Dictyostelium discoideum: A useful model system to evaluate the function of queuine and of the Q-family of tRNAs

G. Ott, H. Kersten and S. Nishimura*

Institut für Physiologische Chemie der Universität Erlangen-Nürnberg, Fahrstrasse 17, D-8520 Erlangen, FRG and **National Cancer Center Research Institute, Tsukiji 5-chome, Chuo-ku, Tokyo, Japan*

Received 22 July 1982

(Dictyostelium discoideum) *Wild-type strain* *Axenic strain* *Development* *tRNA modification* *Queuine supply*

1. INTRODUCTION

The nucleoside queuosine (Q) is found in the first position of the anticodon of $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asp}$ and $tRNA^{Asn}$ of eubacteria and eukaryotes except yeast [1]. In eukaryotes queuine is inserted into the tRNAs in exchange for guanine by the enzyme queuine: guanine tRNA-transglycosylase [2,3]. Experiments with germ-free mice, fed on queuine-free diet, showed that mice do not synthesize queuine de novo [4,5]. The modification queuine in the Q-family of tRNA was found to be incomplete in tumours from various sources and to change in cells and tissues undergoing differentiation or ageing [6–10]. The role of queuine in these processes is unknown.

Here, we show that *Dictyostelium discoideum* can be applied as a model system to evaluate the biological significance of queuine. When myxamoebae of the axenic strain AX-2 are grown in a well-defined synthetic medium supplemented with yeast extract but without peptone their tRNAs comprise guanine in place of queuine. The addition of queuine to the medium, at 10^{-7} M, causes an almost complete modification of the corresponding tRNAs. Analysis of tRNA from the wild-type strain, NC-4, that needs *E. coli* B/r for growth indicates that bacteria supply *D. discoideum* with queuine.

2. MATERIALS AND METHODS

Chemicals were obtained from the following sources: [^{3}H]guanine sulfate (7.7 Ci/mmol), Radiochemical Centre (Amersham); [^{3}H]asparagine (15 Ci/mmol) New England Nuclear Corp. (MA); bacteriological peptone and yeast extract, Oxoid (London). All other chemicals were from sources described in [11].

Dictyostelium discoideum, strain AX-2, was usually cultivated in axenic medium [12]. When indicated the cells were grown in a defined synthetic medium [13] supplemented with yeast extract (7.15 g/l), referred to as peptone-free medium. Amoebae, cultivated in the conventional axenic medium to 4–5 × 10^6 cells/ml were adapted to the new conditions as follows: after dilution to 5 × 10^4 cells/ml with peptone-free medium, they were grown to 3.5 × 10^6 cells/ml. This culture was diluted again and the cells were harvested at 3.5 × 10^6/ml. The generation time was found to be 6.5 h at 22°C. Synthetic queuine [14] was added to the cultures as indicated in the corresponding legends. Wild-type *D.discoideum* NC-4 was grown in liquid medium with *E. coli* B/r [15].

tRNA preparation was according to [16] with the following modifications: after treatment with phenol the aqueous phases were applied to the DEAE-cellulose column. Subsequent desalting was achieved by Sephadex G-25 column chromatography. When tested as substrates in the transglycosylase reaction the tRNAs had to be further purified on a Sephadex G-150 column (85 cm × 1.6 cm diam.). Elution was performed with a buffer containing 10 mM Tris–HCl (pH 7.5), 200 mM NaCl, 10 mM $MgCl_2$ and 6 mM NaN_3 and subsequently tRNAs were desalted on a Sephadex G-25 column.

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75 © 1982 Federation of European Biochemical Societies

Aminoacylation with asparagine and separation of the $tRNA^{Asn}$ isoacceptors was as follows: Frozen cells were disrupted by a Mikrodismembrator II (Braun, FRG) and S-100 supernatant was prepared as enzyme source [16]. The aminoacylation assay, optimized for asparagine, contained: 100 mM sodium cacodylate (pH 7.5), 6 mM ATP, 15 mM $MgCl_2$, 2 mM spermidine, 20 μM [^{3}H]Asn and 3 μg protein/μl of S-100 extract. In a 35 μl assay, 1 A_{260} tRNA was aminoacylated, and the reaction reached the plateau after 30 min incubation at 22°C. The charged tRNA was recovered and separated on an RPC-5 column (30 cm × 0.75 cm diam., 25 ml/h) [16]. A nonlinear gradient from 0.4–1 M NaCl was established by the LKB gradient mixer 11 300 Ultrograd. tRNA in fractions of 1.5 ml was precipitated with 10% trichloroacetic acid on glassfiber filters, washed, dried and counted by conventional methods.

