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Growth inhibition of crown-gall tissues in relation to the structure and activity of DNA

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The growth of crown-gall cells cultured in vitro (*Nicotiana tabacum* L. cv. White Burley and *Parthenocissus tricuspidata* cv. Veitchii) is inhibited by alstonine (BG-8), a plant alkaloid, the anti-cancer effect of which has previously been demonstrated on animals and plants. The growth of normal cells is only slightly affected. The inhibitory effect of BG-8 on crown-gall cells is antagonized by indole-3-acetic acid (IAA) added to the culture medium. Kinetin associated with IAA does not prevent this inhibitory effect. BG-8 present in the culture medium containing the two types of hormones seems to modify the later hormonal requirement of *Parthenocissus* crown-gall tissues.

BG-8 exhibits high binding affinity for crown-gall DNA and, therefore, strongly inhibits its in vitro synthesis. The alkaloid has practically no effect on DNA from healthy cells. The inhibition by BG-8 is dependent on the level of DNA strand separation and on the origin and nature of the tissues (crown-gall DNA is more destabilized than healthy DNA; DNA from habituated tissues is intermediate). IAA and kinetin have opposite effects on the in vitro strand separation of the DNAs from crown-gall cells and, consequently, antagonistic effects on DNA replication (IAA stimulates and kinetin inhibits). It is possible to establish a close relationship between in situ development of crown-gall tissues of the two species studied (in the presence or absence of BG-8 or cell-growth factors), in vitro DNA synthesis and DNA strand separation.

Additional key words – Anti-cancer drug, DNA strand separation, DNA synthesis, IAA, kinetin, *Nicotiana tabacum, Parthenocissus tricuspidata.*

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Introduction

Healthy plant and animal cells may be transformed into cancerous cells and vice versa, which implies the existence of an essentially reversible biochemical mechanism. This hypothesis emerges from different experimental findings. Thus, normal plant tissue cultures maintained in the presence of phytohormones may acquire the specific capacity for autonomous growth of the crown-gall tissue so that they no longer require the addition of auxin (Gautheret 1959) and/or cytokinin (Fox 1963) for growth in vitro; and they are then said to be habituated. On the other hand, the neoplastic state of cloned cell lines from crown-gall teratoma of tobacco may be suppressed under appropriate experimental conditions, leading to a "normalization" of the tumor cells (Braun and Wood 1976). In addition, teratocarcinoma cells of mice lose their malignant characteristics when introduced into healthy embryos (Mintz and Illmensee 1975); and at extremely low concentrations, tumor-promoting agents, some of which have no mutagenic properties, can induce the differentiation of human leukemic cells into normal cells (Huberman and Callaham 1979). All these data are suggestive of altered

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gene expression in healthy cells (or cancerous cells) leading to their transformation into cancerous cells (or normal cells) (McKinnel et al. 1969, Braun and Wood 1976).

All the substances mentioned (plant and animal hormones, carcinogens and carcinogen-like chemical agents) induce a modulated separation of DNA double strands (Beljanski 1983). This phenomenon is unimportant for normal DNA (Mong et al. 1981), but, for cancer DNA, complete DNA strand separation may be observed (Beljanski 1979, Beljanski et al. 1981, Le Goff and Beljanski 1981, 1982). It seems likely that such specific DNA strand separation is an important step which regulates gene expression. Potent carcinogenic substances tend to separate locally the double strands of DNA thus allowing access of DNA and RNA polymerases. This permits an acceleration of DNA synthesis in vitro and of cancerous cell multiplication in vivo (Beljanski 1979, Le Goff and Beljanski 1979, Beljanski et al. 1981). On the other hand, chemical substances opposed to DNA separation prevent the release of genetic information, and therefore the growth of the tissues concerned (Beljanski 1983). Consequently, it is conceivable that the control of the systems involved in activation and inactivation of the genes permits a persistent but potentially reversible suppression of a cancerous state, leading to a normalization of the cancerous cells and vice versa.

The present paper describes experiments to approach this problem by using habituated and crown-gall tissues, which are a model of an unbalanced physiological state in plants. We have studied the correlation between the growth of these tissues in vivo on a synthetic culture medium, and the in vitro DNA replication under the influence of substances acting on double-stranded DNA. We have used two hormones, IAA and kinetin, which induce cell enlargement and division, respectively (Jablonski and Skoog 1954); and a plant alkaloid (alstonine, BG-8) which acts as a selective anti-mitotic drug in cancerous cells of animals (Beljanski and Beljanski 1982) and plants (Beljanski et al. 1982).

