should facilitate a systematic analysis of the biochemistry of error-prone repair.

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A transposon in *Streptococcus faecalis* with fertility properties

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Certain strains of streptococci have been shown to be capable of conjugatively transferring drug resistance determinants in the apparent absence of plasmid DNA[1–6]. The transferrable tetracycline resistance (*Tc*^{r}) marker of *Streptococcus faecalis* DS16 has been located on a transposon, designated Tn *916*[2,4,7]. We show here that when chromosomal Tn *916* is conjugatively transferred it inserts into a number of different sites on the recipient chromosome. Our studies also imply that transfer and transposition share a common step, which may be excision of the transposon from the donor chromosome.

The ability of Tn*916* to transfer from plasmid-free donors (see Table 1 for description of bacterial strains) in overnight filter matings is shown in the data of Table 2. DS16C3 is able to transfer *Tc*^{r} at a frequency of about 10^{-8} to the plasmid-free recipient strain JH2-2. Transconjugants such as CG130 are capable of transferring *Tc*^{r} to the isogenic recipient JH2SS at a similar frequency. To investigate the nature of this transfer event, chromosomal DNA isolated from the donor and transconjugant strains listed in Table 2 was digested with *Hin*dIII restriction endonuclease, resolved by agarose gel electrophoresis, and transferred to nitrocellulose paper using the Southern technique[8]. The transferred DNA was probed with a ^{32}P-labelled *Eco*R1 restriction fragment (F′) of pAM211 (a derivative of pAD1, a resident plasmid of DS16) that contains the entire transposon. There are no *Eco*R1 sites within Tn*916* and about 85% of the F′ fragment consists of Tn*916* sequences. As Tn*916* contains a single *Hin*dIII site, two chromosome–transposon junction fragments should be resolvable after hybridization with the probe. The resulting autoradiogram (Fig. 1*a*) shows that three different transconjugants obtained from the DS16C3 × JH2-2 mating, detailed in Table 2, display hybridization patterns (lanes 2–4) that differ from those seen in the donor (lane 1) as well as from each other. One such transconjugant, CG140, gave rise to two chromosome–transposon junction fragments (X and Y), whereas two other transconjugants, CG110 and CG130, gave rise to four or more bands. Figure 1*a* also shows the hybridization profiles of transconjugants derived from the secondary mating described in Table 2 that had used strain CG130 as the donor. Again the hybridization bands of the transconjugants (lanes (5–7) differed from those of the donor as well as from each other. Strain CG132 gave rise to a multiple band pattern, whereas two other transconjugant strains, CG131 and CG133, exhibited the simpler banding pattern.

The variation in hybridization profiles amongst different transconjugants implies that Tn*916* inserts at different sites on the recipient chromosome. These findings also argue against the possibility that Tn*916* existed on a plasmid which had escaped physical detection. If the latter had been the case, identical patterns should have been observed in the transconjugants.

The patterns of multiple bands observed with transconjugants CG110, CG130, and CG132 were reproducible, and do not represent incomplete digestion products. An autoradiogram of a Southern blot hybridization experiment involving *Eco*R1 digested chromosomal DNA and ^{32}P-labelled Tn*916* probe is shown in Fig. 1*b*. As expected, a single hybridizing fragment was detected in the *Eco*R1 digested chromosomal DNA from strains CG133, CG131, and DS16C3 (strains that gave rise to two hybridizing fragments in the *Hin*dIII digestion experiments—see Fig. 1*a*). By contrast, those transconjugants that gave rise to complex band patterns following *Hin*dIII digestion (CG132, CG130, and CG110) gave rise to more than one hybridizing fragment when digested with *Eco*R1. In every case, the hybridizing fragments following *Eco*R1 digestion of chromosomal DNA were larger than ~15 kilobase. These data strongly suggest the presence of non-tandem multiple copies of the entire transposon in strains CG110, CG130, and CG132; however, the possibility that major rearrangements of the transposon give rise to these data cannot be completely eliminated. We note that restriction enzyme analyses of several pAD1::Tn*916* plasmids formed by transposition from the CG110 chromosome revealed a typical ~15 kilobase insertion (data not shown).

