IWONNA RAHDEN-STARON and WITOLD SENDECKI

EFFECT OF UNILATERAL NEPHRECTOMY ON THE RNA SYNTHESIS IN HYPERTROPHIC RAT KIDNEY

Department of Biochemistry, Institute of Biopharmacy, Medical School,
Banacha 1, 02-097 Warszawa, Poland

1. The hypertrophic rat kidney after unilateral nephrectomy showed a time-
dependent increase in the RNA/DNA ratio. This was associated with the increased
rate of z-amanitin-insensitive RNA synthesis in isolated nuclei.
2. The increased activity of solubilized RNA polymerase was due to polymerase I,
II and III as judged from the DEAE-Sephadex chromatography.
3. Chromatin DNA from hypertrophic kidneys was more susceptible to DNAase I
than DNA from control kidneys.

RNA content in hypertrophic kidneys increases rapidly prior to cell
multiplication (Halliburton & Thompson, 1965; Malt, 1969). Although this
phenomenon has been extensively investigated, the detailed mechanism resp-
sponsible for the elevated RNA content still remains obscure. The increase
might be due to changes in RNA synthesis or/and RNA degradation. In
the first possibility the transcriptional activity could be modulated
by changes in the level of RNA polymerase (RNA nucleotidyltransferase,
EC 2.7.7.6), (Roeder & Rutter, 1970; Roeder, 1974b), mainly of polymerase I
(Chomczyński et al., 1977; Leonard & Jacob, 1977), or to a more “loose”
structure of chromatin template (Foe et al., 1976; McKnight & Miller,
1976). The last finding is supported by the observations indicating that
transcriptionally active genes are more susceptible to exogenous nuclease
e.g. DNAase I (Garel & Axel, 1976; Weintraub & Groudine, 1976) than
inactive genes.

In the present work we found that RNA synthesis is responsible for
the elevated RNA content in the hypertrophic kidney. Both the increase

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in the activity of RNA polymerase and changes in the chromatin template were observed although different time patterns excluded the simple cause-effect relation between these two processes and the increased rate of RNA synthesis.

MATERIAL AND METHODS

**Chemicals.** Uracil-[U-14C]5'-UTP (specific radioactivity 321 mCi/mmole) was from UVVR (Praha, Czechoslovakia); 1,4-dithiothreitol was from LOBA-Chemie (Wien-Fischamend, Austria); DNA from calf thymus (A grade) was from Calbiochem (Los Angeles, Calif., U.S.A.); CTP, GTP, ATP, α-amanitin (A grade) were from Calbiochem (San Diego, Calif., U.S.A.); MgCl₂ (1 M stock solution) was from B.D.H. Chemicals Ltd. (Poole, Dorset, U.K.); RNA ex yeast, Tris, 2,5-diphenyloxazole (POPOP) were from Koch-Light Lab. Ltd. (Colnbrook, U.K.); bovine serum albumin, sucrose, phenylmethyl sulphophenyl-fluoride were from Serva (Heidelberg, F.R.G.); deoxyribonuclease I from bovine pancreas was from Sigma (St. Louis, MO, U.S.A.); DEAE-Sephadex A-25 was from Pharmacia (Uppsala, Sweden); glass-fibre paper Whatman GF/C was from W.R. Balston (Springfield Mill, Maidstone, U.K.). Other chemicals of analytical grade were from Ciech (Gliwice, Poland).

**Animals.** Male albino rats (Wistar) weighing 150 - 180 g were used for the experiments. The animals were given water and fed with standard LSM Mixture (Bacutil, Warsaw) ad libitum. Unilateral nephrectomy and sham-operation were performed as described by Sendecki et al. (1972). At the time intervals indicated the animals were killed by cervical dislocation, the right kidney was removed and placed in ice-cold 50 mM-Tris/HCl buffer, pH 8, containing 0.15 M-NaCl. All further steps were carried out at 0 - 4°C. For each experiment 10 operated and 5 control rats were used.

**Isolation and the assay of RNA polymerase.** RNA polymerase was isolated from total homogenate of rat kidneys according to Roeder (1974a). The homogenate was sonicated in MSE ultrasonic disintegrator (Mk 2) 4 × 15 sec at 0 - 4°C. The amplitude was 12 μ.

