EVIDENCE FOR GLYCOSYL-TRANSFERASES IN RAT LIVER NUCLEI

by

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SUMMARY

Five glycosyl-transferases have been found present in purified hepatocyte nuclei of the rat (mannosyl-, galactosyl -, N-acetyl-glucosaminyl-, N-acetyl-galactosaminyl- and sialyl-transferases) ;these are capable of fixing specific carbohydrates on to endogenous or exogenous protein acceptors.

In the study of biosynthesis of glycoproteins, numerous studies have been undertaken on the subcellular localization of the enzymatic systems responsible for the glycosylation reactions (glycosyl-transferases). These enzymatic activities have been demonstrated in the microsome fraction (1,2,3,4,5), in the fractions containing large amounts of Golgi apparatus (6,7), in the cytoplasmic phase (8,9) and in the mitochondria (10,11). Furthermore, we felt it would be of interest to find out if these enzymatic systems were also present in the nuclei.

MATERIALS and METHODS

The rat livers, perfused via the portal vein, are sampled and fractionated according to the Zalta et al method (12), adapted by Beck et al.(13). This technique is based on the use of tensioactive agents (Cemulsol NPT-6, Cemulsol NPT-12).

The pureness of the fractions is checked on the one hand, by electron microscopy, and on the other, by means of marker enzymes. The enzymes tested for were :

* Attaché de Recherches au C.N.R.S.

- succinate-dehydrogenase (EC. 1.3.99.1) by the method of DER VARTANIAN and VEEGER (14).
- mono-amine-oxidase (E.C. 1.4.3.4.) by the method of TABOR et al.(15)
- 5'-nucleotidase in alkaline medium (E.C. 3.1.3.5) by the method of SCHACHTER et al.(16).
- 5'-nucleotidase in acid medium (E.C. 3.1.3.2) by the method of NACHBAUR et al.(17).
- glucose 6-phosphatase (E.C. 3.1.3.9.) by the method of SCHACHTER et al.(16)
- RNA-polymerase DNA dependent (E.C. 2.7.7.6) by the method of KRAKOW et al. (18)
- lactate -dehydrogenase (E.C. 1.1.1.27) by the kinetic technique at 340 nm

The acellular systems, for glycosyl-transferase activities, are composed of the following :

- 200 µl of nuclear suspension in 0.02M tris-maleate buffer, pH = 6.
- 10 µl of $MnCl_2$ 0.25 M
- 10 µl of a solution containing:
  - either 87 picomoles of CMP-(^{14}C)-N-acetyl-neuraminic acid at 230 Ci/M
  - or 77 picomoles of UDP-(^{14}C)-galactose at 260 Ci/M.
  - or 117 picomoles of GDP-(^{14}C)-mannose at 170 Ci/M.
  - or 435 picomoles of UDP-(^{14}C)-N-acetyl-glucosamine at 46 Ci/M.
  - or 364 picomoles of UDP-(^{14}C)-N-acetyl-galactosamine at 55 Ci/M.
  - or 112 picomoles of GDP-(^{14}C)-fucose at 170 Ci/M, and
- in some cases, included as exogenous protein acceptor, 10 µl of a fetuin solution (200 µg per test) treated according to the technique of KO and RAGUPATHY (19).

The incubations were performed at 30° during 30 minutes. The synthesized glycoprotein macromolecules are precipitated by the addition of (20% w/v) trichloroacetic acid collected on a Whatman GF/B glass filter and washed by a (4/1) methylal-methanol mixture to eliminate the glycolipides. The radioactivity is estimated by liquid scintillation counting according to the technique previously described (4).

RESULTS

1) Verification of quality by electron microscopy

Plates 1 and II demonstrate, in the more purified fraction, a definite predominence of nucleolated nuclei, the peri-nuclear layer of which is dissolved. Contamination through the endoplasmic membranes is practically nil.

2) Verification of quality by specific marker enzymes

Table I shows :

- the total absence of enzymatic contaminants derived from the mitochondria and from the soluble cytoplasmic phase.
- a very slight contamination by the marker enzymes derived either from the plasmic membranes, or from the endoplasmic reticulum.
- a very significant enrichment of DNA dependent RNA-polymerase during purification of the nuclear fraction.

