and episomes in prokaryotes (*9, 22, 23*). Such sequences are known to exist in *Drosophila* (*24, 25*), comprising most of the moderately repetitive DNA—about 17 percent of the *Drosophila* genome. Key properties of these elements, designated "nomadic sequences," are their presence at multiple locations in the genome and the strain variations in these positions. The P factor, defined in terms of the induction of hybrid dysgenic traits, has been shown to possess these properties (*17, 26*), and our results show that breakage sites possess them as well. We suggest that the breakage regions represent points of insertion of one of the nomadic sequences and that the P factor itself is also a nomadic sequence, perhaps the same one. The relation between hybrid dysgenesis and nomadic sequences is also indicated by recent indirect evidence suggesting that nomadic sequences also exist as free circles in the nucleoplasm (*27*). If these circles can replicate there independently of their chromosomal counterparts, they could provide an ideal basis for the unusual mixture of chromosomal and extrachromosomal inheritance followed by cytotype—the other component of hybrid dysgenesis (*17*). One might expect these sequences to be found preferentially in regions of intercalary heterochromatin where they are less likely to be selected against, thus explaining the correspondence between constrictions and the hot spots.

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Tumor Promoter TPA Mimics Irradiation Effects on the Cell Cycle of HeLa Cells

Abstract. *When asynchronous and synchronous HeLa cells were incubated with small doses (10^{-7}M) of tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA), a variety of transient alterations in the replication cycle were detected within 24 hours by the use of independent methods. Especially, a delayed passage through the S phase and influences on the G2 phase resemble x-ray irradiation effects on cell cultures. None of these alterations was observed with the hyperplasiogenic but nonpromoting 4-O-methyl-TPA.*

It is generally accepted that carcinogenesis is usually a multistep process, as illustrated by the two-stage mouse skin model (*1*). Initiation and subsequent promotion are elicited by separate classes of chemicals. Tumor promoters are regarded neither as carcinogenic (*2*) nor as mutagenic (*3*). They markedly increase tumor frequency and shorten the latency period of tumor appearance when administered after an initiator. Despite a number of models (*2*), the mechanism of the promoter action is not well understood, since the effects of the potent tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (*4*) seem to be reversible (*5*) when dealing with cells not previously treated with a carcinogen. We have shown that HeLa cells represent a good system for the study of the TPA action (*6*). These cells respond to a large series of phorbol derivatives, their effectiveness corresponding closely to their promoting ability in mouse skin. We (*7*) and others (*8*) had obtained indications that tumor-promoting TPA might specifically interfere with the replication cycle. This study has now elabo-

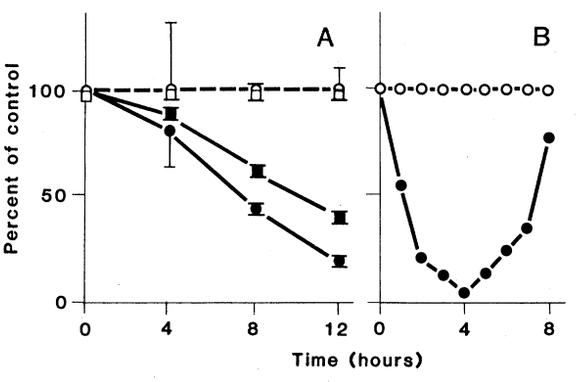

Fig. 1. Response of asynchronous HeLa cells to TPA ($10^{-7}M$) or acetone (0.2 percent) as evaluated by measuring thymidine incorporation rate and labeling index (A), and the mitotic index (B). HeLa cells were cultivated in Eagles minimal essential medium containing Earle's salts supplemented with 10 percent calf serum and were kept in a mixture of humidified air and CO_2 (95:5) at 37°C. Cells were transferred in complete medium to plastic (Falcon) petri dishes (1.45×10^6 cells per dish) 15 hours prior to the experiment. At time 0 TPA or acetone was added. (A) For determination of the incorporation rate and labeling index [*methyl*-^{3}H]thymidine (specific activity, 40 to 60 Ci/mmole; New England Nuclear) (5 μCi/5 ml) was added for 30 minutes prior to the indicated time. The medium was removed, and the cultures were rinsed three times with cold phosphate-buffered saline, fixed in a mixture of ethanol and acetic acid (7:3), and washed twice with cold trichloroacetic acid (5 percent). Some of the dishes were processed for colorimetric determination of DNA (*19*) and scintillation counting. The other dishes were further processed and coated with K2 emulsion (Ilford) for autoradiography. After exposure and development, cells were stained with Giemsa. The labeling index was determined from at least 3×1000 cells per group. ●, ○, Incorporation rate; ■, □, labeling index; ●, ■, TPA; and ○, □, acetone. Bars indicate standard deviation. (B) At the times indicated, photographs were taken (six per group) from undisturbed cultures with the use of an inverted phase-contrast microscope (Leitz Diavert; ×10 lens, ×10 ocular) in order to overcome problems arising from loosely attached mitotic cells. Evaluation was from enlarged prints (13 by 18 cm) for at least 1000 cells per group; ●, TPA; and ○, acetone.