The reaction was carried out at 37°C for 20 min in 0.25 ml of the mixture containing: 100 mM-Tris/HCl buffer, pH 7.9; 8 mM-MgCl₂, 3 mM-MnCl₂, 0.6 mM-ATP, 0.6 mM-GTP, 0.6 mM-CTP, 0.1 mM-UTP, 0.03 mM-[14C]UTP, 0.06 M-(NH₄)₂SO₄, albumin (1 mg/ml), calf thymus DNA (25 μg) and a proper amount of enzyme preparation. Where indicated, 1 μg of α-amanitin was additionally added. The solution of native DNA (1 mg/ml) was prepared as a suspension in 10 mM-Tris/HCl buffer, pH 7.5, containing 10 mM-NaCl, 0.1 mM-EDTA, and stored at 0 - 4°C. Reaction in incubated samples was stopped by rapid cooling on ice. From each sample 2 × 0.1 ml portions

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1 Abbreviations used: DTT, 1,4-dithiothreitol; PMSF, phenylmethyl sulphophenylfluoride.
were taken and placed on filters (1.5 x 1.5 cm) from Whatman GF/C filter paper. Filters were washed for 15 min in 10\% trichloroacetic acid containing 0.1 m-Na_2P_2O_7 and then four times for 10 min in 5\% trichloroacetic acid containing 0.1 m-Na_2P_2O_7. Final wash was for 2 min in ethanol/ethyl ether (1:1) mixture. Filters were air-dried and the radioactivity measured in toluene scintillator (6 g PPO, 0.075 g POPOP per litre) in Nuclear Chicago Scintillation Counter (Isocap 300) with the efficiency of about 80\%. The reaction catalysed by polymerase was linearly dependent on (a) the amount of calf thymus DNA up to 25 \( \mu \)g, and (b) time, up to 20 min.

**Isolation of nuclei from rat kidney.** Nuclei were isolated from kidneys according to the modified method described by Widnell & Tata (1964) and by Chauveau et al. (1956). The procedure was as follows: kidneys were gently homogenized in Potter-Elvehjem teflon-glass homogenizer in 3 vol. of 20 mm-Tris/HCl buffer, pH 7.8, containing 0.32 m-sucrose, 3 mm-MgCl_2, 1 mm-DTT. The homogenate was filtered through two layers of gauze and centrifuged for 10 min at 700 g in a Sorvall centrifuge (SS 34 rotor). The nuclear pellet was suspended in 15 ml of buffer containing 20 mm-Tris/HCl, pH 7.8, 2.2 m-sucrose, 2 mm-MgCl_2, 1 mm-DTT. The suspension was underlayered with 9 ml of 2.2 m-sucrose in the same buffer and centrifuged in the M.S.E. superspeed 65 ultracentrifuge at 4 °C in a 3 x 25 swing-out rotor at 100 000 \( g_{av} \) for 1 h. The nuclear pellet was suspended in 0.25 m-sucrose in the same buffer to the final DNA concentration of about 1 mg/ml and used immediately for determination of the enzymatic activity. The purity of nuclear preparation was checked in phase-contrast microscope.

**Estimation of RNA synthesis in the isolated nuclei.** Standard medium for estimation of the activity of RNA polymerases in nuclei contained 40 mm-Tris/HCl buffer (pH 8.5 or 7.5), ATP, GTP and CTP (0.6 mm each), 1 mm-DTT, 15\% \( v/v \) glycerol, 0.035 mm-[\( ^{14} \)C]UTP.

For the assay of polymerase I (together with polymerase III) the standard medium (pH 8.5) contained 50 mm-(NH_4)_2SO_4, 8 mm-MgCl_2 and 1 \( \mu \)g/ml of \( \alpha \)-amanitin added in a final volume of 0.15 ml.

For the assay of polymerase II the standard medium (pH 7.5) contained 300 mm-(NH_4)_2SO_4 and 3 mm-MnCl_2 in a final volume of 0.15 ml. Polymerase II activity was determined as a difference between estimation without and with \( \alpha \)-amanitin (1 \( \mu \)g/ml).

In both cases, the blank sample contained additionally 2 \( \mu \)g/ml of actinomycin D. Each reaction mixture contained the suspension of nuclei corresponding to 50 \( \mu \)g of DNA. Incubations were carried out in duplicate at 37 °C for 15 min. The reaction was stopped by rapid cooling of the samples in ice and addition of 1 ml of carrier RNA solution (2 mg/ml) and 1 ml of 10\% trichloroacetic acid containing 0.1 m-Na_2P_2O_7. The precipitate was retained on filters (Whatman GF/C), washed five times with 5 ml of 5\% trichloroacetic acid containing 0.1 m-Na_2P_2O_7, and once with
ethanol/ethyl ether (1 : 1) mixture. Radioactivity was measured in the scintillation counter. Under the described conditions the reaction catalysed by RNA polymerase was linear up to 15 min and 50 μg of DNA/sample.

