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*(Invited review paper)*

# Genetic tumors in *Nicotiana*

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**Contents**



## Introduction

The genetic tumors in *Nicotiana* were first discovered in 1930 by Kostoff, more than 50 years after the first report on the occurrence of teratomas on Brassicaceae (Caspary, 1873). The studies on genetic tumors in *Nicotiana* initiated in 1930's, peaked during the 1950's and 1960's. Initially, most of these investigations were focused on its occurrence and cytogenetics. This was an obvious and logical approach for seeking information on a genetic tumor. Results were very uniform and convincing regarding its genetic nature, as reviewed by Kehr (1951), Ahuja (1965), Smith (1972) and Bayer (1982). Subsequently, research was concentrated on the hormonal effects on, and its imbalance in genetic tumors both *in vitro* and *in vivo*. Outcomes on the measurements on the quantity of auxin and cytokinin in the tumorous tissues and on the assessments of their role in tumorigenesis were not always in agreement or encouraging. Attempts to uncover the causes of tumorigenesis failed repeatedly. Furthermore, there is virtually no information on the molecular mechanism of tumorigenesis. This hinders the

advancement of our knowledge of plant genetic tumors in the field of molecular biology as reflected by the decline of research activities in this field in recent years. The last review article on this subject was published as a book chapter almost eight years ago (Bayer, 1982).

The genetic tumors in *Nicotiana* intrigued me when I first encountered and introduced the subject in my plant development class as an example of abnormal development in plants about fifteen years ago. The major attraction of this subject was the possible link between cytokinin and genetic tumor (Kung, 1981). This eventually led me into the study of the molecular basis of genetic tumorigenesis (Xu *et al.*, 1989; Feng *et al.*, 1989). Consequently, a working hypothesis of genetic tumors was formulated (Kung, 1989). In this mini review I intend only to revisit the genetic tumors in *Nicotiana* with an open mind, to assess the role of auxin and cytokinin in tumorigenesis, to search for information in the area of molecular biology and finally to present my somewhat biased view as expressed in the working hypothesis (Kung, 1989). For those researchers who are interested in a more general aspect of this subject, I would suggest several comprehensive review articles (Kehr, 1951; Ahuja, 1965; Smith, 1972; Bayer, 1982). I believe this is an exciting area of research and I would like to generate some excitement for this subject among plant biologists particularly plant molecular biologists.

**Plant Tumors**

Tumorigenesis is one of the major unsolved problems in biology today. In animal systems this problem has been more extensively studied. The progress has been relatively slow due to its complex nature. For this reason, plant tumors have been used as experimental models (Braun, 1967) for studies designed to provide insight into certain fundamental concepts or mechanisms that underlie the tumorous state generally. This, of course, stimulated the studies of plant tumors. However, it also put the study of plant tumors itself in a secondary position. This may explain why the study of plant tumors, except crown gall tumor, has not been very active.

In this review, three of the best understood plant tumors will be briefly described and I will then concentrate on the re-visit of genetic tumors in *Nicotiana*.

1. Crown Gall Tumors: It is undoubtedly the most thoroughly studied and the best understood plant tumors to date at both cellular and molecular levels. Crown gall tumors are caused by the soil bacterium *Agrobacterium tumefaciens* (Schell, 1987). This bacterium infects dicotyledonous plants through wounding where a new wave of phenolic compounds-such as acetosyringon is made in response to injury. The acetosyringon triggers a series of enzymatic reactions on the Ti-plasmid which lead to the excision of a segment of DNA called T-DNA (Holsters *et al.*, 1982). This T-DNA is transferred and integrated into the host genome and expressed. The integrated T-DNA contains both auxin and cytokinin genes, in addition to other genes. Their expression initiates renewed cell division and enlargement, resulting in tumors.
2. Black's Wound Tumors: Black's wound tumor is caused by oncogenic viruses (Black, 1982). Some 45 plant species in 20 families have been found to be susceptible to wound tumor virus. After infection, tumors develop at restricted points of initiation, e.g. on lateral roots.

