Multiplication of Tobacco Mosaic Virus in Tobacco Leaf Disks Is Inhibited by (2′-5′) Oligoadenylate

Abstract. *The oligonucleotide (2′-5′) oligoadenylate that is induced in interferon-treated animal cells protects plant tissue from infection by the tobacco mosaic virus. This inhibition of virus multiplication was obtained at concentrations comparable to those affecting protein synthesis and antiviral activities in animal cells. After treatment with (2′-5′) oligoadenylate, the multiplicability of tobacco mosaic virus was reduced by 80 to 90 percent, as measured by enzyme-linked immunosorbent assay. These results, along with the observation that human interferon protects tobacco tissue from infection by tobacco mosaic virus, indicate that plants and animals may have a common pathway for virus resistance.*

A number of activities are induced in interferon-treated animal cells (*1*). One pathway of such activities is the release of a synthetase activity which, in the presence of double-stranded RNA, polymerizes adenosine triphosphate (ATP) to make a unique series of oligoadenylates, $pppA(2'p5'A)_n$, commonly known as 2-5A (*2*). This nucleotide activates a preexisting nuclease which, in turn, brings about the inhibition of protein synthesis. These activities were also found in intact animal cells after the administration of 2-5A or of its "core," which lacks the 5′-terminal triphosphates (*3–7*). The 2-5A core also inhibited DNA synthesis in mitogen-stimulated lymphocytes (*8*). The induction of 2-5A synthesis in animal cells was correlated with the antiviral activity of interferon (*9*). It was also demonstrated that 2-5A and its cordycepin analog inhibited the Epstein-Barr virus–induced transformation of human lymphocytes (*10*).

The plant *Nicotiana glutinosa* L. produces an antiviral factor (AVF) after infection with RNA-containing tobacco mosaic virus (TMV) (*11*). The properties and some activities of AVF are similar to those of interferon (*12*), among them the induction of AVF-directed ATP polymerization (*13*). Furthermore, a considerable antiviral activity, resulting in more than 90 percent inhibition of TMV multiplicability in plants, was reported for various human leukocyte interferons (*14*). We investigated the possible activity of 2-5A in virus-infected plants and report that TMV multiplication is halted in tobacco tissue treated with this nucleotide.

The effect of the various applications of 2-5A on TMV multiplication was studied by determining the virus content of the tissue after treatment. Leaves of the tobacco variety Samsun, in which TMV spreads systematically, were mechanically inoculated with the purified virus (5 μg/ml in 0.01*M* sodium phosphate, *p*H 7.6). Disks, 6.5 mm in diameter, were punched out of the inoculated leaves into a beaker containing water. The variability in the physiological state of the inoculated leaves and in the actual number of initially inoculated cells in the various disks was taken into account by distributing groups of 20 disks each, selected at random from the common pool, into separate petri dishes, each containing 20 ml of the above-mentioned phosphate buffer. Noninoculated disks were also included for background calibration. The TMV was allowed to multiply for 1 hour before any treatment, after which the solutions to be tested were added. After an additional 72 hours of incubation at room temperature under constant illumination, the disks were frozen and homogenized in the phosphate buffer (0.1 ml per disk). The TMV content of the extracts was determined by an enzyme-linked immunosorbent assay (ELISA) essentially by the method of Clark and Adams (*15*), but with the following order of layers on the microtiter plates: (i) the γ-globulin fraction of a serum obtained from a chicken immunized against TMV was absorbed directly to the plate; (ii) the next layer was the tested sample (at various dilutions) or a series of purified TMV suspensions at known concentrations, which provided internal calibration curves; (iii) the third layer was the γ-globulin from the serum of a TMV-immunized rabbit; and (iv) the final layer was goat antibody to rabbit immunoglobulin G conjugated with alkaline phospha-