Abbreviations – BA, N^6-benzyladenine; BG-8, alstonine; 2,4-D, 2,4-dichlorophenoxyacetic acid; DNA, deoxyribonucleic acid; IAA, indole-3-acetic acid; RNA, ribonucleic acid; 1 x SSC, standard saline citrate (0.15 *M* NaCl, 0.015 *M* Na-citrate, pH 7.0); TCA, trichloroacetic acid; Tris, tris (hydroxymethyl) amino methane; TTP, (methyl-^{3}H) thymidine-5'-triphosphate.

Materials and methods

Cloned tissue lines of *Parthenocissus tricuspidata* cv. Veitchii and *Nicotiana tabacum* L. cv. White Burley, cultured in vitro, were used throughout. Healthy tissues were taken from callus obtained from inverted stem sections of *Parthenocissus* plants grown under axenic conditions. Habituated tissues were obtained in our laboratory from healthy callus tissues after prolonged culture in the presence of hormonal substances (2,4-D and BA), which are initially a prerequisite for tissue proliferation. The habituated tissues appear morphologically similar to crown-gall tumor tissues and may grow on a basal culture medium without exogenous hormones. Cultured crown-gall cells of tobacco came from primary tumors induced by *Agrobacterium tumefaciens* (Smith and Town) Conn. strain B_6, those of *Parthenocissus* originated from primary tumors induced by *A. tumefaciens* strain A_6. Those crown-gall tissue lines were supplied by Prof. R. J. Gautheret (P. and M. Curie Univ., Paris).

Agar-cultured cells

Healthy callus tissues of *Parthenocissus* were grown on the medium of Nitsch (1969) supplemented with 2 μg ml^{-1} (9 μ*M*) BA and 0.02 μg ml^{-1} (0.1 μ*M*) 2,4-D, and crown-gall tissues on the medium of Heller (1953). Auxin-dependent callus of tobacco (healthy control tissues) was excised from inverted stem sections and transferred to a modified Murashige and Skoog medium (Murashige and Skoog 1962) without glycine and casein hydrolysate but containing 1 μg ml^{-1} (3 μ*M*) thiamine supplemented with 0.3 μg ml^{-1} (1.7 μ*M*) IAA and 0.3 μg ml^{-1} (1.4 μ*M*) kinetin. Crown-gall tissues of tobacco were maintained on the modified Murashige and Skoog medium with or without IAA or kinetin (or both) at variable concentrations as noted later in the text. The substance BG-8 was mixed with culture media at concentrations between 1 and 10 μg ml^{-1} (3 to 30 μ*M*, final concentration).

Suspension-cultured cells

Callus tissues crushed with a pair of pliers were placed in the appropriate basal liquid medium (see above) and dissociated by shaking for 48 h. Ten ml of cell suspensions containing single cells and small clumps were pipetted into 50 ml Erlenmeyer flasks. Growth hormones and BG-8 were added to or omitted from the suspension as indicated for solid media [5 μg ml^{-1} (15 μ*M*)]. The flasks were incubated for 8 or 15 days on a gyratory shaker (New Brunswick Scientific Co., 120 rpm) at 24–26°C in continuous light (0.24 W m^{-2}) from Sylvania "lifeline" fluorescent lamps. The cultures were passed through filter-papers (Durieux no. 111), and the filtered tissues dried at 50°C for 48 h. All treatments were carried out in 15 replicates, and the experiments were repeated three times. Growth was measured on a dry-weight basis: we compared the weight of 15 experimental filtered cultures with that of 15 initial cultures.

In some experiments, crown-gall cell clumps incubated in a liquid culture medium containing an aqueous solution of BG-8 (distilled water in control) were transferred to an analogous solid culture medium without BG-8, and aqueous solutions of growth hormones were added to the basal medium at concentrations usually required for normal tissue proliferation (see above).

The investigation was performed under aseptic conditions. Basal media and solutions of growth hormones were sterilized by autoclaving at 110°C for 20 min. Distilled water solution of BG-8 was sterilized by filtration (Millipore 0.45 μm).

