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1 June 1976; revised 20 August 1976

Is There Selection Against Wobble in Codon-Anticodon Pairing?

Abstract. *Among amino acid codons that require a third-position pyrimidine, there is a significant bias favoring the use of cytidine over uracil in MS2 phage RNA. This could arise from selection against wobble pairing in the interaction of transfer RNA and messenger RNA. Among amino acid codons with fourfold degeneracy, there is a bias favoring pyrimidines over purines.*

In 1966, Crick (*1*) proposed the wobble hypothesis which permitted a single anticodon to recognize more than one codon. This immediately explained why there are not 61 transfer RNA's (tRNA's) and why coding sometimes differed depending on whether the third-position nucleotide was a purine or a pyrimidine.

One of the intriguing possibilities is that the (negative) binding energies of the adenine : uracil (A : U) and guanine : cytidine (G : C) base pairs are sufficiently larger than that for the G : U base pair in the binding of the anticodon of a tRNA to its complementary codon in the messenger to bring about, selectively, the preferential usage of codons with nonwobble pairing for some reason (for example, that errors are then less frequent). The presence of multiple tRNA's for a single amino acid is a complicating factor in any simple attempt to determine whether this suggestion is valid by any method that does not examine the binding energies directly. There do not, however, appear to be multiple tRNA's with different anticodon specificities for amino acids where the third position of the codon must be a pyrimidine, that is, where a third-position purine would change the amino acid encoded (*2*, *3*). In these cases, the nucleotide involved in pairing with the third nucleotide of the codon is invariably a guanine, albeit sometimes modified. The codons are AAY (Asn), AGY (Ser), CAY (His), GAY (Asp), UAY (Tyr), UGY (Cys), and UUY (Phe), where Y stands for a pyrimidine (*3*). These will be called the pyrimidine-restricted codons. At least one tRNA anticodon is known for each of these amino acids except asparagine, including, in every case, a tRNA from *Escherichia coli*, the host of MS2 phage.

If the hypothesis of selection against wobble pairing is correct, then one should see a preferential utilization of third position C over U in codons for the above cases. This can now be tested since the messenger RNA for the A protein, coat protein, and replicase for the MS2 phage have all been completely sequenced (*4*). This represents a total of 1071 codons, of which 235 code for the cases given above. They are distributed (142 C's plus 93 U's) as shown in Table 1. Aspartate is the only case where more third-position U than C codons are used. The overall bias is significant. Assuming that an equal number of each of the two codons for any one amino acid is expected, we would anticipate the column totals both to be 117.5, on average. On this basis, we find $\chi_1^2 = 10.22$. This means that the probability of this much deviation from expectation is $< .002$. The conclusion must be that there is a bias operating among pyrimidine-restricted codons that favors codons ending in C over those ending in U. Thus, selection has indeed favored the nonwobble pairing. It should be noticed, however, that energy considerations were the basis upon which the question was originally framed, and these results, while consistent with those considerations, do not prove their validity. Any mechanism that would produce the same bias is, a priori, an equally acceptable explanation of that bias. It would therefore be of interest to determine the binding energies of these triplets to their anticodon.

A similar argument and analysis cannot be made for the purine-restricted codons because of the existence, at least in some cases, of tRNA's that specifically recognize codons ending in G only. Apart from any difficulty this imposes in framing a statistical hypothesis, there is no significant difference between the number of purine-restricted codons ending in G and in A.

