2000), providing a direct evidence for a putative role of ethylene in the stimulation of syncytia TCs identity. In the other hand, mutants compromised in the ethylene signalling cascade (*etr1-1*, *ein2-1*, *ein3-1*, *eir1-1*, and *axr2*) showed a lower susceptibility to the infection by CNs (Wubben et al., 2001; Goverse et al., 2000). Interestingly, the most clarifying study of a functional implication in TCs characteristic of NFCs, as the CIs formation, comes from the analysis of UDP-glucose dehydrogenase (UGD) coding genes. *UGDs* act through oxidation of UDP-glucose producing several cell wall polysaccharides. *UGD2* and *UGD3* are necessary for the production of CIs in syncytia and loss of function in double mutants severely affected nematode development (Siddique et al., 2012).

In conclusion, there are several evidences that nematodes may interfere or partially “hijack” signal transduction pathways used by the plant to initiate and/or maintain developmental processes where auxins and cytokinins play a central role, such as SAM and RAM maintenance, and LR and vascular tissue formation. Additionally, nematodes may also interfere with transduction pathways leading to the differentiation of specialized plant cell types such as TCs. The understanding of the plant molecular components necessary to reprogram normal plant cells into NFCs are an outstanding topic to be deciphered, what would contribute to the basic understanding of the plant-nematode interaction. This will also help to identify the interactions of nematode effectors with plant components, and at the end, their mode of

action while interfering with the plant developmental programs. Furthermore, this knowledge will constitute a powerful tool for engineering nematode resistance in plants and to direct the search for specific nematode control strategies.

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Figures.-

Figure 1.- Transgenic *Arabidopsis* line *DR5::GUS* showing GUS activity in 4 dpi galls induced by *Meloidogyne javanica* at different incubation times in the GUS staining solution. A) After 2h incubation, GUS activity is centred in giant cells. B) After 4h incubation, staining spreads to the adjacent vascular tissue. C) With overnight incubation, the signal covers most of the gall vascular tissues. N: Nematode; *: Giant Cells. Zoom in images of A and B are shown in the panels below. Bars: 100 μ m.

Figure 2.- Transgenic lines of lateral root and XPP pericycle markers and *LBD16* expressing either GFP or GUS in galls formed by *Meloidogyne javanica*. A) J0121>>GFP at 4dpi. B) J0192>>GFP at 4dpi. C) *pLBD16:GUS* at 7 dpi (Cabrera et al., 2014a). Bars: 100 μ m.