Isolation and characterization of DNA

Growing plant tissues, both healthy and crown-gall, were frozen at −20°C and then lyophilized or immediately used for extraction of DNA. They were homogenized in a manual Potter-Elvehjem type homogenizer with a minimum volume (v/w, 2:1) of Lerman's solution, pH 8.0 (Lerman and Tolmach 1957), supplemented with 8-hydroxyquinoline (14 m*M* final concentration) (Thompson and Cleland 1971) and sodium laurylsulfate (7 m*M* final concentration). The gauze-filtered suspension was treated once with a phenol-distilled water solution (v/v, 9:1) containing 14 m*M* 8-hydroxyquinoline and twice with chloroform-isoamylic alcohol (v/v, 19:1). The homogenate was incubated in the mixture defined above for 5 min at 37°C and then treated with phenol and chloroform. After centrifugation at 5000 *g* for 10 min, the upper phases of all tubes were mixed, and two volumes of 96% alcohol were added. RNA, which contaminates DNA preparations, was practically eliminated by incubation with RNase A (aqueous solution preheated at 95°C for 10 min) in a 0.2 x SSC solution (20 μg of enzyme ml^{-1} for 30 min at about 25°C). The reaction was stopped by addition (v/v) of the phenol solution. The enzyme was removed by this phenol treatment followed by three chloroform treatments (see above), each of which was ended by centrifugation. DNA was precipitated by two volumes of 96°C alcohol, dissolved in a minimum volume of 2.0 x SSC solution and dialysed against 1.0 l of the same solution for 18 h. The operations were carried out at 4°C, using oven-sterilized glass and newly distilled alcohol and water. The amount of DNA was determined by absorbance at 260 nm. We ascertained that the purified DNA preparations (A 260/ A 280 = 2.0) contained less than 10% (w/w) of RNA and 1% (w/w) of protein. The integrity of DNA was verified by ultracentrifugation in an alkaline sucrose gradient. Moreover, only DNA preparations were used where the hyperchromic effect after incubation with 0.2 *M* NaOH (or 0.2 *M* KOH) was 35–45%.

In vitro DNA synthesis

DNA-dependent DNA polymerase I, partially purified from *E. coli* strain K 12 as previously described (Beljanski and Beljanski 1974), was used for in vitro DNA synthesis. The incubation mixture (Beljanski 1979) contained per ml: Tris-HCl pH 7.65, 170 μmol; $MgCl_2$, 13 μmol; four deoxyribonucleotide-5′-triphosphates each 33 nmol + labelled TTP 12.5 kBq (of specific activity 30 Ci $mmol^{-1}$); purified DNA 3.5–7 μg; compound to be tested, 0–120 μg depending of the substance used (see Figs); DNA-dependent DNA polymerase I 265–530 μg. Final volume of the incubation mixture was 0.15 ml. After incubation at 36°C (10 min) the acid-precipitable synthesized material was filtered onto a Whatman GF/C glass filter, washed with 5% TCA and dried, and the radioactivity [(^{3}H)-DNA] measured in a Packard spectrometer. Results were expressed as Bq units.

Hyperchromicity of DNA

DNA (10 μg) was dissolved in 0.01 *M* Tris-HCl (pH 7.65). The substances to be tested were dissolved in distilled water and then added to the DNA solution and to the blank (solution without DNA). The mixture (final volume 1 ml) was gently shaken at 20–25°C, and its UV spectrum was determined. Concentrations inducing the maximum increase in A 260 nm were determined for each DNA preparation used. Results were expressed as UV absorbance increase (%).

Results

Action of BG-8 on the growth of healthy and crown-gall callus tissues

Agar-cultured normal tissues were almost insensitive to the action of BG-8 as compared to the responsive crown-gall tissues (Fig. 1). A rapid change in the growth of the crown-gall tissues and the inhibiting effect were observed as a function of the concentrations used. An

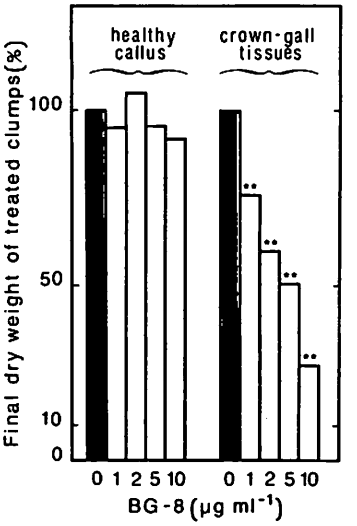


Fig. 1. Effect of BG-8 on the growth of healthy and crown-gall tissues of *Parthenocissus* maintained for one month on a solid culture medium. Percentage weight increase or decrease of BG-8-treated tissues is expressed relative to control without BG-8.
Control values (mg) ± SE of the mean: healthy tissues 284 ± 28; crown-gall tissues 346 ± 20. **, indicates that the difference between growth of treated and untreated tissues is significant at the 1% level (Student's *t*-test).

