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Suppression of a yeast *amber* mutation in *Escherichia coli*

THE complementation of *Escherichia coli* auxotrophs by cloned eukaryotic genes[1–4] makes it possible to use standard bacterial genetic techniques to study these eukaryotic genes. Here, we describe the cloning of a *Saccharomyces cerevisiae* (yeast) gene with an *amber* suppressible allele. Reversion and suppression properties of this allele are examined in growing *E. coli* cells.

The yeast *his3* gene coding for imidazoleglycerolphosphate (IGP) dehydratase was cloned as a hybrid of bacteriophage λ which complemented *hisB* auxotrophs of *E. coli* lacking the analogous activity[1]. The wild type yeast *his3* gene is transcribed and translated in *E. coli* with high fidelity to produce an enzyme activity strongly resembling the activity found in yeast cells[2]. Complementation analysis of many derivatives containing *his3* sequences indicates that the *his3* structural gene is localised to a region of approximately 700 base pairs[5]. The length and location of this *E. coli* complementation unit is similar to the region homologous to the 650 base poly(A)-containing RNA species found in yeast cells (K. S., unpublished).

Genetic analysis in *E. coli* of the yeast *his3* gene depends on the availability of cloned derivatives which are nonfunctional. Mutant *his3* genes which are nonfunctional in $his3^-$ yeast cells are also nonfunctional when cloned and propagated in *E. coli*; that is, they do not complement *E. coli hisB* auxotrophs[2]. The order of two such *his3* lesions has been established by a three-factor cross of the bacteriophage λ hybrids containing the cloned mutant genes[2]. Non-complementing deletions of the yeast *his3* gene spontaneously generated during lytic growth of a bacteriophage λ*his3* hybrid have been isolated[5] by the method of Parkinson and Huskey[6]. The physical locations of the yeast lesions have been determined by deletion mapping in *E. coli*[5]. Deletion mutants which require transcriptional initiation from the λ promoter P_L for *his3* expression have been used to define a yeast DNA sequence which functions in *E. coli* as a promoter[7]. Derivatives of a λ*his3* hybrid which overproduces IGP dehydratase activity in *E. coli* have also been isolated (M. Brennan and K. S., unpublished results).

The demonstration that cloned yeast genes can be reintroduced into yeast cells by transformation and expressed[8] makes it possible to fuse the genetic systems of *E. coli* and *S. cerevisiae*. Recent findings indicate that DNA transformation of yeast can occur by at least three mechanisms (ref. 9 and J. B. Hick, A. Hinnen and G. R. F., unpublished results). Depending on the particular mechanism, transforming DNA can integrate into the yeast chromosomes by homologous recombination and/or replicate autonomously. Introduction of physically and genetically defined cloned *his3* derivatives into yeast cells should be an important tool in elucidating mechanisms of yeast regulation, DNA replication and recombination.

To aid such studies it would be useful to clone a conditionally lethal yeast mutation. Strain constructions would be significantly facilitated and interpretation of experimental results would be strengthened by the conditional nature of the lesion. To isolate a conditionally lethal lesion, 112 independently derived *his3* mutants of yeast[10] were screened for *amber* suppressibility by scoring for histidine prototrophy following mating with *his3*-532 *SUP4*-2. Three mutants (*his3*-14, *his3*-19 and *his3*-X5-21B) presumably containing internal UAG codons suppressible by tyrosine-inserting tRNAs[11] were isolated. DNA from strain *his3*-X5-21B was cloned in λgt4 (ref. 12) by the *Eco*RI-DNA ligase method[2]. From this pool of λgt4 hybrids, a phage containing *his3* sequences was isolated by the plaque filter hybridisation method of Benton and Davis[13]. The 10.1 kb (kilobase pair) *Eco*RI fragment in this phage, Sc2693, is physically indistinguishable from the analogous fragment (Sc2601)· which complements *E. coli hisB* mutations. λgt4-Sc2693 does not complement *hisB*463; therefore, the phage contains a cloned yeast *his3* gene with an *amber* lesion. The *his3* gene is located internally within a 1.7 kb *Bam*Hl fragment of Sc2601 DNA[5]. The equivalent *Bam*Hl DNA fragments of λ hybrids containing *his3* mutant genes were cloned in the yeast vectors YIp5 and YRp7 (ref. 9). The sources of these cloned mutant genes were λgt1-Sc2612 (*his3*-38), λgt6-Sc2679 (*his3*-532) (ref. 2), and λgt4-Sc2693 (*his3*-X5-21B).