Tc^r transconjugants of JH2-2 that exhibit altered transfer frequencies in secondary matings were readily obtainable, and usually represented about 20% of the original transconjugants. Strain CG110 was capable of donating Tn*916* at frequencies of about 10^{-6} (Table 2). To investigate the transposition of Tn*916* in such a strain the conjugative haemolysin plasmid pAD1 was introduced into strains CG110 and CG130, and the frequency of Tn*916* transposition from the chromosome to pAD1 was measured by determining the number of *Tc*^{r}, hyperhaemolytic transconjugants obtained after filter-mating of these

Table 1 *S. faecalis* strains and relevant properties

Strain	Chromosomal genotype	Plasmid content	Reference
DS16C3	*tet*	None	4
JH2-2	*rif fus*	None	21
JH2SS	*str spc*	None	22
CG110	*rif fus tet*	None	This paper
CG130	*rif fus tet*	None	This paper
OG1-RF (pAM211)	*rif fus*	pAM211(Tn*916* inserted into *Eco*R1 F fragment of pAD1)	4
CG180	*rif fus*	pAM180 (pAM81::Tn*916*)	This paper
CG190	*str spc*	pAM190(Tn*917* inserted into *Eco*R1 B fragment and Tn*916* into *Eco*R1 D fragment of pAD1)	This paper

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Table 2 Tn*916* transfer from plasmid-free donors

Donor strain	Recipient strain	Frequency of Tc^r transconjugants per recipients*	Representative transconjugants
DS16C3	JH2-2	1×10^{-8}	CG110 CG130 CG140
CG130	JH2SS	2×10^{-8}	CG131 CG132 CG133
CG110	JH2SS	4×10^{-6}	

* Filter matings were carried out as described in ref. 4, using Difco antibiotic medium no. 3 (AB3), and an initial ratio of one donor per ten recipients. Antibiotics were used in selective agar plates at the following concentrations: tetracycline, 10 μg ml^{-1}; rifampin, 25 μg ml^{-1}; fusidic acid, 25 μg ml^{-1}; and streptomycin, 1000 μg ml^{-1}. For reasons discussed in ref. 4, we generally express frequencies in terms of recipients (rather than donors) in cases where the values are very low. Frequency of spontaneous Tc^r mutations was less than 10^{-10}.

donor strains with JH2SS. The hyperhaemolytic phenotype results from an insertion within or near the haemolysin determinant of pAD1, and can be readily detected by an increased diameter of haemolysis zones on horse blood agar plates[4]. The frequency of hyperhaemolytic, Tc^r transconjugants was found to be more than 200 times higher when CG110 was the donor strain (4.9×10^{-6} per recipient) than when CG130 was the donor strain (1.8×10^{-8}). pAD1, *per se*, transferred equally well ($\sim10^{-2}$) from both hosts. Similar results were obtained with another independently isolated variant, CG100, which transferred Tn*916* at frequencies about 10 times higher than normal; transposition from the CG100 chromosome to pAD1 was similarly increased. These data indicate Tn*916* transfer and transposition may share a common step.

When located on a conjugative plasmid, Tn*916* was found to excise at relatively high frequency upon transfer to a recipient strain. pAM180 is a derivative of the ~26 kilobase erythromycin-resistance (Em^r) plasmid pAM81 with Tn*916* inserted into the *Eco*R1 fragment G. When donor strains containing pAM180 were filter-mated with JH2SS, a high degree of segregation of the Em^r and Tc^r determinants occurred, as can be seen in the data of Table 3. Although both the Em^r and Tc^r determinants transferred at similar frequencies, a significant proportion of erythromycin selected transconjugants (43–77%) were not resistant to tetracycline.

