CYTOLOGICAL EVENTS INVOLVED IN GLYCOPROTEIN SYNTHESIS IN CELLULAR AND SYNCYTIAL TROPHOBLAST OF HUMAN PLACENTA

An Electron Microscope Autoradiographic Study of \(^{3}\text{H}\text{gal}\) Incorporation

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ABSTRACT

Electron microscope autoradiography was used to study glycoprotein synthesis in cellular trophoblast (cytotrophoblast) and syncytial trophoblast of term human placental villi incubated in vitro with D-\(^{1}\text{H}\text{gal}\) (\(^{3}\text{H}\text{gal}\)). Autoradiographs were analyzed using the hypothetical grain analysis of Blackett and Parry (1973. J. Cell Biol. 57:9–15). The results of this study indicated that \(^{3}\text{H}\text{gal}\) incorporation into term placental villi was predominantly localized to cytotrophoblast. Utilization of \(^{3}\text{H}\text{gal}\) by term syncytial trophoblast was extremely low and yielded too few grains for a quantitative grain analysis. This result is in striking contrast to that found in the preceding study of \(^{3}\text{H}\text{leucine}\) incorporation (Nelson, D. M., A. C. Enders, and B. F. King. 1978). Within cytotrophoblast, the rough endoplasmic reticulum incorporated the most \(^{3}\text{H}\text{gal}\) into glycoprotein. The Golgi apparatus was another site of \(^{3}\text{H}\text{gal}\) incorporation. The vast majority of the \(^{3}\text{H}\text{gal}\) incorporated into cytotrophoblast during the pulse incubation remained intracellular through the duration of the experiment. There was little autoradiographic evidence for secretion of tritiated macromolecules. Cytotrophoblast incubated for the longest time period studied (4 h*) showed a substantial concentration of tritiated macromolecules in the Golgi complex and in the ground plasm but not in the rough endoplasmic reticulum.

KEY WORDS placenta  glycopolys  electron microscopy  trophoblast  autoradiography

Tritiated monosaccharides have been used in a number of studies to label glycoproteins whose synthesis and secretion are to be followed by light and/or electron microscope autoradiography. These studies have demonstrated that some glycoproteins that are synthesized remain within the cell (5, 6, 15, 37, 38, 43) while others are added either to the surface coat (3, 4, 6, 23, 28, 29, 41) or to the basal lamina (12, 13). In other cases, the glycoprotein synthesized is destined for secre-
The human placenta synthesizes and secretes at least two glycoprotein hormones, human chorionic gonadotropin (HCG), and human chorionic thyrotropin (HCT). In addition, a glycoprotein basement membrane component has been isolated from placental villi. A number of poorly characterized pregnancy proteins have also been isolated from placental homogenates, and some of these are glycoproteins.

While the placenta therefore appears to synthesize a variety of glycoproteins, little is known of the cytological events involved in their synthesis and secretion by trophoblast.

In the preceding paper, both cellular and syncytiotrophoblast were demonstrated to have marked capacities for the incorporation of [3H]leucine into proteins. To investigate the capacity of both forms of trophoblast to incorporate monosaccharide into macromolecules, term human placental villi were incubated with [3H]galactose and incorporation of radioactivity was followed by electron microscope autoradiography.

MATERIALS AND METHODS

A total of four term human placentas were used. Tissue preparation and incubation conditions were as described in the preceding paper, except that Medium 199 (M199) contained [3H]leucine ([3H]leu) and the radioactive label was 250 μCi/ml (= 42 μM) of [3H]galactose ([3H]gal) (New England Nuclear; lot no. 477-083; sp act, 5.95 Ci/mmol). Chase medium contained 44 mM pHgal. Radiochemical purity of [3H]gal in the stock solution and in medium incubated with tissue was determined by descending paper chromatography as described by Deppert et al. (14).

To determine whether glutaraldehyde nonspecifically bound [3H]gal to tissue, villi were incubated for 5 min in ice-cold M199 with or without 250 μCi/ml [3H]gal, rinsed 3 times with ice-cold M199 (= 8 min total), and prepared for light microscope autoradiographic analysis. All tissues prepared for electron microscope autoradiographic analysis were fixed for 1 h at room temperature in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) containing 0.05% CaCl₂. Subsequent tissue processing was exactly as described in the preceding paper, except that the phosphate buffer rinse was carried out for only 1-4 h.

RESULTS

Chromatography of [3H]Gal-Containing Solutions

Radiochemical purity of stock [3H]gal was determined to be >97% and >97% of the radioactive species present in medium incubated with tissue chromatographed the same as the stock [3H]gal.

