input produced local postsynaptic changes in the target neuron that increased efficiency of VL input heterosynaptically (*18*).

Our results indicate that associative LTP is induced only in neurons in the superficial layers, which contain only noncorticofugal neurons, many of which are local interneurons. These neurons receive nonspecific input from the VL (*19*) but specific input from the SCx (*20*). This result suggests that associative LTP is produced only in intracortical neurons, which then modulate the activity of a small group of cortical efferent neurons.

One possible role of associative LTP in motor learning may be the following: In the early stages of learning a motor skill, a set of movements is practiced slowly, with heavy reliance on sensory feedback. After repeated practice, the movement becomes faster and smoother, and eventually the sensory feedback becomes unnecessary. At this stage, removal of the SCx does not impair the learned motor skills (*8*). It is possible that the function of CC LTP is to facilitate learning and retention of motor skills and the latter function is transferred to VL LTP as the learning proceeds.

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7. In the previous experiment (*6*), eight cells that had membrane potentials of 50 mV or more were used for this examination. The recording periods lasted from 6 to 40 min, and the latencies of VL-induced EPSPs were 2.2 ms or less. Of these cells, three were located in layer III (by micromanipulator reading), but the actual depths of the remaining five cells were unknown. Tetanic stimulation of the VL (200 Hz, 20 s) did not produce any significant change in the amplitudes of VL-induced EPSPs in six cells, but in two cells it produced short-lasting (less than 1 min) increase or decrease (less than 20%) of the EPSP amplitudes. At 2 to 3 min after the tetanic stimulation, the amplitude of VL-induced EPSPs of these eight cells was 95.13 ± 1.57% (mean ± SEM) of the control amplitude.
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11. Glass microelectrodes were advanced through the MCx while the SCx was being stimulated (30 μA, 0.2 ms, 1 Hz) through all the SCx electrodes. Whenever a neuron responded to SCx stimulation with a short-latency EPSP, the electrode responsible for the EPSP was determined. Then the VL was stimulated (100 μA, 0.2 ms, 1Hz) to determine whether the same cortical neuron received short-latency EPSPs from a single electrode in the VL.
12. We estimated the membrane resistances by measuring the difference in the amplifier's bridge balance inside and outside the cells. Brief pulses were occasionally passed during the recordings to determine if there was a change in membrane resistance induced by the tetanic stimulation; in no instance was a change in resistance observed.
13. When the recording was stable, 3-min control was sufficient because further observation (up to 20 min) showed that the amplitude of the EPSPs did not change from the initial amplitude.
14. The intensity of each tetanic pulse was identical to that used to evoke control EPSPs. The interval between each pair of SCx and VL stimuli, during the tetanic stimulation, was set so that the peak of the VL-evoked EPSP was preceded by the peak of the SCx-evoked EPSP by 1 to 2 ms. Because the potentiating effects of tetanic stimulation of the SCx are known to be long-lasting (*6*), each stimulating site in the SCx and VL was used for tetanic stimulation only once in each experiment.
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The Mechanism of DNA Transfer in the Mating System of an Archaebacterium

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The genetic transfer system in the extremely halophilic archaebacterium *Halobacterium volcanii* is the only archaebacterial mating system known. The mechanism of genetic transfer of this archaebacterium was studied by using the immobile plasmids pHV2 and pHV11 as cytoplasmic markers. It was found that the cytoplasms of the parental types do not mix during the mating process, that each parental type can serve both as a donor and as a recipient, and that cytoplasmic bridges, with dimensions of up to 2 micrometers long and 0.1 micrometer in diameter, were formed between the parental types. These bridges appear to be used for the transfer of DNA from one cell to another. If so, this archaebacterial mating system is different from both eubacterial conjugation and eukaryotic sexual cell fusion.

FINDINGS IN COMPARATIVE TAXONOMY led to the division of prokaryotes into distinct urkingdoms, that of the eubacteria and that of the archaebacteria (*1*). This division was based on a comparison of T1 catalogs of 16*S* and 23*S* ribosomal RNAs, characteristic features of the cell envelope, membrane lipids, and the DNA-dependent RNA polymerases. A similar comparison between archaebacteria and eukaryotes appears to indicate several common characteristics. For example, as in yeast, some archaebacteria contain introns in transfer RNA genes (*2*), and the RNA polymerase of archaebacteria shows a considerable immunological cross-reactivity to the RNA polymerase of yeast (*3*). Most of the archaebacteria grow in unusual ecological niches: methanogens are strictly anaerobic, the sulfur-metabolizing archaebacteria are thermoacidophilic, and the halobacteria grow only in concentrated salt solutions.

