References and Notes

1. S. Dähne, *Z. Wiss. Photogr. Photophys. Photochem.* **56**, 71 (1962); *ibid.* **59**, 113 (1966); T. Tani and S. Kipuchi, *Photogr. Sci. Eng.* **11**, 129 (1967); R. W. Berriman and P. B. Gilman, Jr., *ibid.* **17**, 235 (1973).
2. P. B. Gilman, Jr., *Photogr. Sci. Eng.* **18**, 475 (1976).
3. R. Large, in *Photographic Sensitivity*, R. J. Cox, Ed. (Academic Press, New York, 1973), pp. 241-263.
4. L. Peters *et al.*, *J. Pharmacol Exp. Ther.* **95**, 212 (1949); V. M. L. Srivastova, K. K. Chatteyee, S. Chatak, *Ind. J. Biochem. Biophys.* **8**, 97 (1971); M. Banno, *Kanko Shikiso* **34**, 1 (1955); L. G. S. Brooker and L. A. Sweet, *Science* **105**, 496 (1947); H. N. Wright, J. T. Litchfield, Jr., T. Brey, E. M. Cranston, A. C. Cuckler, R. N. Beiter, *Ann. N.Y. Acad. Sci.* **50**, 109 (1948); L. S. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics* (Macmillan, New York, ed. 5, 1975); O. D. Stauden, *Experimental Chemotherapy*, R. J. Schnitzer and F. Hawking, Eds. (Academic Press, New York, 1963), vol. 1, pp. 701-892.
5. K. W. Kinnally and H. Tedeschi, *Biochim. Biophys. Acta* **503**, 380 (1978).
6. S. Zigman and J. D. Hare, *Mol. Cell. Biochem.* **10**, 131 (1976).
7. T. L. Penner and P. B. Gilman, Jr., *Photogr. Sci. Eng.* **20**, 97 (1976).
8. A. Szent-Györgyi, *Electronic Biology and Cancer: A New Theory of Cancer* (Dekker, New York, 1976).
9. E. M. Hodnett, P. Gopolalakrisham, J. Amuiroazzami, *J. Med. Chem.* **21**, 11 (1978).
10. A preliminary report of this work was presented by S. Zigman, B. C. Antonellis, and S. F. Zigman [*Biol. Bull.* **153**, 451 (1977)].
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Phorbol Ester Action Is Independent of Viral and Cellular *src* Kinase Levels

Abstract. *Treatment of normal chick embryo fibroblasts with phorbol myristate acetate causes those cells to express many of the phenotypic properties of virally transformed cells and also enhances the expression of transformed properties in Rous sarcoma virus–transformed chick embryo fibroblasts. We report here that phorbol myristate acetate has little or no effect on the level of protein kinases encoded by the viral* src *or endogenous* sarc *genes.*

Phorbol esters, the most potent class of tumor promoters, have been shown to induce partial mimicry of the transformed phenotype in normal fibroblasts growing in monolayer culture, to cause enhanced expression in transformed cells of properties frequently associated with the transformed state, and to inhibit terminal differentiation and differentiated cell functions (*1-5*). Because the results of phorbol ester action bear a striking resemblance to the effects on cells induced by the transformation (*src*) gene product of Rous sarcoma virus (RSV), it has been hypothesized that the phorbol esters might act through an effect on the amount or activity of the viral *src* or endogenous *sarc* gene product (*6, 7*). Indeed, Bissell *et al.* (*7*) proposed that the *src* gene product of RSV might better be viewed as a promoter rather than as a carcinogen or "initiator." Recently, substantial progress has been made in identifying, assaying, and characterizing the viral *src* and cellular *sarc* gene product (*8, 9*). The *src* gene of RSV appears to code for a protein of molecular weight 60,000, designated pp60^{src} by Erikson and co-workers (*9*), which can be immunoprecipitated from extracts of RSV-infected chick embryo fibroblasts with antiserum derived from rabbits bearing RSV-induced tumors. The pp60^{src} antigen is capable of transferring ^{32}P-labeled phosphate from γ-^{32}P-labeled adenosine triphosphate (ATP) to the immunoglobulin molecule to which it is bound. Uninfected chick embryo fibroblasts also possess a protein kinase of similar molecular weight which can be immunoprecipitated by the same antiserums (*10, 11*). This protein, the product of the endogenous *sarc* gene, is, however, present at 1 to 2 percent of the level of the viral *src* gene product in RSV-transformed chick embryo fibroblasts.

