

CYTIDYLIC AND ADENYLIC ACID INCORPORATION INTO FRAGMENTS OF tRNA

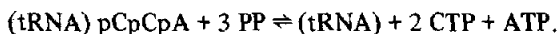
H.OVERATH, F.FITTLER, K.HARBERS, R.THIEBE and H.G.ZACHAU

*Institut für Physiologische Chemie und Physikalische Biochemie
der Universität München, Germany*

Received 16 October 1970

1. Introduction

CCA pyrophosphorylase catalyses the reaction:



It has been purified from *E. coli* [1, 2], rat liver [3], and yeast [4]. These enzymes act on the various amino acid specific tRNAs, from the same and also from different organisms. Also unfractionated mixtures of tRNA fragments were found to accept pA with CCA pyrophosphorylase from *E. coli* [5]. It was recently observed that the enzyme adds pC and pA also to the RNA of Turnip Yellow Mosaic virus, which is probably due to a tRNA^{Val}-like structure at the 3'-end of this RNA [6]. For its normal substrate, namely tRNA, CCA pyrophosphorylase requires certain structural features: the fourth nucleoside from the 3'-end must still be present when the pCpCpA sequence is to be added, the enzyme also seems to require that the tRNA is in its native conformation; denatured tRNA^{Leu}_{III} of yeast is a very poor substrate [7].

In an attempt to define the regions of tRNA which are essential for the recognition by yeast CCA pyrophosphorylase we studied the pC and pA incorporation into defined fragments of yeast tRNA^{Ser} and tRNA^{Phe}. The finding of pC and pA acceptance by half and three quarter molecules alone and by combinations with heterologous counterparts clearly distinguishes the recognition requirements of the CCA pyrophosphorylase from those of the aminoacyl tRNA synthetase (summary e.g. [8]).

2. Materials and methods

Unfractionated tRNA from brewer's yeast was a product of Boehringer Mannheim GmbH. Yeast tRNA^{Ser} [9] and tRNA^{Phe} [10] were prepared as in the references. F 1-34/Sr (pG to anticodon, about 2/5 of the molecule) and F 36-83/Ser (anticodon to acceptor stem, terminal pA and about 1.3 terminal pC missing, about 3/5 of the molecule) were prepared from pCpA-depleted [12] tRNA^{Ser} by partial T1 RNase digestion [9] and subsequent preparative disc electrophoresis (F. Fittler, unpublished). Treatment of tRNA^{Phe} (HCl) with aniline [11] gave F 1-36/Phe and F 38-76/Phe (approximately half molecules). The fragments F 1-15/Phe (pG to hU region, about 1/5 of the molecule) and F 17-73/Phe (hU region to acceptor stem, terminal pCpCpA missing, about 3/4 of the molecule) were prepared by partial T2 RNase digestion of tRNA^{Phe} [13]; F 17-73/Phe had been treated with phospho monoesterase. Splitting in the anticodon region of tRNA^{Phe} with pancreas RNase yielded F 1-33/Phe and F 34-74/Phe [13, 14].

CCA pyrophosphorylase (EC 2.7.7.25) activity was assayed by incubating for 10 min at 37° in 0.1 ml: 0.75 A₂₆₀ units pCpA-depleted [12] yeast tRNA, 0.01 μmole ³H-CTP (100 Ci/mole), 1.5 μmoles MgCl₂, 2.0 μmoles glycine-NaOH pH 9.0, 0.1 μmole glutathione, 1.0 μg albumin, and the specified amount of enzyme; radioactivity was determined on filter discs [15]. 1 unit of enzyme incorporates 1 μmole pC/min under these conditions.

The CCA pyrophosphorylase from brewer's yeast (11 mU/A₂₆₀ unit; 90-100 fold purified) was a side fraction in the procedure for the isolation of Ser tRNA synthetase (modified by Hirsch [4]).

