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THE NOPALINE SYNTHASE PROMOTER AS A MODEL SYSTEM FOR STUDYING PLANT RESPONSE TO UV-B

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The destruction of the stratospheric ozone layer, which is mainly caused by human-produced chlorofluorocarbons (CFCs), has strikingly raised the level of ultraviolet B (UV-B) radiation on the surface of earth. Plants on earth perceive and respond to UV-B present in sunlight. Consequently, increase in the level of UV-B radiation affects plant growth due to genetic alteration. Therefore, the interaction between the perception of UV-B and the alteration of gene expression is of ecological significance. In this paper we discuss the utilization of the nopaline synthase (*nos*) promoter as a model system on the effect of UV-B on plant genes.

Key Words
UV-B, plant, *Nicotiana tabacum*, nopaline synthase promoter, genetic alteration, signal transduction

INTRODUCTION

Most of the land on the earth is covered with green plants, which support life by serving as the link between solar energy and organic matter. The solar energy absorbed by chlorophyll pigments in plants is converted into chemical energy, which is used to fix carbon dioxide into organic matter. As a result of this ability, all living organisms are able to sustain life and to reproduce.

In recent generations, human activities have accelerated the reduction of plant matter on earth. One of the most clearly documented cases is the depletion of ozone layer in the stratosphere. The density of the ozone layer varies with gaseous mixing, solar spot activity, and stratospheric temperature changes; however, ozone depletion has been, mainly, caused by chlorine, bromides, and human-produced chlorofluorocarbons (CFCs) [1,2]. Recently, international negotiations are proceeding to reduce CFC

production. Despite these actions, much still remains to be learned about the potential results of human produced perturbations of ozone layer.

The stratospheric ozone layer absorbs most ultraviolet (UV) light less than 320nm [1,2]. UV light is classified into UV-C (wavelengths below 280 nm), UV-B (280 to 320 nm), and UV-A (320 to 390 nm). UV-C, which is highly energetic, is absorbed by ozone layer and does not reach the surface of earth. UV-A wavelengths are not affected by ozone layer, so all of these wavelengths arrive at the earth's surface.

USE OF THE *NOS* PROMOTER AS A MODEL SYSTEM IN STUDIES OF PLANT RESPONSE TO UV-B

The soil bacterium *Agrobacterium tumefaciens* induces tumors called "crown gall disease" in a variety of plants [9, 10, 11]. The wild type bacteria contains a tumor-inducing (Ti) plasmid, which can transfer a portion of its plasmid DNA into a host plant genome. This region of transferred DNA is called T-DNA and encodes genes which produce plant hormones and opines, derivatives of amino acids, and sugars for autonomous proliferation. The plasmid can be manipulated to delete these genes, resulting in non-tumorigenic bacteria which retain the ability to transfer foreign genes.

The most abundant opine in the nopaline-type tumor tissues is nopaline, which is formed by the condensation of α-ketoglutarate with arginine [11]. This reaction is catalized by a product of the nopaline synthase gene (*nos*). The promoter of the *nos* gene had been used for construction of plant-selective markers, since it was considered that Much attention is paid to UV-B radiation since the amount of UV-B reaching the earth increases as the depletion of ozone layer elevates. Therefore, in this paper, we will concentrate on plant response to UV-B rather than UV-C or UV-A.

There has been much speculation about the relationship between the amount of UV-B radiation and plant response. The production of UV-B photons over the whole spectrum of solar radiation varies at particular temporal and spatial conditions, therefore plants show a great variety of responses in photosynthesis, growth, and gene expression [3]. Furthermore, even in a controlled experimental system, plants respond to UV-B in a quite distinct manner compared to the responses of bacteria and mammalian cells [4, 5]; mammalian cells grow as monolayers, resulting in an equal exposure of UV-B for a brief period of time, whereas plants grow in clusters, which results in heterogeneity in biological responses.

In recent studies, much effort has been made to discover a primary UV-B mechanism that can alter cellular activities, particularly in the level of gene expression. The effects of UV-B on plant genes can be divided in to two types [6]: lethal and non-lethal UV damage. Lethal UV damage, which we will not refer to, results from unrepaired nucleic acid lesions. Non-lethal effects of UV, which are of biological significance, may involve genetic alterations through direct UV absorption by nucleic acids or indirect UV sensitizing cellular signal transduction pathways. Recently, a gene that encodes an early step of flavonoid biosynthesis, chalcone synthase (CHS), has been utilized as a model system for studies of the interaction of UV-B and genetic alteration [7, 8]. However, a few difficulties exist in studies using CHS since large differences in the response to irradiation are shown in various experimental conditions. In consequence, little is known still about the signal transduction pathway between the interception of UV radiation photons and the induction of flavonoid biosynthetic gene expression.

In the present study, we utilize the nopaline synthase (*nos*) gene for the investigation of the molecular mechanism of response to UV-B. The *nos* gene, which comes from an *Agrobacterium* tumor inducing (Ti) plasmid, has regulatory regions that interact with the host regulatory system to express the gene in plant cells [9, 10]. The *nos* transcript is most abundant in tumor cells among the Ti transcripts [11]. The *nos* promoter activity is, nonetheless, very weak in transgenic tobacco plants unless the plants are treated by environmental stresses [12]. Hence, the studies of the *nos* gene will provide much information about the signal pathway mechanism of the response of distressed plants.

