Research review paper

Functions of rol genes in plant secondary metabolism

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For a long time, the Agrobacterium rhizogenes rolA, rolB and rolC oncogenes have been considered to be modulators of plant growth and cell differentiation. A new function of the rol genes in plant–Agrobacterium interaction became apparent with the discovery that these genes are potential activators of secondary metabolism in transformed cells from the Solanaceae, Araliaceae, Rubiaceae, Vitaceae and Rosaceae families. In some cases, the activator effect of individual rol genes is sufficient to overcome the inability of cultured plant cells to produce large amounts of secondary metabolites. Here, I summarize the available evidence that shows that genetic transformation by single Agrobacterium rol genes may be used as a powerful tool to manipulate secondary metabolites in cultured plant cells. Although it is known that the rol genes act via transcriptional activation of defense genes, the mechanism of activation is unclear. In this review, evidence is presented to support the hypothesis that the rol genes mediate uncommon signal transduction pathways in plants.

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1. Introduction

The rolA, rolB, rolC and rolD genes are plant oncogenes, and are carried on plasmids of the plant pathogen Agrobacterium rhizogenes.

Following agrobacterial infection, these genes are transferred into plant genome and cause tumor formation and hairy root disease. Twenty years ago, the rolA, rolC and rolB oncogenes of A. rhizogenes were shown to induce root formation in transformed plant cells (Spina et al., 1987; Cardarelli et al., 1987). This discovery stimulated investigations that aimed to understand the effects of these genes upon plant development. There have been many studies of rol genes; however, the findings from these studies are rather conflicting and therefore the role of the rol genes in oncogenesis is not well understood. This fact is not entirely surprising, since oncogenes cause multiple biochemical and physiological alterations in transformed plants and appear to act in a complex manner, and
in this aspect they are similar to animal oncogenes. "The rol genes saga," written by Costantino et al. (1994) and continued in other review articles (Nilsson and Olsson, 1997; Binns and Costantino, 1998; Meyer et al., 2000), summarized different aspects of growth, development and hormone metabolism in transformed plants. The advantage of using rol genes in forculture was reviewed by Casanova et al. (2005). Over the past decade, novel features of plant cells transformed with the rolA, B and C genes have been revealed, such as increased production of secondary metabolites. This review summarizes these findings and discusses potential biotechnological applications of the rol genes. In particular, the inextricable link between studies investigating the general biochemical function of the rol genes and studies of their effects on secondary metabolism is highlighted.

2. The rol genes and secondary metabolism

2.1. The rolA gene

The RolA protein is probably a member of the DNA-binding proteins, and shares structural similarity with the papillomavirus E2 DNA-binding domain (Ridgen and Carneiro, 1999). The biochemical function of RolA is generally unknown. The rolA gene has been shown to have a stimulatory effect on nicotine production (Palazón et al., 1997). RolA-expressing calli from Rubia cordifolia produced a 2.8-fold higher level of anthraquinones (AQs) compared to control calli (Shkryl et al., 2007). An interesting biotechnological peculiarity of rolA is that the gene expressed in R. cordifolia calli ensured remarkably stable levels of AQs, and rolA expression simultaneously provided conditions for vigorous callus growth. This effect was stable over a 7 year period of observation of the rolA-transformed callus line (Shkryl et al., 2007). However, these data are too limited to provide conclusive information regarding the effect of rolA on secondary metabolism.

2.2. The rolB gene

Of the rol genes, rolB is apparently the most powerful inducer of secondary metabolism and is also the most powerful suppressor of cell growth. In R. cordifolia transformed calli, rolB expression positively correlated with increased expression of a key gene for AQ biosynthesis, isochorismate synthase (ICS) gene and with anthraquinone production (Shkryl et al., 2007). Investigation of the growth parameters of these calli showed that high levels of rolB expression inhibited callus growth in a manner dependent on the strength of expression. Interestingly, the rolA and rolC genes showed no negative effect on callus growth. When combined with the rolA and C genes in a rolABC construct, rolB no longer had an adverse action on growth of rolABC-transformed cells (Shkryl et al., 2007). The rolB gene had the largest effect on AQ accumulation when compared to rolA and rolC. In a high-rolB-expressing culture, a 15-fold increase in AQs was detected. However, the stimulatory effect of the rolB gene on AQ formation was weaker when the gene was combined with the rolC and rolA genes in a rolABC construct.

