Long-Term Memory Requires PolyADP-ribosylation

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PolyADP-ribose-polymerase 1 is activated in neurons that mediate several forms of long-term memory in *Aplysia*. Because polyADP-ribosylation of nuclear proteins is a response to DNA damage in virtually all eukaryotic cells, it is surprising that activation of the polymerase occurs during learning and is required for long-term memory. We suggest that fast and transient decondensation of chromatin structure by polyADP-ribosylation enables the transcription needed to form long-term memory without strand breaks in DNA.

The formation of long-term memory requires new gene expression (*1*). Transcription is initiated by alteration of chromatin structure (*2*) induced by posttranslational modification of DNA-bound proteins through phosphorylation, acetylation, methylation, and polyADP-ribosylation (*2*, *3*). PolyADP-ribosylation, a transient modification of nuclear proteins that regulates their binding to DNA (*4–7*), is catalyzed primarily by polyADP-ribose-polymerase-1 (PARP 1), a highly conserved and abundant nuclear enzyme (*4–6*). PolyADP-ribosylation can modify histones, transcription factors, RNA polymerase II, topoisomerases, and high-mobility group proteins (*6*). Activation of PARP 1 is initiated by stressful stimuli that damage DNA (*6–10*), but it can also be induced by other stimuli (*9*, *11*): Depolarization of rat brain cortical neurons activates PARP 1 in the absence of DNA damage (*11*), which suggests that PARP 1 can be activated in neurons by physiological activity.

Does polyADP-ribosylation play a role in forming long-term memory? To answer this question, we examined two learning tasks in *Aplysia*. One task, governed by the pleural-pedal ganglia, is the sensitization of defensive withdrawal reflexes by noxious stimuli (*1*). The other, controlled by the cerebral and buccal ganglia (*12*), is the conditioning of feeding responses by pairing with negative reinforcing stimuli (*13*, *14*).

A single noxious stimulus to an intact animal produces short-term sensitization of withdrawal reflexes lasting minutes; four or more spaced stimuli produce long-term sensitization lasting days to weeks (*1*). Sensitizing stimuli induce the release of serotonin (5-HT) from modulatory neurons. Release of the neurotransmitter that results from either short- or long-term sensitization facilitates sensory-to-motor neuron synapses in the pleural and pedal ganglia (*1*, *15*). These synapses can also be facilitated by administering pulses of 5-HT to isolated ganglia (*16*). One pulse produces short-term facilitation (*1*, *15*); five spaced pulses produce the long-term form (*1*, *15*). To determine whether stimulation by 5-HT induces the activation of PARP 1, we first examined polyADP-ribosylation in isolated pleural-pedal ganglia. Because activated PARP 1 is itself polyADP-ribosylated (*4–7*), activation of the polymerase in stimulated ganglia was assayed by its auto-polyADP-ribosylation measured by a shift in isolelectric point (pI) toward acidic pH values (*16*).

PARP 1 was activated in ganglia stimulated by five spaced pulses of 5-HT (Fig. 1A). The change in pI was due to polyADP-ribosylation, because it was prevented by inhibiting PARP 1 activity with 3-aminobenzamide (3-AB; 0.5 to 1.0 mM) and with 6(5H)-phenanthridinone (80 μM) (*17*) (Fig. 1A). The 3-AB specifically blocked polyADP-ribosylation without inhibiting mono-ADP-ribosylation (*18*) (Fig. 1B). PARP 1 was not activated during short-term facilitation, as shown by the lack of polyADP-ribosylation after a single application of 5-HT (Fig. 1A).

Plasticity of sensory-to-motor neuron synapses in the pleural-pedal ganglia is bidirectional, also showing long-term depression, (*19*, *20*). Depression is elicited by five spaced applications of the inhibitory neuropeptide Phe-Met-Arg-Phe-NH_2 (FMRFamide) to the ganglia (*19*). Treatment with FMRFamide did not activate PARP 1 (Fig. 1A), indicating that polyADP-ribosylation is not correlated with all forms of long-term synaptic plasticity.

Long-term facilitation of sensory-to-motor neuron synapses can also be demonstrated in the intact animal after training that causes long-term sensitization (*21*, *22*). Four spaced noxious stimuli to one side of an *Aplysia* result in long-term behavioral sensitization of withdrawal reflexes in response to ipsilateral stimuli, without affecting the response to stimulation of the other side. Underlying this behavioral sensitization is the long-term facilitation of sensory-to-motor synapses in the ipsilateral pleural-pedal gan-

