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.

BRURIA GAZIT
HOWARD CEDAR
Departments of Molecular Biology and Cellular Biochemistry, Hebrew University, Jerusalem, Israel
ISRAELA LERER, RUTH VOSS
Department of Human Genetics, Hadassah Medical Center, Jerusalem, Israel

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).
16. This work was supported by grant GM 20483 from the National Institutes of Health.

11 January 1982; revised 6 May 1982

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

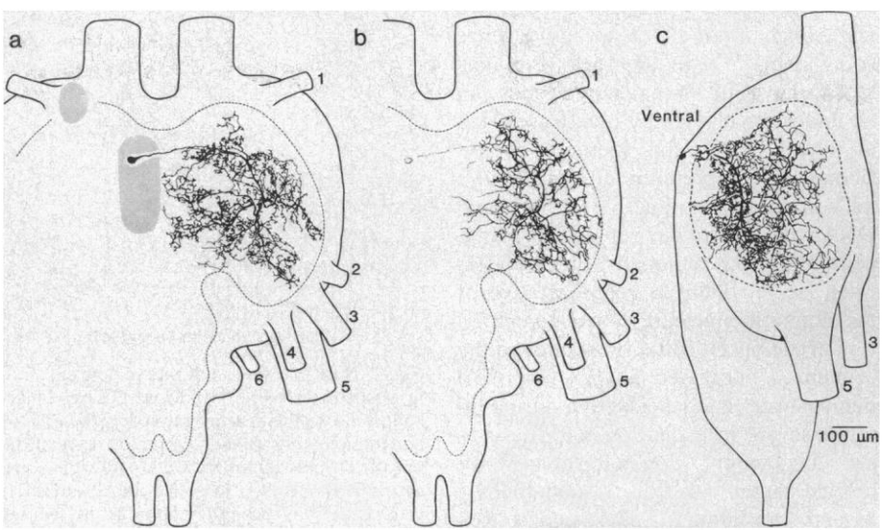

Fig. 1. Drawings of a spiking local interneuron in the metathoracic ganglion of the locust, stained by the intracellular injection of cobalt with subsequent silver intensification. An interneuron with this morphology and with the same physiological properties has been stained in four locusts. (a) The 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.

0036-8075/82/0813-0650$01.00/0

arthropods and characterized according to their responses to particular sensory stimuli (*9*).

Spiking local interneurons that are involved in the same local circuits as the nonspiking local interneurons have, nevertheless, not yet been described. Such a description is essential if we are to understand why some local interneurons are spiking and some nonspiking. Here, we describe a population of spiking local interneurons with similar morphological and physiological properties in the metathoracic ganglion of the locust. We also show that one of these interneurons mediates a local postural reflex of a hind leg. Finally, we compare the properties of the spiking local interneurons with those of the nonspiking local interneurons involved in the same movements.

Locusts were minimally dissected and capable of making vigorous movements so that activity in individual neurons could be related to behavior. The metathoracic ganglion, which controls the hind legs and hind wings, was exposed by a ventral dissection to allow intracellular recording from the somata of interneurons and motor neurons, and the subsequent staining of interneurons with cobalt (*10*).

Intracellular staining of individual spiking local interneurons revealed two ventrally located groups of cell bodies in each half-ganglion (Fig. 1). Each interneuron has a distinctive morphology, although all have features in common. They ramify widely within one-half of the ganglion, both dorsally, among the branches of motor neurons, and ventrally, among the axons of primary afferents. Their monopolar cell bodies, 10 to 20 μm in diameter, are contralateral to the major neuropilar branching. The process between a cell body and the neuropilar branches traverses the ganglion in one of two commissures. While no branches emerge from a process as it crosses the ganglion, some interneurons, with cell bodies in the lateral group (Fig. 1), have short and fine branches in the neuropil ipsilateral to the cell body.

Spikes (*11*) can be elicited in the spiking local interneurons by proprioceptive and mechanosensory inputs that originate from the hind leg ipsilateral to their major neuropilar branches. For example, the interneuron in Fig. 1 spikes when the ipsilateral tibia is extended about the femur and when a group of hairs on the distal part of the tibia is touched (Fig. 2, a and b). Similar stimuli to the contralateral hind leg, or stimuli elsewhere on the body, are without effect, unless they elicit extension of the ipsilateral tibia. Other local spiking interneurons respond preferentially to: touching of particular hairs (the movement of a single hair is a sufficient stimulus to elicit spikes), pressure on particular campaniform sensilla, flexion of the tibia, and depression or elevation of the tarsus. Those that respond to movements of the tibia or tarsus do so whether the leg is moved forcibly by the experimenter, or actively by the animal itself.

