cal activity for the isopentenylphenols remains to be determined, we have found them to be active on selected Hemiptera and Orthoptera. The development of additional suicide substrates with selective cytotoxic actions on the insect corpus allatum could provide new approaches to insect control.

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References and Notes

1. W. S. Bowers, in *The Juvenile Hormones*, L. I. Gilbert, Ed. (Plenum, New York, 1976), pp. 394–408.
2. ———, T. Ohta, J. S. Cleere, P. A. Marsella, *Science* **193**, 542 (1976).
3. W. S. Bowers, *Am. Zool.* **21**, 737 (1981).
4. ——— and R. Martinez-Pardo, *Science* **197**, 1369 (1977).
5. W. S. Bowers and J. R. Aldrich, *Experientia* **36**, 362 (1980).
6. W. S. Bowers and M. F. Feldlaufer, *Gen. Comp. Endocrinol.*, in press.
7. P. J. Muller, P. Masner, M. Kalin, W. S. Bowers, *Experientia* **35**, 704 (1979).
8. G. C. Unnithan, K. K. Nair, W. S. Bowers, *J. Insect Physiol.* **23**, 1081 (1977).
9. G. C. Unnithan, K. K. Nair, A. Syed, *Experientia* **36**, 135 (1980).
10. H. Schooneveld, *ibid.* **35**, 363 (1979).
11. L. Liechty and B. J. Sedlak, *Gen. Comp. Endocrinol.* **36**, 433 (1978).
12. W. S. Bowers, in *Natural Products and the Protection of Plants*, G. B. Marini-Bettolo, Ed. (Pontifical Academy of Science, Scripta Varia 41, Vatican City, Italy, 1977), pp. 129–156.
13. W. S. Bowers and D. M. Soderlund, in *Regulation of Insect Development and Behavior* (Wroclaw Technical University, Wroclaw, Poland, 1980), part 1, pp. 309–322.
14. D. M. Soderlund, A. Messeguer, W. S. Bowers, *J. Agric. Food Chem.* **28**, 724 (1980).
15. G. T. Brooks, G. E. Pratt, R. C. Jennings, *Nature (London)* **281**, 570 (1979).
16. R. C. Jennings and A. P. Ottridge, *Chem. Commun.* **1979**, 920 (1979).
17. G. E. Pratt, R. C. Jennings, A. F. Hammett, G. T. Brooks, *Nature (London)* **284**, 320 (1980).
18. D. M. Soderlund, M. F. Feldlaufer, S. Y. Takahashi, W. S. Bowers, in *Juvenile Hormone Biochemistry*, G. E. Pratt and G. T. Brooks, Eds. (Elsevier, Amsterdam, 1981), pp. 353–362.
19. L. Jurd and G. D. Manners, *J. Agric. Food Chem.* **28**, 183 (1980).
20. L. Jurd, K. Stevens, G. D. Manners, *Phytochemistry* **11**, 2535 (1972).
21. Molar equivalents of the appropriate phenol and 3-methyl-2-butene-1-ol (for example, prenol or its equivalent, 3-methyl-1-butene-3-ol) were reacted in 20 percent phosphoric acid with stirring at 80°C for 1 hour. Work-up and recrystallization or open-column chromatography over Florisil, yields 40 to 60 percent of the desired isopentenylphenol. Proton nuclear magnetic resonance ($CDCl_3$) for 5-ethoxy-4-methoxy-2-(3′-methylbut-2′-enyl)phenol: 1.45 (3H, triplet), 1.8 (6H, singlet), 3.3 (2H, doublet, J = 7), 3.85 (3H, singlet), 4.05 (2H, quartet), 5.0 (1H, singlet), 5.35 (1H, triplet, J = 7), 6.45 (1H, singlet), 6.65 (1H, singlet).
22. Second-instar nymphs were continuously exposed to residues of the test compounds, as detailed in Table 1. No precocious adults developed on treatment with methylenedioxyprecocene (MDP) alone (1 μg/cm²) or with combined treatments of MDP (2 μg/cm²) and precocene 2 (1 μg/cm²) or MDP (2 μg/cm²) and 3-ethoxy-4-methoxy-6-*iso*-pentenylphenol (1 μg/cm²).
23. We thank the Rockefeller Foundation for their support of these investigations.

