partially capable of compensating for the loss of *Ddc* regulatory elements. However, no regulatory elements necessary for normal tissue-specific expression are located upstream of −208 (*12*), nor have we found any normal flanking sequences duplicated within 200 bp upstream of this point. Supporting the possibility of intragenic regulatory elements is the finding (*12*) that severely deleted *Ddc* genes retaining only 24 bp of upstream flanking sequences appear to show some components of normally regulated expression when stimulated by elements from adjacent vector sequences.

Our results precisely localize specific elements that determine the tissue-specific expression of a higher eukaryotic gene. Previous studies have implicated both enhancer elements (*17*) and other elements without detectable enhancer activity (*18*) as determinants of tissue-specific expression. Although protein-binding assays have detected factors binding to specific sequences within both the immunoglobulin and insulin enhancer regions (*19*), the precise identification of the functional elements within these regions is unknown.

Our deletions provide tools for further investigating the physiological role of *Ddc* expression in the CNS. We have constructed genes with phenotypically normal expression in the hypoderm that are not expressed at detectable levels in the CNS. Flies carrying these genes will allow a more complete assessment of the role of potential neurotransmitters synthesized by the *Ddc* gene product in normal behavior and learning (*6*).

Note added in proof: Recent immunohistochemical experiments (*20*) have shown that at least one other previously undetected element is required in addition to element I for correct cell-specific expression of *Ddc* in the larval CNS.

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From Stimulation to Undulation: A Neuronal Pathway for the Control of Swimming in the Leech

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Initiation and performance of the swimming movement in the leech (*Hirudo medicinalis*) are controlled by neurons organized at at least four functional levels—sensory neurons, gating neurons, oscillator neurons, and motor neurons. A paired neuron, designated as Tr1, in the subesophageal ganglion of the leech has now been shown to define a fifth level, interposed between sensory and gating neurons. Cell Tr1 is activated by pressure and nociceptive mechanosensory neurons, which mediate body-wall stimulus–evoked swimming activity in intact leeches. In the isolated leech nervous system, brief stimulation of cell Tr1 elicits sustained activation of the gating neurons and triggers the onset of swimming activity. The synaptic interactions between all five levels of control are direct. Discovery of the Tr1 cells thus completes the identification of a synaptic pathway by which mechanosensory stimulation leads to the swimming movements of the leech.

RHYTHMIC MOVEMENTS OF ANIMALS are generated and regulated by neurons organized at several interdependent functional levels, including sensory input, two levels of control neurons ("trigger" and "gating"), oscillator neurons, and motor neuron output (*1*). The central level is that of the oscillator, which consists of neuronal networks interconnected to generate rhythmic timing cues (*2*). The oscillator provides phasic excitatory and inhibitory output to effector muscles via motor neurons. The expression of rhythmic movement is controlled by excitatory or inhibitory inputs to the central oscillator from two types of neurons: trigger neurons, that when transiently activated initiate prolonged rhythmic output, and gating neurons, which elicit rhythmic motor patterns only while they are active (*1, 3*). Input to these trigger or gating neurons can arise from many sources, including sensory neurons. Although progress has been made in describing many of these functional levels, especially in various invertebrate species, the neuronal elements that form and link all of the functional levels have not been identified for any rhythmic movement in any species (*4*).

For the neuronal circuits that underlie swimming movements in the medicinal leech (*Hirudo medicinalis*), four functional levels—sensory, gating, oscillator, and motor neurons—have been identified (*5*). Here we describe a paired neuron, cell Tr1, which defines a fifth, or trigger, functional level.