All mutant *his3* genes cloned in these yeast vectors were introduced into the *E. coli* strain *hisB*463 by selection for the plasmid-coded gene for ampicillin resistance. The spontaneous reversion rates in *E. coli* of these yeast lesions were measured and compared to the spontaneous reversion rates in yeast (Table 1). The comparison is complicated by the fact that in these *E. coli* cells, the *his3* genes are presumably present in about 20 copies, and that the expression is only about four times above the single copy level[7]. The reversion rates are measured as His^+ colonies obtained per cell. It is unclear how these numbers are related to number of reversion events per DNA molecule. Multiple copies of *his3*-38 in *hisB*463 cells allow some growth in the absence of histidine. The reversion rate of this lesion in *E. coli* as determined from plasmid hybrids (2×10^{-8}) agrees well with the previous results obtained with phage hybrids[2]. The reversion rate of this lesion in yeast is significantly lower ($<10^{-9}$). *his3*-532 is a very stable lesion in both organisms, though revertants are detected in *E. coli* but not in yeast *his3*-X5-21B reverts in *E. coli* at a significantly lower rate than it reverts in yeast. These results indicate that spontaneous reversion of a given yeast lesion occurs at different rates in yeast and in *E. coli*.

Table 1 Suppression and reversion characteristics of yeast *his3* lesions

his3 lesion	λ Hybrid	Plasmid hybrid	*hisB*(*supF*)	his^+ colonies *hisB*	Yeast
his3-38	λgt1-Sc2612	YIp5-Sc2719	2×10^{-8}	3×10^{-8}	$<10^{-9}$
		YRp7-Sc2719	1×10^{-8}	1×10^{-8}	
his3-532	λgt6-Sc2679	YIp5-Sc2720	2×10^{-10}	1×10^{-10}	$<10^{-10}$
his3-X5-21B	λgt4-Sc2693	YIp5-Sc2721	1	3×10^{-9}	2fl10^{-7}
		YRp7-Sc2721	1	1×10^{-9}	

0028-0836/79/0279—0078$01.00

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As *his3*-X5-21B is suppressed in yeast by a tyrosine-inserting tRNA, suppression in *E. coli* was examined with an analogous tRNA species (supF). *hisB*463 cells containing the cloned yeast *his3* lesions were lysogenised by a phage ($\phi 80$ *supF*) containing the *E. coli* gene coding for a tRNA species capable of suppressing amber (UAG) codons[14]. Introduction of the *supF* allele into these *hisB*463 derivatives was determined by their ability to plate $\lambda cI857Sam7$. *hisB*463 cells containing both the *his3*-X5-21B and the *supF* alleles grow in the absence of histidine (Table 1); that is, the *his3* lesion is suppressed by *supF*. This result is not due to an artefact of selection, as both the *his3*-X5-21B and the *supF* alleles were introduced into the *hisB*463 host by non-selective means. Furthermore, it confirms the *amber* suppressibility of the *his3*-X5-21B allele. It is now possible to introduce DNA containing this conditionally lethal yeast gene back into yeast and *E. coli*.

These results indicate that an *E. coli amber*-suppressing tRNA species suppresses a yeast *amber* lesion in growing *E. coli* cells. It has been previously established that purified yeast suppressor tRNAs, when present in heterologous, eukaryotic, *in vitro* translation systems, suppress prokaryotic ($Q\beta$) *amber* mutations[15,16]. Taken together, these *in vivo* and *in vitro* experiments provide evidence that translational termination and tRNA suppression of nonsense codons are processes which are conserved between prokaryotic and eukaryotic organisms.

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Received 27 December 1978; accepted 12 March 1979.

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Genotype control of the dystrophia muscularis gene in mice

MUTANTS of the dystrophia muscularis gene (*dy*) in mouse suffer from progressive skeletal muscle loss, together with inadequate repair or regeneration[6]. In 1974 I reported a distinctive phenotypic difference between two dystrophic mutant alleles with respect to myogenesis *in vitro*[1]. Homozygous dy^{2J} mice gave cultures in which there was apparently normal myogenesis, whereas mice homozygous for *dy* gave cultures which contained large numbers of grouped uninucleate myoblasts (pseudostraps) in place of multinucleate syncitial myotubes[2]. MacPike, however, showed[3] that muscular dystrophies caused by these two alleles carried in the same genetic background (C57BL/6J) were similar when assessed histologically. I have, therefore, investigated how the process of myogenesis *in vitro* (which can be considered to be another independent function of the muscle) is affected not only by the two allelic mutants but also by the genetic background. The *dy* dystrophy was known to be histologically similar in either the C57BL/6J or 129/ReJ background[3] but surprisingly C57BL/6J *dy*/*dy* cultures gave normal myogenesis while 129/ReJ *dy*/*dy* cultures again produced pseudostraps.

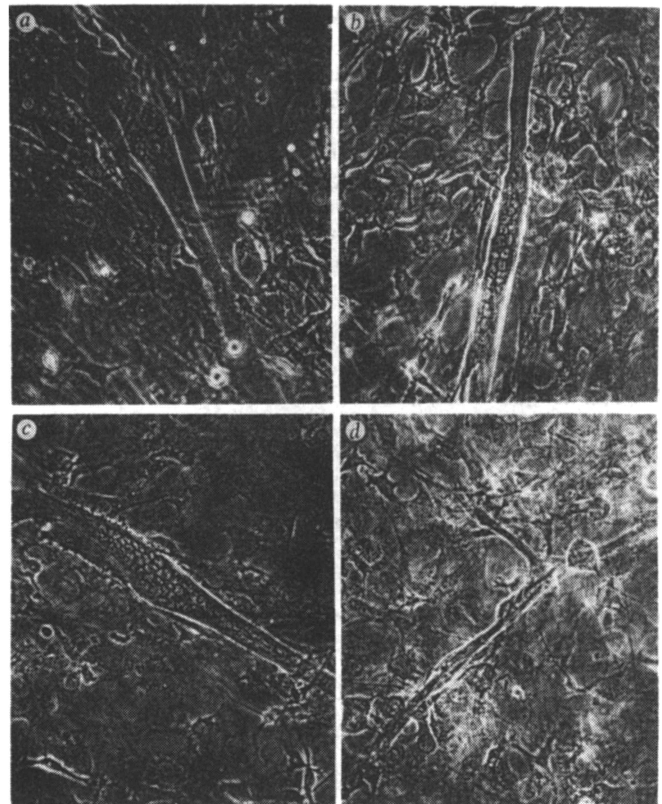

Fig. 1 Unstained living cultures photographed with phase contrast-optics. The structures are typical and the illustrations representative of the experimental results, *a*, C57 +/+, normal multinucleate myotube; *b*, C57dy^{2J}/dy^{2J}, large syncitial myotube; *c*, C57*dy*/*dy*, a definite multinucleate myotube; *d*, 129*dy*/*dy*, characteristic pseudostraps with discrete uninucleate cells. All photographs were taken at 4 d *in vitro* in identical conditions.

The mice used in this series of experiments were C57BL/6J dy^{2J}/dy^{2J}, C57BL/6J *dy*/*dy*, C57BL/J6 +/+ and 129/ReJ *dy*/*dy* at 2, 3, 4 and 5 months of age. Cultures were obtained from 4 day crush lesions as described previously[1]. Daily examination for a period of 8 days was carried out blind; 48 cultures of each genotype were assessed. As before, the pattern of growth during the first 3 days was one of proliferation and explant spreading. Myotubes, increasing in size and number, developed by days 4 to 8 in all three C57BL/6J genotypes including the *dy* mutant (Fig. 1*a*, *b*, *c*). The 129/ReJ dy/dy showed without exception the characteristic pseudostraps (Fig. 1*d*) and the occasional small myotybe during the first 3 days.

In vitro the 129/ReJ *dy* muscle attempts to form myotubes but does not succeed[1]. However, the same muscle in a more complex organotypic culture of fetal spinal cord and muscle exhibits some regeneration[4] although it is much less than normal muscle in the same conditions. Furthermore, the *dy* muscle cells cultured as a low density monolayer[5] produce apparently normal myogenic colonies which contain well differentiated muscle fibres. Clearly, myogenesis from dystrophic muscle is extremely susceptible to environmental conditions. But it is also

0028–0836/79/0279—0079$01.00

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