powered drill (designed by the Polar Ice Coring Office, Lincoln, Nebraska) used on Quelccaya in 1983 to obtain two cores, one to bedrock. We are indebted to numerous scientists, engineers, and technicians from Electroperú (Lima and Huaraz offices) who provided both scientific and logistical assistance essential to the success of this 8-year effort. Logistical support was also provided by the Inter American Geodetic Survey (U.S. Defense Mapping Agency) office in Lima, and we especially thank W. Berk. Over 8 years many people have participated in the annual field programs, and their collective efforts are gratefully acknowledged. We thank J. Bolzan and P. D. Kruss for reviewing the manuscript and R. Tope for the illustrations. Contribution No. 509 of the Institute of Polar Studies, Ohio State University.

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Temperature Effects on the Rate of Ty Transposition

Abstract. *An assay has been developed to measure the rate of transposition of the transposable element Ty in* Saccharomyces cerevisiae. *The assay is based on the altered expression of the glucose-repressible alcohol dehydrogenase gene of yeast upon insertion of a Ty in front of this gene. By this assay the transposition rate of Ty elements was found to increase approximately 100-fold at temperatures lower than 30°C, the optimum growth temperature for* Saccharomyces cerevisiae.

Ty elements comprise a family of homologous transposable DNA sequences present in about 30 copies in most laboratory yeast strains (*1, 2*). These sequences are about 5.9 kilobases (kb) long with direct repeats of 330 base pairs called δ sequences at each end. Their structure has many features in common with the *Drosophila* transposable element *copia* and with retroviruses (*1, 2*). Attempts to measure transposition frequencies with the use of marked elements have not been successful because of the difficulty of distinguishing transposition events from gene conversion of endogenous Ty elements (*3*). To avoid this difficulty, we used a method for detecting transposition of Ty elements based on the ability of Ty elements to alter the expression of adjacent genes (*1, 2*) and, in particular, the alcohol dehydrogenase 2 (*ADH2*) gene. *Saccharomyces cerevisiae* has three ADH isozymes: ADHI, the fermentative isozyme; ADHII, an isozyme repressible by glucose; and ADHIII, an isozyme associated with mitochondria (*4*). Medium containing antimycin A can be used to select for cells with ADH activity because it eliminates respiration, thus requiring cells to use their fermentative pathway and therefore ADH activity to grow (*4*). Mutants that express ADHII constitutively because of mutations at *ADH2* (the ADHII structural gene) or at regulatory loci for *ADH2* have been selected from a strain lacking ADHI by their ability to grow on medium containing glucose and antimycin A (*4*). Seven of the nine ADHII constitutive mutants selected that were linked to *ADH2* were shown to carry Ty insertions upstream from this gene (*5*).

By determining the rate at which antimycin A–resistant mutations occur and the percentage of such mutations which are the result of the insertion of a Ty element adjacent to *ADH2*, the rate at which Ty elements transpose into this region was estimated. A potential problem in the determination of mutation rates in the ADH system is that cells expressing ADH on glucose-containing medium have a shorter doubling time than cells that do not have ADH activity (2 hours and 4 hours per doubling at 30°C, respectively). To circumvent this problem we used the P_0 method, which bases mutation-rate calculations on the proportion of cultures with no mutations (*6*) (see legend to Table 1). Even though use of this method leads to larger variations in mutation rate than estimates by other methods, it eliminates biases due to growth rate differences of mutants.