The most prominent example of the effectiveness of rolB transformation was recently demonstrated for Vitis amurensis cells, where transformation led to more than a 100-fold increase in resveratrol production (Kiselev et al., 2007). Resveratrol is an important stilbene, which prevents carcinogenesis at tumor initiation, promotion and progression. In this study, normal cell cultures of wild-growing grape (V. amurensis) were developed. The cultures produced low levels of resveratrol, up to 0.026% dry wt., which was comparable to the levels reported for other grape cell cultures. The application of methods commonly used to increase secondary metabolite production (cell selection, elicitor treatments and addition of a biosynthetic precursor) only led to a slight enhancement in cell productivity. The rolB-transformed calli were capable of producing up to 3.15% dry wt. of resveratrol, and levels of the stilbene were correlated with the abundance of rolB mRNA transcripts.

Growth suppression is a limiting factor for the practical application of rolB-transformed cells. Since rolB encodes a protein that possesses tyrosine phosphatase activity (Filippini et al., 1996), the authors of two studies attempted to block rolB effects by using tyrosine phosphatase inhibitors. The inhibitors partially prevented the toxic effects of rolB on cell growth and lowered the production of secondary metabolites (Kiselev et al., 2007; Shkryl et al., 2007).

2.3. The rolC gene

By itself, the rolC gene is capable of stimulating the production of tropane alkaloids (Bonhomme et al., 2000a), pyridine alkaloids (Palazón et al., 1998a), indole alkaloids (Palazón et al., 1998b), ginsenosides (Bulgakov et al., 1998) and anthraquinones (Bulgakov et al., 2002a, 2003; Shkryl et al., 2007) in transformed plants and plant cell cultures.

Transformation of Nicotiana tabacum leaf sections with pPCV002-ABC (rol genes A, B and C together, under the control of their own promoters) or pPCV002-CaMV (rolC transcribed through the CaMV 35S promoter) constructs yielded two (rolABC and rolC) transgenic root lines, which were then examined for their nicotine productivity relative to growth rate and amounts of rolC gene product (Palazón et al., 1998a). Changes in rolC expression were positively correlated with the capacity of the transgenic roots to grow and produce nicotine. In another study, the authors hypothesized that the rolC gene stimulates alkaloid production by stimulating growth, but not by directly increasing the biosynthetic activity of transformed roots (Palazón et al., 1997). However, in Catharanthus roseus rolC-transformed roots, rolC stimulated the production of indole alkaloids, and the amount of the rolC gene product correlated with the accumulation of these secondary metabolites (Palazón et al., 1998b).

The extent of activation was different in tested plant species. In transformed roots of Atropa belladonna, rolC caused, on average, a 12-fold increase in hyoscyamine and scopolamine content when compared with untransformed roots (Bonhomme et al., 2000a). A 4-fold difference in alkaloid content among independently transformed root clones was detected. Transformed roots of Panax ginseng were established by transformation of the 1c callus line with rolC (Bulgakov et al., 1998). These roots contained high ginsenoside concentrations that exceeded those of the 1c parent calli by 1.8–3 times (Bulgakov et al., 1998). A 3-fold difference in ginsenoside content was observed among independently rolC-transformed root cultures. The maximal content of ginsenosides in the rolC-transformed ginseng root cultures was very high (6.58% dry wt.), and represented almost the upper limit of the biosynthetic capacity of ginseng cells. This study showed that the activator effect of the rolC gene is independent of the level of cell differentiation, because equal levels of ginsenosides were observed in transformed roots and calli derived from them (Bulgakov et al., 1998).