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rated a variety of transient changes that were induced by TPA in different phases of the cell cycle of HeLa cells and that resemble those published for x-ray irradiated cell cultures (*9*).

Our experiments were carried out with random as well as synchronous HeLa cells. Cell cycle passage was analyzed by determination of thymidine incorporation rates into DNA and of labeling and mitotic indices, as well as by high-speed flow cytometry for up to 24 hours after synchronization or addition of phorbol esters. (The phorbol esters were a gift from Dr. E. Hecker, German Cancer Research Center.) They were kept as a stock solution in dimethyl sulfoxide (DMSO) or acetone at −20°C.

Earlier studies had shown that the rate of thymidine incorporation into asynchronous HeLa cells decreased with time specifically after application of small nontoxic doses of tumor-promoting phorbol derivatives (*7*). The data in Fig. 1A show a drop in the incorporation rate of thymidine into DNA upon exposure to $10^{-7}M$ TPA as well as a decrease in the number of labeled cells. The latter indicates a blocking (probably incomplete) of cells in the G1 phase prior to entering the S phase. In addition, comparison of both curves shows that more cells incorporated thymidine than expected from the incorporation rates alone. Thymidine uptake was not significantly altered by TPA when compared with DMSO or the nonpromoting 4-*O*-methyl-TPA (*10*). Thus, the decreased incorporation of thymidine per cell pointed to a delay in the passage through the S phase of those cells exposed to TPA during this period. This was confirmed independently by flow cytometry. The rapid decline of the mitotic activity of asynchronous cultures treated with TPA (Fig. 1B) indicated a block in G2 immediately before entering mitosis. Cells recovered, to some degree, from this blockage after about 8 hours. The mitotic index, however, remained lower than that in the control group. The interpretation of all these observations was supported by DNA histograms obtained through flow cytometry, which were combined to form flow diagrams (Fig. 2). The percentages of cells in different cell cycle phases are plotted versus time. Data were calculated by using the equations described (*11*), but were additionally fitted with results obtained from autoradiography. Despite the immediate onset of the G1 block in the TPA group, as evident from the data in Fig. 1A, the G1 fraction seemed to remain constant for about 8 hours. This is explained by the G2 block at the same time. No cells entered or left G1. After the recovery from the premitotic blocking, the G1 fraction started to increase at 8 hours. The constancy of the cell cycle stages of cultures treated with DMSO is the result of the continuous passage of cells through the different phases. The G2-M fraction in the TPA group (representing mainly G2 cells because of the low mitotic index observed) expanded up until 8 hours, and the largest G2-M/S proportion was reached after 12 hours (1.2 versus 0.6 at time 0). These data are interpreted as a delayed passage through the G2 phase of a portion of cells that were in early S at the addition of TPA.

Results obtained with HeLa cells synchronized by Amethopterin for DNA synthesis (*12*) supported this idea. Addition of TPA in the early S phase caused a

