cell lines used in this study appear to belong to different complementation groups for apoptosis (*22*), thus illustrating the redundant complexity of the mechanisms regulating cell death.

REFERENCES AND NOTES

1. Y. Ionov, M. A. Peinado, S. Malkhosyan, D. Shibata, M. Perucho, *Nature* **363**, 558 (1993).
2. S. N. Thibodeau, G. Bren, D. Schaid, *Science* **260**, 816 (1993); L. A. Aaltonen *et al.*, *ibid.*, p. 812.
3. L. A. Loeb, *Cancer Res.* **54**, 5059 (1994); M. Perucho *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **59**, 339 (1994).
4. G. Streisinger *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 77 (1966).
5. R. D. Kolodner, *Trends Biochem. Sci.* **20**, 397 (1995); G. Marra and C. R. Boland, *J. Natl. Cancer Inst.* **87**, 1114 (1995).
6. S. Markowitz *et al.*, *Science* **268**, 1336 (1995).
7. H. Kim, J. Jen, B. Vogelstein, S. R. Hamilton, *Am. J. Pathol.* **145**, 148 (1994); M. Konishi *et al.*, *Gastroenterology* **111**, 307 (1996).
8. L. A. Donehower and A. Bradley, *Biochim. Biophys. Acta Rev. Cancer* **1155**, 181 (1993); A. J. Levine, *Annu. Rev. Biochem.* **62**, 623 (1993).
9. E. Yonish-Rouach *et al.*, *Nature* **352**, 345 (1991); M. B. Kastan, O. Onyekwere, D. Sidransky, B. Vogelstein, R. W. Craig, *Cancer Res.* **51**, 6304 (1991).
10. T. Miyashita and J. C. Reed, *Cell* **80**, 293 (1995).
11. E. White, *Genes. Dev.* **10**, 1 (1996); S. Cory, *Annu. Rev. Immunol.* **13**, 513 (1995).
12. Z. N. Oltvai, C. L. Milliman, S. J. Korsmeyer, *Cell* **74**, 609 (1993).
13. Somatic insertion or deletion mutations in $(G)_8$ or $(C)_8$ tracts were found in the same MMP$^+$ colon tumors in the following genes: 12 of 40 in the coding region of the *hMSH6* DNA mismatch repair gene (*14*); 8 of 40 (coding) in the insulin-like growth factor II receptor gene (*IGFR-II*) [D. O. Morgan *et al.*, *Nature* **329**, 301 (1987)]; 2 of 41 in the 3′ noncoding region of the *IGFR-I* gene [A. Ullrich *et al.*, *EMBO J.* **5**, 2503 (1986)]; 2 of 41 in the promoter region of the muscle glycogen synthase (*GS*) gene [European Molecular Biology Laboratory (EMBL) and GenBank number HSGSYG1]; 2 of 40 (coding) in an uncharacterized human repair DNA protein (EMBL and GenBank number HUM51CP); 0 of 40 (coding) in a human nuclease-sensitive element DNA binding protein (*NSEP*) [R. Kolluri and A. J. Kinniburg, *Nucleic Acids Res.* **19**, 4771 (1991)]; and 0 of 41 (3′ noncoding) in the nerve growth factor receptor gene (*NGFR*) [D. Johnson *et al.*, *Cell* **47**, 545 (1986)]. The statistics (Fisher exact test) were as follows: *BAX* versus *hMSH6*, $P = 0.043$; versus *IGFR-II*, $P = 0.0032$; versus *IGFR-I*, *GS*, or *HUM51CP*, $P < 10^{-6}$; versus *NSEP* or *NGFR*, $P < 10^{-7}$.
14. S. Malkhosyan, N. Rampino, H. Yamamoto, M. Perucho, *Nature* **382**, 499 (1996).
15. The heterozygous or homozygous nature of these mutations cannot be easily distinguished in primary tumor specimens because of the presence of variable amounts of contaminating normal tissue. The approximate amount of normal tissue could be estimated in some tumors by comparing the proportion of nonaltered versus altered sequences at several mononucleotide microsatellite loci. In this manner, we estimate that some tumors (73, 442, and 453 of Fig. 3A) harbored more mutant than normal *BAX* alleles. Microdissection experiments to select regions enriched in tumor cells revealed the homozygous nature of some mutations, which appeared heterozygous before microdissection (tumor 43, Fig. 3A). In several primary tumors (61, 211, and 437 of Fig. 3A) and in three MMP$^+$ cell lines (LS411N, HCT116, and SKUT1B), the *BAX* frameshift mutations appeared heterozygous, or present in only a fraction of the tumor cells.
16. C. M. Knudson, K. S. K. Tung, W. G. Tourtellote, G. A. J. Brown, S. J. Korsmeyer, *Science* **270**, 96 (1995).
17. S. J. Korsmeyer, *Trends Genet.* **11**, 101 (1995).
18. J. P. P. Meijerink, T. F. C. M. Smetsers, A. W. Sloetjes, E. H. P. Linders, E. J. B. M. Mensink, *Leukemia* **9**, 1828 (1995).
19. E. R. Fearon and B. Vogelstein, *Cell* **61**, 759 (1990).
20. D. Shibata, M. A. Peinado, Y. Ionov, S. Malkhosyan, M. Perucho, *Nature Genet.* **6**, 273 (1994).
21. S. Lee, B. Elenbaas, A. Levine, J. Griffith, *Cell* **81**, 1013 (1995).
22. S. Casares, Y. Ionov, H. Y. Ge, E. Stanbridge, M. Perucho, *Oncogene* **11**, 2303 (1995).
23. Tumor cell lines were obtained from the American Cell Type Collection and characterized as MMP$^+$ by PCR analysis of single-cell clones (*20, 22*). There were striking associations between *BAX* frameshift mutations and mutations in members of the *Mut S* and *Mut L* families of mutator genes, respectively. This is in line with their differences in the corresponding mutation spectra in microsatellites and cellular genes [S. Malkhosyan, A. McCarty, H. Sawai, M. Perucho, *Mutat. Res.* **316**, 249 (1996)].
24. A 94–base pair region encompassing the $(G)_8$ tract in *BAX* was amplified by PCR with primers 5′-ATC CAG GAT CGA GCA GGG CG-3′ and 5′-ACT CGC TCA GCT TCT TGG TG-3′. PCR with Vent DNA polymerase (New England Biolabs) was carried out for one cycle of 94°C for 4 min followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s in the presence of 0.2 mCi of [^{32}P]deoxycytidine triphosphate. PCR products were separated in a denaturing 6% polyacrylamide gel and subjected to autoradiography. P7, P8, and P9 are the PCR products amplified from plasmids containing seven, eight (wild-type), or nine G's in *BAX*, determined by nucleotide sequencing (see Fig. 2B).
25. Protein cell lysates (10 mg) were separated in a 12% SDS-polyacrylamide gel. Proteins were electroblotted onto a nylon membrane and incubated with rabbit antibodies to human BAX and F1β ATPase. Alkaline phosphatase–conjugated secondary antibodies were used for detection. S. Krajewski *et al.*, *Cancer Res.* **55**, 4471 (1995); S. Krajewski, J. M. Zapata, J. C. Reed, *Anal. Biochem.* **236**, 221 (1996).
26. The PCR products were eluted from the gels, amplified, and subcloned into pCRTM2.1 (Invitrogen). Recombinant plasmids were sequenced by the dideoxy chain termination method, with a Sequenase DNA sequencing kit (United States Biochemical). DNA was also reamplified and purified with QIAquick PCR purification kit (Qiagen) and sequenced with the ABI PRISMTM dye terminator cycle sequencing kit (Perkin-Elmer). The DNA sequences obtained by the two methods were identical.
27. Tissues were obtained from the Southern Division of the Cooperative Human Tissue Network (University of Alabama at Birmingham) as frozen specimens after surgical resections. Detailed family histories for these cases were not available. MMP$^+$ tumors were defined as those with deletion mutations in mononucleotide microsatellite loci and deletion or insertion mutations of more than one repeated unit in dinucleotide microsatellite sequences. Tumors exhibiting sporadic dinucleotide microsatellite mobility shifts of only one repeated unit without TGF-β receptor mutations were considered MMP$^-$. The properties of a subset of these tumors, were described in (*1*).

