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Possible role of markers synthesized during cancer evolution: II. Markers in crown-gall tissues

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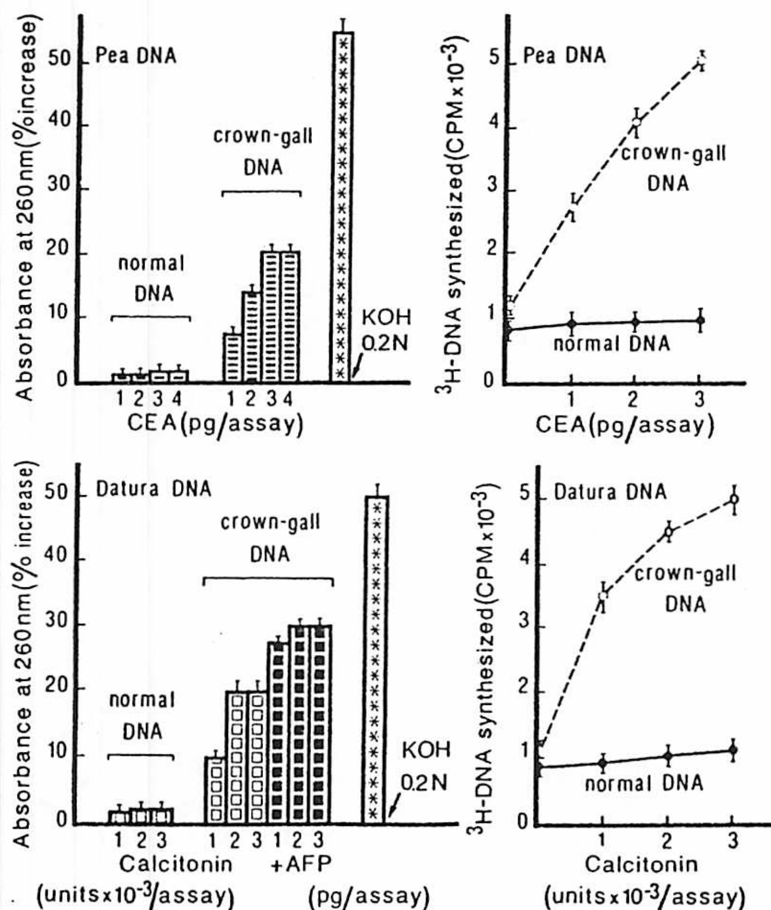
Our previous studies have shown that different trigger molecules induce plant crown-gall DNA chain separation, thu accelerating DNA *in vitro* synthesis and multiplication of tumour cells *in vivo* (1–3). Above a certain threshold, increase cancer DNA relaxation (more areas of unpaired DNA chains) leads to unscheduled DNA synthesis (4) and transcriptio (5). Plant crown-gall tumour cells, the DNA of which is destabilized (1, 2, 6), synthesize opines, commonly considered a incidental metabolites apparently characteristic of plant tumours (7). Octopine (an opine usually found in *Agrobacteriu tumefaciens* B6-induced tumour (8), and the plant hormone auxin, play a part in plant tumour cell development (9–11) b modifying DNA conformation (3). Since fetal antigens recognize mammalian destabilized cancer DNA (12) w determined the effect of these antigens on the behaviour of plant tumour cell DNA and compared it with the effect o octopine and auxin. In the present report, evidence is presented showing that mammalian fetal antigens accelerate *in vitr* crown-gall DNA chain relaxation and DNA synthesis and *in vivo* tumour cell multiplication.

Materials and methods: Human α-fetoprotein (AFP), carcinoembryonic antigen (CEA), and pig calcitonin are th same as those used with mammalian DNAs (12). Human ferritin, octopine and auxin indole-3-acetic acid (IAA) wer respectively obtained from Hoechs (Germany), Sigma (USA) and Prolab (France). Two-day-old etiolated decapitate epicotyls of *Pisum sativum* L. (cv. Annonay) were infected with *A. tumefaciens* strain B (about 10^8 bacteria/wound). Conditions fo introduction of different substances into the developing pea tumour tissues (24 h afte bacterial infection) have been previousl described (1, 13). The tumours were excise and weighed on the 12th day. Taking int account the length of the lateral shoots adjusted values for tumour weights wer calculated according to the method o Manigault (14). Light grown pea shoots age 2 weeks were used to provide the health plant cells. Crown-gall cells of *Datur stramonium* (cv. Tatula) originate from primary tumours induced by *A. tumefacien* B6 on greenhouse plant stems and corresponding normal material was composed of apical segments of healthy plan stems. Healthy and crown-gall cloned tissue lines of *Nicotiana tabacum* (cv. White Burley) and *Parthenocissus tricuspidata* (cv. Veitchii), cultured *in vitro* (6) were supplied b Oncogenese Vegetale Laboratory (P.M Curie University, Paris). DNAs from crown-gall tumour tissues and from health plant cells were purified as describe elsewhere (1, 3, 6). DNA chain relaxatio (hyperchromicity) was measured by th increase in absorbance at 260 nm as describe (3, 6, 12, 15, 16) before and after addition o tested markers, and the results wer expressed as percent increase in UV absorbance induced by a given compoun compared to control (without marker). Conditions for evaluating DNA *in vitro* synthesis measured as [^{3}H]-TN (cpm × 10^{-3}) into acid-precipitable material using DNA as template have been reported (3, 6, 12, 15, 16).



