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# Selective Associations of Hormonal Steroids with Aminoacyl Transfer RNAs and Control of Protein Synthesis*

**(testosterone/progesterone/estradiol/yeast tRNA/*E. coli* tRNA)**

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**ABSTRACT The hormonal steroids progesterone, estradiol, testosterone, and 5α-dihydrotestosterone bind to aminoacyl-tRNA, but not to deacylated tRNA, implying that a change in conformation of tRNA occurs on aminoacylation. Binding is restricted to a few tRNA species and depends on the structure of both tRNA and steroid. There is one binding site per aminoacyl-tRNA molecule, the specificity of which appears to depend on a restricted, single-stranded loop sequence and on the tRNA conformation. By binding to an aminoacyl-tRNA, a steroid can control polypeptide synthesis in a model *in vitro* system by inhibiting chain elongation under conditions where aminoacyl-tRNA concentration is rate-limiting.**

Certain hormonal steroids bind to single-stranded regions of polynucleotides and, in so doing, exhibit a specific requirement for guanine [except for estradiol, which will also bind to inosine (1)]. These associations require two steroid functional groups (2), occur internally along polynucleotide chains (3), and appear to depend on the formation of two hydrogen bonds (2, 4), in addition to hydrophobic forces. Construction of models (1, 2, 4) indicates that each steroid can fit well along a trinucleotide in which guanine residues occupy one or both terminal positions, depending on the steroid in question. For the optimal binding of progesterone and testosterone, close stacking of the bases is favorable (1, 3); if the contribution to stacking energies of nearest-neighbor bases and the observed role of polymer conformation (3) is taken into account, it appears that a longer nucleotide sequence may more completely define a binding site (4).

To assign any biological role to this type of interaction ideally requires the use of nucleic acid molecules of defined structure and function. For this reason transfer RNAs are attractive as a class; in addition, they represent potential control points in the regulation of gene translation. In our early experiments (1), no binding was observed between steroids and crude tRNA, since steroids bind to aminoacyl-tRNA, but not to deacylated tRNA, and binding is restricted to only a few tRNA species. Selectivity in these associations depends on both tRNA and steroid structures; by our use of partially purified tRNAs of known primary structure, we are able to suggest possible steroid-binding sites. A correlation was found between steroid binding and inhibition of aminoacyl transfer from the charged tRNA in an *in vitro* protein-synthesizing system under conditions where the concentration of the aminoacyl-tRNA was rate-limiting. Only progesterone, estradiol, testosterone, and 5α-dihydrotestosterone have been examined; other steroids also bind to certain nucleic acids (1, 4).

## MATERIALS AND METHODS

*Radiochemicals.* The following steroids and amino acids were obtained from the New England Nuclear Corp.: [1,2-$^3$H]testosterone (50 Ci/mmol), [1,2-$^3$H]progesterone (50 Ci/mmol), [6,7-$^3$H]17β-estradiol (50 Ci/mmol), [1,2-$^3$H]5α-dihydrotestosterone (46 Ci/mmol), [U-$^{14}$C]L-phenylalanine (384 Ci/mol), [U-$^{14}$C]L-tyrosine (367 Ci/mol), [U-$^{14}$C]L-serine (137 Ci/mol), [U-$^{14}$C]L-alanine (123 Ci/mol), [U-$^{14}$C]L-valine (200 Ci/mol).

*Nucleic Acids.* Yeast tRNA was obtained from Miles Laboratories; tRNA was isolated by phenol extraction from *Escherichia coli* K-12 and from the pH-5 fractions of rat liver (5). tRNA was fractionated by chromatography on BD-cellulose (6); some of these studies have used tRNA fractions, fractionated from brewer's yeast (Boehringer) by Dr. I. C. Gillam. Yeast tRNA$^{Phe}$ without the *Y* base (fraction EF-*Y*), prepared by mild acid hydrolysis of fraction EF (7), was also prepared by Dr. Gillam. Concentrations of tRNA were determined spectrophotometrically ($E^{1\%}_{1cm}$ = 240 at 260 nm). Poly(U) was obtained from Miles Laboratories.