Of those transconjugants selected for tetracycline resistance, however, greater than 90% were also erythromycin resistant. Interestingly, in some of the erythromycin selected transconjugants that were tetracycline resistant Em^r and Tc^r were no longer linked, as implied by a great reduction in the transfer frequency of Tc^r, but not Em^r, in secondary matings. The loss of the Tc^r determinant from the plasmid possibly relates to a zygotic induction of one or more Tn*916*-related recombination enzymes.

To determine the structural integrity of pAM81 DNA sequences in the various types of transconjugant, DNA from representative plasmids was digested with *Hin*dIII and analysed by agarose gel electrophoresis (Fig. 2*a*). Plasmid DNA isolated from two Em^rTc^r transconjugants in which Em^r and Tc^r were no longer linked (CG184 and CG186) exhibit a restriction pattern identical to that seen for pAM81. (Note the two fragments of pAM180 that had contained Tn*916* sequences were lost, and pAM81 fragment G reappeared). It is presumed that Tn*916* transposed to the bacterial chromosome in these strains since they were tetracycline resistant and capable of transferring Tn*916* at very low frequency. Plasmid DNA from strain CG187 (Em^rTc^s) also gave rise to a restriction pattern identical to that of pAM81. In contrast, plasmid DNA from strain CG181 (Em^rTc^r), in which both resistance determinants remained linked, displayed a restriction pattern identical to that of the pAM180 donor. These data support the view that transposition involves an excision followed by insertion, but that insertion is not necessarily coupled to excision—in which case the excised element is lost.

Analogous results were obtained in matings that used as a donor strain CG190. This strain harbors a pAD1 derivative (pAM190) with two transposon insertions: Tn*917* (Em^r) and Tn*916* (see Table 1). The Tn*916* insertion gave rise to a nonhaemolytic phenotype, yet the strain displays a hyperhaemolytic phenotype on blood plates that contain tetracycline (10 μg ml^{-1}). This drug-influenced phenotype has been observed previously in certain pAD1::Tn*916* derivatives containing transposon insertions into or near the haemolysin determinant[9]. CG190 transferred Em^r at a frequency of $\sim2\times10^{-4}$ per donor to JH2-2 in overnight broth matings; and Tc^s derivatives (for example CG191) as well as Tc^r derivatives in which the Em^r and Tc^r determinants were no longer linked (CG194) were found among the transconjugants at a frequency of about 4%. Agarose gel electrophoresis of *Eco*R1 digested plasmid DNA from such transconjugants (Fig. 2*b*) revealed the presence of a fragment (*Eco*R1 D fragment) that had been missing in the original pAM190 DNA as a result of Tn*916* insertion. Most significant was the additional observation that transconjugants CG191, CG192, and CG194 all regained the haemolytic phenotype, implying that the excision of Tn*916* was precise.

