J. Biosci., Vol. 1, Number 2, June 1979, pp. 135-141.

Uterine RNA synthesis during protein deficiency and steroid-maintained pregnancy in rats*†

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MS received 22 November 1978; revised 10 March 1979

Abstract. Some aspects of uterine RNA synthesis including [^{3}H]-uridine incorpo ration into RNA, activities of RNA polymerases and ribonucleases were studied. It was observed that both normal and pregnant animals, kept on protein-free diet for 15 and 20 days, showed a significant increase in *in vivo* uptake of [^{3}H]-uridine into total RNA. Activities of RNA polymerase I and polymerase III increased two-fold in animals kept on a protein-free diet; however, RNA polymerase II activity was unaffected by protein restriction. In animals kept on protein-free diet where pregnancy was maintained by exogenous estrogen and progesterone, specific activity of nuclear RNA was further increased and the activities of RNA polymerases I, II and III markedly increased. Levels of RNase were also increased significantly during protein deficiency, thus showing a rapid turn-over of uterine RNA. These observations indicate that during protein restriction, uterine RNA synthesis is regulated at transcriptional level by a selective stimulation of RNA polymerase and RNase also plays an important role.

Keywords. Protein deficiency; steroid-maintained pregnancy; RNA synthesis; rat uterus; RNA polymerases.

Introduction

Failure of pregnancy due to decreased levels of pituitary gonadotropins and ovarian hormones, and its maintenance by exogenous estrogen and progesterone during protein deficiency have previously been reported by several groups (Fisher and Leatham, 1965; Hazelwood and Nelson, 1965). It is also known that cells undergo several molecular responses and adaptations under the influence of malnutrition. Increasing lines of evidence suggest that a low protein diet (5-6%) results in increased incorporation of labelled precursors into liver RNA in rats (Wannemacher *et al.*, 1971; Kawada *et al.*, 1977; Shaw and Fillios, 1968). However, the precise mechanism involved in regulation of RNA synthesis under these conditions is still vague. It has been postulated that increased uptake of precursors

*Central Drug Research Institute Communication No. 2495.
†Part of this work was presented at the 47th Annual General Meeting, Society of Biological Chemists, Delhi, 1978,

into RNA is an early event in the action of steroid hormones in rat uterus (Miller and Baggett, 1972; Gorski and Nicolette, 1963).

In the present investigation an attempt has been made to elucidate the changes in uterine RNA synthesis at the transcriptional level, during protein deficiency and steroid-maintained pregnancy. It was also examined whether the changes are due to changes in RNA synthesis or degradation or a combination of the two.

Materials and methods

Composition of diet

Two types of semi-synthetic diets (18% casein and protein-free diet) were prepared according to the method of Leatham and Wolf (1954) with the substitution of hydrogenated oil made by Hindustan Lever Ltd., Bombay, instead of ' Crisco', made in USA. Vitamin mixture per 100 g diet contained: 0·8 mg thiamine-HC1; l·6 mg riboflavin; 4 mg each, of nicotinamide, *p*-aminobenzoic acid and α-tocopherol; 4·4 mg calcium pantothenate; 21·6 mg inositol; 1 mg vitamin K and 0·8 mg pyridoxine. All the constituents of diet were sufficient for normal growth and met the energy requirements of animals.

Animal groups

Albino rats bred at the Institute colony, weighing 140-150 g, were used. A group of virgin rats was transferred to 18% casein diet and another to protein-free diet. Some rats were mated on day 1 of pregnancy (when spermatozoa were found in vaginal levage). They were divided into 3 groups, viz., animals fed with (i) 18% casein diet, (ii) protein-free diet and administered 0·2 ml oil vehicle subcutaneously (s.c.) and (iii) protein-free diet + 1 *μg* estrone + 4 mg progesterone in 0.2 ml oil (s.c.) daily from day 3 to 20 of pregnancy.

