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DISTRIBUTION OF ISOACCEPTING tRNAs AND CODONS FOR PROLINE AND GLYCINE IN COLLAGENOUS AND NONCOLLAGENOUS CHICKEN TISSUES

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SUMMARY. The relation between codon usage and tRNA content for proline and glycine, the major constituents of collagen, was studied in two tissues : the magnum of laying hen oviduct and the leg tendons of chick embryo where collagen is produced. Although the relative contents of $tRNA^{Gly}_{GCC}$ and $tRNA^{Pro}_{IGG}$ in tendons, as compared to magnum indicate a specialization of the tRNA population for collagen synthesis, the distribution of the preponderant codons in collagen mRNA is correlated but at a lesser extent to that of their cognate tRNAs.

The adjustment of tRNA populations to the needs of protein synthesis has already been described in the case of multicellular organisms (1). A well documented example of the so called functional adaptation of tRNA comes from studies performed on the silkworm Bombyx mori using electrophoretic mapping of tRNA and structural characterization of major isoacceptor species (2,3). Indeed in the posterior silk gland, the relative amounts of the main tRNAs needed for decoding fibroin mRNA strictly correlates with the frequency of the corresponding codons. In vertebrates, a correspondance was already observed between the levels of aminoacid acceptance of tRNA and the aminoacid composition of abundantly synthetized proteins (4,5,6,7). The occurrence of codons in globin mRNA was also shown to be well correlated with the codon recognition properties of iso-tRNAs separated by reverse-phase chromatography (5). However a quantitative relationship between the tRNA content and the codon frequency could not be deduced from the latter studies.

The question was, whether the phenomenon observed in Bombyx mori could be generalized to vertebrates or not ? In the present work, we attempted to evaluate how closely codon usage and tRNA content are related to each other in two highly specialized tissues, the magnum of laying hen oviduct and the leg tendons of chick embryo. The main proteins synthesized in magnum display a fairly average aminoacid composition. In contrast, chick tendon cells devote approximately 60 % of their protein synthesis to procollagen (8), the collagenic region containing 33 % of glycine, 23 % of proline and 11 % of alanine residues. We studied therefore the relation between the abundance of codons

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for proline and glycine and that of their cognate tRNAs in overall cellular and polysome bound tRNAs from magnum and tendon cells.

MATERIALS AND METHODS

Leg tendon cells were isolated from 16-day-old chick embryos by a modification (9) of the Dehm and Prockop (8) procedure.

Total tRNA was isolated from (i) chicken liver and from the magnum of hen oviduct as described by Rogg et al. (10), (ii) leg tendon cells and (iii) polysomal particles as previously described (11). The preparation of polysomes was as described (12) except that 10 mM vanadyl ribonucleoside complexes were used as ribonuclease inhibitor.

To improve the separation of tRNA species, several modifications were made to the electrophoresis conditions previously described (11). The two dimensional polyacrylamide gel electrophoresis (2D gel) was performed at 6°C on 9.6 % acrylamide in presence of 7.5 M urea (first dimension) and on 20 % acrylamide in presence of 4 M urea (second dimension) both containing 20 mM Tris-borate buffer pH 8.3, 2.5 mM EDTA.

Aminoacid accepting activity of tRNAs extracted from the gel, 3' end labelling of tRNAs and quantification of ^{32}P tRNAs in gel spots were performed as previously described (11). ^{32}P tRNA was submitted to 2D gel electrophoresis in presence of unlabelled carrier bulk cellular tRNA. Gel spots were precisely located by staining with methylene blue. In order to avoid major errors in the quantitative estimation of individual ^{32}P labelled tRNA species due to the close location of some spots, tRNA was extracted from 2D gel spots and submitted to a further electrophoresis on one dimensional denaturing gel as described. The relative amounts of tRNA species were determined by calculating the amount of an individual spot as percent of total radioactivity recovered in the 2D gel spots.