*Preparation of Aminoacyl tRNAs.* Aminoacyl-tRNA synthetases from *E. coli* K-12 (early-log) were prepared from the S-100 fraction by DEAE-cellulose chromatography (8) and stored at −170°C in 50% glycerol. Aminoacyl-tRNA synthetases from baker's yeast (Fleishman) were prepared according to Hoskinson and Khorana (9). Assay of amino-acid acceptor activity was prformed according to Berg *et al.* (10); the charged tRNAs were recovered by phenol extraction and repeated ethanol-precipitation (11). For preparation of aminoacyl-tRNA for steroid binding analysis, this procedure was scaled up using one or more [$^{12}$C]aminoacids, the extent of charging being assessed from preparations made in parallel using [$^{14}$C]aminoacid(s). Aminoacyl-tRNA from rat liver was prepared by the method of Moldave (5). All aminoacyl-tRNA preparations were stored at −20°C.

*Steroid Binding Assays.* Equilibrium dialysis was performed as described (1), in a pH 7.3 buffer containing 0.01 M Tris·HCl–0.01 M $MgCl_2$–1 mM $Na_2EDTA$. Apparent binding constants $nK$ ($M^{-1}$) were expressed on the basis $n$ = the number

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TABLE 1. *Binding of steroids to unfractionated tRNA*

| tRNA source | $nK(M^{-1})$* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Progesterone | | Estradiol | | Testosterone | | 5α-Dihydrotestosterone | |
| | tRNA | aminoacyl-tRNA | tRNA | aminoacyl-tRNA | tRNA | aminoacyl-tRNA | tRNA | aminoacyl-tRNA |
| *E. coli* | 237 | 560 | 267 | 552 | 151 | 272 | 125 | 300 |
| Yeast | 106 | 775 | 100 | 688 | 240 | 388 | 0 | 438 |
| Rat liver | 0 | 600 | 0 | 316 | 0 | 468 | 0 | 425 |

* The apparent binding constant, $nK(M^{-1})$, is expressed per mole of tRNA, assuming an average molecular weight for tRNA of 25,000. No corrections have been made for the extent of charging of the various tRNAs.

of binding sites per tRNA molecule. Values of $K$ and $n$ were derived from binding isotherms by the method of Scatchard (12), using a polymer concentration of about 1 mg/ml and a series of steroid concentrations ranging from $10^{-10}$ to $10^{-5}$ M. The extent of deacylation of the aminoacyl-tRNA during the equilibration period was assessed by following, in experiments run in parallel but without steroid, the release of [$^{14}$C]-aminoacid from the relevant [$^{14}$C]aminoacyl-tRNA. The appropriate correction was applied in the determination of [$^{12}$C]-aminoacyl-tRNA concentration. The extent of deacylation depended on the amino acid concerned: e.g., Val-tRNA$^{Val\ 1}_{coli}$ remained 87% charged, while Phe-tRNA$^{Phe}_{yeast}$ remained only 65% charged after the 48-hr dialysis.

*Assay of [$^{14}$C]Aminoacid Incorporation.* Ribosomes and supernatant enzymes were isolated from early-log phase *E. coli* MRE 600. Ribosomes were prepared according to Nirenberg *et al.* (13); 100,000 × *g* supernatant enzymes were prepared (13) and used as such, or purified further by streptomycin sulfate and ammonium sulfate precipitation (14). In most instances, the amino acid incorporating system consisted of the following components, in a total volume of 0.5 ml: 50 μmol Tris·HCl (pH 7.8), 5 μmol Mg acetate, 25 μmol KCl, 3 μmol 2-mercaptoethanol, 0.15 μmol GTP, 2.5 μmol phosphoenolpyruvate, 1 μg of pyruvate kinase, 60 μg poly(U), 5–200 pmol [$^{14}$C]L-aminoacyl-tRNA, 22 $A_{260}$ units of ribosomes, 50 μl of supernatant enzymes. Steroids were added as indicated. Incubation was at 37°C for 15 min (13).

## RESULTS

### Steroid associations with unfractionated tRNA

All four steroids examined bound to aminoacylated tRNAs, but less well, or not at all, to the corresponding uncharged tRNAs from *E. coli*, yeast, and rat liver (Table 1). This result implies that aminoacylation of tRNA favors association with steroids. Higher concentrations (5 mg/ml) of uncharged tRNA were used in the present experiments than in those reported previously where no binding was found (1). The low binding with uncharged tRNA observed here may reflect incomplete deacylation or binding of steroids to contaminant RNA fragments (detectable by acrylamide gel electrophoresis).

