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7. Fish were anesthetized with MS-222 (67 to 200 mg/l) during surgery. Data were collected within 1 to 4 days of cannula implantation.
8. The fiberoptic endoscope (0.7 mm in diameter, 0.5 m long, Instrument Technology, Inc.) was attached to a charge-coupled device camera (Sony XC-37) and a 0.5-inch VHS recorder (Panasonic AG-1950). Videotaped images were digitized (24-bit color) with the use of a RasterOps FrameGrabber 324 connected to an Apple Macintosh IIcx. Digitized images were Wiener filtered to reduce noise and blur and then contrast stretched to improve overall visibility. The thermistor flow probe (1.09 mm in diameter) was heated by a feedback logarithmic amplifier [modified from that in M. LaBarbera and S. Vogel, *Limnol. Oceanogr.* **21**, 750 (1976)], and the output was sampled at 100 Hz by a microcomputer-controlled A/D convertor (GW Instruments MacADIOS 411). The probe was calibrated from 0 to 147 cm/s and temperature-compensated from 20° to 30°C. Experiments were conducted at 24° to 25°C.
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20. We thank Instrument Technology, Inc., for loan of the endoscope and D. Reeves for technical advice regarding its use; H. Fischer and L. Matteson for preparing Fig. 1; J. Brandt and V. R. Algazi, Center for Image Processing and Integrated Computing, University of California, Davis, for help with image processing of Fig. 2; and A. Cheer, R. Wassersug, K. Liem, W. Hoogenboezem, D. Rueb, V. Chow, D. Erickson, D. Castleberry, M. Choi, T. Hopkins, and P. Young for advice and assistance. Supported by a National Science Foundation Postdoctoral Research Fellowship in Environmental Biology to S.L.S. (BSR-8800190).

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A Molecular Genetic Classification of Zooxanthellae and the Evolution of Animal-Algal Symbioses

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Zooxanthellae are unicellular algae that occur as endosymbionts in many hundreds of marine invertebrate species. Because zooxanthellae have traditionally been difficult to classify, little is known about the natural history of these symbioses. Zooxanthellae were isolated from 131 individuals in 22 host taxa and characterized by the use of restriction fragment length polymorphisms (RFLPs) in nuclear genes that encode small ribosomal subunit RNA (ssRNA). Six algal RFLPs, distributed host species specifically, were detected. Individual hosts contained one algal RFLP. Zooxanthella phylogenetic relationships were estimated from 22 algal ssRNA sequences—one from each host species. Closely related algae were found in dissimilar hosts, suggesting that animal and algal lineages have maintained a flexible evolutionary relation with each other.

ANIMAL-ALGAL SYMBIOSES ARE UBIQuitous and typically dominant features of shallow tropical seas. Of the several kinds of unicellular algal participants (*1*), the most abundant are coccoid, yellow-brown dinoflagellates that are known as zooxanthellae (*2, 3*). Photosynthetic production by these symbioses, especially the reef-building corals, is largely responsible for the life and growth of tropical reef communities (*4*). Zooxanthella hosts include various invertebrate taxa, but zooxanthella diversity (*3, 5–7*) is far less obvious and has been difficult to evaluate. Most zooxanthellae are referred to the genus *Symbiodinium* Freudenthal (*3, 8*), which presently includes four species described as in vitro cultures and many cryptic forms (*8, 9*). *Symbiodinium* taxonomy has been hindered by a paucity of informative morphology, especially in the vegetative (symbiotic) state (*10*), by the possibility of host-associated phenotypic plasticity (*5*), by difficulties in obtaining in vitro cultures (*5*), and by the absence of sex, which precludes formal genetic analyses (*3*). The poor status of *Symbiodinium* taxonomy has limited our understanding of symbiosis ecology and evolution. We classified 22 zooxanthella isolates using the polymerase chain reaction (PCR) (*11*) to amplify small ribosomal subunit RNA (ssRNA) genes from *Symbiodinium* (*12*), and ssRNA sequence data.