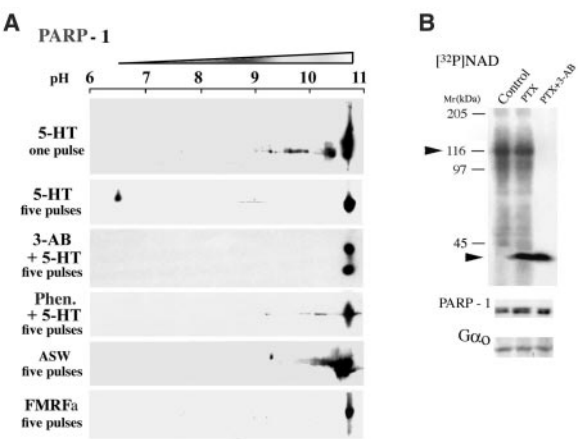

Fig. 1. The effect of neurotransmitters on PARP 1 activation in isolated *Aplysia* pleural-pedal ganglia. (**A**) PolyADP-ribosylation of PARP 1, immunolabeled with a monoclonal antibody against human PARP 1 (Serotec, Oxford, UK), was assayed by a shift in pI toward acid pH values on two-dimensional polyacrylamide gel electrophoresis. The enzyme was extracted from nuclei of pleural-pedal ganglia (10 for each sample). Treatment with 5-HT that produces long-term facilitation (5-HT, five pulses) resulted in polyADP-ribosylation of PARP 1, which is blocked by 3-AB (3-AB + 5-HT, five pulses) and by 6 (5H)-phenanthridinone (Phen. + 5-HT, five pulses). Treatment with 5-HT that produces only short-term facilitation (5-HT, one pulse) and with FMRFamide (FMRFa, five pulses), which produces long-term synaptic depression, did not result in the polyADP-ribosylation of the polymerase. The immunoblots shown are representative of three independent experiments; 20.3 ± 3.4% of PARP 1 in extracts of the stimulated ganglia was polyADP-ribosylated. (**B**) [^{32}P]PolyADP-ribosylation of PARP 1 (M_r 116,000) is unaffected by ADP-ribose transferase (pertussis toxin, PTX), which catalyzes monoADP-ribosylation of Gαo protein (M_r 39,000). Treatment with 3-AB (1 mM) blocked polyADP-ribosylation, but not monoADP-ribosylation (lane PTX + 3-AB). Proteins immunolabeled in each sample for either PARP 1 or Gαo are shown below. Similar results were obtained in three independent experiments (*16*).

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glia. Noxious stimuli eliciting unilateral long-term sensitization (*16*) also induced unilateral polyADP-ribosylation in the ganglia. PARP 1 was activated only in pleural-pedal ganglia ipsilateral to the sensitizing stimuli (Fig. 2). Thus, application of 5-HT to isolated ganglia in vitro and the training procedures in the intact animal produce similar activation of PARP 1.

PolyADP-ribosylation is a well-characterized response to tissue damage, and long-term sensitization is elicited by potentially damaging noxious stimuli. PolyADP-ribosylation initiated by sensitizing stimuli is unlikely to be related to generalized stress, however, because the effects were unilateral. It also occurs during the formation of memory produced by stimuli that are not noxious. We trained animals using an operant-conditioning training procedure that affects feeding. Feeding responses governed by the cerebral and buccal ganglia were paired with reinforcing stimuli signaling whether animals have successfully swallowed food (*13*). Training animals with inedible food that cannot be swallowed (*16*) causes a decrease in feeding responses and initiates long-term (24 hours to 7 days) memory (*14*). PARP 1 was activated in cerebral and buccal ganglia sampled from *Aplysia* just after training: The pI of PARP 1 was shifted because of polyADP-ribosylation (Fig. 3A). This activation was not attributable to the exposure to food or to feeding movements that accompany the training, because the polymerase was not polyADP-ribosylated in animals that had eaten freely for a time equal to that of the training (Fig. 3A). When 3-AB was applied just before the training (*16*), it blocked the activation of PARP 1 (Fig. 3A). Treatment with 3-AB did not affect the animals' survival or behaviors such as locomotion and feeding (*16*). These observations indicate that PARP 1 can also be activated as a result of an associative training procedure that elicits long-term memory.

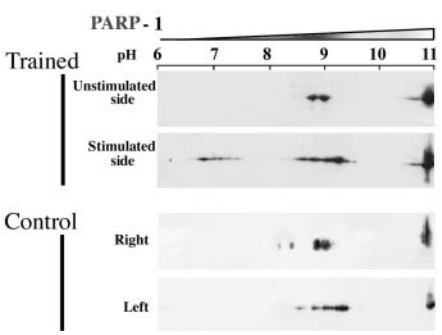

Fig. 2. Activation of PARP 1 in intact *Aplysia*. Activation was assayed by a shift in pI of the polymerase from pleural-pedal ganglia nuclei sampled immediately after unilateral electrical stimulation that produces long-term sensitization of the siphon-tail withdrawal reflex (Trained). Stimuli were randomly delivered to the right or to the left side of 13 animals (13 ganglia were taken from the stimulated sides and 13 from control sides), and the experiment was replicated twice; 23.6% and 22.7% of PARP 1 in samples of the stimulated ganglia was polyADP-ribosylated. In other experiments (Control), we found no difference in PARP 1 activity between the right and left ganglia of unstimulated animals (12 ganglia for each sample).

