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* J.R. is a scholar of the Leukemia Society of America

25 September 1980; revised 21 November 1980

Suppressible Four-Base Glycine and Proline Codons in Yeast

Abstract. *Five ICR-170–induced mutations at the* His*4 locus in yeast are +1 G·C (G, guanine; C, cytosine) additions in DNA regions that contain multiple G·C base pairs. These mutations represent both nonsuppressible and suppressible alleles. All externally, suppressible frameshift mutations occur in glycine and proline codons to produce the four-base codons GGGU (U, uracil), GGGG, and CCCU. This implies that suppression of these four-base codons in yeast, as in bacteria, involves a four-base anticodon or its functional equivalent. Two identical four-base codons (CCCU) at widely separate regions within* His*4 are not suppressed equally.*

Frameshift mutations result from the addition or deletion of bases to the DNA (*1*). The most complete information on frameshift mutations comes from studies on enteric bacteria (*2*) and their bacteriophages (*3*). In these systems, planar aromatic hydrocarbons have been shown to induce frameshift mutations in regions of the DNA that contain sequences of repeated bases. Reversion of frameshift mutations can occur by a second, compensating frameshift within the same gene, or by a suppressor mutation in a different gene. A class of compounds, the ICR series (*4*), induces frameshift mutations at several different loci in *Salmonella typhimurium* and *Escherichia coli* (*2, 5*). Mutations caused by one of these compounds, ICR-191, have been most extensively characterized in the *hisD* gene of *S. typhimurium*. In this gene, ICR-191 causes base additions or deletions in monotonous runs of G·C base pairs (G, guanine; C, cytosine) (*6*). Two major groups of ICR-191–induced, suppressible mutations have been characterized. One group of mutations generates the four-base codon GGGG (code for glycine) (*7*) and the other group generates the four-base codon CCC· (proline) (*8*). In most instances the suppression results, respectively, from an alteration in a glycine or a proline transfer RNA (tRNA) (*9, 10*). The suppressor mutation at *sufD* has been shown conclusively to involve the mutation of the anticodon of the glycl-tRNA from CCC → CCCC (*9*). Suppression by *sufD* is presumed to involve recognition of a four-base glycine codon by the mutant tRNA.

ICR-170 (*11*) is a powerful mutagen in eukaryotes including *Drosophila*, ascites tumor cells, yeast, and other fungal organisms (*4, 12*). Mutations at the *his*4 locus of *S. cerevisiae* (*13*) are similar to those induced by ICR-191 in bacteria. Three groups of ICR-170–induced *his*4 mutations were identified on the basis of reversion and suppression analysis. Group I mutations failed to revert with ICR-170 and were not suppressed by external suppressors. Group II and group III mutations revert at high frequency with ICR-170 and are suppressed by external suppressors. Suppressors of group II mutations fail to suppress group III mutations, and similarly suppressors of group III mutations fail to suppress group II mutations. Nineteen ICR-170–induced *his*4 mutations are group II and two are group III. One of the suppressors of group II frameshift mutations alters the chromatographic behavior of a glycyl-tRNA species (*13*). These early studies on the ICR-170–induced mutations in yeast led to the suggestion that ICR-170 was making frameshift mutations in runs of G or C and that the ICR-170–induced suppressors were acting at the level of translation to suppress a four-base code word.

We now report the DNA sequence of five ICR-170–induced mutations at the *his*4 locus in yeast. One of the mutations, *his*4-506, is representative of the nonsuppressible group I mutations. Four of the mutations, two from group II, *his*4-38 and *his*4-519, and two from group III, *his*4-712 and *his*4-713, are representative of the two mutually exclusive classes of suppressible mutations. The DNA sequence data show conclusively that ICR-170 causes frameshift mutations in G·C regions of yeast DNA.

ICR-170–induced mutations at the *his*4 locus were cloned by the "integration and excision" method (*14*). Haploid yeast strains were genetically constructed to contain the ICR-170–induced mutation, as well as the *ura*3 mutation *ura*3-52. Strains were transformed with a pBR322 plasmid containing a functional *ura*3 gene and the proximal Eco RI restriction fragment from the *his*4 locus (*15*), selecting for the Ura$^+$ phenotype.

