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Plant Tumorigenesis: Different Ways for Shifting Systemic Control of Plant Cell Division and Differentiation

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ABSTRACT

Investigation of plant tumorigenesis is a way to clarify the mechanisms of systemic control of plant cell division and differentiation. Two spacious groups of plant tumors are exist. The first are tumors which are induced by different pathogens. Most known tumorigenic plant pathogens are the representatives of the *Agrobacterium* genus which use a specific way to genetically colonize and insert a plasmid DNA fragment (T-DNA) into the host genome. The second group includes spontaneous tumors that form on plants with certain genotypes: interspecific hybrids in some genera (most known of them are *Nicotiana* interspecific hybrids), inbred lines of some cross-pollinating species (e.g. tumorous lines of radish *Raphanus sativus* var. *radicula* Pers.), tumorous mutants and transgenic plants (e.g. *pas* and *tsd* mutants of *Arabidopsis thaliana*, *CHRK1*-supressed transgenic *Nicotiana tabacum* plants). The cause for all types of plant tumorigenesis is deviations in the metabolism and/or signaling of two main groups of phytohormones – cytokinins and auxins. These hormones take part in the control of the plant cell cycle via regulation of cyclins and cyclin-dependent kinase gene expression. In this review we examine different ways in which the cytokinin/auxin balance shifts for some types of plant tumors.

Keywords: inbred lines, interspecific hybrids, oncogenes, transgenic plants, tumor-producing mutants
Abbreviations: IAA, indole-3-acetic acid, **QC**, quiescent centre; **RAM**, root apical meristem; **SAM**, shoot apical meristem

CONTENTS

INTRODUCTION: SYSTEMIC CONTROL OF PLANT CELL DIVISION

Proper control of cell division and differentiation is necessary for the development of all multicellular organisms. As active proliferation is the natural character of stem cells and cell division rate dramatically decreases in specialized cell types, cell division and differentiation are often considered as two opposite processes (Jakoby and Schnittger 2004). The re-activation of cell proliferation, e.g. during the initiation of neoplastic growth in differentiated tissues includes de-differentiation of specialized cells which might consider as the return of them to stem cell state. The mechanisms of this process are not fully understood for today (Jakoby and Scnittger 2004). Many regulatory systems supporting the homeostasis between cell division and differentiation have been worked out during eukaryotes evolution. Tumor formation is a result of the exit of some cell or cell population from this systemic control resulting in uncontrolled cell proliferation. Disturbance in the work of any component of cell division control may cause tumor formation. One important peculiarity of plant development is post-embryonic organogenesis that occurs over the entire life cycle and relies on the existence of stem cell pools in the meristems (Ramirez-Parra *et al*. 2005). A second character specific for plant development is high regeneration potential that is based on the ability of differentiated cells to dedifferentiate and proliferate. Finally, complex regulatory pathways that control plant development and regeneration depend on the concerted action of plant hormones (Ramirez-Parra *et al*. 2005). Thus, plant cell proliferation seems to be under several levels of control. A first level includes mechanisms involved in the direct control of the cell cycle that are highly conserved among all eukaryotes (**Fig. 1**). Mutation in any key component of the cell cycle machinery may lead to defects in cell cycle progression across the whole plant body – arrest of

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Invited Review

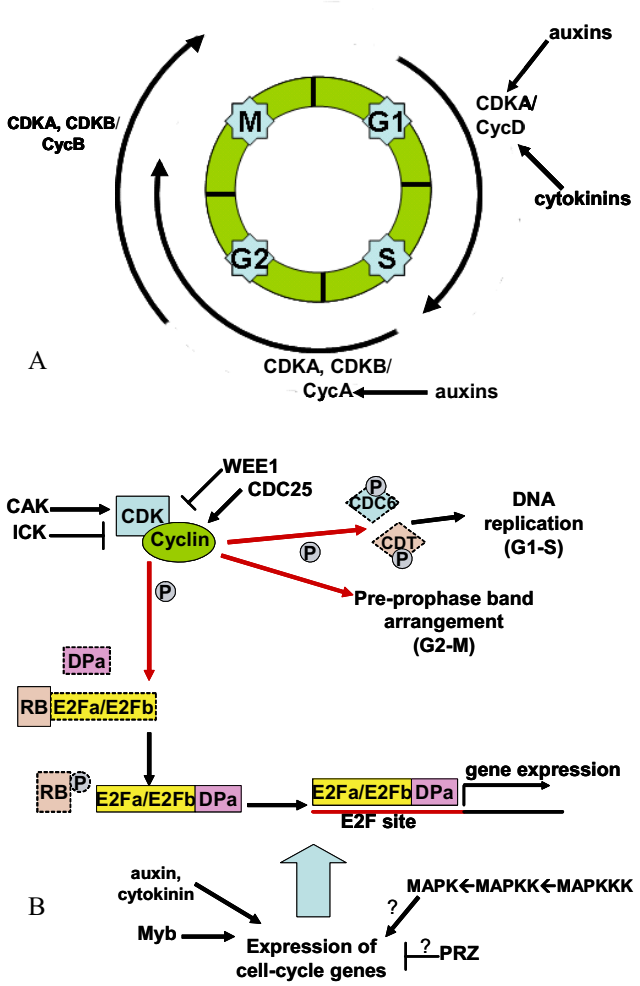

Fig. 1 Plant cell cycle regulation. (**A**) Regulation of cell cycle phases by different *CDK*s and cyclins. *CDKA-CycD* complex regulates G1-S transition, its activity is under regulation of mitogenic signals especially cytokinins; *CycA* forms complex with *CDKA* or *CDKB* and regulates G2-M transition; *CycB* regulates G2-M transition and proper courcе of mitosis interacting with *CDKA* or *CDKB*; (**B**) Interactions between the plant cell cycle regulators. *CDK*-cyclin complexes regulate three major processes in the control of cell cycle. The phosphorylation of *RB* protein causes the dissociation of *RB-E2F* complexes, *E2F* transcriptional factors form the dimers with *DP* proteins and regulate the expression of certain genes. The phosphorilation of proteins of pre-replication complex by *CDK*-cyclin is a key moment in the initiation of replication. Phosphorilation by *CDK*-cyclin causes pre-profase band arrangement that is necessary for G2-M progression. The activity of *CDK*-cyclin complexes is under regulation of *WEE1* kinase and *CDC25* phosphatase and also *CAK* and *ICK* proteins. The expression of cell-cycle genes is under control of phytohormones, *MAP*-kinase cascade and *Myb* transcription factors.

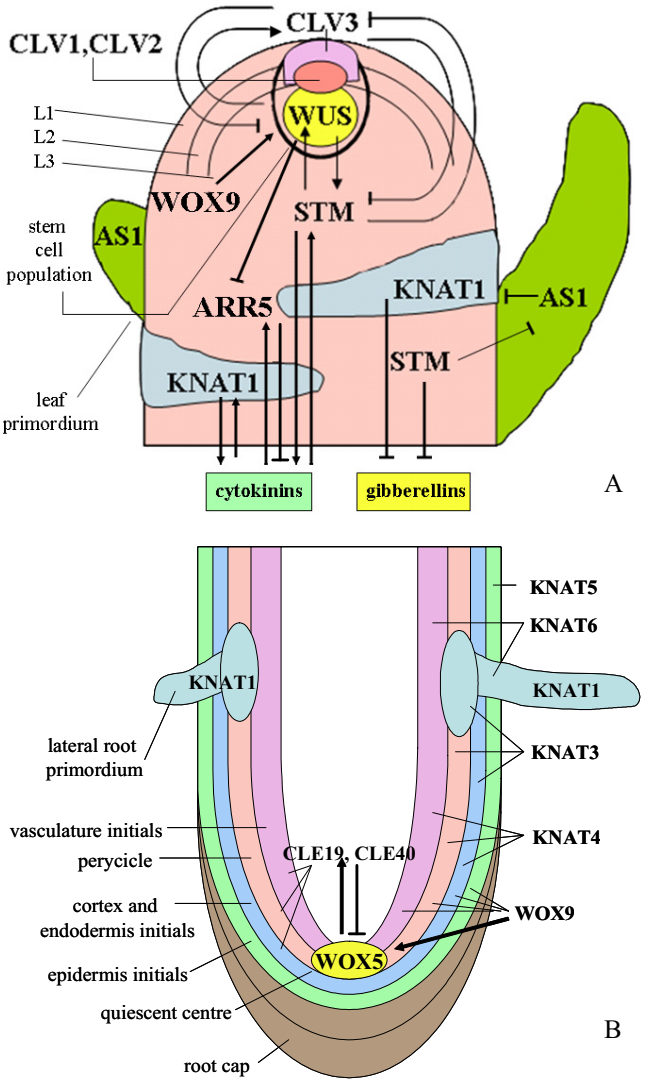

Fig. 2 Plant meristem regulation. (**A**) Shoot apical meristem. SAM consists of three distinct cell layers, L1, L2 and L3, each of them has a certain cell division plane and gives rise to different tissues. *STM* expresses specifically in the SAM and is essential for SAM maintenance. The expression of *Arabidopsis KNAT1* occurs in the base of leaf primordia. The *WUS* gene expresses in a small group of cells in the centre of SAM and is essential for SAM stem cell identity. *WUS* induces the expression of *CLV3* (*CLAVATA3*) secreted protein which acts as a ligand for *LRR*-receptor kinase comprised of *CLV1* and *CLV2* proteins. The *CLV* signaling pathway restricts *WUS* expression. Several regulator proteins such as *AS1* repress the expression of *KNOX* genes in leaf primordia. *WOX9* gene expression positively influence on *WUS* activity. Cytokinins positively regulate the expression of *KNOX* (e.g. *STM* and *KNAT1*) genes, the expression of these genes positively influence on the level of cytokinin and negatively regulate the biosynthesis of gibberellins. *WUS* protein also activates cytokinin signalling through the repression of *ARR5*, the repressor of cytokinin response. (**B**) Root apical meristem. RAM includes several cell layers which consist of different tissues initials. A small slowly dividing stem cell population, the quiescent centre (QC) in the central region of the RAM gives rise to initials of specialized cell types. The expression of *KNAT1*, *KNAT3*, *KNAT4*, *KNAT5* and *KNAT6* genes in the root show a cell type specific pattern. *KNAT1* express in the base of lateral root primordia supporting the role in their formation. *KNAT6* functions as the negative regulator of lateral root formation. *WOX5* gene expresses in the QC and plays a major role in the control of RAM stem cell identity. *WOX9* is suggested to influence on the *WOX5* expression. *CLE19* and *CLE40* genes homologous to *CLV3* also express in the root and positively regulate the RAM development.

mitosis, delayed or accelerated cell proliferation (de Jager *et al*. 2005). At the second level the control of meristem function takes place (**Fig. 2**). Mutations in meristem regulatory genes may lead to the development of reduced or enlarged meristems, ectopic meristem formation or disturbance between cell proliferation and organogenesis in the meristems (Reiser *et al*. 2000). Phytohormones are regulatory substances which are essential for control of many processes in plant development including the regulation of cell division and differentiation. Many experimental data showed that phytohormones, especially cytokinins and auxins, act in the control of both cell cycle progression and meristem function. Thus, the third level of plant cells division control is the phytohormonal regulation of this process (**Figs. 1, 2**).

Plant cell cycle regulation

Numerous cell cycle regulators are required for the control of cell cycle transitions as well as in coordination between cell proliferation and differentiation (Ramirez-Parra *et al*. 2005). The elements of cell cycle machinery are conserved among eukaryotes. Moreover, the mechanisms controlling the cell cycle in plants have more similarity with animal cell cycle control than with the same components in yeast

(Dewitte *et al*. 2003). Cell cycle transitions are under the control of cyclin-dependent kinases (*CDK*) and their catalytic subunits named cyclins. *CDK*/cyclin complexes in plant cells seem to participate in at least three major cell cycle control points. One such *CDK*/cyclin function is the phosphorylation of *RB* (retinoblastoma) protein resulting in dissociation of complexes which are formed by the *RB* protein together with *E2F* transcription factors (Ramirez-Parra *et al*. 2005). Another pathway which involves CDK/cyclin activity is the initiation of DNA replication during the G1-S transition. CDK/cyclin complexes perform the phosphorylation of members of pre-replication complex such as *CDC6* and *CDT* proteins and so target them for proteasome-mediated proteolysis (Castellano *et al*. 2004). Thirdly, *CDK*/cyclin function is a control of pre-prophase band arrangement that is necessary for G2-M progression (Weingartner *et al*. 2001). Several *CDK* types were characterized in plants (Vandepoele *et al*. 2002). The main plant *CDK* is *CDKA* which is homologous to *Sacharomyces cerevisiae cdc2* kinase and controls all stages of the plant cell cycle (Vandepoele *et al*. 2002). In addition, the plant-specific *CDK–CDKB* takes part in the control of the G2-M transition (Sorrel *et al*. 2001). *CDKD* and *CDKF* function as *CDK*-activating kinases (*CAK*) (Yamaguchi *et al*. 2003). A large family of cyclins has been identified in plants. Plant cyclins which regulate the kinase activity of *CDK* at different stages of cell cycle are divided into six classes (Wang *et al*. 2004). Some plant cyclins are conserved between plants and animals while other cyclin groups are specific to plants. The functions of many cyclin classes in plant cells differ from their functions in animals (Wang *et al*. 2004). The main function of *D*-class cyclins in plants is a control of the G1-S transition (Dewitte *et al*. 2003). *A*-class cyclins in plant cells take part in the control of both G1-S and G2-M transitions: *Arabidopsis CYCA3;2* seems to be a functional homologue of animal cyclin E, a main regulator of the G1-S transition which is lacking in plants (Yu *et al*. 2003). *B*-class cyclins are essential for the G2-M transition and for the proper mitosis course (Weingartner *et al*. 2004). Transcriptional regulation of cyclins and *CDK* genes as well as post-translational modification of *CDK*/cyclin complexes that affects their stability and activity is essential for cell cycle control (Ramirez-Parra *et al*. 2005). Transcriptional regulation of genes encoding components of the cell cycle machinery may depend on several signal path-ways. Many cell-cycle genes, such as *CDKB1* and *CycD3* have *E2F*-binding sites in their promoters (de Jager *et al*. 2001). On the other hand, there is data that suggests the participation of *Myb* family transcription factors (Araki *et al*. 2004) and *MAP*-kinase cascade (Krysan *et al*. 2002) in the transcriptional regulation of several cell cycle genes. Finally, the phytohormonal regulation of transcription was shown for several cell-cycle genes (Riou-Khamlichi *et al*. 1999; Roudier *et al*. 2003). The most studied mechanisms for post-translational regulation of *CDK*/cyclin complexes involve protein phosphorylation and protein-protein interactions. *CDK*/cyclin complexes are reversibly inactivated via the phosphorylation of *CDK* by *WEE1* kinase (Sorrell *et al*. 2002). *CDC25* phosphatase causes dephosphorylation and activation of *CDK* (Landrieu *et al*. 2004). Another way for modulation of *CDK*/cyclin complexes activity in plant cells is the interaction with *ICK* (*I*nhibitors of *CDK*) and *CKS* (*CDK S*ubunits) proteins (Vandepoele *et al*. 2002). Single *RB* protein is the most conserved part of the cell cycle controlling pathway (Huntley *et al*. 1998; Ebel *et al*. 2004). Dissociation of *RB-E2F* complexes releases *E2F* transcription factors which form dimers with *DP* (*D*imerization *P*artners) proteins for interacting with a specific motif in the gene promoters (de Jager *et al*. 2001). *E2F*-sites were detected in the promoters of many plant genes controlling DNA replication and chromatin dynamics, as well as in the promoters of genes which take part in the regulation of different metabolic processes and signal transduction pathways (Ramirez-Parra *et al*. 2003; Vandepoele *et al*. 2005). Thus, a large group of proteins which the control plant cell cycle at different stages were identified. In addition, many unidentified factors can influence the plant cell cycle. However, a cyclic expression pattern which is peculiar for cell-cycle genes was detected for more than 500 genes in tobacco synchronized cell culture (Menges *et al*. 2003). In animals, deregulation of the components involved in the cell cycle control triggers ectopic cell proliferation leading to tumorigenesis. Similarly, several genes working in the control of the cell cycle and meristem maintenance were reported as plant oncogenes. For example, overexpression of the *CycD3* gene under the constitutive promoter *35S* of *Cauliflower mosaic virus* (*CaMV 35S*) in transgenic *Arabidopsis* plants led to the production of rapidly proliferating tumor-like calli from leaf explants on hormone-free medium. As the *Agrobacterium tumefaciens*-induced crown gall tumor (Escobar and Dandekar 2005) these calli failed to regenerate shoots (Riou-Khamlichi *et al*. 1999). Overexpression of the *CycD2* gene leads to an increased cell division rate in meristems (Cockroft *et al*. 2000). Expression of the tobacco *CycA3;2* gene in transgenic *Arabidopsis* plants induced a high ability to form callus and lacked tissue differentiation from the callus (Yu *et al*. 2003).

