complex than simple reciprocal regulation, since in vivo studies indicate that SS alone has predominantly inhibitory actions on pyramidal neurons but is excitatory in the presence of ACh (*13*). This unusual interaction could result from the closing by ACh of the same M-channels opened by SS; in the presence of SS, more M-channels are open for ACh to act upon (perhaps at lower ACh concentrations). Our data (Fig. 2) suggest that ACh will then predominate over the SS effect. However, more direct studies are needed to determine if the other effects of ACh, for example, the voltage-independent depolarizations (*5*) and reduction of Ca^{2+} currents (*6, 23*), are involved in the ACh-SS interaction.

Several other inhibitory agonists (opioid peptides, α-adrenergic agonists, serotonin, baclofen, and adenosine) may also activate voltage-dependent K^+ (inward-rectifying) channels, perhaps through a common mechanism involving a G-protein (*24*). Our studies (*25*) suggest that SS can also open inward-rectifying K^+ channels in hippocampus. Some of the other agonists could also augment the M-current, although our preliminary data on baclofen, serotonin, and adenosine suggest otherwise. In terms of function the M-current will act to clamp the membrane potential at rest (*8, 18*), thus braking regenerative phenomena such as spike bursts. Somatostatin should accentuate this function. Thus, the selective SS-ACh interaction could be involved in epilepsy or in the well-known role of the hippocampus and other cortical areas in behaviors such as memory and learning. The pronounced cortical loss of these two transmitters in dementia of the Alzheimer's type (*26*) further broadens such implications.

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16. Transverse hippocampal slices (from male Sprague-Dawley rats of 100 to 170 g in body weight) 350 to 400 μm thick were cut on a brain slicer, incubated (*15*) and placed in gassed (95% O_2 and 5% CO_2) ACSF that had the following composition, in millimoles per liter: NaCl, 130; KCl, 3.5; NaH_2PO_4, 1.25; $MgSO_4 \cdot 7H_2O$, 1.5; $CaCl_2$, 2.0; $NaHCO_3$, 24; and glucose, 10. Other ions and agents were added to this ACSF medium. The slices were completely submerged and continuously superfused with warm (28° to 37°C) ACSF at a constant rate (2.0 to 4.0 ml/min). Most of the voltage-clamp studies were done at 28° to 29°C to slow M-current kinetics; four cells studied at 35° to 37°C showed the same SS augmentation of the M-current as at lower temperatures. Glass micropipettes filled with potassium chloride (3*M*; tip resistances of 60 to 80 megohms) were used to penetrate CA1 pyramidal neurons. Methods of perfusion, current-clamp recording, cell identification, drug administration, and data analysis were as described (*9, 15*). In the single electrode voltage-clamp mode (*17*), the switching frequency between current injection and voltage sampling was 3 to 4 kHz, and electrode "settling time" was monitored continuously from the headstage input on a separate oscilloscope. For measurement of the instantaneous portion of the M-current with hyperpolarizing commands, the resulting current relaxation was extrapolated back to zero time; this method is valid because of the logarithmic nature of the M-current relaxation (*8, 18*). The various problems (for example, space-clamping) associated with voltage-clamping of neurons with extended processes are discussed elsewhere (*8, 17, 19*). However, these problems are less acute when dealing with relative, qualitative changes after drug or peptide application (*5*), as in the present study.
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A General Method for the Chromosomal Amplification of Genes in Yeast

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The yeast retrotransposon Ty can be used to insert multiple copies of a gene at new sites in the genome. The gene of interest is inserted into a *GAL1*-Ty fusion construct; the entire "amplification cassette" is then introduced into yeast on a high copy number plasmid vector. Transposition of the Ty element carrying the gene occurs at multiple sites in the genome. Two genes, a bacterial neomycin phosphotransferase gene and the yeast *TRP1* gene, were amplified in this way. Although the amplified genes were about 1 kilobase in length, they were amplified to about the same extent as a 40–base pair segment. The benefit of this "shotgun" approach is that amplification can be achieved in one set of manipulations.