In addition to eliciting long-term memory, this training procedure also produces a short-term (30 min) memory (*14*). Only the long-term memory requires activation of PARP 1, however. We treated animals with 3-AB before training (Fig. 3B) and then tested for either the short- or the long-term form. Inhibiting PARP 1 during training blocked only long-term memory, without affecting short-term memory (Fig. 3B) or behavior during the training (*16*).

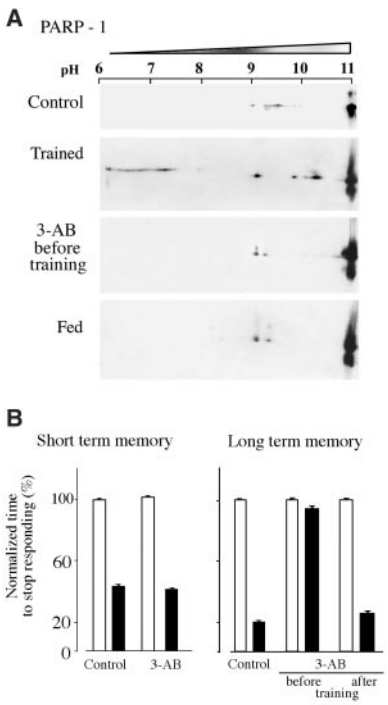

Fig. 3. PARP 1 is activated in conditioned *Aplysia*. (**A**) Activation was assayed in buccal and cerebral ganglia by a shift in pI under the conditions indicated. The results shown are representative of three independent experiments (seven animals for each experiment; 15 ± 1.4% of PARP 1 was polyADP-ribosylated in nuclear extracts from trained animals). (**B**) The effects of blocking PARP 1 activity on memory were examined by treating intact animals with 3-AB (1 mM) before or after training. Memory is indicated by a decrease in the time for an animal to stop responding to netted, inedible seaweed. Experimental data (filled bars) are normalized to the average time by which an animal stopped responding in the first training session (empty bars). Tests of short-term memory showed significant decreases in the time to stop responding in control animals [$P < 0.001$, $t(6) = 8.30$] and in animals treated with 3-AB before training [$P = 0.008$, $t(6) = 4.21$]. Tests of long-term memory showed a significant decrease in the time to stop responding in control animals [$P < 0.001$, $t(6) = 9.64$] and in animals treated with 3-AB after the training [$P < 0.001$, $t(6) = 6.84$]. In animals treated with 3-AB before the training, however, there was no long-term memory [$P = 0.52$, $t(6) = 0.68$]. All tests are two-tailed, paired t tests.

Learning that food is inedible requires a period of consolidation during which blocking the synthesis of mRNA or protein prevents formation of long-term memory (*23*). Long-term memory in *Aplysia* treated with 3-AB after the training period was not impaired (Fig. 3B), which suggests that polyADP-ribosylation of nuclear proteins is critical only during the training and is no longer needed during the consolidation phase.

How might polyADP-ribosylation contribute to the formation of long-term memory? Many nuclear proteins that might function to regulate gene expression involved in memory formation may be modified by polyADP-ribosylation (*6*). One protein of particular interest is linker histone H1, which has been found to undergo polyADP-ribosylation along with the activation of PARP 1 (*6*, *24*). H1 was polyADP-ribosylated in pleural-pedal ganglia as a result of treatments with 5-HT that elicit long-term facilitation (Fig. 4). The histone was not modified by treatments producing short-term facilitation or long-term depression, indicating that polyADP-ribosylation is specific for the formation of long-term facilitation. PolyADP-

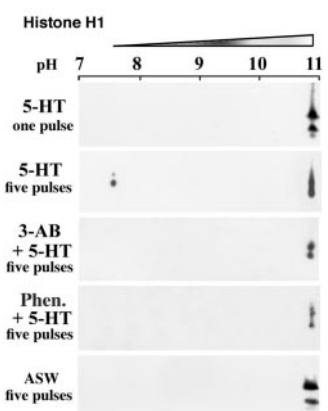

Fig. 4. PolyADP-ribosylation of histone H1 in ganglia stimulated by 5-HT. As indicated by the shift in pI, linker histone H1 [immunolabeled with a monoclonal antibody against the human histone (Upstate, Milton Keynes, UK)] was polyADP-ribosylated in isolated pleural-pedal ganglia after treatment inducing long-term facilitation of sensory-to-motor synapses (5-HT, five pulses). H1 was not polyADP-ribosylated after treatment inducing only short-term facilitation (5-HT, one pulse), nor when PARP 1 activity was suppressed with 1 mM 3-AB (3-AB + 5-HT, five pulses) or with 80 mM 6 (5H)–phenanthridinone (Phen. + 5-HT, five pulses). For each sample, proteins were extracted from 10 pleural-pedal ganglia. The immunoblots shown were replicated in three independent experiments; 8.5 ± 0.7% of H1 was polyADP-ribosylated in nuclear extracts from the ganglia stimulated by five pulses of 5-HT.