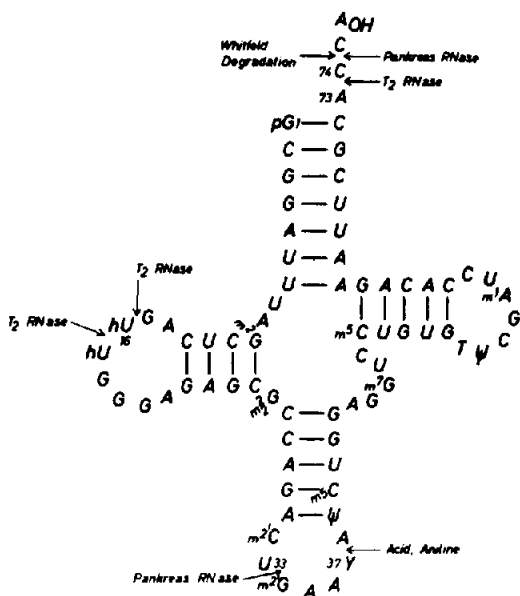


Fig. 1. Sites of splits in a cloverleaf model of yeast tRNA^{Phe} [16].

Assays of pC and pA acceptance by tRNA fragments were carried out as follows (see also figure legends): mixtures of about 30 pmoles of the acceptor fragment and various amounts of the pG fragments were heated to 70° for 1 min in 0.1 ml containing 0.01 μmole ¹⁴C-ATP (100 Ci/mole) and 0.02 μmoles ³H-CTP (100 Ci/mole); other ingredients as above, without enzyme; the solution was then kept at 37° for 15 min, brought to 20° and, after addition of 36 μU enzyme, incubated for the times indicated in the legends; immediately before stopping the reaction, 0.5 A₂₆₀ units carrier RNA were added; the radioactivities were determined on filter discs [15].

3. Results

The CCA pyrophosphorylase experiments were performed with 2 fragments from tRNA^{Ser} which had been prepared by a chain scission in the anticodon and with 5 fragments from tRNA^{Phe} (fig. 1). Only fragments which were well separated from other fragments and from total tRNA on column chromatography and/or preparative disc electrophoresis had been selected.

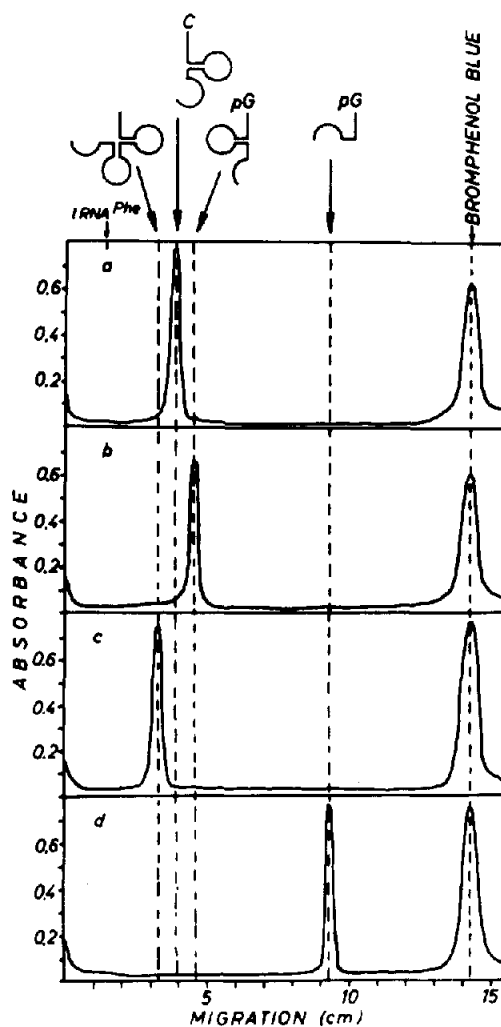


Fig. 2. Densitograms of parallel disc electrophoretic separations [10] of a) F 34-74/Phe, b) F 1-33/Phe, c) F 17-73/Phe, and d) F 1-15/Phe.

All fragments were pure according to disc electrophoresis (fig. 2) and oligonucleotide analyses; 1 and 5%, respectively, of contaminating nucleotide material would have been detected with these methods. Mixtures of pCpA- or pCpCpA-depleted half or three quarter molecules with the corresponding pG halves accepted 55-90% of the theoretical amount of pC and pA (fig. 3-5). When one quarter is missing, as in the three quarter molecule derived from tRNA^{Phe} (fig. 4)

