As another enzyme example, we selected phosphofructokinase (PFK, rabbit muscle), which requires ATP to form fructose 1,6-diphosphate. At low concentrations of εATP and ATP, the K_m values were determined to be 0.030 mM and 0.013 mM, respectively, with the V_{max} for εATP being 95 percent of that of ATP.

Production of ADP (or εADP) in the PFK reaction was followed by utilizing the coupled assay involving pyruvate kinase and lactate dehydrogenase, after it was found that εADP was an excellent substitute for ADP in the pyruvate kinase system, with a K_m approximately equal to that of ADP (0.30 mM) and a V_{max} equal to ~ 80 percent of that of ADP (*9*). These data allow the use of the coupled assay for εADP not only with PFK, but also with many other kinases. We also tested εATP as an allosteric effector in the PFK system. At high concentrations of ATP (and uridine triphosphate) PFK is significantly inhibited. Other nucleoside triphosphates (guanosine, cytidine, and inosine triphosphate) also serve as phosphoryl donors, but do not serve as allosteric effectors (*10*). The εATP was found to inhibit PFK at about twice the concentration of ATP required to produce the comparable inhibition.

In addition to our studies, several other groups at the University of Illinois have determined the effect of substitution of εATP for ATP in other enzyme systems and have made their preliminary results available to us. With phosphoribosylpyrophosphate synthetase (*11*) which catalyzes pyrophosphoryl transfer from ATP to ribose 5-phosphate and which exhibits high specificity for the nucleotide substrate, εATP was found to perform with about 5 to 10 percent of the activity of ATP (*12*). The activation by εATP for attachment of tyrosine to yeast transfer RNA was comparable to that of ATP. The catalyst used in the adenylyl transfer was pig pancreas tyrosine activating enzyme (*13*).

The sampling of enzymes presented here indicates that εATP is an exceptionally versatile replacement for ATP. It shows activity in phosphoryl, pyrophosphoryl, and adenylyl transfer systems, and it also shows the allosteric interaction with PFK. In those cases where it shows appreciable activity, it may be assumed that free N at position 1 and free NH_2 at position 6 of ATP are not required for binding since these sites are concealed in εATP. This modified coenzyme derives further value from its fluorescence characteristics, which include excitation at long wavelength, detection at low concentration, and a long fluorescence lifetime. A great variety of applications involving εATP can be foreseen.

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4. The abbreviation εATP is derived from the name given in the text, where ε stands for etheno and is also suggestive of the molar absorbance term and of fluorescence emission. According to the nomenclature based on the ring system, compound **1** is 3-β-D-ribofuranosylimidazo[2,1-*i*]purine 5′-triphosphate. The modified compounds εADP (adenosine diphosphate) and εAMP (adenosine monophosphate) were readily prepared by the reaction of chloroacetaldehyde (*5*) with the corresponding adenine ribonucleotides.
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Crayfish Muscle Receptor Organ: Role in Regulation of Postural Flexion

Abstract. *Recordings were made from postural-control motor nerves with antagonistic functions in the crayfish abdomen through flexible suction electrodes. During unrestrained flexion of the abdomen, output from the tonic stretch receptor neuron of RM_1 (SR_1) is sufficient to drive an extensor motor unit in the same segment. However, this intrasegmental "resistance" reflex is normally blocked by an intersegmental reflex in which output from SR_1's in caudal segments inhibits SR_1 discharge in more anterior segments.*

"Resistance" reflexes are used to stabilize postural systems in space. During active movements these often must be overcome, particularly when a movement sequentially involves several body parts, each of which has a passive mechanical effect upon others. In the crayfish abdomen, commands for such movements can be generated by stimulating single central neurons. Segmental "resistance" reflexes and intersegmental inhibitory reflexes have been found. Both have been described in terms of the activity of identified sensory, motor, and inhibitory neurons. We now report that an inhibitory intersegmental reflex

