In vitro translation of human placental pregnancy-specific beta$_1$-glycoprotein (SP$_1$)[1]

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Summary. Synthesis of SP$_1$-glycoprotein by the human placenta was directly demonstrated, by in vitro translation of RNA extracted from full term and from early placentas in a cell-free wheat germ system followed by specific immunoprecipitation of the radioactively labeled nascent peptides. De novo synthesized SP$_1$-glycoprotein in both RNA preparations accounted for 1-1.3% of total protein synthesis.

The presence of the pregnancy-specific beta$_1$-glycoprotein (or SP$_1$), the biological acitivity of which is 1 unknown, in the plasma of normal pregnant women, has long been characterized[3]. During mid-term and throughout the last stage of gestation, SP$_1$ plasma concentration is highest among the proteins specifically associated with pregnancy. Recently, SP$_1$ has been proved potentially applicable as a marker for pregnancy complications[4,5] and for prognosis of some particular malignancies[6].

Although the significance of SP$_1$ has become increasingly evident, the origin of the protein is still being widely debated. The evidence that SP$_1$ originates from the placenta is good, but not overwhelming. Localization of the protein in the placental syncytiotrophoblast by immunofluorescent staining[7-9] does not definitely show that it is produced by these cells because the protein may be secreted by maternal tissue and merely absorbed by the placenta[10]. Recently, more concrete evidence has been produced by Actis et al.[11], who showed that full-term placental tissue slices incubated in vitro were capable of synthesizing and secreting SP$_1$. In the present studies, biosynthetic capacities for SP$_1$ of both full-term and early placentas were confirmed by using in vitro cell-free translation of the mRNA extracted from the placental tissues.

Materials and methods. Human placental tissues were obtained from either normal delivery at full-term or 10-12 week therapeutic abortions. Total RNA was prepared by the methods similar to those previously described by Fiddes and Goodman[12]. Futher enrichment of the poly(A)-RNA was performed using oligo(dT)-cellulose columns (Collaborative Research Lab). RNA samples were translated by wheat germ cell-free extracts[13] in the presence of human placental ribonuclease inhibitor (ENZO Biochemicals, New York)[14] to obtain maximum incorporation of radioactivity. Reaction mixtures (100 µl) containing 14 mM Hepes (pH 7.4), 2.37 mM ATP, 1.5 mM Mg^{++}, 0.118 mM of 19 L-amino acids (omit L-leucine), 0.8 mM spermidine and 20 µCi ^{3}H-L-leucine (New England Nuclear) were incubated at 22 °C for 120 min. Total radioactivity incorporation was measured in 5 µl aliquots on a 3 MM Whatman filter paper following trichloroacetic acid precipitation. De novo synthesized SP$_1$ was detected by immunoprecipitation with homologous antiserum (Dako, Denmark) and formaldehyde-treated *Staphylococcus aureus*[15]. Reticulocyte RNA was prepared from rabbits with phenylhydrazine-induced anemia.

In vitro translation of SP$_1$-glycoprotein by RNA from human placentas

Source of RNA	Total protein (cpm-^{3}H-L-leu)	Incorporation into SP$_1$ (% total)
Background	15,176	-
Total RNA:		
Rabbit reticulocyte	135,126	0.14
Early placenta	155,763	1.16
Term placenta	107,289	0.98
Poly(A)-RNA:		
Early placenta	120,900	1.26
Term placenta	135,948	1.32

The radioactive SP$_1$ was eluted by sodium dodecylsulphate from the immunoprecipitate and the radioactivity counted on a 3 MM Whatman filter paper following trichloroacetic acid precipitation. Results are expressed as the mean values of duplicate experiments after subtraction of the background in which no RNA was added. Total RNA added in 100 µl incubation mixture were 0.57, 0.6, 0.6 A_{260} for reticulocyte, early, term and poly(A)-RNA were 0.17, 0.18 A_{260} for early and term placentas respectively.

Results and discussion. RNA extracted from full term and early placentas were translated in the wheat germ cell-free system with ^{3}H-L-leucine. The efficiencies of stimulation of incorporation of radioactivity by both RNA preparations were similar, and about 10-15-fold greater than wheat germ endogeneous activity in the presence of the placental RNase inhibitor. Translational efficiency without the inhibitor, as has previously been reported by many investigators[12,16,17], was markedly decreased to about ½ in our experiment. Moreover, the inhibitor could also prolong the linear incorporation of radioactivity for up to 120 min at 22 °C. Radioactively labeled de novo synthesized SP$_1$ was detected by specific immunoprecipitation following translation. Maximal immunoprecipitation of radioactive SP$_1$ was obtained with 2 µl of the undiluted antiserum and 50 µl of 10% (w/v) *S. aureus* suspension. The synthesis of SP$_1$ accounted for only about 1-1.3% of the total protein in both full term and early placental RNAs; a relatively insignificant amount of radioactivity was observed as a result of the similar translation using rabbit reticulocyte RNA (table).