$tRNA^{Gly}$ was purified by benzoylated diethylaminoethyl cellulose column chromatography, using the procedure of naphtoxyacetylation as described by Gillam et al. (14). The RPC-5 system (15) was used to purify $tRNA^{Pro}$. Further purification and separation of isoacceptors were performed by successive electrophoresis on 2D gel and one dimensional denaturing gel as described above. The materials and methods used to determine the nature of the anticodons of iso-$tRNAs^{Gly}$ and iso-$tRNAs^{Pro}$ were described elsewhere (13).

For high performance liquid chromatography (HPLC) analysis of nucleosides tRNA was hydrolyzed as described by Gerhke et al. (16). HPLC conditions were as described by Buck et al. (17), using a LKB system connected to a Merck D 2000 integrator.

RESULTS

Estimation of codons frequency in mRNAs from hen oviduct magnum and chick embryo tendon cells. In magnum of hen oviduct, ovalbumin, ovomucoïd, lysozyme and conalbumin mRNA represent respectively 50.0, 6.6, 3.4 (18) and 10.0 % (19) of the total poly A containing mRNA, whereas, type I procollagen mRNAs (2/3 α_1, 1/3 α_2) represent about 60 % of mRNA from tendon cells (20). In all cases, the synthesis level of mRNA is well correlated with that of protein (20,21). The relative proportions of each codon could be deduced from the percentages of the main mRNAs synthetized in magnum and in tendon. They were calculated from the published sequences of magnum mRNAs (22-25) and procollagen mRNA (26) or genes (27) and from the mean distribution of codons in vertebrates (Genbank release 38) for the 30 to 40 % of the mRNA that remains (Table 1).

From all main mRNAs mentionned above, only the nucleotide sequence of pro α_1 (I) mRNA is not completely known yet. Indeed, only the 3' region including all of the C terminal region and one fifth (201 codons) of the helical coding region have been

Table I. Codon usage for proline and glycine in vertebrates and chicken tissues and relative content of isoaccepting species in chicken tissues

Codons	Codons %[a]			Anti-codons	Iso-tRNAs %[c]				
	Verte-brates	Magnum	Tendon[b]		Total tRNA			Polysomal tRNA	
					Liver	Magnum	Tendon	Magnum	Tendon
Proline									
CCC/U	2.7	2.1	11.5,9.6	IGG	2.5	2.5	5.4	3.2	11.5
CCA	1.0	1.0	1.3,2.1	U*GG	0.6	0.4	0.6		
CCG	0.5	0.2	0.4,0.8						
Glycine									
GGC/U	3.8	2.7	15.7,13.1	GCC	3.0	2.8	4.0	3.2	4.8
GGA	1.4	1.4	2.1, 3.1	UCC	2.1	2.4	2.7	2.6	3.6
GGG	1.4	0.8	1.0, 1.9	CCC	1.1	1.3	1.3	1.0	0.9

(a) Relative amounts of codons in coding sequence of vertebrates and in mRNA from chicken tissues.

(b) The percent values in tendon mRNA were calculated by assigning to the non sequenced region of procollagen α_1 (I) helical domain either the codon choice observed for the sequenced region (1st value) or that characteristic for vertebrate (2nd value) (see the text).

(c) Relative amounts of iso-tRNAs expressed as a percentage of total radioactivity recovered from electrophoretic 2D gels. For magnum and tendon tRNAs, each given value represent the mean of five determinations derived from separate gels. The relative amounts of $tRNA^{Pro}_{IGG}$ and $tRNA^{Pro}_{U*GG}$ in total tRNA were calculated by correcting the percentage of total $tRNA^{Pro}$ by the relative proportions of I and U* as determined by HPLC analysis (Fig. 2).