   Although our current knowledge on the structure of the wound tumor virus is quite extensive, there is no detailed account of how such tumor is formed at the molecular level.
3. Genetic Tumors: Another best studied tumor is genetic tumor. Many plant tumors have been loosely referred to as genetic tumors when no obvious external cause was identifiable. Spontaneous arising tumors in sweet clover (Littau and Black, 1950), pea (Snoad and Mathews, 1969) sorghum (Lin and Ross, 1969) and tea (Shaw and Burnett, 1968) have been termed genetic tumors although adequate genetic tests were not conducted (Smith, 1972). On the other hand, the genetic tumors occurring in interspecific hybrids may all have genetic origin. In mustard, for instance, the frequency of tumor occurrence decreased from one generation to the next. (Voskresenkaya *et al.*, 1959), although the parental plants grown under the same conditions did not form tumors. Tumors also developed on the interspecific hybrids of datura (Satina *et al.*, 1951), Japanese morning glory (Takenak and Yaneda, 1965), lily (Emsweller *et al.*, 1962), tomato (Martin, 1966) and tobacco (Kostoff, 1930),

the best studied genetic tumors.

These three types of plant tumors described are caused by three different factors; bacterial and viral infection or spontaneous formation. Although the causal factors are quite diverse the resulting abnormal growth is very similar. This underlies an important and fundamental concept in tumor biology, i.e. there exists a common mechanism which can be triggered by different devices leading to similar consequences -tumorigenesis.

## Genetic Tumors in *Nicotiana* Revisited

Genetic tumors in *Nicotiana*, first discovered by Kostoff in 1930, represent the best studied plant genetic tumors to date and is often referred to as Kostoff genetic tumors. By review the major work on this subject one may gain different insight and perspectives leading to different approaches for future research. This alone constitutes a compelling reason for this re -visitation.

### *Occurrence*

In *Nicotiana*, genetic tumor occurs only on some well-defined interspecific hybrids. This genus consists of 64 species (Goodspeed, 1954). About 300 interspecific hybrids have been made and, of these, 30 produce tumors spontaneously throughout the plant. It is well established that tumors occur only on the interspecific hybrids derived from a cross between two distantly related species. In general, parental species of the tumor-forming interspecific hybrids have been divided into two groups which have been termed "plus" and "minus" (Näf, 1958). A cross between a plus species and a minus species results in a tumorous hybrid and crosses within the plus group or minus group results in normal non-tumorous hybrids. Thus, a species-specific combination is required. Such combination supports the genetic origin of these tumors.

In addition to the 30 interspecific hybrids which produce tumor throughout the entire plant regularly, there are at least 22 others that develop similar, but restricted or irregular growth abnormalities resembling tumors (Kehr and Smith, 1954). Among the frequently studied tumorous hybrids, the hybrid of *N. glauca*×*N. langsdorffii* is highly tumorous. Much of the important information accumulated in the literature has been derived from the study of this particular hybrid.

### *Morphology*

The morphology of the genetic tumors of *N. glauca*×*N. langsdorffii* was first described by Kostoff (1930). Tumors usually develop on mature plants. Under normal field conditions and in the greenhouse, tumors appear regularly at the end of the vegetative growth phase of plants and often interfere with the flowering and fruiting. They appear on all parts of the mature plant; on the roots, stems, inflorescence, most frequently at the area of stress such as at leaf scars, leaf nodes, wounds, and the stem at the ground line. Tumors occur less frequently on leaves and when they do a distinct green island of tumorous growth is observed while the remaining portion shows signs of senescence.

