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Synaptic Activity Results in Increased Transcription as Detected by the Brain-Specific Probe pEL-48 in *Torpedo marmorata*

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Abstract

The cloning of sequences expressed exclusively in neurons that use acetylcholine as a neurotransmitter resulted in multiple probes. One of them, pEL-48, was used to detect changes in transcription patterns in the electric lobes of *Torpedo marmorata*. Fish were stimulated either electrically or mechanically to induce discharge of the electric organs; they were killed after different poststimulation time intervals. The RNA isolated from the electric lobes was quantified using Northern blots and pEL-48 as a probe; distinct changes in the quantity of pEL-48 complementary RNA could be detected. Partial sequencing of the clone pEL-48 revealed an unusual primary sequence with numerous short open reading frames, coding for hitherto unknown polypeptides.

Index Entries: Acetylcholine; Northern blot; transcription; *Torpedo marmorata*; mRNA.

Introduction

It has been shown in many cases that neurons are capable of altering their gene expression patterns in response to extracellular stimuli. Since 1985, the expression of immediate early genes (IEG) in neurons is developing into a major field in molecular neurobiology (*see* the article by Abraham et al., this vol., and references therein). Moreover, evidence accumulates that specific genes involved in neuronal function are selectively activated by multiple factors, such as stress, osmotic pressure, salt concentration, and stimulation of preganglionic nerves (Biguet et al., 1989; Bondy et al., 1989; Murphy et al., 1989; Weisinger et al., 1990). These approaches rely on the utilization of probes that are already identified as neuron-specific sequences; yet there might well be other, up to now unknown genes whose expression is stimulus-dependent. An attempt to isolate this class of genes is described in this article: As a biological model the electromotor system of *Torpedo marmorata* was used, with the electric lobes representing a brain part built up exclusively by neuons using acetylcholine as a neurotransmitter (Whittaker, 1984). From this tissue, mRNA was isolated and a cDNA library constructed; a differential screen was used to identify clones that represent sequences expressed exclusively in the cholinergic electromotor neurons (Schmid and Girou, 1987). One of the so-identified clones, pEL-48, was used as a probe for Northern blot hybridization, with RNAs isolated from electric lobes from fish having undergone stimulation of their electromotor system.

The results obtained indicate that preganglionic as well as direct stimulation of the electromotor neurons give rise to a dramatic increase of an RNA recognized by the probe pEL-48. Partial sequencing of the pEL-48 DNA insert indicates an unusual primary structure coding for multiple small polypeptides.

Methods

Electrical Stimulation of the Electromotor System of Torpedo marmorata

Adult fish (obtained from the Institut de Biologie Marine, Arcachon, France) were anesthetized in 0.5% MS-222 (Sigma, Munich, FRG), followed by electrical stimulation. The skull was opened and a platinum electrode was applied to the dura mater of the electric lobes. Stimulation conditions were 10 V, 5 Hz for 10 min.

Mechanical Stimulation

This was performed for 10 min on anesthetized animals by a pushing device working at 5 Hz, triggering the sensory neurons in the lateral line organ of the fish.

Molecular Cloning of Electromotor Neuron-Specific Sequences

This was done as described by Schmid and Girou (1987).

RNA Isolation

Immediately after the end of stimulation, or after given times of recovery (30 min to 15 h), fish were killed and RNA was isolated by the guanidinium-isothiocyanate method (Sambrook et al., 1989).

Probe Preparation, Northern Blots

These were done according to standard techniques (Sambrook et al., 1989).

DNA Sequencing

This was performed with a Sequenase sequencing kit (USB), Toutart et Matignon, Paris, France) following the manufacturer's instructions. The

DNAsis (Pharmacia, Freiburg, FRG) software was used to treat the primary sequence data. Sequence comparisons were done using GenBank® entries.