The remaining codons are, mostly, characterized by fourfold degeneracy. I had no hypothesis regarding them but, for the sake of completeness, they too were examined. They too showed a bias, but in this case it was a preference for third-position pyrimidines over purines (Table 2). Nearly 60 percent (326) of the 565 fourfold degenerate codons contained a third-position pyrimidine. This gives $\chi^2 = 13.4$. The probability of this many pyrimidines in a random sample assuming equal expectations of each is $< .0003$. It is hardly clear why this should be so. It is certainly not the case that the freedom from coding constraint in the fourfold degenerate codons is being used to bring toward random the frequencies of the four necleotides in the third position since that would require a bias in favor of purines rather than pyrimidines in order to compensate for the opposite bias in the restricted codons. There is a slight excess of purines over pyrimidines in the first two codon nucleotide positions, and one might suggest an optimization of secondary structure could be achieved by a corresponding rel-

Table 1. The distribution of pyrimidine restricted codons in MS2 phage. In the first colum shown, the first two nucleotides of those codons that must end in a pyrimidine in order to code for the amino acid in the last column. The center two columns show the frequency of utilization of these codons in MS2 phage according to whether the third position base is cytidine or uracil.

Codons	--C	--U	
AA-	28	17	Asn
AG-	16	8	Ser
CA-	9	6	His
GA-	22	28	Asp
UA-	32	9	Tyr
UG-	6	6	Cys
UU-	29	19	Phe
Total	142	93	

Table 2. The distribution of fourfold degenerate codons in MS2 phage. In the first column, the first two nucleotides of those codons whose amino acid, shown in the last column, is specified regardless of the third nucleotide. The center two columns show the frequency of utilization of these codons in MS2 phage according to whether the third position base is a pyrimidine (Y) or a purine (R).

Codons	--Y	--R	
AC-	39	27	Thr
CC-	28	22	Pro
CG-	41	21	Arg
CU-	41	24	Leu
GU-	47	44	Ala
GG-	53	28	Gly
GU-	42	35	Val
UC-	35	38	Ser
Total	326	239	

ative excess of pyrimidines over purines in the third position. The expected distribution in that case does not, however, match the observed distribution very well ($\chi_1^2 = 7.7$, $P < .01$). Thus secondary structure is unlikely to be the principal determinant of the observed bias.

One remaining possibility is that the frequency of purines and pyrimidines in the fourfold degenerate codons is necessarily positively correlated to the frequency of amino acids whose third codon position is necessarily a purine or pyrimidine. This could presumably arise through a kind of equilibrium between those amino acids that must be coded by third-position purines or pyrimidines and those that are indifferent to the nature of the third nucleotide. There are 235 pyrimidine restricted codons in MS2 phage and 169 purine restricted codons (the numbers do not include tryptophan, methionine, or isoleucine codons). Thus 0.582 of the restricted codons are pyrimidine restricted, and 0.582 of 565 fourfold degenerate codons is 328.7 codons—a value not significantly different from, indeed it is remarkably close to, the 326 actually observed. One cannot, however, prove a null hypothesis statistically. Failure to reject may simply mean that the sample size was too small or the test too ill-suited to discriminate among alternatives. Moreover, there is no reason why, if the basic concept is correct, all four nucleotides individually ought not to display a similar distribution in the two classes of codons, restricted and fourfold degenerate. Since there is, among fourfold degenerate codons ending in pyrimidine, a 10 percent excess of U over C in the third position (171 as compared to 155) these two nucleotides vary significantly in frequency from that expected on the basis of their frequency in the pyrimidine restricted codons.

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3. The abbreviations for the amino acids in the order mentioned are Asn, asparagine; Ser, serine; His, histidine; Asp, aspartic acid; Tyr, tyrosine; Cys, half-cystine; Phe, phenylalanine; Ala, alanine; Pro, proline; Gly, glycine; and Val, valine.
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5. Supported by NSF grant BMS 75-20109.