Like totipotency, every cell of the tumorous hybrid is capable of forming tumor (see Section 5). At times, the entire stem can develop into encrustation of tumor tissue. The tumors first appear as small, light -colored protuberances that gradually grow into different forms of tumors. They may continue to grow into unorganized masses of stem or of root, or they may be partly organized as teratomatous growths or masses of fasciated stems. Some may grow into small plantlet. In most cases genetic tumors can be described as a cluster of tiny plantlets. Necrosis in older tumors results from an insufficient nutrient supply due to the abnormal tissue development (Smith, 1972).

In general, there are morphological similarities between genetic and crown gall tumors. However, close analysis reveals that genetic tumors exhibit more differentiated growth than crown gall tumors.

### *Cytogenetics*

Evidence is very convincing that the spontaneous tumor formation in certain *Nicotiana* interspecific hybrids has genetic origin. This first indication for genetic tumor has come from the fact that the occurrence of tumors is restricted only to certain genotypic combinations in *Nicotiana*. The gene(s) responsible for such tumor formation is located on nuclear genome since the reciprocal interspecific hybrids develop identical tumors (Smith, 1972).

The genetic origin of these tumors has been further supported by the repeated failure of many workers to detect any foreign factors as the causative agent in-

cluding bacteria, viruses or fungi (Kehr, 1951). Grafting experiments have been conducted by several investigators and no causative agent was transmitted across the graft union (Kehr and Smith, 1954).

There is considerable cytogenetic evidence to define the genetic tumors. In the case of the *N. glauca* (G) × *N. langsdorffii* (L) hybrid, both G and L genomes are required for tumorigenesis. The ratio of the genomic combinations can vary from GLL to GGGL without effecting the tumor-forming potential of the hybrids (Kehr and Smith, 1954). However, the combination of GGL formed a stronger tumor than that of GLL indicating a differential contribution of G and L genomes to the size of tumor of these species (Ahuja and Hagen, 1967). The contribution of *N. glauca* to tumor expression was far exceeding that of *N. langsdorffii*. Cheng and Smith (1973) went as far as to suggest that adding a *glauca* genome (GGL) has a stimulating effect; adding a *langsdorffii* genome (GLL) has an inhibitory effect.

Apparently, the genetic information for tumorigenesis resides on a specific chromosome. When one or a few *N. glauca* chromosomes were randomly introduced to the *N. langsdorffii* background by repeated backcrossings of the *N. glauca* × *N. langsdorffii* with *N. langsdorffii*, tumors failed to appear in the aerial parts of the plant (Kehr and Smith, 1954). On the other hand, when a single or even a fragment of a given chromosome from *N. langsdorffii* was combined with that of amphiploid *N. debneyi-tabacum* also by repeated backcrossings, tumor formed (Ahuja, 1965). Recently, Smith (1988) was able to introduce a single (specific) chromosome from *N. glauca* into the *N. langsdorffii* background to induce tumor. The evidence suggests that gene or genes responsible for tumorigenesis in *Nicotiana* species is located on a specific chromosome. The identity of this specific chromosome has not been revealed.

*Physiology*

As in crown gall tumors, genetic tumors exhibit many physiological, morphological and biochemical features that distinguish them from the non-tumorous genotypes. Among these unique features are their response to a wide range of physiological stresses which trigger tumorigenesis and their independence on hormones in tissue culture.

*Environmental stresses*: Genetic tumors are usually developed spontaneously late in the growing phase or when the young plants are exposed to stress conditions. These stress factors can be radiation, temperatures, crowding, wounding and even sexual reproduction. Applying radiation to various parts of genetic tumor-prone plants accelerates tumor formation. Tumors appear earlier than unirradiated control plants (Smith and Stevenson, 1961). The other environmental factor which accelerates tumor formation on young plants is elevated temperature at 24-27°C (Schaeffer *et al.*, 1966). Crowding also accelerates tumor formation on seedlings (Smith, 1972). The most severe and effective stress is wounding. Wounding on any part of the plant including leaf will induce tumor formation at such site (Kung *et al.*, 1989).