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Involvement of Pre- and Postsynaptic Mechanisms in Posttetanic Potentiation at *Aplysia* Synapses

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Posttetanic potentiation (PTP) is a common form of short-term synaptic plasticity that is generally thought to be entirely presynaptic. Consistent with that idea, PTP of evoked excitatory postsynaptic potentials at *Aplysia* sensory-motor neuron synapses in cell culture was reduced by presynaptic injection of a slow calcium chelator and was accompanied by an increase in the frequency but not the amplitude of spontaneous excitatory postsynaptic potentials. However, PTP was also reduced by postsynaptic injection of a rapid calcium chelator or postsynaptic hyperpolarization. Thus, PTP at these synapses is likely to involve a postsynaptic induction mechanism in addition to the known presynaptic mechanisms.

Posttetanic potentiation, an increase in synaptic strength for several minutes following high frequency stimulation of the presynaptic fibers (*1*), has been observed and extensively studied at a number of synapses including *Aplysia* sensory-motor neuron synapses (*2, 3*). *Aplysia* sensory neurons release the neurotransmitter glutamate which acts through *N*-methyl-D-aspartate–like receptors on the motor neurons, and thus these synapses may share properties with glutamatergic synapses in vertebrates (*4*). At *Aplysia* sensory-motor neuron synapses, PTP is accompanied by an increase in the frequency of spontaneous miniature excitatory postsynaptic potentials (mEPSPs) or currents (mEPSCs) with no change in their amplitude, indicating that the expression of PTP is presynaptic (*3*). In addition to PTP, repeated tetanic stimulation of the sensory neuron or pairing high-frequency stimulation of the sensory neuron with strong depolarization of the motor neuron can produce long-term potentiation (LTP) at these synapses (*5, 6*). This potentiation is reduced by postsynaptic hyperpolarization during the tetanic stimulation or infusion of the Ca^{2+} chelator BAPTA [1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid] into the postsynaptic neuron, indicating that the induction of LTP involves postsynaptic events. Therefore, we