7 June 1976; revised 3 September 1976

Bone Compressive Strength: The Influence of Density and Strain Rate

Abstract. *The compressive strength of bone is proportional to the square of the apparent density and to the strain rate raised to the 0.06 power. This relationship is applicable to trabecular and compact bone, and provides clinical guidelines for predicting bone strength on the basis of x-ray and densitometric examination.*

A typical long bone is composed of bone tissue in two forms of structural organization. Compact bone forms the cortex of the central shaft (diaphysis) and the thin outer wall of the flared end (metaphysis). Trabecular bone is continuous with the inner surface of the cortex and exists as a three-dimensional lattice of bony plates and columns (trabeculae). The trabeculae divide the interior volume of the bone into intercommunicating pores, which are filled with a variable mixture of red and yellow marrow. The characteristic dimensions of the pores vary considerably throughout the bone interior, resulting in a structure of variable density (*1*).

The differences in morphology and mechanical behavior of compact and trabecular bone have prompted many researchers to investigate these two bone types as if they were different materials (*2*). Recently, however, some workers have attempted to view trabecular bone as a porous structure comprised of bone tissue with the same microscopic mechanical properties as compact bone (*3–8*). The purpose of the study reported here was to further explore the hypothesis that all bone can be mechanically viewed as a single material. We were also interested in deriving a simple expression to describe the compressive strength of all bone as a function of apparent density and the applied strain rate.

In order to determine the compressive strength of trabecular bone spanning a large density range, we examined both human and bovine bone. In addition to specimen density, the effect of strain rate and the effect of marrow in situ were examined. Specimens of compact bone were not tested since there are adequate data on the mechanical properties of compact bone in the literature. One hundred cylindrical specimens of human trabecular bone and 24 specimens of bovine trabecular bone were machined under continuous irrigation. The specimens were 5 mm thick and 10.3 mm in radius, were removed from human tibial plateaus and bovine femoral condyles, and were oriented with their axes paral-

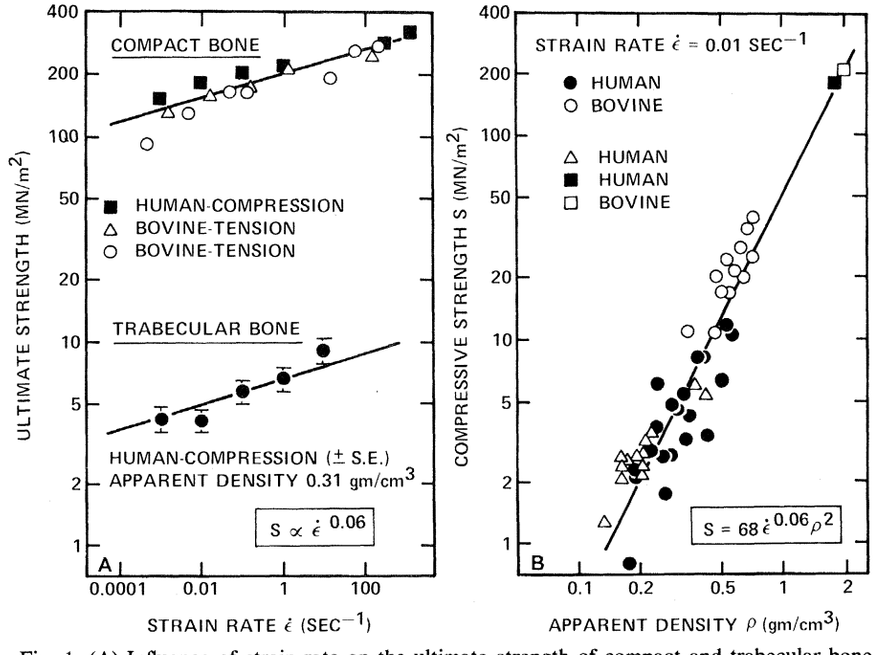

Fig. 1. (A) Influence of strain rate on the ultimate strength of compact and trabecular bone tested without marrow in situ. Data denoted by filled circles are from this study (± S.E.), filled squares are from (*11*), open triangles from (*12*), and open circles from (*13*). (B) Influence of apparent density on the compressive strength of trabecular and compact bone. Data denoted by filled and open circles are from this study, open triangles are from (*10*), filled and open squares from (*11*).