Results and Discussion

The stimulation of the *Torpedo* electromotor system, a model for the mammalian neuromuscular junction, causes characteristic decline in the response of the electric organ to 1% or less of the original response if stimulation is continued for a sufficiently long timecourse. The response can be monitored to check for effective stimulation. If RNA that is prepared from the electric lobes of fish killed immediately or after different intervals of recovery is translated in vitro and the translation products analyzed by two-dimensional electrophoresis, distinct changes in the protein pattern obtained can be observed (Schmid, 1989). These RNAs were used for a Northern blot hybridization, with the electric-lobe specific clone pEL-48 as a probe. As shown in Fig. 1, a stimulation-dependent increase in the expression of a pEL-48 complementary RNA takes place. This increase peaks at the end of the stimulation period (time = 0) with a value 12X higher than that of the control; during the recovery period the signal falls gradually to reach the control level at about 15 h. The probe detects in all cases two bands; the upper band shows most changes in expression. This might reflect the presence of a precursor RNA molecule. The rapid increase of RNA during the 10-min period of stimulation, as well as the gradual decrease back to control levels during a period of recovery lasting several hours has a timescale similar to that observed for other, stimulus-inducible genes. These changes take place only in the electric lobes; the cerebellum showed no stimulation-induced changes detectable with pEL-48. Could the electrical stimulation give rise to artefactual transcription? The experiments were repeated with fish whose lateral line organ was stimulated mechanically,

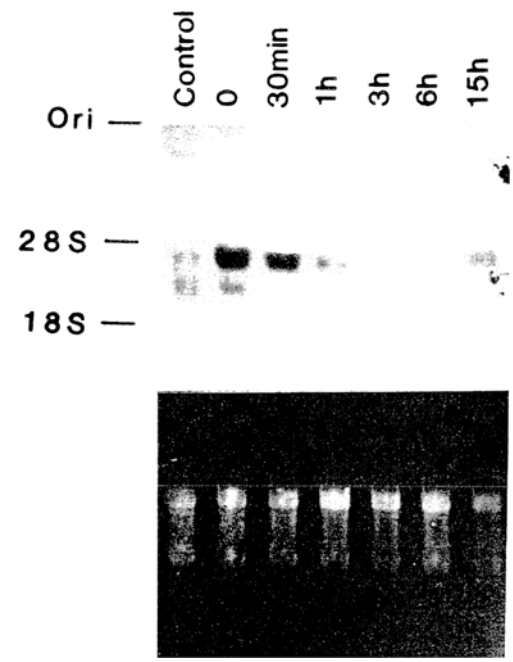

Fig. 1. (Upper panel) Northern blot showing the hybridization of pEL-48 to RNA isolated from stimulated *Torpedo* electric lobes. RNA was prepared from electrically stimulated fish directly after the stimulation (= 0) or after different periods of recovery (30 min to 15 h). The control is RNA prepared from the electric lobes of an anesthetized but unstimulated fish. Densitometric scanning of the upper band resulted in the following relative intensities (times control value): 0 = 12; 30 min = 11.8; 1 h = 5.1; 3 h = 4.1; 6 h = 1.5; 15 h = 1. (Lower panel) Ethidium bromide stain of the RNAs after electrophoresis and before transfer to the membrane; 10 µg total RNA/lane were applied.

therefore mimicking the in vivo flux of stimuli leading to the electric organ discharge. Two time points, 0 and 1 h poststimulation, were assayed and the same results as with the electrical stimulation were obtained (results not shown). Therefore, it seems plausible that preganglionic stimulation of the electromotor neurons gives rise to transcriptional changes, at least in the case detected by pEL-48.

The insert of clone pEL-48 was partially sequenced. The primary sequence of the insert from pEL-48 reveals, in all three reading frames,

several polypeptides encoded on the message. Recently, a very short protein, PEP-19, was described, a 7.2 kDa polypeptide whose RNA is transcriptionally regulated by afferent stimulation of Purkinje cells (Sangameswaran et al., 1989). Considering the most plausible reading frame of the pEL-48 sequence, eight polypeptides between 0.5 and 4.8 kDa could be synthetized from this message, but because the message is at least 3000 nucleotides long, there could be much more information that is unknown to date. The comparison of the primary nucleotide sequence with the sequences contained in GenBank did not lead to any conclusive similarity. Further studies, e.g., the full-length sequencing of the pEL-48 complementary RNA, are needed to fully understand the function of this transcriptionally regulated molecule. Furthermore, it is now possible to isolate the genomic sequence of the pEL-48 insert. This could lead to the identification of regulatory sequences involved in the coupling of stimulation and transcription events.

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