4 June 1982

Active Genes Are Sensitive to Deoxyribonuclease I During Metaphase

Abstract. *The active exogenous murine leukemia virus sequences of mouse cells growing in culture are preferentially digested by deoxyribonuclease I in metaphase chromosomes. As determined by nuclear nick translation, all of the gene sequences of these cells active during interphase are in a deoxyribonuclease I–sensitive conformation during metaphase. This method of nick translation can therefore be used to label chromosomes in situ in order to visualize the active regions of the genome.*

When nuclei from many types of organisms are partially digested with deoxyribonuclease I, the active genes are found to be in a special sensitive conformation (*1–3*). The nuclei are usually examined in cells at interphase when these genes are actively being transcribed. It was of interest to investigate the deoxyribonuclease I sensitivity of active genes at different stages in the cell cycle and, in particular, during metaphase when most active genes are usually silent.

To determine whether potentially active genes are sensitive to deoxyribonuclease I during metaphase, we examined the nuclease sensitivity of the exogenous murine leukemia virus (MuLV) sequences in mouse cells growing in monolayer cultures. These genes are known to be active, as determined by the production of viral RNA (*4*) and by the observation that they are three to four times more sensitive to deoxyribonuclease I than the total nuclear DNA is (*5*). Cells in mitosis were isolated from growing cultures, and the nuclei of these cells were treated with deoxyribonuclease I until about 10 percent of the DNA was digested. The undigested DNA was then hybridized to an MuLV-specific probe and compared to DNA from total unsynchronized cultures. Figure 1A shows