Generally, tumors develop in mature hybrid plants. Maturation in this case implies the transition of plant development from vegetative to reproductive growth. Sexual reproduction can also be considered as one form of stress. Thus, tumors usually appear at the flowering stage (Smith, 1972). In addition to these stressing factors mentioned above several chemical compounds such as phenol benzene derivatives, mercaptoethanol and even paint mixture can also initiate tumor formation prematurely (Kehr and Smith, 1954; Ames and Smith, 1969). Smith (1972) theorized that the tumor-prone hybrids maintain a precarious balance between normal differentiated and tumorous growth. Without external stress the plants will eventually reach a metabolic condition such as reproduction conducive to tumor formation. With a variety of stress conditions the balance can be tipped off early in development and triggers abnormal growth.

*Hormonal imbalance*: Cytogenetics and hormonal imbalance are the two most intensively studied areas in genetic tumor. While the evidence for genetic origin of the spontaneous tumors in *Nicotiana* has been convincing the information regarding the role of hormones in tumorigenesis remains unclear. It was widely believed that the tumorous hybrid produced higher than normal amounts of both auxin and cytokinins. This notion was derived from the hormonal independence of the tumorous cells in tissue culture and their ability to engage in abnormal and profuse cell division and growth. Leaves of the tumorous hybrids were reported to contain more extractable auxin than those of non-tumorous parental

species (Kehr and Smith, 1954). The highly tumorous $F_1$ (GL) and amphidiploid (GGLL) hybrids of *N. glauca*×*N. langsdorffii* contain ten times and two times, respectively, higher levels of auxin than the parental species (Bayer, 1969). On the other hand, the triploid (GLL) contained only about 10% of the extractable auxin of of $F_1$ hybrid (Bayer, 1967). Although the results showed no clear trend of auxin effect and tumorigenesis, a later report revealed correlation between the degree of tumorigenicity and endogenous auxin levels (Bayer and Ahuja, 1968). The potentially tumorous tissues showed higher levels of auxin than tissues of parental species and non-tumorous segregants. These results led to the inevitable assumption that the genetic basis for tumor formation and the regulation of hormone synthesis coincide in interspecific tumorous crosses (Bayer, 1982). However, this assumption has not been substantiated with solid evidence. The involvement of auxin in the induction of genetic tumor is still an issue surrounded with conflicting reports. Schaeffer (1962) demonstrated that cytokinin in the presence of auxin exerted a strong influence on tumor induction in *Nicotiana* seedlings. Eighty-six percent of the treated seedlings produced early tumors. Treatment with auxin alone had no effects whereas cytokinins alone triggered tumor formation in 44% of the seedlings.

The role of auxin in genetic tumor formation in *Nicotiana* was the subject of investigation by Ames and his colleagues for many years. It was observed that there is a close correlation in time between a decline in endogenous auxin levels and the onset of tumor formation in hybrids (Ames and Mistretta, 1975; Ames, 1974). This was in agreement with an earlier report, also by his group that auxin treatment inhibited tumor formation. When high concentration (10%) of auxin was applied to the decapitated plants no tumors or teratomas developed (Ames *et al.*, 1969). This experimental observation is compatible with the natural occurrence of genetic tumors in hybrids plants; tumors develop most frequently in plants at the end of their vegetative and during the reproductive phases. The endogeneous levels of auxin are low at this stage of plant life (Schwabe, 1973). This was in direct conflict with earlier assumptions that there is a close correltion between high auxin levels and tumor formation.

The nature of auxin involvement in tumorigenesis as indicated in literature is complicated, if not confusing. Initial observations favor the direct involvement of auxin in tumor formation; there exists a positive correlation between higher levels of auxin and frequent occurrence of tumors (Kehr and Smith, 1954; Bayer and Ahuja, 1968). This was tempered by later reports that auxin had no effect on tumor induction (Schaeffer, 1962). To complicate this issue further, Ames' group demonstrated that it was the decline of auxin level which was associated with tumor formation (Ames, 1974). It is likely that auxin alone does not play a significant role in tumor formation in *Nicotiana*. This concept gained a strong support recently from the evidence provided by Chen *et al.* (1987). By using a more defined and accurate approach they detected no significant difference in the amount of measurable auxin between the tumorous hybrids, the non-tumorous mutant and their normal parental species. Because of the advancement in technology and instrumentation this may represent the most accurate and reliable measurement on the content of auxin in those plants to date.