**Column chromatography on DEAE-Sephadex A-25.** The polymerase preparations obtained according to Roeder (1974a) were chromatographed on DEAE-Sephadex A-25 columns (1.6 × 20 cm). (A) The sample from control kidney containing 43 mg protein (spec. act. 102 pmol UMP incorporated/20 min per mg protein) was loaded on the column equilibrated with 0.02 M-(NH₄)₂SO₄ in the standard TGMED buffer (0.05 M-Tris/HCl, pH 7.9; 25% (v/v) glycerol; 5 mM-MgCl₂; 0.1 mM-EDTA; 0.5 mM-DTT). The column was washed with TGMED buffer until E₂₈₀ < 0.1. The enzyme was then eluted with the linear gradient of 0.02 → 0.5 M-(NH₄)₂SO₄ in standard buffer. (B) The sample from hypertrophic kidney, 48 h after the operation, containing 40 mg protein (spec. act. 144 pmol UMP/20 min per mg protein) was chromatographed under the same conditions except that the gradient was 0.00 → 0.5 M-(NH₄)₂SO₄.

**Digestion of nuclear DNA with DNAase I.** Nuclei were prepared as described on p.193, except that PMSF was present both in the homogenization buffer (0.5 mM) and in the centrifugation buffer (0.1 mM). The nuclear preparation was washed with the solution containing 0.25 M-sucrose, 1 mM-MgCl₂, 10 mM-Tris/HCl, pH 7.8, and 0.1 mM-PMSF, and centrifuged in the Sorvall centrifuge at 600 g for 10 min. The obtained nuclear pellet was suspended in the same solution to a final concentration of DNA 50 - 100 μg/ml. After 5 min preincubation of nuclei at 37 °C DNAase I was added. The reaction was carried out at 37 °C and was stopped by addition of an equal volume of cold 0.8 M-perchloric acid containing 0.8 M-NaCl. After 60 min at 0 °C the samples were centrifuged at 1000 g for 15 min and the pellets were used for estimation of DNA.

**Isolation of nucleic acids.** Nucleic acids were isolated by the method of Henrichs et al. (1964).

**Measurement of (NH₄)₂SO₄, DNA, RNA and protein.** Concentration of ammonium sulphate was determined at room temperature with the use of RL-1 Refractometer. RNA was determined by the orcinol method according to Campbell & Sargent (1969), and DNA by the method of Burton (1968) using calf thymus DNA as a standard. Protein was measured according to Lowry et al. (1951) with bovine serum albumin as a standard.

**RESULTS**

**RNA/DNA ratio in hypertrophic kidney.** Unilateral nephrectomy in rats resulted in the increased RNA/DNA ratio in the remaining kidney. A significant increase in the RNA/DNA ratio was observed as early as 12 h after the operation and after 48 h the ratio reached 130% of the control value. This is in agreement with the earlier observations of Halliburton & Thompson (1965) and Malt (1969).
RNA synthesis in the isolated nuclei. The increase in the rate of RNA synthesis in the nuclei isolated from hypertrophic kidney reached the maximum on the 24th h after the operation; later a slight decrease of the rate was observed. These changes were due to the action of α-amanitin-insensitive polymerase I. Similar, although much less pronounced changes were found in the sham-operated animals. Figure 1 illustrates the net effect of unilateral nephrectomy on the rate of RNA synthesis as calculated from the difference between the rate of RNA synthesis in nuclei from hypertrophic kidneys and kidneys from sham-operated animals.

Fig. 1. Effect of unilateral nephrectomy on the rate of RNA synthesis in nuclei isolated from rat kidney. Each preparation was obtained from 10 animals. The results are expressed as the difference between the rate of RNA synthesis in the nephrectomized and sham-operated animals related to the control value. Synthesis mediated by: ●, polymerase I, and ×, polymerase II.

Changes in the rate of RNA synthesis could be due either to the increased activity of RNA polymerases or to the increased susceptibility of the template to these enzymes. To check these possibilities we analysed the activity of solubilized RNA polymerases and the susceptibility of chromatin DNA to the nucleolytic enzyme.