Guanine exchange at position 34 of Q-deficient tRNA was tested by the transglycosylase reaction [6], in a standard assay of 100 μl containing 0.5 A_{260} units of tRNA and 2 units of *E. coli*-tRNA-guanine transglycosylase [17].

3. RESULTS AND DISCUSSION

Two major isoacceptors for asparagine have been found in tRNA preparations from axenic strains of *D. discoideum* [11,16]. The isoacceptor $tRNA_2^{Asn}$ contains queuine whereas $tRNA_3^{Asn}$ possesses a guanine residue at position 34 [11]. These isoacceptors were chosen as representatives to elucidate the extent of Q-modification in the Q-family of tRNA and referred to as (Q^+) $tRNA^{Asn}$ or (Q^-) $tRNA^{Asn}$, respectively.

In tRNA preparations of the wild type strain NC-4, grown on *E. coli* as food supply, only one $tRNA^{Asn}$ isoacceptor is present. This $tRNA^{Asn}$ elutes from RPC-5 columns at exactly the same salt concentration as (Q^+) $tRNA^{Asn}$ (fig.1). When treated with periodate (Q^+) $tRNA^{Asn}$ from the axenic strain and the $tRNA^{Asn}$ from the wild-type also coelute, but at higher salt concentrations. This is a characteristic property of (Q^+) tRNAs [18] (not shown). The result suggests that bacteria serve as natural source of queuine for the myxamoebae. Therefore exogenous queuine is probably essential for the biosynthesis of Q-containing

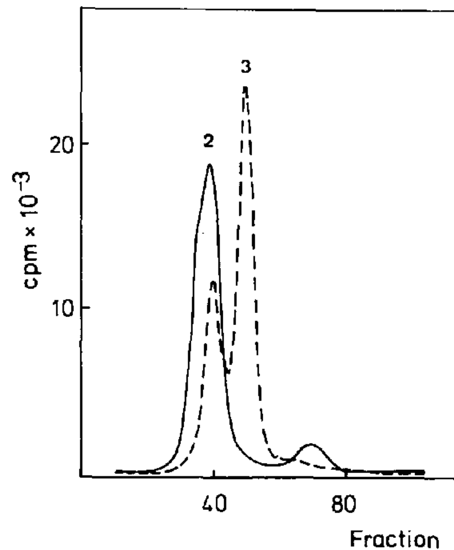

Fig.1. RPC-5 chromatographic profiles of asparaginyl-tRNA from the wild-type and the axenic strain of *D. discoideum*. The wild-type strain NC-4 was grown on *E. coli* B/r (——) and strain AX-2 in the conventional axenic medium containing peptone (- - -): tRNA was aminoacylated with [^{3}H]ASN and separated on RPC-5 (pH 4.5). The tRNA in each fraction was precipitated, filtered and counted as described in the method section. The elution profiles of two subsequent runs are shown. $tRNA_2^{Asn}$ is the Q-containing isoacceptor, $tRNA_3^{Asn}$ contains guanine at position 34 [11].

tRNA in axenic strains of *D. discoideum*. When cells are supplied with the conventional axenic medium, comprising yeast extract and peptone, queuine might become limiting.

To test this hypothesis the axenic strain of *D. discoideum* was cultivated in a defined synthetic medium [13]. Since the cells grew very slowly the medium was supplemented with yeast extract. Under these conditions the cells duplicate at a highly reproducible generation time of 6.5 h, which is even lower than the growth rate supported by the conventional axenic medium (8 h). The tRNA from cells grown in peptone-free medium was isolated, charged with Asn and chromatographed on RPC-5 columns. The elution profile shows only one isoacceptor for Asn, having exactly the same retention time as (Q^-) $tRNA^{Asn}$ (fig.2a). Upon addition of 10^{-7} M queuine to the peptone-free medium the cells synthesize $tRNA^{Asn}$ completely modified with respect to queuine (fig.2b). Analo-