The archaebacterial genome resembles the eubacterial genome in its size and complexity (1×10^3 to 3×10^3 kb) (*4*). However, the lack of a well-developed genetic analysis system has restricted functional studies of the archaebacterial DNA. The extremely halophilic archaebacteria of the genus *Halobacterium* are probably the best model system for the study of the genome organization in archaebacteria. These bacteria are easy to grow and maintain, and auxotrophic mutants are readily obtainable.

The existence of a natural genetic transfer system in *Halobacterium volcanii* has been demonstrated (*5*). This transfer system has the following characteristics: it requires physical contact between the two parental cells, the two parental cells have to be alive, and no distinction can be made between donor cells and recipient cells. This system is the only genetic transfer system known so far for archaebacteria.

Eukaryotic organisms and eubacteria use different basic strategies for cell contact–dependent DNA transfer. In all eukaryotes, two haploid cells fuse to form one diploid cell. In eubacteria, cell contact–dependent DNA transfer (conjugation) involves pore formation between juxtaposed donor and recipient cells (*6*). We examined the basic strategy of cell contact–dependent DNA transfer in archaebacteria.

Cytoplasmic markers are used to distinguish between the two DNA transfer mech-

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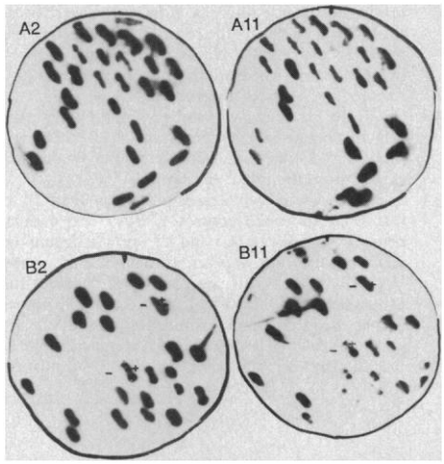

Fig. 1. Colony hybridization analysis of the presence of pHV2 (A2 and B2) and pHV11 (A11 and B11) in 96 recombinant colonies generated by crossing WR358 and WR344 (*13*). The location of the parental type controls is indicated by (+) for the positive control WR358 and by (−) for the negative control WR344. Colonies that hybridize strongly with pHV2 and faintly with pHV11 were further tested for the presence of pHV11 by plasmid preparations. In all cases, the colonies contained both pHV2 and pHV11. *Halobacterium volcanii* strains were crossed by a previously described protocol (*5*), with minor modifications. Cells were grown to absorbance of 0.8 to 1.2 (measured at 550 nm) in complete medium; then 0.5 ml of each parental type was mixed and filtered through a nitrocellulose filter (diameter 25 mm, BA85, Schleicher & Schuell, Keene, New Hampshire). The filters were placed on complete medium agar plates and incubated for 18 to 24 hours at 42°C. For segregation, the filters were removed and placed in 5 ml of complete medium, and the cultures were suspended by vigorous shaking. The suspended cultures were then grown for 24 to 36 hours in a shaker at 37°C. Before being plated for selection, the cells were washed twice with SMT (3.5*M* NaCl, 0.15*M* $MgSO_4$, and 10 m*M* tris-HCl, *p*H 7.2). Recombinants were recovered after 8 days of incubation. Both parental types were tested for reversion in the same way as the recombinants.

anisms. If cells fuse before recombination, all the recombinants have the cytoplasmic markers of both parents. If, however, the chromosomes are transferred by conjugation, half of the recombinants have the markers of one parent and half have the markers of the other parent. When plasmids are used as cytoplasmic markers, the results of the crossing experiment can be conclusive only if the plasmids are immobile and the bacteria use a conjugation-like mechanism. If all the recombinants are found to contain the plasmids (apparently proving the fusion mechanism), it could be argued that the plasmids are conjugative and move with the chromosome through the conjugation "bridge."

The strains WR335 (*7*) [containing the plasmids pHV2 and pHV11 (*8*)] and WFD11 [which does not contain these plasmids (*9*)] were mutagenized, and auxotrophic strains were isolated. The auxotrophic strains WR358 (containing the two plasmids and requiring serine for growth) and WR344 (requiring purines for growth) were crossed and recombinants were recovered, at a normal frequency (*10*), on minimal selective plates. When 96 recombinants were analyzed for the presence of pHV2 and pHV11 by in situ colony hybridization, only 50 of the recombinants contained both plasmids pHV2 and pHV11; the other 46 recombinants did not contain either of these plasmids (Fig. 1). Similar results were found when other strains were used for the same type of experiment [WR359 (containing the two plasmids and requiring methionine for growth) crossed with WR338 (requiring arginine for growth)].