Regulation of the plant meristem

The second level of plant cell division control is based on the specific plant body organization. During post-embryonic development of plants most of the mitotic activity is restricted to specific meristematic zones which are a source for different organ primordia. Shoot and root apical meristems (SAMs and RAMs) and also lateral and intercalary meristems exist in flowering plants (Scofield and Murray 2006). The plant meristem includes a pool of active proliferating stem cells in the central region being replaced by differentiating cells which form organ primordia at the periphery. Proper function of the meristem involves the coordination between division and differentiation of cells. The disturbance of this balance can lead to hyperproliferation of undifferentiated cells and tumor formation. The SAM and RAM play a crucial role in plant development. Numerous genes controlling cell proliferation and organogenesis in the SAM and RAM have been identified. Despite the existence of common ways in SAM and RAM regulation, both apical meristems strongly differ in their regulator sets. Homeobox-containing genes play the main role in the control of SAM and RAM development. Homeobox genes encode homeodomain transcriptional regulators that are involved in pattern formation in all multicellular organisms. Several homeobox gene subfamilies such as *KNOX*, *WOX*, *BELL* and *HD-ZIP* play a major role in the development of higher plants (Reiser *et al*. 2000; Scofield and Murray 2006). The essential function of homeobox genes in the meristems was shown for different plant species and studied in detail in SAM and RAM regulation (for review see: Veit 2004; de Jager *et al*. 2005; Scofield and Murray 2006).

The SAM consists of three distinct cell layers, L1, L2 and L3, each of them has a certain cell division plane and gives rise to different tissues. The central zone (CZ) of the SAM represents the population of slowly dividing stem cells, the peripheral zone (PZ) surrounding the CZ consists of fast dividing cells which form lateral organs (leaves or flowers depending on the developmental stage), or primordia (for review see: Aida and Tasaka 2006). Class 1 and class 2 *KNOX* homeobox genes play a crucial role in SAM development and maintenance in dicots and monocots (Reiser *et al*. 2000). So, the *STM* (*Shoot Meristemless*) gene of *Arabidopsis thaliana* (Long *et al*. 1996) and its ortologue *kn1* (*knotted1*) of maize (Jackson *et al*. 1994) are normally expressed only in the SAM and their functions are essential for initial shoot meristem formation and subsequent SAM maintenance. The ortologues of *STM* and *kn1* genes were found in tobacco, tomato, soybean, rice, barley (for review see: Reiser *et al*. 2000). All these genes demonstrate an expression pattern similar to *STM* with the exception of tomato *Tkn2/LeT6* that is also expressed in the compound

leaves (Janssen *et al*. 1998). Loss-of-function mutations in the *STM* and *kn1* genes lead to the absence or severe reduction of the SAM (Long *et al*. 1996; Vollbrecht *et al*. 2000), while their overexpression prolongs cell proliferation in leaf primodia (Jackson *et al*. 1994; Lenhard *et al*. 2002). Despite the predominant expression in the SAM, other class 1 *KNOX* genes, such as *Arabidopsis KNAT1*, have a less important influence on SAM formation. Within the SAM the expression of *Arabidopsis KNAT1* and its orthologues in other plant species is restricted to the base of leaf primordia (Lincoln *et al*. 1994; Schneeberger *et al*. 1995; Sato *et al*. 1999). *KNAT1* loss-of-function mutations do not seriously affect the SAM development, but overexpression of *KNAT1* in *Arabidopsis* and its orthologues in other plants leads to ectopic meristem formation resulting in a shoot-like outgrowth in the leaves and other organs (Hewelt *et al*. 2000; Frugis *et al*. 2001; Chiapetta *et al*. 2005). The class 2 *KNOX* genes have wider expression patterns in the meristems as well as in the differentiated tissues and are suggested to function not only in meristem control (Pautot *et al*. 2001; Dean *et al*. 2004; Truernit *et al*. 2006). The *WUS* gene encoding a *WOX*-family homeodomain protein expresses in a small group of SAM cells in CZ (Haeker *et al*. 2004). The function of *WUS* is essential for SAM stem cell identity (Gallois *et al*. 2004). *WUS* induces the expression of *CLV3* (*CLAVATA3*) secreted protein which acts as a ligand for *LRR*-receptor kinase comprised of *CLV1* and *CLV2* proteins. The *CLV* signaling pathway restricts *WUS* expression and thereby regulates stem cell homeostasis (Schoof *et al*. 2000). The exclusion of the "meristem" gene expression from developing organs is essential for normal tissue differentiation. Several regulator proteins repress the expression of *KNOX* genes in leaf primordia. Among them are *MYB*-domain transcriptional regulators such as *Arabidopsis AS1* (*Assimetric Leaves 1*; Byrne *et al*. 2002) and also its ortologues *PHAN* (*PHANTASTICA*) from *Antirrhinum majus* and *rs2* (*Rough sheath 2*) from maize (Tsiantis *et al*. 1999), cysteine-repeat protein *AS2* (*Assimetric Leaves 2*; Iwakawa *et al*. 2002), zinc-finger-type transcription factors *FIL* (*Filamentous flower*) and *YAB3* (*Yabbi 3*; Kumaran *et al*. 2002).

The RAM shows a less complex organization than the SAM. It also includes several cell layers which consist of initials of different tissues. A small group of slowly dividing stem cell population, the quiescent centre (QC) is disposed in the central region of the RAM and gives rise to initials of specialized cell types (Jiang and Feldman 2005). In contrast to SAM, lateral organ initiation in the root occurs aside from the RAM, in the upper fully differentiated zone and involves re-initiation of cell division in the pericycle cells (Jiang and Feldman 2005). The *KNOX* and *WOX* homeobox genes are involved in the control of root development. The expression of *KNAT1*, *KNAT3*, *KNAT4*, *KNAT5* and *KNAT6* genes was revealed in the *Arabidopsis* root and show a cell type specific pattern (Dean *et al*. 2004; Truernit *et al*. 2006). For example, the expression of *KNAT1* was observed in the base of lateral root primordia (Truernit *et al*. 2006) supporting the crucial role of this gene in the formation of lateral organs on both the shoot and root. Downregulation of *KNAT6* expression resulted in an increased number of lateral roots, suggesting its function as the negative regulator of lateral root formation (Dean *et al*. 2004). As well as in the SAM, *WOX* homebox genes are essential for the restriction of the stem cell population size in the RAM (Jiang and Feldman 2005). *WOX5* gene expresses in the cells of the QC in the *A. thaliana* RAM (Haecker *et al*. 2004). *WOX5* begins to express in the RAM at an embryonic stage and plays a major role in the control of RAM stem cell identity, analogous to *WUS* function in the SAM (Haecker *et al*. 2004). The homologue of *WOX5* had the same function identified in rice (Kamiya *et al*. 2003). Another *WOX* family gene of *A. thaliana*, *WOX9*, also named *STIP* (*STIMPI*), is suggested to play a role in the development of the SAM, RAM and lateral meristems: *stip* mutants demonstrate a dramatic reduction of the SAM and RAM and also the absence of lateral roots (Wu *et al*. 2005). The expression of *WUS* and *CLV* genes is abscent in the SAM of *stip* mutants, suggesting the function of *WOX9* in the regulation of *WUS* expression (Wu *et al*. 2005). The expression of genes homologous to *CLV3* was also revealed in the *Arabidopsis* root (Hobe *et al*. 2003). It was shown that overexpression of some of these genes, e.g. *CLE19* and *CLE40*, leads to a reduction of the RAM (Casamitjana-Martinez *et al*. 2003; Hobe *et al*. 2003), so that in the RAM *CLV3*-like genes carry out the same function as in the SAM. Also the activity of *WUS* and *CLV3* homologues was revealed in the RAM, but the existence of a *CLV/WUS*-like regulatory system was not found in the root (Jiang and Feldman 2005).

Thus, numerous genes involved in SAM and RAM regulation are known today. Deregulation of some of these genes expression leads to increase of existing meristems or ectopic meristem formation. However, loss- or gain-of-function mutations in neither of genes involved in the meristem regulation lead to the formation of disorganized tumors in plants. At the same time, the expression levels of some of *KNOX* genes were shown to increased in several types of spontaneous plant tumors, e.g. in the tumors of *Nicotiana* interspecific hybrids, *CHRK1*-downregulated *Nicotiana tabacum* transgenic plants and *A. thaliana* tumor-producing mutants (Franc *et al*. 2002; Harrar *et al*. 2003; Lee *et al*. 2004). Ectopic expression of *KNOX* genes in the tumors corresponds with ectopic zones of cell proliferation and might be a cause of formation of secondary SAMs leading to the development of teratomas (Lee *et al*. 2004).

Phytohormonal regulation of plant cell division

Phytohormones, especially auxins and cytokinins, play a crucial role in many aspects of plant development e.g. cell division and differentiation. The function of cytokinins as stimulators of cell proliferation was shown for several mutants and transgenic plants. Decreased cytokinin levels in transgenic tobacco plants that overexpressed cytokinin-oxidases resulted in a reduced rate of cell division that led to a reduced SAM and small leaves (Werner *et al*. 2001). The opposite phenotype was observed in the *Arabidopsis amp* mutant which displayed an increased zeatin-type cytokinin level (Helliwell *et al*. 2001) and transgenic tobacco plants carrying the *ipt* gene from T-DNA of *A. tumefaciens* (Medford *et al*. 1989). The expression of several genes encoding key regulators of the plant cell cycle is under phytohormonal control. Among them are *Arabidopsis CDKA;1* (Chung and Parrish 1995) and alfalfa *CycA2;2* (Roudier *et al*. 2003) genes whose expression is induced by auxin; the *Arabidopsis CycD3;1* gene is also up-regulated by the cytokinins zeatin and 6-benzylaminopurine (BAP) (1 μM) (Riou-Khamlichi *et al*. 1999). The expression of cell-cycle genes is altered in phytohormonal mutants. For example, *Arabidopsis prz1* (*proporz1*) mutant that forms rapidly proliferating calli in response to either auxin or cytokinin shows increased expression levels of *CDKB1;1* and *E2Fc* genes (Sieberer *et al*. 2003). The presumable role of *PRZ1* is to mediate a hormonal signal into a cell cycle control (Sieberer *et al*. 2003). Moreover, auxins and cytokinins are involved in the regulation of activity and stability of key cell cycle regulators. It was recently reported that auxin positively regulates the accumulation and stability of *E2Fb* transcription factor in *Arabidopsis* cells (Magyar *et al*. 2005). In addition, the cyclic expression patterns which are peculiar for cell-cycle genes were shown for several genes participating in the transcriptional response to cytokinin, auxin and ethylene (Menges *et al*. 2003) suggesting the role of these genes in the control of the plant cell cycle. The functions of phytohormones in the control of plant tissue differentiation and organogenesis partially depend on the hormonal control of meristem development. Two groups of phytohormones, cytokinins and gibberellins were shown to play antagonistic roles in the control of meristematic gene expression (Jasinski *et al*. 2005). Many experimental data

show that cytokinins increase the expression of *KNOX* genes and enlarge their expression zone (Rupp *et al*. 1999). At the same time, overexpression of *KNOX* genes leads to the accumulation of cytokinins and cytokinin-autotrophic tissue growth (Hewelt *et al*. 2000; Frugis *et al*. 2001; Hamant *et al*. 2002; Chiapetta *et al*. 2005; Yanai *et al*. 2005). On the other hand, *KNOX* gene expression represses the biosynthesis of gibberellic acid in the SAM (Hay *et al*. 2002). It was recently shown that the *WUS* gene, which acts as a positive regulator of stem cells, directly represses the transcription of several A-class *ARR* (*Arabidopsis Response Regulator*; Leibfried *et al*. 2005) genes that act in the negative feedback loop of cytokinin signaling (D'Agostino and Kieber 1999).

During the transition from normal to neoplastic growth, some cells or cell populations become independent of regulatory systems and begin uncoordinated proliferation. Logically, mutations in the genes acting at each level in cell division control may lead to uncontrolled cell proliferation and tumor formation. In fact, several examples of plant tumors show alterations in the work of many genes acting at different levels of plant cell division control (Wang *et al*. 2001; Frank *et al*. 2002; Harrar *et al*. 2003; Lee *et al*. 2004). However, plants seem to be strikingly resistant to unrestricted growth similar to mammalian hyperplasia or cancer (Ramirez-Parra *et al*. 2005). Only few examples of plant tumors are known and the role in the control of plant tumorigenesis was documented for only small range of genes. The reason for this may be the lethality of mutants with a loss-of-function in key regulators of plant cell division. So, the loss-of-function mutation in the gene encoding the *RB* protein in *Arabidopsis* results in restricted mitosis in the haploid nuclei of female hametophytes and in endosperm nuclei leading to a lethal phenotype (Ebel *et al*. 2004). Another reason for plant "resistance" to tumorigenesis is the high plasticity of the plant genome.

PLANT TUMORS CAUSED BY DIFFERENT PATHOGENS

Plant neoplastic diseases are caused by bacteria, such as species of the genus *Agrobacterium* (Escobar and Dandekar 2003) and several other pathogenic bacteria (Glickmann *et al*. 1998; Vandeputte *et al*. 2005; Chapulowicz *et al*. 2006), double-stranded RNA viruses (Moriyashi *et al*. 2005), the obligate parasitic protist *Plasmodiophora brassica* (Devos *et al*. 2005), fungi (Tavares *et al*. 2004; Fernando *et al*. 2005), nematodes (de Meutter *et al*. 2003) and gall-forming insects (Mapes and Davies 2001). Among tumor-inducing plant pathogens, gall-forming bacteria, especially *Agrobacterium*, are the most well studied. At the same time, the mechanisms of tumor formation caused by fungi, nematodes, insects and viruses are less understood.

Despite different pathogenic strategy of very heterogenous plant pathogens, most of them demonstrate the ability to shift phytohormonal balance in the host plants producing IAA and cytokinins (Jameson 2000).

PLANT TUMORS INDUCED BY PATHOGENIC BACTERIA

Several plant pathogenic bacteria can induce hyperplasia of host plant tissues during pathogenesis. The most well known of these bacteria are represantives of the genus *Agrobacterium* which induce formation of crown gall tumors with unlimited growth like *A. tumefaciens* (Escobar and Dandekar 2003) or multiple roots on the infection site like *A. rhizogenes* (Aoki and Syono 1999b), and also gall-inducing bacteria, such as *Pseudomonas savastanoi* (Glickmann *et al*. 1998), *Pantoea agglomerata* (Chapulowicz *et al*. 2006) and *Rhodococcus fascians* (Vandeputte *et al*. 2005), which cause the formation of gall tumors with limited growth. The common character of all tumor-inducing bacteria is the ability to synthesise cytokinin and auxin, which is determinated by plasmid or chromosomal genes (Morris 1986). Production of high amounts of IAA and cytokinins in the infected plants leads to uncontrolled cell proliferation and tumor formation (Morris 1986; Crespi *et al*. 1992; Clark *et al*. 1993; Lichter *et al*. 1995; Vandeputte *et al*. 2005). Genes controlling cytokinin and auxin biosynthesis in different tumor-inducing plant pathogenic bacteria were shown to have structural and functional similarity (Morris 1986; Glass and Kosuge 1988; Crespi *et al*. 1992; Clark *et al*. 1993; Lichter *et al*. 1995; Vandeputte *et al*. 2005). Since "phytohormonal" genes exist in distantly related plant pathogenic bacteria (Glickmann *et al*. 1998), common evolutional origin (Glickmann *et al*. 1998) or horizontal gene transfer (Bird and Koltai 2000) of these genes between different bacteria species are likely. *Agrobacterium* is an unical group of pathogenic bacteria which can induce a certain part of its virulent plasmid (which includes "phytohormonal" genes) into the host genome (Chilton 1977). Other tumor-inducing bacteria can not transform host plant cells (Clark *et al*. 1989).