In R. cordifolia transformed calli, expression of rolC led to a stable increase in anthraquinone content. The extent of activation was 1.3–1.8 times that of non-transformed cells (Bulgakov et al., 2002a). After prolonged periods of cultivation (more than 5 years), the rolC-transformed calli of R. cordifolia accumulated 2.0–4.3 times more AQs than normal calli, demonstrating that the acquired increased productivity is highly stable over time (Shkryl et al., 2007). Production of anthraquinones and the expression of the ICS gene were dependent on the strength of rolC expression: the higher the expression of the rolC gene, the higher the observed expression of the ICS gene and accumulation of AQs (Shkryl et al., 2007).

Considering all these data, one can conclude that rolC provides a signal that activates secondary metabolic processes. It is likely that rolC may confer a wider spectrum of defense reactions in addition to secondary metabolite stimulation. RolC expression in ginseng cells correlated with activation of β-1,3-glucanase, which belongs to the PR-2 family of plant defense proteins (Kiselev et al., 2006). This effect was due to transcriptional activation of the corresponding β-1,3-glucanase gene.
2.4. The rolD gene

The effects of rolD on secondary metabolism have never been investigated. RolD is the only Rol protein which biochemical function is clearly determined. Based on bioinformatic data and direct biochemical methods, Trovato et al. (2001) determined that rolD encodes ornithine cyclodeaminase, an enzyme that converts ornithine to proline. Decreasing the ornithine pool and increasing the proline pool in transformed cells is believed to contribute to the morphological effects of rolD, such as the maintenance of hairy root growth and abundant flowering of transformed plants (Trovato et al., 2001). Interestingly, expression of rolD in tomato plants enhanced the competence of cells to mount defensive responses, as measured by determination of PR-1 gene expression (Bettini et al., 2003).

2.5. The combined effects of the rolA, B and C genes

In principle, the combined effect of the rol genes has been studied in numerous investigations using hairy root cultures, where the genes were expressed under the control of their own promoters, together with other T-DNA genes. These investigations showed that there was an increase in the production of secondary metabolites in hairy roots in most transformed plant species (reviewed by Giri and Narasu, 2000; Sevon and Oksman-Caldentey, 2002). The ability of hairy roots to produce high amounts of secondary metabolites is an interesting natural phenomenon, which raises the question of which gene loci of T-DNA are responsible for this effect. Considering that T-DNA rol gene loci have a large impact on diverse biochemical processes in transformed plant cells, it is reasonable to propose that the essential genetic determinants are the rol genes of A. rhizogenes.

It was found that the accumulation of secondary metabolites in rolABC-transformed roots of different plants was similar to that found in wild-type transformed hairy roots (Palazón et al., 1997; Bonhomme et al., 2000b; Hong et al., 2006). A recent investigation of rolA, rolB, rolC, rolABC and pRiA4-transformed cells of Rubia cordifolia revealed that each of the rol genes appears to have its own individual mechanism of AQ activation (Shkryl et al., 2007). Collectively, these genes play a major role in the pathway that leads to high levels of secondary metabolites. Although the possibility that additional T-DNA genes may also have an impact on secondary metabolism in hairy roots cannot be excluded, the influence of additional genes seems to be less prominent.

This investigation also yielded interesting results that indicated that rolC (and perhaps rolA) has an antagonistic effect on the rolB-induced increase in AQs. This observation is not surprising, because the antagonistic effects of the rol genes have previously been demonstrated at different levels. Constitutive rolB expression suppressed the growth of tobacco cells, and the rolC gene was able to attenuate this growth inhibition (Schmülling et al., 1988). Likewise, rolC diminished the rolB-induced high sensitivity to auxin in transformed cells (Maurel et al., 1991) and the severity of the rolB-induced transformed phenotypes (Capone et al., 1989; Vanaltvorst et al., 1992). There is some data that suggest that the antagonistic effects of rolB and rolC may be mediated at the level of calcium signaling (Bulgakov et al., 2003). It is probable that rolB is a major player in secondary metabolism activation by wild-type transformation, while rolC has its own role in secondary metabolism and provides a signal that tunes the action of rolB.