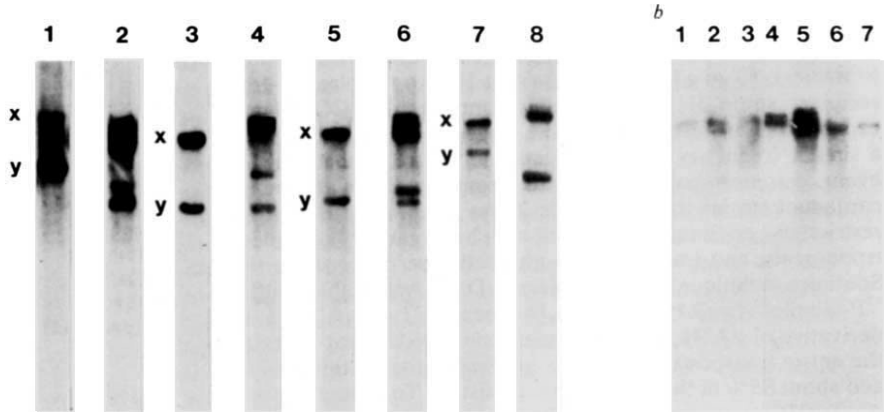

Fig. 1 Autoradiograms obtained from filter-blot hybridization analyses of chromosomal DNA from Tc^r transconjugants. *a*, *Hin*dIII digested DNA (1–2 μg) from transconjugants CG110, CG140, and CG130, (lanes 2–4 respectively) which were obtained using DS16C3 (lane 1) as donor. Lanes 5–7 contain *Hin*dIII digested DNA from transconjugants CG131, CG132, and CG133, respectively, which were obtained using CG130 as donor. Fragments marked x and y denote presumed chromosome–transposon junction fragments. Lane 8 contains *Hin*dIII digested pAM211 DNA and hybridizing bands are approximately 18.5 Kb and 7.5 kb. *b*, *Eco*R1-digested DNA (1–2 μg) from strains CG133, CG132, CG131, CG130, CG110, and DS16C3, (lanes 1–6 respectively). Lane 7 contains *Eco*R1 digested pAM211 DNA from which the probe had been made. Isolation of DNA by buoyant density gradient centrifugation and agarose gel electrophoresis was as described in ref. 4. DNA transfer and hybridization were essentially according to Southern[8] as modified by Wahl *et al.*[23], and used a BRL blot transfer system. The *Eco*R1 F′ fragment of pAM211 was purified by electroeluting the DNA from an agarose gel slice placed in a dialysis bag filled with electrophoresis buffer that had been embedded in 1% agarose. Following electrophoresis[4] the DNA was recovered by ethanol precipitation. Nick translation of pAM211 *Eco*RI F′ probe DNA with either ^{32}P-dCTP (670 Ci mmol^{-1}) or ^{32}P-dATP (410 Ci mmol^{-1}) was carried out using a nick-translation kit.

