inhibition began to decrease. In the case of the pigmented first, albino second matings, however, the offspring were primarily albino (second mating) even when the stimulation came as late as an hour after the first mating. The long-lasting inhibitory effect in the pigmented first, albino second mating may have been the result of less hardy sperm being ejaculated by the highly inbred pigmented male (*7*). In a competitive situation, therefore, it appears a male can cancel the effects of a previous male's copulation if the second male begins intromitting soon enough.

We previously demonstrated that the copulatory intromissions were necessary to initiate both sperm transport and the hormonal conditions of pregnancy (pseudopregnancy) (*1*, *2*). We now suggest that copulatory behavior has a disruptive effect on pregnancy if the intromissions occur too soon after a prior ejaculation. There are other situations in which reproductive processes are blocked: the odor of strange males inhibits implantation in female mice (*8*); sperm seem rejected by the bursa copulatrix of drosophila females when an interspecific mating has occurred (*9*); prolonged auditory stimulation reduces fertility in rats (*10*); and social crowding reduces reproductive success in rats and house mice (*11*, *12*).

The phenomenon discussed here, the inhibitory effect of copulation, may be related to the social structure of rodents. After ejaculation, there is a period during which the male is sexually unresponsive (*13*). This interval varies between 4.6 and 11.9 minutes, depending on which ejaculatory series is being considered. (In this study, the female was most susceptible to inhibitory cervical stimulation during the first 4 minutes after a male's first ejaculation). Although precise data are needed in order to describe the nature of rodent copulatory behavior in settings more natural than the laboratory, it may be that the normal pacing of sexual behavior and the periods of sexual refractoriness ensure the tranquility necessary for sperm transport.

It is, however, already known that under conditions of crowding, laboratory rats show persistent social pathology (*12*). One type of behavioral abnormality is a kind of pansexual behavior in which males mount at a much higher rate than usual. In these crowded colonies, reproduction decreases; part of this decrease may result from the mechanism described in this report. Even among normal colonies, colonies of rats with low social rank have more males than groups of higher social rank; and the low ranked groups have a reduced number of pups born (*14*). One of the mechanisms responsible for the decreased reproductive performance in low-ranking normal groups, as in the abnormally crowded groups, might be copulatory interference with sperm transport. In short, sexual behavior not only stimulates pregnancy, but under certain circumstances inhibits it.

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Partial Reversion in Yeast: Genetic Evidence for a New Type of Bifunctional Protein

Abstract. *Two kinds of phenotypic expression in purine biosynthesis result from recessive mutation to* ade12 *in baker's yeast. The mutants are adenine-specific, blocked in the conversion of inosine 5′-phosphate to adenylosuccinic acid; their response to inhibition of pathway activity by adenine is considerably reduced. Allelic partial reversions can restore prototrophy without correcting the regulatory defect imparted by the primary mutation. The separation of the two properties of the locus by allelic mutation supports the hypothesis that the locus specifies a protein of two independent functions, enzymatic and regulatory.*

A new physiological role for proteins was established with the identification and isolation of protein repressors as regulators of metabolic activity. Our studies on the genetic regulation of purine biosynthesis in yeast now raise the possibility that a single protein may combine within itself the dual roles of enzyme and repressor.

Single-event mutations at the *ade12* locus (*1*) of *Saccharomyces cerevisiae* produce two distinct phenotypic effects. (i) There is a loss of adenylosuccinate synthetase (AS), the penultimate step in adenosine 5′-phosphate (AMP) biosynthesis, as indicated by (a) the appearance of a nutritional requirement for adenine that is not satisfied by hypoxanthine, (b) the presence of inosine as a characteristic accumulant in cell extracts, and (c) growth-dependent excretion of hypoxanthine (*1*). (ii) The normal regulation of purine biosynthetic activity is modified toward constitutive synthesis. This effect of *ade12* is demonstrable (*1*) in the presence of either of the red pigment-forming, adenineless mutations, *ade1* or *ade2* (*2*, *3*). Strains that are mutant only at *ade1* or *ade2*, or both, are white when grown with excess adenine sulfate [75 μg/ml in GBHA (*4*)], although they routinely develop pigment at the customary supplemental levels of adenine (10 to 20 μg/ml), or on yeast extract–peptone medium (YEP) (*4*). With the addition of the *ade12* mutation to make the combinations *ade1 ade12* or *ade2 ade12*, the cells turn pink, then

red, as the intermediate purine precursor 4-amino-5-imidazole ribotide accumulates despite the high adenine content of the medium. Thus the development of color in the presence of excess adenine is an indicator of constitutive purine biosynthetic activity. In purine prototrophs, constitutive pathway activity is shown by hypoxanthine excretion.