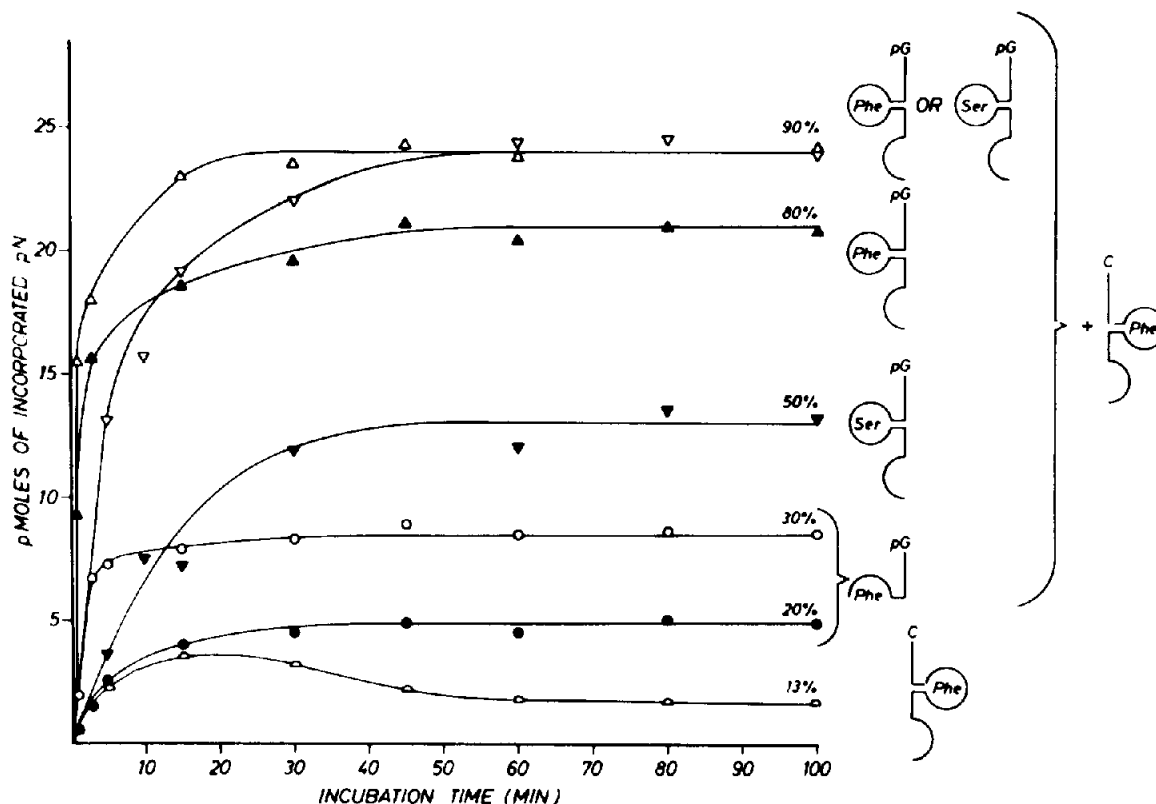


Fig. 3. pC and pA acceptance (open and full symbols, respectively) of 27 pmoles = 0.01 A_{260} unit F 34-74/Phe (\square), and of combination of 27 pmoles F 34-74/Phe with 60 pmoles = 0.011 A_{260} units F 1-15/Phe (\circ , \bullet), with 60 pmoles = A_{260} units F 1-36/Phe (Δ , \blacktriangle), and with 60 pmoles = 0.022 A_{260} units F 1-34/Ser (∇ , \blacktriangledown).

or in mixtures of a half molecule with a quarter (fig. 3), up to 35% pC and 20% pA acceptance was found. 20-90% pC and pA acceptance was observed when the acceptor fragment was derived from tRNA^{Phe} and the pG half from tRNA^{Ser} (figs. 3 and 4) while in the reverse case, 7-15% incorporation was obtained (fig. 5). pC incorporations into the depleted CCA halves alone were small (10-12%) but significant (figs. 3 and 5). Analogous results (not shown here) were obtained with a fragment of tRNA^{Phe}, F 38-74/Phe, which was prepared by chemical methods (chain scission with acid and aniline [10], isolation F 38-76/Phe [11], two Whitfield degradations [17]); 25% pC and 12% pA acceptance was found with that fragment alone. Less than 0.2% pC and pA acceptance was observed when the pG halves alone, F 1-36/Phe and F 1-34/Ser, were assayed. In some experiments the pC and particularly the pA acceptance of F 34-74/Phe, F 17-73/Phe, and

F 36-83/Ser alone decreased on prolonged incubation while, in most cases, it stayed at a plateau level when these fragments were combined with their counterparts. This is explained best by a nuclease contamination in the CCA pyrophosphorylase preparation.