One action of spiking local interneurons is to mediate local postural reflexes. When the tibia of a hind leg is extended forcibly about the femur, the tarsus of that leg is depressed. The depressor tarsi motor neurons are excited, while the single antagonistic levator tarsi motor neuron (*12*) is inhibited (Fig. 2c). Motor neurons innervating muscles of other joints are also affected by the imposed movement (*13*), but tarsal motor neurons of the opposite hind leg are not (Fig. 2c). The reflex is local, persisting when the metathoracic ganglion is isolated from the rest of the central nervous system (*13*). Large and distinctive inhibitory postsynaptic potentials (IPSP's) of two classes are responsible for this inhibition of the levator tarsi motor neuron (*14*). These potentials also may occur at a low frequency in the absence of an externally applied stimulus, or at a high frequency if certain tibial hairs are touched (Fig. 2b). The spiking local interneuron in Fig. 1 shares these response properties and evokes one class of IPSP in the levator motor neuron. Each spike in this interneuron is followed at a constant delay by an IPSP in the levator motor neuron (Fig. 2, d and e). Paired recordings from the interneuron and motor neuron in seven locusts show that the average delay is 0.97 msec (standard error, ± 0.08 msec) (*15*). Spikes are followed by IPSP's whether they are elicited by sensory stimulation or by injecting depolarizing current into the soma of the interneuron.

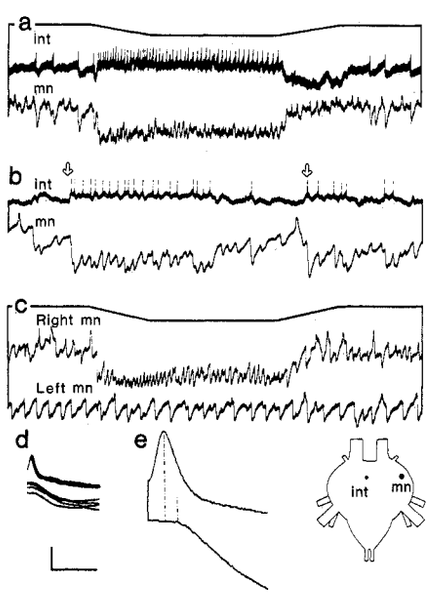

Fig. 2. The spiking local interneuron drawn in Fig. 1 mediates a local postural reflex. (a) The right tibia is forcibly extended from a femoral-tibial angle of 90° to 140°, held there, and then returned to 90° (top trace). The local interneuron (middle trace) is depolarized and spikes, while the right levator tarsi motor neuron (bottom trace) is hyperpolarized. (b) Spikes are also elicited in the interneuron when distal hairs on the tibia are stroked (open arrows). (c) When the right tibia is forcibly extended (top trace), the right levator tarsi motor neuron (middle trace) is hyperpolarized, but the left levator tarsi motor neuron (bottom trace) is not. (d) Superimposed single sweeps. Each spike in the local interneuron is followed by an IPSP in the right levator tarsi motor neuron. (e) Average of 64 sweeps. The peak of the spike and the onset of the IPSP are marked by dotted lines. The delay is 0.9 msec. Schematic drawing of the ganglion indicates locations of recording electrodes in the somata of the interneuron (*int*) and motor neuron (*mn*). Vertical calibration, interneuron: (a) 2.5 mV; (b) 4 mV; (d) 2 mV; (e) 0.6 mV; motor neuron: 4 mV. Horizontal calibration: (a) to (c) 400 msec; (d), 12.5 msec; (e) 1.9 msec.

Two lines of evidence suggest that the motor output of this interneuron is restricted. First, depolarization of the interneuron to elicit spiking at frequencies up to 50 Hz causes only a depression of the tarsus (provided the tarsus has been actively elevated). Extracellular recordings from muscles of the hind legs reveal no other effect. Second, intracellular recordings made simultaneously from the levator motor neuron and from motor neurons to other tarsal and tibial muscles, or nonspiking interneurons with connections to these motor neurons, have failed to reveal any which share this distinctive pattern of IPSP's. Similarly, intracellular recordings from depressor motor neurons reveal no complementary excitatory connection. The motor effect of this interneuron is thus explained by its inhibitory connection to the levator motor neuron. Other connections are unknown, but judging from the interneuron's profuse branching, some might be expected.