We are particularly interested in the signal pathway of the primary genetic response of plants to UV-B irradiation. We have induced *nos* promoter activity by exposing transgenic tobacco plants to a low fluorescence of UV-B (300 nm) at a non-lethal level. We expect that biochemical and cell physiological studies of the response of *nos* promoter will undoubtedly make progress towards discovering the possible mechanisms of one or more cellular signal transduction pathway which may be transducing, amplifying, and storing the light signal. We will discuss the relationship between UV-B light signal transduction and

other basic mechanisms operative with some positive factors, such as salicylic acid, methyl jasmonate, and hydrogen peroxide, affecting *nos* promoter activity.

UV-B IRRADIATION AND DNA DAMAGE

To understand how UV-B may affect *nos* promoter activity, we have investigated the effects of UV-B on expression of the *nos* promoter using transgenic tobacco plants. The transgenic plants were obtained by the cocultivation of tobacco leaf segments with *A. tumefaciens* carrying the chimeric gene, which is a fusion between the *nos* promoter and coding region of the chloramphenicol acetyltransferase (CAT) reporter gene. The third generation of the plants were grown under green house conditions for all experiments.

Since the *nos* promoter shows a great variety of expression patterns depending on environmental and developmental conditions, it was necessary to establish a condition that provides reliable data. We dealt with this problem by incubating leaf segments on a liquid medium. This treatment is considered to be a mild wounding since the *nos* promoter activity is slightly induced under this condition. The promoter responses to UV-B irradiation was monitored using the leaf segments. The results indicated that the CAT activity was increased by the irradiation with UV-B (300 nm) (Fig. 1). It was reported that UV-B can cause DNA damage in plant cells to result in the formation of cyclobutane type pyrimidine dimers and pyrimidine(6.4)pyrimidinone dimers (6.4 photoproducts), which is reversed by irradiation with blue (370 to 450 nm) or white light [17]. This "light" repair or photoreactivation occurs within a few hours and requires specific photolyase enzyme together with blue light [18]. In this study, we showed that the *nos* promoter induction by UV-B was observed under both high levels and low levels of visible light. These results imply that *nos* promoter induction by UV-B occurs through some other mechanisms rather than through photoreversible DNA damage.

Although plants receive UV-B radiation every day, up to 60 fold more dosage than is actually observed in the laboratory, they do not accumulate UV damage over time in normal conditions [19]. This is because pigments in the epidermal cells, such as anthocyanines, shield UV-B irradiation by about 90 %, and photoreactivation activity is sufficiently high enough to repair all of the DNA damage. If DNA damage accumulates over time in vegetative tissues, it can be transferred to gametophytes that emerge from vegetative tissue after the short period of meiosis. Therefore, the DNA damage caused by increasing UV-B irradiation may result in the potential mutations and genetic aberrations in plant germinal cells and finally affect the plant's biological fitness in the ecosystem.

PATHWAYS OF TRANSDUCING UV-B SIGNAL TO GENE

Apart from inducing DNA damage, the UV-B induction of gene expression involves alteration of metabolic processes that affect plant development and growth. It has been widely suggested that UV-B induction of gene expression is meditated by photoreceptors that specifically absorb UV-B and that photoreception is coupled to transcription through a cellular signal transduction pathway. In many higher plants, a specific UV-B photoreceptor seems to exist [20, 21]. However, there is little information on the nature of the UV-B photoreceptor and on the UV-B signal transduction.

Recent studies indicate that reactive oxygen species (ROS) are involved in the UV-B signal transduction pathway in plants [22]. We have obtained evidence that the *nos* promoter induction by UV-B is mediated by ROS (data not published). ROS may increase the level of the *nos* transcript as a diffusible signal or through the activation of transcriptional factors. It is also possible that oxidative burst may enhance the turnover rates of signaling molecules, such as salicylic acid or hydrogen peroxide. The *nos* promoter could be defined as an early gene or a primary-response gene since it has a

stimulus responsive *cis* element [23]. The *cis* element enables a short and direct path between stimulus perception and gene activation. As found in animals, this *cis* element may be activated through ROS which was induced by UV-B irradiation [24, 25]. How ROS activate the gene still remains to be solved.

CONCLUSION

As a primary-response gene, the *nos* promoter serves as a valuable model system to identify cellular factors involved in the UV-B signal transduction pathway such as transduction, amplification, intracellular communication, and regulation of late gene expression. This approach will greatly aid in serving as the link between UV-B perception and UV-B induction of gene expression which is, later, responsible for the metabolic processes that affect plant development, growth, and stress adaptation.

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FIGURE LEGEND

Figure 1. Induction of *nos* promoter by UV-B in the presence of low and high fluorescence of white light. Leaf segments carrying the *nos* -CAT fusion gene were subjected to 2 h treatment with 0.1 µmol $m^{-2}sec^{-1}$ UV-B (UV-B: under the dim white light). After UV-B treatment, leaf segments were incubated for further 10 h under low (**LWL**; 80 µmol $m^{-2}s^{-1}$) or high (**HWL**; 400 µmol $m^{-2}s^{-1}$) fluorescence of white light. CAT activity was assayed by measuring conversion of chloramphenicol (**cm**) to acetylchloramphenicol (**ac**). Results of CAT activity from a representative sample are shown in the TLC autoradiograph.

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