### Steroid associations with aminoacyl-tRNA species

In view of the known specificity of binding-site requirements, it is possible that only certain species of tRNA will bind steroids. If aminoacylation of tRNA is necessary for steroid binding, any such specificity can be examined by charging only one tRNA species at a time. Experiments of this kind were per-

TABLE 2. *Binding of steroids to partially purified tRNA species*

| tRNA, Column fraction† | Amino acid present in aminoacyl-tRNA | $K(M^{-1})$* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Progesterone | | Estradiol | | Testosterone | | 5α-Dihydrotestosterone | |
| | | tRNA | aminoacyl-tRNA | tRNA | aminoacyl-tRNA | tRNA | aminoacyl-tRNA | tRNA | aminoacyl-tRNA |
| *Yeast* | | | | | | | | | |
| C | Ala | 0 | 0 | 926‡ | 900 | 0 | 0 | 0 | 0 |
| H | Ser | 0 | 82,000 | 0 | 43,000 | 0 | 0 | 0 | 0 |
| J | Ser | 0 | 26,000 | 0 | 5,000 | 0 | 0 | 0 | 0 |
| J | Tyr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| EF | Phe | 0 | 109,000 | 0 | 84,000 | 0 | 32,000 | 0 | 85,000 |
| EF-Y§ | Phe | 0 | 172,000 | 0 | 166,000 | 0 | 105,000 | 0 | 208,000 |
| *E. coli* | | | | | | | | | |
| A | Val (*1*) | 0 | 0 | 0 | 0 | 0 | 5,000 | 0 | 0 |
| C | Val (*2*) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | Phe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The calculation of binding constants assumes that there is only one binding site per aminoacyl-tRNA molecule. This assumption appears to be justified (see Fig. 1).

† Fractions obtained by BD-cellulose chromatography (6).

‡ This calculation assumes that only tRNA$^{Ala}$ is binding the steroid.

§ In this case, the *Y*-base of tRNA$^{Phe}$ was removed from the ethanol-eluted fraction (EF) by mild acid hydrolysis (7).

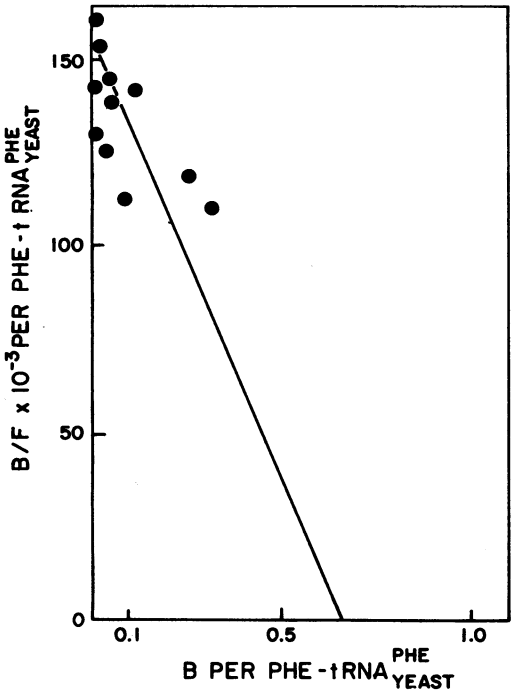


FIG. 1. Scatchard plot of binding of progesterone to Phe-tRNA$^{Phe}_{yeats}$. Equilibrium dialysis at 4°C for 48 hr.

formed with tRNA fractions derived by chromatography on BD-cellulose, which reduced background and allowed analysis of steroid interactions with separable isoaccepting tRNA species. We examined only tRNAs (from yeast and *E. coli*) whose primary sequences have been established, to allow for correlation of binding sites with nucleotide sequences.

Marked selectivity of association was indeed observed, being dependent both on tRNA species and on steroid structure (Table 2); in all cases where binding did occur aminoacylation was a prerequisite. All four steroids bound to Phe-tRNA$^{Phe}_{yeast}$; binding was not associated with the lipophilic *Y* base, since it was not eliminated by excision of this residue (Table 2). Binding to the aromatic amino acid was excluded by the absence of steroid association with Phe-tRNA$^{Phe}_{coli}$ and Tyr-tRNA$^{Tyr}_{yeast}$, and by the observed binding in cases where the amino acid is not aromatic. Only progesterone and estradiol bound to Ser-tRNA$^{Ser}_{yeast}$ species in two different chromatographic-column fractions. Testosterone (but not the other three steroids) bound to Val-tRNA$^{Val\ 1}_{coli}$, but not to the isoaccepting Val-tRNA$^{Val\ 2}_{coli}$.