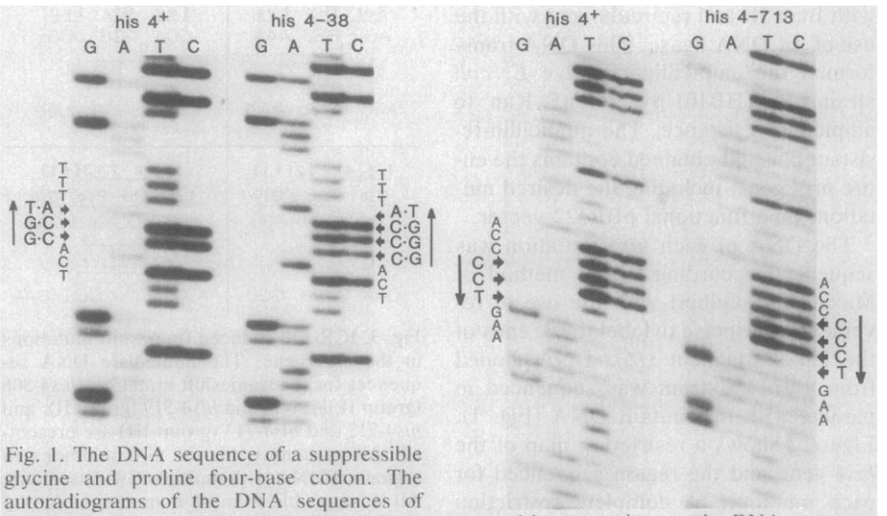

Fig. 1. The DNA sequence of a suppressible glycine and proline four-base codon. The autoradiograms of the DNA sequences of frameshift mutations *his*4-38 and *his*4-713 are presented in comparison to the DNA sequence from the corresponding wild-type region. The antisense strand of *his*4-38 was sequenced and is presented, base-paired, to emphasize the suppressible four-base codon, GGGT (T, thymine); *his*4-713 is the suppressible four-base codon CCCT. Note the +1 addition, as well as the +1 shift in the sequence ladder.

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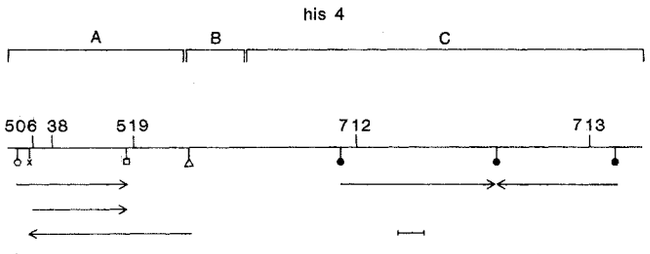

Fig. 2. The sequencing strategy for *his*4 frameshift mutations. The restriction sites used for sequencing the *his*4 frameshift mutations are Sau 3A (○), Xho I (X), Sal I (□), Eco RI (△), and Hind III (●). The arrows define the restriction fragments isolated by polyacrylamide gel electrophoresis. The orientation of the arrows represents the direction of sequencing from the 3′ end which was labeled with reverse transcriptase (*16*). Mutations *his*4-712 and *his*4-713 were strand-separated prior to sequencing. Identical restriction fragments isolated from the wild-type *his*4 gene were similarly labeled and sequenced in parallel with the mutations. The mutations are presented relative to their positions in the *his*4 gene, as defined by genetic data and sequence analysis of *his*4 point mutations and deletions. The scale of the *his*4 map is 100 base pairs.

The Ura$^+$ transformants always represent integration at the *his*4 locus directed by the two *his*4 homologies between the donor and recipient. This results in a tandem duplication of the partial Eco RI restriction fragment of *his*4 from the donor and an intact *his*4 gene from the recipient. These two *his*4 regions are separated by the pBR322 vector sequence which contains the functionally expressed *ura*3 gene. No Ura$^+$ transformant has ever been observed to integrate at the *ura*3-52 locus. Presumably the *ura*3-52 allele does not support an integration event which yields a functional *ura*3 gene. The orientation of the *his*4 fragment on the plasmid was chosen so that restriction of the yeast DNA with Bam HI would cleave once at the far end of the pBR322 plasmid and again on the other side, beyond the complete *his*4 region. For this reason, Ura$^+$, His$^-$ transformants were picked for study as the *his*4 auxotrophy assured the presence of the frameshift mutation within the complete *his*4 region. DNA prepared from the transformants was restricted with Bam HI and recircularized with the use of T4 DNA ligase. This DNA transformed the ampicillin-sensitive *E. coli* strain 6507 (HB101 *pyr*23::Tn5, Kan^r to ampicillin resistance. The ampicillin-resistant plasmid obtained contains the entire *his*4 gene, including the desired mutation, on a functional pBR322 vector.

The DNA of each *his*4 mutation was sequenced according to the method of Maxam and Gilbert with the use of reverse transcriptase to label the 3′ ends of the DNA fragment (*16*). DNA cloned from a *His*4$^+$ strain was sequenced in parallel with the mutant DNA (Fig. 1). Figure 2 shows a restriction map of the *his*4 gene and the region sequenced for each mutation. A complete restriction map and the sequence of the wild-type *his*4 gene have been established (*17*).