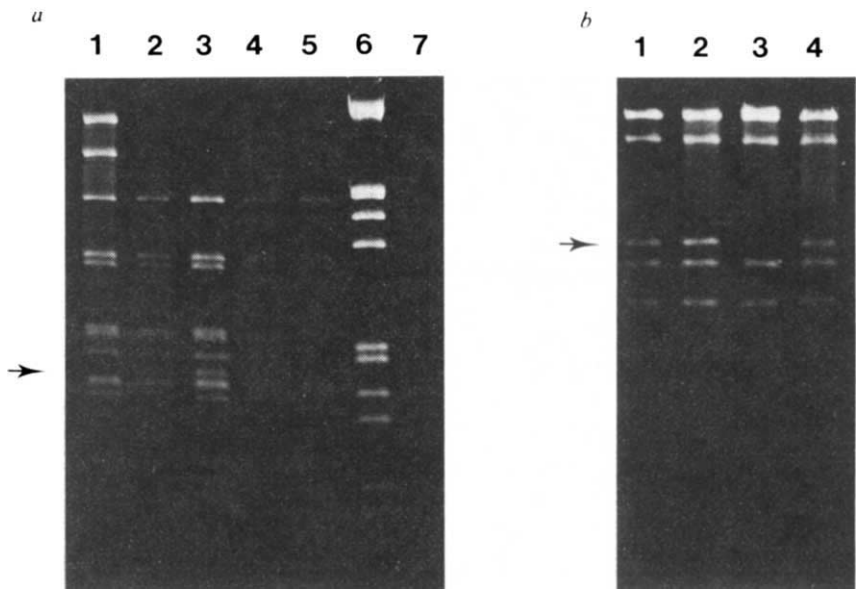

Fig. 2 Excision of Tn*916* from plasmid DNA after transfer to recipients. *a*, *Hin*dIII digests of transconjugant plasmid DNAs derived from the CG180 donor. Lanes 1 and 2 contain plasmid DNA from CG180 and CG130 (pAM81), respectively. The arrow marks fragment G, no longer present after Tn*916* insertion into pAM81. Lanes 3–5 contain plasmid DNA from the Em^rTc^r transconjugants CG184 and CG186, and the Em^rTc^s transconjugant CG187. Lane 7 contains plasmid DNA from the Em^rTc^r CG181 strain, and lane 6 contains λ DNA digested with *Eco*R1 and *Hin*dIII. *b*, *Eco*R1 digests of transconjugant plasmid DNAs derived from the CG190 donor. Lanes 1–4 contain plasmid DNA from the Em^rTc^s transconjugants CG191 and CG192, and the Em^rTc^r transconjugants CG193 and CG194. The restriction pattern obtained for pAM190 is identical to that of CG193 (data not shown). The arrow marks the *Eco*R1 fragment D of pAD1, lost as a result of Tn*916* insertion and regenerated after excision. The highest molecular weight band in lanes 1, 2 and 4 represents a doublet and includes the pAD1 *Eco*R1 A and B::Tn*917* fragments; whereas the highest molecular weight material in lane 3 represents a triplet which includes the *Eco*R1 D::Tn*916* fragment. (Broth matings were performed as in ref. 24.) Agarose gel electrophoresis utilized a 1% (Fig. 2*a*) or 0.7% (Fig. 2*b*) gel and Tris-borate buffer[25].

Transconjugants in which the Em^r and Tc^r markers remained linked, such as CG193, displayed an *Eco*R1 restriction pattern like the donor and were, as expected, nonhaemolytic.

On the basis of information known thus far we suggest the following model for Tn*916* behaviour. When located on the bacterial chromosome Tn*916* may spontaneously excise at low frequency. Although there is no direct evidence for the form the excised element might take, we think that it is most probably a circular molecule incapable of vegetative replication. Assuming that induction of key enzymes accompanies the excision step, the element now has the potential to: (1) insert back into the chromosome at a new, or perhaps the original site; (2) insert into a resident plasmid; (3) transfer into a recipient strain (if conditions warrant it); or (4) segregate. Conjugation could be viewed as occurring by a plasmid-like process, with zygotic induction of appropriate enzymes facilitating insertion into the recipient chromosome. The generation of multiple copies of Tn*916* in some transconjugants could arise by secondary transpositions (still via excision-insertion) from a replicated portion of the chromosome to an unreplicated region. Completion of replication thus results in one of the daughter chromosomes having two transposons. Conceivably when Tn*916* enters a new cell, a certain amount of time (a generation or so?) may be required to achieve levels of 'repressor' sufficient for stable maintenance of the element at one site. During this time there could be a tendency to move from one site to another.

In view of the increasing reports of 'plasmid-free' transfer of resistance determinants, especially among the streptococci, Tn*916* may represent a prototype of a new class of genetic elements that could be referred to as 'conjugative transposons'. In this regard Smith and Guild[10] have recently obtained evidence suggesting that the conjugative multiple resistance element originating in *S. agalactiae* B109 may be located on a large transposon.

It is important to note that homology has recently been demonstrated between Tn*916* and the transferable Tc^r determinants (classified as *tetM*) of *S. pneumoniae* and *S. agalactiae*[11,12] as well as with a chromosomal Tc^r determinant (not yet shown to be transferable) in *S. mutans*[13]. Recent reports of transferable nonplasmid elements in *Clostridium difficile*[14] and *Bacteroides fragilis*[15–18] suggest that such elements may exist in a variety of prokaryotic genera (including Gram negative bacteria). Although certain R-plasmids in *Escherichia coli* (IncJ)[19] and *Haemophilis*[20] prefer to be integrated in the chromosome, their possession of replication functions and dependence on host recombination functions to integrate distinguishes these elements from Tn*916*.