The involvement of cytokinins in genetic tumorigenesis has not been as intensively studied as that of auxin. Thus, the role of cytokinins in genetic tumor formation remained obscure for a long time. The first indication that cytokinins may be involved in genetic tumors was the hormonal independence of tumorous tissues grown *in vivo*. Schaeffer (1962) was the first to show that cytokinins induced tumor formation on otherwise normal growing hybrid seedlings. This cytokinin effect was confirmed by Ames ten years later by showing that cytokinin accelerates the tumor incidence in several tumor-prone *Nicotiana* interspecific hybrids (Ames, 1972). Although there is virtually no further investigation on the role and function of the cytokinins in triggering the genetic tumor, a close link between cytokinin and tumor formation was proposed (Kung, 1981). This is supported by the evidence that the high level expression of isopentenyl transferase (ipt) gene in crown gall tumors resulted in correspondingly high level of cytokinin in these tissues (Akiyoshi *et al.*, 1983). This phenomenon drew considerable attention among the workers who have an interest in studying genetic tumors. Careful analysis of the morphology of genetic tumors points to the fact that it resembles a cytokinin effect (Kung *et al.*, 1989). In most cases, genetic tumors can be considered as a cluster of mini plantlets. Leaf tumors can delay senescence as cyto-

kinin can (Kung *et al.*, 1989). Both biological titrations and analytical determination illustrate that tumorous tissues contain additional level of cytokinin than their parental species. The total cytokinin level is over three times higher in the tumorous hybrid than their parental species (Palni, personal communication). Similar results were obtained by Feng *et al.* (1989) who reported that the cytokinin level was seven times higher in the tumorous hybrid than in the non-tumorous mutant. Upon the genetic transformation of the non-tumorous mutant by ipt gene, the cytokinin level increased almost twenty times (Feng *et al.*, 1989). Accompanying the increase of cytokinin content was the restoration of the tumorous phenotype of the non-tumorous mutant, thus suggesting a close correlation between cytokinin level and tumor formation.

Apparently there is a drastic increment in cytokinin level whereas the auxin level remains unchanged in genetic tumors. This, of course, tips off the natural balance of auxin and cytokinin in plants. Regardless of the molecular basis of genetic tumorigenesis, it is clear that it is the cytokinin that plays a pivotal role in genetic tumors.

*Protoplast Fusion and Tissue Culture*

The genetic origin of the spontaneous plant tumors was further demonstrated in 1972 by Carlson *et al.*, who fused protoplasts from *N. glauca* and *N. langsdorffii*. Only the fused products between those two species could grow on the MS medium without exogeneously added auxin and cytokinins (Carlson *et al.*, 1972). This biochemical and physiological feature constitutes a useful selective marker for fusion products. More importantly, the protoplast fusion experiment illustrated the cellular location of the inheritable material substance of genetic tumors. All somatic hybrids, grown to maturity, were fertile, tumorous, and produced tumorous hybrids. In spite of a divergent chromosome number among the hybrid progenies ranging from 56 to 64 instead of 42, tumor formed in all cases. This again provides excellent evidence for the genetic nature of the spontaneous tumors and the requirement of hybridity.