3. How the rol genes can be integrated into defense signaling pathways

3.1. Biochemical functions of rol genes

It is clear that the effects of rol genes on secondary metabolism cannot be elucidated without understanding their biochemical function and their involvement in existing transduction pathways. Studies concerning the biochemical functions of Rol proteins have been reviewed by several authors (Costantino et al., 1994; Nilsson and Olsson, 1997; Binns and Costantino, 1998; Meyer et al., 2000). Early studies suggested that the RolB and RolC proteins are glucosidases liberating auxin and cytokinins, respectively, from their bound forms (Estruch et al., 1991a,b). However, other investigations have not provided evidence to support this hypothesis, and argue that changes in the levels of free hormones observed in some rolC and rolB-transformed plants reflect non-specific effects of the oncogenes (Nilsson et al., 1993, 1996; Schmülling et al., 1993; Delbarre et al., 1994). More recently, Fais et al. (1996) proposed that RolC could act as a non-specific glucosidase that hydrolyzes the plant cell wall, thereby interfering with plant development via the release of oligosaccharides. To date, there have been no additional studies to elucidate the biochemical function of RolC. The molecular mass of the rolC gene product is 20.1 kDa (Slighsom et al., 1985). However, it is of note that known plant and bacterial glucosidases are high-molecular mass proteins (50–200 kDa, reviewed by Sinnott, 1990), and the molecular masses of these proteins are much greater than the molecular mass of RolC. These arguments led us to study β-D-glucosidase activity in the 1c non-transformed callus culture from P. ginseng and several 1c-derived rol-transformed cultures with different levels of rolC expression using p-nitrophenyl-β-D-glucopyranoside as a substrate. When compared with the activities of other carbohydrases, β-D-glucosidase activity was unexpectedly low in the 1c culture and all of the rolC-transgenic cultures. Instead, a 3–10-fold increase in the activities of the β- and α-β-galactosidases (Bulgakov et al., 2002b) and 1,3-β-D-glucanase (Kiselev et al., 2006) were detected in rolC-transformed cells compared to control cells. Gel-permeation experiments showed that there was no β-D-glucosidase activity in the fractions containing proteins with molecular masses of around 20 kDa (Bulgakov et al., 2002b). Therefore, if the rolC-encoded product is indeed a glucosidase, it would be expected that it is a quite specific enzyme, representing a new, as yet uncharacterized class of glucosidases.

In contrast, two breakthroughs have revealed important new insights into the mechanism of RolB action. RolB was shown to exhibit tyrosine phosphatase activity (Filippini et al., 1996) and to interact with 14–3–3 proteins (Moriuchi et al., 2004). In turn, these studies highlight an interesting parallel between plant and animal oncogenes, since both Tyr phosphatases and 14–3–3 proteins are known to play pivotal roles in mammalian onogenesis, and to participate in the complex processes of cell growth, differentiation and death (Clevenger, 2004; Dougherty and Morrison, 2004). The tyrosine phosphatase function for RolB needs to be examined further, since the CX5R motif of the pRiA4 RolB protein, which is a characteristic motif of the tyrosine phosphatase family, is not found in most other RolB proteins (Lemcke and Schmülling, 1998). However, there is evidence to support the involvement of the proposed tyrosine phosphatase function of RolB in secondary metabolism. Tyrosine phosphatase inhibitors have been shown to abolish the rolB-mediated stimulation of resveratrol (Kiselev et al., 2007) and anthraquinones (Shkryl et al., 2007) in transformed cells.