The constitutive activity of *ade1 ade12* strains is unlikely to result from induction by accumulated intermediates, since the substrate of AS, inosine 5′-phosphate (IMP), does not accumulate extensively in these doubly blocked cells (*1*). Moreover, IMP is itself an inhibitor of the first enzyme of the pathway (*5*) and of pathway activity in vivo (*6*). Since exogenous adenine supports the growth of these cells there cannot be a serious deficit of intracellular adenylnucleotides, substances which would normally function to reduce AMP biosynthetic activity (*5, 7*). In the illustrative parallel case of *ade1* combined with the alternate adenine-specific mutation, *ade13* (*1, 3*), the cells respond inversely to the amount of adenine available by forming pigment exactly as if *ade1* were present alone. The dual role of the *ade12* mutation is therefore unexplained by changes in nucleotide pool sizes, and the role of macromolecular gene products must be considered.

It was proposed (*1*) that the *ade12* locus specifies a bifunctional protein with enzymatic and regulatory capacities. In regulation, the protein may act as a repressor controlling the synthesis of one or more enzymes in the pathway. A recent comparable model (*8*) suggests that nitrate reductase of *Aspergillus nidulans* functions both as corepressor and coinducer in the regulation of its own synthesis.

One prediction from the hypothesis of bifunctionality is that the affected protein would be internally differentiated with respect to its two distinguishable functions; therefore it should be possible to generate *ade12* mutants which have lost either one of the normal properties, but not both. This report presents the results of a search for enzyme-active but regulation-defective alleles at the *ade12* locus by reversion from known *ade12* mutants.

Accessibility to allelic secondary reversion of only one of the *ade12* functions must be an allele-specific property. It would partly depend on the primary mutational site and the way in which the affected protein is consequently modified. We assumed that the alleles most likely to yield partial reversions from second-site mutations would be missense mutants, a class with an intermediate frequency of allelic complementation (*9*). Several alleles were chosen for mutagenic treatment by this criterion.

Table 1. Reversions to prototrophy from adenine-specific *ade12* auxotrophs. Yeast cells were grown overnight in 5 ml of YEP and transferred at 10^7 cell/ml to fresh YEP made 1.0 percent in diethyl sulfate. After 5 minutes at 30°C the cells were removed to 6 percent sodium thiosulfate for 10 minutes, then diluted and plated on adenineless medium, or medium supplemented with hypoxanthine (20 mg/ml) when *ade1* was present. Relative cross-feeding, from undetectable (−) to strong (+++), was judged by the zone of *ade1* cell growth (*10*) around each colony on adenineless medium.

Genotype	*ade12* allele number	Viable cells plated	Percent survival	Number of revertants	Relative cross-feeding ability				Number tested for cross-feeding
					−	+	++	+++	
α *ade1*	60	3.0×10^6	28	82	27	5	12	6	50
a ade1 lys2	64	4.1×10^5	38	58	1	0	0	57	58
a ade1	71	4.3×10^4	4	0					
		4.4×10^5	41	13					0
a ade1 lys2	79	5.1×10^6	47	109	1	0	0	25	26
α *ade1*	109	5.2×10^5	48	133					0
a ade1 lys2	221	3×10^6	~ 40	6					0
α *lys2 trp5*	223	3×10^6	~ 40	21	1	6	4	10	21

Partial revertants to prototrophy at the *ade12* locus were sought in strains with and without the *ade1* marker. Some *ade12* alleles have been recovered as the only adenineless gene in recombinant ascospores. After mutagenic treatment of these strains, population samples were plated on adenineless medium. Colonies which grew were bioassayed for their ability to cross-feed homozygous *ade1* diploid yeast cells (*10*) as a measure of hypoxanthine excretion (*1*), and hence of hyperactivity in AMP biosynthesis. Such excretion signals retention of the *ade12*-associated regulatory defect.

Many of the *ade12* alleles, however, have not been obtained in a form free of *ade1* or *ade2* because such recombinants are commonly inviable. In such cases partial revertants were selected in cells of genotype *ade1 ade12* by growth on a synthetic medium supplemented with hypoxanthine. Retention of the regulatory defect was tested (i) by the pigment developed in 2 days on a high adenine medium, and (ii) by crossing the hypoxanthine-utilizing colonies to a noncomplementing *ade12* allele and noting the ability of the resulting prototrophic diploids to cross-feed *ade1* tester cells.

Mutagenic studies with 24 *ade12* alleles demonstrate the occurrence of the expected variability among different alleles of the locus in response to the mutagen diethyl sulfate. No revertants were recovered from 14 of the alleles; three yielded one revertant each; and from the remaining seven (Table 1), 422 revertants were collected, excluding phenotypically complete revertants.

The *ade12* alleles are further distinguished from each other by the types of revertants produced from any one allele with respect to the amount of hypoxanthine excreted. Thus, with the exception of one non-cross-feeder each, all of the revertants tested from alleles 64 and 79 are strong cross-feeders, whereas the revertants obtained from alleles 60 and 223 differ greatly in the amount of hypoxanthine they excreted (Table 1).