YEAST GENOMES TYPICALLY CONTAIN 30 to 40 copies of a transposable element, Ty (*1*). These transposable elements consist of a central region containing two long open reading frames and is flanked by two δ sequences (*2*). New copies of the transposon arise by a replicative transposition process in which the Ty transcript is converted to a progeny DNA molecule by a Ty-encoded reverse transcriptase (*3*). The

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complementary DNA can transpose to many sites in host DNA. The mechanism for limiting Ty copy number is not entirely clear, although deletion of Ty elements via their homologous δ sequences (*4*) is probably a major mechanism.

We have described the transposition of a Ty element, TyH3, which had been modified in vitro (*3*). Its δ promoter sequence was replaced with the yeast *GAL1* promoter, resulting in a *GAL1*-TyH3 fusion. A high copy number plasmid vector carrying the fusion (pGTyH3) directs the synthesis of TyH3 RNA when yeast transformants are grown in galactose-containing (inducing) medium (referred to as transposition induction). Subsequently, marker DNA segments were inserted into a nonessential site within the TyH3 sequence. Marker sequences transpose along with TyH3 sequences in transformants bearing marked pGTyH3 plasmids.

Because transposition induction results in the accumulation of large numbers of virus-like particles (Ty-VLPs), containing Ty RNA and Ty-encoded reverse transcriptase, the Ty-VLP is probably a transposition intermediate [Ty-VLP preparations contain full-length linear Ty DNA (*5*)]. We wanted to determine whether entire genes could be inserted into Ty elements and thus transposed to new sites in the genome and, if so, whether such genes would be expressed. Such constructs might be incapable of transposition if the inserted gene interfered with Ty RNA packaging, a process likely to be essential for transposition. Moreover, any termination signals would have to be removed from the inserted gene segment; otherwise the 3′ δ sequences normally present in the Ty transcript and essential for transposition (*6*) would not be transcribed.

Two constructs of this type have been tested for transposition, one containing the bacterial neomycin phosphotransferase gene (*neo*) of 1 kb, the other containing yeast *TRP1*, of about 700 bp. In both constructs, the gene of interest was inserted into the Bgl II site at position 5561 of the TyH3 sequence (*2*). The two plasmids, pGTyH3-*neo* and pGTyH3-*TRP1*, are shown in Fig. 1. The 3′ end of the *TRP1* gene had been removed with BAL-31 nuclease prior to insertion into the pGTy plasmid to prevent transcription termination within *TRP1*.

Both recombinant constructions and control constructions were transformed into yeast to assay transposition. The transformants were grown for 5 days at 22°C on SC-ura–2% galactose plates to induce transposition of the marked Ty elements. In pGTyH3-*neo*, transposition of the Ty-*neo* cassette into host chromosomes was assayed as follows. Colonies from plates containing galactose were chosen at random and purified on SC-ura–2% glucose plates at 30°C to stop transposition and to select cells retaining the plasmid. The plasmids were allowed to segregate by growth on rich media. Plasmid-free colonies were replica-plated to dishes containing G418 (500 μg/ml), a drug to which the parent strain is sensitive but to which cells containing the *neo* gene (whose product inactivates G418) are resistant (*7*). The fraction of the plasmid-free (Ura^-) colonies that became resistant to G418 is shown in Table 1. The presence of Ty-*neo* cassettes in genomic DNA was confirmed by Southern blotting (see below). Only constructs bearing the *neo* gene at position 5561 can transpose. Galactose induction is required for transposition; glucose-grown cells did not produce G418-resistant derivatives. The position of insertion of the *neo* fragment is also critical; insertion in either orientation in two sites lying within coding regions did not result in transposition. A 1.7-kb Pvu II fragment containing the same *neo* gene (derived from bacterial transposon Tn903) inserted at position 5561 was unable to transpose. That this construct fails to transpose is apparently not due to a termination signal within this segment, as transcription runs through it (*8*), but probably results from the presence of long inverted repeats (from Tn903) flanking the *neo* gene, which would form a long hairpin in the Ty-*neo* RNA. Its 1.7-kb size is unlikely to exceed a packaging limit because we and others have inserted much larger fragments into Ty without preventing its transposition (*9, 10*). We have also inserted bacterial miniplasmid πan7 (885 bp) into pGTyH3 without disrupting Ty transposition (*5*).