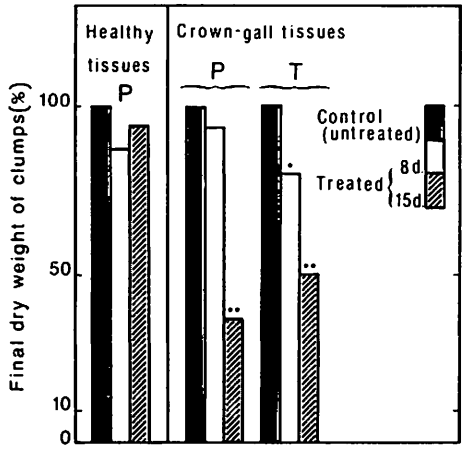


Fig. 2. Effect of the duration of BG-8 (5 μg ml^{-1}) treatment on the growth of healthy and crown-gall cell clumps maintained on liquid culture medium. Percentage weight decrease of BG-8-treated tissues is expressed to control without BG-8.
Control values (mg) ± SE of the mean: healthy tissues (P = *Parthenocissus*) 16 ± 3 (8 days) and 46 ± 6 (15 days); crown-gall tissues (P = *Parthenocissus*) 43 ± 3 (8 days) and 40 ± 4 (15 days); crown-gall tissues (T = tobacco) 19 ± 3 (8 days) and 171 ± 7 (15 days).
*,** indicates that the difference between growth of treated and untreated tissues is significant at the 5% and 1% levels, respectively (Student's *t*-test).

amount of 10 μg BG-8 per ml of culture medium leads to about 75% inhibition of tumor growth. Results obtained with suspension-cultured tissues (Fig. 2) corroborate the above observations. The growth-inhibiting effect depends on the duration of the treatment: it is two or three times greater after a 15 day-contact period than after an 8 day-contact period. Similar results were obtained for both *Parthenocissus* and tobacco (Fig. 2).

Interaction between growth substances and the anti-tumor drug

The growth-inhibiting effect of BG-8 on crown-gall tissues, which was large in the absence of IAA and kinetin for a 15 day-culture period (Fig. 2), was relieved when these two hormones were present in the culture medium (Fig. 3). Furthermore, in the presence of the anti-tumor drug in the culture medium, IAA used alone increased cell proliferation as compared to the control (Fig. 3). Among various concentrations used, 0.1 μg ml^{-1} (0.6 μ*M*) of IAA was the most effective. Similar results were obtained with tobacco crown-gall tissues (results not shown).

After transfer of clumps of suspension-cultured cells to a solid culture medium with or without IAA and ki-

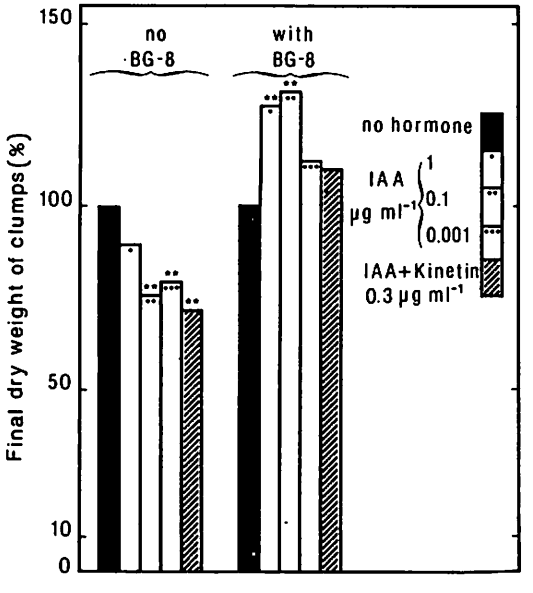


Fig. 3. Interaction between IAA or (IAA + kinetin) and BG-8 on the growth of *Parthenocissus* crown-gall. Cell clumps were maintained for 15 days on liquid medium supplemented with or without (control) BG-8 (5 μg ml^{-1}), IAA (0.001, 0.1, 1 μg ml^{-1}) or IAA (0.3 μg ml^{-1}) with kinetin (0.3 μg ml^{-1}). Results are expressed as percentage weight increase in relation to the appropriate "no hormone" control. Control values (mg) ± SE of the mean: no BG-8 (untreated) 58 ± 3; treated with BG-8 49 ± 2.
**, indicates that the difference between growth of treated and untreated cell clumps is significant at the 1% level (Student's *t*-test).