Symbiodinium-like zooxanthellae were isolated from hosts (Table 1) and their nuclear-encoded ssRNA genes were PCR-amplified with zooxanthella-specific primers as described (*12*). The amplified DNA, which corresponds to the ssRNA molecule less 103 nucleotides at either end (*12*), was first analyzed with restriction enzymes. Three common and three unique Taq I digestion patterns [restriction fragment length polymorphisms (RFLPs)] were observed (Fig. 1). Taq I RFLPs A and B are predicted by the ssRNA sequences from cultured *Symbiodinium* isolates (*13*), and all six RFLPs can be related to one another by a single Taq I site gain or loss (*14*). This implies that individual host samples contain only one of six detectable zooxanthella genotypes (*15*). Zooxanthellae obtained from different individuals of the same host species (*16*) always exhibited the same RFLP genotype (in a total of 129 algal isolates from 20 host taxa collected from nature) (Table 1). Since these genotypes apparently represent biologically distinct algae that associate specifically with one or more host species, algal taxonomy was investigated in greater detail.

Phylogenetic relationships among zooxanthellae were estimated from ssRNA gene sequences. Four cultured (*13*) and 18 fresh isolates were compared. PCR-amplified DNA was digested with Xba I and cloned into the vector M13mp18 (*17*), and two relatively variable regions (*13*) were sequenced, yielding a total of 472 to 476 nucleotide positions from each isolate. These sequences are obtained from a single Xba I fragment and should represent about one-half of the variability contained in entire *Symbiodinium* ssRNA molecules (*13*). Sequences were identical to one another or differed by up to 35 nucleotides in 83

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variable positions (Fig. 2). Differences are not distributed randomly among individual sequences, indicating that their origin is not in PCR artifact (*11, 18*). Taq I RFLPs A, B, and C are recognized by several apparent synapomorphies (underlined boldly in Fig. 2), five of which also associate two of the unique Taq I RFLPs with RFLP C–containing algae. There is an obvious consensus nucleotide at every variable position. Within each group, sequences that differed from the consensus at three or fewer positions were scored as indistinguishable from one another, to allow for methodological imprecision (*18*), leaving ten distinct taxa that were grouped by genetic distance. The estimated phylogeny (Fig. 3) contains the three common RFLP groups (group C includes the unique RFLPs D1 and D2) and a single taxon corresponding to one unique Taq I RFLP.