Mutation rates to antimycin A resistance were determined in several independent experiments for cultures of a strain carrying a deletion in *ADH1*, the structural gene for ADHI (*7*), grown at 15°, 20°, 30° and 37°C. A total of 83 independent antimycin A–resistant mutants was examined. DNA was isolated from each of these mutants, and transfers (Southern) of genomic DNA cut with Bam HI were examined after hy-

Table 1. Determination of mutation rates with the use of strain 315-1D (*adh1–Δ1 ADH2 trp1 his4*). Construction of a yeast strain carrying a deletion of *ADH1* (mutation *adh1–Δ1*) has been described (*7*). Each line in the table represents an independent experiment. For each estimate of the mutation rate to antimycin A resistance, 16 to 18 cultures were grown for six to eight generations at the experimental temperatures, and a small portion of cells from each culture was plated on YEPD medium (yeast extract, peptone, and dextrose) to determine the total number of cells per culture. The remaining cells were plated on YEPD medium containing glucose and antimycin A and incubated at 30°C (*4*). Colonies were counted after growth at 30° for 5 days. Mutation rates are reported as the number of mutations per cell per generation ± the 95 percent confidence interval (*6*). The increase in transposition rate at low temperatures was not due to a burst of Ty transposition when the cells are first placed at low temperatures because only a small number of mutants per culture was seen (the median number of mutants per culture is between 0 and 7 for all experiments). The cells were plated at approximately 10^6 cells per milliliter for cultures grown at 20° and 15°C and at approximately 10^7 cells per milliliter for cultures grown at 30° and 37°C. For 315-1D cells grown at 37°C the estimates of the number of cells per milliliter from hemocytometer counts was ten times higher than estimates from the number of cells that grew on YEPD plates, indicating that only 10 percent of the cells were viable. Hemocytometer counts were only about 10 percent higher than viable counts for 315-1D grown at the other temperatures and for three independent mutants carrying Ty insertions adjacent to *ADH2* grown at 37°, 30°, and 15°C. The detection of Ty insertions is described in the text. DNA was isolated as described (*17*). Transposition rates are reported as the number of transpositions per cell per generation. These transposition rates are minimum estimates because only one mutant from each culture was tested by Southern blot analysis (*8*) for insertion of a Ty element to ensure that all the mutants were independent. The mean number of mutations per culture was less than 3 for all experiments so that sampling errors should not be a major problem. Also, mutants carrying insertions at *ADH2* appeared to grow as fast (or faster than) as most other antimycin A–resistant mutants. Reconstruction experiments in which three mutants carrying Ty insertions adjacent to *ADH2* and strain 315-1D were grown at 37°, 30°, and 15°C and then mixed at appropriate densities (10 to 100 cells per plate for mutants carrying Ty insertions adjacent to *ADH2* and 10^7 to 10^8 cells per plate for 315-1D) showed nearly 100 percent plating efficiency for all three Ty mutants grown at all three temperatures.

Temperature (°C)	Mutation rate to antimycin A resistance*	Mutants with Ty insertions		Transposition rate†
		Fraction	Percent	
15	$(1.1 \pm 1.0) \times 10^{-7}$	5/12	42	2.4×10^{-8}
20	$(1.6 \pm 1.5) \times 10^{-7}$	5/18	28	1.7×10^{-8}
30	$(6.6 \pm 7.4) \times 10^{-9}$	0/7	3	$<7.4 \times 10^{-10}$
	$(6.8 \pm 12.4) \times 10^{-9}$	0/5		$<9.3 \times 10^{-10}$
	$(4.9 \pm 5.4) \times 10^{-9}$	0/11		$<2.7 \times 10^{-10}$
	$(4.7 \pm 3.0) \times 10^{-9}$	1/12		2.1×10^{-10}
37	$(2.4 \pm 3.6) \times 10^{-7}$	0/2	0	$<1.2 \times 10^{-7}$
	$(1.7 \pm 1.2) \times 10^{-7}$	0/16		$<4.9 \times 10^{-9}$

*Mutation rates were estimated by the P_0 method ($P_0 = e^{-m}$, where P_0 is the number of cultures without mutants and m is the mean number of mutations per culture; m is then divided by the mean number of cells per culture to give the mutation rate) (*6*). †Transposition rates were calculated whereby P_0 is equal to the number of cultures without antimycin A–resistant mutations plus the number of cultures in which the mutant analyzed did not contain a Ty insertion as P_0.