What can we conclude about the presence of both spiking and nonspiking local interneurons in the same local circuits? There are similarities in the morphology of the interneurons and in their roles in local reflexes but differences in the extent of their inputs and outputs. Both types of interneuron branch extensively, typically within one-half of the bilaterally symmetrical ganglion, although the branches of the spiking interneurons are more clearly segregated into ventral and dorsal regions (*16*). The presence or ab-

sence of spikes does not, therefore, appear to be related to the morphological distance over which information must be conducted by an interneuron. A comparison of the roles of spiking and nonspiking local interneurons in local reflexes reveals further similarities. First, interneurons of both types have apparently direct synaptic effects on hind leg motor neurons. Second, interneurons of both types are excited by movements of the hind legs. For example, during movements of the femoral-tibial joint, a series of spikes or excitatory postsynaptic potentials (EPSP's) can be elicited in some spiking or nonspiking interneurons, respectively (*17*). When the joint is held flexed or extended some spiking local interneurons spike tonically, whereas some nonspiking interneurons show tonic shifts in their membrane potential (*18*). Third, interneurons of both types can cause either transient or sustained changes in the membrane potentials of postsynaptic motor neurons. Discrete synaptic potentials in motor neurons may be caused by single spikes in spiking local interneurons, or by single EPSP's in nonspiking local interneurons (*17*). Sustained postsynaptic changes may be caused by repetitive spikes in spiking local interneurons, or by sustained depolarization of nonspiking local interneurons (*18*). However, when the outputs and inputs of the two types of interneuron are compared, two differences emerge. First, spiking local neurons can, when stimulated individually, produce only a limited motor effect. By contrast, individual nonspiking local interneurons can cause vigorous and well-coordinated movements about several joints of a hind leg (*4–6*). Second, spiking local interneurons respond to more restricted sensory inputs than do nonspiking local interneurons. Further study may reveal differences between the two types of interneuron in the relative locations of input and output synapses, or in the electrotonic distances over which information must be transmitted intracellularly (*19*).

We do not yet know enough about the connections of local spiking interneurons to provide any general rationale for the presence of both spiking and nonspiking local interneurons. Nonetheless, the most reasonable assumption on which to base further experiments is that the use of particular types of local interneurons is related to their different functions in the behavior of the animal. For example, one role of spiking interneurons is to effect local reflexes in response to specific sensory inputs, and one role of nonspiking interneurons is to coordinate the output of groups of motor neurons. These functions may, in turn, account for the need to conduct a particular type of signal intracellularly, or the need to effect the release of transmitter in a particular way.