To determine whether tumor promoters modulate the level of the product

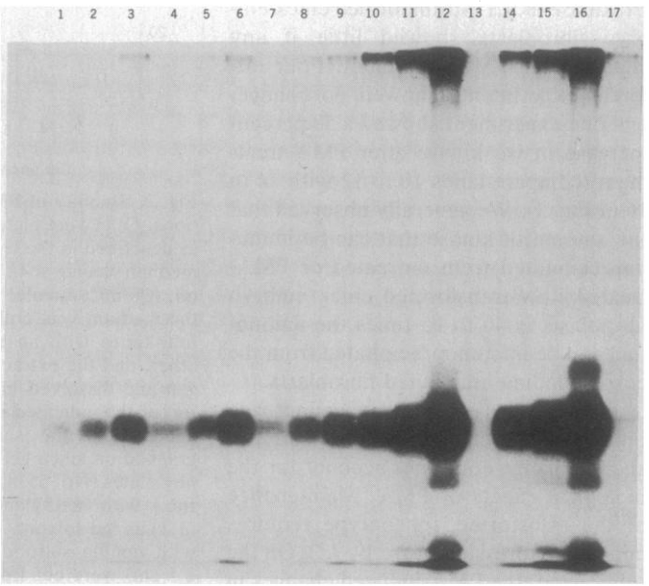

Fig. 1. Effect of PMA on the level of viral *src* kinase or cellular *sarc* kinase. Normal or Schmidt-Ruppin RSV-transformed secondary chick embryo fibroblasts were plated at 4×10^6 or 5×10^6 cells, respectively, per 100-mm dish and grown with daily medium changes in medium 199 supplemented with 10 percent tryptose phosphate broth, 4 percent calf serum, 1 percent heat-inactivated chicken serum, penicillin G (75 U/ml), streptomycin (50 μg/ml), and either dimethyl sulfoxide (DMSO) (0.1 percent, final concentration) or PMA (100 ng/ml, 0.1 percent DMSO, final concentration). Cells were then lysed in buffer containing 1 percent Triton X-100 as described in (*15*). Cell extracts were immunoprecipitated with antiserum from tumor-bearing rabbits (*8*). Antiserums from several rabbits showed broad cross-reactivity with pp60^{src} encoded by several strains of RSV. These serums recognized a protein in uninfected chick embryo fibroblasts that possessed protein kinase activity (*11*). Immune complexes were adsorbed to protein A–Sepharose beads (Pharmacia), washed with Triton buffer, and then assayed for kinase activity. Reaction mixtures were incubated at room temperature for 10 minutes in buffer containing 20 m*M* tris, *p*H 8.1, 5 m*M* $MgCl_2$, 14 m*M* 2-mercaptoethanol, and either 6.6×10^{-8}*M* [γ-^{32}P]ATP (3000 Ci/mmole) when transformed cells were assayed or 2.3×10^{-7}*M* [γ^{32}-P]ATP (3000 Ci/mmole) when uninfected cells were assayed. After incubation, the Sepharose beads were washed and adsorbed proteins were released by boiling in sodium dodecyl sulfate (SDS) and mercaptoethanol and were analyzed by electrophoresis on an 8 percent SDS-polyacrylamide gel. Gels were dried and processed for autoradiography. Tracks 1 to 3 show 100, 200, and 600 μg of protein extracted from quiescent normal chick cells immunoprecipitated with serum from tumor-bearing rabbits (TBR); tracks 4 to 6 show 100, 200, and 600 μg of protein extracted from serum-stimulated normal chick cells immunoprecipitated with TBR serum; tracks 7 to 9 show 100, 200, and 600 μg of protein extracted from normal cells that had been serum-stimulated and treated with PMA; tracks 10 to 12 show 50, 100, and 300 μg of protein extracted from RSV-transformed cells immunoprecipitated with TBR serum; track 13 shows 300 μg of protein extracted from RSV-transformed cells immunoprecipitated with normal rabbit serum; tracks 14 to 16 show 50, 100, and 300 μg of protein from PMA-treated RSV-transformed chick cells immunoprecipitated with TBR serum; track 17 shows 300 μg of protein from PMA-treated RSV-transformed cells immunoprecipitated with normal rabbit serum. In all lanes, ^{32}P-labeled phosphate had been transferred to the 52,000-dalton reduced form of immunoglobulin. For quantitation, this band was cut out from the gel and radioactivity was determined. Under the conditions of the assay, radioactivity was proportional to the amount of active kinase rather than its specific activity (*15*).