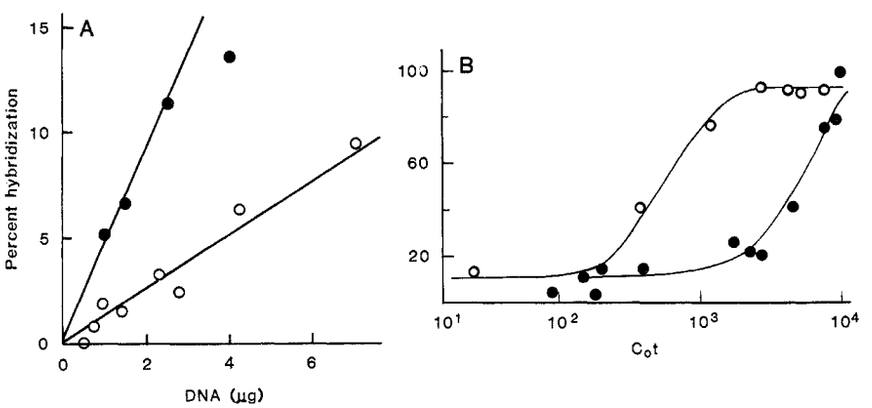

Fig. 1. Deoxyribonuclease I sensitivity of active genes in mitotic chromosomes. (A) NIH Swiss mouse cells were maintained in culture as described (*5*). These cells were synchronized by treatment with thymidine and Colcemid (*13*), and mitotic cells were selected by gentle shaking of the monolayer cultures. Metaphase cells were then purified by differential centrifugation in detergent containing hypotonic medium (*14*). We regularly obtained cells with a mitotic index of 90 percent by this procedure. The resulting metaphase chromosomes were treated with deoxyribonuclease I to obtain 10 percent digestion as determined by acid solubility (*5*). Total DNA (●) and deoxyribonuclease I–treated DNA (○) were purified by phenol and chloroform extractions (*5*) and hybridized for 72 hours at 70°C to a tritium-labeled MuLV complementary DNA (cDNA) probe (10,000 cpm/ng) with an excess of cDNA (*5*). The results are expressed as percent hybridization, determined by S1 nuclease analysis (*1*), after subtraction of 2 percent background hybridization observed in the absence of added DNA. (B) Mitotic chromosomes were nick-translated essentially as described previously for interphase nuclei (*7*). These chromosomes (1 mg/ml) were incubated with deoxyribonuclease I (0.1 μg/ml) in 50 m*M* tris-HCl (*p*H 7.9), 5 m*M* $MgCl_2$, 10 m*M* β-mercaptoethanol, and bovine serum albumin (50 μg/ml) for 15 minutes at 37°C. DNA polymerase (Boehringer Mannheim, 10 unit/ml), 4 μ*M* deoxyadenosine triphosphate, deoxycytidine triphosphate, and deoxyguanosine triphosphate and 0.3 μ*M* 3H-labeled thymidine triphosphate (New England Nuclear, 40 to 50 Ci/mmole) were then added, and the reaction was incubated at 15°C for 5 minutes. The resulting DNA (5000 cpm/μg) was purified by phenol extraction and Sephadex G-50 chromatography and hybridized to itself to remove highly repeated sequences (*7*). This radioactive probe representing the deoxyribonuclease I–sensitive regions of the metaphase chromosomes was then hybridized to excess total DNA (○) or DNA obtained from 10 percent deoxyribonuclease I digestion of interphase nuclei (●) (*5*). The conditions for hybridization and analysis of DNA duplex formation were exactly as described by Levitt *et al.* (*7*). C_0t, concentration of the probe (moles per liter) multiplied by the time of digestion (seconds).

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that the viral genes in metaphase nuclei are about three times more sensitive to digestion than total DNA is. These results are identical to those obtained with unsynchronized cells (*5*). When the same DNA was hybridized to a mouse β-globin probe, this gene was not preferentially digested by deoxyribonuclease I. Furthermore, the inactive endogenous mouse viral sequences that cross-hybridize to the MuLV hybridization probe were insensitive to deoxyribonuclease I in metaphase nuclei of NIH Swiss mouse cells (*5*) (data not shown). These experiments indicate that the chromatin conformation of both active and inactive genes as measured by nuclease sensitivity is the same in metaphase and interphase chromosomes. The high mobility group proteins HMG 14 and 17 are directly involved in maintaining the sensitivity of active genes to deoxyribonuclease I in interphase nuclei (*6*). Removal of these proteins from metaphase nuclei by treatment with 0.4*M* NaCl caused these viral genes to revert to a nonsensitive conformation (results not shown), an indication that the factors controlling the structure of active genes are similar in interphase and metaphase cells.

While this finding suggests that the active viral gene remains exposed to enzymatic probes during metaphase as well as during interphase, it was deemed necessary to show that this is true for a wide range, if not all, of the active genes being expressed in any cell. Previous studies have shown that active genes can be uniformly labeled by nick translation of nuclei (*7*). In this method, deoxyribonuclease I preferentially introduces nicks in the DNA at active regions, and DNA polymerase then replaces the adjacent DNA with radioactive nucleotides. Such labeled DNA is representative of the deoxyribonuclease I–sensitive fraction of the nuclei and corresponds to the sequences expressed as RNA (*7*). To demonstrate that genes active during interphase are in a deoxyribonuclease I–sensitive conformation in metaphase, we labeled metaphase chromosomal DNA by nick translation to high specific activity and hybridized the labeled DNA to total DNA and to DNA isolated from interphase nuclei after minimal deoxyribonuclease I digestion. The latter DNA presumably lacks active gene sequences. The labeled DNA representing the deoxyribonuclease I–sensitive regions of metaphase chromosomes did not hybridize well to this deoxyribonuclease I–resistant DNA, indicating that the sensitive regions of metaphase chromosomes are equivalent to the sensitive regions of the interphase chromosomes (Fig. 1B). This observation was confirmed by a converse hybridization experiment with nuclear nick-translated interphase DNA and an excess of total and deoxyribonuclease I–resistant fractions from metaphase nuclei (results not shown). These experiments show that the active genes expressed during interphase are in a deoxyribonuclease I–sensitive conformation during metaphase.