The *ade12-223* revertants vary individually in amount of cross-feeding, but they differ collectively from those recovered from other alleles in retaining a characteristically slow growth rate in the absence of adenine. This indicates that AS activity is only partially restored, remains rate-limiting, and consequently affects progeny spore viability as well. In crosses of the fast-growing cross-feeder revertants from *ade12-64* and *ade12-79* by wild type the ungerminated spore in three-spored asci carried either a wild type or a revertant strand with equal frequency, whereas the corresponding absent spore in progeny of the slow-growing revertants derived from *ade12-223* generally carried the revertant strand (28 of 32 asci).

Fifteen partial revertants of alleles *ade12-64*, *ade12-79*, and *ade12-223* were backcrossed to wild type. No adenine auxotrophs were recovered among the 669 viable spores obtained from 193 asci (viability 86.5 percent). However, this result might be attributed

to the typically poor germination of *ade12* spores unattended by other adenine markers. The hybrids of parental mutants × wild type gave only 53 viable spores from 21 asci (viability 63.0 percent) including only a single instance where an *ade12* spore survived. To contend with this explanation for the lack of *ade12* segregants from the backcrosses, seven partial revertants were crossed to a noncomplementing *ade12* allele, *ade12-55*. The diploid hybrids were adenine independent and excreters of hypoxanthine. The dissection of 60 asci gave 9 with two viable spores, 32 with three viable spores, and 19 with all four spores surviving. No more than two adenine-specific auxotrophs were found in an ascus and these were invariably identified as *ade12-55* by complementation tests. Thus, although we are assessing the allelism of several independent events of mutation simultaneously, when the data for any one of them is moderate, we conclude from the total absence of recombinants that these reversions are allelic to the *ade12* locus.

The recovery of allelic partial revertants demonstrates that the enzymatic and regulatory functions associated with the *ade12* locus are mutationally separable, a condition that would not obtain if either property were merely the physiological consequence of a monofunctional mutation. Our results support the hypothesis that this locus encodes a novel type of protein with catalytic and repressor functions.

Note added in proof: A current report by Lomax and Woods (*11*) demonstrates that the prototrophic, hypoxanthine-excreting mutants designated *pur1* (*10*, *11*) are allelic to *ade12*. Such mutants, by single mutation from wild type, are the functional equivalent of our enzyme-restored partial revertants *ade12E*. The finding is consistent with our hypothesis.

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Homing Behavior, Orientation, and Home Range of Salamanders Tagged with Tantalum-182

Abstract. *Using radioactive tags, we recorded movements of salamanders* (Plethodon jordani) *in their home areas and during homing. Males occupied home areas about three times larger than those of females and made occasional excursions into outlying regions. Homing after 22- to 60-meter displacements was direct and rapid, once initiated. Course headings at 1 meter from release were random; those at 2 meters and more were home-oriented. Males initiated homing movements sooner than females, although both sexes traveled at similar rates. Increased incidence of climbing on vegetation after displacement suggests olfactory mechanisms of orientation. These observations give direct evidence of homing orientation in caudate amphibians.*

Homing ability and size of the home range has been studied in *Plethodon jordani* (*1*) and other species of salamanders, and general course and time estimates of homing have been given (*2*). However, more detailed records of home range and homing movements on substantial percentages of experimental animals are needed before homing success can be attributed to any system(s) of homing orientation. Animals displaced beyond the limits of their home range can theoretically return to the home area by random or patterned movements alone without the operation of homing orientation mechanisms. Although patterned movements can be oriented with respect to the release point, neither patterned nor random movements have any consistent spatial relationship to the position of the home area, the characteristic of the homing orientation system. The lack of continuous tracking devices has greatly limited homing studies in salamanders. By using radioactive tagging methods, we obtained the first direct evidence of continuous homing orientation in salamanders which were displaced outside their normal home areas (*3*).

The study was conducted near Highlands, North Carolina, during August and early September 1969, in areas of mixed hardwood-hemlock forest with mostly rhododendron–mountain laurel understory. Herbaceous cover ranged from sparse to medium dense, and most areas contained a deep layer of fallen leaves and logs. Adult *P. jordani* (*4*) were collected by hand at night (between 2000 and 2330 E.S.T.), usually from burrow entrances. The size of each salamander was measured and the sex was determined; each salamander was marked by toe-clipping and was tagged by the injection of a 3- to 5-mm long, 18-gauge ^{182}Ta wire (20 to 48 μc) into the abdomen. Salamanders thus treated could be located in the habitat at about 2 m distance with a scintillation detector system (*5*). Direct observations were often made under subdued lantern light while the salamanders were on the surface at night. Each tagged salamander was either released at the point of capture (control) or displaced in an opaque container (*6*). Release usually occurred within 20 minutes of capture. Displacements were made in parallel, two salamanders of either sex being displaced in the same or opposite directions with sufficient spatial separation to avoid confusion over identification. Searches were made from two to eight times each night (between 1930 and 0600 E.S.T.) and once during the day