Determination of RNA synthesis

A few preliminary experiments were performed to determine the maximum incorporation and optimal dose of [^{3}H]-uridine (sp. activity 1250 mCi/mmol from Bhabha Atomic Research Centre, Bombay). Each animal received an injection of 50 μCi [^{3}H]-uridine intraperitoneally (i.p.) in 0·5 ml saline, 20 min before sacrifice. Uteri were removed and the number of implantations and features was recorded. Labelled RNA was determined according to Trams *et al.* (1973).

Radioactivity was measured in 10 ml of ' Diatol' scintillation fluid (3·25 g PPO, 6·5 mg POPOP, 52 g naphthalene, 250 ml each of toluene and dioxane and 150 ml methanol). Nucleotide pool in each experiment was measured and specific incorporation was calculated for per million counts per minute present in perchloric and soluble fractions (Sälaman *et al.,* 1972).

Determination of RNA polymerases

Assay of RNA polymerase I, II and III was based on the principle that α-amanitin inhibits specifically RNA polymerase II at low concentration *in vitro* and a Mg^{2+} low salt system and Mn^{2+}-high salt system can be used for assay of RNA polymerases I and III (Kedinger *et al.,* 1970; Roeder and Rutter, 1969).

Animals were sacrificed after 15 and 20 days feeding and uteri were pooled. Procedure for isolation, purification of uterine nuclei by sucrose gradient was essentially the same as described by Webster and Hamilton (1976). Each assay system contained 0·3 μmol each ATP, GTP, UTP; 3 nmol (^{3}H)-CTP. (sp. act. 35 mCi/mg, New England Nuclear, Boston, USA); 50 μmol tris-buffer pH (8·5 or 7·5); 0·1 μmol EDTA, 1 μmo1 2-mercaptoethanol, 30% v/v glycerol, 2·5 μmol $MgCl_2$ or 2 μmol $MnC1_2$ plus 200 μmo1 sodium sulphate in presence or absence of 100 ng α-amanitin. Incubation and pre-imcubation were carried out at 37° C.

Measurement of ribonuclease activity

Animals were sacrificed and uteri were homogenised in 10 mM trisHCl buffer (pH 7·0) and centrifuged at 10000 g for 30 min at 0-4° C. Activity of RNase in the homogenate and 10000 g supernatant was determined by the method of De Duve *et al.* (1955). The reaction mixtures containing 0·2 ml enzyme solution, 0·1 ml Triton X-100 (1·0%) and 0·5 ml dialysed RNA (3 mg/ml in 0·1 M sodium acetate buffer pH 5·0) and 0·2 ml water were incubated at 37° C for 30 min. The reaction was stopped by the addition of 2·0 ml cold 10% perchloric acid containing 0·25% (W/V) uranyl acetate. Tubes were kept in cold for 1 h and centrifuged at 2000 rpm for 10 min. In control tubes, RNA was added after perchloric acid addition. Enzyme activity (1 unit) was expressed as the amount of enzyme required to produce an absorbance change of 1 at 260 nm.

Other analyses

Uterine RNA and DNA contents were measured by the orcinol method (Albaum and Umbreit, 1947) and the method of Burton (1956) respectively. Protein was estimated by the procedure of Lowry *et al.* (1951).

Internal efficiency of the liquid scintillation counter (Packard-3320, USA) used was calculated and the radioactivity count was corrected to 100% efficiency by external standardisation. Significance of mean values was determined by student's *t*-test.

Results

In preliminary experiments, maximum incorporation of [^{3}H]-uridine into total RNA was observed at 50 μCi dose, injected 20 min before sacrifice.

Uterine RNA, DNA and protein (mg/uterus) contents decreased markedly during protein deficiency as shown in table 1. The decrease in RNA and protein (mg/g wet wt) is highly significant ($P<0·01$) as compared to DNA concentration.

The incorporation pattern of [^{3}H]-uridine in different groups is summarised in table 2. A significant increase ($P < 0·01$) is observed in normal and pregnant rats kept on protein-free diet for 15 and 20 days. In normal pregnancy (group 3), the animals show a marked rise in nuclear specific activity of RNA ($P < 0·05$) as compared to virgin controls. Under steroid maintained pregnancy (group 5) we observed a much higher degree of incorporation ($P < 0·01$) of [^{3}H]-uridine into total RNA,

Table 1. Effect of protein deficiency on protein, RNA and DNA content of rat uterus.