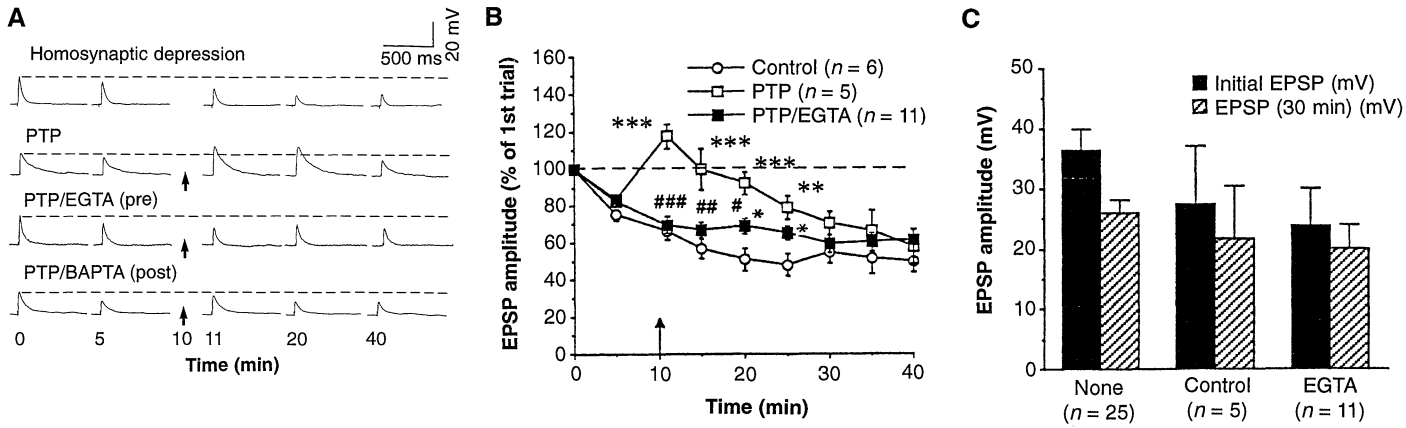

Fig. 1. PTP is reduced by presynaptic EGTA. (**A**) Original recordings from four representative experiments at LFS synapses, showing homosynaptic depression of EPSPs, PTP of EPSPs caused by a tetanus (20 Hz for 2 s) applied at 10 min (arrow), PTP 30 min after the sensory neuron was injected with EGTA (presynaptic, 50 mM in the electrode), and PTP after an LFS cell was loaded with BAPTA (postsynaptic, 200 mM in the electrode) for 30 min. (**B**) Presynaptic EGTA substantially reduced PTP at LFS synapses. For PTP and PTP/EGTA groups, a tetanus was given at 10 min (arrow). In both control and PTP groups, the sensory neuron received injection of vehicle solution. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus control; $\#P < 0.05$, $\#\#P < 0.01$, and $\#\#\#P < 0.001$ versus PTP. (**C**) Presynaptic EGTA had no effect on basal synaptic transmission at LFS synapses. None indicates that no injection was performed.

have examined whether postsynaptic infusion of BAPTA or hyperpolarization has similar effects on PTP at *Aplysia* sensory-motor neuron synapses in dissociated cell culture (*7*), using conventional intracellular recording techniques (*8*).

At a variety of synapses, PTP results from presynaptic residual Ca^{2+} that accumulates during tetanic stimulation (*9–11*). To test whether PTP at *Aplysia* sensory-motor neuron synapses requires presynaptic Ca^{2+}, we injected (*12*) sensory neurons with EGTA, a Ca^{2+} chelator with slow kinetics that buffers residual Ca^{2+} without affecting transmitter release (*13*). In control cells, the evoked EPSPs (eEPSPs) underwent homosynaptic depression; they declined over time at synapses onto either L7 or LFS motor neurons, even when the stimulation interval was as long as 5 min (Fig. 1, A and B). Tetanic stimulation (20 Hz for 2 s) of the sensory neuron induced PTP of the eEPSPs at LFS or L7 synapses (Fig. 1, A and B, and Fig. 2). Compared to test-alone homosynaptic depression, PTP lasted for about 20 min (Figs. 1B and 2). Presynaptic EGTA (50 mM in the electrode) substantially reduced PTP at LFS synapses (Fig. 1, A and B) but did not affect the baseline EPSPs (Fig. 1C) or the area under the summed EPSPs during tetanic stimulation [$F(2,28) = 0.722$, $P = 0.4954$] (*14*). These results are consistent with the conventionally held view that PTP is presynaptically mediated and requires presynaptic Ca^{2+} accumulation.

Aplysia sensory-motor neuron synapses also exhibit LTP, which is blocked by postsynaptic infusion of the Ca^{2+} chelator BAPTA (*6*). To test whether PTP is also dependent on postsynaptic Ca^{2+}, we injected BAPTA tetrapotassium salt (200 mM in the electrode) into the motor neuron. BAPTA injected into the motor neuron significantly reduced PTP (Fig. 1A and Fig. 2, A and B) (*15*). The effects of BAPTA seemed to be somewhat larger at LFS than at L7 synapses (compare A and B in Fig. 2), probably because there was a higher concentration of BAPTA in LFS cells because

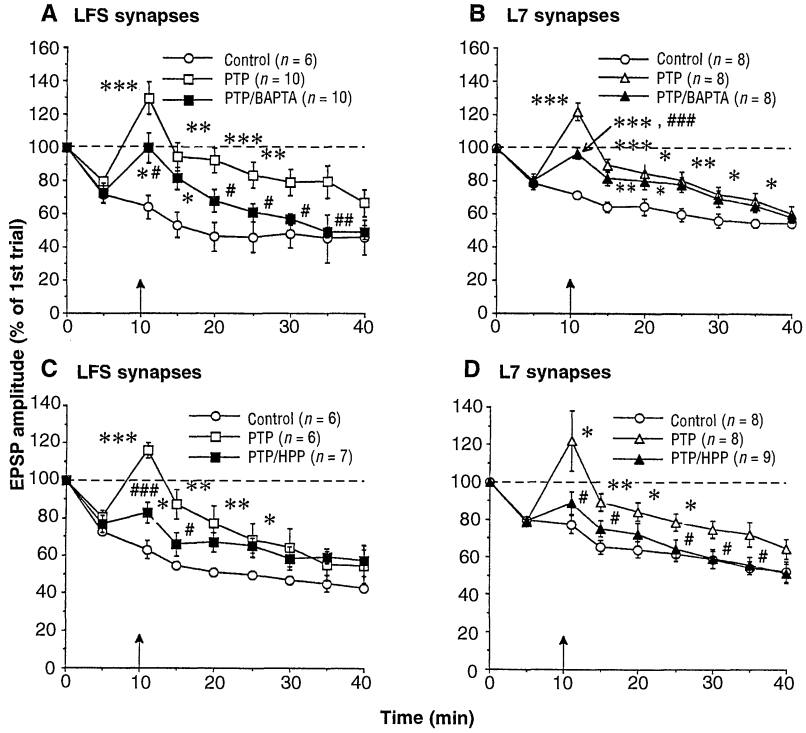

Fig. 2. PTP is reduced by postsynaptic BAPTA or hyperpolarization during the tetanus. (**A** and **B**) Effect of postsynaptic BAPTA (200 mM in the electrode) at LFS (A) or L7 (B) synapses. (**C** and **D**) Effect of postsynaptic hyperpolarization (HPP) at LFS (C) or L7 (D) synapses. Hyperpolarization (2 nA for LFS and 4 nA for L7) was given from approximately 20 s before to 20 s after the tetanus.