M. BURROWS
M. V. S. SIEGLER
Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, England

References and Notes

1. Local interneurons are those in an anatomically restricted region of the nervous system [see (*2*)]. In the ventral nerve cord of arthropods, a local interneuron is confined to a segmental ganglion; in the brains of arthropods and vertebrates, the distinction is less easy to make.
2. P. Rakic, *Local Circuit Neurons* (MIT Press, Cambridge, Mass., 1976).
3. G. M. Shepherd, *The Synaptic Organization of the Brain* (Oxford Univ. Press, New York, 1979); F. O. Schmitt and F. G. Worden, Eds., *The Neurosciences Fourth Study Program* (MIT Press, Cambridge, Mass., 1979); A. Roberts and B. M. H. Bush, Eds., *Neurones Without Impulses* (Cambridge Univ. Press, Cambridge, 1981).
4. M. Burrows and M. V. S. Siegler, *J. Physiol. (London)* **298**, 213 (1980).
5. M. Burrows, *J. Neurophysiol.* **42**, 1108 (1979).
6. K. G. Pearson and C. R. Fourtner, *ibid.* **38**, 33 (1975).
7. W. J. Heitler and K. G. Pearson, *Brain Res.* **187**, 206 (1980); A. J. Simmers and B. M. H. Bush, *ibid.* **197**, 247 (1980).
8. G. B. Casady and R. R. Hoy, *J. Comp. Physiol.* **121**, 1 (1977); A. V. Popov, A. M. Markovitch, A. S. Andjan, *ibid.* **126**, 183 (1978); D. W. Wohlers and F. Huber, *ibid.* **127**, 11 (1978).
9. For example, for olfactory stimuli, see R. Selzer, *ibid.* **134**, 159 (1979); S. G. Matsumoto and J. G. Hildebrand, *Proc. R. Soc. London Ser. B* **213**, 249 (1981).
10. The methods are described in detail by G. Hoyle and M. Burrows [*J. Neurobiol.* **4**, 3 (1973)] and in (*4*). The femora of the hind legs were fixed and the tibiae moved by mechanical actuators controlled by a microprocessor. Microelectrodes were filled with either 2*M* potassium acetate (d-c resistances of 40 to 60 megohms) or with a cobaltous lysine complex made as described by F. Gallyas, L. Lenard, and G. Lazar [*Neurosci. Lett.* **9**, 213 (1978)]. The interneurons were stained with cobalt and silver, as described by R. M. Pitman, C. D. Tweedle, and M. J. Cohen [*Science* **176**, 412 (1972)] and J. P. Bacon and J. S. Altman [*Brain Res.* **138**, 359 (1977)]. Adult *Schistocerca americana gregaria* (Dirsh) of either sex were obtained from a crowded laboratory culture.
11. Spikes in the somata of the interneurons were typically 1 to 5 mV in amplitude and were of briefer duration than those recorded in the somata of leg motor neurons. The site of their initiation is unknown.
12. Two levator tarsi motor neurons were originally described, one fast and one slow, in M. Burrows and G. Hoyle [*J. Neurobiol.* **4**, 167 (1973)]. Present evidence indicates that there is usually only one excitatory motor neuron (M. V. S. Siegler, *J. Exp. Biol.*, in press).
13. M. Burrows and G. A. Horridge, *Philos. Trans. R. Soc. London* **269**, 49 (1974).
14. Passing current into the motor neuron reverses the polarity of these potentials at a membrane potential more negative than the normal resting one. The synaptic potentials are therefore both chemically mediated and inhibitory. The IPSP's often occur in a pattern that includes beats, indicating that they are derived from two sources. The larger IPSP class is mediated by the interneuron described here.
15. At the giant synapse of the squid, the synaptic delay is 1.2 msec at 15°C [A. Takeuchi and N. Takeuchi, *J. Gen. Physiol.* **45**, 1181 (1962)]. Our measurements made at 22°C are therefore consistent with the connection being monosynaptic, but physiological evidence alone cannot rule out the possibility of an interposed nonspiking interneuron.
16. The morphology of nonspiking interneurons is described by M. V. S. Siegler and M. Burrows, *J. Comp. Neurol.* **183**, 121 (1979).
17. M. Burrows, *Science* **204**, 81 (1979).
18. M. V. S. Siegler, *J. Neurophysiol.* **46**, 296 (1981); *ibid.*, p. 310.
19. W. Rall, in *Neurones Without Impulses*, A. Roberts and B. M. H. Bush, Eds. (Cambridge Univ. Press, Cambridge, 1981), p. 223.
20. We thank our Cambridge colleagues for their helpful comments on the manuscript. Supported by NIH grant 1R01 NS16058-01 to M.B. and a project grant from the Medical Research Council (United Kingdom) to M.V.S.S.

30 November 1981; revised 4 March 1982

Force-Sensitive Interneurons in the Spinal Cord of the Cat

Abstract. *The input-output properties of interneurons mediating spinal reflexes were investigated by extracellularly recording the response of interneurons to excitation from muscle receptors in the ankle extensor muscles of decerebrated, spinal cats. A population of interneurons in the intermediate region of the spinal cord is potently excited by increases in muscle force. Unlike the discharge of Golgi tendon organs, which accurately encodes moment-to-moment variations in the force of a single muscle, the discharge of these interneurons depends in a dynamic and usually nonlinear way on the force in several muscles. Powerful input from unidentified mechanoreceptors in muscle, presumably free nerve endings, is at least partly responsible for these properties. These force-sensitive interneurons are more likely to mediate clasp knife–type inhibition than simple negative force feedback.*

Golgi tendon organs, sensory receptors located at the musculotendinous junction in vertebrate muscle, are accurate force transducers (*1*). Since electrical stimulation of muscle nerves at strengths sufficient to excite Golgi tendon organ afferents (Ib) inhibits homonymous motoneurons (*2*), the Ib pathway may provide negative force feedback. This feedback would act to oppose changes in muscle force, such as those associated with stretch, fatigue, or the length-regulating actions of spindle afferent pathways. Although several investigators report that the strength, or gain, of force feedback in decerebrated cats is small or negligible (*3*), their findings may be peculiar to the decerebrated preparation. Because measurement of reflex gain in alternative preparations is technically difficult, we chose to investigate directly the processing of force information in the spinal cord by extracellularly recording the response of spinal inter-

0036-8075/82/0813-0652$01.00/0