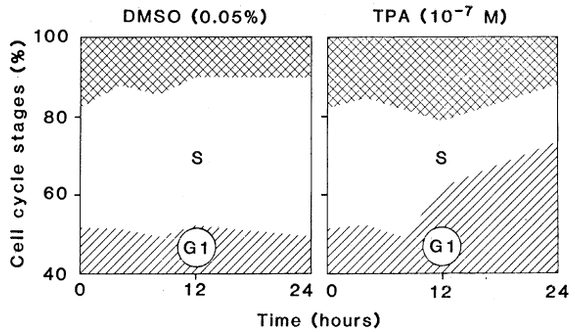

Fig. 2. Flow diagrams of cell cycle distributions of asynchronous HeLa cells developing in the presence of TPA ($10^{-7}M$) or DMSO (0.05 percent). HeLa cells were cultivated in petri dishes for 2 days. At time 0 the medium was replaced by fresh medium containing TPA or DMSO. Some of the cultures were used for determination of the labeling index (see Fig. 1). The other part was processed for flow cytometric measurement of DNA histograms. Cells were washed with 0.1*M* tris-Cl (*p*H 7.5) containing 0.1*M* NaCl, fixed with 70 percent ethanol, and collected at this stage at 4°C. After removal of ethanol, the above buffer containing the fluorochrome 4′,6-diamidino-2-phenylindol (3 μg/ml; compound from SERVA, Heidelberg) was then added. Flow cytometric analysis was carried out with a computerized FACS II cell sorter (Becton Dickinson) which was equipped with an ultraviolet laser beam. The laser was tuned at 363 nm; cellular fluorescence was collected above 390 nm. In order to control the G2-M fraction for doublets, repeated probes were sorted on glass slides and checked microscopically. Calculation of the cell cycle stages was done according to the equations described (*11*) but was fitted in addition with the data obtained by autoradiography. In view of the 30-minute [^{3}H]thymidine pulses given, the S phase may have been slightly overcalculated. The cell cycle stages (measured at 0, 4, 8, 12, and 24 hours) were plotted against time. The G1 fraction (hatched) is partly shown (above 40 percent); the G2-M fraction (crosshatched) is shown on top of each diagram.

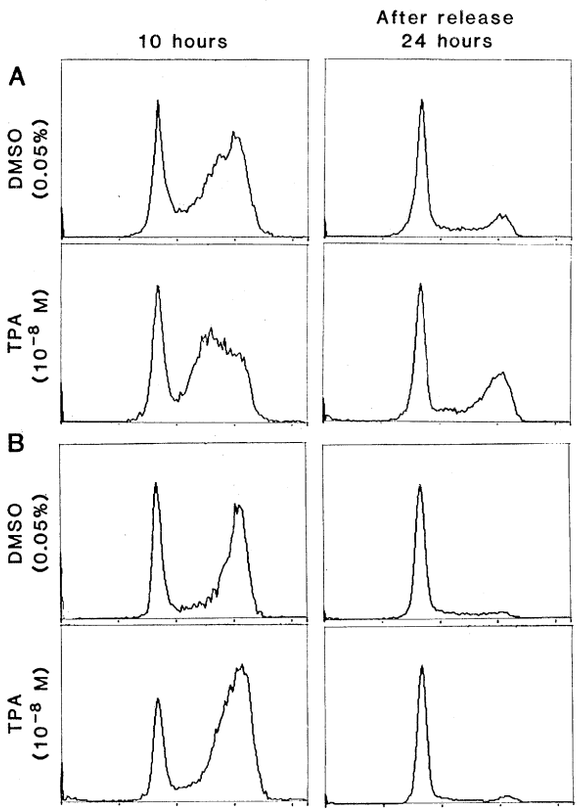

Fig. 3. DNA histograms of synchronous HeLa cells in the presence of TPA ($10^{-8}M$) or DMSO (0.05 percent). Two-day-old HeLa cultures were synchronized by the addition of Amethopterin ($10^{-6}M$; from Lederle, Munich) and adenosine ($5 \times 10^{-5}M$) for 16 hours (*12*). The blockage was released by the addition of thymidine (10 μg per 10^6 cells). TPA or DMSO were given with fresh medium either at the time of release (A) or 6.5 hours later (B). DNA histograms were measured, as in Fig. 2, at times after release as indicated (abscissa, relative fluorescence intensity equivalent to DNA content; ordinate, relative cell number).

large G2 peak after 24 hours (Fig. 3A) when compared with the DMSO control. However, TPA given 6.5 hours after release from synchronizing blockage (that is, in the second half of S) did not cause a larger G2 peak (Fig. 3B). Data obtained 10 hours after release show, in addition, the delayed passage of TPA-treated cells through the S phase (Fig. 3, A and B), thus confirming the interpretation of the incorporation data.