Figure 3 displays the DNA sequence of the group I, II, and III mutations as compared with their parental DNA sequences. Each mutation represents an addition of a G·C base pair in a region of multiple tandem G·C base pairs. All frameshift mutations are polar since they destroy the normal *his*4 reading frame. In every case the shift in reading frame introduces premature termination signals soon after the frameshift mutation.

The nonsuppressible group I mutation *his*4-506, is a +1 C addition between the serine codon AGC (A, adenine) and the leucine codon CUG (U, uracil) (Fig. 3). ICR-170 does not induce either internal or external suppressors of this mutation. Apparently, no potential four-base codon generated by the *his*4-506 frameshift mutation is suppressible by tRNA's with four-base anticodons. Either the cell will not tolerate a four-base anticodon in these tRNA's or the efficiency of suppression is too poor to permit isolation of His$^+$ revertants. Furthermore, ICR-170 does not induce internal suppression of this mutation. Although a compensating −1 deletion at the same site or at the proximal glycine codon could restore the correct reading frame, revertants containing these changes have not been isolated by ICR-170 mutagenesis. The possibility also exists that ICR-170 preferentially induces +1 frameshift-type mutations.

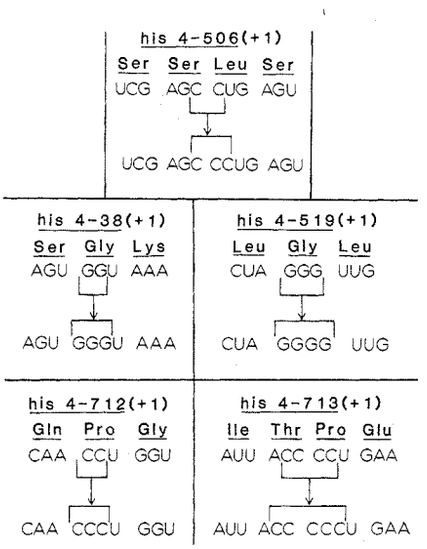

Fig. 3. ICR-170–induced frameshift mutations in the *his*4 gene. The immediate DNA sequences for the frameshift mutations *his*4-506 (group I), *his*4-38 and *his*4-519 (group II), and *his*4-712 and *his*4-713 (group III) are presented. All mutations are +1 G·C additions in regions of DNA with multiple G·C base pairs. All suppressible frameshift mutations (group II and group III) are either glycine or proline four-base codons. The shift in reading frame for each mutation eventually results in a premature termination signal (only shown for *his*4-38 and *his*4-713).

The group II mutations, *his*4-38 and *his*4-519, are each a +1 G addition to the glycine codons GGU and GGG, respectively (Fig. 3). Suppressors of group II mutations have been found in five unlinked genes (*13*, *18*). Each of these suppressors suppresses both *his*4-38 and *his*4-519 and is therefore capable of suppressing both GGGU and GGGG. A likely mechanism of suppression based on suppressor analysis of four-base glycine codons in bacteria (*9*) is a mutation of a glycyl-tRNA gene to a form capable of reading a four-base anticodon and thereby restoring the normal frame. This mechanism is supported by studies on the ICR-170–induced *SUF*5 mutant, which has a glycyl-tRNA with an altered chromatographic profile as compared with that of glycyl-tRNA from wild-type (*13*). Since all group II mutations are suppressed by the same suppressors, including the *SUF*5 suppressor, we propose that all 19 group II mutations are also +1 additions in glycine codons.

Both group III mutations, *his*4-712 and *his*4-713, are +1 C additions in the proline codon CCU (Fig. 3). The finding of the identical four-base codon, CCCU, in both mutations was unexpected as *his*4-712 is not suppressed by some external suppressors that suppress *his*4-713. Six suppressor loci of group III mutations have been identified (*13*, *19*). The suppressors *SUF*7, -8, -9, and -11 suppress only *his*4-713 and not *his*4-712; *SUF*2 and *SUF*10 suppress both *his*4-712 and *his*4-713. The *SUF*2 mutation is induced at high frequency with ICR-170 and is a candidate for a mutation creating a four-base anticodon in a prolyl-tRNA.

The differential suppression of the identical CCCU four-base proline codon of *his*4-712 and *his*4-713 could be explained by several mechanisms. One explanation is that the CCCU codon is suppressed by either *SUF*7, -8, -9, or -11 with low but equal efficiency in *his*4-712 and *his*4-713. The amount of full-length protein produced by this suppression is insufficient to give a His$^+$ phenotype in

either case. However, *his*4-713 is suppressed to a His^+ phenotype because of the ability of the *his*4-713 mutant protein to form an active dimer with the small amount of full-length protein produced as a result of suppression, whereas the *his*4-712 mutant protein cannot form such a dimer. The relative positions of *his*4-712 and *his*4-713 within the *his*4 gene support this explanation. The *his*4-713 mutation is only 134 base pairs from the end of the *his*4 gene and can make a protein that is 95 percent the size of wild-type. The *his*4-712 is more than 1000 base pairs from the end of the gene and would make only a small fragment of the *his*4 protein (less than 62 percent the size of the wild-type protein).