This observation is potentially important since it may permit the detection of active gene sequences on mitotic chromosomes present during metaphase. Cell preparations rich in mitotic cells were fixed and spread on microscope slides for visualization of mitotic chromosomes. The chromosome mounts were incubated in buffer with various concentrations of deoxyribonuclease I, then with DNA polymerase in the presence of tritiated deoxynucleoside triphosphates. The slides could then be autoradiographed, stained, and examined with the light microscope. Grains were observed in both interphase nuclei and in mitotic chromosomes. As expected, the number of observed grains was proportional to the extent of deoxyribonuclease I digestion before labeling (Fig. 2A), indicating that this assay is indeed specific to deoxyribonuclease I–sensitive regions, which presumably represent the active fraction of the genome. At every deoxyribonuclease I concentration, the number of grains seen in interphase chromosomes was equivalent to the number of grains appearing for each set of mitotic chromosomes on the same slide preparation. This supports the biochemical evidence that deoxyribonuclease I sensitivity is quantitatively preserved during metaphase.

The specificity of the nick translation labeling reaction was analyzed by labeling mitotic chromosome preparations at low deoxyribonuclease I concentrations in order to obtain approximately one or two grains per chromosome. Since each mitotic chromosome consists of two identical chromatids, most of the label might be expected to be symmetrically placed on the two sister chromatids of each chromosome. In fact, more than 70 percent of the grains were found side by side (Fig. 2B).

Active genes in dividing cells have generally been found in a deoxyribonuclease I–sensitive chromosomal conformation (*5, 8–10*). Since most genes are expressed during the interphase stage of