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of the cellular *sarc* or viral *src* gene, we grew chick embryo fibroblasts and Schmidt-Ruppin RSV-transformed chick embryo fibroblasts for 3 days in medium containing the potent tumor promoter phorbol 12-myristate 13-acetate (PMA). Medium was changed daily to ensure similar growth rates for the untreated and treated cells and to minimize the problem of phorbol ester degradation. The concentration of PMA used in these experiments was 25 to 44 times the concentrations previously found to give half-maximal responses for mitogenesis, stimulation of 2-deoxyglucose uptake, induction of plasminogen activator, decrease of fibronectin [large external transformation-sensitive (LETS) protein], and morphological alteration (*2, 4, 5, 12*). A 3-day incubation time was chosen since a number of the effects of PMA—such as fibronectin loss or the decrease in collagen synthesis—are only slowly expressed (*3, 4*).

Under these conditions, PMA has little or no effect on the level of endogenous *sarc* kinase (compare lanes 4 to 6 with lanes 7 to 9 in Fig. 1) in uninfected chick embryo fibroblasts. Two different experiments were performed. One showed no difference in *sarc* kinase levels and the other showed a 50 percent increase after PMA treatment. The level of *sarc* kinase in quiescent cells was approximately two-thirds that observed in serum-stimulated cells 24 hours after serum addition. Similarly, the level of *src* kinase in RSV-transformed chick embryo fibroblasts showed little if any change after PMA treatment. Four different experiments showed no change, and one experiment showed a 50 percent increase in *src* kinase after PMA treatment (compare lanes 10 to 12 with 14 to 16 in Fig. 1). We generally observed that the amount of kinase that can be immunoprecipitated from untreated or PMA-treated RSV-transformed chick embryo fibroblasts is 40 to 65 times the amount that can be immunoprecipitated from the corresponding uninfected fibroblasts.

Although our experiments cannot rule out a transient change in kinase activity, such a change could not account for the biological effects of PMA. Maintenance of the transformed phenotype requires constant expression of *src* (*9, 13*). On the other hand, the PMA-induced changes in morphology and fibronectin represent a new steady state rather than transient expression.

To determine whether the lack of effect of PMA on kinase levels in normal and transformed cells was due to degradation of the phorbol esters, we performed the following experiment. Cells were grown as described above, except that radioactive PMA was added to the growth medium for the last 24-hour incubation period, when cell number was highest and degradation of the phorbol ester might be expected to be maximal. At the conclusion of the experiment, the phorbol esters were extracted from the medium and the amount of intact PMA was determined. Recovery of radioactivity was 80 percent. Of this, PMA accounted for 29 percent (equivalent to a final concentration of 23 ng/ml) after incubation with normal chick cells (Fig. 2A) and for 53 percent (equivalent to a final concentration of 42 ng/ml) after incubation with RSV-transformed chick cells (Fig. 2B). These levels of PMA are substantially above that required for expression of the characteristic biological effects of PMA in normal and transformed chick embryo fibroblasts. Altered cell morphology typical of PMA-treated monolayer cultures (*2, 5*) and decreased cell volume, measured with a model ZBI Coulter counter equipped with a C1000 Channelyzer, provided further evidence that PMA was effective in these cell cultures.