TABLE 3. *Effects of steroids on the incorporation into protein of [$^{14}$C]phenylalanine from E. coli and yeast [$^{14}$C]phenylalanyl tRNA*

| [$^{14}$C]Phe-tRNA$^{Phe}$ source | Steroid* addition | Incorporation of [$^{14}$C]Phe, % of control |
|---|---|---|
| *E. coli* | None | 100 |
| | Progesterone | 85–100 |
| | Testosterone | 100 |
| Yeast | None | 100 |
| | Progesterone | 43–75† |
| | Testosterone | 60–80† |

* Steroid concentration was about $10^{-6}$ M and Phe-tRNA$^{Phe}$ concentration was about $5 \times 10^{-8}$ M.

† The extent of inhibition varied among experiments and depended on parameters such as the concentrations of ribosomes, enzymes, and Phe-tRNA$^{Phe}$. The effects of varying some of these parameters are considered for yeast Phe-tRNA$^{Phe}$ in Fig. 2.

### Binding isotherms

Scatchard analysis of the association between progesterone and Phe-tRNA$^{Phe}_{yeast}$ in the presence of 10 mM $Mg^{+2}$ is shown in Fig. 1. On the basis of a molecular weight of 25,000, the number of binding sites, *n*, was found to be between 0.2 and 1.0 per tRNA molecule, and the binding constant *K* was found to be of the order of $10^5$ ($M^{-1}$). There is thus a maximum of one progesterone-binding site per Phe-tRNA$^{Phe}_{yeast}$ molecule. Taken together with the overall selectivity of binding and the requirement of the aminoacyl form of the tRNA, this suggests a very specific fit of the steroid with the tRNA molecule.

### Steroids and aminoacylation of tRNA

The fact that steroids bind only to the aminoacyl form of tRNA makes it unlikely that these molecules would influence the aminoacylation reaction, except perhaps by association with the reaction product. In experiments using each of the above four steroids in concentrations up to $10^{-6}$ M, no effect was observed on the extent or rate of aminoacylation by the synthetases from *E. coli* or yeast of any of the tRNAs listed in Table 2.

### Steroid effects on polypeptide synthesis

It is possible that steroid binding might influence the subsequent participation of an aminoacyl-tRNA in polypeptide synthesis. Although poly(U)-directed polyphenylalanine synthesis at 10 mM $Mg^{+2}$ permits analysis only of chain elongation (16), it provides a useful model for answering this question in a simple fashion. This system has the advantage of clarity: poly(U) does not bind steroids (1), eliminating any possible steroid effect on the message and Phe-tRNA$^{Phe}_{yeast}$, which binds steroids and Phe-tRNA$^{Phe}_{coli}$, which does not, can be compared. Incorporation of phenylalanine from Phe-tRNA$^{Phe}_{yeast}$ was inhibited most effectively by progesterone, and less so by testosterone, whereas incorporation from the corresponding *E. coli* tRNA was essentially unaffected (Table 3). Although some parameters of the incorporation system were varied over a series of experiments of this type, the two aminoacyl-tRNAs were compared in parallel in each case. The steroid effects are consistent with the observed differences in binding with the two aminoacyl-tRNAs (Table 2). Inhibition was evident only in the range over which the concentration of yeast Phe-tRNA$^{Phe}$ is rate-limiting (16) (a Phe-tRNA$^{Phe}$ concentration of $1 \times 10^{-8}$ to $2 \times 10^{-7}$ M, to give a Phe-tRNA$^{Phe}$ to ribosome ratio of 0.02–0.24), and the effect diminished as the Phe-tRNA$^{Phe}$ concentration and the Phe-tRNA$^{Phe}$ to ribosome ratio increased (Fig. 2). The steroid inhibition curve shifts to the left or to the right according to steroid concentration.