Tumors induced by *Agrobacterium* are the most investigated examples of plant tumorigenesis. The genus *Agrobacterium* includes facultative parasitic soil bacteria which cause neoplastic tissue growth in the host plants. Two *Agrobacterium* species – *A. tumefaciens*, a causative agent of crown gall disease, and *A. rhizogenes* which causes hairy root syndrome – are the most well studied species of *Agrobacterium*. *A. tumefaciens* causes the formation of crown gall tumors on the base of stems of infected plants (**Fig. 3**). This is typical tumor tissue with rapid cell proliferation, hormone-independent growth and lacking regeneration ability (Ahuja 1998). Crown gall tumors are induced as an undifferentiated cell mass, while older tumors begin to differentiate some tissue types, especially phloema and xylema (Ullrich and Aloni 2000; Veselov *et al*. 2003). *A. rhizogenes* causes the formation of numerous adventitious roots at the infection site. These roots demonstrate rapid agravitropic growth and decreased hormone requirement in culture. Plants with specific phenotypic deviations can be easily regenerated from hairy roots (David *et al*. 1984). A unique peculiarity of *Agrobacterium* is its unusual strategy for bacteria pathogenesis consisting in the integration of certain DNA segment – transfer DNA or T-DNA – into the plant genome. The first evidence that the neoplastic tissue outgrowth during *Agrobacterium* pathogenesis is caused by the incorporation of a specific part of a virulent plasmid into plant chromosomes was presented by Chilton (1977).

All pathogenic *Agrobacterium* species contain a big (200-800 kbp in size for natural strains) virulent plasmid named *Ti* (*T*umor *i*nducing) in *A. tumefaciens* and *Ri* (*R*oot *i*nducing) in *A. rhizogenes*. In both *Ti* and *Ri* plasmids, T-DNA represents a 10-30 kbp fragment delimited by 25-bp direct repeats which are necessary for T-DNA processing. In some plasmids T-DNA is composed of TR (right) and TL (left) segments separated by the nontransferred region (Huffman *et al*. 1984). The processing of T-DNA from *Agrobacterium* plasmid and its subsequent export into the plant cell

Fig. 3 Tumor induced by *Agrobacterium tumefaciens C-58* strain on the stem of radish (*Raphanus sativus* var. Radicula Pers.).

are under the control of genes from the *Vir* (virulence) region. *Vir* regions of *Ti* and *Ri* plasmids are very similar and contain 8 virulence loci (*VirA-VirG*). In addition, several chromosome genes and specific "overdrive" sequences near the T-DNA right border also take part in the control of T-DNA transfer. The functions of *Vir* genes in the control of all stages of T-DNA transfer were studied in detail (for review see Escobar and Dandekar 2003; Gelvin 2006). It was shown that all cells of hairy roots carry a T-DNA insertion (David *et al*. 1984; Tepfer *et al*. 1984) while crown gall tumors consist of only transformed cells (Rezmer *et al*. 1999) or they may be chimeric (Azmi *et al*. 2001). Expression of T-DNA genes in the host plant cells causes the outgrowth of tissues and also the synthesis and secretion of opines and amino acid derivates, which are utilized by *Agrobacterium* as a carbon/nitrogen source. Natural *Agrobacterium* strain classification is based on the synthesis of certain opine types: so, *A*. *tumefaciens* strains and Ti-plasmid were classified into four types according to octopine, nopaline, agropine and succinamopine synthesis (Dessaux *et al*. 1986). Apart from opine synthesis genes, *Ti* and *Ri* T-DNA contain oncogenes which cause neoplastic plant tissue growth. The development of *A*. *tumefaciens*-induced crown gall tumor primarily depends on the production of high amounts of auxin and cytokinin by the enzymes encoded in several *Ti* T-DNA genes (Barry *et al*. 1984; Kemper *et al*. 1985). Moreover, the endogenous concentrations of some other phytohormones such as ethylene, abscisic acid and jasmonic acid change during crown gall development suggesting the role of these hormones in the control of tumorigenesis (Veselov *et al*. 2003). The cause of hairy root induction by *A*. *rhizogenes Ri* plasmid is less understood.

T-DNA of *Ti* plasmid carries several genes encoding enzymes which catalyze cytokinin and auxin bosynthesis. Deletion analysis of T-DNA indicated that *tmr*, *tms* and *tml* loci have a major role in the determination of crown gall tumorigenesis (Garfinkel *et al*. 1981). Of particular importance is the *tms* (tumor morphology shoot) locus containing *tms1* (*iaaM*) and *tms2* (*iaaH*) genes which encode, correspondingly, tryptophan monooxigenase and indolacetamide hydrolase, the enzymes for two crucial steps of indole-3-acetic acid synthesis (Kemper *et al*. 1985) and the *tmr* locus which contains the *ipt* gene encoding isopentenyl transferrase (*IPT*), a key enzyme of cytokinin biosynthesis (Barry *et al*. 1984). Additional gene responsible for bacterial zeatin secretion, *tzs* (*trans*-zeatin synthase), is carried only on nopaline *Ti*-plasmid (Akiyoshi *et al*. 1985). It is remarkable that plant *IPTs* were isolated from *A*. *thaliana* significantly later than bacterial *ipt* (Takei *et al*. 2001). *Agrobacterium IPT* has 33-45% similarity at the amino acid level with *Arabidopsis AtIPT* enzymes (Takei *et al*. 2001). Overexpression of the bacterial or plant *ipt* genes in the transgenic plants leads to cytokinin overproduction resulting in so-called cytokinin syndrome: dwarfism, reduced apical dominance, weakened root development, delayed leaf senescence, and spontaneous shoot regeneration from callus (Medford *et al*. 1989; Sun *et al*. 2003).

The direct functions of other *A*. *tumefaciens* T-DNA genes are still unknown. Expression of these genes in plant tissues does not lead to an increase of phytohormone levels (Delbarre *et al*. 1994). At the same time they also cause different morphogenic abnormalities connected with the disturbance in cell division and differentiation (Aoki and Syono 1999b; Gorpenchenko *et al*. 2005). Some *A*. *tumefaciens* T-DNA genes, together with most of *A*. *rhizogenes* T-DNA genes belong to a gene family named *plast* or *rolB-like* family which obviously has a common evolutionary origin (Levesque *et al*. 1988). The influence on tumor formation has been reported for *tml* (tumor morphology large) locus which contains a *6b* gene with unknown function. Based on sequence analysis, the *6b* gene belongs to the *plast* genes family (Levesque *et al*. 1988). Overexpression of the *6b* gene in transgenic tobacco plants leads to tumor formation, hormone-independent callus proliferation and specific graft-transmissible enation syndrome similar to the phenotypes of some plants with overexpressed *KNOX* genes (Helfer *et al*. 2003). Recently it was reported that the *6b* gene interacts with the novel nuclear protein, *NtSIP1* which presumably functions as a transcription factor in *N*. *tabacum* cells. The interaction of *6b* and *NtSIP1* proteins is necessary for determination of the phenotype of *6b*-transgenic plants (Kitakura *et al*. 2002). In addition, the *Ti* plasmid contains *5*, *6a*, *e* and *f* genes which also belong to the *plast* gene family (Levesque *et al*. 1988). At least some of these genes also take part in the establishment of crown gall tumor growth. For example, a mutation in the *e* gene leads to the formation of very small tumors on the infected plants (Broer *et al*. 1995). At the same time, no influence on crown gall development was revealed for the *f* gene (Broer *et al*. 1995). The product of gene *5* was shown to convert tryptophan into indole-3-lactate which might act as an auxin antagonist by competing with IAA for auxin-binding proteins (Hamill 1993).

Little is known about the functions of *A*. *rhizogenes* T-DNA genes. The *Ri* plasmids of mannopine-type strains contain a single T-DNA, however, the best studied agropine-type *Ri* plasmids carry two parts of T-DNA – TL and TR. *Ri* TL-DNA contains 18 open reading frames (Slightom *et al*. 1986). TR-DNA of agropine-type strains contains *aux1* and *aux2* genes homologous to *A*. *tumefaciens* and *P*. *savastanoi iaaM* and *iaaH* genes (Camilleri and Jouanin 1991). The influence on the hairy root phenotype and on the phenotype of transgenic plants was documented for 10 ORFs from *Ri* TL-DNA and also for *aux1* and *aux2* genes (Schmulling *et al*. 1988; Capone *et al*. 1989; Hansen *et al*. 1993; Lemcke and Schmulling 1998).

Several *rol* (rooting locus) genes named *rolA*, *rolB*, *rolC* and *rolD* (White *et al*. 1985) which are essential for the hairy root phenotype were identified during the deletion studies of *Ri* TL-DNA. The *rolB* gene seems to be most important for hairy root induction. The introduction of the *rolB* gene alone induced adventitious root formation in explants and whole transgenic tobacco plants (Schmulling *et al*. 1988; Capone *et al*. 1989; Aoki and Syono 1999b). On the other hand, overexpression of the *rolB* gene under the *35S CaMV* constitutive promoter led to suppression of rooting and necroses on transgenic *Nicotiana tabacum* plants (Schmulling *et al*. 1988) – and so, the proper level of *rolB* expression is necessary for hairy root formation. Despite the correlation between *rolB* expression and the cleavage of IAA conjugates (Estruch *et al*. 1991b) or IAA binding on the membranes (Filippini *et al*. 1994) the direct function of the *rolB* gene remains unknown. No proteins homologous to the *rolB* proteins are known except for those encoded by other genes from the *plast* family (Levesque *et al*. 1988). It was recently reported that the *rolB* protein interacts with *14-3-3* proteins in transgenic tobacco cells (Moriuchi *et al*. 2004). *14-3-3* is a family of proteins, which presumably function in the regulation of different signal pathways via protein-protein interactions. These proteins determine the nuclear localization of *rolB* protein and the *rolB*-associated phenotype (Moriuchi *et al*. 2004). However, no data on the interactions of *rolB* and *14-3-3* proteins with auxin metabolism or cell division control pathways are available.

Inoculation experiments revealed that the *rolC* gene stimulates (Aoki and Syono 1999b; Piispanen *et al*. 2003) or inhibits (Capone *et al*. 1989) *rolB*-induced adventitious root formation. Extracts from transgenic plants which overexpress *rolC* were shown to have cytokinin-glucosidase activity (Estruch *et al*. 1991a), but the *rolC* protein is not homologous with known cytokinin-glucosidases. Overexpression of the *rolC* gene in transgenic tobacco plants causes a specific phenotype: reduced internode length, pale green lanceolate leaves and very small flowers (Schmulling *et al*. 1988). Transformation of *Panax ginseng* callus cultures by the *rolC* gene under the *35S CaMV* promoter caused spontaneous somatic embryogenesis of calli in hormone-free medium (Gorpenchenko *et al*. 2005). No proteins interacting with the *rolC* protein are known to date.

Transgenic tobacco plants carrying the *rolA* gene had

shortened internodes and a decreased gibberellic acid content suggesting the role of this gene in the control of gibberellin metabolism. It was shown that the *rolA* protein has DNA-binding activity and demonstrates homology with the DNA-binding domain of papillomavirus transcription factor suggesting that this protein can act as a transcriptional regulator (Rigden and Carneiro 1999).

The *rolD* gene was revealed to encode a functional ornithin-cyclodeaminase – the enzyme which catalyzes NAD^+ dependent conversion of ornithine to proline. It was supposed that the *rolD* enzyme may have an influence on the synthesis of hydroxiprolin-rich proteins of cell walls which can in turn play a role in the growth and division of the cells. However, overexpression of the *rolD* gene in transgenic plants has no phenotypic effect (Bettini *et al*. 2003).

ORF3, *ORF8*, *ORF13* and *ORF14* genes from *Ri* T-DNA were also shown to promote *rolB*-induced root formation (Capone *et al*. 1989; Aoki and Syono 1999b). *ORF13* is the most conserved of *Ri* T-DNA genes (Brevet and Tempe 1988). Transformation of carrot root discs with the *ORF13* gene leads to the formation of dense green and rapidly proliferating callus on the disc surface (Capone *et al*. 1989; Frundt *et al*. 1998). Transgenic tobacco plants, which have the *ORF13* gene expressed under the constitutive *35S CaMV* promoter demonstrated a specific phenotype similar to that of cytokinin-treated plants and *ipt*-transformants of tobacco but did not differ from the wild-type in cytokinin content in the tissues (Lemcke and Shmulling 1998). These plants were short, with an increased level of cell division in the SAM, a reduced apical dominance and dark-green abnormally shaped leaves. The observation that this phenotype can be transmitted by grafting of transgenic shoots onto wild type plants is of particular interest and suggests that *ORF13* may cause the production of a diffusible factor with cytokinin-like activity (Hansen *et al*. 1993). An increased level of expression of class1 and class2 *KNOX* genes in *ORF13*-transgenic tomato plants can partially explain the *ORF13*-associated phenotype (Stieger *et al*. 2004). It has been recently revealed that *ORF13* protein contains a conservative *LxCxE* motif for binding with the *RB* protein and interacts with *Zea mays RB* protein *in vitro* (Stieger *et al*. 2004). Thus *ORF13* protein may influence the plant cell cycle via interaction with its crucial component. Functional *RB*-binding motif is essential for the acceleration of cell divisions in the SAM of transgenic plants, but the influence of *ORF13* on *KNOX* gene expression was not dependent on *RB*-binding motif (Stieger *et al*. 2004). It is interesting that geminiviruses, small DNA viruses whose replicative cycle relies on the host cell factors, also can promote the G1-S transition and cell cycle activation by the interaction with the *RB* protein. Some of these viruses cause hyper-proliferation of plant cells (Gutierrez 2000).

The *ORF3* gene causes late flowering, altered leaf shape and reduced internode length in transgenic tobacco plants (Lemcke and Schmulling 1998). The formation of callus from explants of *ORF3* transgenic plants on auxin- or cytokinin-containing media was significantly reduced. Thus, it was proposed that the function of *ORF3* suppress the dedifferentiation of tissues during hairy root development (Lemcke and Schmulling 1998). The protein encoded by the *ORF8* gene is a natural fusion protein consisting of the N-terminus homologous to *A. rhizogenes rolB* protein and the C-terminus homologous to the *A. tumefaciens iaaM* gene (Levesque *et al*. 1988). The tryptophan-monooxygenase activity was conserved in the C-terminal part of the *ORF8* protein (Lemcke *et al*. 2000) while the N-terminal part of this protein functions in carbohydrate metabolism (Otten, Helfer, 2001). Moreover, a physical connection between N- and C-regions of *ORF8* protein is nessesary for the appearance of a specific phenotype in *ORF8*-transgenic plants suggesting the distinct specific function of the whole protein (Umber *et al*. 2005). The *ORF14* gene stimulates *rolB*-induced root formation (Capone *et al*. 1989) but the transformation by the *ORF14* gene under *35S CaMV* or its own promoter did not cause any phenotypic alterations in transgenic plants (Lemcke and Schmulling 1998).