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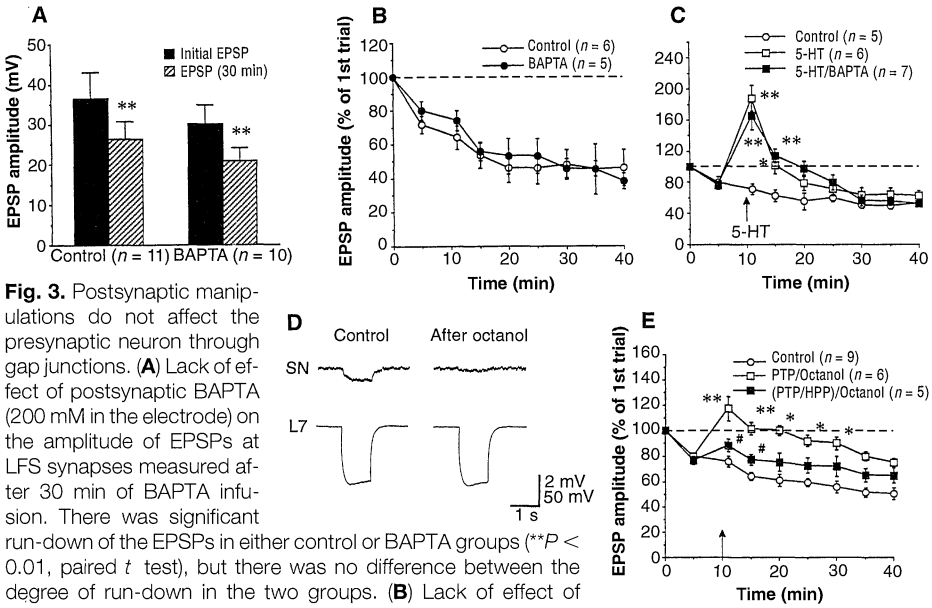

Fig. 3. Postsynaptic manipulations do not affect the presynaptic neuron through gap junctions. (**A**) Lack of effect of postsynaptic BAPTA (200 mM in the electrode) on the amplitude of EPSPs at LFS synapses measured after 30 min of BAPTA infusion. There was significant run-down of the EPSPs in either control or BAPTA groups (**$P < 0.01$, paired t test), but there was no difference between the degree of run-down in the two groups. (**B**) Lack of effect of postsynaptic BAPTA on homosynaptic depression at LFS synapses. (**C**) Lack of effect of postsynaptic BAPTA on short-term facilitation induced by 5-HT at LFS synapses. We applied 5-HT (10 μl, 50 μM) at 10 min (arrow). (**D**) Perfusion with L-octanol (1 mM) for at least 30 min blocked the electrical coupling between the sensory neuron (SN) and motor neuron (L7). (**E**) L-octanol did not affect PTP or the effect of postsynaptic HPP (4 nA) on PTP at L7 synapses.

of their smaller size. Thus, postsynaptic Ca^{2+} was also involved in PTP induction.

BAPTA might have interfered with PTP by diffusing through gap junctions to the presynaptic terminals. However, BAPTA injected into the motor neuron did not significantly affect the amplitude of eEPSPs measured 30 min after BAPTA injection (Fig. 3A), the rate of homosynaptic depression (Fig. 3B), or the short-term heterosynaptic facilitation produced by serotonin [5-hydroxytryptamine (5-HT)] (Fig. 3C) (*16*). Thus, postsynaptic BAPTA did not affect basal synaptic transmission or other forms of plasticity that are thought to be presynaptic (*17*, *18*), suggesting that it did not diffuse from the motor neuron to the sensory neuron, but rather acted primarily or exclusively to lower the Ca^{2+} concentration in the postsynaptic cell.

LTP at these synapses is also reduced by postsynaptic hyperpolarization during tetanic stimulation (5). Similarly to LTP, strong postsynaptic hyperpolarization during the tetanus also reduced PTP at both LFS and L7 synapses (Fig. 2, C and D) (*19*). It is possible that postsynaptic hyperpolarization interfered with PTP by damaging the postsynaptic cell or by hyperpolarizing the presynaptic terminals through electrical coupling. However, we found that postsynaptic hyperpolarization did not have deleterious effects on basal synaptic transmission (control cells in Fig. 2, C and D). Under our experimental conditions, there was small but detectable coupling between sensory and motor neurons (*20*), which was blocked by perfusion for 20 to 30 min with L-octanol (1 mM), an inhibitor of electrical coupling in other systems ($n = 5$) (Fig. 3D) (*21*). In the continuous presence of 1 mM L-octanol, tetanic stimulation of the sensory neuron still produced normal PTP of eEPSPs, and strong postsynaptic hyperpolarization (4 nA) still produced significant reduction of