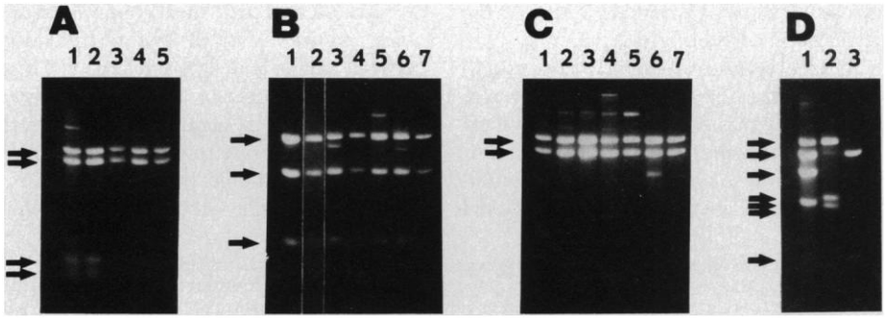

Fig. 1. Taq I RFLP analysis of ssRNA-encoding DNA from different *Symbiodinium* isolates. Common RFLPs designated A, B, and C are in panel (A), (B), and (C), respectively; (D) shows three distinct RFLPs. The algae were isolated from the following hosts: (**A**) lane 1, *Cassiopeia xamachana* (A1); lane 2, *Zoanthus sociatus* (A2); lane 3, *Stoichactis helianthus* (A3); lane 4, *Acropora palmata* (A4); lane 5, *Acropora cervicornis* (A5); (**B**) lane 1, *Aiptasia pulchella* (B1); lane 2, *Aiptasia pallida* (B2); lane 3, *Anthopleura elegantissima* (B3); lane 4, *Plexaura* A (B4); lane 5, *Astrangia danae* (B5); lane 6, *Favia fragum* (B6); lane 7, *Madracis mirabilis* (B7); (**C**) lane 1, *Zoanthus pacificus* (C1); lane 2, *Palythoa tuberculosa* (C2); lane 3, *Phymanthus crucifer* (C3); lane 4, *Porites compressa* (C4); lane 5, *Pavona duerdeni* (C5); lane 6, *Cyphastrea ocellina* (C6); lane 7, *Montipora verrucosa* (C7); (**D**) lane 1, *Pocillopora damicornis* (D1); lane 2, *Palythoa vestitus* (D2); and lane 3, *Montipora patula* (D3). Portions of individual amplifications in Taq polymerase buffer (50 mM KCl, 1.5 mM $MgCl_2$, and 10 mM tris-HCl, pH 8.3, at 25°C) were adjusted to contain 10 mM $MgCl_2$ and digested with the restriction endonuclease Taq I at 65°C. Digests were separated by electrophoresis through a 3.5% NuSieve/1% SeaKem agarose (FMC BioProducts) gel that was stained with ethidium bromide. Restriction fragments that contribute to the scored RFLPs are indicated by arrows.

Speculations on zooxanthella taxonomy have ranged from the idea of a single pandemic zooxanthella species, *Symbiodinium (Gymnodinium) microadriaticum* (*2, 19*), to the concept of one (cryptic) zooxanthella taxon for every host taxon. Our data suggest an intermediate hypothesis (*3*), with ten putative algal taxa distributed among 22 hosts. Ten taxa is a minimum estimate because closely related species might have similar or identical ssRNA sequences (*20*). For example, the cultured zooxanthellae from *Zoanthus sociatus (Symbiodinium pilosum)* and from *Cassiopeia xamachana (S. microadriaticum)* were scored as indistinguishable, but they are different organisms (*9*). The conclusion that the ten distinguishable isolates are distinct taxa is a corollary to this caveat and is supported by other comparative studies (*20*). The ssRNA sequences from free-living dinoflagellates (*13*) root the zooxanthella tree (Fig. 3) on the branch that connects group A algae with all other isolates, and indicate that group A algae are as closely related to some free-living species as they are to other zooxanthellae. We conclude that ssRNA sequences, like other data (*3, 5–7*), demonstrate considerable variability within the genus *Symbiodinium*.

Table 1. Cnidarians from which zooxanthellae were isolated. Specimens of *Aiptasia pulchella* and *Aiptasia pallida* were from laboratory cultures; all other specimens were collected from nature at the location given. Numbers in parentheses indicate the number of individual symbioses of each host type that were analyzed for zooxanthella RFLPs (Fig. 1).

Host organism	Geographic origin
Class Scyphozoa: Order Rhizostomeae:	
Cassiopeia xamachana (2)	St. Croix, U.S.V.I.
Class Anthozoa: Order Actiniaria:	
Aiptasia pulchella (1)	Oahu, HI
Aiptasia pallida (1)	Bermuda
Anthopleura elegantissima (30)	Pacific Grove, CA
Phymanthus crucifer (6)	St. Croix, U.S.V.I.
Stoichactis helianthus (6)	St. Croix, U.S.V.I.
Class Anthozoa: Order Alcyonaria:	
Plexaura A (4)	Bahama Islands
Class Anthozoa: Order Zoanthiniaria:	
Zoanthus sociatus (5)	St. Croix, U.S.V.I.
Zoanthus pacificus (5)	Oahu, HI
Palythoa tuberculosa (5)	Oahu, HI
Palythoa vestitus (3)	Oahu, HI
Class Anthozoa: Order Scleractinia:	
Acropora cervicornis (5)	St. Croix, U.S.V.I.
Acropora palmata (5)	St. Croix, U.S.V.I.
Astrangia danae (11)	Woods Hole, MA
Cyphastrea ocellina (5)	Oahu, HI
Favia fragum (5)	St. Croix, U.S.V.I.
Madracis mirabilis (4)	St. Croix, U.S.V.I.
Montipora patula (6)	Oahu, HI
Montipora verrucosa (8)	Oahu, HI
Pavona duerdeni (4)	Oahu, HI
Pocillopora damicornis (5)	Oahu, HI
Porites compressa (5)	Oahu, HI