Nothing is known about the involvement of tyrosine phosphorylation processes and processes mediated by 14–3–3 proteins in plant secondary metabolism. Recent studies suggest that protein Tyr phosphorylation performs critical functions in plants, regulating the activity of MAP kinases, transcription factors and ROS signaling (Laloi et al., 2004). The existence of Tyr phosphatasas in plants was controversial until recently, when several members of the protein Tyr phosphatase family were characterized in Arabidopsis (Gupta and Luan, 2003). The inability of plant cells to produce high levels of resveratrol, and the low efficiency of standard biotechnological methods pose major problems to resveratrol production in vitro. The ability of rolB to stimulate the production of high levels of resveratrol overcomes this problem (Kiselev et al., 2007), and demonstrates the great potential of engineering plant Tyr phosphorylation-mediated pathways. This pathway may potentially be exploited to establish cell lines that are capable of producing particular groups of secondary metabolites at high yields.
3.2. Initial assessment of signal transduction

The plant defense hormones methyl jasmonate, salicylic acid and ethylene play important roles in the establishment of plant defense reactions (Zhao et al., 2005). The involvement of the oxidative burst generated by NADPH oxidase during phytoalexin stimulation is well known (Zhao et al., 2005), although examples exist where the NADPH oxidase pathway does not participate in phytoalexin stimulation (Sasabe et al., 2000). Pharmacological experiments revealed that the rolC and rolB genes act on anthraquinone production independently of MeJA-responses of normal and rolC-expressing cells. These results indicate that the rolC gene acts as a powerful ROS mediator and calcium-dependent NADPH oxidase pathways (Bulgakov et al., 2003, 2004, 2005). This also appears to be the case for ethylene-and SA-mediated pathways, since ethylene had no effect on AQ production in either normal or rolC-transformed cells, while SA and the rolC gene had an additive stimulatory effect, suggesting that there are parallel signaling pathways that stimulate AQ production (Bulgakov et al., 2002a).

Cantharidin, an inhibitor of protein phosphatases 1 and 2A, is the only known inhibitor that has differential effects on secondary metabolism in normal and rolC- and rolB-transformed R. cordifolia cultures (Bulgakov et al., 2002a, 2003), as well as in normal and rolC-transformed root cultures from E. sericeum (Bulgakov et al., 2005). A significant body of evidence indicates that a protein kinase/phosphatase cascade is part of the signal transduction pathway between the molecular recognition of elicitors and activation of plant defense reactions (Shirasu et al., 1997; Zhao et al., 2005). The fact that a specific protein phosphatase inhibitor can stimulate a response means that a protein phosphatase plays an important role in the stimulus-response system. Since cantharidin strongly activates secondary metabolism in rolC and rolB-transformed cells, but not in corresponding control cells (Bulgakov et al., 2002a, 2005), it may be proposed that oncogene expression is essential in order to stimulate the expression of a cantharidin-sensitive subset of Ser/Thr phosphatases in transgenic cultures. These phosphatases probably mediate the stimulatory effect of the oncogenes on phytoalexin production. In any case, there is a significant involvement of protein phosphorylation/dephosphorylation processes in the signal transduction pathways initiated by rolC and rolB. Studying these processes by direct genetic and biochemical methods is necessary in order to unravel the activator function of the rol genes.

3.3. A role for ROS in rolC-mediated effects

The production of reactive oxygen species (ROS) plays a key role in plant defense reactions (Hammond-Kosack and Jones, 1996). Experiments aimed at understanding the relationship between secondary metabolism activation and the increased production of reactive oxygen species were performed with R. cordifolia cells. Single-cell assays based on confocal microscopy and fluorogenic dyes showed reduced steady-state levels of ROS in rolC-expressing madder cells when compared to normal cells. ROS inducers caused significant ROS elevation in normal cells, but had little effect on rolC-transformed cells. These results indicate that the rolC gene acts as a powerful ROS suppressor. In addition, 2- to 3-fold increases in the tolerance of rolC-transformed cells to salt, heat and cold treatments were observed. These data indicate that the oncogene provoked multiple effects, and ROS production and phytoalexin production can clearly be dissociated (Bulgakov et al., submitted for publication-a).