The effects of TPA on the cell cycle of HeLa cells comprise (i) a G1 block prior to entering S, (ii) a delayed passage through S, (iii) a transient blocking in G2, and (iv) a G2 delay of a portion of cells treated with TPA in the early S phase. Since none of these effects were observed with 4-*O*-methyl-TPA (*10*)—a nonpromoting but hyperplasiogenic (*13*) derivative—we conclude that HeLa cell cultures do not offer a target for the mitogenic capacity of phorbol esters. These data and our earlier observations (*6, 7*) seem to be specific for tumor-promoting phorbol derivatives. The effects are reminiscent of results reported from x-ray irradiated cultures. Ionizing radiation can influence the transition from G1 to S in a variety of cells (*14, 15*). Cells in the S phase have been shown to be susceptible to the induction of modification in G2 in their immediate life-span (*9*), and they respond principally with a reduced rate of DNA synthesis and lengthening of the S phase (*9, 15, 16*). A direct blockage of G2 cells by irradiation has also been reported (*9*).

In view of the possibility that TPA may induce sister chromatid exchanges (*17*), although this has been questioned (*18*), the effects of TPA on cells in S as well as in G2 seem to be of particular interest, since sister chromatid exchanges can occur only in these phases of the cell cycle. In that respect also, TPA would mimic irradiation. It remains to be established whether both effects are related.

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Chemical Mimicry as an Integrating Mechanism: Cuticular Hydrocarbons of a Termitophile and Its Host

Abstract. *The staphylinid beetle* Trichopsenius frosti *Seevers has the same cuticular hydrocarbons as those of its host termite* Reticulitermes flavipes *(Kollar) and it biosynthesizes them. These cuticular hydrocarbons probably serve as the primary mechanism by which* Trichopsenius frosti *integrates itself into the termite colony.*

The paramount problem of termite-termitophile biology is determining how termitophiles integrate themselves into the social structure of termite colonies. Various authors have suggested that termitophiles use "appeasement chemicals" (*1*), pass as morphological mimics (*2*), or engage in behavioral mimicry (*3*) (or all). Little evidence, however, has been presented to substantiate these hypotheses.

We have reported (*4*) that several species of termites possess caste- and species-specific mixtures of cuticular hydrocarbons, and that these mixtures may be used by the termites as important, perhaps even primary, species recognition cues. We subsequently reasoned that termitophiles associated with these termites may have evolved cuticular hydrocarbon compositions similar enough to those of their host termites to allow them to be perceived as termites. We now report that the staphylinid beetle *Trichopsenius frosti* Seevers, a highly integrated, host-specific termitophile, has cuticular hydrocarbon components identical to those of its termite host *Reticulitermes flavipes* (Kollar). Furthermore, the beetle biosynthesizes its hydrocarbons.

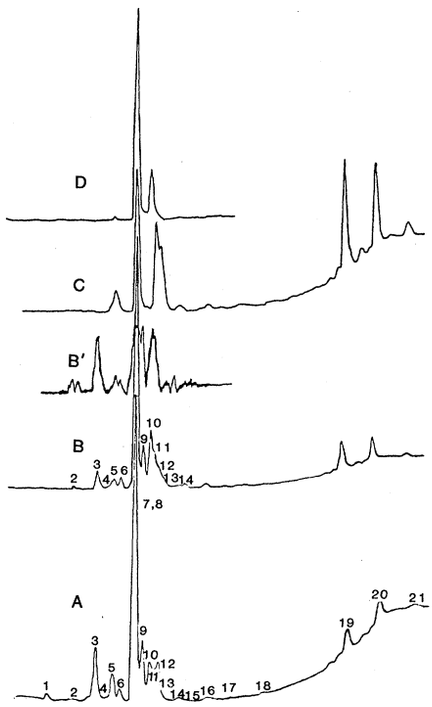

Fig. 1. Gas chromatograph profiles of the total cuticular hydrocarbons of *Reticulitermes flavipes* (A) and *Trichopsenius frosti* (B), the branched hydrocarbons of *T. frosti* (C), and the olefins of *T. frosti* (D); B′ is the radio-GLC trace obtained after incorporation of [1-^{14}C]acetate into hydrocarbons of *T. frosti*.

Portions of several colonies of *R. flavipes* containing *T. frosti* were collected during 1978 from pine logs in Harrison, Jackson, or Stone counties, Mississippi. The beetles were separated from the termites, counted, and stored at −20°C until used. From 715 *T. frosti* (98.69 mg, wet weight), we isolated cuticular hydrocarbons and separated them as described (*4*). Hydrocarbons were characterized by electron impact (EI) and chemical ionization (CI) gas chromatography–mass spectrometry (GC-MS) (*5*). Alkenes

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