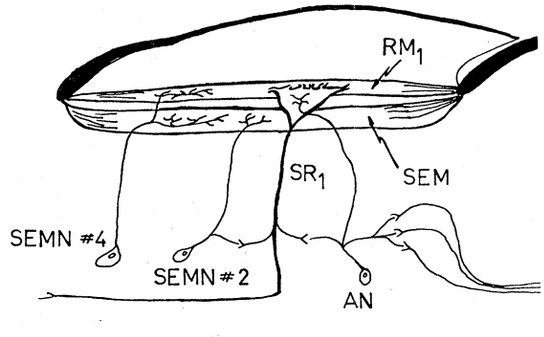

Fig. 1. Diagram of sensory and motor reflex organization of crayfish abdominal postural control system. AN, accessory nerve; RM_1, tonic receptor muscle; SEMN, slow extensor motor neurons; SR_1, sensory neuron of RM_1. Output of SR_1 is directed to SEMN #2 and AN in the same segment and to AN's in more anterior segments. Axons from the right which end on AN are from SR_1's in more posterior segments. [Adapted from Kennedy (*2*, figure 2)]

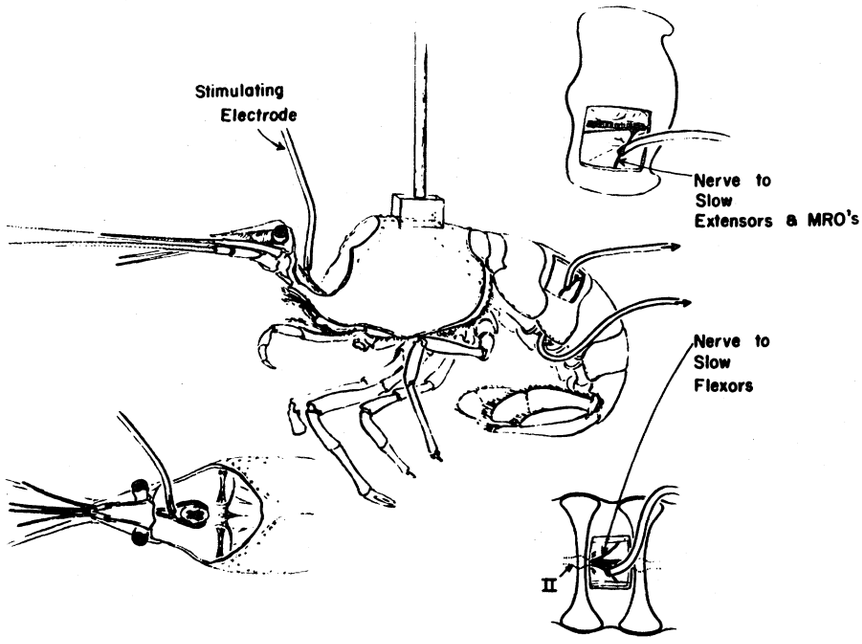

Fig. 2. Rigidly tethered crayfish preparation. Each animal was prepared for recording by cutting windows in the dorsolateral and medioventral cuticle of the second abdominal segment to expose the dorsal branch of the second root and the third superficial root of ganglion II (inserts, upper and lower right). The cephalothorax of "voluntary" animals remained intact. In other animals the stomach, gonads, and green glands were removed, exposing the circumesophageal connectives for command fiber stimulation (insert, lower left). After suspension of the animal in an upright position, suction electrodes were applied to the dorsal and third superficial nerves. Small axon bundles containing command fiber interneurons were stimulated with a third suction electrode.

is used specifically to disable a resistance reflex during abdominal flexion.

Recordings from identified elements in the crayfish abdomen such as those comprising the MRO_1 [the tonic receptor muscle (RM_1) and its sensory neuron (SR_1)] motor units innervating the superficial slow extensor muscle (SEM) and motor units innervating the slow flexor muscles (SFM) have provided considerable information about the reflex organization of the sensory, interneuronal, and motor elements which control abdominal posture in the crayfish (*1*, *2*). Sensitivity of SR_1 to RM_1 stretch is decreased by an inhibitory efferent, the thick accessory nerve (AN) (*3*) (Fig. 1). A specific excitatory slow extensor motor neuron (SEMN #4) innervates RM_1 and increases SR_1 sensitivity (*4*). Activity of SR_1 reflexly excites the AN (*5*) and a specific motor neuron (SEMN #2) which innervates almost all SEM fibers (*4*). Reflex activation of the AN spreads several segments anteriorly while SR_1 excitation of SEMN #2 is confined to the stimulated segment (*6*).