## DISCUSSION

### Nature of steroid-binding sites on aminoacyl-tRNAs

The requirement of the aminoacyl form of tRNA, the presence of a single tRNA binding site, and the selectivity with respect to tRNA and steroid structures imply a very specific fit of a steroid with a tRNA molecule. Our present data do not permit precise identification of the binding site on a given tRNA,

but correlation of binding data with primary structure suggests certain possibilities. Binding requires a single-stranded nucleotide sequence (1). tRNA is thought to have the form of a folded clover-leaf, with the side loops folded in a somewhat uncertain manner and the anticodon loop relatively free. These loops contain sequences that could be single-stranded under certain conditions. The sequences of these regions of those tRNAs where binding studies have been done are shown in Table 4. With the exception of Ala-tRNA$^{Ala}_{yeast}$ (discussed below), in all cases where binding was observed at least one sequence of the type GXG is present; in no case where steroid was not bound is such a sequence present.

Since all four steroids bind to poly(G), and model building indicates that all could fit to the sequence GGG (2, 4), we suggest that the binding site on Phe-tRNA$^{Phe}_{yeast}$ could be GGG in the dihydrouracil loop, or DGGGA if a pentanucleotide is necessary to define the site, in conformity with stacking requirements (4). However, this same sequence occurs in Val-tRNA$^{Val\ 1}_{coli}$ (Table 4), which binds only testosterone, suggesting that in addition to the requisite primary sequence, tRNA conformation may play a role in determining binding specificity. This degree of specificity is remarkable, since it permits discrimination between steroids as similar in structure as testosterone and 5$\alpha$-dihydrotestosterone.

The thermodynamic data suggest that the binding site is relatively hydrophobic. Thus, binding constants of 5000–100,000 are greater than might be expected from two hydrogen bonds in water: association of the codons AUG or GUG with the anticodon loop of *E. coli* tRNA$^{Met}_{f}$ may involve as many as seven hydrogen bonds, but the $K$ values are only about 1200 (24). In solution the anticodon loop is probably more exposed to water than certain other regions of the tRNA molecule. The finding of a binding constant for the association of estradiol with tRNA$^{Ala}_{yeast}$ of about 900, whether this tRNA is aminoacylated or deacylated (Table 4), suggests that the binding site in this case might be the anticodon GCI$^{me}$, which could be expected to bind estradiol but not other steroids (1). The somewhat different values of the binding constants of progesterone and estradiol with two Ser-tRNA$^{Ser}_{yeast}$ fractions (*H* and *J*, Table 4), probably reflect the differing proportions

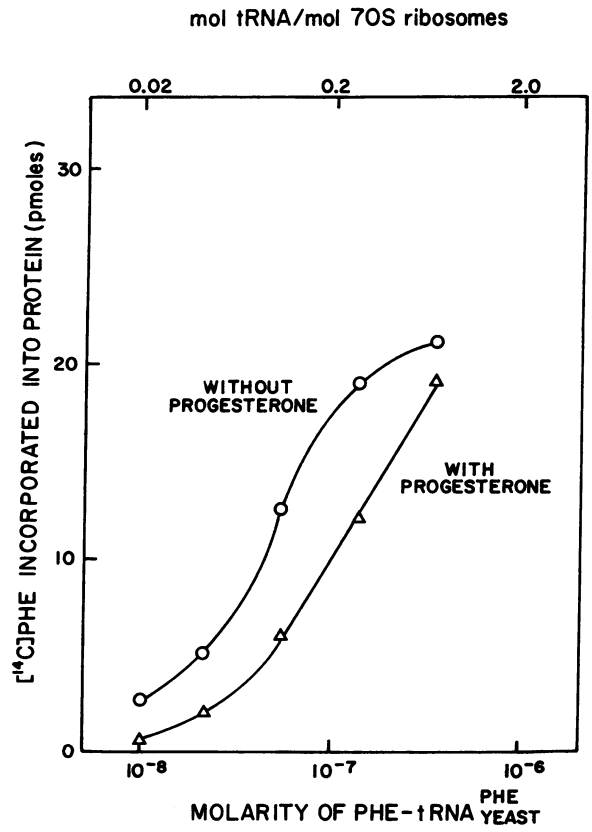


FIG. 2. Effect of progesterone on the incorporation of [$^{14}$C]-phenylalanine into protein as a function of Phe-tRNA$^{Phe}_{yeast}$ concentration. The ribosome concentration was 2 $\mu$M, and progesterone was 5 $\mu$M.

in each fraction of tRNA$^{Ser\ 1}$ and tRNA$^{Ser\ 2}$. These tRNAs contain one or two copies of a conceivable binding sequence, GAG; possibly they differ in conformation as well as having a small difference in primary structure.