Control Experiment for Fixative Binding of [3H]Gal

Both sets of light microscope autoradiographs prepared from the fixation control experiment exhibited background grain levels, indicating that glutaraldehyde did not nonspecifically bind [3H]gal to tissues.

Light Microscope Autoradiography

Light microscope autoradiographs of tissue incubated with [3H]gal had silver grains located predominantly over the perinuclear cytoplasm of cytotrophoblast, with no apparent concentration in any portion of the cytoplasm. There were also clusters of grains that were not adjacent to a cytotrophoblast nucleus, but were located adjacent to the trophoblast basement membrane and appeared to overlie cytoplasm of syncytiotrophoblast. However, electron microscope autoradiography demonstrated these clusters to be overlying processes of cytotrophoblast (see below). The number of grains overlying syncytiotrophoblast was very low in all cases, even in autoradiographs from the longest exposure times (2 wk). Although the endothelium of fetal vessels and other cells in the villous core had some overlying grains, connective tissue in the villi had only background grain levels at all time-points studied.

Electron Microscope Autoradiography:
Cytotrophoblast

The compartments analyzed as sources and sites in the hypothetical grain analysis are presented in Table 1 along with the numerical distribution of hypothetical grains actually recorded. Since the distribution of activity was assumed to be random, the total number of grains in each row represents the relative area occupied by the organelle (7).
Table I

Distribution of Hypothetical Grains for Uniform Activity throughout the Tissue

| Position of Grid Point (Source of Hypothetical Grain) | SITE OF HYPOTHETICAL GRAIN (POSITION OF CIRCLE) |
|------------------------------------------------------|-------------------------------------------------|
|                                                      | GP      | RER    | Mit   | Golgi | RER/   | Mit   | Golgi/ | Mit   | BM   |
| Ground Plasm (GP)                                    | 20      | 28     | 12    | 12    | 0      | 2     | 19     | 1     | 17   |
| Basement Membrane (BM)                               | 3       | 1      | 1     | 1     | 0      | 2     | 0      | 0     | 18   |
| Rough Endoplasm Reticulum (RER)                      | 2       | 35     | 20    | 25    | 4      | 2     | 0      | 5     | 9    |
| Mitochondria (Mit)                                   | 2       | 1      | 21    | 2     | 1      | 0     | 2      | 2     | 3    |
| Golgi                                                | 1       | 3      | 6     | 15    | 0      | 5     | 8      | 52    | 8    |
| Nucleus (N)                                          | 0       | 4      | 0     | 0     | 11     | 59    | 0      | 2     | 0    |
| Plasma Membrane (PM)                                 | 1       | 0      | 0     | 0     | 0      | 0     | 2      | 0     | 0    |
| Total                                                | 29      | 72     | 60    | 55    | 20     | 68    | 12     | 82    | 21   |

Table I presenting the sites and sources used in the hypothetical and real grain analyses of cytotrophoblast and the numerical totals for each compartment. The column on the right shows the total number of hypothetical grains analyzed and the total number of real grains recorded from autoradiographs of tissue incubated for the time periods indicated. In the hypothetical grain analysis, the total number of grains in each row provides an estimate for the volume occupied by the organelle assumed to be a source of radioactivity in the cell. The total of each column provides the circle analysis for a grain distribution obtainable from cells assumed to contain radioactivity uniformly distributed. All “chase” incubations followed a 20-min “pulse” incubation in radioactive medium.

The summation of grains in each column provides the circle analysis for a distribution of radioactivity assumed to be uniform (7; see also reference 54). By use of sequential computer analysis of the data obtained from the hypothetical grain distribution, it was possible to make estimates of the relative areas of the organelles (Table II). This is an estimate of the relative volumes occupied by each organelle in the cytotrophoblast represented by the micrographs used in the analysis. As can be seen in Table II, ground plasm, Golgi complex occupy approximately equal volumes in the cytotrophoblast, and the nucleus somewhat less than these. Table I presents the total number of grains recorded and the distribution of real grains within each organelle compartment at each time-point. The number of hypothetical grains associated with each compartment when the tissue was assumed to contain a uniform distribution of radioactivity was adjusted to fit the real grain data of each time-point. This alteration allowed for the assignment of a “relative activity” for organelles assumed to be a source. Table II presents the relative activity for each organelle at various time-points.

3 "Ground plasm” is a compartment used in the Blackett and Parry analysis (7), and can be considered synonymous with the phrase “cytoplasmic matrix” or “ground substance.”