sequenced (26). 14 exons (395 codons) had also been sequenced in the helical domain of the mouse pro α_1 (I) gene (28). Apart from 9 residues (other than proline or glycine), the sequences of the first 6 exons (132 codons) are the same as that of the homologous region of the chicken gene. Moreover the pattern of glycine and proline codon usage in the sequenced chicken region is very close to that observed for the fourteen mouse exons. Supposing that the codon choice would be the same in the sequenced as in the nonsequenced region, we calculated the percentage of glycine and proline codons in pro α_1 (I) mRNA by extrapolating the values obtained for the sequenced region to the whole helical domain. However, the possibility of an overestimation of the main codons for glycine and proline in pro α_1 (I) could not be ruled out because of the strong U/C bias of the sequenced helical region. This led us to another calculation, by assigning to the aminoacid residues of the non sequenced region (based on its aminoacid sequence (29)) the codon choice characteristic of vertebrates. The relative values of GGC/U and CCC/U are then only slightly different, they are lesser by a factor of 1.2 as compared to those previously calculated. The results for codon frequency thus calculated show that the predominant glycine and proline codons (GGC/U, CCC/U) in collagen are about 5 times higher in tendon cells than in magnum mRNA, the codon usage pattern of the latter being close to that of vertebrates.

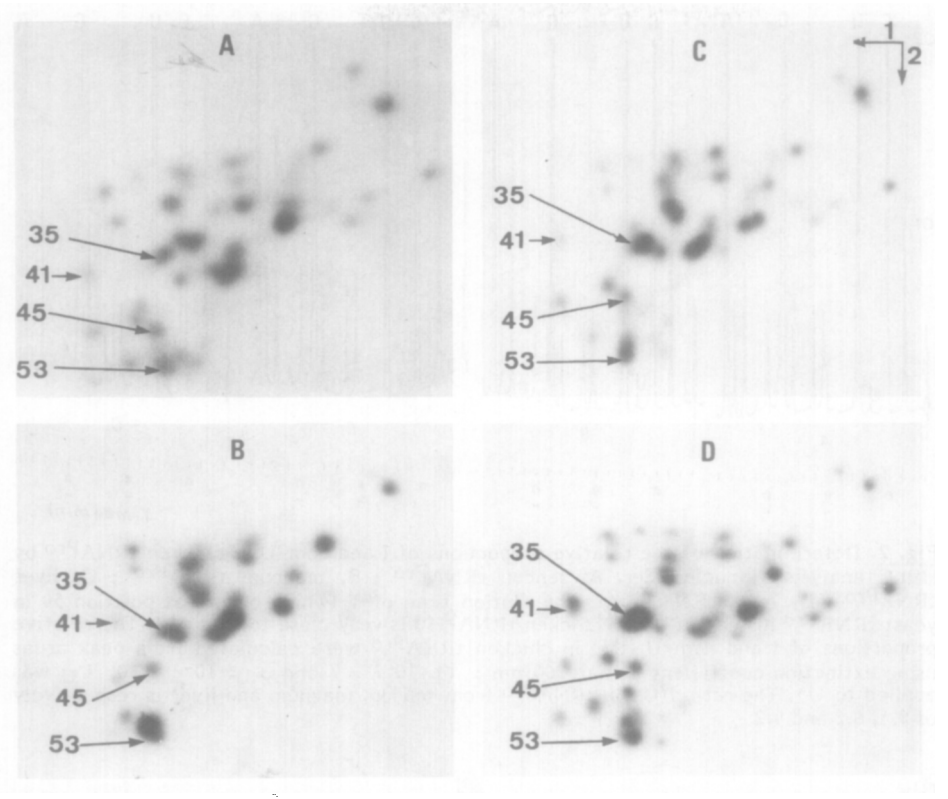

Fig. 1. Autoradiograms of 2D gel electrophoresis fractionation of ^{32}P labelled chicken tRNAs. A,B : tRNA from magnum of hen oviduct ; C,D : tRNA from chick embryo tendon. A and C are total tRNA ; B and D are polysome-bound tRNA. Spot 35, $tRNA^{Pro}$; 41, $tRNA^{Gly}_{CCC}$; 45, $tRNA^{Gly}_{UCC}$; 53, $tRNA^{Gly}_{GCC}$.