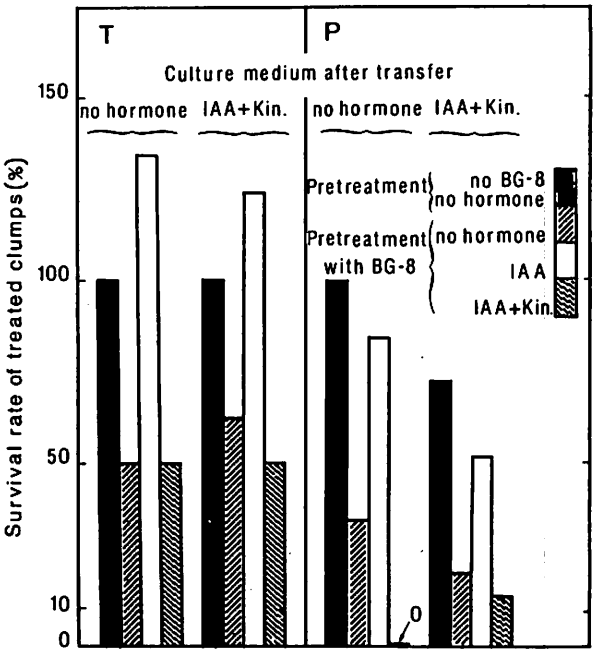


Fig. 4. Survival rate of crown-gall tissues of tobacco (T) and *Parthenocissus* (P) after BG-8 treatment. Clumps were pretreated with 5 μg ml^{-1} for 15 days in liquid medium in the presence or absence of 1 μg ml^{-1} IAA or of 0.3 μg ml^{-1} IAA + 0.3 μg ml^{-1} kinetin. The clumps were then transferred to a BG-8 free solid medium with or without hormones. Results are expressed relative to the control (no hormone, no BG-8 during the pretreatment and no hormone in the culture medium after transfer). All differences between experimental values and control values are significant at the 0.1% level (χ^2-test).

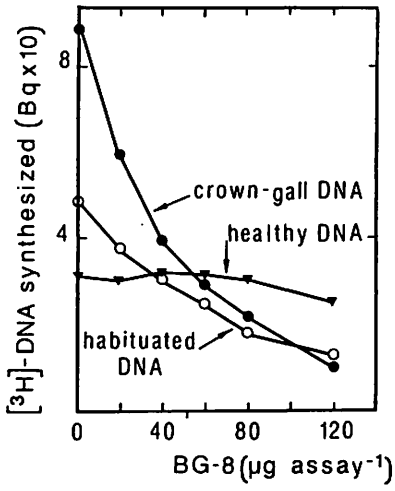


Fig. 5. Effect of added BG-8 on in vitro synthesis of DNA from healthy, habituated and crown-gall tissues of *Parthenocissus*. Each type of DNA was used as template (see methods) in the absence or presence of different concentration of BG-8, 20–120 µg assay^{-1} (370 µ*M* – 2.2 m*M*, final concentration).

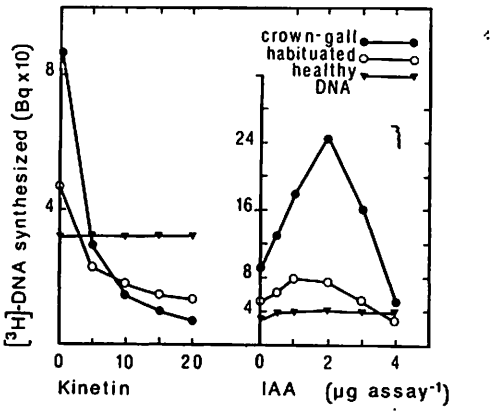


Fig. 6. Effects of kinetin and IAA on in vitro synthesis of DNA from healthy, habituated and crown-gall tissues of *Parthenocissus*. Each type of DNA was used as template (see methods) in the absence or presence of kinetin or IAA as indicated.

netin, the survival rate of the BG-8 pretreated tissues was lower than the untreated ones (Fig. 4). This decrease was less for clumps of crown-gall cells from tobacco than from *Parthenocissus*, which would therefore appear more sensitive. If the pre-culture had been carried out in the presence of both BG-8 and 1 µg ml^{-1} (6 µ*M*) of IAA, the hormone always alleviated the inhibitory effect of BG-8. The survival rate of *Parthenocissus* cell clumps thus treated was, therefore, nearly the same as for the control. In the case of tobacco tissues, IAA associated with BG-8 increased the survival rate of treated clumps as compared to the control. When crown-gall suspensions were treated by BG-8 in the presence of both IAA and kinetin, the effects observed after transfer to a solid medium depended on the tissue line used. For tobacco tissues, the association of (IAA + kinetin) and BG-8 during the pretreatment did not alter the growth-inhibitory effect of the anti-tumor drug. For *Parthenocissus*, the effect of pretreatment with IAA and kinetin associated to BG-8 was a further decrease in the survival rate after transfer, independently of the presence or absence of IAA and kinetin in the culture medium after transfer. These results emphasize the high sensitivity of *Parthenocissus* tumor clumps to exogenous growth substances, whereas tobacco tissues are less sensitive.