Table 3 Segregation of Em^r and Tc^r in filter matings involving CG180 donors

	$Em^r\ Tc^r$ transconjugants		
Experiment	Erythromycin selected*	Tetracycline selected	Erythromycin selected $Em^r\ Tc^r$ transconjugants with unlinked markers
1	27/47 (57%)	41/43 (95%)	6/12
2	16/48 (33%)	46/48 (96%)	1/12
3	11/48 (23%)	44/48 (92%)	1/10

Tn*916* was inserted into pAM81 to generate pAM180 via overnight filter matings between CG110 (pAM81) and JH2SS followed by selection for tetracycline resistant transconjugants capable of transferring Tc^r linked to Em^r in secondary matings. (The plasmid pAM81, originally identified in *S. faecalis* DU81 is capable of conjugal transfer in filter matings at frequencies of about 10^{-4} per donor, Y. Yagi and L. Dempsey, personal communication.) Each donor was derived from a single colony isolate and was filter mated with JH2SS (JH2-2 in secondary matings) as described in Table 2. Frequency of Em^r and Tc^r transconjugants was $0.6–1.5 \times 10^{-5}$ per donor.

* Although Em^rTc^s transconjugants appeared readily, random analyses of the CG180 donor failed to detect any Tc^s colonies (135/135 were Em^rTc^r) implying dissociation of Tc^r occurred after, or during, transfer.

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Regulatory sites for *his3* gene expression in yeast

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The expression levels of many genes are regulated in response to particular environmental or developmental cues. A simple regulated gene can conceptually be divided into three elements: structural components that encode the gene product, promoter elements that are essential for gene expression, and regulatory elements responsible for changing the level of expression after a specific stimulus[1,2]. When cells of the yeast *Saccharomyces cerevisiae* are subjected to amino acid starvation, *his3* and many other amino acid biosynthetic genes are expressed threefold above the basal level[3,4]. Previously, I isolated mutations mapping outside the structural gene that severely reduce or eliminate *his3* expression[5,6]. These mutations define two distinct *his3* promoter elements located 115–155 and 32–52 base pairs (bp) from the site of transcriptional initiation. Here I describe 18 small deletion mutations, some of which express *his3* at the basal level but are unable to increase the level of expression in the appropriate physiological conditions. These define two regulatory sites located 32–41 and 80–100 bp upstream from the site of transcriptional initiation, and they strongly suggest that these regions are necessary for the positive regulation of *his3* expression. I consider the results in terms of models for the general control of amino acid biosynthesis.

his3 encodes imidazoleglycerol phosphate dehydratase (IGPD), an enzyme involved in histidine biosynthesis. Its expression is regulated coordinately with the genes involved in amino acid biosynthesis. When yeast cells are starved of histidine, methionine or tryptophan by adding appropriate inhibitors to the growth medium, the levels of IGPD increase about threefold (Table 1). In addition, *his3* expression is controlled in the expected manner by the *aas2* and *tra3* genes.

It is probable that *his3* is regulated solely as a function of general amino acid biosynthesis: first, IGPD levels do not depend on the presence or absence of histidine in the growth medium (Table 1). Second, growth conditions resulting in the selective induction of histidine biosynthetic enzymes have never been found. Third, mutations selected for defective regulation of histidine enzymes always affect other amino acid biosynthetic enzymes also[3]. These experiments, which fail to demonstrate histidine-specific regulation in yeast, are identical to those that clearly establish such control in *Salmonella typhimurium*.

The mapping positions of 18 mutations of the cloned *his3* gene are presented in Table 2. Each mutation deletes sequences (ranging between 9 and 66 bp) within the untranscribed region located 6–138 nucleotides upstream from the 5′ end of the gene. Using standard techniques, the mutant genes were introduced back into yeast cells so that the resulting strains contained one copy of the cloned DNA per cell at the normal *his3* chromosomal location (for experimental details see Fig. 1 legend). Strains harbouring these cloned mutant alleles were always grown in medium containing histidine so that *his3* expression was gratuitous for cell growth; inducing conditions were achieved by starving the cells of tryptophan. The IGPD levels are shown in Table 2. Two strains were examined for each *his3* allele. One strain contained all the original transforming DNA sequences; the other lacked the vector sequences and is equivalent to replacement of the original *his3*-532 mutation by the allele of interest (see Fig. 1 legend). The IGPD levels for both strains of any given mutant were always the same. Of the 18 mutations tested, 9 regulate *his3* expression properly. However, strains with any of the nine mutations produce IGP constitutively, that is, the enzyme levels are the same in normal conditions and during amino acid starvation. Thus, these nine mutations have the expected properties of *his3* regulatory mutations.