Characterization of iso-tRNAs^{Gly} and iso-tRNAs^{Pro}. Iso-tRNAs^{Gly} and iso-tRNAs^{Pro} were detected by aminoacylation on spots extracted from 2D gel electrophoresis of total cellular tRNA (Fig. 1, A and C). The nature of their anticodon was determined by primary sequence study.

$tRNA^{Pro}$ (spot 35) has a unique primary sequence except the first nucleotide of the anticodon, I or U*, as was previously found for murine $tRNA^{Pro}$ (30). U* has been found to be ncm^5U and is identical to the modified U in Torulopsis utilis $tRNA^{Pro}$, Saccharomyces cerevisiae $tRNA^{Val}2A$ (31), Saccharomyces cerevisiae and beef liver $tRNA^{Pro}$ (G. Keith, to be published). $tRNA^{Pro}_{IGG}$ was thus not separated from $tRNA^{Pro}_{U*CG}$ on 2D gel. However, their relative proportions in total $tRNA^{Pro}$ could be determined by HPLC analysis of the constituent nucleosides. HPLC patterns of $tRNA^{Pro}$ digests are shown in Fig. 2, whereas yeast $tRNA^{Pro}_{U*GG}$ was used as a reference for the elution time of U*.

The iso-tRNAs^{Gly} were located in spots 41, 45 and 53 (Fig. 1). The sequence of $tRNA^{Gly}_{CCC}$ (spot 41) determined from nucleotides 1 to 53 and that of $tRNA^{Gly}_{GCC}$ (spot 53) determined from nucleotides 1 to 10, 13 to 44 and 54 to 75 are similar to the corresponding sequences in tRNAs^{Gly} from human placenta (32a and b). In the case of

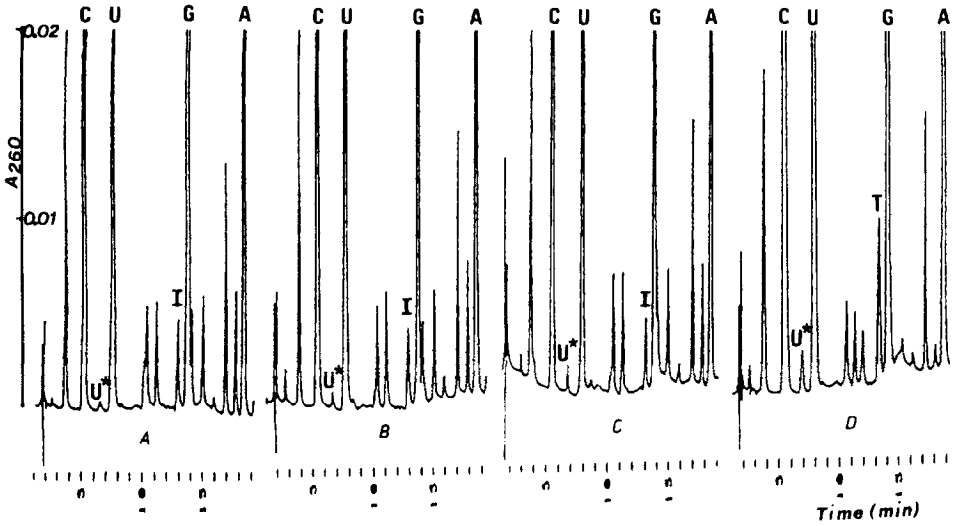

Fig. 2. Determination of the relative proportions of I and ncm^5U in chicken $tRNA^{Pro}$ by HPLC analysis of nucleosides. A, tendon $tRNA^{Pro}$; B, magnum $tRNA^{Pro}$; C, liver $tRNA^{Pro}$; D, yeast $tRNA^{Pro}_{\Psi^*GG}$. The elution time of T (which occurs at position 54 in yeast $tRNA^{Pro}$ instead of Ψ in chicken $tRNA^{Pro}$) is very close to that of I. The relative proportions of I and ncm^5U (U*) in chicken $tRNA^{Pro}$ were calculated from peak areas using extinction coefficient (ε) at 260 nm : $\varepsilon_I \cdot 10^{-3} = 7$ and $\varepsilon_U \cdot 10^{-3} = 10$. ε_U was applied to U*. The ratio I/U* in $tRNA^{Pro}$ from tendon, magnum and liver is respectively of 9.1, 6.2 and 4.2.