These results demonstrate that the plasmids pHV2 and pHV11 are not mobilized by the DNA transfer system and therefore are suitable cytoplasmic markers; that cell fusion is not involved in the DNA transfer, and therefore only chromosomal genetic information moves from one cell to the other; and that both parental types can serve as donor and as recipient. In 50% of the cases the cells that contained the two plasmids were the recipients and in 50% of the cases the cells that did not contain any plasmid were the recipients.

The natural DNA transfer system of *H. volcanii* is most efficient when the cells are mated on solid support (*5*). In the original description of this archaebacterium, Mullakhanbhai and Larsen (*11*) showed that cells that grow on solid support appear to be connected to each other by intercellular bridges. These bridges maintain cytoplasmic continuity between the two connected cells and are made of the regular *H. volcanii* membrane and cellular envelope. They interpreted this phenomenon as a failure in the completion of cell division. If so, we should not find more than one bridge connecting any two cells. However, if these bridges are made de novo, more than one bridge might be formed between adjacent cells. Scanning electron microscopy (SEM) showed that many pairs of cells are connected by more than one bridge (Fig. 2, A and B). Moreover, many *H. volcanii* cells form a network of intercellular bridges (Fig. 2C). These results suggest that solid support or contact between cells induces differentiation of the unicellular *H. volcanii* cells into the network form.

We further predicted that if *H. volcanii* cells grown on solid support are connected by continuous cytoplasmic bridges, destabilization of the bridges would cause cells to fuse. Cell fusion could be monitored by using the plasmids pHV2 and pHV11 as cytoplasmic markers, as in the first experiment. If cell fusion occurred, all the recombinants would contain the two plasmids. The cytoplasmic bridges were destabilized by lowering the Mg^{2+} concentration to remove the cell envelopes (*12*).

Two auxotrophic mutants, WR358, which contains the plasmids pHV2 and pHV11, and WR344, which does not contain them, were crossed on two nitrocellulose filters. The culture on the filter that was used as a control was suspended and plated

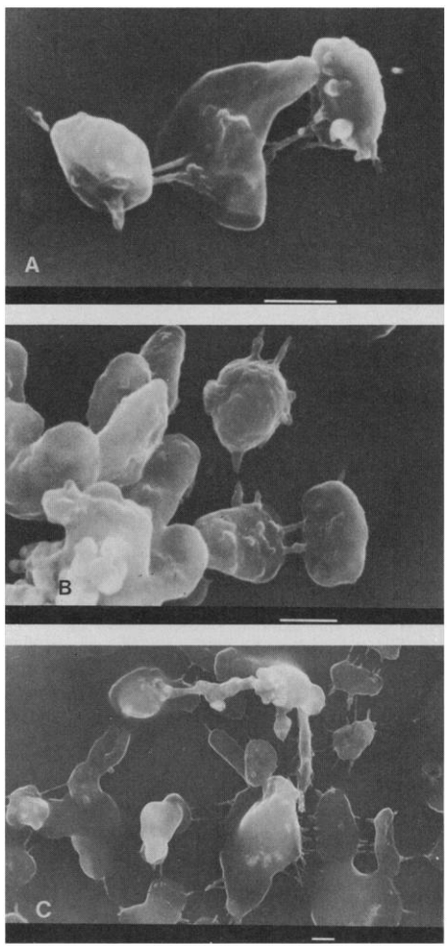

Fig. 2. Scanning electron microscopy of the intercellular bridges. (**A** and **B**) Pairs of cells that are connected by more than one bridge. (**C**) A network of cells conncted by intercellular bridges. Scale bar in (A), (B), and (C), 1 μm. *Halobacterium volcanii* cells grown on solid support (agar plates) were scraped from the plates and were suspended in SMT; 100 μl of suspension was mounted on cover slips coated with polylysine (*14*). After 1 hour of incubation at room temperature, excess cells were removed and replaced with 100 μl of 2% glutaraldehyde in SMT. The preparations were incubated for 1 hour at room temperature and then washed with water. The fixed cells were dehydrated through a gradient series of ethanol-water and ethanol-Freon TF and coated with carbon. A scanning electron microscope (JEOL-840; JEOL, U.S.A., Peabody, Massachusetts) operating at 30 kV was used for examining the cells.