In vitro synthesis of cultured plant cell DNA

DNA purified from crown-gall cells behaves in vitro very differently from DNA of corresponding healthy cells (Fig. 5). In vitro DNA synthesis was greatest when crown-gall DNA was used as template. DNA isolated from habituated cells exhibited an intermediate template activity and DNA from healthy tissues the least. Moreover, BG-8 strongly inhibited the in vitro synthesis of *Parthenocissus* crown-gall DNA, while it was almost without effect on DNA synthesis from the auxin-dependent callus of the same plant species and intermediate when habituated callus DNA was used.

Figure 6 demonstrates the very different effects of IAA and kinetin on the in vitro synthesis of DNA from *Parthenocissus* tissues. When crown-gall DNA was used as template, low concentrations of IAA strongly stimulated DNA synthesis. The stimulating action of IAA on synthesis of DNA from habituated tissues was detec-

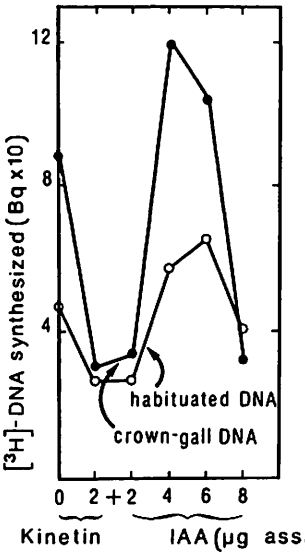


Fig. 7. Opposite effects of IAA and kinetin on in vitro synthesis of DNA from crown-gall and habituated tissues of *Parthenocissus*. Each type of DNA (0.5 µg) was used as template (see methods) firstly in the absence of hormone (O), secondly in the presence of kinetin only (2 µg assay^{-1}), and thirdly in the presence of increasing concentrations of IAA added to kinetin.

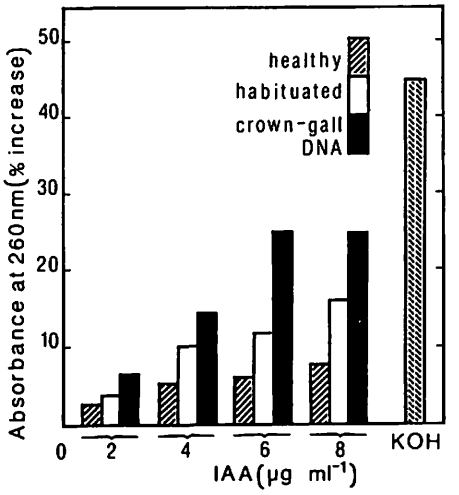
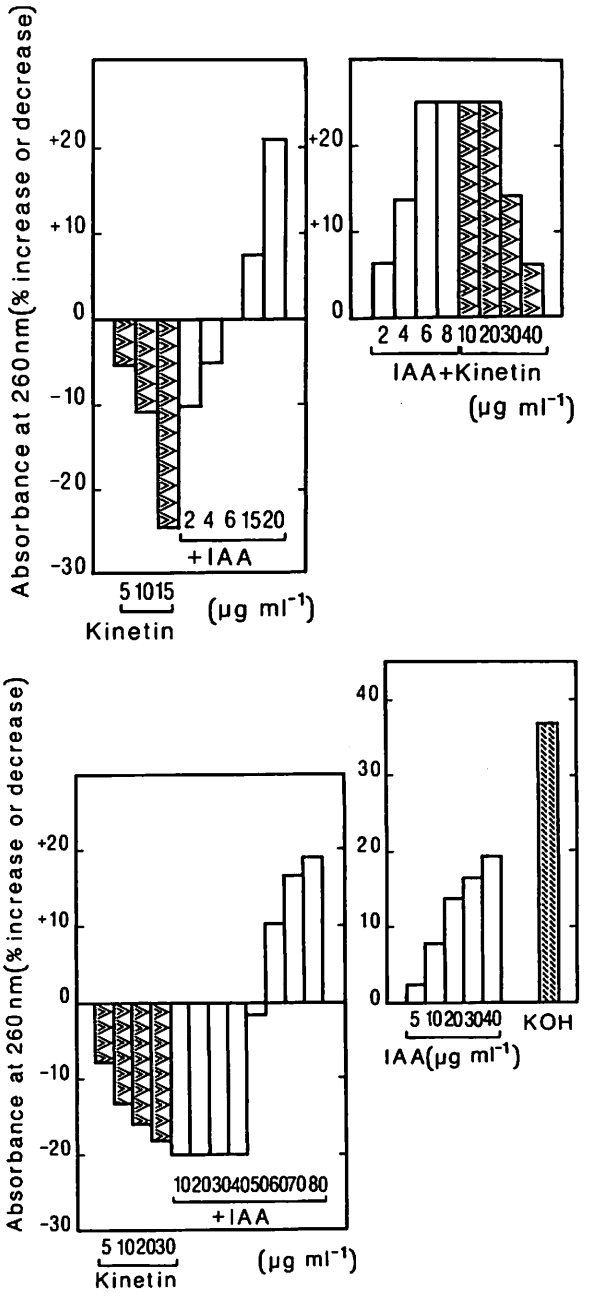