$tRNA^{Gly}_{UCC}$ (spot 45) the determined sequences (nucleotides 1 to 19 and 32 to 46) are identical to those of the corresponding regions of the murine $tRNA^{Gly}_{UCC}$ gene (33). The sequences include the anticodon regions.

Comparison between the distribution of codons for glycine and proline and that of the cognate tRNAs in the magnum of hen oviduct and in chick embryo tendon cells. Autoradiograms of 2D gels of total and polysome-bound tRNA from magnum and tendon cells are shown in Fig. 1. The relative proportions of total radioactivity of iso-$tRNAs^{Gly}$ and iso-$tRNAs^{Pro}$ (located by staining) are presented in Table 1. These tRNA species were also quantified in total tRNA from chicken liver. As determined by 2D gel mapping of total tRNA (not shown), the patterns of tRNA distribution in magnum and liver are similar and ressemble to the pattern of tendon tRNA with only one noticeable exception : $tRNA^{Pro}$ (spot 35) which is found in higher amounts in tendon tRNA.

$tRNA^{Pro}_{IGG}$: As determined by nucleoside analysis on HPLC (Fig. 2), the high level of $tRNA^{Pro}$ in tendon, twice that in other tissues (magnum and liver) depends on a higher level of $tRNA^{Pro}_{IGG}$. Indeed, it is the most abundant species in tendon, although its relative amount (5.4 %) is lesser by a factor of about 2 than that of the corresponding major triplets CCC/U coding for proline in collagen.

$tRNA^{Gly}_{GCC}$: In tendon, the relative content of $tRNA^{Gly}_{GCC}$ (4 %) is about 40 % higher than in magnum, but lesser by a factor of about 3.5 as compared to the major triplets GGU/C coding for glycine in collagen.

It is obvious that in magnum, the relative contents of $tRNA^{Pro}_{IGG}$ and $tRNA^{Gly}_{GCC}$

closely fit the codon usage. In addition, the remaining tRNA species, $tRNA^{Pro}_{U*GG}$, $tRNA^{Gly}_{UCC}$ and $tRNA^{Gly}_{CCC}$ have about the same relative values in tendon as in magnum. Nevertheless the most significant difference between the distribution of those tRNAs and that of their cognate codons was observed in the case of $tRNA^{Pro}_{U*GG}$. Indeed, this $tRNA^{Pro}$ is relatively less abundant by a factor of 2 (or more) than its cognate codon CCA. Moreover $tRNA^{Pro}$ with a CGG anticodon has not been found in chicken. If $tRNA^{Pro}_{CGG}$ is really lacking, $tRNA^{Pro}_{U*GG}$ should recognize CCG in addition to CCA, although some modifications of U at the anticodon were shown to produce a preference for codons ending in A over codons ending in G (34,35).

Moreover, in polysomal tRNA from tendon, the relative amount of $tRNA^{Pro}$, which is twofold higher than in total tRNA is closely related to that of the corresponding codons. But the amount of $tRNA^{Gly}_{GCC}$ is only slightly higher in polysomal than in total tRNA and lower by a factor of about 3 from the corresponding codons. The possibility that a spot containing a $tRNA^{Gly}_{GCC}$ species could have escaped from our analysis cannot be completely ruled out. However, none of the spots in the gel map of tendon tRNA is abundant enough to fit, even when added to spot 53, the GGC/U codon usage.