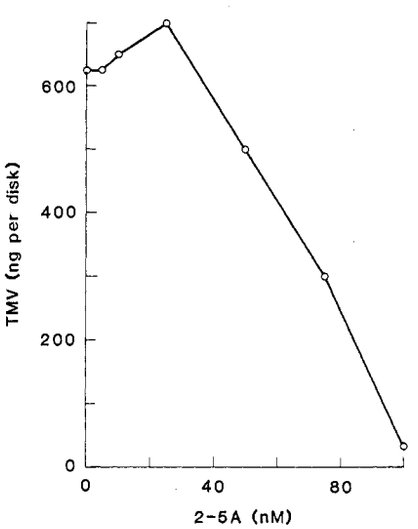

Fig. 1 (above). The effect of 2-5A on TMV multiplication. The phosphate buffer was aspirated from the disk-containing plates 1 hour after inoculation with TMV and replaced by 2-5A at the indicated molarities in the application buffer. After incubation with 2-5A for 1 hour, the 2-5A–containing buffer was replaced by a buffer lacking 2-5A for another hour. The disks were then washed with 0.01*M* sodium phosphate, *p*H 7.6. The incubation continued in the phosphate buffer until 72 hours after inoculation, at which time the TMV content of the disks was determined by ELISA.

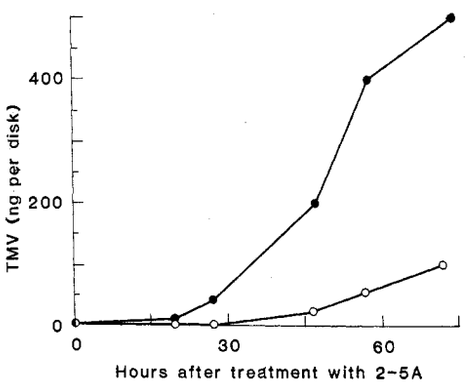

Fig. 2 (top right). Kinetics of TMV multiplication in the (●) absence and (○) presence of 2-5A (200 n*M*). Application of 2-5A was as in the legend to Fig. 1. Sample disks were removed at various times after TMV inoculation and their TMV content was determined by ELISA.

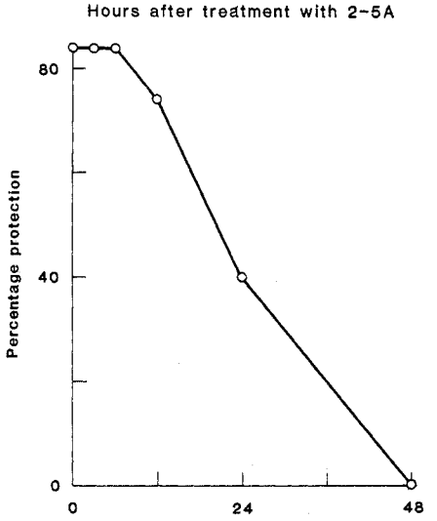

Fig. 3 (bottom right). The development of resistance to TMV infection in tobacco leaf disks as a function of time of 2-5A application. The 2-5A (200 n*M*) and the respective buffer without 2-5A were added for 1 hour at the indicated time after TMV inoculation. The disks were then transferred back into 0.01*M* sodium phosphate, *p*H 7.6, and treated as described in the legend to Fig. 1. Percentage protection was calculated as [1 − (nanograms of TMV in 2-5A–treated disk/nanograms of TMV in application buffer–treated disk)] × 100.

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tase (Sigma). The release of phosphate from *p*-nitrophenyl phosphate, due to the antibody-conjugated alkaline phosphatase, was monitored spectrophotometrically in a special reader (Microelisa Auto-Reader, Dynatech) and was proportional to TMV concentration in a log linear manner for about two logs (< 2 ng to > 200 ng).

The 2-5A used throughout this work was a core preparation of the trimer (A2′p5′A2′p5′A) (*16*). This 2-5A core was checked by thin-layer chromatography (polyethyleneimine plates developed with 0.1*M* LiCl) along with reference 2-5A samples (obtained from P-L Biochemicals) treated with alkaline phosphatase to remove their 5′-triphosphate. Phosphorylated 2-5A requires the calcium phosphate coprecipitation technique to penetrate into animal cells (*5*), whereas the 2-5A core penetrates if introduced in a regular medium for cell growth (*10*). Since there was no such experience with plant tissues, the 2-5A core in this series of experiments was applied with the calcium phosphate coprecipitation buffer (*5*).