Fig. 8. Effect of IAA on strand separation on DNA from healthy, habituated and crown-gall tissues of *Parthenocissus*. The DNA samples (20 μg dissolved in 1 ml of 0.01 *M* Tris-HCl pH 7.65 containing increasing concentrations of IAA as indicated) were read against a blank cuvette containing IAA at corresponding concentrations. Maximum possible DNA strand separation was obtained by using 0.2 *M* KOH.

table, but less significant than that observed for synthesis of crown-gall DNA. In both cases, high concentrations of IAA were supraoptimal. Kinetin strongly inhibited the in vitro synthesis of crown-gall DNA and less strongly that of habituated DNA. As for synthesis of DNA from healthy cells, both IAA and kinetin were without effect. These results would be consistent with an antagonistic effect of kinetin and IAA at the level of crown-gall and habituated cell DNA. Effectively, Fig. 7 confirms that synthesis of DNA from crown-gall tissues is inhibited by kinetin. The inhibiting efficiency is high, approximately 65% with 2 μg (60 μ*M*) of kinetin per assay. The inhibitory action of kinetin is counteracted if IAA is added to the reaction mixture at concentrations of between 2 and 6 μg (75 and 225 μ*M*) per assay. At 8 μg (300 μ*M*) per assay, IAA is again supraoptimal for DNA synthesis. Similar results were obtained for synthesis of DNA from habituated tissues.

DNA strand separation in the presence of IAA and kinetin

The IAA-induced increase in in vitro synthesis of DNA from crown-gall and habituated tissues of *Parthenocissus* and tobacco must be preceded by DNA strand separation, a necessary prelude to replication. Thus, one might expect an enhancement of absorbance when DNA is incubated with IAA, and the degree of enhancement ought to depend on the origin of the tissue. Figure 8 shows that there was a large increase in UV absorbance at 260 nm for crown-gall DNA, less for habituated DNA and only a slight increase for healthy DNA when they were incubated with IAA. Also, a progressive increase in UV absorbance of the different DNA types was induced by increasing concentrations of IAA. At 8 μg ml^{-1} (45 μ*M*) of IAA this led to 25% hyperchromicity in crown-gall DNA and 15% in habituated DNA, while with healthy DNA the effect was about 8%. Maximal increase in UV absorbance was obtained in the presence of KOH.



Figs 9 and 10. Opposite effects of kinetin and IAA on strand separation of DNA from crown-gall tissues of *Parthenocissus* (Fig. 9) and tobacco (Fig. 10). The absorbance of DNA was measured in the presence of increasing concentrations of kinetin (or IAA) then of kinetin + IAA (or IAA + kinetin). Maximum possible DNA strand separation was obtained by the presence of 0.2 *M* KOH.

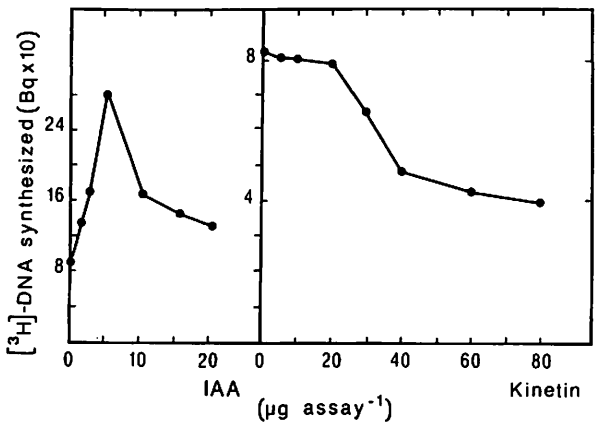


Fig. 11. Effects of IAA and kinetin on in vitro synthesis of DNA from crown-gall tissues of tobaccco.

The IAA-kinetin interaction observed in vitro on the synthesis of DNA from crown-gall derived from *Parthenocissus* (Fig. 7) was evident also for DNA strand separation (Fig. 9). Kinetin decreased the UV absorbance at 260 nm (Fig. 9A) and this effect was reversed by IAA. In fact, after the contraction due to the effect of kinetin on the double strands of DNA, the addition of IAA at increasing concentrations led to an increase in UV absorbance (Fig. 9A), explained by a large separation of the DNA strands. Conversely, when IAA was used first, it caused DNA hyperchromicity and thus hyperseparation of the double strands. This hyperseparation may be suppressed after addition of kinetin, which progressively decreases UV absorbance (Fig. 9B) as a result of a progressive contraction of DNA double strands. This IAA-kinetin interaction is thus independent of the sequence of hormone addition to the reaction mixture.