This information was obtained from dissected, immobilized preparations. It is not possible to predict from these findings the relative "strength" of the various synergistic and antagonistic reflexes. The reflex coordination of abdominal movement must be examined in an unrestrained preparation. Such a preparation, which utilizes fine Tygon suction electrodes (*7*) has been developed and used to examine MRO_1 regulation of flexion.

Very flexible suction electrodes were made by pulling Tygon tubing into 2-cm-long tips whose inner diameter ranged from 100 μm at the shank to 60 μm at the tip. A 50-μm silver wire was inserted through the shank to within 50 μm of the tip. To obtain high signal-to-noise recordings the nerve was sucked into the tip to make physical contact with the wire. Units were usually easily identified on the basis of the relative size of their impulses and their patterns of discharge (*4*).

Crayfish were perfused with saline, and most appendages were removed. A rigid support was cemented to the thoracic carapace, and the animal was suspended in a chamber filled with aerated saline at 14° to 16°C (Fig. 2). The dorsal nerve (to SEM and the muscle receptor organ) and the superficial posterior nerve (to SFM) were exposed. A mirror was used to permit visual observation of the superficial posterior nerve during electrode placement. Unit recordings from both nerves were amplified, displayed on an oscilloscope, and stored on magnetic tape.

Two types of flexions were examined: command-fiber elicited and "voluntary." Command fibers were isolated in small bundles from the circumesophageal connective. Bundles which evoked reproducible flexions when stimulated at low voltage at frequencies between 10 and 80 hertz were used to obtain data. "Voluntary" flexions were elicited in other individuals by touching cephalothoracic hairs and the uropods.

Abdominal flexion almost invariably elicited an increase in SR_1 discharge (Figs. 3 and 4). In flexions evoked by command fiber stimulation at high frequency (Fig. 3A) the inhibitory SEMN (#5) is the only actively discharging extensor motor neuron; all excitatory SEMN activity (including SEMN #4) is centrally suppressed. The absence of SEMN #4 activity during flexion suggests that the increased SR_1 activity results from passive stretch (rather than active contraction) of RM_1.

When flexion command fibers were stimulated at lower frequencies, central suppression was insufficient to inhibit completely all excitatory SEMN activity. A low-frequency impulse discharge of SEMN #2 was usually observed (Fig. 3B). Since all other excitatory SEMN activity is here suppressed, we presume that the SEMN discharge results from direct SR_1 reflex activation. This conclusion is strengthened by the observation that each SEMN #2 impulse occurs approximately 30 msec after an SR impulse (*4*).

Similar recordings in which the rate of SR_1 discharge increased with subsequent driving of SEMN #2 were obtained during voluntary flexion (Fig. 3C). Thus, during both "command-initiated" and "voluntary" abdominal movements, flexion can evoke the reflex activation of SEMN #2 despite central inhibition of all other excitatory SEMN activity.

From observations with restrained abdominal preparations three control mechanisms have been described which might be expected to have an inhibitory effect on the SR_1-SEMN #2 reflex activation during unrestrained abdominal flexion. These are (i) central inhibitory suppression of the excitatory SEMN's (*8*), (ii) activation of the inhibitory SEMN (*8*), and (iii) the anteriorly directed intersegmental AN reflex (*6*).

As indicated by the results reported thus far, central inhibition is often inadequate to suppress the SR_1-SEMN #2 reflex pathway. Since the inhibitory SEMN #5 innervates only 30 percent of the SEM fibers innervated by SEMN #2 (*1*), SEMN #5 activity is clearly insufficient as a mechanism for nullifying the excitatory effects of the SR_1-SEMN #2 reflex activation. Instead, suppression of the SR_1-SEMN #2 reflex pathway is the result of SR_1 reflex activation of the AN.