Another explanation for the difference in suppressor efficiency of identical four-base codons could be the effect of "context" (*20*). Studies of suppression of *his*D mutations in *S. typhimurium* suggest that the codons in the neighborhood of a suppressible mutation may affect the efficiency of suppression (*21*). A UAG nonsense mutation in *his*D was not suppressed by a known amber tRNA suppressor. A selection for suppression uncovered a strain that contained a single base change in a codon immediately adjacent to the original UAG mutation. The interpretation of these results was that the amber tRNA suppressor could not be accommodated in a side-by-side interaction with the original neighboring tRNA during translation as a result of steric hindrance. More efficient suppression of the UAG mutation occurs in the revertant because the codon adjacent to the UAG has been altered so that it can be read by a tRNA species that does not interfere with the suppressor tRNA. In a similar way the failure of some suppressors of *his*4-713 to suppress *his*4-712 could be related to the codons flanking the *his*4-712 mutation.

A third explanation for the different suppressibility patterns of *his*4-713 and *his*4-712 is that some suppressors of *his*4-713 do not act at the level of suppression of the CCCU codon but at another four-base codon, within a limited distance, which is not present in the *his*4-712 region. For example, a +1 mutation in the *his*D gene of *S. typhimurium* is suppressed at a site downstream from the original mutation by a suppressor of four-base codons (*21*). Although the four-base codon suppressed was not the one affected by the mutation, the suppression restored the reading frame, permitting expression of the gene. Since a nonsense codon (UGA) immediately follows the *his*4-713 mutation, no suppression can occur distal to the mutation as a result of premature termination of translation. However, "indirect" suppression could still occur at a four-base codon proximal to the *his*4-713 frameshift site, which would restore the normal reading frame.

There may be reading of nontriplet codons even in wild-type yeast strains. Frameshift mutations both +1 and −1 in a monotonous run of T's in the *oxi*-1 gene of the mitochondrion have a leaky phenotype (*22*). The leaky phenotypes could result from nontriplet reading by normal tRNA's. Several reports concerning this phenomenon in bacteria suggest that nontriplet codons are read at low levels both in vitro and in vivo (*23–25*). In vitro translation studies indicate that specific wild-type tRNA's are responsible for the level of nontriplet reading (*24*). The translation of nontriplet codons in vivo may be important for the growth of some bacteriophages (*25*). No evidence of a physiological role for nontriplet reading has been reported in eukaryotes.

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26. We thank F. Chumley, J. Jackson, and G. S. Roeder for their critical review of this manuscript, and F. Ormsbee for help in preparation. Supported by NIH grant CA23441 (to G.R.F.) and a fellowship from the Jane Coffin Childs Memorial Fund for Medical Research (to T.F.D.).

8 November 1980

Influence of Calcium Ion on the Binding of Fibrin Amino Terminal Peptides to Fibrinogen

Abstract. *The affinity of the amino terminal tetrapeptide of the β chain of fibrin, Gly-His-Arg-Pro, for fibrinogen dramatically increases in the presence of 2 millimolar calcium ion. In contrast, there is no significant increase in the affinity of peptides beginning with the amino terminal sequence of the fibrin α chain, Gly-Pro-Arg, in the presence of calcium ions, although the number of binding sites increases. In the latter case, the increased number of sites is due to the α chain analogs binding to the site ordinarily occupied by the β chain analogs. These results indicate that structures at the amino terminus of the fibrin β chain play a more important role in fibrin polymerization when calcium ions are present.*

The formation of a fibrin clot involves removal of small polar peptides (fibrinopeptides A and B) from the amino termini of the α and β chains of fibrinogen by thrombin, polymerization of the resultant fibrin monomers to form a noncovalently bound gel, and covalent cross-linking in the presence of activated factor XIII and calcium ion. Peptides beginning with the sequence Gly-Pro-Arg . . . (Gly, glycine; Pro, proline; Arg, arginine), which corresponds to the fibrin α chain amino terminus that is unveiled when thrombin removes fibrinopeptide A, bind to fibrinogen and inhibit the polymerization of fibrin (*1*). The peptide Gly-His-Arg-Pro (His, histidine), the first four amino acids of the fibrin β chain exposed by the release of fibrinopeptide B, binds to separate sites on fibrinogen and does not inhibit polymerization (*2*). Both types of peptide bind to

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