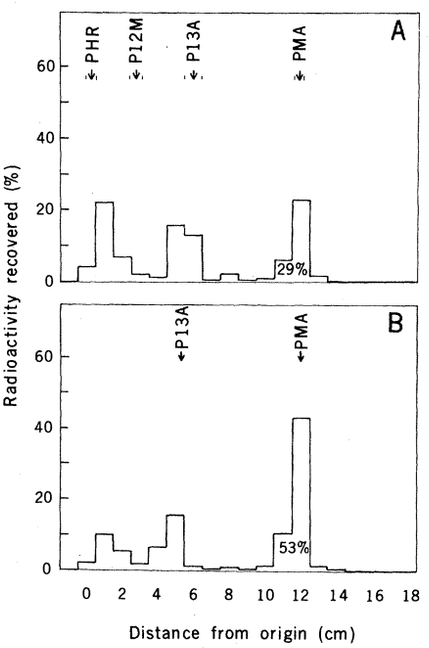

Fig. 2. Stability of PMA under culture conditions. Cells were plated and treated with PMA as described in the legend to Fig. 1. After the medium change at 48 hours, [^{3}H]PMA (100 ng/ml, 630 mCi/mmole) was added. At 72 hours the medium was collected. It was extracted four times with an equal volume of diethyl ether, and the extract was dried under nitrogen and dissolved in methanol. Portions (30 μg) of the indicated marker compounds were added, and the samples were chromatographed on silica gel thin-layer chromatography plates (No. 5538, E. Merck Laboratories, Inc.) with methylene chloride and acetone (3:1) as the solvent. Markers were visualized with vanillin-sulfuric acid spray. Strips (1 by 2.5 cm) were cut from the plates and eluted overnight with 1 ml of methanol. Scintiverse (0.9 ml; Beckman) was added to each vial and radioactivity determined. The results shown for each cell type are the average of four separate plates. Marker compounds are phorbol (*PHR*), phorbol 12-myristate (*P12M*), phorbol 13-acetate (*P13A*), and phorbol 12-myristate 13-acetate (*PMA*). (A) Normal chick embryo fibroblasts. (B) RSV-transformed chick embryo fibroblasts.

In separate experiments, the activity of the viral *src* gene product isolated by immunoprecipitation was assayed in the absence or presence of PMA (100 ng/ml). Rate assays were performed by incubation of immunoprecipitates for varying periods of time in an ice-water bath at limiting substrate concentrations, $10^{-8}M$ [γ-^{32}P]ATP (3000 Ci/mmole). We observed that PMA had no noticeable effect on either initial rate or yield of activity (data not shown).

The lack of significant effect of PMA on the viral *src* gene in chick cells contrasts with the enhanced expression of Epstein-Barr viral antigens observed in lymphoblastoid cell lines by zur Hausen *et al.* (*14*). Induction of viral genes by PMA is thus not a general phenomenon. The findings with the endogenous *sarc* and viral *src* gene products indicate, moreover, that the mechanism by which the phorbol esters modulate transformation-sensitive functions does not involve direct action of these kinases. The results thus provide additional evidence for the existence of multiple means of control of transformation-sensitive cellular properties. How these control pathways are differentially affected by tumor promoters and by direct transformation remains to be determined.