Almost all flexions that we observed begin in the most posterior segment (the sixth) and proceed anteriorly. Recording in the second segment during such a flexion, we invariably observe that the increase in SR_1 discharge elicited by flexion is suppressed by AN activity.

In Fig. 4A the SR_1 discharge is interrupted by an AN burst which is followed by a secondary increase in SR_1 firing resulting from continuation of the evoked flexion movement. A similar pattern can be observed during voluntary flexions (Fig. 4B). Flexion of the abdomen (coincident with increased activity in the flexor excitors) leads to suppression of second-segment SR_1 discharge as a result of AN activation.

It has been suggested (*9*) that AN bursts during flexion originate centrally and are not reflexly activated by SR_1 activity. Three observations support our conclusion that the activation of the AN which we have observed is the result of SR_1 activity in more posterior segments: (i) flexion of caudal segments elicits vigorous AN activity in anterior segments (see *6*); (ii) stimulation of flexion command fibers in restrained preparations does not evoke AN activity (*8*); and (iii) central interneurons which selectively drive the AN have a much weaker effect than that required to evoke the vigorous accessory discharge observed in our experiments (*10*).

Although our observations of an anteriorly directed AN reflex activation during flexion are supported by previous observations (*6*), they are in contrast with the recent conclusion (*11*) that the anterior bias of the AN reflex is too small to be significant in coordinating segmental abdominal movements. This difference in interpretation may result from our use of unrestrained preparations in which AN activation resulted from flexion-induced SR_1 activity rather than being activated by electrical stimulation of both SR_1 and SR_2 (the phasic stretch receptor neuron of RM_2) in an isolated, immobile abdomen (*11*).

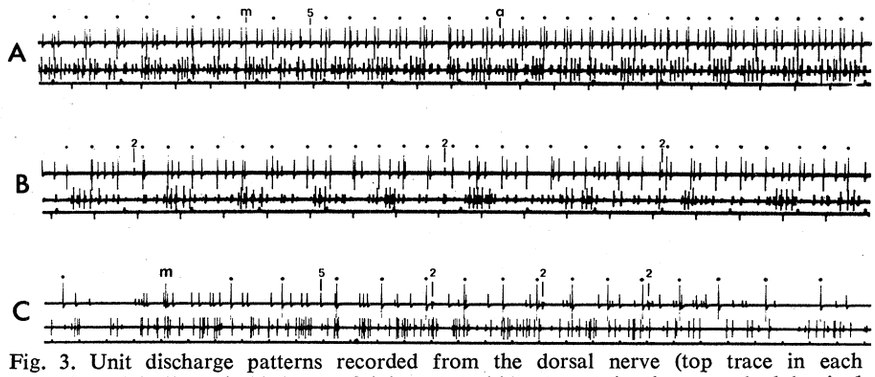

Fig. 3. Unit discharge patterns recorded from the dorsal nerve (top trace in each record) and ipsilateral third superficial root (middle trace) in the second abdominal segment. These records do not show the activity of direct antagonists since they are taken from nerves in the same segment. The SEM is the antagonistic of the SFM in the next posterior segment (*6*). The lower trace in each record contains a 100-msec time mark (positive deflections) and, during command fiber stimulation, a stimulus mark (negative deflections). Dots have been placed above SR_1 spikes to aid visualization. (A) Record of activity during stimulation of a flexion command fiber at 20 stimuli per second. Flexor motor neurons (middle trace) and extensor peripheral inhibitor (#5) (top trace) are strongly driven. The SR_1 (*m*) and the accessory nerve (*a*) are also activated by the unrestrained flexion. (B) Segment of record during stimulation of the same flexion command fiber at 15.8 stimuli per second. Flexor motor neurons and extensor motor neuron #5 are less strongly driven. However, a low rate of excitatory motor neuron #2 discharge is observed. (C) Voluntary abdominal flexion from a different animal on a slower time base. SR_1 discharge increases during flexion and drives extensor motor neuron #2 with a latency of 28 msec. Spontaneous activity in a smaller extensor motor neuron is also apparent.