There thus appear to be two general requirements for selective binding of steroids: a particular single-stranded nucleo-

TABLE 4. *Steroid binding and nucleotide sequences of tRNA loop regions*

| Steroid binding | | | | Aminoacyl-tRNA | Nucleotide sequence | | | Ref. |
|---|---|---|---|---|---|---|---|---|
| T‡ | DT‡ | P‡ | E‡ | | D‡ Loop | Ac‡ Loop | $\psi$‡ Loop | |
| + | + | + | + | $Phe_{yeast}$ | AGDDGGGA | *CUGAAYA | T$\psi$CGAUC | 18 |
| − | − | − | − | $Phe_{coli}$ | AGDCGGDA | UUGAAA | T$\psi$CGAUU | 19 |
| − | − | − | ± | $Ala_{yeast}$ | GUAGDCGGDA | UUIGCI$\psi$ | T$\psi$CGAUU | 20 |
| − | − | + | + | $Ser^{2}_{yeast}$ | GAGD*GGDDAA | $\psi$UIGAAA | T$\psi$CGAGU | 21 |
| − | − | + | + | $Ser^{1}_{yeast}$ | GAGD*GGDDAA | $\psi$UIGAAA | T$\psi$CAAAU | 21 |
| − | − | − | − | $Tyr_{yeast}$ | AAGDD*GGDDDAA | CUG$\psi$AAA | T$\psi$CG*ACU | 22 |
| + | − | − | − | $Val^{1}_{coli}$ | AGCDGGGA | CUXACAA | T$\psi$CGAUC | 23 |
| − | − | − | − | $Val^{2}_{coli}$† | | | | |

‡ T = Testosterone, DT = 5$\alpha$-dihydrotestosterone, P = progesterone, E = estradiol, D = dihydrouracil, Ac = anticodon, $\psi$ = pseudouridine.

* Modified bases or sugars, usually methylated.

† Although the complete sequence of this tRNA is not published, the D loop is known to contain only GG, not GGG (B. G. Barrell, personal communication). Possible binding-site sequences are underlined.

tide sequence and a particular tRNA tertiary structure. It will be important to determine if there is a linear nucleotide code for each steroid.

**Structural differences between tRNA and aminoacyl-tRNA**

The observed binding of steroids to aminoacyl-tRNA and not to deacylated tRNA implies that a change in structure of a tRNA molecule occurs on aminoacylation, since a previously absent steroid binding site is then made available. In the example that has been examined most thoroughly, Phe-$tRNA^{Phe}_{yeast}$, not all the G residues of the dihydrouracil loop sequence GGG are available in the deacylated tRNA. While $G_{20}$ reacts with kethoxal, $G_{18}$ and $G_{19}$ do not (25), implying that the latter residues are involved in base-pairs elsewhere in the tRNA molecule or are protected by tertiary folding. If this is indeed the binding site, $G_{18}$ and $G_{19}$ must become available as the consequence of altered conformation after aminoacylation. The present data support the existence of structural differences between deacylated and aminoacyl-tRNA (26–31).

**Steroids in the control of protein synthesis**

The negative control by steroids of polyphenylalanine synthesis from Phe-$tRNA^{Phe}_{yeast}$ *in vitro* can only be considered as a model. However, the correlation with aminoacyl-tRNA binding, the dependence of the extent of control on steroid and aminoacyl-tRNA concentrations, and the observation that this control is operative only when the aminoacyl-tRNA concentration is rate-limiting, suggest the possibility that protein synthesis could be regulated by such a means.

Anderson (16) has demonstrated that the rate of translation of poly(A, G) in an *in vitro* protein-synthesizing system derived from *E. coli* is limited by the amount of the $tRNA^{Arg}$-recognizing code words, AGA and AGG. He suggested that the rate of protein synthesis may be regulated by the slowing of polypeptide-chain propagation at certain codons due to the presence of rate-limiting tRNA species. This concept implies the existence of regulatory codons in addition to the initiator (AUG, GUG), and terminator (UAA, UAG, UGA) codons.

It will be of interest to search for steroid-binding, minor eukaryote tRNA species that recognize regulatory codons. The end result of a primarily negative control would depend on the functions of those families of proteins whose synthesis is immediately regulated by steroids: if these had repressor functions, the ultimate effect on net protein or specific enzyme synthesis would be stimulation.