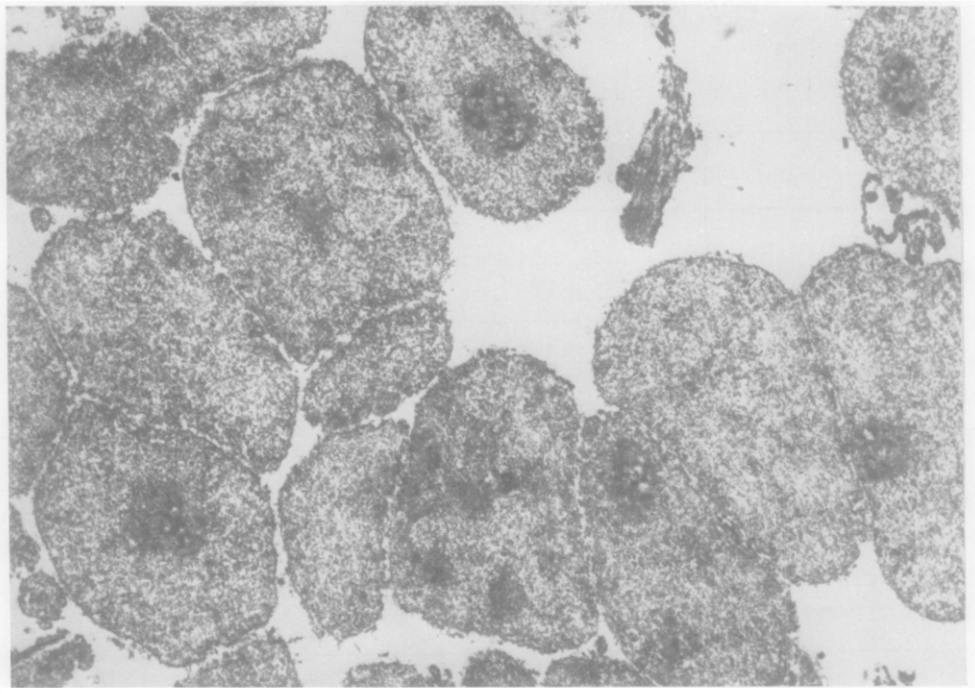
3) Importance of nuclear glycosyl-transferases.

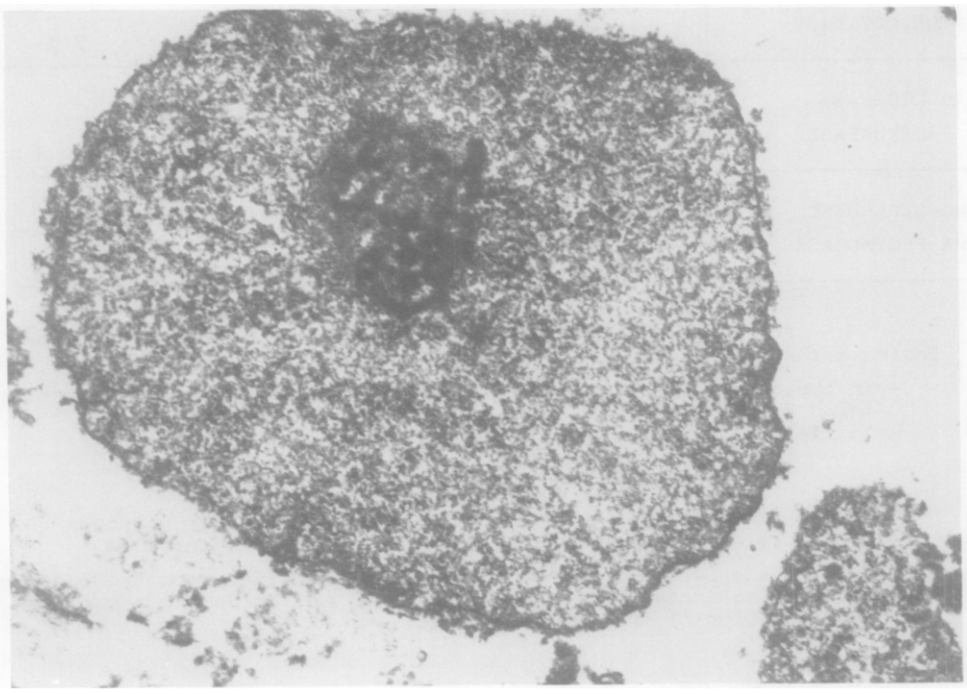
Table II demonstrates in the nuclear fraction :

