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Effects of Organ Preservation on the Transport of RNA from the Nucleus (41050)

HARRISON M. LAZARUS AND ARLENE HOPFENBECK†

Department of Surgery, University of Utah; Veterans Administration Medical Center, and LDSH-Deseret Foundation, Salt Lake City, Utah 84132

Abstract. Earlier we demonstrated that injury to tissue following kidney preservation causes decreased nuclear content of HnRNA and ribosomal percursor RNA. Others have shown that cytoplasmic protein synthesis decreases along with these nuclear changes. We have investigated the effects of organ preservation on the connecting link of RNA transport from the nucleus to the cytoplasm utilizing a cell-free system. The transport of kidney nuclear RNA from the nucleus to the cytoplasm in the *in vitro* system was determined before and after ischemic injury to the tissue. The release of RNA from purified kidney nuclei in a cell-free medium was dependent on energy (ATP) and on cytosol. The transport of the [^{3}H]RNA was decreased to approximately half of that of control level by 1 hr of warm storage or by 4 hr of cold preservation. After prolonged cold storage, 24 to 72 hr, the release of [^{3}H]RNA from the nucleus to the cytoplasm increased. This increased release of RNA that occurred with cold storage was less energy dependent and presumably not representative of *in vivo* transport mechanisms. Biochemical changes induced by organ storage at 37° were not equal to changes caused by cold storage, 0–4°, even for prolonged periods. The death of tissue during preservation may occur through different biochemical pathways depending on the storage temperatures.

Earlier, we demonstrated that injury to tissue following ischemia causes decreased nuclear content of HnRNA and ribosomal precursor RNA (1); others have shown that cytoplasmic protein synthesis decreases along with these nuclear changes (2). We wanted to investigate the effects of ischemic tissue injury on the connecting link of RNA transport from the nucleus to the cytoplasm. Recently, a cell-free system for studying the important post-transcriptional events in the regulation of the flow of RNA from the nucleus to the cytoplasm has been developed (3–6). The regulation of post-transcriptional ribosome formation involves the transportation to the cytoplasm (7). A cell-free system has been used to study the transport of RNA to the cytoplasm. The system is dependent on energy (ATP) and on the presence of macromolecules in the cytosol (3); the cell-free system appeared to provide a reliable system for studying the processing and transport of RNA (3–6). After a 30-min *in vivo* labeling of RNA precursors, the transport of m-RNA can be studied (6). The RNA transport system has been shown to reflect physiological and pathological changes in other tissues (3–6). We evaluated a similar *in vitro* system for the kidney so that we could examine post-transcriptional events and we studied the effect of ischemic tissue injury on the post-transcriptional system of transport of RNA from the nucleus to the cytoplasm.

Materials and Methods. The source of material used was as follows: 6 to 10-week-old white mice, Charles River Laboratories, Wilmington, Massachusetts; [^{3}H]orotic acid (500 μCi/ml), sp act 19 Ci/m*M;* calf thymus DNA, Worthington Biochemical Corp., Freehold, New York; ATP, PL Biochemical Corporation, Milwaukee, Wisconsin; yeast RNA, (Type XI), phospho(enol)pyruvate, spermidine trihydrochloride, Sigma Chemical Company, St. Louis, Missouri; dithiothreitol, Calbiochem, San Diego, California; Tris, ultrapure, Schwartz/Mann, Orangeburg, New York; Soluene 100, Packard Instrument Company, Downers Grove, Illinois. All other chemicals are reagent grade.

Mouse RNA was labeled *in vivo* for 30 min by incorporation of [^{3}H]orotic acid, 100 μCi, injected intraperitoneally. After being flushed with 0.9% NaCl, the kidneys were

† Deceased.

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removed, cleaned of capsule and hilar vessels, and while covered with 0.5 ml of 0.9% NaCl, subjected to various ischemic storage conditions. One kidney from each mouse acted as a control to the other test kidney. Warm ischemia (37°) was tested at 1 hr and at 2 hr; the control kidneys were kept at 0–4° for the 1- and 2-hr storage periods. The cold storage periods tested were 4, 24, 48, and 72 hr at 0–4°, with the control kidneys processed immediately.

After homogenizing the kidneys in 2.2 *M* sucrose, 3.3 m*M* calcium acetate (1:15 w/v), nuclei were isolated using the method of Muramatsu and Busch (8). The nuclei were suspended in 1 *M* sucrose, containing 1 m*M* calcium acetate; and a purified nuclear pellet was obtained by centrifugation at 2000*g* for 10 min. DNA content was determined by the method of Burton (9).

Cytosol was prepared from mouse kidneys homogenized (1:2.5, w/v) in 0.05 *M* Tris (pH 7.5), 0.0025 *M* $MgCl_2$, 0.025 *M* KCL, 0.25 *M* sucrose; the homogenate was centrifuged at 13,000*g* for 10 min. The supernatant was centrifuged at 105,000*g* for 1 hr and the remaining supernatant was dialyzed overnight at 0–4° against two changes of the homogenizing solution. A 10-min 2000*g* centrifugation was required to remove a cloudy flocculence prior to measuring the protein concentration by the method of Lowry *et al.* (10).

The incubation mix (2.05 ml) contained the following: Nuclei (150–500 μg of DNA); cytosol–protein 6.1 mg/ml, 2 m*M* ATP, 2.5 m*M* phospho(enol)pyruvate, 1000 μg yeast RNA, 0.06 *M* Tris–HCL (pH 7.5), 55 units pyruvate kinase, 25 m*M* KCL, 2.5 m*M* $MgCl_2$, 0.5 m*M* $CaCl_2$, 5.0 m*M* NaCl, 0.3 m*M* $MnCl_2$, 2.5 m*M* Na_2HPO_4, 5 m*M* spermidine, 2 m*M* dithiothreitol. One millimolar of phospho(enol)pyruvate was added every 10 min during the incubation period at 30°, and the nuclei were centrifuged out at 8000*g* for 2 min.

The RNA released in the supernatant was precipitated on Whatman No. 3 paper squares (2 cm^2) with cold 5% TCA as described by Warnick and Lazarus (11) and Bollum (12). The dried squares were placed in scintillation vials and the RNA was solubilized by adding a mixture of 0.5 ml Soluene and 10.5 ml of toluene scintillation counting fluid. Samples were incubated overnight at 37° prior to counting in a Packard scintillation counter with an efficiency of 40%. The resultant counts are reported as the percentage of the acid-precipitated counts in an equivalent aliquot from the original reaction mixture. The results were not normally distributed; therefore, the Wilcoxon rank sum test was used to demonstrate the statistical significance of the difference between the control and the stored kidneys. The standard deviation was computed for descriptive purposes only.

Results. The release of messenger RNA (informosomes) from purified rat liver nuclei into a cell free medium is dependent on energy (ATP) and on cytosol (3). The experiments reported here were designed to investigate the release of RNA from the nuclei of the mouse kidney, both before and after the kidneys were injured by being kept in different storage conditions. Figure 1 shows that the transport system for kidney nuclear RNA is energy (ATP) dependent. When ATP and phospho(enol)pyruvate

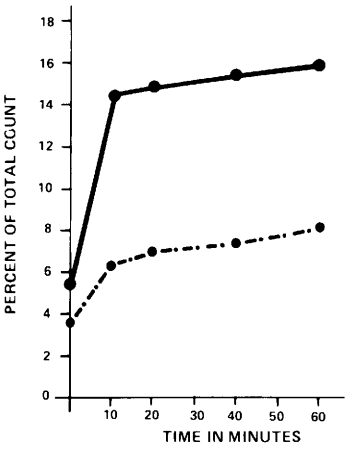

FIG. 1. Transport dependence of mouse kidney nuclear [^{3}H]RNA on ATP in the presence of cytosol. Normal mouse kidney nuclei prelabeled *in vivo* for 30 min with 100 μCi [^{3}H]orotic acid were incubated at 30° for varying times up to 60 min in an incubation medium. At each time period aliquots were removed from the incubation medium and assayed for acid-precipitable radioactivity outside the nucleus. The incubation medium contained energy (ATP) (——) or no energy (– · – · –). The counts per minute for 100% transport would equal 5293 with energy and 5553 without energy.

(PEP) are removed from the system, a distinct reduction in the ability of the nuclei to release RNA is seen. When cytosol was not present in the transport media, the release of [^{3}H]RNA from the nucleus was large, 42% at 20 min, and it was not significantly influenced by the presence of ATP and PEP being 52%. This release of RNA in the absence of cytosol was not due to nuclear lysis as determined by phase contrast microscopy or measuring the DNA in the postnuclear supernatant.

To determine the optimum concentration of nuclei (DNA) the transport experiment was set up with DNA varying from 30 to 510 μg/ml. Satisfactory results were obtained when the DNA content was above 125 μg/ml, with the transport being proportional to DNA concentration.

Warm ischemia (37° for 1 hr) reduced the ability of the nuclei to release RNA to the cytoplasmic media (Fig. 2, Table I). The decrease was apparent at all times during the transport reaction. The rate of release of RNA to the cytosol was lower after tissue storage. Also, the maximum level obtained was lower. An additional hour of warm storage did not cause any further deterioration in the transport of RNA.

Figure 3 shows that cold ischemic storage for four hrs results in a similar reduction of RNA released as was seen in the warm storage experiments. However, 24 hr of cold ischemic tissue injury caused no reduction in the release of [^{3}H]RNA from the nucleus to the transport media (Fig. 3B). Moreover, after 48 or 72 hr of cold ischemic renal storage, the nuclei rapidly lost RNA

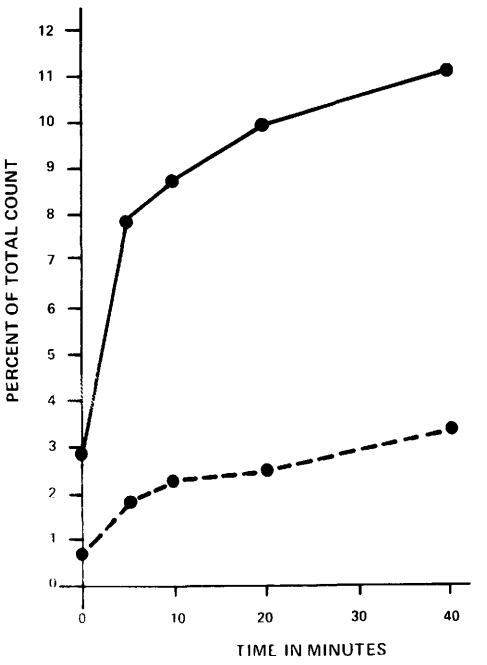

FIG. 2. RNA transport from nuclei after ischemic tissue injury. After 30-min *in vivo* labeling, mouse kidneys were perfused, removed, and covered with 0.5 ml saline, 2 kidneys/tube. From each mouse one kidney was used as a control to the other test kidney. The ischemic kidneys were kept in a 37° H_2O bath for the designated time; the control kidneys remained on ice. Purified nuclei prepared by the method of Muramatsu and Busch (8) were combined with incubation medium and incubated at 30°. Aliquots removed at 0, 5, 10, 20, and 40 min were centrifuged and the supernatants assayed for acid-precipitable radioactivity. 37° 1-hr ischemia reduces the ability of the nuclei to transport RNA (– – –) compared to the control (——). The counts per minute for 100% transport would equal 2681 for the ischemic kidney and 2601 for the control.

TABLE I. ALTERATIONS OF RNA TRANSPORT FROM NUCLEI AFTER ORGAN STORAGE

Storage condition	No. of expt	Percentage of released nuclear counts ± SD		Percentage change	*P* value of difference
		Control	Stored		
1 hr, 37°	6	9.33 ± 1.84	3.57 ± .618	−61	<0.002
2 hr, 37°	4	6.15 ± 1.03	2.75 ± .58	−55	<0.03
4 hr, 0°	8	9.2 ± 2.42	5.3 ± .58	−42	<0.002
24 hr, 0°	12	11.2 ± 4.84	14.08 ± .67	+25	<0.4
48 hr, 0°	10	8.62 ± 1.77	27.24 ± 7.05	+216	<0.001
72 hr, 0°	12	7.25 ± 1.58	33.69 ± 5.83	+365	<0.001

Note. Nuclei purified from stored kidneys were incubated for 40 min in a cell-free cytosol mix which was then assayed for acid-precipitable radioactivity after removing the nuclei. The Wilcoxon rank sum test was used to determine the *P* value for significance. The standard deviation was computed for descriptive purposes only.

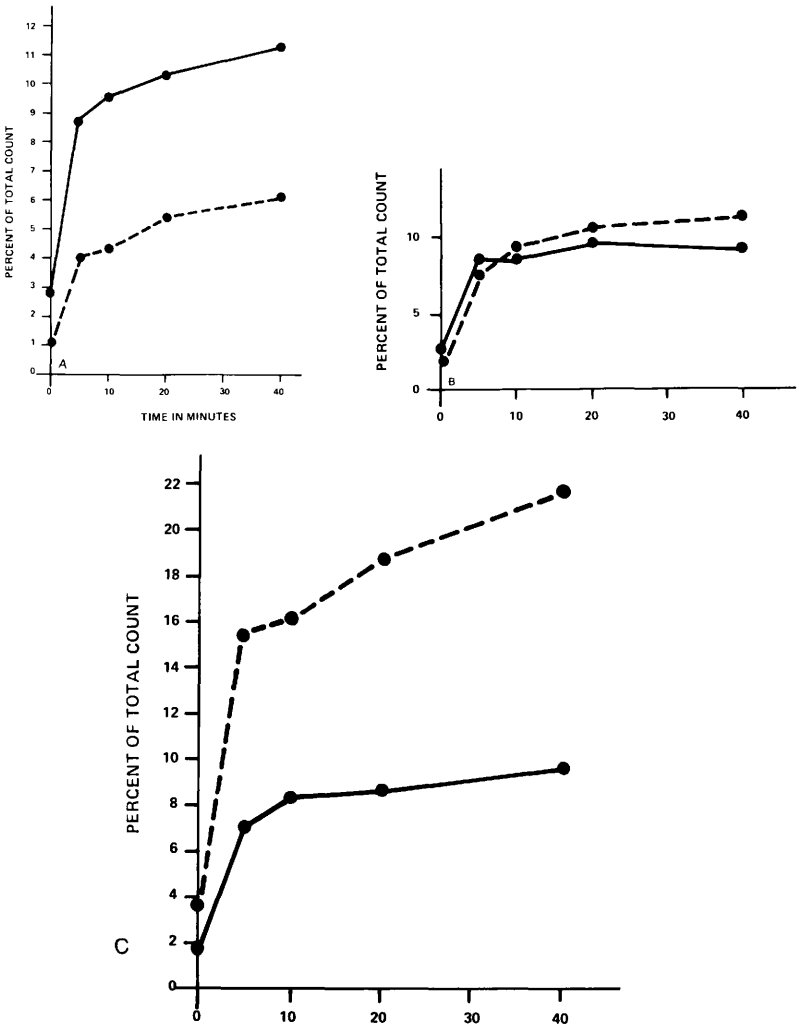

FIG. 3. RNA transport from kidney nuclei after cold organ storage. After 30 min of *in vivo* labeling, test kidneys were stored at 0° for 4, 24, 48, and 72 hr before nuclei were isolated. