and receptors of the atrial peptides can be explored with the preparation of synthetic analogs. The continued investigation of this unique family of atrial peptides may provide insight into the properties of a major endocrine regulatory system.

Note added in proof: Synthetic atriopeptins I and II exhibit the same chromatographic and biological activity as the authentic biologically derived peptides. The synthetic peptides required oxidation to elicit their biological effect, thereby supporting the requirement for the cysteine disulfide ring.

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Activation of Antitumor Agent Gilvocarcins by Visible Light

Abstract. *Gilvocarcins that are antitumor agents are activated by low doses of visible light to induce bacteriophage lambda in* Escherichia coli. *This result is dependent on interaction with DNA. Gilvocarcin M, an analog without antitumor activity, failed to induce the prophage after light exposure, thus demonstrating a correlation between photosensitizing and antitumor activities. These results raise several possibilities regarding the mode of action of gilvocarcins as antitumor agents in vivo, involving light or enzymatic activating systems, which could be exploited in human cancer therapy.*

Current strategies for the development of new anticancer drugs involve the design or selection of agents that are tumor specific and less toxic than those used in the past. Several groups of investigators (*1–5*) have reported the isolation from Streptomycetes of antitumor active compounds with the gilvocarcin chromophore (*4–9*) which exhibit little toxicity in animals (*4, 9, 10*). A similar compound, chrysomycin A, was isolated 25 years ago (*11*), but was not completely characterized until recently (*9*). In view of the antimicrobial activity and mammalian cell cytotoxicity exhibited by gilvocarcins (*1–5, 10, 11*) the tolerance of animals for high doses (LD_{50} ~ 1000 mg/kg) (*4, 9, 10*) has been a mystery.

In our program, gilvocarcin-producing fermentation broths were detected as inducers of bacteriophage lambda, utilizing a colorimetric assay (*5*). However, tests of the purified active component (gilvocarcin V) here and elsewhere yielded conflicting results regarding its prophage-inducing activity (*4, 5*). These results, along with experimental observations on DNA strand break formation after irradiation of gilvocarcin V–DNA complexes (*12*), led us to examine the role of light in the prophage-inducing activity of this class of compounds. We now present data demonstrating the critical role of light in the activity of antitumor active gilvocarcins, a role previously unsuspected, we believe, because of their extreme sensitivity to normal fluorescent light. Comparison of the activity of gilvocarcins with that of related chemicals, including psoralens (Fig. 1), provides clues to the structural requirements for their activation and serves to emphasize the special characteristics of these compounds.

Prophage induction experiments were performed with *Escherichia coli* strain BR 513, a strain designed for screening of carcinogens and antitumor agents (*13*). These bacteria contain a chromosomally integrated lambda-lacZ fusion phage under control of the lambda repressor. Treatment with DNA-damaging agents leads to induction of the "SOS response," derepression of prophage, and synthesis of β-galactosidase, product of the *lacZ* gene. β-Galactosidase was detected by reaction with substrates giving colored products that could be quantitated or observed in spot tests on agar. In the latter case, comparisons could be made among many chemicals irradiated together on the same plate.

Figure 2a shows the dose response for prophage induction with chrysomycin A and gilvocarcin V in the presence and absence of light. Whereas both compounds showed strong prophage-inducing activity after a 15- or 20-minute exposure of solutions to fluorescent light, no activity was seen when the experiment was performed in the dark (or under yellow lights). These results accounted for the experimental variation seen in two laboratories at Frederick.

Other experiments indicated that prophage induction occurred only when the bacteria and chemical were present together during light exposure, a result reminiscent of the behavior of psoralens. Incubation of chemical alone in the light, followed by addition of bacteria, did not result in prophage-inducing activity (Fig. 2a). In a control experiment, irradiation of bacteria alone did not induce the pro-

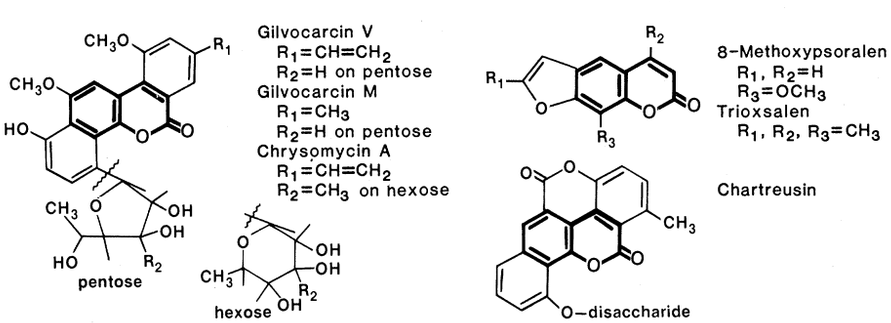


Fig. 1. Structures of gilvocarcins and related compounds. Bold-faced structure is coumarin.

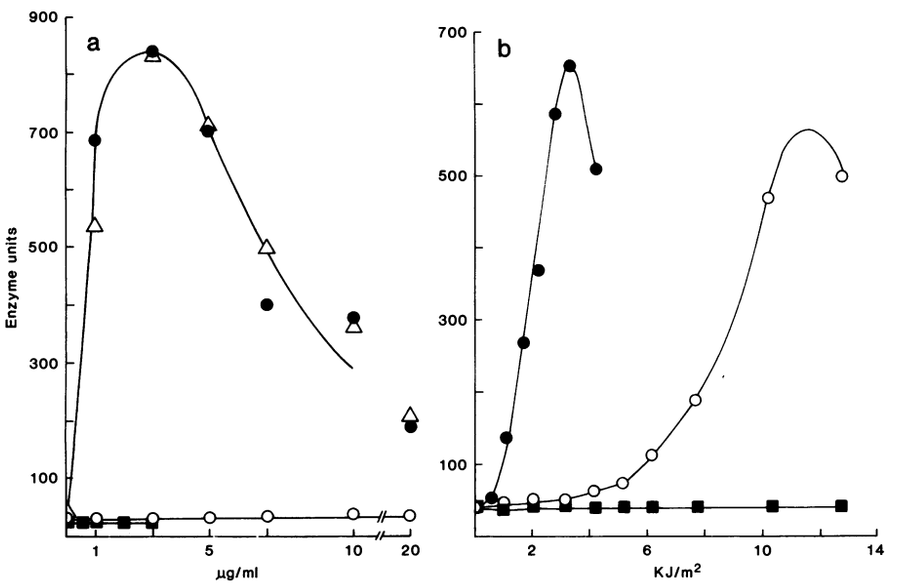
phage (Fig. 2b). Gilvocarcin V and chrysomycin A, differing only in the sugar moiety, gave identical responses when tested together on the same plate (data not shown).

Induction of prophage was dose-dependent for light exposure as well as for concentration of chemical (Fig. 2b). The presence of a screen eliminating wavelengths below 390 nm did not eliminate prophage-inducing activity mediated by radiation from a Coolwhite (visible) lamp. However, induction was more efficient when the UVA lamp (with a stronger emission in the 320- to 420-nm "A" region of the ultraviolet) was used (Figs. 2b and 3). From these results, the limited absorption of radiation above 430 nm by gilvocarcins (*7*), and the absence of activity under yellow lights, we conclude that the action spectrum for gilvocarcin V extends into both the near ultraviolet (below 400 nm) and visible (above 400 nm) wavelength regions.

Other compounds with related structures were also tested for light-mediated phage-inducing activity (Fig. 3). Gilvocarcin M, the methyl analog of gilvocarcin V (vinyl), has not shown antitumor activity (*4, 10*). Likewise, it was negative in our prophage induction experiments under several conditions of illumination, even though its absorption spectrum was similar to that of gilvocarcin V (*7*). This result provides a positive correlation between light-mediated activation and antitumor activity. Chartreusin, another antitumor agent bearing a structural resemblance to gilvocarcins (Fig. 1), showed only slight prophage-inducing activity that was independent of irradiation. These results implicate the vinyl group, present also in ravidomycin (*8*), which is another antitumor-active gilvocarcin (*1*), as a critical structural element for activity of gilvocarcins.

Following these observations, we were interested in comparing the activity of gilvocarcins with that of the psoralens, well-known phototoxic compounds (*14*). As expected, 8-methoxypsoralen (8-MOP) and trioxsalen were active as inducers of bacteriophage in our system after irradiation with ultraviolet but not visible light (Fig. 3). The psoralens and gilvocarcins are both coumarin derivatives (Fig. 1), but they differ sufficiently in size and structure that the mode of DNA interaction of gilvocarcins is probably different from that known to occur with psoralens (*15*). For example, the psoralens are bifunctional, whereas the gilvocarcins appear to have only one reactive site, the vinyl group. Under optimum conditions of illumination for detection of the psoralens (UVA lamp), we found that we could detect gilvocarcin V at concentrations 10^3 times lower than trioxsalen and 10^5 times lower than 8-MOP (Fig. 3). The reasons for the high activity of gilvocarcins relative to the psoralens remain to be elucidated.

Numerous reports on the activity of gilvocarcin-type compounds against microbes, viruses, and mammalian cells



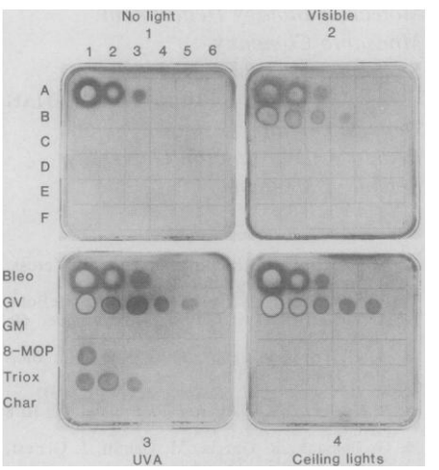


Fig. 2 (left). Induction of bacteriophage lambda by photoactive gilvocarcins. (a) Induction as a function of dose of chemical. The *E. coli* strain BR 513 (λ *lacZ ΔuvrB envA*) (*13*) was treated with chemicals in the presence or absence of light, and then incubated (with shaking, in the dark) for 3 hours. Expression of β-galactosidase was then quantitated by absorbance at 420 nm after the addition of substrate *O*-nitrophenyl-β-D-galactopyranoside, yielding the colored product *o*-nitrophenol. Enzyme units are expressed as 100 A_{420}/t_h, where t_h is time of color development in hours (*20*). △, Exposure of 0.5-ml portions of bacteria (2×10^8 per milliliter) plus gilvocarcin V to light from a single fluorescent lamp (General Electric F40CW, Mainlighter Coolwhite) for 20 minutes at a distance of 12 cm; the dose at wavelengths from 400 to 800 nm was 1.0×10^4 J/m². ●, Exposure of bacteria plus chrysomycin A to fluorescent light from ceiling fixtures at a distance of 1.2 m. ■, Exposure of chrysomycin A to light as above (●), followed by the addition of bacteria. ○, Incubation of bacteria with chrysomycin A in the absence of light. (b) Induction as a function of light exposure. Treatment of BR 513 with aqueous solutions of chrysomycin A (1 μg/ml) in the presence of light from different sources. ●, UVA lamp (Sylvania Lifeline FR40T12) plus Mylar screen (emission in the 320- to 400-nm wavelength range, 3.6 W/m² at a distance of 12 cm; screen eliminates wavelengths below 310 nm). ○, Daylight fluorescent lamp, as in (a), plus weathered Mylar screen (emission in the 400- to 800-nm wavelength range was 8.5 W/m² at a distance of 12 cm; the screen eliminates wavelengths below 390 nm). ■, UVA or visible light, no chemical. After treatment, bacteria were incubated at 38°C while shaken in the dark for 3 hours for expression of β-galactosidase. Fig. 3 (right). Induction of bacteriophage lambda by related coumarin compounds in the presence of light from different sources. Bacterial lawns containing BR 513 were spotted with 5-μl portions of chemicals dissolved in water (bleomycin) or dimethyl sulfoxide (all other chemicals). After light exposure, plates were incubated in the dark at 38°C for 4 hours for enzyme expression, followed by addition of chromogenic substrate 6-bromo-2-naphthyl-β-D-galactopyranoside plus fast blue. Red spots developed within 10 minutes [see (*20*) for details]. Light sources were as described (Fig. 2). Plate 1, no light. Plate 2, 10-minute exposure to daylight lamp (Coolwhite) plus weathered Mylar screen from a distance of 12 cm. The incident dose (400 to 800 nm) was 5.1 kJ/m². Plate 3, 10-minute exposure to UVA lamp plus Mylar screen from a distance of 12 cm. The incident dose (320 to 400 nm) was 2.8 kJ/m². Plate 4, 20-minute exposure to fluorescent light from ceiling fixtures at a distance of 1.8 m. Row A, bleomycin at 100, 10, and 1 μg/ml in columns 1 to 3; column 4, water; column 5, dimethyl sulfoxide. Row B, gilvocarcin V at 100, 10, 1, 0.1, 0.01, and 0.001 μg/ml in columns 1 to 6, respectively. Row C, gilvocarcin M, same as row B. Row D, 8-MOP at 1000, 100, 10, 1, 0.1, 0.01 μg/ml in columns 1 to 6, respectively. Row E, trioxsalen, same as row D. Row F, chartreusin, same as row D.

have appeared (*1–5, 10, 11*). However, the role of light in these effects was not considered. Very low concentrations of gilvocarcins (0.01 μg/ml) are detected as DNA damaging agents following illumination under normal laboratory conditions (Fig. 3, plate 4). Experiments performed by our colleagues on other microorganisms have indicated no instance in which gilvocarcin V exhibited toxicity in the absence of light. It seems likely therefore that similar effects reported by others were light-mediated.

Among antitumor agents, gilvocarcins appear to be unusual compounds. Unlike the hematoporphyrin derivative (HPD), an agent of current clinical interest that is retained by tumors and activated by laser light (*16*), the antitumor activity of gilvocarcins in mice was demonstrated in the absence of intentional irradiation. Other antitumor agents (such as daunorubicin and bleomycin) also demonstrate photodynamic effects (*17, 18*). However, unlike gilvocarcins, these agents have cytotoxic activity that is independent of light. An interesting case is that of camptothecin, the photoactivation of which appears to have been discovered 8 years after its clinical trials (*19*). The effect of light on the activity of this compound in humans can only be surmised, but the clinical experience with psoralens demonstrates that such effects can be dramatic.

Whether the observed in vivo antitumor activity of gilvocarcins was mediated by ambient light is not known. Gilvocarcin V showed activity against tumor cells administered intraperitoneally, a site not exposed to light, as well as against tumor cells transplanted under the skin (*6*); however, gilvocarcins are extremely light sensitive and some light penetration to internal organs in animals as small as mice might occur. Activation at the skin seems unlikely because of the absence of systemic toxicity (*1, 4, 9, 10*) and the requirement for activation at the target DNA (*12*) (Fig. 2a).

In vivo, activation could be enzymatic. The lack of systemic toxicity in animals may indicate that gilvocarcins undergo selective distribution into or activation at specific target tissues. Gilvocarcins might thus provide opportunities for several modes of cancer therapy, involving in vivo activation at the sites of tumors, or activation via external irradiation.

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The Human Homologs of the *raf* (*mil*) Oncogene Are Located on Human Chromosomes 3 and 4

Abstract. *Two human genes that are homologous to both the murine transforming gene (oncogene) v-*raf *and the chicken transforming gene v-*mil *have been mapped by means of human-rodent somatic cell hybrids to human chromosomes previously devoid of known oncogenes. One gene, c-*raf*-2, which appears to be a processed pseudogene, is located on chromosome 4. The other gene, c-*raf*-1, which appears to be the active gene, is located on chromosome 3 and has been regionally mapped by chromosomal in situ hybridization to 3p25. This assignment correlates with specific chromosomal abnormalities associated with certain human malignancies.*

Transforming genes, or oncogenes, are a class of evolutionarily conserved genes that have been associated with the development of tumors in various vertebrate species (*1*). In some cases they are sufficiently conserved that invertebrate homologs have been identified (*2*). The fact that these genes are so well conserved suggests that they have an important role in the normal cell and that their tumorigenic properties arise from their abnormal expression. Most of the approximately 20 known oncogenes were originally identified as genes of nonprimate origin that were incorporated into acutely transforming retroviruses and have yet to be directly implicated in human disease. One possible approach to defining their involvement in human cancer is based on the observation (*3*) that certain types of human tumors are associated with specific chromosomal rearrangements. This observation led Klein (*4*) to suggest that such tumors arise from the transposition of transforming genes to a chromosomal location where their regulation is altered. This hypothesis has received considerable support from reports (*5*) that the characteristic translocation in Burkitt lymphoma of a portion of chromosome 8 to chromosome 14, t(8;14) (q24;q23), places the *myc* gene in proximity to the immunoglobulin heavy chain genes. If tumor-specific rearrangements activate oncogenes, it might be possible to use the chromosomal location of an oncogene to focus on a few types of human tumors as the most probable candidates for involvement of the oncogene. In this report, we apply this concept to the recently characterized *raf* and *mil* oncogenes, which are of murine origin (*6*) and avian origin (*7*), respectively.

The *raf* oncogene was isolated as the transforming gene of 3611-MSV, an acutely transforming, replication-defective, murine retrovirus. This virus transforms fibroblasts and epithelial cells in culture and induces fibrosarcomas in newborn mice. The v-*raf* does not encode a tyrosine-specific protein kinase, which is common to several oncogenes (*6, 8*). The *mil* oncogene has been identified as a second oncogene in the avian retrovirus MH2, which contains the *myc* oncogene. In contrast to other *myc*-containing retroviruses, the MH2 virus is associated with a high incidence of liver and kidney carcinomas. This carcinoma-inducing potential could be a characteristic of the *mil* oncogene or a cooperative