bridization with an *ADH2*–containing probe [see (*5*)]. In 10 of the 30 mutants examined from cultures grown at 15° or 20°C, the size of the Bam HI fragment containing *ADH2* increased from approximately 7.2 kb to 13 kb, suggesting that an insertion about the size of Ty (5.8 kb) occurred. In an 11th mutant, obtained from cultures grown at 30°C, the size of the Bam HI fragment containing *ADH2* increased to 9.5 kb and a new Bam HI fragment of about 3.5 kb appeared, indicating that an insert about the size of Ty (5.8 kb) containing a Bam HI site had occurred. This probably indicates the insertion of a Ty2 element, a subclass of Ty elements that contains a Bam HI site (*1, 2*). The other 72 mutants showed no alterations in the size of the Bam HI fragment containing *ADH2*. Six mutants with alterations in the band containing *ADH2* were studied further by Southern blot analysis (*8*) and shown to carry insertions within a 1-kb region upstream from *ADH2*, the same region where five Ty insertions were previously mapped (*5*). Restriction maps of the six insertions were consistent with restriction maps of known Ty elements (Fig. 1). Polymorphisms among the restriction patterns obtained indicated that all six Ty sequences analyzed were different. Southern blot analysis of four of these mutants with the use of an internal Ty fragment as a probe indicated that all four had an extra band when compared to the parent strain at the correct location for a new Ty next to *ADH2* (*9*). Thus all 11 mutants presumably carried a Ty element adjacent to *ADH2*.

The mutation rates to antimycin A resistance and information obtained from Southern blot analysis were used to calculate transposition rates (Table 1). The mutation rate was not by itself a sufficient indicator of the transposition rate (Table 1). The mutation rate at 30°C, the optimum growth temperature for yeast, was about 50 times lower than the mutation rate at 15°, 20°, and 37°C, but the percentage of mutations due to insertion of a Ty element decreased as temperature increased. Although the mutation rate at 37°C was high, none of the 18 mutants examined were due to Ty insertions near *ADH2* even though reconstruction experiments showed that mutants carrying a Ty adjacent to *ADH2* were viable and plated efficiently after growth at 37°C (see legend to Table 1). The fraction of Ty insertions at 15°C (42 percent) was high considering that the antimycin A–resistant mutations selected by this technique would include any mutations in unlinked genes that act to regulate *ADH2* expression (*4*). Only one transposition event to the *ADH2* locus was detected among the 35 antimycin A–resistant mutants isolated from cultures grown at 30°C, and this was the only Ty2 transposition event detected. The transposition rates were reproducible in a number of independent experiments (see Table 1), showing that this assay can provide valuable information on eukaryotic transposition rates. Our data clearly show that the transposition rate to this locus is about 100 times higher at 15° and 20° than at 30°C.

Several factors prevented us from using the rates we obtained to estimate the rates of transposition of Ty elements throughout the yeast genome. One factor