Abdominal movements usually begin at the most posterior abdominal segment and continue anteriorly. Consequently, flexion-induced activity of the SR_1's in the more posterior segments will, through intersegmental reflex connections, activate the AN's in the more anterior segments. An AN discharge will suppress SR_1 activation in all segments except the most caudal. This will block the reflex activation of SEMN #2 during the initial portion of flexion when central inhibition is presumed to be weak. During continued flexion both

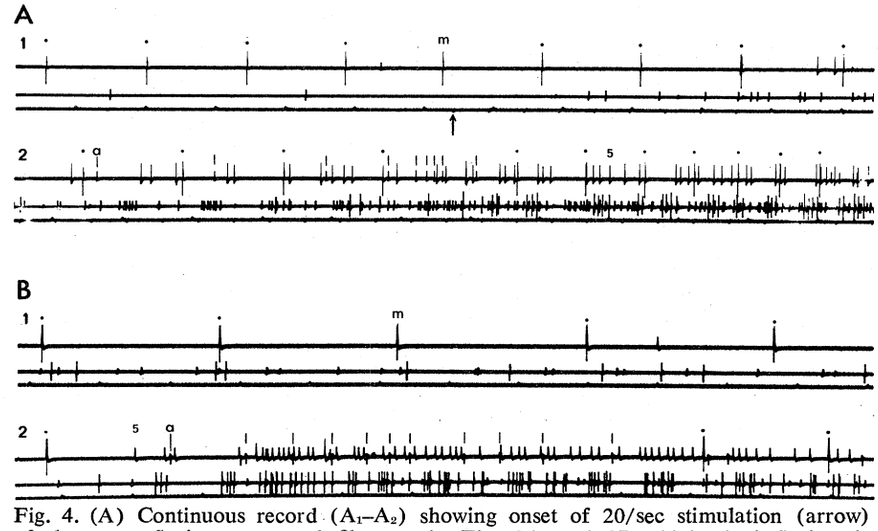

Fig. 4. (A) Continuous record (A_1–A_2) showing onset of 20/sec stimulation (arrow) of the same flexion command fiber as in Fig. 3A and 3B. Abdominal flexion is accompanied by a burst in the accessory nerve (*a* and vertical marks) which interrupts SR_1 firing (*m*) prior to strong driving of the extensor inhibitor (#5). Dots have been placed above SR_1 spikes in A and B. (B) Record of a voluntary abdominal flexion in another animal. SR_1 discharge is interrupted by an accessory nerve burst at the beginning of the movement.

central inhibition and the anteriorly directed AN reflex will act synergistically, preventing the SR_1-SEMN #2 segmental resistance reflex.

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Externally Suppressible Proline Quadruplet CCC$^{U}_{\bullet}$

Abstract. *Three (+1) frameshift mutations located at different genetic sites respond with high specificity to the same external suppressor. In each case, the suppressor restores small amounts of protein that is normal in electrophoretic mobility and heat stability. One of these proteins has been shown to have the wild-type amino acid sequence. The messenger RNA quadruplet CCC$^{U}_{\bullet}$ appears to be common to all three frameshift sites and to be translated by the suppressor as proline. A likely suppressor agent is a proline transfer RNA with a quadruplet anticodon or its functional equivalent.*