4. Discussion

Since amino acid acceptance has been found up to now only when the 5'-part of the acceptor stem was present and when the fragments were derived from tRNAs specific for the same amino acid [8], the CCA pyrophosphorylase from yeast, apparently, has less stringent requirements in tRNA or fragment recognition than the aminoacyl tRNA synthetases. It cannot be excluded that small amounts of complementary oligonucleotides in the half and three quarter mole-

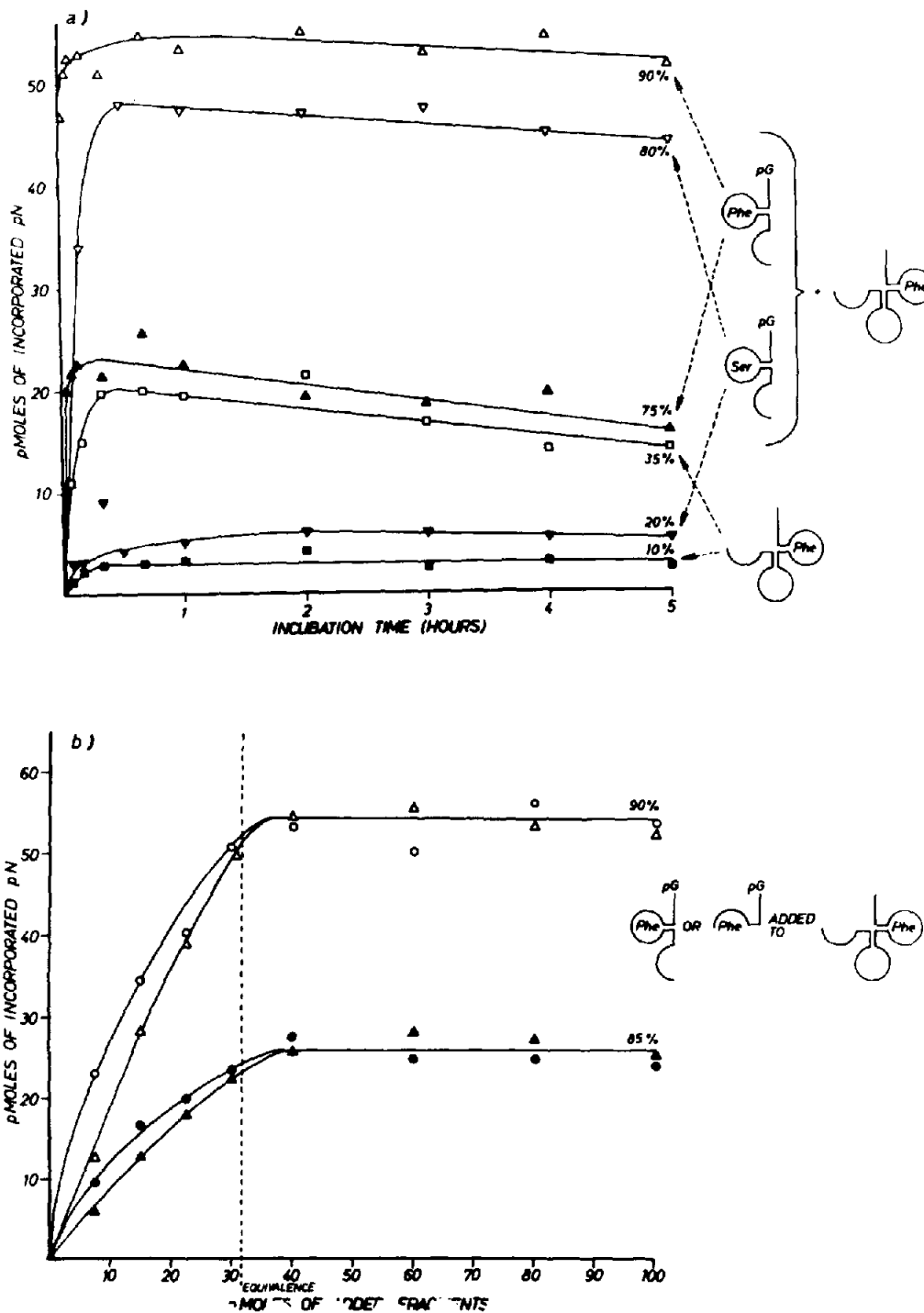


Fig. 4. pC and pA acceptance (open and full symbols, respectively) of (a) 30 pmoles = 0.015 A₂₆₀ units F 17-73/Phe (□, ●); 30 pmoles F17-73/Phe plus 60 pmoles F 1-36/Phe (△, ▲); F 17-73/Phe plus 60 pmoles F 1-34/Ser (▽, ▼); (b) combination of 30 pmoles F 17-73/Phe with increasing amounts of F1-15/Phe (○, ●) and F 1-36/Phe (△, ▲) after 45 min incubation. Incorporation values of F 17-73/Phe alone were subtracted throughout.