In the symbioses that we examined, the zooxanthella taxonomy is largely independent of host taxonomic status (Fig. 3). Indistinguishable algae were repeatedly isolated from hosts of ordinal or greater dissimilarity: anemones, corals, and a gorgonian (group B algae); corals, a zoanthid, and an anemone (group C algae); corals, a zoanthid, an anemone, and a jellyfish (group A algae). Of these hosts, the fossil record of the scleractinian corals provides a superior phylogenetic taxonomy (*21*). Corals in the same family [namely *Madracis mirabilis* and *Pocillopora damicornis* (Pocilloporidae); *Favia fragum* and *Cyphastrea ocellina* (Faviidae); and *Montipora verrucosa, M. patula*, and *Acropora* (Acroporidae)] have relatively dissimilar zooxanthellae, whereas similar algae were isolated from corals in different families (*Astrangia* and *Madracis; Porites, Pavona*, and *Cyphastrea*). Corals in the same genus have dissimilar (*Montipora*) or indistinguishable (*Acropora*) algae. These conclusions refer to algal taxa that are defined by ssRNA RFLPs (Fig. 1) (*22*). RFLP data are especially informative because host-species specificity of algal genotypes was documented with these characters. Overall, the lack of correlation between host similarity and zooxanthella similarity in our sample is

too complete to be explained by errors in the classification of either group.

These observations—similar zooxanthellae with dissimilar hosts and possibly vice versa—imply that symbioses evolve by shuffling (recombining) algal and animal lineages. A contrasting hypothesis, that symbiosis evolution occurs by the concerted diversification of permanently associated lineages, predicts a much better correlation between animal and algal similarities. These two scenarios are not mutually exclusive however, and because our data represent so few taxa (with a bias toward higher ranks), both processes should be considered. Cnidarian-zooxanthella symbioses have probably existed since the Jurassic period or earlier (*23*), so extant associations may have long and complex histories. Molecular data on *Nostoc*, cyanobacterial partners of lichen symbioses, suggest that *Nostoc* symbioses are also taxonomically flexible (*24*).

Symbiosis recombination is not without precedent, having been achieved in the laboratory by allowing different zooxanthella strains to infect animals that had been rendered nonsymbiotic by experimental methods (*3, 6*). Hosts that are briefly nonsymbiotic in nature, such as planula larvae that do not inherit their parent's algae (*3*) and adults affected in bleaching epidemics (*25*), might provide a natural opportunity for symbioses to recombine. Since animal and zooxanthella metabolisms probably interact to their mutual benefit (*4*), mechanisms for recombining hosts and symbionts should be favored in the same way that genomic recombination is favored (*26*). Many Cnidarian life histories are dominated by clonal phases that should facilitate the propagation of new combinations (*27*). This could occur along geographical or ecological, as well as temporal, scales. Our samples from Hawaii and St. Croix included no apparent recombinants (all individuals of a given host taxon exhibited the same zooxanthella ssRNA RFLP). Since distinct zooxanthellae co-occur in these environments, we conclude that these symbioses do not recombine promiscuously.

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A1: T ACAAACAAAAAGCCG  ACTCTTCTTGGACACATTAGCAGTCTTGTCGTCTTCA  CAGCATCTGCTTAGACTATTCAGCT (0)
A2: T ACAAACAAAATGCTG  ACTCTTCTTGGACACATTAGCAGTCTTGTCGTCTTTA  CAGCATCTGCTTAGACTATTCAGCT (3)
A3: T ACAAACAAAAAGCCG  ACTCTTCTTGGACACATTAGCAGTCTTGTCGTCTTCA  CAGCATCTGCTTAGACTATTCAGCT (0)
A4: T ACAAACAAAAAGCCG  ACTCTTCTTGGACACATTAGCAGTCTTGTCGTCTTCA  CAGCATCTGCTTAGACTATTCAGCT (0)
A5: T ACAAACAAAAAGCCG  ACTCTTCTTGGACACATTAGCAGTCTTGTTGTCTTCA  CAGCATCTGCTTAGACTATTCAGCT (1)