DISCUSSION

In the case of the magnum of hen oviduct in which the most abundantly synthetized mRNAs display a fairly average codon composition, the content of the iso-tRNAs studied mainly fits the codon choice. In the case of embryonic chick leg tendons, mRNA synthesis is dominated by type I procollagen genes sharing an unusual codon composition. However tRNA distribution does not converge in the same highly unbalanced pattern. The relative increases of $tRNA^{Pro}_{IGG}$ (100 %) and $tRNA^{Gly}_{GCC}$ (40 %) in tendon as compared to other tissues may be associated with collagen synthesis, but they are far from reaching that of their corresponding codons in mRNA, 5 times more abundant in tendon than in magnum. The very close correlation observed in Bombyx mori between the relative amounts of the main tRNAs in the silk gland and their cognate codons in mRNA (2,3) would thus not appear as a generalized characteristic.

It should further be pointed out that the relative amounts of total $tRNA^{Pro}$ and total $tRNA^{Gly}$ in tendon are very similar to those previously determined by aminoacylation of total tRNA in the same tissue by Christner and Rosenbloom (7). Moreover these authors reported an increase in tendon of about 33 % for $tRNA^{Gly}$ and 90 % for $tRNA^{Pro}$ over the average acceptor activity of tRNA from other embryonic tissues. These values are of the same order of magnitude that the differences observed here between tendon and magnum tRNA.

The low relative amount of $tRNA^{Gly}_{GCC}$ as compared to the corresponding codon usage in mRNA was also found in the polysomal fraction from tendon cells, whereas that of $tRNA^{Pro}$ closely fits the codon usage. Such a result does not agree with the assumption that an efficient translation needs a close correlation between the relative abundance of

polysome-bound tRNAs and that of their corresponding codons. Nevertheless, as previously discussed by others (4) the extent of tRNA attachment to ribosomes would be a function not only of the frequency of codons but also of the life time of ribosome interaction itself related to tRNA availability. However it is obvious that $tRNA^{Gly}_{GCC}$ must occur in an amount compatible with a high translation level.

In addition, the amount of specific tRNAs in the cell is probably dependent on at least two factors : (i) the efficiency of promoter elements and (ii) the copy number of iso tRNA genes. It could furthermore be modulated by variations in the rate of tRNA degradation and of pre-tRNAs processing and maturation.. In an attempt to evaluate the role of the copy gene number we analyzed the correlation between the relative amount of each tRNA species hybridized to DNA and that of mature species in magnum and tendon tRNA as determined from their 2D gel maps (unpublished results). Although,the correlation degree was somewhat lower in embryonic tendon ($\tau = 0.84$) than in magnum ($\tau = 0.90$), the strong positive correlation found in both cases is suggestive of a tRNA gene distribution similar to the average tRNA distribution in the organism. The gene copy number would thus be an essential factor in determining the content of tRNAs. However, as shown here, tRNA content can be somewhat modulated in relation with tissue specificity. We are now investigating at what extent the relative amounts of specific tRNAs are modified as a function of the level of collagen synthesis in tendon cells.