ALLAN R. GOLDBERG
Rockefeller University, New York 10021

K. BARRY DELCLOS
PETER M. BLUMBERG
Department of Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

References and Notes

1. R. K. Boutwell, *CRC Crit. Rev. Toxicol.* **2**, 419 (1974); I. B. Weinstein, M. Wigler, C. Pietropaolo, in *Origins of Human Cancer*, H. H. Hiatt, J. D. Watson, J. A. Winsten, Eds. (Cold Spring Laboratory, Cold Spring Harbor, N.Y., 1977), p. 751; I. B. Weinstein and M. Wigler, *Nature (London)* **270**, 659 (1977); I. B. Weinstein, H. Yamasaki, M. Wigler, L.-S. Lee, P. B. Fisher, A. Jeffrey, D. Grunberger, in *Carcinogens: Identification and Mechanisms of Action*, A. C. Griffin and C. R. Shaw, Eds. (Raven, New York, 1979), p. 399.
2. R. H. Goldfarb and J. P. Quigley, *Cancer Res.* **38**, 4601 (1978).
3. K. B. Delclos and P. M. Blumberg, *ibid.* **39**, 1667 (1979).
4. P. M. Blumberg, P. E. Driedger, P. W. Rossow, *Nature (London)* **264**, 446 (1976).
5. P. E. Driedger and P. M. Blumberg, *Cancer Res.* **37**, 3257 (1977).
6. P. M. Blumberg, *CRC Crit. Rev. Toxicol.*, in press; N. H. Colburn, in *Carcinogenesis*, vol. 7,

Modifiers of Carcinogenesis, T. J. Slaga, Ed. (Raven, New York, in press).
7. M. J. Bissell, C. Hatié, M. Calvin, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 348 (1979).
8. J. S. Brugge and R. L. Erikson, *Nature (London)* **269**, 346 (1977); A. F. Purchio, E. Erikson, J. S. Brugge, R. L. Erikson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1567 (1978).
9. M. S. Collett and R. L. Erikson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2021 (1978); A. D. Levinson, H. Oppermann, L. Levintow, H. E. Varmus, J. M. Bishop, *Cell* **15**, 561 (1978).
10. M. S. Collett, J. S. Brugge, R. L. Erikson, *Cell* **15**, 1363 (1978); J. S. Brugge, M. S. Collett, A. Siddiqui, B. Marczynska, F. Deinhardt, R. L. Erikson, *J. Virol.* **29**, 1196 (1979); H. Oppermann, A. D. Levinson, H. E. Varmus, L. Levintow, J. M. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1804 (1979); R. Karess, W. Hayward, H. Hanafusa, personal communication.
11. A. R. Goldberg and J. G. Krueger, unpublished observations.
12. M. Wigler and I. B. Weinstein, *Nature (London)* **259**, 232 (1976).
13. G. S. Martin, *ibid.* **227**, 1021 (1970).
14. H. zur Hausen, F. J. O'Neill, U. K. Freese, E. Hecker, *ibid.* **272**, 373 (1978); H. zur Hausen, G. W. Bornkamm, R. Schmidt, E. Hecker, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 782 (1979).
15. J. G. Krueger, E. Wang, A. R. Goldberg, *Virology* **101**, 25 (1980).
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Photoluminescent Thermometer Probes: Temperature Measurements in Microwave Fields

Abstract. *Based on luminescence, the photoluminescent thermometer can in principle function in electromagnetic fields without field coupling and perturbation. Several prototypal probes that have been constructed and tested demonstrate the feasibility of the concept. Temperature resolution of approximately 0.3°C has thus far been achieved. The probes are needle-shaped, sturdy and less than 1 millimeter in size. This thermometer system has excellent potential application in monitoring and controlling tissue temperatures when microwaves are used to induce hyperthermia for cancer treatment.*

Hyperthermia is currently being used either alone or in conjunction with radiation and chemotherapy for the treatment of cancer (*1*-*5*). Accurate thermal dosimetry is essential for the objective evaluation and control of this form of therapy (*6*). Electromagnetic (EM) fields (radiofrequency and microwave) and ultrasonic waves are considered practical for inducing local hyperthermia in tissue (*7*, *8*). However, there are unique problems associated with these methodologies with regard to thermometry. This is particularly true with EM fields. Conventional probes (thermocouples and thermistors) give erroneous results in EM fields because of field coupling and perturbations. Temperature measurements with these probes, therefore, require attention with regard to the probe-field orientation and, often, intermittent turning off of the EM fields during therapy while readings are taken (*9*, *10*). Considerable effort has been made to overcome this problem. Several new probes, using liquid crystals, birefringent crystals, optical etalon sensors, fluid viscosity, and so forth, have been explored for specific use in EM environments (*11*).