Apart from *Agrobacterium*, induction of neoplastic growth in host plants was also documented for several Gram-negative and Gram-positive pathogenic bacteria (Glickman *et al*. 1998; Vandeputte *et al*. 2005; Chalupowicz *et al*. 2006). These bacteria are known as gall-inducing bacteria because of the induction of smaller galls with limited growth during their pathogenesis in contrast with crown gall tumor with practically unlimited growth (Chalupovicz *et al*. 2006). However, tumors induced by *A. tumefaciens* and gall-forming bacterium *Pantoea agglomerata* are very similar in their anatomy (Chalupovicz *et al*. 2006). In contrast with *Agrobacterium*, other tumor-inducing bacteria are not able to transform host plant cells (Clark *et al*. 1989).

Pseudomonas savastanoi causes tumor formation on olive (*Olea europea* L.) and oleander (*Nerium oleander* L.). Tumor formation caused by *P. savastanoi* infection is dependent upon the bacterial synthesis and secretion of IAA and zeatin-type cytokinins (Morris *et al*. 1986). Moreover, it was shown that the level of IAA production can influence pathogenesis efficiency of *P. savastanoi* on oleander (Silverstone *et al*. 1993). IAA and cytokinin production by *P. savastanoi* depends on two genes, *iaaM* (tryptophan monooxigenase) and *iaaH* (indole acetamide hydrolase) which encode enzymes catalysing two following steps of IAA biosynthesis (Glass and Kosuge 1988) and also the *ptz* gene which encodes isopentenyl transferase (*IPT*), the enzyme catalysing cytokinin biosynthesis (MacDonald *et al*. 1986). In *P. savastanoi* strains isolated from the oleander galls, *iaaM* and *iaaH* genes are disposed on the plasmid named *pIAA* and in the strains isolated from olive galls these genes are located on the chromosome (Glass and Kosuge 1988). The *ptz* genes in both olive- and oleander-associated strains are located on a separate plasmid *pCK2* (MacDonald *et al*. 1986). Remarkably, the auxin and cytokinin biosynthetic genes from *P. savastanoi* are closely homologous to the *tms1*, *tms2* and *tmr* genes from *A. tumefaciens* which carry out the same functions (Morris *et al*. 1986). In addition, the plasmid of oleander gall isolates of *P. savastanoi* carry the *iaaL* gene, encoding IAA-lysine synthase, an enzyme which catalyses the conversion of free IAA to an IAA-lysine conjugate (Glass and Kosuge 1988).

Another plant pathogenic bacterium, *Pantoea agglomerata* (previously known as *Erwinia herbicola*) was also reported as a tumor-producing agent in several plant hosts such as beet and gypsophila (Chalupowicz *et al*. 2006). The pathogenicity of *P. agglomerata* depends on a plasmid named *pPATH* which harbors genes necessary for virulence and also genes encoding enzymes for IAA and cytokinin biosynthesis (Clark *et al*. 1993; Lichter *et al*. 1995). The *iaaM* and *iaaH* genes of *P. agglomerata* were shown to have structural and functional similarity with corresponding genes of *Pseudomonas savastanoi* (Clark *et al*. 1993). Mutations in IAA biosynthesis genes of *P. agglomerata* led to the formation of very small tumors on *Gypsophila paniculata* (Clark *et al*. 1993). The cytokinin byosinthesis gene of *P. agglomerata* designated *etz*, has significant homology with the *ptz* gene of *Pseudomonas savastanoi* and the *ipt* gene of *A. tumefaciens* (Lichter *et al*. 1995). Mutations in the *etz* gene also caused a reduction of tumor size but not the full inhibition of tumor formation (Lichter *et al*. 1995). Simultaneous mutation in *iaaM*, *iaaH* and *etz* genes also did not fully eliminate *Pantoea*-induced tumor formation suggesting the existence of other tumorigenesis-related genes in the genome of *P. agglomerata* (Manulis *et al*. 1998). Thus, tumor formation caused by *Pseudomonas savastanoi* and *Pantoea agglomerata* have common mechanisms and similar genetic control. A very similar anatomical structure was shown for tumors caused by *A. tumefaciens* and *Pantoea agglomerate* on *Gypsophila* (Chalupovicz *et al*. 2006). The most significant difference between *Agrobacterium*- and *Pantoea*-induced tumors consisted in limited growth of the latter in contrast with crown gall caused by *A. tumefaciens* (Chalupovicz *et al*. 2006). Limited growth also is the character of tumors induced by *Pseudomonas savastanoi* (Glass

and Kosuge 1988). Such a difference might be caused by different pathogenesis strategies of *Agrobacterium* and other plant pathogenic bacterium (Chalupowicz *et al*. 2006). However, tumor formation induced by *Pseudomonas savastanoi* or *Pantoea* agglomerata does not involve the transformation of host cells (Clark *et al*. 1989) and therefore requires the constant present of living bacteria in the tumor (Chalupowicz *et al*. 2006).

The gram-positive bacteria *Rhodococcus fascians* infects a wide range of plant species resulting in the formation of so-called leafy gall tumors (Vereecke *et al*. 2000). When *R. fascians* infects plant tissue, adventitious meristems that gave rise to multiple abnormal shoots are formed; the process of *de novo* meristem formation was shown to be caused by re-activation of cell division in the stem cortical cells (de O Manes *et al*. 2001). Infection of tobacco seedlings by *R. fascians* resulted in growth inhibition, arrest of root development and slowed leaf formation (Crespi *et al*. 1992). Studies on molecular mechanisms of *R. fascians*-plant interactions revealed that essential virulence determinants of this bacterium are located on a *pFi* plasmid (Crespi *et al*. 1992). The leafy gall formation is caused by the alterstion of cytokinin/auxin balance in the host plant tissues (Crespi *et al*. 1992; Vandeputte *et al*. 2005). Zeatin- and isopentenyl-type cytokinins overproduction was revealed in the leafy galls (Crespi *et al*. 1992), moreover, production of 11 different cytokinin compounds was found in the culture of *R. fascians* irrespective of their pathogenicity (Eason *et al*. 1996). Cytokinin production by *R. fascians* was shown to depend upon the *fas* locus of the *pFi* plasmid. The *fas* locus is an operon of six genes; one of these genes encodes *IPT*, a key enzyme of cytokinin biosynthesis (Crespi *et al*. 1992). The *fas1* mutant of *R. fascians* carrying the insertion into the *ipt* gene is completely avirulent indicating the essential role of cytokinin in *R. fascians* pathogenesis (Temmerman *et al*. 2001). Study of cell-cycle genes expression during *R. fascians*-induced leafy gall formation on *Arabidopsis thaliana* (Vereecke *et al*. 2000) and *N. tabacum* (de O Manes *et al*. 2001) revealed that tumor formation correlated with a strong induction of *CDKA1* (Vereecke *et al*. 2000), *CycB1* and *CycD3;2* (de O Manes *et al*. 2001) gene expression. Incubation of synchronized tobacco *BY-2* (Bright Yellow-2) cells resulted in a broader mitosis index peak and this effect was linked with the expression of the *fas* locus (Temmerman *et al*. 2001). At the same time, exogenous supply of the cytokinin BAP (10^{-6}-10^{-7} M) on synchronized *BY-2* cells did not result in such broadening of the mitotic peak, and simultaneous cocultivation with *R. fascians* and BAP supply did not lead to a cumulative effect suggesting that the effect of *R. fascians* on mitosis of *BY-2* cells is not depend upon bacterial cytokinin production (Temmerman *et al*. 2001). It was recently reported that *R. fascians* also produces and secretes IAA (Vandeputte *et al*. 2005). IAA production by *R. fascians* increased in the infected tobacco when tryptophan is not limiting (Vandeputte *et al*. 2005). Production of IAA occurred even in plasmid-free *R. fascians* strains indicating that the genes controlling IAA biosynthesis are located in the chromosome (Vandeputte *et al*. 2005).

Thus, plant tumors which are induced by different species of bacteria with a different pathogenesis strategy have one common character: production of high amounts of IAA and cytokinins caused by the expression of bacterial genes encding the enzymes of IAA and cytokinin biosynthesis. These genes express in the bacteria persisting in the tumor in the case of gall-inducing bacteria (MacDonald *et al*. 1986; Glass and Kosuge 1988; Crespi *et al*. 1992; Clark *et al*. 1993; Lichter *et al*. 1995; Vandeputte *et al*. 2005) or can be introduced into the host plant genome in the case of *Agrobacterium* (Escobar and Dandekar 2003). Interestingly, the production of small amounts of cytokinin was found in avirulent strains of *Pseudomonas savastanoi* and *Pantoea agglomerata* (Lichter *et al*. 1995). Moreover, the production of cytokinins was reported for many non-pathoghenic bacteria species suggesting the role of these hormones in the bacteria's life irrespective of pathogenicity (Jameson *et al*. 2000). Production of bacterial cytokinins might be a result of the presence of *ipt* genes as was predicted in the genomes of many bacteria, according to GenBank data (Takei *et al*. 2001).

PLANT TUMORS CAUSED BY VIRUSES, PROTISTS, FUNGI, NEMATODES AND INSECTS

Apart from bacteria, plant neoplastic growth can be caused by viruses, protists, fungi, root-knot and cyst nematodes ang gall-forming insects (for review see: Armstrong 1995). In several cases of pathogen-induced plant neoplasia the shift of phytohormonal balance in the infected plant tissues was reported (for review see: Jameson 2000).

Among numerous species of plant pathogenic viruses, the represantives of the *Phytoreovirus* genus induce neoplastic growth of plant tissues (Kudo *et al*. 1991). Phytoreoviruses are insect-transmitted viruses which are transmitted by *Diptera* insects such as *Agalia constricta*, *Nephotettix nigropictus*, *N. cincticeps*, *N. malayanus*, *N. virescens*, *Recilia dorsalis* (Streissle and Maramorosch 1963). Isects are used by *Phytoreovirus* both as a vector and intermediate host, i.e. the virus multiplies in their tissue, too. More than 50 plant species are known to be susceptible to wound tumor viruses. The genus *Phytoreovirus* includes six species and five of them, with the exception of rice dwarf virus (RDV), are able to induce tumor formation on host plants (Streissle and Maramorosch 1963). Most known of them are wound tumor virus (WTV) (Anzolla *et al*. 1987) and rice gall dwarf virus (RGDV; Moriyasu *et al*. 2000). Among the symptoms caused by these viruses are small tumors at the stem and larger and more numerous ones at the roots and the development of organs from otherwise normal organs, e.g. a leaf can develop at the surface of another leaf (Streissle and Maramorosch 1963). The genomes of *Phytoreovirus* species consist of 10-12 segments of double-stranded RNA and each virus particle contains one copy of each segment. Each of the 12 segments can be transcribed and it is assumed that each of them encodes one protein (Ichihama and Barbier 1994; Moriyasu *et al*. 2000; Miyazaki *et al*. 2005). Among proteins encoded in the genomes of *Phytoreovirus* is the transcriptase enzyme which transcribes single-stranded RNA or, in other words, produces an mRNA complementary to the transcribed strand (Ichihama and Barbier 1994). Despite the study of mechanisms of structural and non-structural peptide formation and host specifity of phytoreoviruses (Moriyasu *et al*. 2000; Miyazaki *et al*. 2005), the mechanisms of plant tumor induction by these viruses is still unclear.

Plasmodiophora brassica Woronin is an obligate pathogenic protist which causes clubroot disease of plants from the *Brassicaceae* family. Clubroot gall formation on the hypocotyl and root of infected plants is a result of enhanced proliferation and subsequent elongation of cortex and stele cells (Devos *et al*. 2005). Such enormous cell elongation is caused by the remodeling of cellulose microfibrills in the cell wall. Cell wall remodeling is a result of xyloglucan hydrolases enzyme activity which was shown to increase in the plant tissues as a result of infection by *Plasmodiophora* (Devos *et al*. 2005). Enhanced cell proliferation in the infected plant tissues might be a result of a shift in phytohormonal balance caused by *P. brassica*. The increase of free IAA and IAA conjugates levels was detected in the infected tissues of *Brassica rapa* beginning from the 6th day after inoculation (Devos *et al*. 2005). The concentrations of zeatin and zeatin riboside in the clubroots on *Brassica* was several-fold higher than in uninfected roots; in addition, infected root and stem explants showed cytokinin-independent growth *in vitro* (Dekhuijzen *et al*. 1981). Several-fold increase of ABA concentration was also reported for *Plasmodiophora*-infected *Brassica rapa* (Devos *et al*. 2005). At present, no data on the genetic control of phytohormone production in *P. brassica* is available.

Among the gall-forming plant pathogenic fungi the

most well known are *Ustillago esculenta* which incites the edible galls on the grass *Zizania latifolia* (Chung and Tzeng 2004), *Taphrina deformans* which causes the formation of tumors on peach leaves (peach leaf curl disease) (Tavares *et al*. 2004), and *Dibotryon morbosum* which forms so called black knot galls on the stems of *Rosaceae* plants (Fernando *et al*. 2005). Some plant pathogenic fungi are able to produce phytohormones (Perley and Stove 1969; van Staden and Nicolson 1989; Ozgan and Topcuoglu 2001; Chung and Tzeng 2004). For example, *Taphrina deformans* was shown to produce tryptamine suggesting the possibility to produce IAA from tryptophan via tryptamine (Perley and Stove 1969). *Ustillago esculenta* can convert indole-3-acetamide, indole-pyruvate and indole-lactic acid into IAA *in vitro* (Chung and Tzeng 2004). *Fusarium moniliforme*, a fungus which causes leaf and flower malformation was shown to produce and metabolise cytokinins (van Staden and Nicolson 1989). The involvement of cytokinins in the formation of "green islands" on the leaves of plants infected by different pathogenic fungi was reported (Jameson 2000). In addition, gibberellin, abscisic asid and cytokinin production was reported for a wide range of plant-pathogenic and saprophytic fungi species (Ozgan and Topcuoglu 2001). Thus, numerous fungal species are able to produce phytohormones irrespective of pathogenicity.

Cyst nematodes, including the genera *Globodera* and *Heterodera*, and root-knot nematodes, icluding the genus *Meloidogyne* are the most studied of plant parasitic nematodes (Bird and Koltai 2000; de Meutter *et al*. 2003). The pathogenesis strategy specific for nematodes includes the formation of so-called feeding sites in the roots of infected plants. In the case of root-knot nematodes feeding sites consist of giant cells with numerous nuclei, and cyst nematodes form syncytia. In both types feeding site initiation includes the activation of the cell cycle in the pericycle and in xylem parenchyma cells (Goverse *et al*. 2000). One of the first events in the plant-nematode interaction is the injection of nematode pharyngeal gland secretion into the initial feeding site (Bird and Koltai 2000). Since several studies demonstrate the presence of cytokinins in the lysates of different root-knot and cyst nematodes (Bird and Loveys 1980; de Meutter *et al*. 2003) the secretion of phytohormones by parasitic nematodes into the infected roots was suggested. For examples, in the extracts of juvenile stages of *Heterodera schachtii* and *Meloidogyne incognita* 10 zeatin- and isopentenyl type cytokinins were detected (de Meutter *et al*. 2003). The mechanism of cytokinin production by nematodes is still unknown but their synthesis migh be carried out by a functional *IPT* enzyme which, as predicted by Takei *et al*. (2001) in different bacteria and eucaryotic species, including *Caenorhabditis elegans*, basing on GenBank data. In fact, the presence of cytokinins was also found in the extracts of *C. elegans* (de Meutter *et al*. 2003). An obvious role of cytokinins as activators of the cell cycle allows one suggest that the cell cycle activation in nematode feeding sites is caused by the sectretion of cytokinins into plant tissues by nematodes. At the same time, the mapping of auxin levels in *Meloidogyne*-infected white clover roots revealed the decline of auxin levels in the initials of feeding sites by the disruption of polar auxin transport (Hutangura *et al*. 1999). The mechanism of this phenomenon is still unclear. The possible cause of such alteration in polar auxin transport might be the production of flavonoids which can act as auxin transport inhibitors, as shown for nematodes (Hutangura *et al*. 1999). Increased meristematic activity in *Meloidogyne* feed site initials was shown to coincide with the ectopic expression of the class 1 *KNOX* gene *LeT6/Tkn2* in the tomato root (Koltai and Bird 2000). The expression of the *Le-phan* gene (tomato homologue of *PHANTASTICA* gene of *Antirrhinum*) which encodes a *Myb* transcriptional factor suppressing *KNOX* gene expression (Tsiantis *et al*. 1999) was also increased in the *Meloidogyne*-induced giant cells (Bird and Koltai 2000).