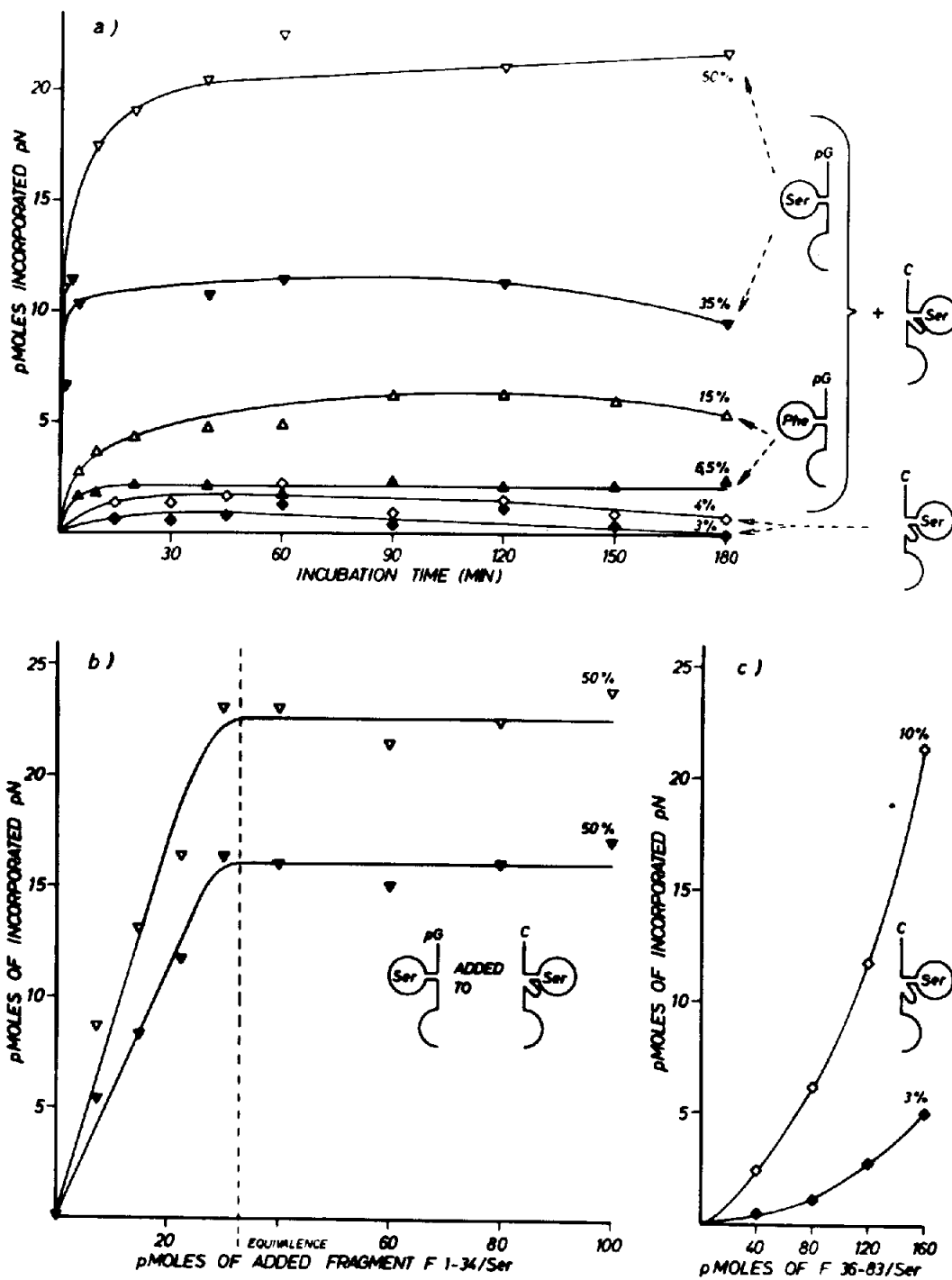


Fig. 5. pC and pA acceptance (open and full symbols, respectively) of (a) 33 pmoles = 0.015 A_{260} units F 36-83/Ser (\diamond, \bullet); 33 pmoles F 36-83/Ser plus F 1-34/Ser (∇, \triangledown); 33 pmoles F 36-83/Ser plus 60 pmoles F 1-36/Phe (Δ, \blacktriangle); (b) combinations of 33 pmoles F 36-83/Ser with increasing amounts of F 1-34/Ser (∇, \triangledown) after 60 min incubation. Incorporation values of F 36-83/Ser alone subtracted throughout; (c) increasing amounts of F 36-83/Ser (\diamond, \bullet) after 60 min.

cules, which escaped our methods of analysis, contributed to the pC and pA acceptance by these fragments. But since this explanation is rather unlikely, we consider for the time being the activity of the fragments as a fact. According to differential melting curves (analogous to [18]; F.Fittler and R.Römer, unpublished) and disc electrophoresis (similar to [19]), F 1-36/Phe and F 36-85/Ser associate in solution while no association between F 1-34/Ser and F 38-76/Phe was detected. The finding that CCA pyrophosphorylase recognizes also the latter combination may be explained by assuming that a small amount of association products present in the equilibrium reacts with the enzyme. The enzyme may also promote the association and/or stabilize the complex between the fragments.

Acknowledgements

We thank Miss B.Alzner for expert technical assistance. The work was supported by Deutsche Forschungsgemeinschaft, SFB 51, and by Fonds der Chemischen Industrie.

References

- [1] J.Preiss, M.Dieckmann, and P.Berg, *J. Biol. Chem.* 236 (1961) 1748.