his3 regulatory sites are defined by comparing the DNA sequences of mutations that either regulate or fail to regulate *his3* expression. The simplest interpretation of the data assumes that: (1) a subset of the sequences deleted by a regulatory mutation is essential for normal regulation; and (2) sequences deleted by correctly regulated derivatives are unimportant. Such analysis, when applied to all the derivatives, leads to the simple and internally consistent description of *his3* regulatory elements illustrated in Fig. 2 and discussed below. It is difficult to disprove alternative explanations for the phenotype of any given mutation, and more complex models can be advanced to explain the data.

The extent of *his3*-Δ22 indicates a regulatory site that includes a sequence between 32 and 41 bp upstream from the start of transcription (nucleotides −32 to −41). Deletion of this region accounts for the regulatory defects of five other mutations (*his3*-Δ20, Δ21, Δ23, Δ24 and Δ25). As yet, the DNA sequences sufficient to constitute this regulatory site are not well mapped. However, sequences further upstream than −52

Table 1 Regulation of the *his3* gene

Strain	Genotype	Complete	−His	−Trp	+AT	+Eth
KY29	*trp1*-289 *his3*$^+$	1.0	1.0	3.0	3.0	3.2
KY137	*trp1*-289 *his3*-532	<0.1	NG	<0.1	NG	NT
KY107	*aas2*-5039 *his3*$^+$	1.0	1.1	NT	1.5	1.3
KY108	*tra3*-1 *his3*$^+$	3.0	3.0	NT	3.5	3.4

The yeast strains used were: KY29 (*mat-a ura3*-52 *trp1*-289) isolated by M. Thomas as strain M1-2B; KY137 (*mat-a ade2*-1 *ura3*-1 *ura3*-2 *trp1*-289 *his3*-532 *can1*$^-$) isolated by S. Scherer; KY107 (*mat-a aas2*-5039) and KY108 (*mat-a tra3*-1) both obtained from G. Fink[3]. Because *tra3*-1 renders strains temperature-sensitive for growth, all cultures were incubated at 27 °C with shaking. Complete medium contained 2% glucose, 0.67% yeast nitrogen base without amino acids (Difco), and 0.1 mg ml^{-1} each of histidine, tryptophan, uracil and adenine. In these conditions, the doubling time was 2 h for all strains except KY108, which doubled every 3 h. Strains incubated in complete medium lacking histidine (−His) grew at the same rate, except for KY137 which did not grow (NG). Cells grown in either of these non-starvation conditions were collected in the middle of exponential growth (A_{600} = 2 or ~2 × 10^7 cells ml^{-1}). To achieve amino acid starvation, cells were first grown in complete medium until A_{600} was ~1.0, concentrated by centrifugation, washed once with distilled water, and then resuspended in complete medium that either lacked tryptophan (−Trp), lacked histidine but contained 10 mM aminotriazole (+AT), or contained 20 μM ethionine (+Eth). The resuspended cells were incubated for a further 6–9 h; the cells undergo an average of one more cell division before their growth is arrested as a result of Trp, His or Met starvation. To determine the levels of IGPD activity, cells were collected, washed twice with water, permeabilized with 1.5% chloroform at 37 °C for 20 min, pelleted by centrifugation, and resuspended in 0.1 M triethanolamine, *p*H 7.7. The enzyme assay was performed as described previously[12] with minor modifications (to be published elsewhere). The enzyme levels are normalized to both the number of cells assayed and to the amount of absorbance at 290 nM released after chloroform treatment. The activity levels shown are relative to those of wild-type yeast grown in complete medium (defined as 1.0). For enzyme levels around 1.0, the error is ~10%.

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