In tissue culture the tumorous tissue from the hybrid of *N. glauca*×*N. langsdorffii* exhibited remarkable hormonal independence. For example, the leaf disks from this tumorous hybrid produced callus vigorously on MS medium without added auxin and cytokinin which are essential for normal plant tissues to grow under similar conditions. Addition of auxin or cytokinin alone or in combination at a wide range of concentrations altered very little the pattern of growth and the shooty morphology of the callus (Kung *et al.*, 1989). This is in conflict with earlier report that addition of auxin at all levels inhibited the differentiation of tumor tissues (Ahuja and Hagen, 1966). Concomitantly with the shooty morphology, the tumorous tissue contains a higher level of cytokinin. This was demonstrated by adding excess amounts of auxin (12 mg/L) to the MS medium without causing the leaf disks from tumorous hybrid to initiate root systems. While the leaf disks from the non-tumorous mutant or the parental species developed root systems when less auxin (1-6 mg/L) was added (Kung *et al.*, 1969). Again, this illustrates that the tumorous tissue contains higher than normal amounts of cytokinin. Current evidence indicates that the tumorous tissues contain several fold higher cytokinin than the non-tumorous mutant or parental species whereas the auxin level remains constant in tissues of tumorous as well as non-tumorous plants.

## Molecular Biology of Plant Genetic Tumors

Since there are only a few molecular biologists who are familiar with genetic tumors, there is very little information on the molecular biology of genetic tumors. Based on the knowledge gained from studying crown gall tumors, T-DNA was at one time suspected to have a role in genetic tumors. There was no evidence on T-DNA's involvement. Ri T-DNA was found to share sequence homology with nuclear DNA of several *Nicotiana* species but no correlation with tumor formation was established. The genetic transformation of non-tumorous mutant with ipt gene opens the door of studying molecular biology of genetic tumors. It is hoped that this approach together with others (Durante *et al.*, 1986) will bring us a step closer to understanding the molecular mechanisms of genetic tumors.

*Ti-DNA*

Genetic and crown gall tumors share many physiological, morphological, histological and biochemical similarities (Ahuja, 1965; Smith, 1972; Bayer, 1982). Because of these similarities, they could also share

similar causes. Since T-DNA has been identified as the cause of crown gall tumors, the T-DNA was suspected to cause genetic tumors. Many workers have tested this possibility and found no plant DNA sequence homologous with T-DNA. When the pTi B6S3 T-DNA was used as probe no sequence homology with nuclear DNA could be detected from thirteen species and nine tumorous hybrids in *Nicotiana* (Xu *et al.*, 1989). The genetic tumors are caused by a different mechanism than that which caused the crown gall tumors. This supports the notion that not every tumorous cell contains T-DNA and not every T-DNA containing cell proliferates (Holsters *et al.*, 1982).

*Ri-DNA*

The discovery of sequence homology of Ri T-DNA in several *Nicotiana* species (Furner *et al.*, 1986; Xu *et al.*, 1989) raised the possibility that it may be involved in genetic tumors in *Nicotiana*. Xu *et al.* (1989) reported that of the thirteen *Nicotiana* species tested only three; *N. glauca*, *N. tomentosiformis*, *N. tabacum* contain DNA sequence homologous with the left arm of Ri T-DNA. This sequence was found in all six cultivars of *N. glauca*. However, there is no causative relationship between the Ri T-DNA and tumor formation (Xu *et al.*, 1989). It is present in both the tumorous and non-tumorous hybrids of *N. glauca*×*N. langsdorffii*. Furthermore, it is absent in the tumorous hybrids of *N. suaveolens*×*N. plumbaginifolia* and *N. gossie*×*N. longiflora* (Xu *et al.*, 1989). The origin and function of Ri T-DNA in plants is unknown.