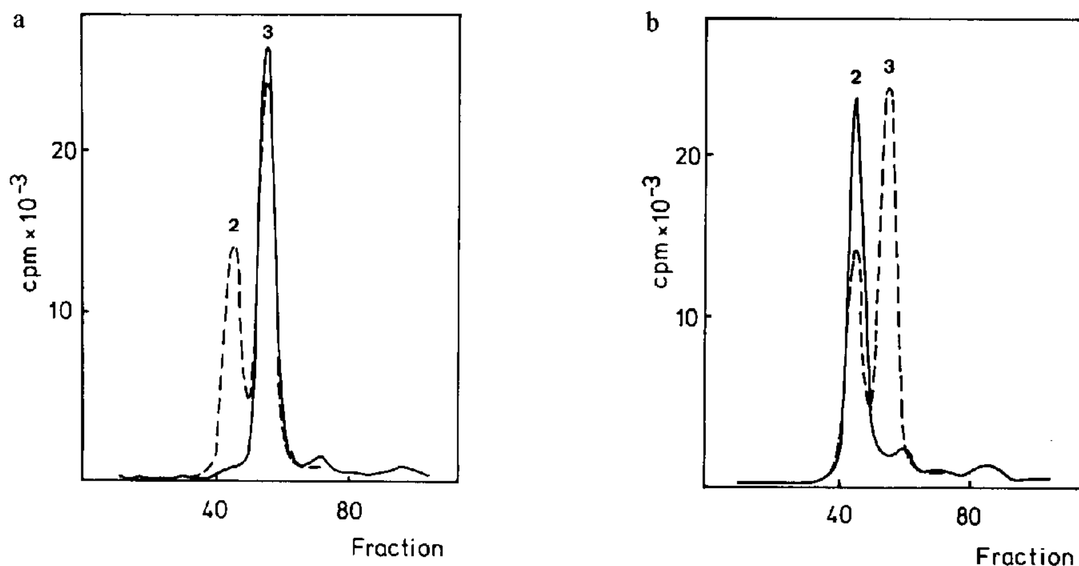

Fig.2. RPC-5 chromatographic profiles of asparaginyl-tRNA from the axenic strain *D. discoideum*. Cells of AX-2 were grown in peptone-free medium (——) without (a) or with the addition of 10^{-7} M queuine (b). A subsequent run with tRNA from cells, grown in the conventional medium is shown for comparison (- - -), for details see (fig.1).

Table 1

Relative amounts of (Q−) tRNAs in wild-type and axenic strain of *D. discoideum* grown in different media

Strain and food supply	Relative portion (%) of (Q−) tRNA calculated from:			
	[^{3}H]guanine insertion into bulk tRNA by the *E. coli* tRNA-guanine transglycosylase at:			RPC-5 profiles of asparaginyl-tRNA
	1 h	2 h	4 h	
AX-2: peptone-free medium no queuine added	100	100	100	100
AX-2: conventional axenic medium (contains peptone)	67	71	68	63
NC-4: *E. coli* B/r	10.1	8.8	7.6	Traces[a]
AX-2: peptone-free medium 10^{-7} M queuine added	2.0	2.2	2.3	None
AX-2: peptone-free medium 10^{-5} M queuine added	1.6	1.6	1.8	None

+Traces of (Q−) $tRNA^{Asn}$ are detectable after periodate treatment. This was not the case for tRNAs from strain AX-2 grown in peptone-free medium with the addition of queuine

Values are averages from 2 determinations. With tRNA from cells grown in synthetic medium (no queuine added) 1.6×10^{4} cpm were found in a sample of 15 μl after 1 h incubation. Blank values (tRNA omitted) were below 230 cpm

gous results were obtained for $tRNA^{Asp}$ (not shown). The growth rate is not affected by queuine.

In a second series of experiments the relative amount of Q-deficient tRNA was determined in bulk tRNA from the wild-type, grown on *E. coli*, and in bulk tRNA from the axenic strain grown on various nutrient sources. The guanine exchange in (Q^-) tRNA by the *E. coli* tRNA-guanine transglycosylase was applied to label the (Q^-) tRNA at position 34 specifically with [^{3}H]guanine [6]. In these experiments tRNA preparations were first purified by Sephadex G-150 column chromatography.

