may reach 20-30 μ in diameter. In ultrathin sections, on the basis of all their ultrastructures taken together, these AML can be classed as old or dying cells, in which, besides changes in the other intracellular organelles, reduction of the cytoplasmic outgrowths is taking place by a decrease in their number and size.

On the basis of these results the formation of the folded-nodular surface of AML after intragastric injection of NDMA and alcohol can be explained by collapse, curving, and fusion of the cytoplasmic outgrowths or frills. This change probably reflects destabilization of the cell membrane and of the membranes of the intracellular structures, described by Merkur'eva, et al. (1983) on the basis of a biochemical study of the membrane-damaging effect of NDMA.

Transformation of the surface of AML, a response of the cell to the unfavorable action of chemical agents, can thus be used as a criterion for evaluation of the cytotoxic action of various biologically active environmental factors.

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# IMMUNOCYTOCHEMICAL LOCALIZATION OF TRYPTOPHANYL-tRNA-SYNTHETASE IN A BOVINE KIDNEY CELL LINE AND IN SUBSTRAINS WITH ELEVATED ENZYME LEVELS


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Tryptophanyl-tRNA-synthetase (TRS) is an enzyme which catalyzes the addition of tryptophan to specific tRNA. The study of the intracellular localization of TRS is important in order to elucidate the topography of the structural and functional components of the protein-synthesizing system of the cell.

Previously [2, 9], in an immunomorphologic investigation using mono- and polyclonal antibodies, the writers demonstrated the distribution of TRS in the cytoplasm of a bovine kidney cell culture (line MDBK). Sublines of MDBK cells resistant to competitive TRS inhibitors – the tryptophan analogs tryptamine and tryptophanol [3] – have now been obtained. According to the results of biochemical analysis, the TRS content in these sublines is 10-50 times greater than that in the original culture [3]. However, morphological data on possible differences in the intracellular distribution of TRS, connected with the higher enzyme levels in these sublines, are not available.

One unexpected result of the biochemical investigation [3] was the discovery of TRS in the detergent-insoluble fraction of MDBK cells. We know that this fraction includes several structural cellular components: polysomes, fragments of membranes of the rough endoplasmic reticulum (RER), nucleus, cytofilaments, and lipid inclusions [7]. The immunocytochemical study of the cells after detergent treatment could give additional information on the connection between TRS and these intracellular structures.

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Fig. 1. Immunolocalization of TRS in cells of line MDBK (a, b, c) and of a subline resistant to tryptamine (TN 200-24, d), not treated with Triton X-100. a) Variations in degree of staining of different cells; b) deposits of reaction product in cytosol of two adjacent cells: left – as small granules; right – as amorphous collections; c) staining of polysomes bound with membranes of RER (arrows): no reaction product is present in the cisterns of RER; d) staining of polysomes bound with outer nuclear membrane (arrows). [sic]. Magnification: a) 260×, b) 16,600×, c) 30,000×, d) 29,000×. Here and in Figs. 2 and 3: ultrathin sections without additional staining.

This paper describes a comparative immunocytochemical analysis of the localization of TRS in cells of the original culture of MDBK and of sublines with an elevated TRS level, treated and not treated with the nonpolar detergent Triton X-100.

## EXPERIMENTAL METHOD

MDBK cells and cells of sublines resistant to tryptamine (TN 200-24, TN 200-60) and to tryptophanol (TL) were cultured in medium RPMI (Flow Laboratories, England) with the addition of 10% calf serum, 0.045% glutamine, 75 U/ml of penicillin, and 75 mg/ml of streptomycin. For immune microscopy, coverslips with the cell culture were fixed in a mixture of 6% paraformaldehyde and 0.1% saponin in 0.15 M cacodylate buffer, pH 7.3, for 30 min. In the experiments with detergent, the cells were incubated before fixation in PBS solution (10 mM Na-phosphate buffer in 0.15 M NaCl, pH 7.2), containing 0.5% Triton X-100 (Merck, West Germany) and 3 mM di-isopropyl fluorophosphate (Fluka, Switzerland) for 15 min at 0°C. The preparations were then washed twice with solution of the same composition and twice with PBS, fixed, and treated by the indirect immunoperoxidase method [8]. Monospecific rabbit antibodies to TRS [2] and Fab-fragments of donkey antibodies to rabbit IgG, labeled with horseradish peroxidase, were used. The cell cultures on coverslips were embedded in a mixture of Epon and Araldite for electron microscopy.

The immunologic controls included incubation of the fixed cells only with anti-rabbit Fab-fragments or with diaminobenzidine.