Figures 1 and 2: Stimulation of in vitro *synthesis and strand separation of crown-gall DNAs by CEA, calcitonin and AFP (Figure 1 (upper): pea DNA. Figure 2 (lower):* Datura *DNA). In both figures, the values reported are the means ± SEM of three experiments. DNA isolated from four different tissue samples was pooled before use. DNA originating from* Nicotiana tabacum *and* Parthenocissus tricuspidata *were tested with concordant results (not shown here).*

Results and discussion: AFP, CEA, ferritin and calcitonin, induced *in vitro* crown-gall cancer DNA chain relaxation measured by UV absorbance increase at 260 nm, and strongly enhanced tumour cell DNA *in vitro* synthesis (Figures 1 an 2 and Table 1). The destabilizing effects of these markers were very low with healthy cell DNA. Introduced into an *A tumefaciens* preinfected pea wound, the human fetal antigens stimulated the multiplication of tumour tissues *in vivo* (Tabl 2). Tested under the same conditions the effects produced by fetal antigens on crown-gall cancer DNA and cancer cel multiplication were similar to those of IAA and octopine, but the concentrations necessary to obtain the same effects wer considerably lower for human antigens (10^{-12}, in the 'homeopathic' range) than for the naturally occurring plan substances IAA and octopine (10^{-6}).

It should be emphasized that in mammals, where they originate, each of the various markers AFP, CEA, ferritin and calcitonin, is usually rather specific to a different type of cancer (12), but that in the present experiments, on the contrary, they were all active on crown-gall cell multiplication, i.e., on a single type of cancer which is always induced by the same bacterial agent. It must also be noted that enhancement of cell multiplication by the above markers, when injected into developing plant crown-gall tissue (pea tumour) was all the more efficient because they operated in a closed system, from which they were not eliminated as they may sometimes be in mammals. Antigens devoid of action on DNA from normal plant cells have no effect either on healthy cell multiplication.

Table 1: Effect of IAA, octopine and ferritin on in vitro *DNA strand separation and DNA synthesis in healthy and crown-gall pea tissues (means ± SEM; n = 3)*

Markers	Healthy tissue	Crown-gall tissue
DNA chain relaxation		
No marker	0	0
IAA	4.2 ± 0.4	20.2 ± 0.8**
Octopine	7.2 ± 0.5	14.2 ± 0.4**
Ferritin	1.5 ± 0.2	20.0 ± 0.5**
DNA *in vitro* synthesis		
No marker	7.9 ± 0.3	11.5 ± 0.4*
IAA	8.8 ± 0.1	42.1 ± 1.4**
Octopine	8.6 ± 0.4	36.0 ± 1.6**
Ferritin	7.5 ± 0.2	46.7 ± 2.8**

*Concentrations of markers/assay: IAA, 5 µg; octopine, 10 µg; ferritin, 3 ng. Concentration of DNA: 10 µg (chain relaxation) or 0.5 µg (*in vitro *synthesis). Results obtained with DNAs from healthy and cancerous tissues are statistically compared using Student's* t *test,* p <: **0.01; **0.001.*

Table 2: Effect of various destabilizing compounds on in vivo *pea tumour development*

Compound	Relative adjusted tumour weight[a]	Compound	Relative adjusted tumour weight[a]
Control 1 (distilled water)	100 ± 4[b]	Control 2 (distilled water)	100 ± 5[b]
Calcitonin (units × 5/wound)		CEA (pg/wound)	
10^{-2}	106 ± 7	50	110 ± 5
10^{-3}	113 ± 5*	15	131 ± 6***
10^{-4}	120 ± 3***	0.15	139 ± 5***
10^{-5}	126 ± 6***		
10^{-6}	136 ± 4***	AFP (pg/wound)	
		100	130 ± 4***
IAA (1 µg/wound)	133 ± 4**	15	149 ± 5***
		0.015	147 ± 5***
Octopine (µg/wound)		Ferritin (pg/wound)	
10	134 ± 3**	1000	111 ± 4
5	144 ± 5***	100	135 ± 4***
1	108 ± 9	10	127 ± 5**

[a]See reference 14. Each value is the mean ± SEM of four sets of experiments including 50–60 tumours per group.
[b]Control values (mg; means ± SEM): 187 ± 7 (1) and 187 ± 9 (2). In comparison with control value, p <: **0.05; **0.02; ***0.01 (Student's* t *test).*

Opines and IAA, when interacting with plant cancer DNA, behaved as do carcinogens in animals and plants (2, 13, 17), yet they are plant specific and have no effect on DNAs originating from mammalian cancer tissues. In contrast mammalian fetal antigens which, at very low doses, selectively induced crown-gall DNA relaxation and increased its *in vitro* synthesis were as active as IAA or octopine in the acceleration of the plant *in vivo* carcinogenic process. They kept the unregulated mechanism running in plant crown-gall tissues.

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