B1: T ATCGGTGCAAACCCGCAATCCTCCCTTGACACGATATCAGTCTTGTCGTCCATA  TGGCATGCGCATGGACTCACTAGCT (1)
B2: T ATCGGTGCAAACCCGCAATCCTCCCTAGACACGATATCAGTCTTGTCGTCCTTA  TGGCATGCGCATGGACTCACTAGCT (1)
B3: T ATCGGCGCGAACCCGCAATCCTTCCTTGACACGATATCAGTCTTGTCGTCCTAT  TGGCATGCGTATGGCCTGACTAGCT (8)
B4: T ATCGGTGCAAACCCGCAATCCTCCCTTGACACGATATCAGTCTTGTCGTCCTTA  TGGCATGCACATGGACTCACTAGCT (1)
B5: T ATCGGTGCAAACCCGCAATCCTCCCTTGACACGATATCAGTCTTGTCGTCCATA  TGGCATGCGCATGGACTCACTAGCT (1)
B6: T ATCGGTGCAAACCCGAAATTCTCCCTTGACACGATATCAGTCTCGTCATTCTTA  TGGTATGCGCATGGACTCACTAACT (7)
B7: T ATCGGTGCAAACCCGCAATCCTCCCTTGACACGATATCAGTCTTGTCGTCCTTA  TGGCATGCGCATGGACTCACTAGCT (0)

C1: T ATCGGCGAATACTCACAATCCTTCCTTGACACGATATCAGTCTTTTCGTCCTCA  CGGCGTGCGCTTGGACTTGCTAGCT (2)
C2: T ATCGGCGAATACTCACAATCCTTCCTTGACACGATATTAGTCGTGTTGTCCTCA  CGACATGCGCTTGGACTTGCTAGTT (5)
C3: A ATCGGCGAATACTCACAATCCTTCCTTGACTCGATATCAGTCTTGTCGTCCTCA  CGGCATGCGCTTGGACTTGCTAGTT (3)
C4: T ATCGGCGAATACTCACAATCCTTCCTTGACACGATATCAG_CTTGTCGTCCTCA  CGGCATGCGCTTGGACTTGCTAGCT (1)
C5: TTATCGGCGAATACTCACAATCCTTCCTTAACACGATATCAGTCTTGTCGTCCTCA  CGGCATGCGCTTGGACTTGCTAGCT (2)
C6: T ATCGGCGAATACTCACAATCCTTCCTTGACACGATATCGGTCTTGTCGTCCTCA  CGGCATGCGCTTGGACTTGCTAGCT (1)
C7: T TTCGGTGAATACTCACAATCCTCCCTTGACACAATATCAGTTTTGTCGTCCTCA  CGGCAAGTGCTAGGACTTGCTGGCT (9)
D1: T ATCGGTGAATACTCACAATCCTTCCTTGATACGATATCAATCTTGTCGCTCTCA  CGGCATGCGCTTGGACTTGCTAGCG (6)
D2: T ATCGGTGAATACTCACAGTCTTTCCTTGACACGACGTCAGTCTTGTCGTCCTCATGCGGCATGCGCTTGGATTTGCTAGCT (7)

D3: T ATCGGTGAAAACCCGCAACCCCCTTCCGGCATGATATCAGTCTTGCCGTCCTCA  CGGCATGTGCTTGAACCCACTAGCT
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Fig. 2. Compilation of the nucleotide substitutions in *Symbiodinium* ssRNA sequences. We aligned 22 sequences and removed the invariant positions, that is, each row represents one sequence and each column represents one variable position. A1 to A5, B1 to B7, C1 to C7, and D1 to D3 indicate from which host the algae were isolated, as given in Fig. 1. Four sequences (A1, A2, B1, B2) are from cultured algae (*13*); the ssRNA RFLPs of these cultured algae and of algae freshly reisolated from their host species of origin were indistinguishable (*12*). All other sequences were obtained from freshly isolated algae. Differences from the consensus at each position are underlined. Shared substitutions (synapomorphies) that substantiate RFLP groupings A, B, and C (Fig. 1) are underlined boldly; these place two of the unique RFLPs (D1 and D2) with algae that exhibit RFLP C. Individual sequences differ from the group (A, B, or C + D1 + D2) consensus at the indicated number of positions (numbers in parentheses at the end of each row).