Fig. 2. Immunolocalization of TRS in detergent-extracted preparations of MDBK cells (a, c) and cells of the TL subline, resistant to tryptophanol (b). a) Staining of nucleoli, peripheral zone of nuclei, and also fibers, granules (arrows), and cortical region of cytoplasm can be seen; b) increase in number of cells containing intensely stained granules (arrows); c) staining of bundles of thin fibrils in cortical zone of cytoplasm (F), elements of cytoplasm (EC), peripheral zone of nuclei (N), and nucleoli (No) with reaction product [Labels omitted in Russian original – Publisher]. Magnification: a, b) 380×, c) 8700×.

## EXPERIMENTAL RESULTS

Light-optical investigation of MDBK cells and cells of resistant sublines, not treated with detergent, showed the presence of TRS, which was identified by the diffuse dark brown color of the cytoplasm. In all lines studied the intracellular content of TRS varied considerably. Besides intensely stained cells there were others whose cytoplasm was only weakly stained (Fig. 1a).

Electron-microscopic study of preparations of the original and resistant cultures revealed no definite qualitative differences in the intracellular distribtuion of TRS. When all the lines were examined, the feature first noted was the presence of granular or amorphous deposits of reaction product in the cytosol (Fig. 1b). High electron density and the spread-out character of these deposits make it difficult to decide whether TRS was present on the subjacent ultrastructures and, in particular, on polysomes. For this reason, the discovery of positive staining of polysomes bound with membranes of RER (Fig. 1c) and the outer nuclear membrane (Fig. 1d) becomes possible only because of the characteristic location of the polysomes. However, specific staining of free polysomes is only a matter of probability, for the possibility cannot be ruled out that TRS macromolecules may be located actually in the cytosol [2]. In agreement with previous data [2, 9], the reaction product was absent in the nucleus, the lamellar complex, and the lumen of the cisterns of RER.

Neither light nor electron microscopy enabled comparative visual evaluation of differences between the lines as regards their TRS content because of the considerable variability of staining of different cells of the same population.

The light-optical study of MDBK cells and cells of the resistant sublines after detergent treatment revealed positive staining of the detergent-insoluble elements of the cytoplasm and nucleus (Fig. 2a, b). In most cells staining of the nucleoli, peripheral zone of the nuclei, and also of fibers, of solitary small granules, and of the cortical region of the cytoplasm could be seen. Single cells of the original MDBK culture had numerous intensely stained, large, confluent cytoplasmic granules (Fig. 2a). The number of cells with similar granules in sublines resistant to tryptamine or tryptophanol, was visually increased (Fig. 2b).

Parallel immunoelectron-microscopic analysis of MDBK cells and cells of the distant sublines confirmed the data of light microscopy. Under low power of the electron microscope, staining of oriented bundles of thin (diameter 5-8 nm) fibrils in the cortical region of the cytoplasm, in preserved elements of the cytoplasm, nucleoli, and a narrow peripheral zone of the nucleus, was found in the majority of cells treated with Triton X-100 (Fig. 2c). Under high power of the electron microscope, deposits of reaction product became distinctly visible in the peripheral zone of the nucleus, on polysomes associated with remnants of membranes of RER and cytofilaments, and also directly on the loosely arranged cytofilaments. Dark cytoplasmic granules found under the light microscope correspond to coils of intensely stained cytofilaments, 15-20 nm thick.

These results confirm those of previous investigations [2, 9] and are evidence that TRS is present in polysomes of RER, on the outer nuclear membrane, and on free polysomes in cells of the MDBK and of its sublines resistant to tryptophan analogs. Treatment of the cells with the nonpolar detergent Triton X-100, which enables the majority of cytoplasmic proteins to be extracted from the cells, not only made it possible to localize TRS on free polysomes, but also to confirm existing evidence on the association of ribosomal clusters with cytofilaments [5, 6], thereby emphasizing the conventional nature of the term "free polysomes." Discovery of TRS bound with ribosomes is in agreement with immunomorphologic and biochemical data on a similar localization of other enzymes of the aminoacyl-tRNA-synthetase group [4, 7, 10]. The problem of localization of TRS actually in the cytosol, despite the results of biochemical investigations [1], must remain open because of the limited resolving power of the immunoperoxidase method in electron microscopy [2]. To solve this problem it will be necessary to use other discrete markers of antibodies, such as colloidal gold.

The cytochemical reaction product could be found in cell preparations treated with detergent in the detergent-insoluble nuclear matrix and on cytofilaments. Probably after treatment of the cells with Triton X-100 the nuclear membrane undergoes partial lysis and becomes more permeable for antibodies than after the treatment with saponin that is usually used [2]. The fact that TRS is present in the nucleus is in agreement with immunofluorescence data on the discvoery of phenylalanyl- [7], methionyl- [10] and, probably, histidyl-tRNA-synthetases [11] in nuclei of cells of higher eukaryotes.

The nature and functional significance of the association of TRS with cytofilaments are not clear and require further study.

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