Formation of galls on different plant species was known for numerous herbivor insects, particularly flies, wasps and aphids (Armstrong 1995). Most known examples of galls caused by insects are "tootsie-pop" gall caused by the gall midge (*Asphondylia auripila*), the spruce pineapple gall caused by the spruce gall aphid (*Adelges cooleyi*), the ubiquitous oak apple gall caused by the California gall wasp (*Andricus californicus*), the oak saucer-gall caused by the gall wasp (*Andricus gigas*), the spined-turban gall caused by the gall wasp (*Antron douglasii*), and the unusual Oregon oak "ping-pong" ball gall caused by the gall wasp (*Cynips maculipennis*) (for review see Armstrong 1995). Hovewer, only in several cases the formation of insect-induced galls was shown to be caused by the alteration of phytohormonal balance in host plant tissues because of the production of cytokinins by the insect larvae. For example, the "nipple galls" induced by *Pachypsylla celtidis-mamma* (insect from *Psyllidae* family), on hackberry (*Celtis occidentalis*) leaves showed 50-fold higher levels of isopentenyl adenosine than the uninfected leaf tissues (Mac Dermott *et al*. 1996). The larvae of the tephritid fly *Eurosta solidaginis* which forms ball galls on *Solidago altissima* were shown to produce zeatin, zeatin-riboside and isopentenyladenine and secret these cytokinins into host plant tissues (Mapes and Davies 2001). Genetic control of cytokinin production by gall-forming insects was not investigated.

GENETIC TUMORS IN HIGHER PLANTS

Genetically determined, or genetic, tumors develop spontaneously, in the absence of pathogens or other casual agents, and their formation depends on the genetic constitution of the organism. Studying plant genetic tumors is a way to identify the genes involved in the systemic control of cell divisions (plant oncogens). The phenomenon of spontaneous tumorigenesis in higher plants has been known for about a century. Formation of spontaneous tumors was described for many higher plant species (Ahuja 1998). In addition, tumor formation was reported for interspecific hybrids (Ahuja 1998) and inbred lines (Narbut 1967). The disturbance of proper cell-cycle genes functions in the altered genetic background can be the cause of tumor formation in interspecific hybrids and inbred lines. Several monogenic mutations leading to tumor-formation were identified in *Arabidopis thaliana* (Vittorioso *et al*. 1998; Frank *et al*. 2000, 2002). Tumorous mutants of *Arabidopsis* demonstrate severe abnormalities of morphogenesis and decreased viability. Several novel genes involved in plant cell division control were identified during the study of *Arabidopsis* tumorous mutants (Bellec *et al*. 2002; da Costa *et al*. 2006; Smyczynski *et al*. 2006). In addition, a novel plant oncogene *CHRK1* was identified in the transgenic assay (Lee *et al*. 2003, 2004).

Spontaneous tumorigenesis in interspecific hybrids of *Nicotiana* and other genera

Genetic control, structure, development, hormonal regulation and molecular biology of spontaneous tumorigenesis in higher plants have been extensively investigated using *Nicotiana* interspecific hybrids as a model. Tumor formation in nine interspecific tobacco hybrids was firstly described by Kostoff in 1930. Tumors appear during the flowering stage in all organs of tobacco hybrid plants, and most frequently on the stem. In addition, tumor-formation in tobacco hybrids can be induced at earlier stages by wounding or irradiation (Ahuja and Cameron 1963). Tumor tissue in the tobacco hybrids originates from stem cortex parenchyma and is histologically similar to crown gall induced by *A. tumefaciens* (Ahuja 1998). In contrast with crown gall, tobacco tumors tend to differentiate and become teratomas. They usually demonstrate the features of second differentiation and develop rudimentary leafy or floral buds. It is possible to regenerate whole plants from the shoot-like structures of these teratomas (Ahuja 1998). About 300 interspecific hybrids of *Nicotiana* were obtained but only 30 of them were able to form tumors (Ahuja 1998). Tumors

develop in the hybrids from reciprocal crosses, suggesting that tumor-formation is under the control of nuclear genes. Some cells of tobacco tumors demonstrate cytological instability with aberrant mitoses and a variable number of chromosomes (Palni *et al*. 1969). However, RAPD analysis of genomic polymorphism in tumors and normal leaves of *N. glauca* × *N. langsdorfii* plants showed only slight genomic differences between tumor and leaf tissues (Wang *et al*. 2001; Qu *et al*. 2006). These data suggest that spontaneous tumors in tobacco hybrids are caused by aberrant gene regulation rather than by gene mutations (Ahuja 1998). Based on extensive hybridization experiments, Naf (1958) divided the *Nicotiana* genus into "plus" and "minus" groups. Hybrids between *Nicotiana* species from different groups are tumor-producing in contrast with hybrids between the species which belong to the same group. The "plus" group includes species from *Alatae* section which contain 9 or 10 chromosome pairs, the "minus" group consists of *Nicotiana* species from different sections, generally with 12 chromosome pairs. Extensive investigations of *N. longiflora* × *N. debney-tabacum* and *N. glauca* × *N. langsdorfii* hybrids with different amounts of chromosomes from each parental species supporting the hypothesis that the genome of the "plus" parent contains the Initiator (*I*) – a single gene or a block of genes involved in the initiation of tumor-formation and the genome of the "minus" parent – several *ee* loci which act in the *e*nhansement of *e*xpression of the tumorous phenotype (Ahuja 1998). The experiments on backcrossing of tumor-producing *Nicotiana* hybrids showed that tumorous phenotype maintains in the hybrids which contain full genome of "plus" parent plus certain chromosome from "minus" parent (Smith 1988). Therefore, tumor-formation in *Nicotiana* interspecific hybrids depends on the expression of certain genes from "minus" parent species on the specific genetic background of "plus" species. The participation of genes, which control phytohormonal metabolism and the response to plant hormones in tobacco tumor formation appears very probable. The hypothesis about the role of *I* and *ee* elements in the control of spontaneous tumorigenesis in tobacco hybrids corresponds with an idea that "plus" and "minus" species differ in their phytohormonal status. Different regeneration peculiarities of calli derived from the leaves of tobacco species belonging to different groups support the "plus-minus" hypothesis. It was shown that the species from *Paniculatae* section ("minus" group), including *N. glauca*, tend to differentiate many roots from the callus unlike species from the *Alatae* section ("plus" group), including *N. langsdorfii*, which have a high capacity for shoot regeneration (Bogani *et al*. 1997). Differentiation of roots from explants normally takes place when the concentration of auxins in the medium exceeds the concentration of cytokinins, the differentiation of shoots – when cytokinin/auxin ratio shifts in favor of cytokinin (Skoog and Miller 1957). Thus the root-forming capacity of "minus" group species may reflect the increased sensitivity to auxin and shoot-forming capacity of "plus" species – increased sensitivity to cytokinin. The combination of these characteristics in one organism via interspecific hybridization may lead to tumor formation. The molecular basis of high root-forming capacity of the species of "minus" group might be the homologues of *rol* genes which were revealed in the genomes of 14 "minus" *Nicotiana* species (Furner *et al*. 1986; Meyer *et al*. 1995; Frundt *et al*. 1998; Intrieri and Buiatti 2001). The ability of tobacco homologues of *rol* genes to stimulate root formation was demonstrated on different plant species (Aoki and Syono 1999a; Frundt *et al*. 1998; for more detailed information see below).

Some types of stress, such as the supply of exogenous phytohormones, wounding and X-ray irradiation can enhance tumor-formation in *Nicotiana* hybrids (Ahuja and Cameron 1963). Numerous data suggest that spontaneous tumorigenesis in tobacco interspecific hybrids is caused by shifting of the phytohormonal balance. Tobacco tumor tissues are capable of growing *in vitro* on hormone-free medium. Hormone-autotrophic proliferation of tissues is a feature of plants which overproduce phytohormones, especially auxins and cytokinins. Isolated tobacco tumors form shooty callus with many aberrant buds when grown on hormone-free medium, but never produce roots suggesting that the auxin/cytokinin balance in tumor tissue is shifted in favor of cytokinins (Ahuja 1971). Cultivation on medium with auxin (IAA, 1 mg/l) or cytokinin (benzyladenine, 1 mg/l) had only very slight effects on the growth of tumor tissue, indicating that the tumors lost their sensitivity to phytohormones (Qu *et al*. 2006). Enhanced levels of auxins and cytokinins in tobacco genetic tumors were first reported in early studies. In 1944 Skoog indicated that the SAM of the *N. glauca* × *N. langsdorfii* hybrid containe increased IAA levels. Furthermore, increased free IAA and cytokinin levels were reported for different tissues of *N. glauca* × *N. langsdorfii* and other tumor-producing hybrids (Ames 1972; Ames and Mistretta 1975). The amounts of cytokinin and auxin bound forms are also significantly higher in the tumorous hybrids. For example, the level of cytokinin nucleotide was approximately 5-fold higher in the tissues of tumorous *N. glauca* × *N. langsdorfii* hybrid than in the tissues of parental species (Nandi *et al*. 1990); the level of IAA conjugates in the tissues of tobacco hybrids also increased (Liu *et al*. 1978). It is probable that the fission of auxins and cytokinin conjugates which occurs during flowering leads to the shift of the auxin/cytokinin balance and results in tumor-formation. Treatment of *N. glauca* × *N. langsdorfii* seedlings (Qu *et al*. 2006) or isolated buds (Ames and Mistretta 1975) by exogenous auxins or cytokinins leads to the contrasting effects on tumor formation: cytokinins promote tumor-formation and auxins inhibit this process. The supplying of exogenous cytokinins or transformation by the *ipt* gene restored the shooty callus morphology of the *N. glauca* × *N. langsdorfii* non-tumorous mutant (Feng *et al*. 1990). Thus, high levels of cytokinins, but not auxins evoke tumor formation in tobacco interspecific hybrids Therefore, the shift of the auxin/cytokinin balance in favor of cytokinin must occur at some plant development stage leading to the start of tumor formation. In the *in vitro* culture of *N. tabacum* stem tissues, the competence of phloem and pith cells to dedifferentiate and reenter into the cell cycle is associated with the accumulation of cytokinins and increased expression levels of some cell cycle genes (Boucheron *et al*. 2002). Thus, cytokinins play the main role in the control of cell dedifferentiation which is a crucial step in tumor induction. Studying the kinetics of auxin and cytokinin levels during tumor formation in *Nicotiana* hybrids, the main role of cytokinin to auxin ratio shifting in tumor induction was observed (Ichikawa and Syono 1991). A correlation between a decreasing in the IAA level and induction of tumors in *N. glauca* × *N. langsdorfii* hybrid was reported in several studies (Ahuja *et al*. 1971; Ames and Mistretta 1975; Ichikawa and Syono 1991).

A temporary decrease in the IAA level in the tissues of hybrid *Nicotiana* at the flowering stage took place followed by a dramatic increase in the IAA level, some 30- to 80-fold within five days after tumor induction (Ichikawa and Syono 1991). Taken together, these results suggest that the crucial step in the induction of tumor-formation in *Nicotiana* hybrids is the temporary shift of auxin/cytokinin balance in favor of cytokinin which happens at the beginning of the flowering stage. The increase of auxin level at the later stages of tumor formation may play a role in the further development of tumors, for example in the differentaition of vasculature in the teratomas (Ullrich and Aloni 2000).

Several genes which are presumably involved in the control of genetic tumor formation in *Nicotiana* hybrids have been identified. These genes may be divided into three groups based upon their functions: 1) stress-related genes; 2) the *GTcyc* gene encoding a novel type of cyclin; 3) genes homologous to *A. rhizogenes* T-DNA oncogenes and 4) The *CHRK1* gene encoding a novel type of receptor kinase with a chitinase-like domain. Genes which are preferentially expressed in the tumor tissue of the *N. glauca* × *N. langsdorfii* hybrid were identified during the screening of a genetic tumor cDNA library (Fujita *et al*. 1994). 17 cDNA clones

which are expressed in tumor tissue were isolated, 15 of which were also expressed in callus *in vitro* suggesting the participation of the same genes in the control of callus and tumor formation. Among tumor-specific cDNA clones several genes encoding stress-related proteins were identified. Those were glucan-endo-1,3-β-glucosidase, osmotin, pathogenesis-related (*PR*) proteins and the novel genetic tumor-related proteinase inhibitor I (*GTI*) – protein which was firstly isolated from *Nicotiana* genetic tumors. The expression of many stress-related genes in the genetic tumors of tobacco corresponds to the activation of hypersensitivity response (HR), which is typical for reaction of plant organism to pathogenesis, wounding and some other stresses, in tumor tissue (Fujita *et al*. 1994). In the case of tobacco interspecific tumors, the plant organism may react on genomic instability and on alterations in the hormonal balance as well as on the specific kind of stress. This was verified by Teixeira da Silva (2005) in transformation experiments conducted on *N. tabacum* 'Samsun SS' in which mere injury caused by particle-bombardment of tungsten or gold particles not coated by any plasmid resulted in massive callusing and tumor formation without subsequent differentiation into shoots or roots, hinting at a dedifferentiation program as a result of the HR response.

Plant cell dedifferentiation and hyperproliferation is often associated with an increase in the expression of some genes encoding the elements of the cell cycle machinery (Riou-Khamlichi *et al*. 1999; Boucheron *et al*. 2002; Dewitte *et al*. 2003). However, only one gene which expresses at a high level in the tumor tissue may be involved in the direct control of the cell cycle – it is a gene encoding an unusual cyclin, *GTcyc*. This cyclin was firstly isolated from tumors of the *N. glauca* × *N. langsdorfii* hybrid. *GTcyc* differs from other groups of plant cyclins and should be considered as a new group, distantly related to mammalian D-type cyclins (Wang *et al*. 1999). It was shown that the expression level of *GTcyc* in the genetic tumors of *N. glauca* × *N. langsdorfii* was significantly higher than in the differentiated tisssues (Wang *et al*. 1999). Moreover, the expression of *GTcyc* in the tissues of *N. glauca* × *N. langsdorfii* was about 14-fold higher than in the parent species and non-tumorous hybrids (Wang *et al*. 2001). Overexpression of several plant cyclins, e.g. *CycD3* (Riou-Khamlichi *et al*. 1999), *CycA3;2* (Yu *et al*. 2003) and *CycB1;1* (Boucheron *et al*. 2002) was shown to cause cell dedifferentiation and enhanced cell proliferation in *Arabidopsis* and tobacco tissue cultures. Thus, unusual cyclin *GTCyc* may participate in the control of cells proliferation in *Nicotiana* genetic tumors.