In order to determine whether SP$_1$ synthesis was directed by poly(A)-mRNA, placental RNAs obtained from oligo(dT)-cellulose chromatography were also translated in vitro. The total activity of SP$_1$ synthesis was found in the poly(A)-mRNA fraction (table), and the values appeared to be always higher than that found in total RNA. This result is not easily explained, but could be due to the removal of some non-poly(A)-mRNA by the oligo(dT)-column which consequently increased the proportion of SP$_1$-directed mRNA in the poly(A)-mRNA fraction.

The concentration of SP$_1$ in serum in full-term pregnancy has been previously reported to be 3-4 times higher than the level in early stage of gestation[3]. Equal translational activity of the RNA extracted from tissues from both stages (table) indicated that the serum concentration of SP$_1$ at different times of gestation was probably determined by the total mass increase of the tissue rather than increase of cellular mRNA, respectively.

The relative amount of SP$_1$-mRNA assessed in the present study was slightly different from the 3-5% which has been previously demonstrated using in vitro full term placental tissue slices[11]. The lower value in this report might be due to the precence of RNase activity in the wheat germ extract[18]. Immunoprecipitation of SP$_1$ following in vitro translation in the absence of the placental RNase inhibitor markedly reduced the amount of de novo synthesized radioative SP$_1$ (unpublished observation).

In conclusion, the present study directly demonstrates the capacity of both full-term and early human placentas for the synthesis of placenta-specific SP_1 glycoprotein.

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Day and night levels of hormones in male rhesus monkeys kept under controlled or constant environmental light[1]

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Summary. The marked differences between day and night levels of testosterone, cortisol, prolactin and bioactive luteinizing hormone observed in adult male rhesus monkeys maintained at 12 h light:12 h dark schedule are not changed by exposing monkeys to constant illumination for a period of up to 15 days. These findings suggest that the photoperiodicity of environmental light may not be the principal determinant for the occurrence of the diurnal rhythms observed in the circulating levels of the 4 hormones studied.

The photoperiodicity of environmental light is known to bear a causal relationship with the timing of many reproductive events, including the circadian rhythmicity of the circulating levels of hormones in most of the lower mammals so far studied. This relationship makes it possible to predict occurrence of certain reproductive events in animals maintained under well controlled light:dark schedules. This is particularly true for the albino rat which is amongst the most commonly used experimental animal.

Rhesus monkeys *(Macaca mulatta)* maintained under controlled environmental lighting conditions also exhibit distinct diurnal rhythms in their circulating levels of testosterone (T)[3-5], cortisol (C)[6,7] bioactive luteinizing hormone (bLH)[8] and prolactin (PRL)[8]. It is not known whether the photoperiodicity of environmental light plays a role in determining these daily rhythms. Such information would be of pertinent relevance to the better evaluation of any experimental procedure aimed at altering the endocrine function of rhesus monkeys. The need for such information becomes obvious in view of monkeys constituting an important experimental animal model in the preclinical evaluation of drugs and contraceptives.

Materials and methods. The present studies were carried out in 6 adult male rhesus monkeys (9-14 kg, b.wt) maintained

Table 1. Characteristics of RIA of T, C, PRL and bioassay of LH

Hormone	No. of assays	% coefficient of variation Intrassay	Interassay	Sensitivity
T	22	3.62	6.42	10pg
C	18	6.50	11.38	50pg
bLH	19	8.30	13.52	2ng
PRL	8	4.62	6.89	10μIU

Table 2. Mean levels (± SEM) of hormones in blood samples taken during day and night from adult male rhesus monkeys kept under either 12 h light: 12 h dark schedule or constant light

Hormones	12 h light: 12 h dark Day	Night	Constant light Day	Night
T (nmole/l)	28.11± 4.23	72.82± 4.84***	28.96± 2.08	65.14± 3.30***
C (nmole/l)	817.40±24.20	366.80±21.10***	779.10±34.11	563.12±28.87***
bLH (μg/ml)	1.46± 0.09	2.79± 0.07***	1.58± 0.05	2.46± 0.28**
PRL (mIU/l)	326.51±23.39	522.11±32.12**	362.50±37.71	478.60±44.00*

*$p<0.05$; **$p<0.005$; ***$p<0.001$. Comparisons between day and night levels done by Student's t-test[13].