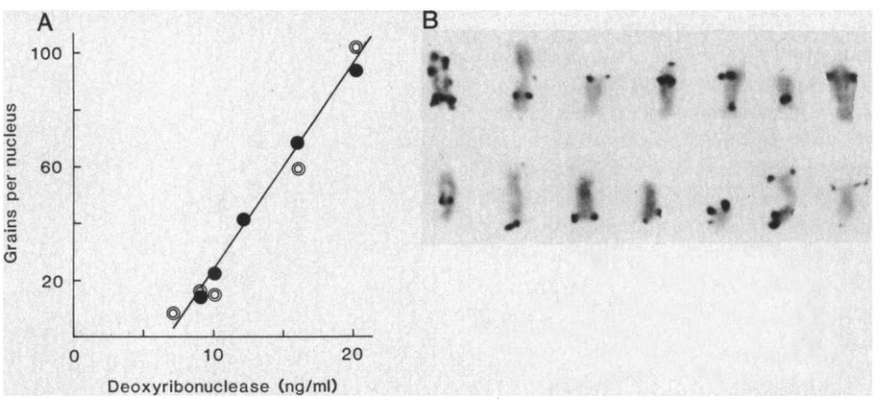

Fig. 2. Nick translation of metaphase chromosomes in situ. (A) Human fibroblast cells growing in suspension were synchronized by Colcemid, treated with hypotonic KCl (0.5 percent) solution, fixed with a mixture of methanol and acetic acid (20:1), dripped on microscope slides, and immediately washed with nick translation buffer (see Fig. 1). These slides, which contained mitotic chromosomes as well as interphase nuclei, were treated with several concentrations of deoxyribonuclease I in nick translation buffer for 15 minutes at room temperature. The slides were then washed and incubated in the same buffer containing DNA polymerase and the deoxynucleoside triphosphates, as described in the legend to Fig. 1B. After this labeling procedure, slides were dipped in emulsion and autoradiographed after 1 or 2 days (*15*). The number of grains appearing in nuclei (●) or on chromosome spreads (○) was then determined, and the background level of four grains per cell obtained without deoxyribonuclease I treatment was subtracted from the data. The fact that this curve does not pass through the origin probably indicates that the generation of nicks in chromosome DNA is not linear with deoxyribonuclease I concentration. (B) Slides rich in metaphase chromosomes were prepared and nick-translated with deoxyribonuclease I at a concentration of 7 ng/ml to obtain approximately one or two grains per chromosome. After autoradiography, the preparations were stained with Giemsa and photographed. The chromosomes shown were selected from two separate cells. Many chromosomes in these cells were completely unlabeled and some chromosomes contained isolated grains. Seventy percent of the grains from each cell were found to be symmetrically placed on sister chromatids. The chromosome shown in the upper left position was from a cell labeled with a higher concentration of deoxyribonuclease I to a level of about ten grains per chromosome.

the cell cycle, it was not clear whether the active conformation is present exclusively during the interphase or also during cell metaphase, when most genes are temporarily silent. It had been demonstrated earlier that metaphase chromosomes have a nucleosomal structure similar to that of interphase cells (*11*). Our studies show that the deoxyribonuclease I–sensitive conformation associated with gene expression is an integral part of the gene environment during all stages of the cell cycle regardless of the activity of the gene. Although we have shown this only for cells growing in culture, it is probably true for somatic cells in general.

The deoxyribonuclease I–sensitive conformation is transferred to newly synthesized DNA during the process of replication, and in this way, this special structure is carried on from generation to generation (*12*). Once formed, this conformation appears to be quite stable and is retained even during metaphase, when genes are relatively inactive. This is consistent with the idea that deoxyribonuclease I sensitivity is correlated with potential gene activity and is retained even when the gene is temporarily silent. The hemoglobin gene, for example, is deoxyribonuclease I–sensitive in avian erythrocytes, even though the cells have ceased to transcribe hemoglobin RNA (*1*). Furthermore, erythroleukemia cells have deoxyribonuclease I–sensitive hemoglobin genes both before and after induction with dimethyl sulfoxide (*8*). All active genes have been shown to be sensitive to nuclease action to the same degree regardless of the extent of activity of the particular gene (*3*). Thus, while this conformational marker indicates potential activity, factors other than deoxyribonuclease I sensitivity appear to be involved in the regulation of gene expression.

The technique of nick translation of mitotic chromosomes should be useful for identifying and mapping active regions of the genome. In preliminary experiments, we have succeeded in using this technology to identify the active and inactive X chromosomes in female fibroblast cells and have characterized several specific locations on homologous autosomal human chromosomes that appear to be in an active conformation.

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References and Notes

1. H. Weintraub and M. Groudine, *Science* **193**, 848 (1976).
2. A. Garel and R. Axel, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3966 (1976).
3. A. Garel, M. Zolan, R. Axel, *ibid.* **74**, 4867 (1977).
4. H. Fan and D. Baltimore, *J. Mol. Biol.* **80**, 93 (1973).
5. A. Panet and H. Cedar, *Cell* **11**, 933 (1977).
6. B. Gazit, A. Panet, H. Cedar, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1787 (1980).
7. A. Levitt, R. Axel, H. Cedar, *Dev. Biol.* **69**, 496 (1979).
8. D. N. Miller, P. Turner, A. Nienhuis, D. C. Axelrod, T. V. Gopalkrishnau, *Cell* **14**, 511 (1978).
9. S. J. Flint and H. Weintraub, *ibid.* **12**, 783 (1977).
10. M. Groudine, R. Eisenman, H. Weintraub, *Nature (London)* **292**, 311 (1981).
11. M. Wigler and R. Axel, *Nucleic Acids Res.* **3**, 1463 (1976).
12. H. Weintraub, *ibid.* **7**, 781 (1979).
13. D. F. Peterson and E. C. Anderson, *Nature (London)* **203**, 642 (1964).
14. K. Willecke and F. H. Ruddle, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1792 (1975).
15. B. M. Stannlis, S. Sheldon, G. L. Grove, Z. J. Cristofals, *J. Histochem. Cytochem.* **27**, 1303 (1979).
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Spiking Local Interneurons Mediate Local Reflexes