REFERENCES

1. Garel, J.P. (1974) J. Theor. Biol. 43, 211-225.
2. Garel, J.P., Garber, R.L. and Siddiqui, M.A.Q. (1977) Biochemistry 16, 3618-3624.
3. Chevallier, A. and Garel, J.P. (1979) Biochimie 61, 245-262.
4. Smith, D.E., and McNamara, A.L. (1974) J. Biol. Chem. 249, 1330-1334.
5. Hatfield, D., Varricchio, F., Rice, M. and Forget, B.G. (1982) J. Biol. Chem. 257, 3183-3188.
6. Hentzen, D. (1976) Cancer Res. 36, 3082-3085.
7. Christner, P.J. and Rosenbloom, J. (1976) Arch. Biochem. Biophys. 172, 399-409.
8. Dehm, P. and Prockop, D.J. (1971) Biochim. Biophys. Acta 240, 358-369.
9. Schwarz, R. and Bissell, M.J. (1977) Proc. Natl. Acad. Sci. USA 74, 4453-4457.
10. Rogg, H., Wehrhand, W. and Staehelin, M. (1969) Biochim. Biophys. Acta 195, 13-15.
11. Reinisch, F. and Heyman, T. (1982) Mol. Cell. Biol. 2, 1247-1257.
12. Keith, G., Nys, Y., Fix, C. and Heyman, T. (1986) Biochem. Biophys. Res. Commun. 138, 1405-1410.
13. Keith, G.., Pixa, G., Fix, C. and Dirheimer, G. (1983) Biochimie 65, 661-672.
14. Gillam, I., Blew, D., Warrington, R.C., Von Tigerstrom, M. and Tener, G.M. (1968) Biochemistry 7, 3459-3468.
15. Pearson, R.L., Weiss, J.F. and Kelmers, A.D. (1971) Biochim. Biophys. Acta 228, 770-774.
16. Gerhke, C.W., Kuo, K.C., McCune, R.A., Gerhardt, K.O. and Agris, P.F. (1982) J. Chromatogr. 230, 297-308.
17. Buck, M., Connick, M. and Ames, B.N. (1983) Anal. Biochem. 129, 1-13.
18. Hynes, N.E., Groner, B., Sippel, A.E., Nguyen Huu, M. and Schütz, G. (1977) Cell 11, 923-932.
19. Lee, D.C., McKnight, G.S. and Palmiter, R.D. (1978) J. Biol. Chem. 253, 3494-3503.
20. Rowe, D.W., Moen, R.C., Davidson, J.M., Byers, P.H., Bornstein, P. and Palmiter, R.D. (1978) Biochemistry 17, 1581-1590.
21. Edwards, N.A., Luttrell, V. and Nir, I. (1976) Comp. Biochem. Physiol. 53B, 183-186.
22. McReynolds, L., O'Malley, B.W., Nisbet, A.D., Fothergill, J.E., Givol, D., Fields, S.,

Robertson, M. and Brownlee, J.E. (1978) Nature 273, 723-728.
23. Jeltsch, J.M. and Chambon, P. (1982) Eur. J. Biochem. 122, 291-295.
24. Catterall, J.F., Stein, J.P., Kristo, P., Means, A.R. and O'Malley, B.W. (1980) J. Cell. Biol. 87, 480-487.
25. Jung, A., Sippel, A.E., Grez, M. and Schütz, G. (1980) Proc. Natl. Acad. Sci. USA 77, 5759-5778.
26. Fuller, F. and Boedtker, H. (1981) Biochemistry 20, 996-1006.
27. Boedtker, H., Finer, M. and Aho, S. (1985) Ann. N.Y. Acad. Sci. 460, 85-116.
28. Monson, J.M., Friedman, J. and McCarthy, B.J. (1982) Mol. Cell. Biol. 2, 1362-1371.
29. Highberger, J.H., Corbett, C., Dixit, S.N., Yu, W., Seyer, J.M., Kang, A.H. and Gross, J.C. (1982) Biochemistry 21, 2048-2055.
30. Harada, F., Peters, G.G. and Dahlberg, J.E. (1979) J. Biol. Chem. 254, 10979-10985.
31. Yamamoto, M., Yamaizumi, Z., Yokohama, S., Migazaiva, T. and Nishimura, S. (1985) J. Biochem. 97, 361-364.
32. Gupta, R.C., Roe, B.A. and Randerath, K. a/ (1979) Nucl. Acids Res. 7, 959-970 ; b/(1980) Biochemistry 19, 1699-1705.
33. Sprinzl, M., Hartmann, T., Meissner, F., Moll, J. and Vorderwülbecke, T. (1987) Nucl. Acids Res. 15, (supplement) r53.
34. Nishimura, S. (1978) in Transfer RNA, pp. 168-195, S. Altman, ed. MIT Press, Cambridge, Mass.
35. Weissenbach, J. and Dirheimer, G. (1978) Biochim. Biophys. Acta 518, 530-534.