*Genetic Transformation of Non-tumorous Mutant with ipt Gene*

With the availability of a non-tumorous mutant, the genetic tumors of *Nicotiana* provide a valuable system for gaining further insight into the molecular mechanisms leading to spontaneous tumor formation. These non-tumorous mutants of the hybrids of *N. glauca*×*N. langsdorffii* have been obtained by x-ray treatment (Izard, 1957) as well as by spontaneous mutation (Smith, 1988). Cultivation of leaf disks from the non-tumorous mutant showed that they required both auxin and cytokinin in the medium for callus formation. However, when an excess of cytokinin (0.8 mg/L) was added to the MS medium, the callus from the leaf disks of the mutant mimicked the morphology of that of the tumorous growth (Feng *et al.*, 1989). This points to the possibility that the mutation may have affected cytokinin production. One way to remedy this defect is to transform this mutant with the cytokinin biosynthesis gene from T-DNA. Insertion and expression of this ipt gene from T-DNA in the mutatn leaf disk cells restored the tumorous morphology (Feng *et al.*, 1989). They demonstrated that the tumorous phenotype of a non-tumorous mutant can be resotred by cytokinin, whether exogeneously applied or endogenously produced by transformation. Under no conditions did auxins alone confer this effect (Feng *et al.*, 1989). This argues that it was the presence of excess cytokinin not auxin that contributes to the tumorous growth. It should be noted that it is the excess or additional level of cytokinin which induces the tumor formation. The mutant like the parental species, contains the normal or physiological amount of cytokinin for normal growth and development.

*Comparison of the Physicochemical Properties Between the Tumorous and Non-tumorous Genomes*

In 1982, Durante *et al.*, reported that DNA heterogeneity exists in *Nicotiana* tumorous and non-tumorous genotypes. Subsequently, they expanded the study to provide data and show that both qualitative and quantitative difference in DNA complexity can be correlated with the tumorous transformation (Durante *et al.*, 1986). They went on to suggest that the non-tumorous mutant hybrid of *N. glauca*×*N. langsdorffii* may carry a deletion responsible for the phenotype. This deletion is on the *N. langsdorffii* genome and is responsible for the surprisingly high degree of mismatching found in reassociation experiments between DNA's extracted from tumorous and non-tumorous tissues (Durante *et al.*, 1986). In the non-tumorous mutant there is a high percentage of highly repetitive sequences than in the tumorous hybrid. Furthermore, DNA bound ion contents were found to be higher in tumorous than non-tumorous tissues. This posts an interesting perspective since the preserve of metal-DNA complexes at the regulatory sequences may induce conformational variations and modifications in the binding of effector molecules. All those observations need further study at the molecular level for functional confirmation. Since the materials used in these comparative studies were long-term *in vitro* cultures, the variations induced by such practice deserves serious consideration.

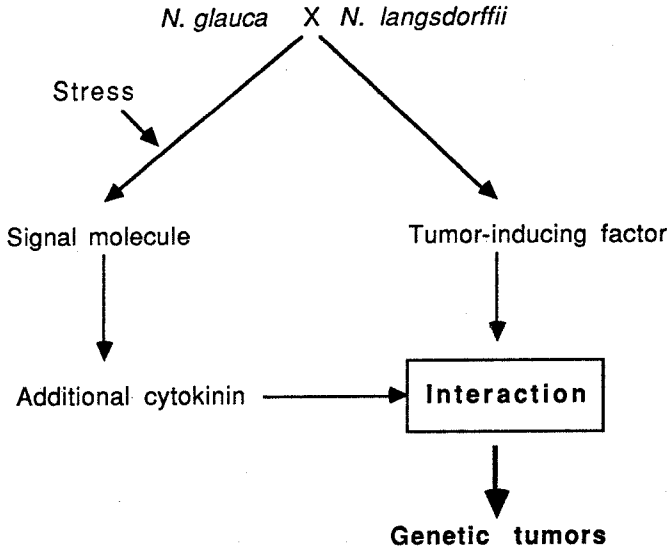


Fig. 1. The essential components and their sequential arrangement in the development of genetic tumor in *Nicotiana* hybrids. (Kung, 1989).