Abstract. *A local spiking interneuron in the locust is excited by particular sensory stimulation of a hind leg and forms an inhibitory connection with one hind leg motor neuron. Its behavioral effect is to mediate a local postural reflex. This interneuron is one of a population of interneurons with similar morphology and physiology that participate in the same local circuits as the better known nonspiking local interneurons.*

Local interneurons outnumber principal interneurons within the central nervous systems of vertebrates and arthropod invertebrates, but relatively little is known about their physiological properties (*1–3*). Recently, however, it has become possible in some arthropods to study the same local interneurons in different individuals, and in the locust in particular to record simultaneously from these interneurons and from other neurons pre- or postsynaptic to them (*4, 5*). In the segmental ganglia of arthropods, most local interneurons described are nonspiking ones, which coordinate and control patterned motor activity (*4, 6, 7*). Only one spiking local interneuron has been characterized, the omega neuron in the prothoracic ganglion of the cricket, which responds to auditory stimuli (*8*). Many more spiking local interneurons have been described in the brains of

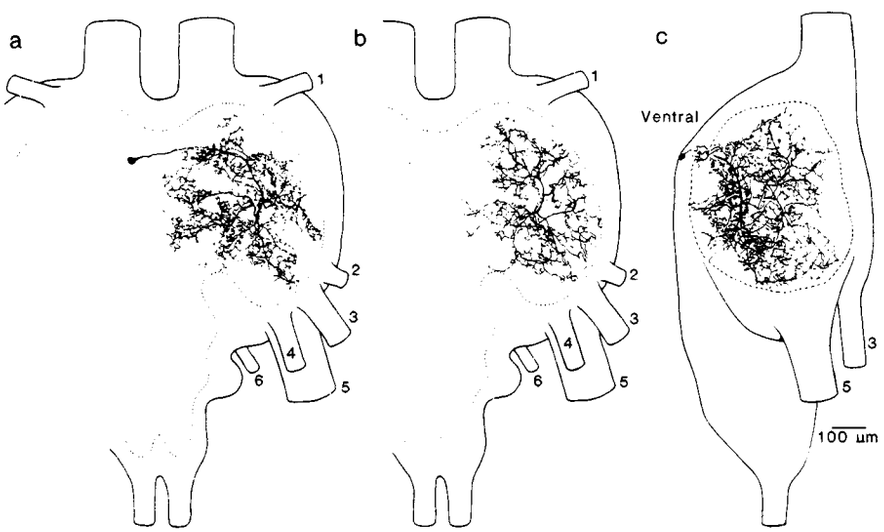

Fig. 1. Drawings of a spiking local interneuron in the metathoracic ganglion of the locust, stained by the intracellular injection of cobalt with subsequent silver intensification. An interneuron with this morphology and with the same physiological properties has been stained in four locusts. (a) The right half of the ganglion, viewed dorsally, showing the more ventral branches of the interneuron. Stippling indicates the two regions in which cell bodies of spiking local interneurons have been found that are affected by the right leg. (b) The same view of the ganglion, showing the more dorsal branches of the interneuron; the major ventral branches are stippled. (c) The ganglion viewed from the side, showing the ventral and dorsal extent of the branches. The extent of the neuropil is indicated by the dashed line. The edge of the most posterior-medial branching coincides roughly with the boundary between the thoracic and abdominal portions of this fused ganglion. Lateral nerves 1 to 6 are numbered; more posterior ones are omitted. The paired anterior connectives to the next segmental ganglion are at the top. The drawings were made of the whole ganglion with the aid of a camera lucida attached to a compound microscope.

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