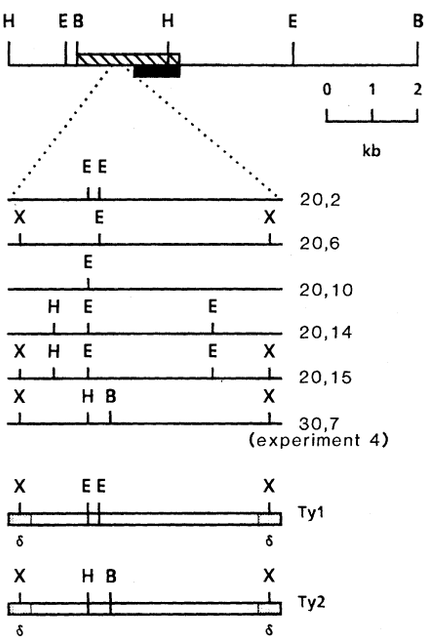

Fig. 1. Restriction maps of genomic DNA surrounding the wild-type *ADH2* gene and six different Ty elements inserted upstream from the *ADH2* structural gene. The six Ty elements are identified by the temperature (°C) and number of the culture from which they were isolated, respectively, to the right of the map. The solid black line indicates the position of the *ADH2* coding sequence (*5*). The dotted lines indicate the region upstream from *ADH2* into which these elements were inserted. These restriction maps were derived from analysis of Southern blots of genomic DNA cut with various enzymes (*8*); thus the sizes of the fragments are approximate. Because the hybridization probe, indicated by cross-hatching, does not contain Ty sequences, sequences wholly internal to the Ty element would not be detected. Thus for enzymes having two cleavage sites within the Ty element it is possible that another site is present between the two shown. The restriction maps are consistent with the restriction maps of known Ty elements. The restriction pattern of a Ty1 element (*1, 2*) and a Ty2 element (*1, 2*), are shown for comparison. The restriction enzymes used were Bam HI (B), Eco RI (E), Hind III (H), and XhoI (X).

was evidence for some specificity in transposition target sites. For example, few cases exist in which a Ty element has inserted into the coding region of a gene, even though such mutations have been actively sought (*10, 11*). In addition, the rates we observed were limited to insertion events that produced constitutive expression of ADHII. All Ty insertion sites near *ADH2* that have been determined (*12*) were within 100 base pairs of each other, and all the Ty elements were inserted in the same orientation. This may reflect a site specificity for transposition, or it may indicate that insertion into this region and in this orientation is necessary for constitutive expression of ADHII. Transposition frequencies ranging from 10^{-7} to 10^{-9} have been estimated for insertion of Ty elements at the *His4* (*2*) and *Lys2* (*11*) loci as well as at a *His3* gene carried on a plasmid (*13*). These differences in transposition frequencies could involve strain differences, target differences, or differences in the physiological conditions of the cell.

Transposition of the bacterial transposon Tn3, insertion sequences IS1 and IS2, as well as the eukaryotic Ty element increases at low temperatures (*14*). The possibility, suggested for bacterial systems (*15*), that an enzyme acting in the Ty transposition process may be sensitive to temperature is intriguing. In addition, in *Drosophila* and in higher plants there is suggestive evidence that the frequency of mutations associated with transposable elements is affected by temperature (*16*). However, little is known about the mechanism of transposition of Ty elements or other eukaryotic transposable elements. Our finding that Ty tranposition is temperature-sensitive will greatly simplify the investigation of this phenomenon by providing conditions that increase both the rate at which transposition occurs and the percentage of observed mutations due to transposition events. For example, our finding that six different Ty elements transposed to a particular locus shows that many of the Ty elements in the yeast strain we are studying are active and capable of transposing to the *ADH2* locus. This result would have been difficult to obtain if all experiments were conducted at 30°C. The ADH system provides a powerful and perhaps unique method of investigating the eukaryotic transposition process.

CHARLOTTE E. PAQUIN
VALERIE M. WILLIAMSON
ARCO Plant Cell Research Institute, Dublin, California 94568

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Differential Development of Two Classes of Acetylcholine Receptors in *Xenopus* Muscle in Culture

Abstract. *Physiological properties of acetylcholine receptors on muscle cells at very early stages of ontogeny were compared with those of cells at later stages. Two changes were observed that contributed to an overall shortening of the mean open time of single-channels. First, there was a shift in the relative proportions of two receptor types with different conductances and mean open times, such that the contribution of receptors with large conductance and short open time increased as development proceeded. Second, there was a sharp reduction in the mean open time of channels having small conductance, with no similar change in channels having large conductance.*

During development the mean open time of nicotinic acetylcholine receptor (AChR) channels gradually shortens (*1–3*). In rat muscle this shortening is associated with innervation (*4*), but in cultured *Xenopus* muscle it can occur in the absence of innervation (*3*). Not much is known, however, about the mechanism underlying these changes. It has been suggested that replacement of receptors of one class (small conductance, long open time) by receptors of another class (large conductance, short open time) is responsible for the developmental changes in mean open time (*1, 3, 5*). There is indirect evidence suggesting that this conversion is due to a modification of the receptor molecules (*1*).