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Table II presenting the values of the relative area of each organelle assumed to be a source of radioactivity in cytotrophoblast and the relative activity of each organelle for each incubation period. The relative activity of each organelle as a function of time can be studied by reading across each row. All "chase" incubations followed a 20-min "pulse" incubation in radioactive medium.

| Organelle | Relative Area | 5 min Pulse | 20 min Pulse | 40 min Pulse | 20 min Chase | 40 min Chase | 4 h Chase |
|-----------|--------------|-------------|--------------|--------------|--------------|--------------|----------|
| GP        | 26±1         | 14±16       | 10±12        | 27±10        | 37±17        | 32±17        | 48±14    |
| PM        | 3±1          | 10±6        | 11±5         | 2±5          | 9±5          | 8±4          | 0±2      |
| BM        | 5±1          | 1±2         | 2±2          | 11±4         | 5±4          | 7±4          | 18±6     |
| RER       | 21±2         | 43±9        | 61±8         | 40±8         | 24±13        | 27±12        | 8±7      |
| Mit       | 8±1          | 0±1         | 0±4          | 0±4          | 0±5          | 3±4          | 0±1      |
| Golgi     | 21±1         | 32±7        | 16±5         | 19±5         | 21±5         | 21±7         | 26±8     |
| N         | 16±2         | 0±1         | 0±1          | 1±2          | 4±3          | 2±2          | 0±1      |

Table II presenting the values of the relative area of each organelle assumed to be a source of radioactivity in cytotrophoblast and the relative activity of each organelle for each incubation period. The relative activity of each organelle as a function of time can be studied by reading across each row. All "chase" incubations followed a 20-min "pulse" incubation in radioactive medium.

The relative activity estimates in each organelle as a function of time, i.e., reading across each row of the table, indicate that the labeling of ground plasm increased through the entire incubation period, becoming the highest labeled compartment in cytotrophoblast (Fig. 1). Golgi complex labeling was highest in tissue incubated for 5 min, then decreased slightly to a plateau which was maintained throughout the incubation period in chase medium (Figs. 2 and 3). At any one time-point, not all Golgi complexes were labeled (Figs. 2 and 4). Interfaces between RER and Golgi complex were frequently observed to have overlying grains (Figs. 2 and 3). RER had its peak labeling at 20 min (Figs. 2 and 3). The relative activity of RER decreased in cytotrophoblast incubated in chase medium, being lowest after the longest incubation. The basement membrane underlying the cytotrophoblast exhibited virtually no labeling after short incubations, increased its relative activity with longer periods of incubation, and had its highest activity in tissue incubated for 4 h in chase medium (Fig. 5). Compared to the relative activity after a 20-min pulse, the relative activity of the basement membrane increased ninefold during the 4-h chase incubation. The connective tissue underlying the basement membrane exhibited background grain levels at all times (Fig. 5). Mitochondria, plasma membrane, and nuclei showed very low levels of labeling at all time-points (Fig. 5).

Table III presents the results of the chi-squared test. Chi-squared values < 19 are reasonable data fits at P values of 0.05 or greater. The high value at 20-min chase (45 ± 20) is probably not significant because the variation in X^2, i.e., SE is very high, indicating that lower values were obtained in the calculation of the standard error.

Electron Microscope Autoradiography: Syncytial Trophoblast

Electron microscope autoradiographs of tissue incubated with [3H]gal exhibited too few grains overlying syncytiotrophoblast to allow a grain analysis to be performed. Even after exposure periods that yielded high grain densities over cytotrophoblast cytoplasm, very few grains were located overlying syncytiotrophoblast (Figs. 1 and 6). The clusters of grains observed in the light microscope autoradiographs apparently overlying syncytiotrophoblast adjacent to base-
DISCUSSION

This study has demonstrated that [H]gal incorporation into term placental villi was predominantly localized to cytotrophoblast while utilization of [H]gal by syncytial trophoblast was extremely low. This result is in striking contrast to that found in the preceding study of [H]leucine incorporation by trophoblast where both cellular and syncytial trophoblast exhibited marked capacities for incorporation of the radioactive amino acid (35). Within cytotrophoblast, the RER incorporated the most [H]gal into glycoprotein. The Golgi apparatus was another active site of [H]gal incorporation. Some of the newly synthesized molecules labeled during the pulse incubation eventually appeared in the cytotrophoblast cytoplasm. A substantial concentration of glycoproteins remained associated with elements of the Golgi apparatus, but not with the RER, in cytotrophoblast incubated for the longest time period studied (4 h). There was little autoradiographic evidence for glycoprotein secretion by cytotrophoblast and no evidence on which to propose a pathway for processing secretory product.

There is evidence to indicate that the carbohydrate chains of glycoproteins are synthesized by the sequential addition of monosaccharides either directly to a polypeptide backbone or to a lipid intermediate that subsequently transfers a growing oligosaccharide chain to the polypeptide (10, 31, 40, 49). Cell fractionation studies have shown that glycosyltransferases are found in high concentration in the Golgi complex especially (21, 33, 44, 45). Electron microscope autoradiographic studies have localized the Golgi complex as a principal site for the incorporation of tritiated monosaccharides into the oligosaccharide chains of glycoproteins (3-6, 18, 22-25, 32, 34, 36-39, 43, 50, 53, 56). The role of the Golgi complex in this aspect of glycoprotein synthesis has been reviewed in detail (52). The addition of monosaccharides to the oligosaccharide chains of glycoproteins occurs in a specific order determined by the sequence of sugars in the oligosaccharide chain itself and is not exclusively the result of Golgi glycosyltransferases. Electron microscope autoradiographic studies of thyroglobulin (25, 53) and immunoglobulin G (56) synthesis have indicated that monosaccharides located proximal to the polypeptide backbone, e.g., mannose, N-acetylglucosamine, are added to the oligosaccharide chains of the glycoprotein molecule while it is still in the RER, while sugars located distally in the...
FIGURE 2 Electron micrograph of a portion of a cytotrophoblast cell in an autoradiograph of tissue incubated for 5 min in [3H]gal-containing medium. A high grain concentration overlies the tangentially sectioned RER in the upper left of the figure. Some Golgi areas have a few overlying grains. Several profiles of RER apparently fusing with Golgi membranes are present. Exposure, 2 mo. Bar, 0.5 μm. × 30,000.

FIGURE 3 Electron micrograph of cytotrophoblast in an autoradiograph of tissue incubated for 20 min in [3H]gal-containing medium and then an additional 20 min in nonradioactive medium. The numerous Golgi-associated vesicles have overlying grains, but the RER/Mit of this cells is unlabeled. Exposure, 4 mo. Bar, 0.5 μm. × 30,000.
oligosaccharide side chains, e.g., galactose, fucose, are incorporated at the level of the Golgi complex. Recent biochemical studies by Waechter and Lennarz (see reference 49 for review) suggest that a lipid intermediate is important in receiving some monosaccharides, e.g., mannose, N-acetylglucosamine, from nucleotide-sugar donors. The sequential addition of specific sugars to such lipid carriers serves to build at least a part of the oligosaccharide side chain of some glycoproteins before the time when the chain is transferred to the polypeptide backbone of the glycoprotein molecule. Galactose transfer to lipid intermediates has not been studied in detail (49). Autoradi-
Table III giving the results of the chi-squared analysis of the grain data for cytotrophoblast.

| Incubation Time | X²±SE | Degrees of Freedom |
|-----------------|-------|--------------------|
| 5 min Pulse     | 16±10 | 10                 |
| 20 min Pulse    | 13±10 | 10                 |
| 40 min Pulse    | 28±14 | 10                 |
| 20 min Chase    | 45±20 | 10                 |
| 40 min Chase    | 26±11 | 10                 |
| 4 h Chase       | 17±10 | 10                 |

Table III giving the results of the chi-squared analysis of the grain data for cytotrophoblast.

Glycoprotein Synthesis in Cellular and Syncytial Trophoblast

The incorporation of [3H]gal by syncytial trophoblast was at such a low level is somewhat of an enigma. On the basis of its cytologic appearance and presumed secretory functions, syncytial trophoblast was expected to be the predominant location for the incorporation of a glycoprotein precursor such as [3H]gal. It is unlikely that the low level of incorporation is due to poor viability of syncytium since syncytial trophoblast incorporates [3H]leucine actively when incubated under the same conditions used in this study (see companion to this paper). In addition, the small number of grains overlying syncytium can not be attributed to grain loss during processing of the autoradiographs since the adjacent cellular trophoblast cells had numerous grains present.

Only term placental tissue was used in this study, and, thus, the glycoprotein synthetic activity of syncytial trophoblast at the end of its normal lifespan is reflected by this study. Syncytial trophoblast from placentas obtained earlier in gestation may be more active in glycoprotein synthesis.
In addition, secretion by cellular and/or syncytial trophoblast may be more active earlier in gestation so that an autoradiographic study of villi obtained earlier in gestation might provide the information about intracellular pathways followed by placental secretory products that this study was unable to illustrate. One glycoprotein secretory product, HCG, has been localized to the syncytiun and is known to contain galactose (47). However, placental secretion of this glycoprotein hormone is highest in first trimester and lowest in term placentas. Thus, a small amount of synthesis by the large mass of syncytiun present in the term placenta could provide the serum concentrations present at the end of gestation and yet synthesis of HCG molecules might not be detected by an electron microscope autoradiographic study of glycoprotein synthesis.

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