ribosylation of H1 has been shown to cause fast and transient relaxation of the highly condensed structure of chromatin, rendering DNA accessible briefly to transcription (*6*, *25–27*).

PolyADP-ribosylation is regarded as an early response to DNA damage. In this context, the modification allows repair enzymes access to broken ends of DNA (*5–8*). We propose that second-messenger cascades that are evoked by experiences that cause long-term memory can also initiate polyADP-ribosylation. We have not yet determined how PARP 1 is activated in *Aplysia* neurons to produce long-term memory. Activation by Ca^{2+} released into the nucleus through the activation of phospholipase C, which operates in rat cortical neurons depolarized by electrical stimulation (*11*), is a possibility, but it is uncertain whether 5-HT can increase nuclear Ca^{2+} in sensory neurons. Hegde *et al.* (*28*) found that 5-HT induces ubiquitin C-terminal hydrolase as an early response gene; the hydrolase enhances ubiquitin-mediated proteolysis of regulatory subunits of the cAMP-dependent protein kinase, producing a persistently active kinase, which is required for long-term facilitation in sensory cells. Activation of PARP 1 also stimulates proteolysis by nuclear proteosomes (*29*), suggesting the possible coordinate action of the hydrolase and PARP 1. In the operant-conditioning task that we used, there is a plausible role for the generation of NO (*30*), possibly through its action on guanylyl cyclase (*31*). Experiments are in progress to identify the mechanisms or mechanisms of activation, which may involve different second-messenger pathways in different neurons.

PolyADP-ribosylation of chromatin would provide quick access to DNA, enabling the transcription needed for long-term memory, while nonspecific DNA transcription by RNA-polymerase II is blocked (*32*). Satchell *et al.* (*33*) recently reported that polyADP-ribosylation contributes to spatial memory measured in a Morris water maze. That PARP 1 plays a role both in learning and in the response to DNA damage reinforces the idea that molecular mechanisms underlying learning might have evolved from the cell's response to stress or injury (*34*).

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Supporting Online Material

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Materials and Methods

References

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Involvement of Mammalian Mus81 in Genome Integrity and Tumor Suppression

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Mus81-Eme1 endonuclease has been implicated in the rescue of stalled replication forks and the resolution of meiotic recombination intermediates in yeast. We used gene targeting to study the physiological requirements of Mus81 in mammals. *Mus81*$^{-/-}$ mice are viable and fertile, which indicates that mammalian Mus81 is not essential for recombination processes associated with meiosis. Mus81-deficient mice and cells were hypersensitive to the DNA cross-linking agent mitomycin C but not to γ-irradiation. Remarkably, both homozygous *Mus81*$^{-/-}$ and heterozygous *Mus81*$^{+/-}$ mice exhibited a similar susceptibility to spontaneous chromosomal damage and a profound and equivalent predisposition to lymphomas and other cancers. These studies demonstrate a critical role for the proper biallelic expression of the mammalian *Mus81* in the maintenance of genomic integrity and tumor suppression.

Homology-directed DNA repair is a major pathway that facilitates the accurate removal of chromosomal damage resulting from exogenous stimuli, stalled replication forks (RFs), or genetically programmed processes (*1–3*). Homologous recombination during mitosis and meiosis is believed to use a four-stranded DNA structural intermediate known as the Holliday junction (HJ) (*4*, *5*). HJs are also thought to be intermediates in the repair of stalled RFs. HJ processing in mammalian cells has recently been linked to RAD51C and XRCC3 (*6*).

Studies have demonstrated a role for the DNA endonuclease Mus81-Eme1/Mms4 in the processing of branched DNA structures associated with stalled RFs and HJ processing (*7–19*). Yeast *mus81* mutants are sensitive to agents that collapse RFs but not those that cause double-strand breaks (*7–9*). As in yeast, mammalian Eme1/Mms4 and Mus81 constitute a structure-specific endonuclease (*16*, *18*, *19*). *Schizosaccharomyces pombe* and human Mus81 complexes cleave HJs in vitro (*10*, *12*), with yeast Mus81-Eme1 show-