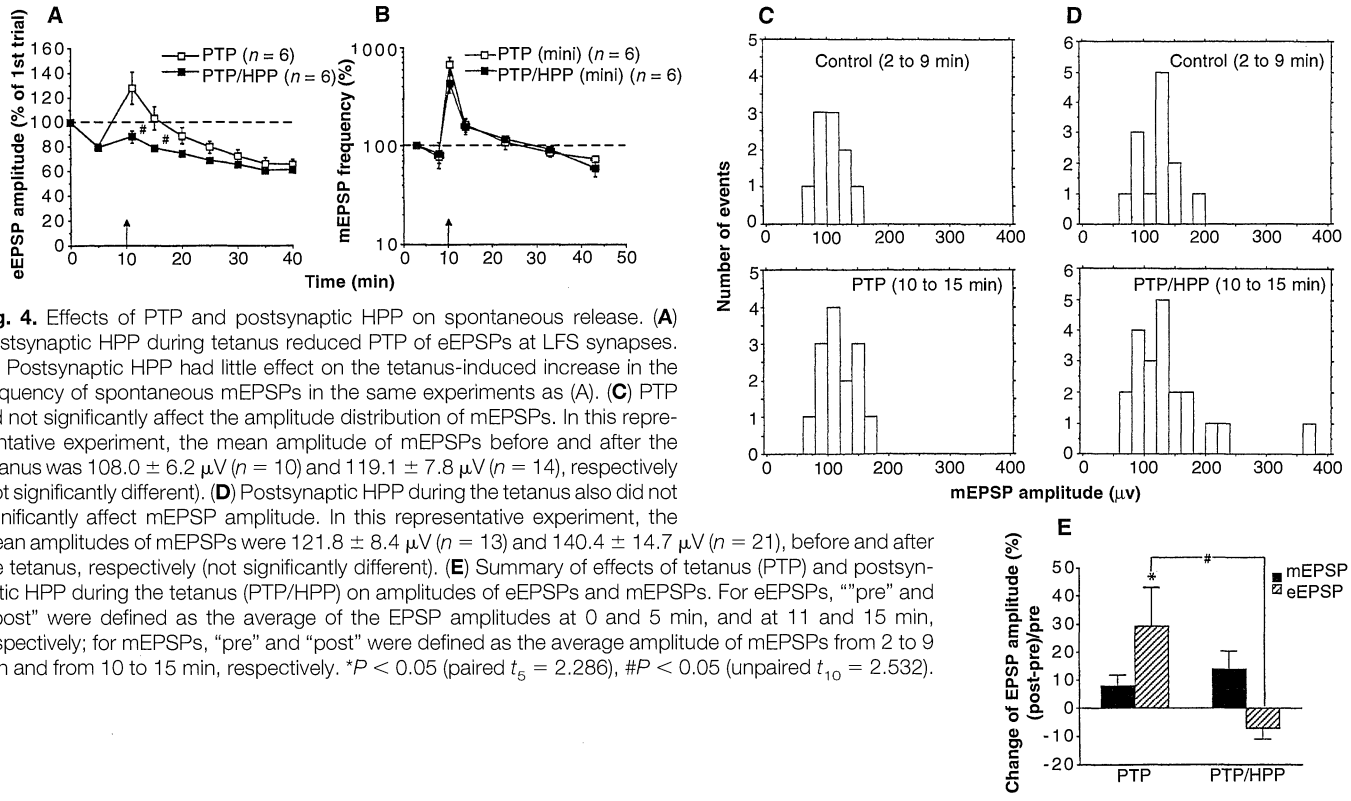

Fig. 4. Effects of PTP and postsynaptic HPP on spontaneous release. (**A**) Postsynaptic HPP during tetanus reduced PTP of eEPSPs at LFS synapses. (**B**) Postsynaptic HPP had little effect on the tetanus-induced increase in the frequency of spontaneous mEPSPs in the same experiments as (A). (**C**) PTP did not significantly affect the amplitude distribution of mEPSPs. In this representative experiment, the mean amplitude of mEPSPs before and after the tetanus was 108.0 ± 6.2 μV ($n = 10$) and 119.1 ± 7.8 μV ($n = 14$), respectively (not significantly different). (**D**) Postsynaptic HPP during the tetanus also did not significantly affect mEPSP amplitude. In this representative experiment, the mean amplitudes of mEPSPs were 121.8 ± 8.4 μV ($n = 13$) and 140.4 ± 14.7 μV ($n = 21$), before and after the tetanus, respectively (not significantly different). (**E**) Summary of effects of tetanus (PTP) and postsynaptic HPP during the tetanus (PTP/HPP) on amplitudes of eEPSPs and mEPSPs. For eEPSPs, ""pre" and ""post" were defined as the average of the EPSP amplitudes at 0 and 5 min, and at 11 and 15 min, respectively; for mEPSPs, "pre" and "post" were defined as the average amplitude of mEPSPs from 2 to 9 min and from 10 to 15 min, respectively. *$P < 0.05$ (paired $t_5 = 2.286$), #$P < 0.05$ (unpaired $t_{10} = 2.532$).

PTP at L7 synapses (Fig. 3E) (*22*). Thus, electrical coupling between the sensory and motor neurons was not required for the induction of PTP, and hyperpolarization of the postsynaptic cell did not reduce PTP by hyperpolarizing the presynaptic terminals through electrical coupling. Taken together, these results suggest that induction of PTP, at least in part, requires postsynaptic depolarization.

Tetanic stimulation of the sensory neuron that induced PTP of eEPSPs also produced a large increase in the frequency of mEPSPs (Fig. 4, A and B), but did not significantly increase the amplitude of mEPSPs (Fig. 4, C and E), suggesting that expression of PTP involves a presynaptic increase in transmitter release. Strong hyperpolarization of the postsynaptic motor neuron during the tetanus reduced PTP of eEPSPs (Fig. 4A). However, it did not affect the increase in the frequency of mEPSPs (Fig. 4B) or their amplitude (Fig. 4, D and E) (*23*). Thus, postsynaptic hyperpolarization may affect some aspect of presynaptic transmitter release that is specific to evoked release.

PTP is accompanied by an increase in the frequency, but not the amplitude, of spontaneous mEPSPs at a number of synapses (*24*) including *Aplysia* sensory-motor neuron synapses in cell culture (Fig. 4) (*3*). Moreover, the time course of PTP at synapses made by L10 cells in *Aplysia* (*9*) and other synapses (*10*) parallels that of the residual presynaptic Ca^{2+} following a tetanus, and presynaptic injection of Ca^{2+} chelators blocks both the rise of residual Ca^{2+} and PTP (Fig. 1B) (*9–11*). Thus presynaptic residual Ca^{2+} that accumulates during trains of action potentials is thought to contribute to the enhancement of both spontaneous and evoked release during PTP. However, PTP was also reduced by either postsynaptic infusion of the Ca^{2+} chelator BAPTA or postsynaptic hyperpolarization (Fig. 2), suggesting that postsynaptic mechanisms (postsynaptic depolarization and a postsynaptic increase in Ca^{2+}) also participate in PTP induction at sensory-motor neuron synapses. One possibility is that the potentiation is composed of two independent components: "conventional" PTP that is entirely presynaptic and depends on presynaptic Ca^{2+}, and short-term potentiation (STP) that requires postsynaptic depolarization and an increase in Ca^{2+}, similar to STP in hippocampus (*25*). However, unlike PTP and STP in hippocampus, the components of potentiation in *Aplysia* that were sensitive to presynaptic EGTA (Fig. 1B) and postsynaptic BAPTA or hyperpolarization (Fig. 2) had roughly similar time courses, which were within the range of PTP at other synapses (*1*). Moreover, presynaptic EGTA reduced maximum PTP by 93% (Fig. 1B), and postsynaptic BAPTA or hyperpolarization reduced it by 58% (the average in Fig. 2, A through D), so that their combined effects would be more than 100% if they were additive. Thus, a more likely possibility is that PTP involves, in part, some interaction between pre- and postsynaptic mechanisms. For example, postsynaptic depolarization and increased Ca^{2+} during the tetanus could lead to formation of a retrograde messenger that interacts with presynaptic residual Ca^{2+} to increase transmitter release. Such a mechanism is thought to contribute to LTP in hippocampus (*26*), and retrograde modulation of transmitter release is also thought to occur in a number of other systems (*27*). Because postsynaptic hyperpolarization did not affect the tetanus-induced increase in spontaneous release, the putative retrograde messenger in *Aplysia* would have to act on some aspect of transmitter release that is specific to synchronized, evoked release. The *Aplysia* cell culture system, with a single presynaptic and postsynaptic neuron in isolation, should be particularly advantageous for studies of the mechanisms of such transsynaptic interactions.