Much experimental data has allowed for a connection between the cause of plant tumor formation and cytokinins and auxins. It seems logical that the expression of "phytohormonal" genes must change during tumor formation in tobacco hybrids. A certain role was shown in the establishment of the tumorous phenotype in tobacco hybrids played by "phytohormonal" genes which are homologous to *Agrobacterium* T-DNA genes involved in the induction of hormonal disbalance in host plant tissues. The sequences which demonstrated a high level of homology with *A. rhizogenes rol* genes were first revealed in the genome of untransformed *N. glauca* (White *et al*. 1982) and named *Ngrol* genes (Furner *et al*. 1986). Furthermore, sequences homologous to *A. rhizogenes ORF13* and *ORF14* genes – *NgORF13* and *NgORF14* – were revealed in the genome of *N. glauca* (Aoki *et al*. 1994). A region of the *N. glauca* genome which is homologous to TL-DNA of *A. rhizogenes Ri*-plasmid was named cT-DNA (cellular T-DNA; Aoki *et al*. 1994). This region contains a portion of genes corresponding to the *rolB*, *rolC*, *ORF13* and *ORF14* genes of the *A. rhizogenes Ri*-plasmid. Further studies revealed the persistence of cT-DNA genes in 15 from 42 studied *Nicotiana* species (Furner *et al*. 1986; Meyer *et al*. 1995; Frundt *et al*. 1998; Intrieri and Buiatti 2001). Restriction mapping showed that the *N. glauca* and *N. cordifolia* cT-DNA region is organized as an imperfect inverted repeat containing *rolB*, *rolC*, *ORF13* and *ORF14* genes in the left arm and *rolC*, *ORF13* and *ORF14* genes in the right arm (Aoki *et al*. 1994; Intrieri and Buiatti 2001; Suzuki *et al*. 2002). Sequence studies of cT-DNA in several *Nicotiana* species showed a high level of similarity (more than 80%) between plant and agrobacterial sequences both in coding and intergenic regions (Furner *et al*. 1986; Aoki *et al*. 1994). However, extensive PCR analysis revealed the deletion of a large portion of cT-DNA in many *Nicotiana* species (Intrieri and Buiatti 2001). Several *Nicotiana* species lost their *rolB*, *ORF13* or *ORF14* cT-DNA genes, but *rolC* gene persisted in 13 out of 15 species (Intrieri and Buiatti 2001). This fact hints at the main role of *rolC* among the other cT-DNA genes in the control of phytohormonal homeostasis and perhaps in tumor formation. Sequence analysis of cT-DNA genes from different *Nicotiana* species showed a range of similarity (from 66.3% to 88.5%) with corresponding *A. rhizogenes* genes. The sequences of *ORF13* and *ORF14* are the most conserved amongst *Nicotiana* cT-DNA genes (Intrieri and Buiatti 2001). A high conservation of cT-DNA genes suggests their physiological role in *Nicotiana* plants.

The hypothesis about the horizontal transfer of TL-DNA region during *A. rhizogenes* infection of the *Nicotiana* ancestor was firstly supported by White *et al*. (1982). Horizontal transfer of genes is a widespread phenomenon among prokaryotes and may play an important role in bacterial evolution as a way to promote genome plasticity. Several examples of horizontal gene transfer between prokaryotes and eukaryotes (Bertolla and Simonet 1999) and even between distant plant species (Diao *et al*. 2006) are known. DNA transfer from *Agrobacterium* to plants and from plant to soil bacteria was demonstrated under laboratory conditions (Tepfer *et al*. 2003). Two additional functional ORFs similar to the mikimopine synthase (*mis*) gene were revealed in the left and right arms of the cT-DNA from four *Nicotiana* species establishing that the T-DNA fragment horizontally transferred into ancestral tobacco genome had originated from the mikimopine-type *Ri*-plasmid like ancestor (Suzuki *et al*. 2002). It is significant that cT-DNA was detected in the species which belong to the "minus" group but not detected in the "plus" group species. Thus, the expression of cT-DNA genes can be the investment of "minus" group parental genotype for the establishment of the tumorous phenotype in tobacco interspecific hybrids. High levels of conservation in coding regions of tobacco cT-DNA genes confirm that these genes take part in the support of the hormonal balance in plant tissues. Some experimental data suggests that these genes take part in tumor development. The expression of tobacco homologues of *A. rhizogenes* oncogenes was detected in teratoma tissues of the *N. glauca* × *N. langsdorfii* hybrid (Ichikawa *et al*. 1990; Aoki *et al*. 1994; Nagata *et al*. 1996) and also in differentiated tissues of hybrid plants (Aoki and Syono 1999b). However, the expression of *Ngrol* genes dramatically enhances after tumor induction by wounding and decreases during regeneration of plants from teratoma tissues (Aoki and Syono 1999b). It was shown that the expression of *NgrolB* occurs presumably in the meristematic regions while *NgrolC* expression is associated with differentiated tissues such as vascular bundles (Nagata *et al*. 1996). The expression of cT-DNA genes was detected not only in tobacco hybrids but also in the different tissues of "minus" parental species (Meyer *et al*. 1995; Frundt *et al*. 1998; Intrieri and Buiatti 2001). Although the amounts of their transcripts are small, these genes express in a tissue-specific manner. *Nicotiana* species from different subgenera distinguished by cT-DNA genes expression levels and pattern suggest their role in the evolution of the *Nicotiana* genus (Intrieri and Buiatti 2001). Hormonal regulation of expression was documented for some cT-DNA genes, for example, exogenous auxins and cytokinins negatively influenced the expression of the *tORF13-1* gene in cultured *N. tabacum* leaf discs (Frundt *et al*. 1998). Transformation studies revealed functional similarities of cT-DNA *rolC*, *ORF13* and *ORF14* genes and their *A. rhizogenes* homologues.

Inoculation of carrot root discs with *A. tumefaciens* strains carrying the *tORF13-1* gene from *N. tabacum* under a *35S CaMV* promoter induced the formation of dense green callus on disc surfaces as well as in inoculation with strains carrying the *RiORF13* gene (Frundt *et al*. 1998). *NgrolC*, *NgORF13* and *NgORF14* genes, similarly to the corresponding genes of the *Ri* plasmid, promoted *rolB*-induced root formation on tobacco leaf discs (Aoki and Syono 1999a). The phenotypes of *P35S-NgORF13* and *P35S-NgrolC* transgenic plants are similar to *P35S-RiORF13* and *P35S-RirolC* plants, respectively (Aoki and Syono 1999a). The *NgORF13* promoter is active in the same tissues of transgenic tobacco plants as the *RiORF13* promoter (Udagawa *et al*. 2004). Similarly to the protein encoded by the *RiORF13* gene, *NgORF13* and *tORF13* proteins contain an *RB*-binding motif (Stieger *et al*. 2004) Therefore, the functions of T-DNA genes are conserved in their tobacco homologues.

However, despite containing *Ngrol* genes, *N. glauca* and other "minus" tobacco species do not exhibit characteristics of the hairy root syndrome. Transformation of tobacco leaf segments by the combination of all four genes of *N. glauca* T-DNA fail to induce extensive root development. At the same time transformation by a combination of corresponding *Ri* T-DNA genes causes root formation (Aoki and Syono 1999b). Whole plants transformed by four genes of *N. glauca* T-DNA did not exhibit any characteristics of the hairy root syndrome but transgenic plants carrying corresponding *Agrobacterium* genes displayed some abnormal features of plants regenerated from hairy roots (Aoki 2004). A transgenic assay showed that only the *NgrolB* gene did not cause phenotypic alterations in plants when overexpressed under the *35S CaMV* promoter (Aoki and Syono 1999b; Aoki 2004). A comparison of the *NgrolB* and *RirolB* sequences revealed that the *NgrolB* gene contains two point mutations. Early termination codons make the ORF of the *NgrolB* gene shorter than that of the *RirolB* gene. Thus, the transferred *rolB* gene was switched off during the evolution of the *Nicotiana* genus. In order to reconstruct an ancestral gene two single base substitutions were introduced into the *NgrolB* gene. As a result, the novel sequence had the same effect on root induction and on transgenic plant morphogenesis as did the *RirolB* gene (Aoki 2004).

Genetic tumors in other interspecific hybrids have been significantly less investigated. Spontaneous tumorigenesis has been reported for the genera *Datura*, *Bryophyllum*, *Gossypium*, *Lilium*, and *Triticum*. Ovular tumors develop from endothelium tissue of hybrids between *Datura stramonium* and certain other *Datura* species (Rietsma *et al*. 1954), on the shoots of hybrids between *Bryophyllum calcinum* and *Bryophyllum daigremontianum* (these tumors originated from the tissues of SAM and cortical parenchyma of the stem) (Resende 1957), on the stems of hybrid *Gossypium hirsutum* × *Gossipium gossipoides* (Phillips and Merrit 1972) and some *Triticum* hybrids (Joshi 1972), and on the SAM and cotyledons of hybrid *Lilium speciosum* × *Lilium auratum* (Emsweller *et al*. 1962). Practically no data about the genetic control, physiology and molecular biology of these tumors are available.

The genetic basis of tumor formation has been investigated the most in tomato hybrid plants carrying the *Frosty spot* (*Frs*) gene from *Lycopersicon chilense* on the genetic background of *Lycopersicon esculentum* (Lang *et al*. 1983). Tumor formation in this hybrid is inherited as a dominant trait. Undifferentiated, chlorophyll-free tumors formed on the leaf veins of hybrid plants. Tumor-producing plants exhibited decreased viability, dwarfism, poor development of vascular tissue, chloroses and necroses on leaves. The tumorous phenotype of the tomato hybrid depended on environmental conditions: strongly manifested in the greenhouse and significantly decreased when plants were cultivated under field condition. *In vitro* culture studies demonstrated that tumorous tissues from tomato hybrids were incapable of phytohormone-autotrophic growth, but required lower amounts of auxin for their growth. These data suggest that the expression of the *L. chilense Frs* gene against the background of *Lycopersicon esculentum* enhanced the level of auxin in hybrid tissues.

Tumorigenesis in *Nicotiana tabacum* transgenic plants with down-regulated *CHRK1* gene expression

CHRK1 – a unique receptor-like kinase, which contains a chitinase-like sequence in the extracellular domain – was isolated from the membrane fraction of *N. tabacum* cells (Kim *et al*. 2000). The C-terminal kinase domain of *CHRK1* protein contains all conserved amino acids of serine/threonine protein kinases. Putative extracellular domain of *CHRK1* is closely related to the class V chitinase of tobacco and microbial chitinases, but lacks the conservative glutamic acid residue essential for chitinase activity. Chitinase-like domain of *CHRK1* protein does not show chitinase activity, but its kinase domain is active and exhibits autophosphorylation (Kim *et al*. 2000). Based on Southern blot analysis it was suggested that *CHRK1* is a single-copy gene in the tobacco genome (Kim *et al*. 2000). *CHRK1* mRNA accumulation is strongly stimulated by fungal and viral pathogens suggesting a role in the defense response. *GUS*-fusion assays showed that the expression of the *CHRK1* gene was restricted to the shoot apex region, leaf primordia and young leaves, and also in the vascular bundles (Lee *et al*. 2003). Transgenic *N. tabacum* plants in which the *CHRK1* gene was suppressed by sense transgene expression exhibited pleiotropic defects in early seedling development including spontaneous formation of rapidly proliferating green shooty callus from emerging embryos in a certain part (about 15%) of transformants (Lee *et al*. 2003). This callus grew rapidly in the absence of phytohormones in contrast to wild-type callus which required cytokinins and auxins for growth. Shoot-like structures which formed on transgenic callus were very similar to those forming on the teratomas of *Nicotiana* interspecific hybrids and were capable of growing on hormone-free medium for more than two years (Lee *et al*. 2003).

Thus, the shooty callus, which originated from a certain part of *CHRK1*-supressed transgenic embryos closely resembled the spontaneous tumors in *Nicotiana* interspecific hybrids. Other *CHRK1*-supressed transgenic plants exhibitted a typical cytokinin response in the absence of cytokinins such as extreme dwarfism, lack of apical dominance, ectopic meristem formation and de-etiolation in the dark. It was shown that *CHRK1*-down-regulated transgenic plants contained a 3-fold higher level of zeatin- and isopentenyl-type cytokinins compared to the wild-type (Lee *et al*. 2003). Therefore, the *CHRK1* gene of *N. tabacum* may be involved in the regulation of cytokinin homeostasis in plant tissues. Cytokinins positively regulate the expression of *D*-type cyclins, especially *CycD3* (Riou-Khamlichi *et al*. 1999) and several class 1 *KNOX* genes (Rupp *et al*. 1999). The expression of several genes involved in the control of the cell cycle (*D*-types cyclins) and meristem function (class 1 and 2 *KNOX* genes) was examined in *CHRK1*-supressed shooty callus and in the wild-type callus and seedlings. The expression level of *CycD3* genes increased dramatically in *CHRK1* transgenic callus compared to wild-type seedlings. However, wild-type callus and seedlings grown in the presence of cytokinin also demonstrate an elevated expression level of the *CycD3* gene (Lee *et al*. 2004). The expression levels of class 1 (*NTH15* and *NTH20*) and class 2 (*NTH23*) *KNOX* genes were enhanced in the *CHRK1* transgenic callus and also in *N. glauca* × *N. langsdorfii* and *N. glauca* × *N. suaveolens* tumor tissues compared to *N. tabacum*, *N. glauca* × *N. langsdorfii* and *N. glauca* × *N. suaveolens* seedlings.

Surprisingly, *N. tabacum* seedlings did not differ from *N. glauca* × *N. langsdorfii* and *N. glauca* × *N. suaveolens* seedlings with respect to the expression of *KNOX* genes. It was previously reported that *Nicotiana* genetic tumors had high levels of stress-related gene expression, which is normally associated with the HR response (Fujita *et al*. 1994). Lee *et*

al. (2004) demonstrated that *CHRK1*-supressed shooty callus of *N. tabacum* together with *N. glauca* × *N. langsdorfii* and *N. glauca* × *N. suaveolens* tumors exhibited the constitutive induction of HR response. Both *CHRK1* transgenic callus and tumor tissues of interspecific hybrids demonstrated a higher level of cell death and ion leakage through cell membranes and H_2O_2 accumulation compared to wild-type seedlings and even to wild-type old leaves. Therefore, the formation of *CHRK1* transgenic shooty callus, similar to the formation of tobacco genetic tumors, is associated with an increased stress response. Study of the expression profile of *CHRK1* transgenic callus revealed that many stress-related *PR* genes were preferentially expressed in transgenic callus tissues (Lee *et al*. 2004). These gene expression profiles in *CHRK1* transgenic callus were very similar to their expression in *N. glauca* × *N. langsdorfii* tumors. In addition, *CHRK1* transgenic callus had increased expression of several genes encoding *EREBP/AP2* and *bZIP* transcription factors (Lee *et al*. 2004). Perhaps, these transcription factors take part in the control of *CHRK1* shooty callus development. Thus, it was demonstrated that *CHRK1*-supressed callus phenocopies the genetic tumors in tobacco interspecific hybrids in its morphology, stress response, hormone-autotrophic growth, increased cytokinin level and expression profile. *N. tabacum* belongs to the "minus" group of *Nicotiana* species whose hybrids with the "plus" group species form spontaneous tumors.

It was supposed, that the spontaneous tumor formation in *Nicotiana* interspecific hybrids depends on some hypothetical factors from "plus" and "minus" parents (Naf 1958). Possibly, *CHRK1* receptor kinase or components of an unknown *CHRK1*-mediated signal transduction pathway may be one of the parental factors contributing to genetic tumor formation in tobacco hybrids. Interestingly, the comparative analysis of *CHRK1* gene structure in *N. glauca* ("minus" species) and *N. langsdorfii* ("plus" species) revealed that *CHRK1* of *N. langsdorfii* had significantly shortened sequences of the extracellular chitinase-like domain (Lee *et al*. 2004). Therefore, it is possible that the *CHRK1*-mediated pathway is repressed in "plus" *Nicotiana* species because of deletion of the *CHRK1* extracellular domain and is active in "minus" species. This fact corresponds with high shoot-forming capacity *in vitro* of "plus" group species (Bogani *et al*. 1997) resembling shooty callus formation in *CHRK1*-supressed transgenic plants. Increased shoot formation on the explants of "plus" tobacco species may be due to the deletion in *CHRK1* and repression of *CHRK1*-mediated signal transduction. However, there were no experimental data on the role of *CHRK1* gene expression in the phenotypes of "minus" and "plus" *Nicotiana* species and in tumor formation of tobacco interspecific hybrids. A putative member of the *CHRK1*-mediated signaling pathway was identified in *N. tabacum*. It is a protein, *NtPUB4*, with an unknown function localized on the plasma membrane and interacts with *CHRK1* (Kim *et al*. 2003). Proteins homologous with *NtPUB4* were isolated from *Arabidopsis* and *Brassica*; protein from *Brassica* interacts with *SRK*-kinase during the self-incompatibility response (Stone *et al*. 1999). The *NtPUB4* protein is likely to be involved in the *CHRK1*-mediated modulation of plant development.