We have reported external suppression of a frameshift mutation, *hisD3018*, in the histidinol dehydrogenase gene of *Salmonella* (*1*). This frameshift was induced with the frameshift mutagen ICR-191 (*2*) by Oeschger and Hartman (*3*). It is revertible not only by ICR compounds but also by certain alkylating agents such as DES (*2*) and NG (*2–4*). The *3018* frameshift is a (+1) type (*2*), most likely containing an extra C in a repeating messenger RNA (mRNA) (*2*) sequence of C residues (*2, 4, 5*). External suppressors of *3018* are efficiently induced by ICR-191 (*1*). Suppression restores small amounts of histidinol dehydrogenase with the wild-type amino acid sequence; this suggests that the suppressors read the (+1) mRNA sequence inefficiently as the correct sequence (*6*). Riddle and Roth showed that most frameshifts which are revertible by alkylating agents as well as ICR compounds, tentatively classified as (+1) types, are externally suppressible. Two classes of externally suppressible frameshift, almost all induced with ICR-191, were delineated. Suppressors of one class of frameshift do not suppress the other (*7*). These frameshifts may be (+1) additions in different types of repeated sequences in mRNA. We have tried to pinpoint the suppressible mRNA sequence and to characterize the proteins that result from suppression of other frameshifts of the *3018* class located at different sites in the histidinol dehydrogenase gene. One such frameshift, *3749*, was found to have an extensive (+1) mRNA sequence of six or seven residues in common with *3018* and to produce histidinol dehydrogenase which is normal in electrophoretic mobility and heat stability (*8*). We have now investigated a third frameshift of this type, *2565*. The accumulated data implicate a suppressor that enables the quadruplet CCC$^{U}_{\bullet}$ (*2*) to be translated as proline.

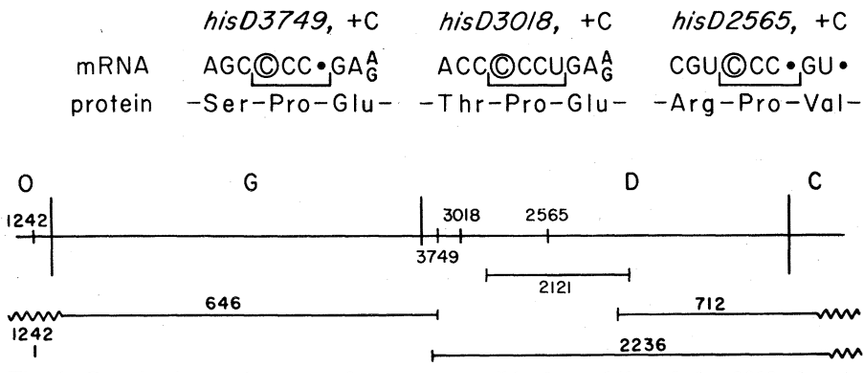

Fig. 1. Genetic sites and nature of cross-suppressible frameshifts of the *3018* class in the *hisD* gene. The *3749* site maps under deletion *646* (*7*). The *3018* site maps between deletions *646* and *2121* (*3*). The *2565* site is yet more internal in the gene and maps under *2121*. The inferred similar sites of suppression in *3018*, *3749*, and *2565* mRNA are shown above the map. An extra C (circled) in a repeated sequence of C residues is considered to be the basis of each (+1) frameshift. To restore normal protein the suppressor reads the quadruplet CCC$^{U}_{\bullet}$ as proline.

Frameshift mutations were induced with ICR-191 in strain *his01242* (*2*) and selected for strong polarity (reduced expression of genes following the mutant gene in the histidine gene cluster) by the temperature method (*9*). Independent cultures of *his01242* in 2 ml of histidine-supplemented 2EM medium (*2*) containing 20 μg of ICR-191 were grown overnight on a shaking device at 37°C; a portion (0.1 ml) of each culture was plated on histidine-supplemented E agar containing 2 percent glucose. ICR-191 (10 μg) was spotted on each plate to enhance mutagenesis further, and the plates were incubated for 72 hours at 43°C. Polar *his* mutants, recognized by their smooth colony morphology, were isolated and examined. Of 24 independent frameshift mutations isolated in the histidinol dehydrogenase gene, 13 showed the reversion and suppression properties of the *3018* class. These map in three different segments of the *hisD* gene, as determined by deletion mapping: three under *his-646*, as *3749*; five between *his-646* and *his-D2121*, as *3018*; and five under *2121*, including *2565* (Fig. 1). It is likely that all are similar (+1) mutations at three hotspots (sites where such mutations easily occur), and that each hotspot contains a repeated sequence of DNA GC pairs (*2*). Frameshifts at the *2565* site represent a previously uncharacterized set of mutations, and *2565* was selected for further study.

The altered mRNA sequence in *2565* was reconstructed with the in vitro codon assignments (*10*) by analysis of