REFERENCES AND NOTES

1. T. P. Feng, *Chin. J. Physiol.* **16**, 341 (1941); R. Waziri, E. R. Kandel, W. T. Frazier, *J. Neurophysiol.* **32**, 509 (1969); R. Epstein and L. Tauc, *J. Physiol. (London)* **209**, 1 (1970); K. L. Magleby and J. E. Zengel, *ibid.* **245**, 183 (1975); H. L. Atwood and J. M. Wojtowicz, *Int. Rev. Neurobiol.* **28**, 275 (1986); R. S. Zucker, *Annu. Rev. Neurosci.* **12**, 13 (1989); *Neth. J. Zool.* **44**, 495 (1994).
2. G. A. Clark and E. R. Kandel, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2577 (1984); E. T. Walters and J. H. Byrne, *Brain Res.* **293**, 377 (1984); S. Schacher, P. G. Montarolo, E. R. Kandel, *J. Neurosci.* **10**, 3286 (1990).
3. L. S. Eliot, E. R. Kandel, R. D. Hawkins, *J. Neurosci.* **14**, 3280 (1994).
4. N. Dale and E. R. Kandel, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 7163 (1993).
5. X. Y. Lin and D. L. Glanzman, *Proc. R. Soc. London Ser. B Biol. Sci.* **255**, 113 (1994).
6. ———, *ibid.*, p. 215.
7. A single identified L7 gill motor neuron or LFS siphon motor neuron was isolated from the abdominal ganglion of a juvenile (1 to 4 g) or adult (70 to 100 g) *Aplysia* respectively (Howard Hughes Medical Institute Mariculture Facility, Miami, FL), and co-cultured with a mechanosensory neuron isolated from the pleural ganglion of an adult animal. Several criteria were used to identify LFS cells [W. N. Frost, thesis, Columbia University, New York (1987), p. 113; L. S. Eliot, R. D. Hawkins, E. R. Kandel, S. Schacher, *J. Neurosci.* **14**, 368 (1994)], although it is possible that some of the putative LFS cells were not motor neurons. The axon stump of the dissociated sensory neuron was placed near the initial segment of the main axon of the motor neuron, where functional synapses are formed. The cells were plated in culture dishes coated with poly-L-lysine (0.5 mg/ml) and left overnight at room temperature, after which the dishes were moved to an incubator where they were maintained at 18°C for 3 to 6 days. The culture medium consisted of 50% filtered *Aplysia* hemolymph and 50% L-15 medium (Flow Laboratories, McLean, VA) supplemented with salts (NaC1, 260 mM; $CaC1_2$, 10.1 mM; KC1, 4.6 mM; $MgSO_4$, 25 mM; $MgC1_2$, 28 mM; and $NaHCO_3$, 2.3 mM) and D-glucose (34.6 mM). Additionally, glutamine (1 mM), penicillin (50 U/ml), and streptomycin (50 μg/ml) were also included in the culture medium. The hemolymph was collected from large animals (usually ~1000 g) and stored at −70°C [S. Schacher and E. Proshansky, *J. Neurosci.* **3**, 2403 (1983); S. Schacher, *ibid.* **5**, 2028 (1985)].
8. A motor neuron was impaled with a microelectrode (6 to 15 megohm) filled with 2.5 M KCl for recording of EPSPs. The electrical signals were amplified by a Dagan 8100 (Dagan, Minneapolis, MN) or Axoclamp 2B (Axon Instruments, Foster City, CA) amplifier and recorded on a four-channel tape recorder. A conventional bridge balance circuit was used to inject current through the recording electrode. Because it is not possible to produce PTP while the sensory neuron is impaled with an intracellular electrode (*3*), an extracellular electrode positioned adjacent to the soma was used to stimulate the sensory neuron with a brief (0.1 ms) depolarizing pulse. The stimulation intensity was gradually increased to reach the threshold for firing an action potential in the sensory neuron and evoking an EPSP in the motor neuron. In order to measure the amplitude of EPSPs accurately and to avoid spike initiation in the motor neuron, we hyperpolarized the neuron 50 mV (for LFS cells) or 30 mV (for L7 cells) below the resting membrane potential throughout the experiment. The EPSP was tested immediately after impalement of the motor neuron, and the culture was discarded if it was smaller than 5 mV. After 30-min rest (or, in some experiments, 30 min after the start of presynaptic injection of EGTA, postsynaptic injection of BAPTA, or bath perfusion of L-octanol), EPSPs were evoked at ~5-min intervals for 40 min. For PTP experiments, a tetanus (20 Hz for 2 s) was applied 10 min after the first EPSP in the series, with a stimulus intensity 20 to 30% above that used to evoke the previous EPSP, which was sufficient to produce one-for-one EPSPs during the tetanus. All experiments were performed at room temperature (20° to 23°C). The culture dish was continuously perfused at a rate of ~0.5 ml/min with 50% supplemented L-15 medium and 50% artificial seawater (NaCl, 460 mM; KCl, 11 mM; $CaCl_2$, 10 mM; $MgCl_2$, 27 mM; and $MgSO_4$, 27 mM). All chemicals were from Sigma (St. Louis, MO), except for BAPTA, which was from Molecular Probes (Eugene, OR).
9. R. Kretz, E. Shapiro, E. R. Kandel, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5430 (1982); J. A. Connor, R. Kretz, E. Shapiro, *J. Physiol. (London)* **375**, 625 (1986).
10. K. R. Delaney, R. S. Zucker, D. W. Tank, *J. Neurosci.* **9**, 3558 (1989); D. Swandulla, M. Hans, K. Zipser, G. J. Augustine, *Neuron* **7**, 915 (1991).
11. H. Kamiya and R. S. Zucker, *Nature* **371**, 603 (1994).
12. For EGTA injection into the sensory neurons, the tip of the injecting electrode was bevelled to have a resistance of 3 to 6 megohms. The electrodes were connected to a Pico-Injector (Medical Systems, Greenvale, NY) and pulses of pressure (800 ms duration; 0.3 to 1.4 psi) were delivered at 2-s intervals for 10 to 60 s. EGTA was first dissolved in KOH, and the pH was adjusted to 7.5 with HCl. Fast green (0.2%) was usually included in the EGTA solution or the control vehicle solution to visually ensure successful injection. The injection electrode was then withdrawn from the cell, and stimulation was begun with an extracellular electrode after a 30-min rest.
13. E. M. Adler, G. J. Augustine, S. N. Duffy, M. P. Charlton, *J. Neurosci.* **11**, 1496 (1991).
14. The data in the text and figures are expressed as means ± SEM (*n* represents the number of cultures) and normalized to the first test EPSP (30 min after the impalement), except where otherwise indicated. Data were analyzed statistically either by *t* test (paired or unpaired) or by two-way analyses of variance (ANOVAs) with one repeated measure (time). If there was a significant overall effect in the ANOVA, comparisons were made at each time point by one-way ANOVAs, followed by Fisher PLSD tests. In the experiments shown in Fig. 1B, there were significant differences between control, PTP, and PTP after EGTA injection (PTP/EGTA) [$F(2,19) = 4.291$ for