The activity of solubilized RNA polymerase. The enzyme preparations used in the experiments described in this section were obtained from kidney homogenate. Such an isolation procedure ruled out the possibility of the loss of soluble enzyme during the preparation of nuclei (Roeder, *1974a). We found additionally that some other precautions were essential for obtaining reproducible results. First, we used native calf thymus DNA as a template, as this allowed to obtain much higher activity than with denatured DNA, irrespective whether the enzyme was crude or partly purified. Since the
activity of the same enzyme preparation varied with different batches of native DNA, we used DNA from the same batch as a template in each series of experiments. Finally, we checked that under our conditions of polymerase assay no measurable degradation of RNA and DNA took place.

Table 1 illustrates changes in the overall activity of RNA polymerases following unilateral nephrectomy or sham-operation. As can be seen, the activity of the enzyme isolated from hypertrophic kidneys, considerably increased shortly after the operation and continued to increase up to 48 h., reaching 183% of control activity. The enzyme from kidneys of the sham-operated animals showed a similar but significantly smaller increase of the activity.

**Table 1**

**Effect of unilateral nephrectomy and sham-operation on the activity of solubilized RNA polymerase**

The activity was measured in the crude homogenate after sonication as described in the Methods and expressed in pmol of [14C]UMP incorporated per mg protein. The results represent a typical experiment out of four performed with 10 animals each; differences between the control and operated animals did not exceed 5%.

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<tr>
<th>Time (h)</th>
<th>Nephrectomy</th>
<th>Sham-operation</th>
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<tr>
<td></td>
<td>[14C]UMP incorporated</td>
<td>% of control</td>
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<tr>
<td>0</td>
<td>52</td>
<td>100</td>
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<td>12</td>
<td>69</td>
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<tr>
<td>24</td>
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<td>48</td>
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In contrast to the changes observed in the rate of RNA synthesis in isolated nuclei the changes observed in the homogenate could not be attributed exclusively to polymerase I. This was demonstrated by fractionation of crude enzyme preparation on DEAE-Sephadex A-25. As can be seen from Fig. 2, chromatography of the polymerase preparation resulted in separation of five fractions designated as polymerase I_A, I_B, II_A, II_B and III (after Roeder & Rutter, 1969). We found that II_A and II_B forms were totally inhibited by α-amanitin at the concentration of 1 µg/ml. The last emerging form corresponding to polymerase III was partly inhibited at this concentration.

The comparison of the activity profiles of the fractions eluted from the column showed differences in the proportion of the particular forms of polymerase isolated from control and hypertrophic kidney (Fig. 2). For instance, a significant change was observed in the ratio of RNA polymerase I_A and I_B. However, contribution of the α-amanitin-insensitive RNA polymerase I_A and I_B in the total polymerase activity remained unchanged.
Fig. 2. Resolution of multiple forms of nuclear RNA polymerase from A, control and B, hypertrophic kidney 48 h after the operation. The polymerase preparation was chromatographed on DEAE-Sephadex A-25 column. It was eluted with (NH₄)₂SO₄ gradient as indicated. Fractions of 1 ml were collected and 0.005 ml aliquots from each fraction were taken for the activity measurements. The activity was measured in the absence (●) and presence (○) of 1 µg/ml of α-amanitin; ———. concentration of (NH₄)₂SO₄. For details see Methods.

Fig. 3. Time-course of hydrolysis with DNAase I of the nuclear DNA from kidneys of the nephrectomized (A) and sham-operated (B) animals. (A). DNA concentration: ●, control, (75 µg/ml); ▲, 12 h after the operation (76 µg/ml), ○, 24 h after the operation (76 µg/ml), △, 48 h after the operation (73 µg/ml). Enzyme concentration: 415 Kunitz units/ml. (B), DNA concentration: ●, control (70 µg/ml); ○, 24 h after the operation (86 µg/ml); △, 48 h after the operation (90 µg/ml). Enzyme concentration: 500 Kunitz units/ml. The results present a typical experiment out of 8 independent experiments; the differences between the control and hypertrophic kidneys (A) varied from 6 - 13%.
Differences in the affinity of the enzymes from the control and hypertrophic kidneys to the DEAE-Sephadex were also apparent (Fig. 2). Polymerase I from hypertrophic kidney was eluted from the column at 0.025 \( I_A \) and 0.06 \( I_B \) M-ammonium sulphate as compared to 0.075 \( I_A \) and 0.15 \( I_B \) M-ammonium sulphate necessary to elute polymerase I from control kidney. At ammonium sulphate concentration of 0.02 M, polymerase I from hypertrophic kidney was not adsorbed on the column.