According to the hypothesis of Naf (1958), tumor formation in *Nicotiana* hybrids is caused by the combination of some unknown factors from "plus" and "minus" parental species in the hybrid organism. However, two groups of factors which are probably involved in the control of tumor formation in *Nicotiana* interspecific hybrids were revealed. It is probable that the "minus" factor is represented by cT-DNA genes which persist in the genome of only "minus" species. Many experiments revealed that the expression of some of these genes in transgenic plants leads to an increase of tissue sensitivity to phytohormones, dedifferentiation and proliferation of cells (Frundt *et al*. 1998; Aoki and Syono 1999b). It is interesting to suppose that the repression of the *CHRK1* kinase pathway caused by the deletion in the gene encoding *CHRK1* receptor kinase is the "plus" factor contributing to tumor formation in tobacco hybrids. The normal structure of the *CHRK1* protein and the deletion of the *CHRK1* extracellular domain were revealed in *N. glauca* ("minus" species) and *N. langsdorfii* ("plus" species), respectively (Lee *et al*. 2004). The repressive role of *CHRK1* in the induction of tumor formation was demonstrated in the work with transgenic *N. glauca* (Lee *et al*. 2003). The repression of the hypothetical *CHRK1*-mediated pathway in combination with factors of hormonal imbalance caused by the expression of cT-DNA genes may lead to tumor formation in *Nicotiana* hybrids.

Other putative oncogene of tobacco is partially dominant gene *Hl-1* (*H*abituated *l*eaf) that was identified in *N. tabacum* (Meins and Foster 1986; Meins and Tomas 2003). Leaf and pith tissues of *Hl* homozygous plants exhibit long-term cytokinin autotrophic growth *in vitro*, but the phenoltypes of these plants do not differ from the wild type. The *Hl-1* gene can partially restore the tumor phenotype of tobacco cells transformed with *A. tumefaciens Ti*-plasmid carrying a mutation in the *ipt* gene. Moreover, the transformation of *Hl-1/Hl-1* plants with *A. tumefaciens tms* genes causes tumor formation in the base of the stem of transgenic seedlings (Meyer *et al*. 1997). These tumors exhibit a close resemblance to *Agrobacterium*-induced crown gall and spontaneous tumors in *Nicotiana* hybrids. These findings suggest that *Hl-1* has an oncogenic function similar to that of the *A. tumefaciens ipt* gene. However, no sequence and expression data on tobacco *Hl* genes are available.

Spontaneous tumorigenesis within species and in inbred species

Spontaneous tumor formation has been reported for some plant species. Tumors observed on the cotyledons of *Pharbitis nil* (Takenaka and Yoneda 1965), branches of *Picea glauca* (de Torok and White 1960), pods of *Pisum sativum*, as well as on the ovaries of *Sorgum bicolor* (Lin and Ross 1969). Genetic data are available only for spontaneous tumors in *P. sativum*. Genetic tumorigenesis was observed in certain pea cultivars. Tumor development depends on partially dominant gene *Np* located in the chromosome IY. The intensity of light influences tumor development on pea pods (Nuttall and Lyall 1964).

Inbreeding causes homozygotisation of many recessive mutations in cross-pollinating species, including the mutations of genes participating in the control of cell division and differentiation. Genetic tumor formation in the inbred lines was reported for *Melilotus alba* (Littau and Black 1952), and *Raphanus sativus* (Narbut 1967).

Genetic data are available for genetic tumors in radish inbred lines (**Fig. 4**). The genetic collection of radish (*Raphanus sativus* var. *radicula*) inbred lines was created in the 1960s by the compulsory inbreeding of three radish cultivars of different origin. At present the genetic collection of radish includes 33 highly inbred (33-35 progenies) lines with different phenotypic abnormalities, spontaneous tumor formation being of most interest. Tumor formation was detected in some radish lines at the level of 2d or 3d inbred generation (Narbut 1967. Tumors forming with a frequency of ≥50% in 10 out of 33 inbred stocks, which originated from different radish cultivars (Narbut *et al*. 1995). Tumors appeared on radish crop-roots (the structures which form from roots and lower part of stem) at the beginning of the flowering stage and consisted of undifferentiated parenchyma cells and abnormally organized vascular bundles (Narbut 1967; Ilyina *et al*. 2006). Isolated radish tumors were capable of hormone-independent growth (Buzovkina *et al*. 1993). Wide genetic analysis revealed that tumor formation in radish is a polygenic trait, but in some cross combinations tumor-formation was inherited as a monogenic recessive trait (Matveeva *et al*. 2000, 2004). Many experimental data revealed that the cause of tumor formation in radish inbred lines is a disturbance of phytohormonal balance. All studied tumorous radish lines contain about a 3-fold higher level of zeatin-type cytokinins compared to the relative non-tumorous lines.

Fig. 4 Spontaneous tumor on the radish (*Raphanus sativus* var. Radicula Pers.) croproot.

Fig. 5 Cytokinin-induced tumors on the young radish (*Raphanus sativus* var. Radicula Pers.) plants cultivated on thidiazurone (1 mg/l).

In addition, some tumorous lines exhibit a significant decrease of free IAA level. It was shown that the dramatic increase of cytokinin level occurs at the early flowering stage, the stage when tumor formation is induced (Matveeva *et al*. 2004).

Thus, the mechanism of tumor induction in radish inbred lines resembles that of tobacco interspecific hybrids and is based on a shift of cytokinin/auxin ratio in favor of cytokinin at the flowering stage. *In vitro* studies demonstrated that the explants of tumorous radish lines are more sensitive to exogenous cytokinins and auxins and become necrotic when grown on medium with these phytohormones while the explants from non-tumorous lines remain green (Buzovkina *et al*. 1993; Matveeva *et al*. 2000; Ilyina *et al*. 2006). In addition, the cotyledon explants of tumorous lines can spontaneously form callus on hormone-free medium (Lutova *et al*. 1994). The regeneration of plants from isolated apices grown on medium with cytokinins (benzyladenine (2 mg/l), kinetin (2 mg/l) and thidiazurone (1 mg/l) resulted in the formation of a specific outgrowth in the lower part of the hypocotyl in some radish inbred lines (Buzovkina *et al*. 1993). These structures, crop-root-like structures or CRS are very similar to the crop-roots of intact radish plants in their morphology and anatomy (Ilyina *et al*. 2006). The genetic analysis of this trait revealed two genes whose dominant alleles control the formation of CRS and also one dominant suppressor of CRS formation (Lutova *et al*. 1994). Tumors that are able to grow in a hormone-independent manner can be induced in the CRS of young plants and seedlings of tumorous lines by the long-term cultivation on the cytokinin-containing mediums (**Fig. 5**) (Buzovkina *et al*. 1993). Moreover, non-tumorous radish lines can phenocopy the tumorous phenotype when treated by cytokinins *in vivo* (Ilyina *et al*. 2006) or when transformed by the cytokinin biosynthesis *ipt* gene of *A. tumefaciens* (Frolova *et al*. 2004; Matveeva *et al*. 2004). Thus, an increase in cytokinin level is the main cause for tumor formation in radish inbred lines. On the other hand, the transformation of non-tumorous radish lines by the *rolC* gene of *A. rhizogenes* also leads to tumor formation and the same effect arises when *rolC* acts in combination with the *rolB* gene (Dodueva *et al*. 2005). The increasing sensitivity of host plant tissues to auxin and cytokinin is presumably related to the functions of the *rol* genes (Aoki and Syono 1999b). Therefore, not only an increase in cytokinin level but also increased sensitivity to cytokinins may be the cause of tumor formation in radish lines. Expression analysis revealed that tumorous radish lines exhibit an increased expression level of radish homologues of cytokinin-responsive gene *ARR5* compared with non-tumorous lines. *ARR5* protein belongs to the A-type *ARR*, components of cytokinin signalling; expression of genes encoding A-type *ARR* increased rapidly after the addition of cytokinin (benzyladenine, 5 μM; Rashotte *et al*. 2003) so that the expression of A-type *ARR* may be used as a molecular marker of cytokinin response. The induction of *ARR5* expression in response to exogenous cytokinins occurs in the seedlings of tumorous radish lines earlier than in non-tumorous lines. The expression levels of radish *KNOX* genes homologous to *STM* and *KNAT1* also increased in the tissues of tumorous radish lines, and the expression of the *WUS* homeobox gene decreased significantly (Osipova *et al*. 2006). The level of *CycD3* homologue expression increased in the tumor tissues and also in the CRS, as well as in tumors induced *in vitro* (Osipova *et al*. 2006). Thus, the expression of many genes involved in the control of plant cell division changes in tumorous radish lines. The same characteristic was shown for other cases of plant spontaneous tumorigenesis: *CHRK1*-downregulated *N. tabacum* transgenic plants (Lee *et al*. 2004), and tumor-producing mutants of *A. thaliana* (Frank *et al*. 2000, 2002; Harrar *et al*. 2003). Some of these genes might participate in the direct or indirect control of spontaneous tumor formation in radish. However, no genes directly involved in the control of tumor formation in radish inbred lines have been identified.

Tumor-producing mutants in *Arabidopsis thaliana*

In *Arabidopsis thaliana*, tumors that show hormone-independent growth were first obtained as a consequence of somatic mutations after γ-ray irradiation (Campel and Town 1991).

Later on, after germination of ethylmethane sulfonate (EMS) mutagenised *A. thaliana* seeds, 14 mutant tissue lines with the characteristics of tumors were isolated. Some mutant seedlings showed spontaneous dedifferentiation of organized tissues after germination on hormone-free medium and subsequently grew *in vitro* as rapid proliferating hormone-independent calli (Frank *et al*. 2000). Based on their differentiation behavior, three mutant classes were distinguished: shooty callus (formed roots), rooty callus (formed green shoot-like structures) and undifferentiated callus (fully undifferentiated). Phenotypes of all three classes were stable for more than two years. The attempts to regenerate plantlets from both rooty and shooty calli were unsuccessful. Only one shooty callus line gave a fertile plant. Genetic analyses revealed that a single recessive mutation was responsible for the shooty callus phenotype. The mutated locus was mapped to chromosome 2. Rooty and shooty mutant callus lines of *Arabidopsis* mimic, correspondingly, auxin and cytokinin effects without the presence of phytohormones. However, endogenous IAA and IAA conjugate amounts increased significantly (5- to 33-fold higher than in wild-type) only in 3 out of 6 rooty callus lines. The concentrations of free and bound forms of cytokinins increased radically (81-fold for zeatin O-glucoside) only in one undifferentiated callus line. This cytokinin-overproducing line had severely decreased cytokinin oxidase activity (only 5% of wild type level). Auxin and cytokinin concentrations in the other callus lines did not differ from the wild type (Frank *et al*. 2000). The expression levels of several auxin-responsive genes (*IAA1*, *IAA2*), cell cycle genes (*CDKA*, *CycD3*) and meristem-specific genes (*KNAT1*, *STM*) were analysed in the mutant lines. Auxin primary response genes *IAA1* and *IAA2* encode the members of *Aux/IAA* protein family, which are components of auxin-mediated signal transduction pathway (Liscum and Reed 2004). The change in *IAA* gene expression is a marker of alteration in auxin signaling. Several rooty and undifferentiated callus lines were shown to have elevated mRNA levels of *IAA1* and *IAA2* genes. Several fast growing callus lines demonstrated a slight increase in *CDKA* mRNA levels. All shooty callus lines also showed increased expression of *STM* and *KNAT* homeobox genes.

Two groups of tumor-producing monogenic mutants – *tsd* and *pas* – have been identified in *A. thaliana* (Faure *et al*. 1998; Frank *et al*. 2002). Tumor formation begins soon after germination and affects the SAM, leaf primordia and hypocotyl tissues of seedlings on these mutants. Therefore, *tsd* and *pas* plants demonstrate a severe decrease in viability: the development of *tsd* mutants arrests at the seedling stage, while *pas* mutants may reach the flowering stage *in vitro* but their flowers are infertile. The peculiarity of both groups of mutants is an unchanged level of auxins and cytokinins in their tissues. At the same time, the expression levels of several cytokinin- and auxin-responsive genes in *pas* and *tsd* mutants significantly differ from the wild-type. Thus, the cause of tumor formation in *pas* and *tsd* mutants is probably associated with the alteration of cell responses to cytokinin and auxin. Moreover, both types of mutant exhibit altered expression levels of several genes involved in the control of the cell cycle and meristem formation. The same change in the expression of these genes was demonstrated for several *Arabidopsis* mutant tissue lines which were able to grow on hormone-free medium (Frank *et al*. 2000).

A group of *tsd* (*t*umorous *s*hoot *d*evelopment) mutants was identified among EMS-mutagenised M2 *A. thaliana* seedlings (Frank *et al*. 2002). All three *tsd* mutants belong to different complementation groups: the mutation *tsd1* was mapped to the chromosome 5 linkage group, *tsd2* to chromosome 1 and *tsd3* to the chromosome 3 linkage group. Severe developmental defects of *tsd* embryos were detected at stages before the bent cotyledon stage. The *tsd* mutants developed green callus-like tumor tissue instead of a SAM in the first days after germination. The difference between phenotypes of *tsd* mutants becomes apparent after 5-7 days after germination. The shoot apex of the *tsd1* mutant forms a callus-like structure which develops some aberrant leaf-like organs. Cotyledons of *tsd1* seedlings become necrotic, roots are short and deformed and stop their development soon after germination. Callus-like structure which develops in the place of the SAM in *tsd2* mutants also forms numerous leaf-like structures, and cotyledons of *tsd2* seedlings remain fused and necrotize after germination, while growth of *tsd2* roots is similar to that in the wild type. Callus-type characteristics of *tsd3* mutants differ from the *tsd1* and *tsd2* SAM of *tsd3* seedlings which start to form leaf-like structures from the leaf primordia about 7 days after germination; these structures start to form green callus-like tissue which produces new leaf-like structures from their base. Cotyledons of *tsd3* seedlings are vitreous and demonstrate epinastic growth, and roots stop to develop soon after germination. Histological analysis showed that *tsd* mutants have different defects of SAM development. The main feature of the SAM of the *tsd1* mutant is a degeneration of the L1 layer and an increase e in cell division activity in the L2 and L3 layers. The SAM of *tsd2* mutants demonstrates decreased cell adhesion and an altered cell division plan in the L2 and L3 layers. Tumor tissue of the *tsd3* mutant originnated from the base of leaf primordia. Histological analysis of *tsd1* and *tsd2* mutants proved that interactions between cell layers play a crucial role in SAM organization. It seems that a disturbance of coordinated cell divisions in SAM layers leads to overproliferation of cells and thus the tumor phenotype.

Numerous data permit the proposal that the cause of the *tsd* phenotype is the shift of cytokinin and auxin balance. Tumor tissues of all three *tsd* mutants are capable of unlimited growth on hormone-free medium. However, there is no significant difference in cytokinin and auxin levels between wild-type and *tsd* mutant seedlings. At the same time, wild-type and mutant tissues differ in their reaction to exogenous cytokinins: cytokinin levels which inhibit growth of wild-type seedlings induce enhanced growth of *tsd* mutants (Frank *et al*. 2002). Wild-type and *tsd* seedlings demonstrate no difference in morphogenetic response to auxin and giberrelic acid. These data suggest that tumor formation in *tsd* mutants is caused by a defect in the cytokinin-dependent control of cell divisions in the SAM.

