its adaptability to monitoring column effluents. The required instrumentation includes special prisms for the monochromator, modification of the monochromator and cell housings for nitrogen purging, and a set of thermospacers for the cell compartment connected to a source of refrigerant, all of which are relatively inexpensive. The increase in sensitivity at 187 nm over that at either 205 or 210 nm would seem to justify this modest outlay.

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L-Asparaginase-Deficient Mutants of Yeast

Abstract. *Yeast* L-*asparaginase is a multimeric enzyme for which only a single structural gene has been found. Fourteen mutants deficient in* L-*asparaginase have been isolated, and they have been located at one site on the genetic map of* Saccharomyces cerevisiae. *The* L-*asparaginase gene* (asp1) *is located about 18 centimorgans from a gene governing tryptophan synthesis* (trp4) *on fragment 2 of the map.*

The amino acid L-aspartate is a key intermediate in the synthesis of pyrimidines and of several other amino acids, and it functions as a link between the tricarboxylic acid cycle and amino acid metabolism. Many of the metabolic pathways involving L-asparate have been extensively studied in yeast. One exception is the interconversion between L-asparate and its amide, L-asparagine. In *Escherichia coli,* L-asparaginase [L-asparagine amidohydrolase (E.C. 3.5.1.-1)] catalyzes the conversion of L-asparagine to L-aspartate and ammonia (*1*). The existence of yeast L-asparaginase has been known for many years (*2*). We present here the results of part of our studies of the genes involved in the synthesis of L-asparaginase in yeast.

We wished to isolate L-asparaginase mutants by selecting cells that would not grow when L-asparagine was the only available source of L-aspartate. In yeast, however, two enzymes that generate endogenous L-aspartate can be present, L-aspartate aminotransferase [L-aspartate: 2-oxoglutarate aminotransferase (E.C. 2.6.1.1)], which synthesizes L-aspartate from L-glutamate (*3*), and L-asparaginase. Since both these enzymes can generate L-aspartate, active aminotransferase would be expected to interfere with the isolation of L-asparaginase mutants. Mutants have been isolated, however, in which the activity of the aminotransferase is reduced or absent (*3, 4*). In these mutants (denoted *asp5*) (*5*), growth occurs if L-aspartate is supplied exogenously. We found that *asp5* mutants can use exogenous L-asparagine in place of L-aspartate for growth. Therefore, by selecting mutants of *asp5* strains that are unable to utilize exogenous L-asparagine for growth, we have been able to isolate L-asparaginase-deficient cells. We used these mutants (denoted *asp1*) to determine how many genes are involved in the synthesis of L-asparaginase and to find where these genes are located on the yeast genetic map.

We also tried to isolate mutants that have an obligate requirement for L-asparagine. Such mutants, if deficient in L-asparagine synthetase, would not be able to grow on exogenous L-aspartate alone. We have found no stable mutants that exhibit this characteristic.

L-Asparaginase mutants were induced in heterothallic strains of *Saccharomyces cerevisiae* by treating haploid cells of strains X2902-21B (*α trp4 asp5*) and X2902-67B (*a trp4 asp5*) with 3 percent ethyl methanesulfonate (*6*). Treated cells were plated on enriched agar plates and allowed to grow at 30°C. After colonies had formed, they were replica plated onto minimal medium supplemented with 100 μg of L-asparagine monohydrate per milliliter and then onto minimal medium supplemented with the same amount of L-aspartic acid. The plates with minimal medium were incubated at 30°C for 1 day and then at room temperature (about 24°C) for several more days. The pairs of replicas were compared periodically, and those clones that grew on minimal medium plus L-aspartic acid but not on minimal medium plus L-asparagine were isolated, purified by selecting single colonies, and tested further. Fourteen stable mutants were found among 30,000 clones grown from treated cells.

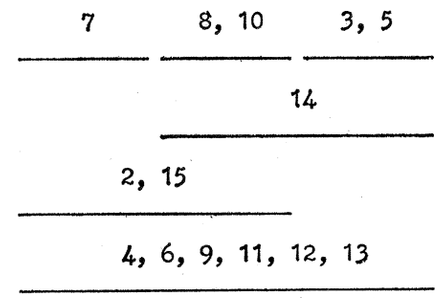

Fig. 1. Complementation map derived from pairwise crosses of 14 L-asparginase mutants of *S. cerevisiae.* Allele numbers of noncomplementing mutants appear on overlapping lines. Mutants that complement are represented by numbers on nonoverlapping lines.

The 14 mutants were crossed to *asp5* strains, the diploid cells were sporulated, and tetrad analysis was performed on the haploid spores formed during sporulation (*7*). In these crosses, the *asp5* mutation was homozygous so that the synthesis of L-aspartate from L-glutamate could not occur. We followed segregation of the inability to utilize L-asparagine by replica plating the haploid segregants onto selective media. All 14 mutations segregate in the 2:2 fashion expected of chromosomal genes

Table 1. Segregation of the *asp1* gene relative to *trp4.* The PD refers to parental ditype asci, NPD to nonparental ditype asci, and T to tetratype asci (*7*). From the totals, the distance between the *asp1* and *trp4* genes, *x*, can be calculated (*9*) from the equation $x = 50(T + 6NPD)/(PD + NPD + T)$.