After eliciting plant responses, amplification of ROS production is dependent on Ca^{2+} influx (Torres et al., 2006; Lecourieux et al., 2006). Data from our group indicate that there is a striking difference in the responses of normal and rolC-transformed R. cordifolia cells to H_{2}O_{2}-induced [Ca^{2+}]_{cyt} elevations. In rolC-transformed cells, an almost complete blockade of H_{2}O_{2}-induced [Ca^{2+}]_{cyt} elevations was detected, and there was a positive correlation between the levels of ROS and H_{2}O_{2}-induced [Ca^{2+}]_{cyt} fluxes (V.P. Bulgakov, D.L. Aminin, T.Y. Gorpenchenko, Y.N. Shkryl; unpublished observation). If ROS and [Ca^{2+}]_{cyt} signaling is impaired in rolC-transformed cells, it is possible that the oncogene bypasses the upstream plant control mechanisms regulated by these important signaling molecules.

3.4. Modulation of the expression of calcium-dependent protein kinase genes

Constitutive expression of rolC in cultured plant cells leads to altered hormone (auxin) sensitivity (Maurel et al., 1991), somatic-to-embryo transition (Gorpenchenko et al., 2006) and calcium imbalance (Bulgakov et al., 2003), and activates the production of PR-2 proteins (Kiselev et al., 2006) and secondary metabolites (Section 2). The complex picture that has emerged from rolC studies raises the question of how rolC can affect such diverse processes. In recent years, several publications have demonstrated that Ca^{2+}/calmodulin-dependent protein kinases and calcium-dependent protein kinases (CDPK) have important regulatory roles during plant–rhizobium interaction (Levy et al., 2004; Harper and Harmon, 2005; Tirschke et al., 2006; Gargantini et al., 2006). As A. rhizogenes is related to rhizobia microorganisms, it is reasonable to hypothesize that A. rhizogenes could develop a similar strategy to affect signaling components of host plant cells. Since CDPKs are commonly accepted as molecules that mediate cross-talk between signaling pathways (Cheng et al., 2002; Ludwig et al., 2004; Harper and Harmon, 2005), modulation of their expression and/or activity could explain the numerous unrelated and largely unexplained effects that have been observed in rolC-transformed cultures. Indeed, it was recently shown that the expression of rolC in cultured cells of P. ginseng, V. amurensis and Eritrichium sericeum resulted in changes in the expression of different CDPK genes and provoked the generation of new transcripts with modified sequences corresponding to catalytic Ser/Thr kinase subdomains (Kiselev et al., 2008; Bulgakov et al., submitted for publication-b). The function of each CDPK gene whose expression was modified in rolC-expressing cell cultures requires further examination. The observation that rolC expression induces the appearance of truncated or long CDPK transcripts (PgCdPK1a-s, VcCdk1a-s, VcCdPK1-L, and EsCdPK1-s) in P. ginseng, V. amurensis and E. sericeum cells is particularly interesting. It is likely that rolC affects multiple biochemical processes in transformed cells by changing the expression of various CDPK genes and by generating new CDPK transcripts.

4. Long-lasting effects in rolC-transformed cells

There is evidence to indicate that the processes of growth, differentiation and secondary metabolism in rolC-transformed cells can be changed over prolonged periods of cultivation. For example, five independently transformed primary rolC-tumors from P. ginseng were sub-cultured over a long period to study their root-forming capability (Gorpenchenko et al., 2006). Roots appeared in these cultures after 3–15-months of cultivation. After 1.5–5 years in culture, all of the independently established primary tumor cultures ceased to form roots, and instead began to form somatic embryos and shoot primordia (Gorpenchenko et al., 2006).