The Tr1 cell pair is located in the most anterior neuromere of the leech subesophageal ganglion (*6*). The neurite of either Tr1 cell crosses the midline of the subesophageal ganglion and projects caudally in the contralateral connective nerve to the posterior

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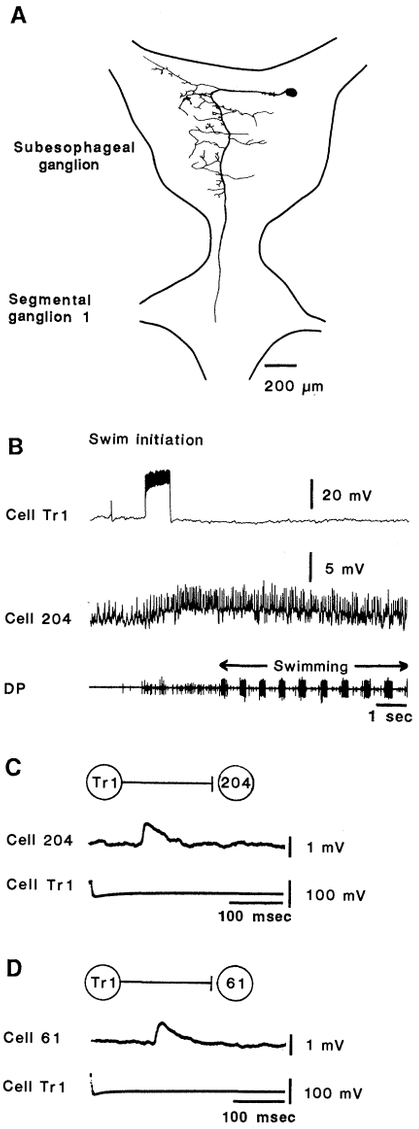

Fig. 1. Morphology and physiology of the Tr1 cells. (A) Tracing of a Tr1 cell stained with horseradish peroxidase. The axon projects in the nerve cord at least to segmental ganglion 17. (B) Interaction between cell Tr1 and a gating neuron, cell 204. Traces represent intracellular recordings from cell Tr1 (top) and cell 204 (middle). Brief stimulation of cell Tr1 by injection of depolarizing current (upward deflection) evokes an excitatory response in cell 204 and culminates in swimming activity, indicated by high frequency impulse bursts in the motor axon of the dorsal posterior segmental nerve (DP, bottom trace) as monitored by extracellular recording. (C and D) Evidence that cell Tr1 is connected directly to cells 204 and 61. Averaged records (Nicolet, 30 sweeps) obtained with simultaneous intracellular recordings from cell Tr1 and either cell 204 or cell 61 in saline containing 10 m*M* Mg^{2+} and 10 m*M* Ca^{2+}. Cell Tr1 spikes (bottom) evoke constant-latency EPSP's in the intracellular records from cells 204 and 61 (top traces). Records were obtained from the isolated leech nervous system extending from the supraesophageal ganglion to segmental ganglion 19 (H-G19 preparation). Details of methods are presented elsewhere (*7, 15*). (Insets) Schematic representation of the connections. T-shaped connection represents an excitatory synapse.

terminus of the ventral nerve cord (Fig. 1A). Either Tr1 cell can trigger swimming activity in the isolated nervous system; a short intracellular current pulse applied to either Tr1 cell is sufficient to elicit a swim episode (Fig. 1B). The two Tr1 cells qualify as trigger cells because the duration of swim episodes they elicit is nearly independent of stimulus duration (*7*).

We examined the pathways by which cell Tr1 might initiate swimming by means of simultaneous intracellular recordings from a Tr1 cell and swim-related neurons in segmental ganglia of the ventral nerve cord. In particular, we investigated connections between cell Tr1 and the two types of neurons in the segmental ganglia that initiate swimming activity, the swim-initiating neurons 204 and 205, and the serotonin-containing neurons 21 and 61. These neurons act as "swim-gating" cells; their activation initiates and maintains swimming activity. However, swimming usually does not continue when these cells are no longer activated (*8*).

We found that activating cell Tr1 by injection of depolarizing current evokes short-latency, long-lasting excitatory responses in both types of swim-gating neurons. For example, brief stimulation of a Tr1 cell excites cell 204, as shown by a sustained depolarization of its membrane and an increase in impulse frequency (Fig. 1B). Stimulation of cell Tr1 elicits similar excitatory responses in the serotonin-containing neurons (*9*). Two physiological tests indicate that the excitatory effects of stimulation of cell Tr1 on the swim-gating neurons are direct (monosynaptic). First, simultaneous intracellular records obtained from cell Tr1 and either cell 204 or 61 revealed the occurrence of constant-latency, excitatory postsynaptic potentials (EPSP's) following cell Tr1 impulses (Fig. 1, C and D). Second, the EPSP's evoked in cells 204 and 61 persisted when the concentrations of divalent cations in the physiological leech saline were elevated to levels (10 m*M* Mg^{2+} and 10 m*M* Ca^{2+}) that block indirect (polysynaptic) pathways (*10*). Hence, cell Tr1 apparently can excite directly both types of swim-gating neurons. Because the structure of the leech nervous system is highly metameric, we expect that all swim-gating neurons (more than 92 cells) receive direct excitatory input from either Tr1 cell (*11*).