It was demonstrated that *tsd* mutants differ from the wild-type in the expression of the *CKI1* hystidine kinase gene (Frank *et al*. 2002). The *CKI1* gene, which encodes a two-component histidine kinase related to cytokinin receptor *CRE1* (D'Agostino and Kieber 1999), demonstrated significantly increased transcription level in *tsd1*, *tsd2* and a little less in *tsd3* seedlings and callus compared to wild-type seedlings and callus. Overexpression of the *CKI1* gene causes cytokinin-independent callus proliferation and mimicked cytokinin effects in transgenic plants (Kakimoto 1996). Therefore, the function of *TSD* genes may consist in the negative regulation of *CKI1* gene expression. The expression levels of *CycD2* and *CycD3* genes in *tsd* mutants are comparable to those in wild-type. It is surprising because *D*-type cyclins, especially *CycD3* play the main role in cytokinin-dependent control of cell cycle (Riou-Khamlichi *et al*. 1999). However, the expression of class1 *KNOX* genes, *STM* and *KNAT1*, increased in *tsd* mutants compared with wild-type seedlings and callus. Expression of class2 *KNOX* gene *KNAT2* also increased in *tsd* mutants (Frank *et al*. 2002). The expression patterns of *KNAT1* and *KNAT2* in *tsd* mutants were investigated by promoter-GUS fusions. It was established that expression patterns of *KNAT1* and *KNAT2* genes changed in the SAM of *tsd* mutants: there were some separate regions of KNAT expression corresponding to separate regions of meristematic activities on the

callus-like aberrant SAM of *tsd* mutants (Frank *et al*. 2002). *KNOX* gene expression levels were enhanced in the response to zeatin and zeatin-*N*-glucosides (Rupp *et al*. 1999). Therefore, increased expression of *KNOX* genes may act in establishing the *tsd* phenotype and enhancing cell division rate when their expression is increased by exogenous cytokinin. Taken together, the expression data allows one to assume that *TSD* genes negatively regulate cytokinin-dependent cell divisions in the SAM.

Another group of tumor producing *Arabidopsis* mutants resembling *tsd* is *pasticcino* (*pas*) mutants (Faure *et al*. 1998). These mutants were selected from EMS- and T-DNA-induced mutant collection on the base of their abnormal reaction to cytokinins. All identified *pas* mutations belong to three complementation groups. Similar to *tsd* mutants the aerial parts of of *pas* mutant tissues showed ectopic cell division that was enhanced in response to cytokinin (Faure *et al*. 1998). However, the morphogenetic defects in *pas* mutants are less severe than those in *tsd*. The phenoltypes of *pas* seedlings are similar to wild-type shoots regenerated from explants on medium with a high cytokinin/auxin ratio (Faure *et al*. 1998). When grown in the dark *pas* seedlings demonstrated a de-etiolated phenotype; in the light these mutants have a short and thick hypocotyl and altered shape of cotyledons. Histological analysis showed that the *pas1-1* hypocotyl had an extra disorganized cell layer, decreased cell adhesion and ectopic cell divisions in the epidermis and cortex. The SAM of *pas* mutants had a variable size ranging from almost no SAM to a very large one (Vittorioso *et al*. 1998). The *pas* mutants could not live under normal growth condition, but *in vitro* some of them could reach the flowering stage. Leaves of *pas* plants were abnormally shaped and hyperhydric and flowers were sterile (Vittorioso *et al*. 1998). All *pas* mutants reacted to exogenous cytokinin by increasing cell division in the SAM and hypocotyl leading to the formation of disorganized tumor tissue (Faure *et al*. 1998). The growth responses of *pas* mutants to other phytohormones (auxin, ethylene, gibberellic acid, abscisic acid and brassinosteroids) are similar to that in the wild-type (Vittorioso *et al*. 1998). The investigation of expression of cell cycle genes (cyclin-dependent kinase *CDKA*, *B*- and *D*-type cyclins), *KNOX* genes and genes involved in the response to cytokinin (*A-type ARRs*) and auxins (*IAA* genes) showed a significant difference in their expression levels and patterns in *pas* mutants and wild-type plants (Harrar *et al*. 2003). Meristems and differentiated tissues of *pas* mutant plants demonstrated enhanced levels of *CDKA* and *CycB1* gene expression in SAMs and RAMs and also the ectopic expression of these genes in cotyledons and the hypocotyl. However, expression of the *CycD3;1* gene is enhanced only in *pas2* but is not modified in other *pas* mutants. The expression levels of meristematic homeobox genes *STM*, *KNAT2* and *KNAT6* are also increased in *pas* mutants, but the expression level of *KNAT1* in *pas* mutants is similar with that in wild-type. *KNAT2* gene demonstrates an enlarged expression pattern corresponding with the enlarged meristematic regions of *pas* mutants. Moreover, a strong allele of *stm* loss-of-function mutation may be suppressed by the *pas2* mutation. Therefore, the *PAS* genes might act as negative regulators of some *KNOX* gene expression. Ectopic expression of *KNOX* genes in *pas* mutants is very similar to their expression patterns in the plants treated by cytokinin (Hamant *et al*. 2002). The expression of *A*-type *ARR* genes (*ARR5*, *ARR6*) after cytokinin treatment of *pas* mutants is enhanced, begins earlier and terminates later after cytokinin influence has been finished. The expression of auxin response genes *IAA1* and *IAA4* are down-regulated in *pas* mutants Therefore it is probable that products of *PAS* genes might influence the response to cytokinins and auxins (Harrar *et al*. 2003). The expression of *PAS* genes mainly takes place in zones with a high level of meristematic activity such as the SAMs and RAMs. *PAS1* gene expression is affected in *pas2* and *pas3* mutants suggesting a possible molecular basis for similarity of *pas* mutant phenotypes (Vittorioso *et al*. 1998). Taken together, the data on expression of cell cycle and "phytohormonal" genes in *pas* mutants suggest that *PAS* genes are involved in the hormonal control of cell divisions in meristems.

PAS1 and *PAS2* genes of *A*. *thaliana* have recently been cloned (Bellec *et al*. 2002; Smyczynski *et al*. 2006). The product of the *PAS1* gene is a high molecular weight immunophillin, a member of the *FK506*-binding protein family (*FKBP*). Immunophillins comprise a large family of proteins with peptidylprolyl *cis-trans*-isomerase activity, which are found in all eukaryotes and have many cellular functions. Immunophilins include cyclophillins and *FKBP* subfamilies. In *A*. *thaliana* a family of 52 immunophillins was revealed. Some of them were shown to play an important role in plant development – for example, mutation in one *FKBP* gene, *TWD* (*TWISTED DWARF*) leads to serious defects in cell elongation and coordinated tissue growth (Sheidt *et al*. 2006). The *PAS1* molecule has three *FKBP12*-like domains at the N-terminal part and the C-terminal domain contains *TPR* motifs for protein-protein interactions (Smyczynski *et al*. 2006). Mutation in the C-terminal region of the *PAS1* gene results in several developmental defects typical for *pas1* mutations such as finger-shaped cotyledons and ectopic cell proliferation in the presence of cytokinin. It was shown that the C-terminal domain of *PAS1* controls the subcellular distribution of this protein and is required for interaction with the *FAN* protein (*FKBP*-associated *NAC*) which is a member of the family of plant-specific *NAC* transcriptiton factors. *NAC* transcription factors play an important role in the control of plant development and meristem function. This protein family was named after *NAM* (*N*o *A*pical *M*eristem) gene of petunia (Souer *et al*. 1996) and the *Arabidopsis ATAF1* and *CUC2* (*Cup Shaped Cotyledons 2*) genes (Aida *et al*. 1997). Expression analysis revealed that the *FAN* gene was expressed at low, constant levels at all developmental stages except in the final stage of embryo development (Smyczynski *et al*. 2006), corresponding to serious defects in embryo development in the *pas1* mutants (Vittorioso *et al*. 1998). It was shown that the C-terminal domain of the *PAS1* protein is necessary for targeting *NAC* transcription factor into the nucleus. Both proteins were detected in the nuclei of proliferating cells and were missing from the nucleus in differentiated cells. Moreover, it was shown that *PAS1* and *FAN* proteins were translocated into the nucleus upon auxin treatment of seedlings and that this process depended on the presence of the C-terminus of *PAS1* protein (Smyczynski *et al*. 2006). Finally, *FAN* protein overproduction can partially suppress the phenotype of the *pas1* mutant (Smyczynski *et al*. 2006). These data suggest that *PAS1* protein can regulate the work of one of the *NAC* transcription factors in the hypothetical pathway of cell proliferation control. This regulation involves the *PAS1*-mediated targeting of *NAC* factor into the nucleus in a hormone- and development-dependent manner.

The *PAS2* gene encodes a member of protein tyrosine phosphatase-like family (*PTPL*) (Bellec *et al*. 2002). *PTPL* proteins are conserved among eucaryotes and their peculiarrity is a mutation in the catalytic site. *PAS2*, like other *PTPL* proteins showed no phosphatase activity. In animals and yeast, *PTPL* proteins are essential for cell viability: the absence of the *PAS2* homologue in yeast has a lethal effect and in mammals leads to severe defects in myofibril differentiation (Neel and Tonks 1997). However, functions of *PTPL* proteins are still unknown. It has been recently demonstrated that the *PAS2* protein interacts with a phosphorylated form of *CDKA*, but cannot interact with its unphosphorylated form. The phosphorylation of conserved Tyr15 in the *CDKA* molecule is essential for its interaction with the *PAS2* protein. At the same time, a loss of *PAS2* function leads to the dephosphorylation of *CDKA1* and increases its kinase activity (da Costa *et al*. 2006). Thus, the tumorous phenoltype of *pas2* mutants is a result of stimulation of cell divisions by the increase of *CDKA* activity. These facts suggest that *PAS2*, like other *PTPL* proteins, may act as the competitors of active phosphatases in the interaction with phos-

phorylated substrates. The essential event for plant cell cycle progression is the dephosphorylation of *CDKA* by *CDC25* phosphatase (Zhang *et al*. 2005). Thus, *PAS2* antiphosphatase may act as an antagonist of *CDC25* in the control of the cell cycle in plants. It was shown that *CDC25* activity is nessesary for cytokinin regulation of the G2-M transition in *Nicotiana plumbaginifolia* cells (Zhang *et al*. 2005). Loss-of-function *pas2* mutants show cell dedifferentiation and hyperproliferation which are specifically enhanced by cytokinins (Faure *et al*. 1998), suggesting that *PAS2* protein and cytokinins could regulate the phosphorylation of *CDKA* by targeting *CDC25* activity (da Costa *et al*. 2006). Overexpression of *PAS2* under the *35S CaMV* promoter leads to slower cell divisions in tobacco suspension cell cultures at the G2-M transition and has a negative effect on cell expansion. *PAS2* overexpressed seedlings exhibit the inhibition of growth and delayed organ development. Therefore, the function of the *PAS2* protein involves the negative control of cell divisions via the interaction with *CDKA* and inhibition of its activity (da Costa *et al*. 2006).

Altogether, these data suggest that the products of the *PAS* and *TSD* genes act in the negative control of cell divisions. The *PAS2* protein regulates cell cycle directly via interaction with *CDKA*; other *PAS* and *TSD* proteins might control the cell cycle indirectly, probably via interaction with any transcriptional factors as was shown for *PAS1*. It is possible that *PAS* and *TSD* proteins negatively regulate cell division in meristems by down-regulation of *KNOX* gene expression. Moreover, these proteins might act as negative regulators of the cytokinin response.

CONCLUSIONS

The disturbance of systemic control of cell divisions is the cause of plant and animal tumor formation. In plants, this systemic control is carried out at three main levels. Genes encoding the components of the plant cell cycle machinery act at the first level of regulation; the second level involves genes controlling meristem development and maintenance; the hormonal regulation of this process constitutes the third level of plant cell division control. The existence of several levels in the regulation of plant cell division is typical for cascade regulation of development in multicellular organisms. The direct or indirect interactions between the components acting at different levels of plant cell division control have been shown. For example, cytokinins and auxins regulate the expression of several cell-cycle genes (Chung and Parrish 1995; Riou-Khamlichi *et al*. 1999; Roudier *et al*. 2003), *KNOX* genes and cytokinins positively regulate each other (Rupp *et al*. 1999; Yanai *et al*. 2005) and the *WUS* gene negatively regulates cytokinin signalling (Leibfred *et al*. 2005). Thus, many relations between these components form the complex network of plant cell division control. The alterations at any level of plant cell division control can lead to abnormalities of morphogenesis including tumor formation. In addition, the interaction with the components of plant cell division control was shown for several genes of *Agrobacterium* T-DNA (Stieger *et al*. 2004). The expression of these genes is essential for the development of *Agrobacterium*-induced hyperplasia (Escobar and Dandekar 2006). Homologues of *Agrobacterium* T-DNA oncogenes that were included in the genomes of *Nicotiana* species by means of horizontal transfer were shown to play a role in the induction of spontaneous tumors in tobacco interspecific hybrids (Aoki and Syono 1999b). Numerous examples of plant tumor formation are being studied at present. Different pathogen-induced tumors as well as spontaneous tumors in the interspecific hybrids, inbred lines, mutants and transgenic plants share many common characteristics. One of these characteristcs is the shift in auxin-cytokinin balance. This shift may consist in an increase of cytokinins level, as was shown for tumors induced by *A. tumefaciens* and several other pathogenic bacteria as well as for spontaneous tumors in *CHRK1*-suppressed *N. tabacum* transgenic plants, *R. sativus* inbred lines and tumorous mutants of *A. thaliana*. Another way of shifting the hormonal balance leading to tumor formation may be associated with an alteration of plant tissue sensitivity to phytohormones. The alterations in the cytokinin and auxin signalling pathways were revealed for several *Arabidopsis* tumorous mutants and tumorous radish lines (Frank *et al*. 2000; Harrar *et al*. 2003; Osipova *et al*. 2006). The other common characteristic of plant tumors is altered expression of genes directly controlling the cell cycle and genes involved in the control of meristem function. Increased expression levels of genes encoding CDKA and cyclins were shown for *Arabidopsis* tumorous mutants, *CHRK1*-suppressed transgenic tobacco plants and radish inbred lines (Frank *et al*. 2000, 2002; Harrar *et al*. 2003; Lee *et al*. 2004; Osipova *et al*. 2006). The expression of unusual *GT* cyclin was shown during the tumor formation in the *Nicotiana* interspecific hybrids (Wang *et al*. 1999, 2001). Enhansed expression of *KNOX* homeobox genes correlates with the tumor formation in *Arabidopsis* mutants, *CHRK1*-suppressed tobacco plants, radish inbred lines (Frank *et al*. 2002; Harrar *et al*. 2003; Osipova *et al*. 2006). Thirdly, increased stress response was revealed in both pathogene-induced and spontaneous plant tumors (Fujita *et al*. 1994; Bird and Koltai 2000; Lee *et al*. 2004; Chalupowicz *et al*. 2006). In the case of plant tumors of pathogenic origin stress response is caused by wounding and secretion of different biologically active compounds by pathogene (Rathjen and Moffett 2003), in the spontaneous tumors chronic stress conditions caused by cellular hormone imbalance lead to the stress symptoms and constitutive expression of stress genes (Lee *et al*. 2004). However, the precise mechanisms of activation of stress signal transduction by hormonal imbalance remains unclear (Lee *et al*. 2004).

Thus, tumor formation in different plant species correlates with a shift in the expression of many genes involved in the cell cycle control network. These circumstances make it difficult to isolate genes directly controlling the tumor formation in cases with polygenic control of this process, tobacco interspecific hybrids and radish inbred lines. To discover such genes is the task for further investigation of these models. Finally, the investigation of mechanisms controlling plant tumor formation revealed several novel proteins involved in the control of plant cell divisions. Those include *CHRK1* receptor kinase (Lee *et al*. 2003), immunofillin *PAS1* (Smyczynski *et al*. 2006), antiphosphatase *PAS2* (da Costa *et al*. 2006), *14-3-3* proteins interacting with the *rolB* protein (Moriuchi *et al*. 2004), and *ORF13* protein that directly interacts with *RB* protein. In conclusion, further study of tumor formation in higher plants would widen the knowledge about the control of plant cell division.

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