Growth of rolC-transformed cell cultures can also be changed during long-term cultivation. A study performed with 7 year old calli of R. cordifolia indicated that the rolC-transformed callus phenotypes were conserved, and that there was stable expression of rolC and high AQ levels during long-term cultivation (Shkryl et al., 2007). However, a contrast difference was revealed in the growth parameters. During long-term cultivation, the growth of the rolC-transformed calli progressively increased, whereas the growth of the control calli remained stable (Shkryl et al., 2007). The highest growth rate was observed in a low-rolC-expressing callus line. A similar observation was also made for low-rolC-expressing P. ginseng calli (2c2 line), which were characterized by a remarkably stable and vigorous pattern of growth over a long period of time (over 10 years) without any selection (Gorpenchenko et al., 2006).
An unexpected result has been reported for rolC-transformed cell cultures from *Etritrichium sericeum* and *Lithospermum erythrorhizon*, where a clear inhibitory effect on secondary metabolism was observed (Bulgakov et al., 2005). Callus cultures from these plants contain large amounts of caffeic acid metabolites rubidosin and rosmarinic acid, which possess profound anti-nephritic activity (Fedoreyev et al., 2005; Inyushkina et al., 2007). The rolC-transformed callus cultures from both plants yielded 2–3-fold less caffeic acid metabolites than the respective control cultures. Conclusions regarding the inhibitory action of the rolC gene on secondary metabolism were made based on chemical analyses performed over a 2 year period of observation (Bulgakov et al., 2005). However, further investigations performed over the next 2–3 years revealed a progressive increase in the production of rubidosin and rosmarinic acid in *E. sericeum* rolC-transformed cells (Inyushkina et al., manuscript in preparation). A similar effect was observed in rolC-transformed roots of *P. ginseng*, where a 6 month lag phase preceded the activation of ginsenoside production (V.P. Bulgakov, unpublished observation).

It is evident that after integration of the rolC gene into the plant genome, the competence for growth, differentiation and secondary metabolism changed in a time-dependent manner, indicating that dynamic processes occurred after transformation. These effects is difficult to unravel, largely because it is unclear to what extent the oncogene rearranges the genetic apparatus of cells, and also to what extent somatic mutations occur in the cells independently of the oncogene. The study of such properties of rol-induced tumors may have a broad biological significance, and be analogous to recent studies that have dealt with the process of somatic mutation in human cancer genomes. In human cancers, some mutations are fortuitous (so-called ‘passenger’ mutations that do not contribute to oncogenesis), but a substantial portion of the mutations are ‘drivers’ that confer a growth advantage of the cell in which they occur (Greenman et al., 2007). Intriguingly, many of the driver mutations occur within the kinase domains of different protein kinase genes (Greenman et al., 2007). Modifications of the kinase domains of CDPK genes in rolC-transformed cells discussed in Section 3.4 are interesting in this respect.

5. Uncommon signal transduction pathways

The possibility that the rolB and rolC genes might function through mechanisms alternative to conventional plant mechanisms is intriguing. Pharmacological experiments suggest that the rolC gene acts on phytoalexin production independently of plant defense hormones and the calcium-dependent NADPH oxidase pathway (Section 3.2). Likewise, the production of AQs in rolC-transformed cells from *R. Cordifolia* is not dependent on oxidative burst (Section 3.3). It is possible that situations occur where both rolC and rolB perform their own critical functions to regulate secondary metabolism by bypassing upstream plant control mechanisms and directing defense reactions via a “short cut”. Considering the natural history of *A. Rhizogenes*-plant interactions, when ancestral T-DNA genes participated in ancient infection processes and played a role in the early events of plant species differentiation (Aoki and Syöño, 1999; Intrieri and Buatti, 2001), one can speculate that rolC influences primitive plant regulatory networks, and does not affect the more recent flexible instruments developed by plants through ROS and defense hormone signaling.