One means of initiating swimming activity in the intact leech is by tactile stimulation of the body wall. Three types of mechanosensory neurons—the touch (T), pressure (P), and nociceptive (N) cells in segmental ganglia and in the subesophageal ganglion (*12*)—mediate this behavioral response (*13*). Mechanical stroking and pinching the leech body wall excites indirectly both types of swim-gating neurons; however, no known neurons link the sensory cells to the swim oscillator network (*14*).

The Tr1 cell pair provides this missing link. Strong mechanical stimulation of body-wall flaps, innervated by either the

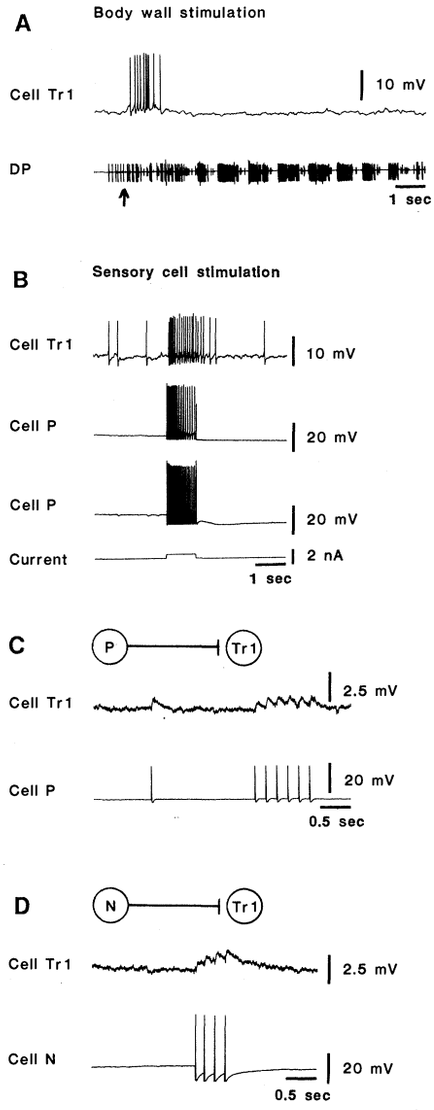

Fig. 2. Mechanosensory input to cell Tr1. (A) Jabbing (or pinching) a body-wall flap (arrow) elicits a burst of spikes in cell Tr1 (top trace) and elicits swimming activity (bottom), as indicated by activity in the dorsal posterior segmental nerve (DP). H-G19 preparation with a flap of body wall attached to the subesophageal ganglion and segmental ganglion 1 via head and segmental nerves, bathed in normal saline. (B) Depolarizing current injected (bottom trace) into two P cells of the subesophageal ganglion (middle traces) elicits P cell spikes and excites cell Tr1 (increased impulse rate, top trace). H-G19 preparation, bathed in normal saline (C and D) Evidence that P and N cells are connected directly to cell Tr1. Each impulse in the P cell (C) and N cell (D) gives rise to a constant latency EPSP in cell Tr1. Cell P was in the subesophageal ganglion and cell N was in the first segmental ganglion. H-G19 preparation, bathed in saline containing 10 m*M* Mg^{2+} and 10 m*M* Ca^{2+}.