- [2] J.J.Furth, J.Hurwitz, R.Krug, and M.Alexander, *J. Biol. Chem.* 236 (1961) 3317.
- [3] V.Daniel and U.Z.Littauer, *J. Biol. Chem.* 238 (1963) 2102.
- [4] P.Lebowitz, P.L.Ipata, M.H.Makman, H.H.Richards, and G.L.Cantoni, *Biochemistry* 5 (1966) 3617.
- [5] H.J.Gross, F.Duerinck, and W.Fiers, *European J. Biochem.*, in press.
- [6] P.Yot, M.Pinck, A.-L.Haenni, H.Duranton, and F.Chapeville, *Proc. Natl. Acad. Sci. U.S.*, in press.
- [7] T.Lindahl, A.Adams, M.Geroch, and J.R.Fresco, *Proc. Natl. Acad. Sci. U.S.* 57 (1967) 178.
- [8] H.G.Zachau, *Angew. Chem.* 81 (1969) 645; *Intern. Edit.* 8 (1969) 711.
- [9] H.G.Zachau, D.Dfütting, and H.Feldmann, *Z.Physiol. Chem.* 347 (1966) 212.
- [10] P.Philippsen, R.Thiebe, W.Wintermeyer, and H.G.Zachau, *Biochem. Biophys. Res. Commun.* 33 (1968) 922.
- [11] R.Thiebe and H.G.Zachau, in: *Methods in Enzymology*, Vol. 20, eds. L.Grossman and K.Moldave, in press.
- [12] G.Zubay and M.Takanami, *Biochem. Biophys. Res. Commun.* 15 (1964) 207.
- [13] K.Harbers, Thesis, Universität München (1970).
- [14] S.H.Chang and U.L.RajBhandary, *J. Biol. Chem.* 243 (1968) 592.
- [15] H.G.Zachau, in: *Methoden der enzymatischen Analyse* 2. ed. Vol. II, ed. H.-U.Bergmeyer (Verlag Chemie, Weinheim) p. 1828.
- [16] U.L.RajBhandary, S.H.Chang, A.Stuart, R.D.Faulkner, R.M.Hoskinson, and H.G.Khorana, *Proc. Natl. Acad. Sci. U.S.* 57 (1967) 751.
- [17] A.Steinschneider and H.Fraenkel-Conrat, *Biochemistry* 5 (1966) 2735.
- [18] W.Wintermeyer, R.Thiebe, H.G.Zachau, D.Riesner, R.Römer, and G.Maass, *FEBS Letters* 5 (1969) 23.
- [19] A.D.Mirzabekov, D.Lastity, E.S.Levina, and A.A.Bayev, *FEBS Letters* 7 (1970) 95.