6. Similarities between some functions of plant and animal oncogenes

Protooncogenes were originally discovered as the cellular cognates of retroviral growth-promoting transforming genes, and have been highly conserved during evolution. This indicates that their protein products have an essential function in the basic regulation of cellular metabolism. Plants contain several animal protooncogene homologues, such as c-myb, c-myc, c-fos, c-jun and ras (Loidl and Loidl, 1996). Some of the protooncogenes are involved in the regulation of secondary metabolism, and perform functions that do not exist in animals (Loidl and Loidl, 1996). After the demonstration that Agrobacterium can transform HeLa cells (Kunik et al., 2001), it was hypothesized that Agrobacterium may be capable of transforming any eukaryotic organism (Lacroix et al., 2006). Recently, an agrobacterial transformation of sea urchin embryos was performed, and the rolC and rolB oncogenes have been shown to promote the formation of teratoma-like structures in animal embryos (Bulgakov et al., 2006). The ability of plant oncogenes to function in animals, and the ability of some animal protooncogenes (such as members of the Bcl-2 family) to function in plants (Kawai-Yamada et al., 2004) suggests that the processes of oncogenesis in plant and animals may share some common features. New data showing functional similarities between animal protooncogenes and rol genes has emerged in recent years. The ROS suppressor function of RolC discussed above is similar to the function that has been demonstrated for NF-κB (Sakon et al., 2003). There is also some functional analogy for the rolC gene with the animal protooncogene Bcl-2, since the expression of this gene in transgenic mice correlated with the inhibition of H2O2-induced intracellular Ca2+ fluxes (Zornig et al., 1995). The ROS-suppressing function of RolC requires further careful examination to evaluate whether or not RolC has a function similar to those animal protooncoproteins (such as NF-κB) that repress apoptosis by ROS suppression, thereby ensuring tumor survival.

7. Perspectives

It has become apparent that the rol genes may be interesting candidates for biotechnological applications. The rolC gene has emerged as a stimulator of growth and secondary metabolism. The rolB gene appears to be the most powerful activator, but its practical use is presently questionable due to its growth-suppressing effect. The rolC gene has been the most studied gene in this respect, and the use of this gene for the activation of secondary metabolism looks promising. Stimulation of secondary metabolism mediated by the rol genes is remarkably stable over long-term cultivation. Further investigation of the activator functions of these genes is highly dependent upon investigation of their general biochemical functions. Such investigation would establish interesting parallels between the defense responses of plant cells and events related to cell differentiation that are triggered by a single rol gene.

It is evident that *A. Rhizogenes* can manipulate defense pathways in transformed plant cells via expression of its T-DNA oncogenes. Are these alterations in secondary metabolism beneficial for *A. Rhizogenes* or for plants? In other words, have the bacteria evolved to cause plant defense reactions (to inhibit competitive microorganisms), or have plants developed the defense mechanisms (a specific type of systemic acquired resistance) in response to the pathogen invasion? Both options are possible. Further investigation of the rol genes may help us to understand why closely related microorganisms such as *A. Rhizogenes*, *A. tumefaciens* and Rhizobium species have evolved to develop such different interactions with plants as pathogenic or symbiotic.

The emerging complexity of the effects of the rolC and rolB genes and the involvement of signals generated by these genes in basic aspects of cell biology, such as calcium and ROS signaling, indicate that the plant oncogenes, like some animal protooncogenes, use sophisticated strategies to affect cell growth and differentiation. These correlations raise the intriguing possibility that some components of plant and animal oncogene signaling pathways may share common features. In this respect, the study of processes that represent “hot spots” in animal cell biology, such as the interplay between oncogene expression, ROS production and cell senescence/apoptosis, calcium signaling and processes of protein phosphorylation/dephosphorylation may shed light upon the functions of plant oncogenes. This review highlights the necessity of using different “omics” methods in order to provide new insights into how plant oncogenes affect different cellular functions. Analysis of the protein interaction networks in plant cells transformed...
with the rol genes will be a valuable tool for a molecular understanding of the functional specificities, regulatory components and pathways mediated by these genes.

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References