Southern analysis of G418-resistant derivatives showed that such strains contain from one to seven copies of Ty-*neo* cassette in genomic DNA (mean = 2.6). As expected, the sizes of fragments hybridizing to a *neo* probe vary in different strains when enzymes generating "junction fragments" (fragments containing *neo*, of TyH3, and variable amounts of flanking DNA) are used to cut genomic DNA.

We have obtained similar results with pGTyH3-*TRP1* constructs. Transposition of the Ty-*TRP1* cassette was assayed in strains mutant at *TRP1*. When the *TRP1* gene was inserted in either orientation in the pGTyH3 plasmid, the Ty-*TRP1* cassette could be mobilized to multiple genomic sites; *TRP1* was expressed because cells that had been grown on galactose-containing medium and subsequently lost the pGTyH3-*TRP1* plasmid were Trp^+. Cells containing one copy of the Ty-*TRP1* cassette often were only weakly Trp^+, whereas those with additional copies were more like wild type. The presence of Ty-*TRP1* cassettes in genomic DNA was confirmed by Southern blotting. Under the transposition-inducing conditions indicated, the number

Table 1. Insertion and transposition of the *neo* gene in pGTyH3.

Structure of plasmid		Carbon source	G418-resistant derivatives
Insertion site*	Orientation†		
2 (3301)	+	GAL	0/12
3 (5561)	+	GAL	10/12
3 (5561)	+	GLU	0/11
2 (3301)	−	GAL	0/12
1 (1702)	−	GAL	0/12

*See Fig. 1 for location of sites. The numbers in parentheses are the positions of the insertions. †Plus (+), direction of transcription the same as that of Ty; minus (−), direction of transcription opposite that of Ty.

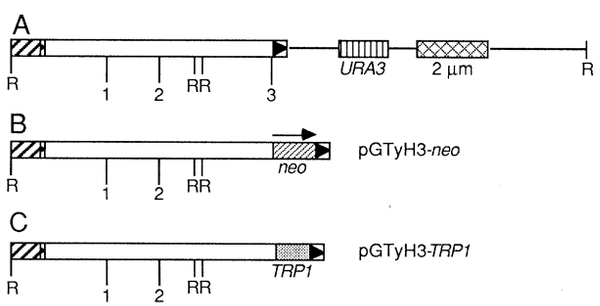

Fig. 1. Derivatives of plasmid pGTyH3 carrying *neo* and *TRP1* genes. (**A**) Plasmid pGTyH3 (*3*) was partially digested with Bgl II (the Bgl II sites are labeled 1, 2, 3); the partial digest was then ligated to either (**B**) the Bam HI fragment containing the *neo* gene from plasmid pGH54 (*13*), resulting in pGTyH3-*neo* or (**C**) the Bam HI fragment containing the *TRP1* coding region derived from pJEF981 [see (*14*) for details of the construction], resulting in pGTyH3-*TRP1*. R, Eco RI sites. The arrow shows the direction of transcription of *neo*. *URA3*, yeast *URA3* gene; 2 μm, yeast 2-μm plasmid sequence (*3*).

Table 2. Transposition rates of various Ty-marker cassettes.

Marker inserted	Length (in bp)	Copies per genome*
neo	950	2.6 (47/18)
TRP1	773	2.3 (77/34)
lacO	40	3.2 (218/68)

*Measured after 5 days at 22°C on SC-ura–2% galactose; mean number of bands hybridizing to an appropriate probe on a genomic Southern blot. The fraction in parentheses is the total number of bands counted divided by the total number of randomly selected colonies analyzed.

of copies of Ty-*TRP1* cassettes per genome ranged from 1 to 10 or more, averaging 2.3.

The number of copies of Ty-*neo*, Ty-*TRP1*, and Ty-*lacO* cassettes incorporated per genome is not very different, although the number of nucleotides inserted into the Ty in these constructs is very different (Table 2). Presumably, the packaging limit for Ty RNA is considerably larger than 7 kb. Inserts as large as 2.3 to 2.7 kb have been successfully transposed (*9, 10*). The size limit to DNA that can "hitchhike" on Ty elements remains to be determined.