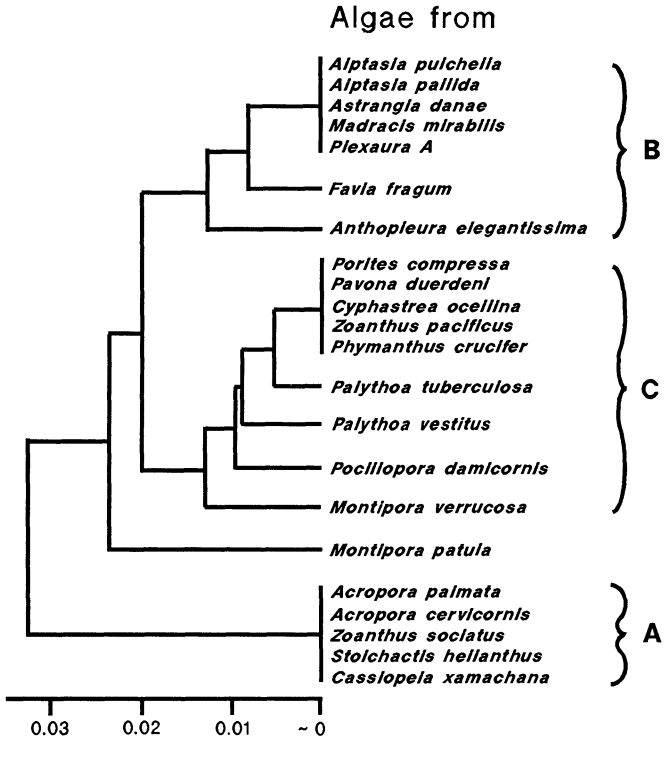

Fig. 3. Phylogenetic relationships of zooxanthellae isolated from different host species. Genetic distances were estimated from ssRNA sequences (472 to 476 nucleotides in length) according to Jukes and Cantor (*28*) using the program DNADIST (*29*), and the unrooted tree was produced from the distance matrix using the program KITSCH (*29*). Distances (the estimated average fraction of nucleotides substituted) are given by the scale. Zooxanthella groups A, B, and C correspond to RFLPs A, B, and C (Fig. 1), except group C also includes RFLPs D1 and D2.

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14. For group C algae, and for the algae from *Pocillopora damicornis* and from *Montipora patula*, the predicted Taq I site gains or losses were verified from the ssRNA sequences that are summarized in Fig. 2.
15. *Pocillopora damicornis* (Fig. 1D, lane 1) and also *P. meandrina* (*12*) are exceptional in appearing to con-

tain two zooxanthella RFLPs: the group C RFLP plus a unique RFLP generated by one nucleotide substitution that creates a Taq I site in the larger of the two RFLP C fragments (*14*). These two RFLPs either represent two distinct algae or a polymorphism within the multicopy ssRNA genes of one alga. Some other digests contain extra fragments that may identify additional ssRNA sequences. These represent small PCR amplification products that cannot be complete ssRNA genes (for example, Fig. 1C, lanes 1 and 6), or they are not interpretable from the available data; where those products occur, they occur in all individuals of that host species.
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30. R.R. thanks L. Brezinsky, C. Kanechika, and T. Humphrys for hospitality and laboratory space on Oahu, and colleagues at the West Indies Laboratory and the National Undersea Research Center on St. Croix for their help in collecting specimens. M. A. Coffroth supplied *Plexaura* A samples. L. Park and C. Patton got the PHYLIP programs running. Supported by an NSF Postdoctoral Fellowship in Marine Biotechnology (R.R.) and by a contract from the National Undersea Research Center, National Oceanic and Atmospheric Administration.