Changes in chromatin DNA structure. Gross changes in the structure of chromatin DNA can be revealed by analysis of the rate and extent of digestion of chromatin DNA on prolonged incubation of nuclei with nucleases. Figure 3 presents the time-course of DNAase I action on the kidney nuclei isolated from the nephrectomized (Fig. 3A) and sham-operated animals (Fig. 3B). One can see that chromatin DNA from the hypertrophic kidney was digested to a slightly larger but reproducible extent, whereas the rate of digestion remained the same (Fig. 3A). Kinetics of digestion of DNA from the sham-operated animals was similar to that from control animals at any time after the operation (Fig. 3B).

**DISCUSSION**

Looking for the reason of the increased content of RNA in hypertrophic kidney we found that this increase was accompanied by an increase of RNA synthesis. It was also proved that unilateral nephrectomy resulted both in an increase of the activity of RNA polymerases and a higher susceptibility of chromatin DNA from hypertrophic kidneys to nuclease digestion. This last observation may indicate higher susceptibility of chromatin template to the action of RNA polymerase. It has been reported by Garel & Axel (1976) and Weintraub & Groudine (1976) that transcriptionally active regions of chromatin are preferentially degraded by nucleases. These regions showing a characteristic “loose” configuration (Franke et al., 1976; Scheer, 1978) represent also r(ribosomal) DNA regions involved in RNA synthesis. However, since the amount of DNA which is activated after unilateral nephrectomy seems to be lower than the observed increase in the “loose” structure, it might be that not the specific regions but the whole chromatin structure is “loosed”.

Data from other laboratories (Hill, 1975; Melvin et al., 1976) suggest that the decreased degradation of cellular RNA can not be excluded as an explanation of the increased content of RNA in hypertrophic kidney.

Although it is clear that all the mechanisms discussed could operate in hypertrophic kidney, the cause and effect relation in elevation of RNA content seems to be complex. The amount of RNA in hypertrophic kidney increased steadily from the time of unilateral nephrectomy up to 48 h after the operation. This is in good agreement with the changes in the activity of solubilized RNA polymerase and reflects to some extent the changes
in template susceptibility to nucleolytic digestion. However, the time-course of changes in RNA synthesis in the isolated nuclei was somewhat different. The maximum rate of RNA synthesis after the unilateral nephrectomy was observed on the 24th h after the operation, and was followed by a small decrease of the rate. It seems possible that the relative increase in the activity of the RNA synthesizing system observed during the first 24 h is high enough to account for the steady increase in RNA content up to 48 h. On the other hand, the increase of the activity of RNA polymerase between 24 - 48 h after the operation could be ascribed to that form of the enzyme which is not actually involved in RNA synthesis (Yu, 1974), and not to the template-bound RNA polymerase.

The increase in the rate of RNA synthesis was restricted to the α-amanitin-insensitive enzyme, whereas the activity ratio of polymerase I and the other forms of RNA polymerase was the same in the preparations from the control and hypertrophic kidneys.

The trend of changes was the same for both the true nephrectomy and sham-operation although these changes were considerably less pronounced in the latter case. A similar phenomenon was observed by Cortes et al. (1976) in the studies on RNA synthesis from labelled orotic acid in the hypertrophic rat kidney. It should be noted that unilateral nephrectomy is not an only way of inducing hypertrophy. This can be also achieved, for instance, by extraintestinal administration of protein. Thus, it can be concluded that the changes described in this work are not due to the lack of one kidney. The similarity of these changes to those occurring in regenerating liver (Organtini et al., 1975) suggests that they are characteristic for the transitory period between the resting state and active division of the cells.

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Wpływ jednostronnej nefrektomii na syntezę RNA w przerastającej nerce szczura

Streszczenie

1. W nerce przerastającej na skutek jednostronnej nefrektomii wzrasta stosunek RNA/DNA. Towarzyszy temu wzrost szybkości syntetyzy RNA katalizowanej przez polimerazę niewrażliwą na α-amanitynę, mierzonej w wyizolowanych jądrach.

2. Zwiększona aktywność upłynniowej polimerazy RNA jest wywołana wzrostem aktywności polimerazy I, II i III, co można stwierdzić po chromatografii na DEAE-Sephadex.

3. Chromatynowy DNA z nerek przerastających jest bardziej dostępny dla DNAazy I niż DNA z nerek kontrolnych.

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