Groups	Uterus wt (mg)		Protein (mg)		RNA (mg)		DNA (mg)	
	15 day	20 day	15 day	20 day	15 day	20 day	15 day	20 day
18% diet, Non-pregnant	362 ±12	385 ±7	52·00 ±2·90	54·37 ±1·83	5·74 ±0·14	5·46 ±0·10	1·82 ±0·04	1·90 ±0·05
Protein-free diet, Non-pregnant	210 ±10	178 ±8	18·66 ±1·08	16·50 ±1·82	1·70 ±0·03	1·44 ±0·06	0·96 ±0·02	0·84 ±0·08
18% diet, Pregnant	1146 ±22	1262 ±27	162·35 ±6·82	170·12 ±9·80	17·48 ±0·26	21·35 ±0·30	5·94 ±1·01	6·33 ±0·02
Protein-free diet Pregnant	196 ±5	180 ±6	16·04 ±0·50	15·22 ±1·38	1·74 ±0·04	1·53 ±0·02	0·86 ±0·03	0·80 ±0·04
Protein-free diet Pregnant + estrone + progesterone	986 ±14	1075 ±18	120·73 ±8·65	128·73 ±5·25	12·53 ±0·43	13·99 ±0·21	5·30 ±0·07	5·78 ±0·04

Values are expressed as mean ± S.E.; number of animals were 5 in each group.

Table 2. *In vivo* incorporation of [^{3}H]-uridine into uterine RNA during short-term protein deficiency.

Groups	Total incorporation dpm × 10^3/100 mg uterus		Specific incorporation dpm × 10^3/mg RNA	
	15 days	20 days	15 days	20 days
Non-pregnant groups				
18% diet	27·40±0·72	24·95±0·51	14·29±0·23	13·66±0·20
Protein-free diet	22·86±0·36	19·64±0·28	23·14±0·68	23·71±0·52
Pregnant groups				
18% diet	36·35±0·71	35·04±0·59	19·48±0·24	15·84±0·25
Protein-free diet	24·88±0·27	27·57±0·26	23·78±0·35	27·61±1·24
Protein-free diet + estrone + progesterone	48·41±0·12	43·15±0·80	27·90±0·28	31·45±0·25

Values are expressed as mean ± S.E.; number of animals in each group, 5.
Each animal received a dose of 50 μCi (^{3}H) uridine in 0·5 ml saline (I.P.), 20 min before sacrifice.

Table 3. Distribution of RNA polymerases I, II and III in rat uterus in response to dietary protein restriction.

Groups	RNA polymerase activity (dpm × 10^3/100 μg DNA)					
	15 days			20 days		
	Type I	Type II	Type III	Type I	Type II	Type III
Non-pregnant groups						
18% diet	11·81±0·42	46·25±1·92	7·85±0·55	10·77±0·32	42·94±2·26	8·07±0·33
Protein-free diet	21·40±1·10	48·68±2·61	16·24±0·83	19·23±0·76	46·29±1·50	16·64±1·22
Pregnant groups						
18% diet	16·70±1·63	60·16±3·71	11·32±0·51	15·83±0·88	63·35±2·82	10·72±0·40
Protein-free diet	31·90±1·68	56·12±4·45	19·65±1·25	33·27±1·39	59·47±2·09	21·40±1·36
Protein-free diet + estrone + progesterone	23·67±2·52	71·80±4·82	16·96±0·92	25·48±1·74	76·22±3·70	17·68±1·18

Values are mean ± S.E.; number of animals in each group, 6.

Effect of dietary protein restriction on activities of RNA polymerases in rat uterus is shown in table 3. DNA was considered as a reference. for expressing enzyme activity determinations. Activities of RNA polymerase I (Mg^{2+}system) and polymerase III (Mn^{2+}-system) are increased significantly ($P < 0{\cdot}01$) whereas RNA polymerase II did not change during protein deficiency (15 and 20 days). On the other hand, in steroid-maintained pregnancy group as well as in normal pregnant animals, activities of all the RNA polymerases are stimulated with a significant rise in type II ($P < 0{\cdot}01$). It is apparent from the above observations that a correlation exists between stimulated specific incorporation and RNA polymerases in different groups.