## Role of Cytokinin in Genetic Tumors-a Working Hypothesis

Based on the available evidence as reviewed here a working hypothesis was proposed (Kung, 1989). It states that the tumorigenesis in *Nicotiana* equires at least three components arranged in a sequential order for inducing tumors (Fig. 1). Since this is a genetic tumor, a genomic combination or complimentation is essential. Such combination is believed to produce an important factor, named tumor-incuding factor (Ti -factor). Although this hypothetical factor has not been identified, the evidence for genomic combination is overwhelming (see cytogenetics section). When this factor interacts with an additional level of cytokinin abnormal growth, e.g. tumor results. The most convincing evidence to support this is the induction of tumor formation on otherwise normal growing hybrid seedlings by cytokinin treatment (Schaeffer, 1962; Ames, 1972). Under no conditions did the parental species produce tumor with the same treatment. The source of this additional level of cytokinin can be either exogenously-applied or endogenously-produced by transformation with ipt gene (Feng *et al.*, 1989). Under natural conditions, this additional level of cytokinin in the tumorous hybrids can be generated by applying physical stresses. In this working hypothesis, it is proposed that the stress triggers the synthesis of small signal molecules, e.g. a phenolic compound (Kung, 1989). Such compound(s) switches on the synthesis of an additional level of cytokinin. This is the weak link in this proposal. No evidence to substantiate this claim, except that the tumorous tissue contains several fold higher cytokinin than the normal plants (Feng *et al.*, 1989). This additional level of cytokinin interacts with the Ti-factor already present in the interspecific hybrids to trigger tumor formation (Fig. 1).

## Remarks

The occurrence of genetic tumors provides plant molecular biologists an excellent opportunity to study the molecular basis for spontaneous tumorigenesis and the regulation of gene expression. In the past, focus has been placed on its occurrence, cytogenetics and hormonal effect. Very little effort has been directed on its molecular mechanisms. Evidence accumulated to date demonstrates beyond any doubt that the spontaneous tumors have genetic origin. However, the involvement of hormones in genetic tumors deserves a close re -examination. Initially, much emphasis was put on the role of auxin in tumorigenesis. The earlier observation on hormonal independence in tissue culture led to the conclusion that tumor tissues possess the capacity to synthesize higher level of auxin and cytokinin. Due to the research interest and techniques available at that time, auxin was intensively investigated for its role in genetic tumors. This led to the belief that auxin was directly involved in genetic tumorigenesis. It was reported that in tumorous tissues there is a higher rate of auxin synthesis than in the non-tumorous tissues (Liu *et al.*, 1978). This is supported by Bayer and Ahuja (1968) who showed correlation between the degree of tumorigenicity and endogenous auxin levels. All these observations are now challenged by the evidence provided by Chen *et al.* (1987). By employing more advanced technology they detected no difference in auxin (free or conjugated) level between the tumorous, non-tumorous hybrids and their parental species. Thus, the concept of auxin involvement in genetic tumorigenesis should be re-evaluated.

In contrast to auxin, the involvement of cytokinin in genetic tumors has not been actively investigated until recently. Current evidence indicates that cytokinin is involved in tumorigenesis in *Nicotiana*. The

earlier reports of Schaeffer (1962) and Ames (1972) demonstrated that cytokinin accelerated the frequency of tumorigenesis. The recent demonstration of restoration of tumor phenotype of non-tumorous mutant by cytokinin treatment and the transformation with cytokinin biosynthesis gene (Feng *et al.*, 1989) supports the role played by cytokinin. This is in agreement with the reports that tissues from genetic tumors contain several fold higher cytokinin than the non-tumorous plants.

The time is now right for us to modify our concept on the hormonal involvement in genetic tumors. It is clear that it is the cytokinin not auxin which plays a pivotal role in genetic tumors. We intend to monitor the cytokinin level during the entire growing period of the tumorous hybrid and the non-tumorous mutant to establish a firm foundation for the cytokinin involvement in genetic tumors. Ultimately, we plan to isolate and identify the cytokinin biosynthesis gene from plant and to study the requlation of its expression.

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