Using the results obtained with the DNA from *Parthenocissus* crown-gall as a model, we established an analogy in target-effector interaction between tobacco crown-gall DNA and IAA and kinetin:
1) IAA destabilizes already "opened" DNA chains and separates kinetin-contracted DNA chains, resulting in an increase of the DNA hyperchromic effect (Fig. 10).
2) IAA, at low concentrations, enhances in vitro DNA synthesis while kinetin lowers it (Fig. 11).

The two lines of cancerous tissues from *Parthenocissus* and tobacco differ on a quantitative point: neutralization of the kinetin-effect on tobacco DNA requires five times more IAA than used to obtain the same effect on *Parthenocissus* DNA strand separation.

Discussion

Results reported here show that the in vitro proliferation of crown-gall tissues is strongly inhibited by BG-8 while, under similar experimental conditions, the growth of healthy tissues is only slightly affected (Figs 1 and 2). The experimental system for analyzing the hormonal requirements induced by BG-8 in cultures in vitro needed a liquid culture medium that allowed the substances tested to be in close contact with the cancerous cell clumps which were consequently more receptive to the action of these substances. Similar growth inhibitory effects of BG-8 were obtained using different techniques (Vervoitte 1984). Tissues from *Parthenocissus* show great sensitivity for the hormones present in the culture medium (Fig. 3), and have a low but steady survival rate if subculturing is performed on a transfer medium containing IAA and kinetin (Fig. 4). The observations seem to indicate that BG-8 initiates the reversion of crown-gall cells to a normal state, so that a fresh requirement for hormonal growth factors is induced. Tobacco crown-gall tissues behave differently, so that if they were treated with BG-8 in the presence of IAA + kinetin and then transferred into a medium without hormone, they showed a survival rate similar to that of tissues treated with BG-8 alone. Furthermore, addition of IAA and kinetin to the new medium did not change the survival rate in tobaccco (Fig. 4).

The different behaviour towards hormones of the crown-gall tissues from the two species was also observed at the molecular level. The action of IAA on the UV absorbance of the DNA from tobacco crown-gall cells and DNA from corresponding *Parthenocissus* tissues was quantitatively different (Figs 9 and 10). The double strands of *Parthenocissus* DNA were separated by low concentrations of IAA that were ineffective on the tobacco crown-gall DNA. The high susceptibility of crown-gall DNA and, to a lower extent, that of habituated DNA (Fig. 8), indicates that the double-helical structure of these DNAs may be more or less destabilized by IAA as compared to the healthy DNA (Le Goff and Beljanski 1982). Kinetin, on the other hand, induced DNA strand contraction (Figs 9 and 10). DNA relaxation of the habituated tissues may result from the persistent action of hormones during the habituation process. The physico-chemical changes in crown-gall DNA are possibly due to the presence of opine genes (or other genes) transferred to recipient cells with the T-DNA (Ream and Gordon 1982, for review see Nester et al. 1984) and/or Ti plasmid-IAA gene required for tumor induction (Liu et al. 1982). It has been shown elsewhere that opines specifically induce in vitro relaxation of crown-gall cell DNA, which parallels crown-gall DNA increased in vitro synthesis (Beljanski 1983) and tumor cell multiplication in vivo (Lippincott et al. 1972).

BG-8 appears to decrease crown-gall DNA replication strongly as compared to habituated DNA and even more as compared to healthy DNA (Fig. 5). This observation suggests a selective affinity of BG-8 for cancerous plant DNA as already demonstrated for mammalian cancerous DNA (Beljanski 1983). One can thus explain the large decrease in growth of BG-8-treated

crown-gall cell clumps, particularly if unstable *Parthenocissus* crown-gall is used.

IAA and kinetin have opposite effects on synthesis of crown-gall DNA, and, to a lesser extent, on synthesis of DNA from habituated tissues; whereas they have no detectable effect on synthesis of healthy DNA (Figs 6, 7 and 11). Moreover, the two hormones have no direct action on the DNA-dependent DNA polymerase enzyme; they act directly on the DNA molecules tested and this action is specific for the plant DNAs. Neither plant hormone had any action on in vitro synthesis of DNA isolated and purified from human or animal cancerous tissues (Beljanski 1983).

Our results provided a basic biochemical model that contributes to the understanding of the essential role of the hormones and BG-8 in the activation or repression of cultured plant cell genes.

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