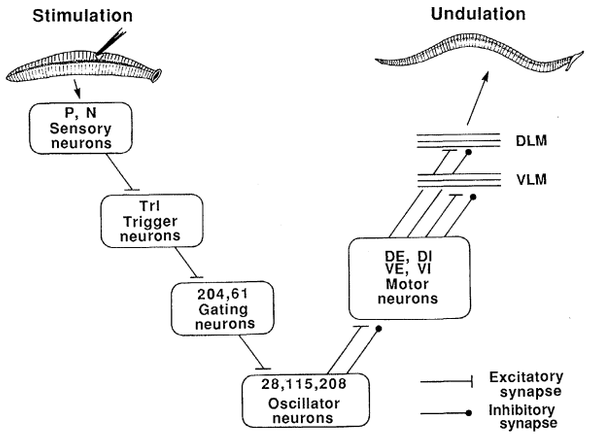

Fig. 3. Schematic overview of the neuronal network which generates and controls the leech swimming rhythm (*17*). Mechanical stimulation of the body wall (top left) activates sensory neurons beginning a cascade of activity that culminates in swimming movements (top right) (*16*). Each box represents one of the five functional levels described in the text. DE, dorsal excitors; DI, dorsal inhibitors; VE, ventral excitors; VI, ventral inhibitors. DLM and VLM denote the dorsal and ventral longitudinal muscles, respectively.

subesophageal ganglion or a midbody ganglion in otherwise isolated nerve cord preparations, activates the Trl cells (Fig. 2A). Direct intracellular stimulation of P cells in the subesophageal ganglion also elicits excitatory responses in Trl cells (Fig. 2B), as does stimulation of N cells. Taken together, these results demonstrate that excitatory connections extend from P and N cells to cell Trl. These interactions appear to be direct because they persist in physiological saline containing 10 m*M* Mg^{2+} and 10 m*M* Ca^{2+} (Fig. 2C). Direct synaptic connections evidently occur also between cell Trl and sensory neurons in the most anterior segmental ganglia (Fig. 2D). Finally, stimulating P and N cells in more posterior segmental ganglia also evokes an excitatory response in the Trl cells, suggesting that P and N cells in every segmental ganglion are connected directly to cell Trl (*15*).

The description of the Trl cells completes the identification of a neuronal pathway for the control and expression of leech swimming movements that extends from mechanosensory input to rhythmic motor output (Fig. 3) (*16*). This pathway includes neurons functioning at five levels: (i) mechanosensory neurons (P and N cells); (ii) trigger cells (Trl cells); (iii) gating cells (cells 204 and 61); (iv) oscillator neurons (cells 28, 115, and 208); and (v) motor neurons (dorsal excitors, dorsal inhibitors, ventral excitors, and ventral inhibitors). This system of neurons controls leech swimming movements by timing the phasic contractions of the dorsal (DLM) and ventral (VLM) longitudinal muscles (*17*). The paired Trl cell forms a node in this schema, receiving converging excitatory signals from more than 150 sensory neurons and providing diverging excitatory drive to at least 92 swim-gating neurons. Although some neuronal elements that participate in the control and generation of the leech swimming movements still are undiscovered, with the identification of the Trl cells and hence the elucidation of a complete neuronal pathway from sensory input to rhythmic motor output, a fundamental goal of neuroethology has been reached—namely, an understanding of the mechanisms responsible for animal behaviors in terms of synaptic interactions between identified neurons.

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6. The leech central nervous system consists of a supraesophageal ganglion, a subesophageal ganglion, a chain of 21 nearly identical segmental ganglia (numbered 1 to 21, anterior to posterior), and a tail ganglion. The segmental ganglia, together with interganglionic connectives, form the ventral nerve cord. Each segmental ganglion and each of the four neuromeres of the subesophageal ganglion, comprises about 400 neuronal somata, many of which are identified cells [K. J. Muller, J. G. Nicholls, G. S. Stent, Eds., *Neurobiology of the Leech* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1981)].
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16. The schema depicted in Fig. 3 does not represent a simple reflex; rather, neuronal mechanisms (unknown at present) convert brief cell Trl excitation into tonic activity in the gating neurons (*15*) and synaptic interactions between these and additional oscillator neurons transform this excitation into phasic output [W. O. Friesen, M. Poon, G. S. Stent, *J. Exp. Biol.* **75**, 34 (1978); W. O. Friesen, *J. Comp. Physiol.* **156**, 231 (1985)].
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