We thank Dr. I. C. Gillam for the gift of yeast tRNA fractions, Dr. M. Singer for *E. coli* strains, and Dr. B. G. Barrell for permission to quote unpublished sequence data. A preliminary report of some of this work has been presented: Chin, R. C., and C. Kidson, *Fed. Proc.*, **29**, 866 (1970).

1. Cohen, P., and C. Kidson, *Proc. Nat. Acad. Sci. USA*, **63**, 458 (1969).
2. Cohen, P., R. C. Chin, and C. Kidson, *Biochemistry*, **8**, 3603 (1969).
3. Kidson, C., A. Thomas, and P. Cohen, *Biochemistry*, **9**, 1517 (1970).
4. Kidson, C., P. Cohen, and R. C. Chin, in *The Sex Steroids: Molecular Mechanisms*, ed. K. W. McKerns (Appleton-Century-Crofts, New York), in press.
5. Moldave, K., *Methods Enzymol.*, **6**, 757 (1963).
6. Gillam, I., S. Millward, D. Blew, B. Tigerstrom, E. Wimmer, and G. M. Tener, *Biochemistry*, **6**, 3043 (1967); Armstrong, D. J., W. J. Burrows, F. Skoog, K. L. Roy, and D. Söll, *Proc. Nat. Acad. Sci. USA*, **63**, 834 (1969).
7. Thiebe, R., and H. B. Zachau, *Eur. J. Biochem.*, **5**, 546 (1968).
8. Muench, K. H., and P. Berg, in *Procedures in Nucleic Acid Research*, ed. G. L. Cantoni and D. R. Davies (Harper and Row, New York, 1966), p. 375.
9. Hoskinson, R. M., and H. G. Khorana, *J. Biol. Chem.*, **240**, 2129 (1965).
10. Berg, P., F. H. Bergmann, E. J. Ofengand, and M. Dieckmann, *J. Biol. Chem.*, **236**, 1726 (1961).
11. von Ehrenstein, G., and D. Dais, *Proc. Nat. Acad. Sci. USA*, **50**, 81 (1963).
12. Scatchard, G., *Ann. N.Y. Acad. Sci.*, **51**, 660 (1949).
13. Nirenberg, M., J. H. Matthaei, and O. W. Jones, *Proc. Nat. Acad. Sci. USA*, **48**, 104 (1962).
14. Nathans, D., and F. Lipmann, *Proc. Nat. Acad. Sci. USA*, **47**, 497 (1961).
15. Lipmann, F., *Science*, **164**, 1024 (1969).
16. Anderson, W. F., *Proc. Nat. Acad. Sci. USA*, **62**, 566 (1969).
17. RajBhandary, U. L., S. H. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson, and H. G. Khorana, *Proc. Nat. Acad. Sci. USA*, **57**, 751 (1967).
18. Barrell, B. G., and F. Sanger, *FEBS. Lett.*, **3**, 275 (1969).
19. Holley, R. W., J. Apgar, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J. R. Penswick, and A. Zamir, *Science*, **147**, 1462 (1965).
20. Zachau, H. G., D. Dütting, and H. Feldman, *Angew. Chem. Int. Ed. Engl.*, **5**, 422 (1966).
21. Madison, J. T., G. A. Everett, and H. Kung, *Science*, **153**, 531 (1966).
22. Yaniv, M., and B. G. Barrell, *Nature*, **222**, 278 (1969).
23. Takemura, S., T. Mizutani, and M. Miyazaki, *J. Biochem. (Tokyo)*, **63**, 277 (1968).
24. Uhlenbeck, O. C., J. Baller, and P. Doty, *Nature*, **225**, 508 (1970).
25. Litt, M., *Biochemistry*, **8**, 3249 (1969).
26. Tissiéres, A., S. Bourgeois, and F. Gros, *J. Mol. Biol.*, **7**, 100 (1963).
27. Gantt, R. R., S. W. Englander, and M. V. Simpson, *Biochemistry*, **8**, 475 (1969).
28. Kaji, H., and Y. Tanaka, *Biochim. Biophys. Acta*, **138**, 642 (1967).
29. Skoultchi, A., Y. Ono, H. M. Moon, and P. Lengyel, *Proc. Nat. Acad. Sci. USA*, **60**, 675 (1968).
30. Hashizume, H., and K. Imahori, *J. Biochem. (Tokyo)*, **61**, 738 (1967).
31. Adler, A. J., and G. D. Fasman, *Biochim. Biophys. Acta*, **204**, 183 (1970).