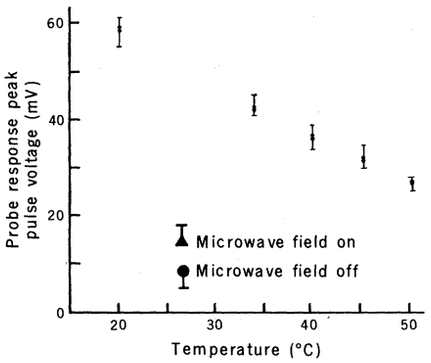

Fig. 1. Temperature response of the calcium sulfide PLT probe with and without 2450-MHz microwave field. Mean response and standard deviations are indicated. The microwave field was generated with a diathermy unit (Burdick MW/225) with a type E corner reflector applicator. The probe tip was positioned in the region of maximum transverse electric field (the transverse plane being that parallel to the plane of the applicator aperture). The probe was inside a glass tube (2 mm inside diameter) through which water from a temperature-controlled reservoir was pumped at a rate of 850 ml/min. The flow was used to keep the probe at the desired temperature while readings were taken. Temperature of the input and output water flow was monitored with thermocouples outside the microwave field region. The estimated power density of the microwave field at the probe was 200 mW/cm^2.

In this report, we introduce the concept of a photoluminescent thermometer (PLT) probe unperturbed by the presence of electromagnetic fields. Figure 1 shows a comparison of temperature measurements with the new PLT probe with and in the absence of 2450-MHz microwave field. Within the current experimental techniques, no perturbation of the probe response as a result of direct coupling with the EM fields can be detected.

The PLT probe is based on the heat-sensitive luminescent response of phosphorescent and fluorescent materials. When excited by light (frequency ν), electrons in these materials can be trapped in higher energy states. The trapped electrons subsequently return to ground states either by producing luminescence (emitting light of lower frequency ν') or by a competing nonradiative process (vibrational quenching). The quantum efficiency (ratio of emitted photons to absorbed photons) depends on the temperature of the material and can be expressed in the form:

$$\eta = [1 + (b/a)e^{-W/kT}]^{-1}$$

where a and b are the radiative and nonradiative transition probabilities, W is the activation energy associated with the nonradiative transition, k is Boltzmann's constant, and T is temperature in degrees Kelvin. In addition, the time dependence of the luminescent response is also temperature-dependent. In a simple single trap model, the lifetime, τ, associated with the rate of luminescent decay is given by $\tau = 1/(se^{-\epsilon/kT})$, where ϵ is the energy associated with the electron trap and s is the frequency at which an electron attempts to escape the trap. This decay time, or lifetime, is an intrinsic property of the luminescent material. Consequently, it allows the measurement of an intensive rather than extensive variable for the purpose of determining temperature. In practice, the temperature dependence of a given luminescent material can be complicated and difficult to pre-

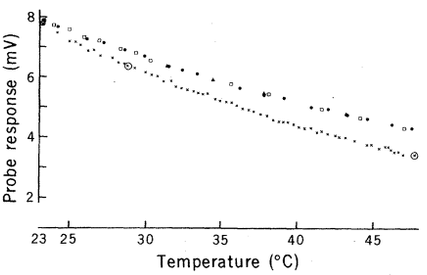

Fig. 2. Temperature calibration curves for prototype probes using different zinc cadmium sulfide phosphors. Xenon light pulses (0.75 msec) were used for excitation, and the time-integrated photoluminescent response was measured. For probe A, the curve of small x's represent individual measurements at the respective temperatures. Approximately 1 minute elapses between each measurement; ⊗ indicates several measurements at selected temperatures. The standard deviation (~ 1 percent) falls within the circle. For probe B, calibration data ● and ▲ were taken 1 and 2 weeks after data □, respectively. Each data point represents the mean of seven separate measurements taken at 1-minute intervals. The standard deviation for each data point is again approximately 1 percent. The resolution for probe A is ~ 0.3° at 40°C, and the resolution for probe B is ~ 0.4° at 40°C.

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