for recombinant selection on minimal plates, as in the first experiment. The envelopes of the cells on the other filter were removed before suspending the cells and plating for selection on minimal medium. A 100-fold increase in recombinant recovery was observed when cell envelopes were removed before plating. All 24 of the recombinant colonies recovered from the second filter contained both pHV2 and pHV11, as shown by colony hybridization (Fig. 3). Of the 24 recombinant colonies recovered from the control filter, 16 contained both plasmids and 8 did not contain any of them. This result shows that (i) removal of the envelopes of cells grown on solid support allowed the cells to fuse, probably as a result of destabilization of the structure of the cytoplasmic bridges; (ii) cells originating from WR344 were fused to cells originating from WR358, suggesting that cytoplasmic connections were formed de novo between cells originating from the two strains, and (iii) the increase in recombination frequency due to cell fusion shows that the bridging frequency is higher than the DNA transfer frequency. The same conclusion could be made by comparing the bridging frequency in the SEM images to the efficiency of the genetic transfer.

From the data described above, the following model for the mechanism of DNA transfer in *H. volcanii* is proposed: cytoplasmic bridges are formed between the cells. In this process the membranes and envelopes of the cells fuse to form cytoplasmic continuity between the two cells. The dimensions of these bridges are up to 2 μm long and 0.1 μm in diameter. The cells are not fully fused and their cytoplasms do not mix, as is the case in the sexual fusion of eukaryotic cells. The cytoplasmic continuity between *H. volcanii* cells seems to be restricted to the movement of certain molecules. Although chromosomal DNA moves through the bridges, the plasmids pHV2 and pHV11 do not move through the bridges.

The *H. volcanii* mating system has a functional resemblance to the eubacterial conjugation system in that cytoplasmic markers cannot be transferred in the process. However, the cytoplasmic bridges are a distinctive property of the halobacterial system.

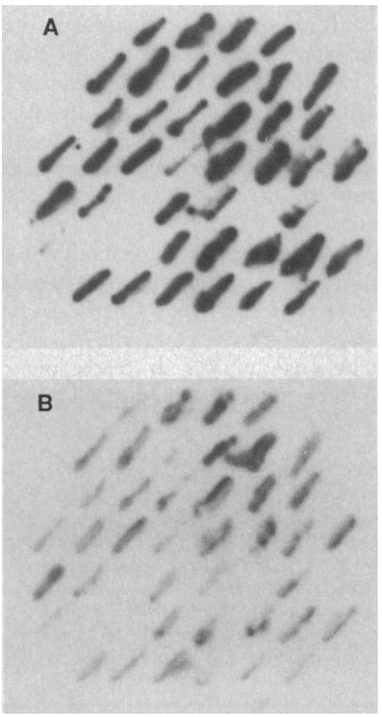

Fig. 3. The presence of (**A**) pHV2 and (**B**) pHV11 in 48 recombinant colonies generated by crossing WR358 with WR344 was examined by colony hybridization (*13*). Crosses were performed as described in the legend of Fig. 1 on two separate filters. The culture on one of the filters was immersed in 1*M* NaCl for 30 min before the cells were suspended and plated. This treatment caused the cell envelopes to dissociate and the cells to become spheroplasts (*8*). The upper 24 colonies in (A) and (B) originated from this treated filter; the lower 24 colonies in (A) and (B) serving as a control originated from the untreated filter.

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14. The cover slips were coated by immersing them for 1 hour in 1% polylysine (BioMakor, Rehovat, Israel) in water.

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Type I and Type II $GABA_A$-Benzodiazepine Receptors Produced in Transfected Cells

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$GABA_A$ (γ-aminobutyric acid A)-benzodiazepine receptors expressed in mammalian cells and assembled from one of three different α subunit variants (α_1, α_2, or α_3) in combination with a β_1 and a γ_2 subunit display the pharmacological properties of either type I or type II receptor subtypes. These receptors contain high-affinity binding sites for benzodiazepines. However, CL 218 872, 2-oxoquazepam, and methyl β-carboline-3-carboxylate (β-CCM) show a temperature-modulated selectivity for α_1 subunit–containing receptors. There were no significant differences in the binding of clonazepam, diazepam, Ro 15-1788, or dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) to all three recombinant receptors. Receptors containing the α_3 subunit show greater GABA potentiation of benzodiazepine binding than receptors containing the α_1 or α_2 subunit, indicating that there are subtypes within the type II class. Thus, diversity in benzodiazepine pharmacology is generated by heterogeneity of the α subunit of the $GABA_A$ receptor.

BENZODIAZEPINES POTENTIATE THE activity of the GABA-gated chloride channel intrinsic to $GABA_A$ receptors (*1*). Although most benzodiazepines

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