- the total absence of fucosyl-transferase.
- the presence of four glycosyl-transferases capable of utilizing endogenous protein as carbohydrate acceptors. These are mannosyl-, galactosyl-, N-acetyl-glucosaminyl and N-acetyl-galactosaminyl-transferases.
- the presence of a sialyl-transferase capable transfering sialic acid on to an exogenous protein acceptor, but incapable of utilizing endogenous proteins for this purpose.
- an increased galactosyl-transferase when an exogenous acceptor is introduced into the system.

DISCUSSION

The presence of glycosyl-transferases, affecting the oses or ose derivatives of biological interest, has been very widely demonstrated, with regard to the microsomes, the plasmic membranes and the Golgi apparatus. The reality of their presence in the mitochondria, and particularly their submitochondrial localization in the inner membrane, has only recently been elucidated (11). The results reported here with regards to the quality of the nuclear fractions, established both by electron microscopy and by enzymatic control, confirm the presence of at least five glycosyl-transferases





Examination under the electron microscope of purified hepatocyte nuclei of the rat.

Plate I : x 6.000
Plate II: x 15.000

TABLE I

	TOTAL CELL HOMOGENATE	NON PURIFIED NUCLEI BEFORE CEMULSOL	PURIFIED NUCLEI
LACTATE DEHYDROGENASE	2	0.53	0
	100 %	2.5 %	0 %
MONO-AMINE-OXIDASE	0.9	2.3	0
	100 %	20 %	0 %
SUCCINATE-DEHYDROGENASE	0.43	0.48	0
	100 %	10 %	0 %
5' ACID NUCLEOTIDASE	635	190	25
	100 %	10 %	0.9 %
BASIC 5'-NUCLEOTIDASE	700	360	90
	100 %	18 %	3 %
GLUCOSE-6-PHOSPHATASE	920	230	40
	100 %	8.5 %	1. 1 %
DNA-DEPENDENT RNA-POLYMERASE	35	145	660
	100 %	35 %	25 %

For each enzyme studied, the numbers given in the top line indicate the specific activities expressed :

- in the case of lactate dehydrogenase as the variation of absorbancy/mn/mg protein.
- in the case of monoamine-oxidase and succinate dehydrogenase :

$$\frac{\text{variation of absorbancy} \times 100}{\text{mn}} \ / \text{ mg protein}$$

- in the case of the (acid or basic) 5'nucleotidases, glucose-6-phosphatase, DNA-dependent RNA-polymerase : dpm/mg protein.

The numbers shown in the bottom line represent the percentages of the total initial activity recovered in each fraction during the course of purification of the nuclei.

in the nuclei accompanied in some cases by endogenous protein acceptors.

This unexpected subcellular localization presents the problem of the biological role of these enzymes and the mechanism

TABLE II

	MANNOSYL Transferase	GALACTOSYL Transferase	N-Acetyl GLUCOSAMINYL Transferase	N-Acetyl galactosaminyl - transferase	SIALYL Transferase	FUCOSYL Transferase
NON PURIFIED NUCLEI BEFORE CEMULSOL	1.86	0.12	1.50	1.15	2.33	0.18
NUCLEI	0.68	1.10	1.25	0.85	0.10	0.00
NUCLEI + EXOGENOUS ACCEPTOR	-	1.68	1.25	0.80	5.50	0.00

The results are expressed in picomoles of ^{14}C - carbohydrate transferred per mg of protein.

The exogenous acceptors used were the following :

- desialylised fetuin in the case of sialyl, fucosyl and galactosaminyl transferases.
- desialylised and degalactosylised fetuin in the case of galactosyl transferase.
- desialysed, degalactosylised and deglucosaminylised fetuin in the case of glucosaminyl-transferase.

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of their mode of functioning. The glycosylation of the glycoproteins present in the nucleus appears to be the normal functional purpose of the nuclear glycosyl-transferases. Their relationship with their cytoplasmic homologues and possible role of a polyprenol intermediary, are in the course of being studied.

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