Because tobacco leaf disks do not contain identical cells, and it is impossible to control the number of cells in each disk that are actually infected on initial inoculation, the level of TMV multiplication varied from one experiment to another. Nevertheless, TMV multiplication was invariably greatly inhibited in 2-5A–treated leaf disks in a concentration-dependent manner (Fig. 1). The kinetics of TMV multiplication in the absence and presence of 2-5A is shown in Fig. 2. Usually, concentrations of 100 to 200 n*M* 2-5A were sufficient to cause near-total inhibition. The 2-5A must be applied to the tissue early in infection to obtain an antiviral effect. A short treatment with 2-5A at any time earlier than 6 hours after inoculation was most effective, but thereafter the antiviral effect gradually diminished with time (Fig. 3).

The above results were corroborated by infectivity tests. Homogenates of buffer-treated and of 2-5A–treated disks were applied to opposite halves of the same *Datura stramonium* L. leaves. The control half-leaves inoculated with buffer-treated disks developed 81 local lesions, whereas the half-leaves inoculated with homogenates of disks treated with 200 n*M* 2-5A core developed only one local lesion.

We assume that 2-5A penetrates the plant cells, in analogy to the animal system. However, there is no indication that this is indeed the case and the 2-5A may exert its effect on plants by affecting cell membranes. The structure of the AVF-induced polymerized ATP has not yet been fully established, although many indications, such as thin-layer chromatography and sensitivity of various enzymes, point to a possible 2-5A nature (*17*). The biological activity of 2-5A in plants suggests a role for this compound in the plant's resistance mechanism.

YAIR DEVASH
SARA BIGGS
ILAN SELA

Virus Laboratory, Hebrew University, Faculty of Agriculture, Rehovot 76 100, Israel

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16. The 2-5A trimer was chemically synthesized by S. Rapoport and Y. Lapidot, and obtained through A. Kimchi.
17. Y. Devash, in preparation.
18. Supported by a grant from the Volkswagen Foundation.

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Oxytocin, Vasopressin, and Estrogen-Stimulated Neurophysin: Daily Patterns of Concentration in Cerebrospinal Fluid

Abstract. *The concentrations of oxytocin, arginine vasopressin, and estrogen-stimulated neurophysin in cerebrospinal fluid of monkeys showed a daily fluctuation with high concentrations occurring during the light period. The patterns of oxytocin and estrogen-stimulated neurophysin in the cerebrospinal fluid were not observed in the plasma nor were they altered after the administration of a dose of estradiol that increased concentrations of estrogen-stimulated neurophysin in plasma. The disassociation between these cerebrospinal fluid and plasma patterns and values suggests that the secretory activity of neurons that release estrogen-stimulated neurophysin and oxytocin into the cerebrospinal fluid is controlled by mechanisms different from those that control their release into the plasma.*

Oxytocin and arginine vasopressin (AVP) originate in neuron cell bodies in the hypothalamus (*1*, *2*). Projections from these neurons extend to many areas of the nervous system, including the posterior pituitary, brainstem, hypothalamus, and spinal cord (*3*). Anatomical and electrophysiological observations suggest that these several projections function independently (*4*). Our data support this concept of independence by demonstrating that the release of oxytocin and its carrier protein estrogen-stimulated neurophysin (ESN) into the cerebrospinal fluid (CSF) of rhesus monkeys is not linked to the release of oxytocin and ESN into plasma. We report here that the concentration of oxytocin and ESN in the CSF, continuously withdrawn from unanesthetized rhesus mon-

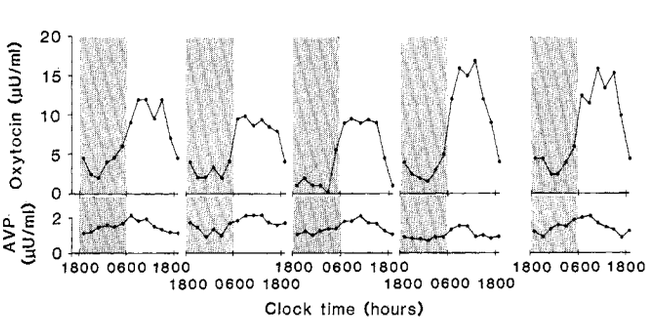

Fig. 1. Patterns of oxytocin and AVP concentrations in the CSF of five male rhesus monkeys studied during diurnal lighting (LD 12:12). Samples of CSF were collected in 2-hour fractions. Shaded areas indicate the time of day when lights were off, and unshaded areas indicate when lights were on.

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