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Recombinase-Mediated Gene Activation and Site-Specific Integration in Mammalian Cells

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A binary system for gene activation and site-specific integration, based on the conditional recombination of transfected sequences mediated by the FLP recombinase from yeast, was implemented in mammalian cells. In several cell lines, FLP rapidly and precisely recombined copies of its specific target sequence to activate an otherwise silent β-galactosidase reporter gene. Clones of marked cells were generated by excisional recombination within a chromosomally integrated copy of the silent reporter. By the reverse reaction, integration of transfected DNA was targeted to a specific chromosomal site. The results suggest that FLP could be used to mosaically activate or inactivate transgenes for analysis of vertebrate development, and to efficiently integrate transfected DNA at predetermined chromosomal locations.

RECENT ANALYSES OF MAMMALIAN development have made use of transfected genes to alter cell interactions and trace cell lineages. This inherently powerful approach could be applied to investigate a broader range of developmental processes if it was possible to restrict transgene expression to specific subsets of the cells, tissues, or developmental stages in which the cis-acting sequences that typically control expression are active. Such mosaic expression is essential for many forms of lineage analyses and would additionally provide a means to assess the effects of transgenes that grossly alter development in small patches of tissue within an otherwise normal embryo. Toward this end, we have characterized a conditional recombination system based on the site-specific recombinase, termed FLP (*1*), from *Saccharomyces cerevisiae*. In this system, gene activation requires prior FLP-mediated excisional recombination, and expression therefore falls under the binary control of the transgene's own cis-acting sequences and those that direct FLP expression. Reversal of this excisional recombination, under different experimental conditions, provides a means for introducing DNA into specific sites in mammalian chromosomes.

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A cotransfection assay was used to characterize FLP-mediated recombination of extrachromosomal DNA in a variety of cell lines. Cells were transfected with an expression construct and a "reporter" plasmid that was a substrate for the recombinase. The activity of the expression construct was assayed either by recovering the transfected reporter and looking for molecular evidence of recombination or by preparing cytoplasmic extracts to measure β-galactosidase activity generated by precisely recombined reporter molecules.

The pNEOβGAL reporter plasmid used in these assays was derived from pFRTβGAL (Fig. 1A). pFRTβGAL contains the bacterial β-galactosidase coding sequence, which has been modified by insertion of an FLP recombination target site (FRT) immediately 3′ to the translational start (*2*). The FRT consisted of the two inverted 13–base pair (bp) repeats and 8-bp spacer that comprise the minimal FLP target (*3, 4*) plus an additional 13-bp repeat that may augment reactivity of the minimal sub-

Table 1. β-Galactosidase activities in cotransfection assays of 293, CV-1, and F9 cells. Positive control transfections (pFRTβGAL) included 1 μg of pFRTβGAL and 18 μg of the pOG28 (*6*) non-FLP control plasmid. Negative control transfections (pNEOβGAL) included 1 μg of pNEOβGAL and 18 μg of pOG28. Experimental transfections (pNEOβGAL + FLP) contained 1 μg of pNEOβGAL and 18 μg of the pOG44 FLP expression plasmid (Fig. 1A). The pNEOβGAL + FLP values are also shown as a percentage of the pFRTβGAL positive control values. Each value represents the mean and SEM of six plates from two experiments. Neither pOG28 nor pOG44 generated β-galactosidase activity when transfected alone (*5*). Transfections and assays were performed as described in the legend to Fig. 1. All transfections contained 1 μg of pRSVL to correct β-galactosidase activities for relative transfection efficiencies.

Cell line	β-Galactosidase activity (units/mg protein)			
	pFRTβGAL	pNEOβGAL	pNEOβGAL + FLP	$\frac{\text{pNEOβGAL + FLP}}{\text{pFRTβGAL}}$ (%)
293	30.4 ± 1.9	0.17 ± 0.02	14.2 ± 2.2	47
CV-1	275 ± 25	0.33 ± 0.06	22.6 ± 1.2	8
F9	24.8 ± 4.3	0.04 ± 0.01	1.88 ± 0.02	8