Levels of RNase activity in total homogenate and 10000 g supernatant, increased sharply ($P<0{\cdot}01$) in animals fed on a protein-free diet of normal and pregnant groups, as represented in table 4. Pattern of RNase activity is quite comparable on 15 and 20 days of protein restriction in different groups. No remarkable changes are obtained in RNase level during steroid-maintained pregnancy over control values.

Discussion

The observed increase in [^{3}H]-uridine incorporation into total uterine RNA of protein deficient animals, is an extension of the earlier reports that ability of liver nuclei to incorporate labelled precursors is enhanced during malnutrition (Shaw and Fillios, 1968; Wannemacher *et al*., 1971).

Table 4. Ribonuclease activity in homogenate and 10000 g supernatant during protein deficiency in rat uterus.

Groups	Specific activity of RNase			
	Homogenate		Supernatant	
	15 days	20 days	15 days	20 days
Non-pregnant groups				
18% Casein	2·03±0·15	2·18±0·08	1·61±0·10	1·73±0·05
Protein-free diet	3·69±0·07	3·80±0·32	3·20±0·32	3·39±0·24
Pregnant groups				
18% Casein	1·82±0·28	1·72±0·16	1·25±0·10	1·25±0·06
Protein-free diet	3·36±0·22	3·53±0·07	3·05±0·30	3·21±0·24
Protein-free diet + estrone + progesterone	2·16±0·15	2·06±0·20	1·75±0·12	1·78±0·09

Values are mean with S.E.; number of animals in each group, 6.

In order to better understand the differential response of uterine RNA synthesis to dietary protein restriction, studies with RNA polymerases were carried out. RNA polymerase I and III, localised in nucleolus, are responsible for preferential synthesis of r-RNA, t-RNA and 5S-RNA (Weinmann and Roeder, 1974; Anderson and Decken, 1975). Our observations on the selective stimulation of RNA polymerase I and III, on experimental protein restriction, would suggest increased r-RNA and t-RNA synthesis in rat uterus. However, activity of RNA polymerase II (m-RNA synthesis) is not affected (table 3). Lewis and Winick (1978) have also recently shown that protein deprivation leads to increased synthesis of nucleolar RNA species.

During normal and steroid-supported pregnancy, specific activity of uterine RNA showed a further increase (table 2) and activity of RNA polymerase II (responsible for transcription of m-RNA) is also stimulated. It can be suggested that estrogen and progesterone during later part of pregnancy exerts a combined or net stimulatory effect on RNA synthesis through RNA polymerases for uterotropic activity. An early stimulation of RNA polymerase II (m-RNA synthesis) followed by other responses by steroid hormone has been reported by Glasser *et al.* (1972). Hormone-induced RNA synthesis (Miller, 1973; Miller and Emmens, 1969) and increased incorporation of precursors during mid-term pregnancy (Heald and O'Hare, 1973) has been well-documented.

Activity of RNase shows a significant increase during protein deficiency over controls showing a higher 'turnover' rate of uterine RNA. Several groups of workers have previously reported that RNA metabolism is regulated by an `RNase-inhibitor' mechanism in malnourished animals (Girija *et al.*, 1965; Kraft and Shortman, 1970; Morgan and Winick, 1977).

We conclude, on the basis of the above observations that a short-term protein restriction leads to increased 'RNA-turnover' in rat uterus while the synthesis of nucleolar RNA is stimulated as shown by stimulated RNA polymerase I and III. During steroid-maintained pregnancy, uterotropic response is preceded through RNA polymerases (specifically polymerase II), which are stimulated by steroid hormones.

It is intriguing to observe how the system is adaptable to utilise its ever-decreasing resources to the maximum and why accumulation of RNA increases during protein deprivation.

Acknowledgement

One of the authors (R. K. S.) is thankful to Indian Council of Medical Research, New Delhi, for an award of Junior Research Fellowship.

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