treatment, $P = 0.029$; $F(16,152) = 5.894$ for treatment × time, $P = 0.0001$]. There were no significant differences between the initial EPSPs or EPSPs 30 min after injection in the three groups shown in Fig. 1C.

15. BAPTA was injected by diffusion or iontophoresis (0.5 to 1 nA, 500 ms at 1 Hz). Similar results were obtained after 20 to 30 min of iontophoretic injection or diffusion. BAPTA injected by these methods into the sensory neuron blocked synaptic transmission, suggesting that it was effective in buffering the Ca^{2+} concentration. In the experiments shown in Fig. 2, A and B, there were significant differences between homosynaptic depression (control), PTP, and PTP/BAPTA [for LFS synapses: $F(2,18) = 6.281$ for treatment, $P = 0.0085$; $F(16,144) = 2.428$ for treatment × time, $P = 0.0029$; for L7 synapses: $F(2,18) = 6.214$ for treatment, $P = 0.0089$; $F(16,144) = 7.191$ for treatment × time, $P = 0.0001$]. For LFS synapses, the average amplitudes of the EPSPs on trial one, 30 min after impalement, were 26.4 ± 4.4 mV (control), 23.3 ± 3.7 mV (PTP), and 20.9 ± 3.3 mV (PTP/BAPTA); for L7 synapses, they were 17.3 ± 3.1 mV, 12.6 ± 3.2 mV, and 16.9 ± 3.9 mV, respectively. There were no significant differences among EPSP amplitudes on trial one for the three groups at either synapse.
16. We manually applied 10 μl of 5-HT (50 μM) with a 50-μl Hamilton syringe to the vicinity of the cells, as previously described (2). Fast green (0.2%), which by itself did not affect synaptic transmission, was included in the 5-HT solution to check the delivery and location of the puff. The solution was washed out in less than 60 s by continuous perfusion of the culture. A two-way ANOVA revealed that 5-HT produced significant facilitation of EPSPs [overall, $F(2,13) = 8.852$ for treatment, $P = 0.0037$ and $F(16,104) = 5.718$ for treatment × time, $P = 0.0001$], but there was no significant difference between the 5-HT and 5-HT/BAPTA groups. The average amplitudes of EPSPs 30 min after impalement were 25.3 ± 5.1 mV (control) and 19.3 ± 4.6 mV (BAPTA) in the experiments shown in Fig. 3B, and 22.7 ± 6.4 mV (control), 28.8 ± 3.6 mV (5-HT), and 20.1 ± 4.2 mV (5-HT/BAPTA) in the experiments shown in Fig. 3C. There were no significant differences among EPSP amplitudes on trial one for the different groups.
17. V. F. Castellucci and E. R. Kandel, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 5004 (1974).
18. N. Dale and E. R. Kandel, *J. Physiol. (London)* **421**, 203 (1990).
19. LFS motor neurons were injected with 2 nA of current and L7 motor neurons with 4 nA, which produced approximately 50 to 100 mV of additional hyperpolarization below the level at which the motor neurons were held throughout the rest of the experiment (*8*), as measured in the soma. However, the hyperpolarization could have been substantially less at the synaptic region. Overall, there were significant differences between control, PTP, and PTP with hyperpolarization during the tetanus (PTP/HPP) [for LFS synapses: $F(2,14) = 6.771$ for treatment, $P = 0.0088$; $F(16,112) = 4.807$ for treatment × time, $P = 0.0001$; for L7 synapses: $F(2,19) = 5.22$ for treatment, $P = 0.0156$; $F(16,152) = 2.951$ for treatment × time, $P = 0.0003$]. For LFS synapses, EPSPs 30 min after impalement had amplitudes of 18.3 ± 2.5 mV (control), 21.3 ± 5.1 mV (PTP), and 22.1 ± 3.8 mV (PTP/HPP); for L7 synapses, they were 14.6 ± 2.6 mV, 19.5 ± 4.0 mV, and 18.1 ± 3.2 mV, respectively. There were no significant differences among EPSP amplitudes on trial one for these three groups.
20. Electrical coupling between the sensory neuron and motor neuron was monitored by impaling both cells with microelectrodes and applying a hyperpolarizing pulse (30 to 120 mV) to one cell (cell 1) and measuring the change of membrane potential in the other (cell 2). The degree of coupling was defined by: (membrane potential change in cell 2/hyperpolarizing potential applied in cell 1) × 100%. The coupling measured from soma to soma was 1.02 ± 0.49% ($n = 5$) from sensory to LFS cell, 0.79 ± 0.38% ($n = 5$) from LFS to sensory cell, 0.32 ± 0.17% ($n = 8$) from sensory to L7 cell, and 0.23 ± 0.12% ($n = 8$) from L7 to sensory cell.
21. M. V. L. Bennett *et al. Neuron* **6**, 305 (1991); R. Yuste, D. A. Nelson, W. W. Rubin, L. C. Katz, *ibid.* **14**, 7 (1995).
22. Overall, there were significant differences between control, PTP in the presence of octanol (PTP/octanol), and PTP with hyperpolarization during the tetanus in the presence of octanol [(PTP/HPP)/octanol] [$F(2,15) = 10.068$ for treatment, $P = 0.0176$ and $F(16,132) = 4.594$ for treatment × time, $P = 0.0001$]. EPSPs 30 min after impalement had amplitudes of 14.9 ± 2.3 mV (control), 11.6 ± 1.5 mV (PTP/octanol), and 12.0 ± 4.1 mV [(PTP/HPP)/octanol]. There were no significant differences among EPSP amplitudes on trial one for these three groups.
23. Recording of spontaneous mEPSPs was made at high gain, filtered at 300 Hz (−3 dB), and AC coupled. In experiments in which both evoked and spontaneous mEPSPs were measured, eEPSPs were recorded at low gain and DC coupled. Signals were digitized off-line with a PC-based ""fetchex" program (part of the ""pclamp" package, Axon Instruments) and analyzed with an ""axobasic" program. We visually identified mEPSPs on the basis of the following criteria: (i) a rise time of 3 to 20 ms, (ii) a half-decay time of at least 5 ms, and (iii) a minimum amplitude of 50 μV, which was usually about 25% greater than the peak-to-peak noise level (*3 18*). The frequency and the peak amplitude of mEPSPs were then automatically determined by the computer. When the dependent variable was the amplitude of eEPSPs (Fig. 4A), There were significant differences between the PTP and PTP/HPP groups [$F(1,10) = 5.244$ for treatment, $P = 0.045$ and $F(8,80) = 6.189$ for treatment × time, $P = 0.001$]. EPSPs 30 min after impalement had amplitudes of 17.8 ± 2.9 mV (PTP) and 25.9 ± 2.3 mV (PTP/HPP) (not significantly different). When the dependent variable was the frequency of mEPSPs (Fig. 4B), there were no significant differences between PTP and PTP/HPP groups. The average frequency of mEPSPs 30 min after impalement was 0.032 ± 0.006 Hz and 0.041 ± 0.007 Hz for PTP and PTP/HPP groups, respectively (not significantly different).
24. A. R. Martin and G. Pilar, *J. Physiol. (London)* **175**, 17 (1964); H. P. Clamann, J. Mathis, H. R. Luscher, *J. Neurophysiol.* **61**, 403 (1989); W. H. Griffith, *ibid.* **63**, 491 (1990).
25. R. Anwyl, D. Mulkeen, M. J. Rowan, *Brain Res.* **503**, 148 (1989); R. C. Malenka, *Neuron* **6**, 53 (1991).
26. T. J. O'Dell, R. D. Hawkins, E. R. Kandel, O. Arancio, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 11285 (1991); M. Zhuo, S. A. Small, E. R. Kandel, R. D. Hawkins, *Science* **260**, 1946 (1993).
27. R. K. Murphey and G. W. Davis, *J. Neurobiol.* **25**, 595 (1994); Y.-J. Lo, Y.-C. Lin, D. H. Sanes, M.-M. Poo, *J. Neurosci.* **14**, 4694 (1994).
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A Mammalian Telomerase-Associated Protein

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The telomerase ribonucleoprotein catalyzes the addition of new telomeres onto chromosome ends. A gene encoding a mammalian telomerase homolog called TP1 (telomerase-associated protein 1) was identified and cloned. TP1 exhibited extensive amino acid similarity to the *Tetrahymena* telomerase protein p80 and was shown to interact specifically with mammalian telomerase RNA. Antiserum to TP1 immunoprecipitated telomerase activity from cell extracts, suggesting that TP1 is associated with telomerase in vivo. The identification of TP1 suggests that telomerase-associated proteins are conserved from ciliates to humans.

Telomerase is an unusual RNA-dependent DNA polymerase that uses an RNA component to specify the addition of telomeric repeat sequences to chromosome ends (*1*). In humans the telomeric repeat is 5′-TTAGGG-3′, and the telomerase RNA contains a sequence complementary to this telomeric repeat (*2, 3*). The telomerase RNA template is required for telomere repeat synthesis in vitro and in vivo (*4–6*). Telomerase activity is differentially regulated in normal and immortalized cells. In germline cells telomeres are maintained, whereas several somatic tissues lack telomerase activity and undergo progressive telomere shortening with increasing age (*1*). In immortalized cells telomere length is stabilized and telomerase activity is often reactivated (*7–9*). Telomerase activity has also been detected in many cancers (*1, 9*).

Telomerase activities have been identified in several organisms, and their RNA components have been cloned from mouse, human, yeast, and several ciliates (*1*). Putative telomerase or telomere-associated proteins have been identified in yeast (*10*). The ribonucleoprotein complex responsible for telomerase activity, however, has been purified only in ciliates (*11–13*). Purified *Tetrahymena* telomerase contains an RNA and two protein components, p80 and p95 (*4, 13*). The p80 component can be specifically cross-linked to

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