The guanine insertion into tRNA of the axenic strain, grown in peptone-free medium without added queuine, was set at 100% (table 1, first row). In tRNA from the axenic strain cultivated in the conventional medium the relative amount of Q-deficient tRNA decreases (table 1, second row) and correlates well with the relative amount of (Q^-) $tRNA^{Asn}$ (fig.2a). The results show that (Q^-) $tRNA^{Asn}$ can be used as indicator for Q-deficiency in tRNAs of the Q-family. The wild type strain grown on *E. coli* contains $<8\%$ of (Q^-) tRNA, showing that *E. coli* serves as a source of queuine for *D. discoideum*. The addition of 10^{-7} M queuine to the peptone-free medium decreases the relative amount of (Q^-) tRNAs in the axenic strain to $<2.5\%$ (10^{-5} M queuine to below 2%). From this result we conclude that all tRNA isoacceptors of the Q-family had been converted almost quantitatively to (Q^+) tRNAs.

Vegetative amoebae of *D. discoideum* can be induced to undergo rapid developmental transition by nutrient starvation. Cells that were grown in the absence of queuine develop spores and the spores germinate showing that queuine is neither essential for development of the strain AX-2 to spores nor for the germination of spores. However, the modified nucleoside accelerates development and stimulates spore germination (Schachner et al., unpublished). The mechanisms of these stimulatory effects of queuine are presently being investigated.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (grant Ke 98/17/6) and Fond der Chemischen Industrie. Queuine was chemically synthesized by Drs H. Akimoto and H. Nomura of Central Research Laboratories, Takada Chemical Ind. Ltd., Osaka, and provided to us as a gift.

REFERENCES

[1] Sprinzl, M. and Gauss, D.H. (1982) Nucleic Acids Res. 10, r1–r5.
[2] Katze, J.R. and Farkas, W.R. (1979) Proc. Natl. Acad. Sci. USA 76, 3271–3275.
[3] Shindo-Okada, N., Okada, N., Ohgi, T., Goto, T. and Nishimura, S. (1980) Biochemistry 19, 395–400.
[4] Katze, J.R. (1980) Biochem. Biophys. Res. Commun. 96, 313–319.
[5] Reyniers, J.P., Pleasants, J.R., Wostmann, B.S., Katze, J.R. and Farkas, W.R. (1981) J. Biol. Chem. 256, 11591–11594.
[6] Okada, N., Shindo-Okada, N., Sato, S., Itoh, Y.H., Oda, K. and Nishimura, S. (1978) Proc. Natl. Acad. Sci. USA 75, 4247–4251.
[7] Shindo-Okada, N., Terada, M. and Nishimura, S. (1981) Eur. J. Biochem. 115, 423–428.
[8] Kersten, H. (1982) in: 33. Mosbacher Colloquium, Biochemistry of Differentiation and Morphogenesis (Jaenicke, L. ed) Springer-Verlag, Berlin, New York in press.
[9] Hosbach, H.A. and Kubli, E. (1979) Mech. Age. Dev. 10, 141–149.
[10] Singhal, R.P., Kopper, R.A., Nishimura, S. and Shindo-Okada, N. (1981) Biochem. Biophys. Res. Commun. 99, 120–126.
[11] Dingermann, Th., Ogilvie, A., Pistel, F., Mühlhofer, W. and Kersten, H. (1981) Hoppe-Seyler's Z. Physiol. Chem. 362, 763–773.
[12] Watts, D.J. and Ashworth, J.M. (1970) Biochem. J. 119, 171–174.
[13] Rahmsdorf, H.J. (1977) Hoppe-Seyler's Z. Physiol. Chem. 358, 527–529.
[14] Okada, N., Noguchi, S., Kasai, H., Shindo-Okada, N., Ohgi, T., Goto, T. and Nishimura, S. (1979) J. Biol. Chem. 254, 3067–3073.
[15] Sussman, M. (1976) in: Methods in Cell Physiology (Prescott, D. ed.) vol. 2, pp. 397–410, Academic Press, New York.
[16] Palatnik, C.M., Katz, E.R. and Brenner, M. (1977) J. Biol. Chem. 252, 694–703.
[17] Okada, N. and Nishimura, S. (1979) J. Biol. Chem. 254, 3061–3066.
[18] Wosnick, M.A. and White, B.N. (1979) Biochim. Biophys. Acta 561, 194–205.