Allele	Number of asci		
	PD	NPD	T
2	35	0	11
3	4	0	4
4	9	1	6
5	8	0	7
6	7	0	2
7	8	0	2
8	9	0	1
9	18	0	8
10	5	0	1
11	4	0	4
12	11	0	5
13	5	0	3
14	8	0	7
15	5	0	7
Totals	136	1	68

(*7*), and all are linked to a marker for tryptophan synthesis (*trp4*) that was carried incidentally in the crosses. The mutations are located about the same distance (averaging 18 centimorgans) from the *trp4* marker on fragment 2 of the *S. cerevisiae* genetic map (Table 1). We also followed the segregation of some of the *asp1* mutations with respect to *trp1*, a centromere-linked gene (*7*). These data indicate that the *asp1* gene is not significantly centromere linked. Therefore, *asp1* is probably not linked to *asp5*, which is located near the centromere of chromosome XII (*4*).

Complementation tests were performed with all pairwise combinations of the 14 mutations to determine whether they are all alleles of the same gene (*8*). From the genetic mapping crosses, we selected segregants that carried the original mutations in combination with the mating type alleles opposite to those of the strains in which the mutations were originally induced. Diploids from 196 pairwise matings were grown and replica plated onto the selective media. All diploids grew well with L-aspartic acid, whereas only certain ones grew with L-asparagine; the pattern of growth was typical of intragenic complementation (*8*) (Fig. 1). The complementation map contains six complementation classes, but only a single functional unit (cistron) is involved, because six of the alleles do not complement any of the others. The fact that complementation does occur indicates that, in *S. cerevisiae*, L-asparaginase is a multimeric enzyme (*8*); the genetic evidence further suggests that only one structural gene for L-asparaginase exists in haploid cells of *S. cerevisiae*.

We also determined some of the biochemical characteristics of yeast L-asparaginase. In crude extracts, the enzyme is stable for at least 2 months when frozen at −25°C and for at least 3 hours at 30°C. Activity decreases logarithmically with time of heating when the extract is held at 50°C and then assayed at 30°C. Enzyme in the crude extract appears to exhibit classical Michaelis-Menten kinetics, the apparent K_m for L-asparagine utilization being about $2.5 \times 10^{-4} M$.

All 14 of the *asp1* mutants have been tested for L-asparaginase activity in vitro and are deficient in the ability to convert L-asparagine into L-aspartate and ammonia. In the presence of unlimited substrate, L-asparaginase activity is usually greater than 5×10^{-2} μmole of ammonia evolved per minute per milligram of protein in extracts from wild-type cells. No activity was detected in extracts from 12 of the mutants, while in extracts of two of them (*asp1-8* and *asp1-10*), the activity was about 10 percent of that measured in the wild-type.

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Adenohypophysial Transmembrane Potentials: Polarity Reversal by Elevated External Potassium Ion Concentration

Abstract. *Incubation of rat adenohypophyses in potassium ion of sufficient concentration to provoke the release of several of the adenohypophysial trophic hormones produces a reversed, positive transmembrane potential in more than half the cells. This finding is consistent with a process of "stimulus-secretion coupling" in which hypothalamic releasing factors act by selective depolarization of their "target" cells. The positive potentials may be due to a prolonged preferential permeability to calcium ions triggered by an initial depolarization of the cell membrane to a threshold value by increased external potassium ion.*

In experiments on the adrenal medulla and neurohypophysis, Douglas (*1*) found that the sequence of events preceding hormone release closely resembles the sequence of events preceding contraction in skeletal muscle. They proposed a general theory of "stimulus-secretion coupling" whereby hormone release is initiated by a decrease in transmembrane potential followed by an influx of Ca^{2+}. As the theory predicts, the release of several adenohypophysial hormones, thyroid stimulating hormone, luteinizing hormone, and adrenocorticotropic hormone (*2*), is increased by exposing adenohypophyses to elevated potassium ion concentration in vitro. We investigated the transmembrane potential changes accompanying the augmented release of these hormones following exposure to elevated potassium ion concentration.

Adenohypophyses were obtained from young adult male Sprague-Dawley rats (Holtzman) which had been maintained in a constant environment for at least 2 weeks (*3*). The animals were killed by decapitation within 20 seconds after their removal from the cage. Adenohypophyses were quickly removed and transferred to a plexiglass bath through which was circulated either Krebs-Ringer bicarbonate alone (*4*) or a "modified" Krebs-Ringer bicarbonate containing 25 m*M* K^+ with sodium ion concentration appropriately reduced. Solutions were kept at 37°C and gassed with 95 percent O_2 and 5 percent CO_2 to maintain a *p*H of 7.2. Transmembrane potentials were measured with glass micropipettes containing 3*M* KCl. Their resistance was greater than 20 megohms, and the tip potential was insensitive to change in potassium ion concentration. The microelectrode was advanced stepwise along a series of penetration tracks with 10 μm increments that allowed 15 seconds between steps, and the resting potentials were recorded on film. Only those potential changes which were abrupt, which were maintained during the entire interval between advances, and which were greater in magnitude than the base-line shift in potential during the recording of a track were recorded.

Of 70 potentials recorded from 24 tracks in six adenohypophyses during perfusion with Krebs-Ringer bicarbonate (Figs. 1A, 2A, and Table 1), 14 were positive. There was no correlation between depth of penetration and