In this study we examined the single-channel properties of AChR's that had just emerged in the surface membrane during ontogeny. We found that the open times of these AChR's were initially long and then shortened as development proceeded in vitro. A new mechanism, in addition to the redistribution of receptor types, must be involved in the maturation of early AChR's. The mechanism decreases the mean open time of channels belonging to a single conductance class of AChR's.

We cultured dissociated muscle cells isolated from myotomes of *Xenopus* embryos. General procedures were similar to those previously described (*6*); however, we dissected the myotomes at earlier stages of development so that the cells stuck to the bottom of the dish and thus became amenable to physiological experiments before first insertion of AChR's into the surface membrane. Myotomes were dissected from *Xenopus* embryos (*7*) at stages 14 to 16, dissociated in calcium-free EGTA Steinberg solution, and plated in culture dishes containing 60 percent L-15 (Gibco) and 1 percent fetal bovine serum, with or without penicillin and streptomycin. Cultures were maintained at 18° to 24°C. Single-channel currents activated by 500 n*M* ACh were recorded by using the conventional patch clamp method (*8*) in the cell-attached mode. Experiments were performed in standard frog Ringer solution containing tetrodotoxin (see legend to Fig. 1) at 11° to 14°C.

We shall express the developmental stage of cultured cells in terms of the corresponding stage of intact embryos maintained at the same temperature as the cultured dishes (*9*). We do not claim that cultured cells develop in the same way as in intact embryos, but we opt for this procedure because of its convenience (*10*).

Acetylcholine receptors first emerge on muscle surface membrane at stage 20 in *Xenopus* embryos (*11*). Recently, Bridgman *et al.* (*9*) showed that, even in culture, the cells start to acquire ACh sensitivity at stage 20. At this early stage overall ACh sensitivity is low, probably reflecting a low density of AChR's, but gradually increases, reaching almost the plateau level at stage 31 (16 hours later at 23°C).

We recorded the single-channel currents of AChR's as early as possible [stages 21 to 24, 1 to 5 hours after stage

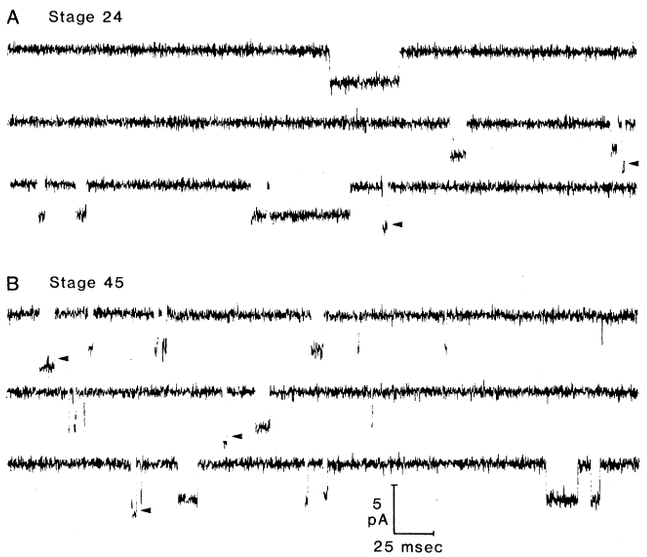

Fig. 1. Acetylcholine–induced single-channel currents recorded from two cells at stage 24 (A) and stage 45 (B). Channel openings produced inward currents, represented as downward deflections. Successive records are not continuous in time. Records at stage 24 were chosen to illustrate the existence of the two populations of conductance events. The frequency of large conductance openings (arrows) was very low at this early stage. Both cells were hyperpolarized from their resting potential by 50 mV. The bathing solution was composed of 115 m*M* NaCl, 2.5 m*M* KCl, 1.8 m*M* $CaCl_2$, 4 m*M* Hepes (*p*H 7.2), and tetrodotoxin (5×10^{-7} g/ml) (~13°C). The recording pipette contained 500 n*M* ACh dissolved in the bathing solution. Data were filtered to 2 kHz and digitized at 100-μsec intervals.