These findings have many potential applications. (i) They provide a convenient phenotypic assay for transposition that does not rely on Southern blotting, allowing many colonies to be screened for genetic transposition defects in both the transposon-carrying plasmid and in the host, particularly when combined with 5-fluoro-orotate selection, which selects against the *URA3* marker in the plasmid (*11*). (ii) This approach may be useful in screening for pharmacological agents that interfere with transposition. (iii) Plasmid pGTyH3-*neo* (and similar plasmids) have been used as transposon tags in cloning yeast genes (*12*). (iv) These pGTy plasmids should be useful in constructing yeast strains bearing multiple dispersed copies of useful genes. Vectors specifically designed for such applications are being developed; a pGTyH3 vector carrying an *ARG3* promoter–*Escherichia coli galK* cassette supported its transposition (*10*). Quantitation of galactokinase activity (performed in *gal1*Δ strains) suggests that these cassettes are expressed well; four different strains suffering single insertions of a TyH3-*ARG3-galK* cassette produced 12,400, 12,300, 23,800, and 20,400 units of galactokinase per milligram of protein; a control cassette (not imbedded in a Ty) on a centromeric plasmid produced 22,200 units per milligram of protein (*10*). (v) Amplification of useful genes with pGTy plasmids may be more desirable than with high copy number plasmids, particularly in large-scale fermentations where instability can cause problems. Preliminary studies indicate that strains containing 20 or more copies of marked chromosomal Ty elements are stable for dozens of generations by Southern blot pattern (*8*); mitotic loss of inserted sequences by δ-δ recombination or gene conversion events does not invalidate this approach. Genes may need special engineering to allow maximal expression within the confines of a Ty element, although *neo* and *TRP1* are expressed after transposition. Their expression could presumably be increased further by fusion to a strong promoter prior to its insertion into the pGTy plasmid.

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Long-Term Facilitation in *Aplysia* Involves Increase in Transmitter Release

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In a variety of vertebrates and invertebrates, long-lasting enhancement of synaptic transmission contributes to the storage of memory lasting one or more days. However, it has not been demonstrated directly whether this increase in synaptic transmission is caused by an enhancement of transmitter release or an increase in the sensitivity of the postsynaptic receptors. These possibilities can be distinguished by a quantal analysis in which the size of the miniature excitatory postsynaptic potential released spontaneously from the presynaptic terminal is used as a reference. By means of microcultures, in which single sensory and motor neurons of *Aplysia* were plated together, miniature excitatory postsynaptic potentials attributable to the spontaneous release of single transmitter quanta from individual presynaptic neurons were recorded and used to analyze long-term facilitation induced by repeated applications of 5-hydroxytryptamine. The results indicate that the facilitation is caused by an increase in the number of transmitter quanta released by the presynaptic neuron.

LONG-LASTING SENSITIZATION OF the gill withdrawal reflex in *Aplysia* involves a corresponding enhancement of the synaptic transmission between siphon sensory and gill motor neurons (*1, 2*). This long-term facilitation of synaptic transmission could be achieved in two ways. (i) The properties of the postsynaptic cell could change so that it could respond more effectively to the same amount of transmitter. Such changes could involve an increase in the affinity or density of the receptors for the transmitter or an increase in the input resistance of the postsynaptic neuron. (ii) The presynaptic cell could be altered to release more transmitter. This could be accomplished by increasing the amount of transmitter released from a constant number of synaptic contact points or by forming, as a result of growth, new release sites between the pre- and postsynaptic neurons. To distinguish between these two possibilities, we have studied the synapses between siphon sensory and gill motor neurons in *Aplysia*. These neurons are involved in mediating the gill withdrawal reflex, and long-lasting facilitation of their synaptic connections contributes to long-term behavioral sensitization, an elementary form of long-term memory (*1, 2*).

To determine whether the change in synaptic strength at this connection involves an increase in transmitter release, we have carried out a quantal analysis. Such an analysis

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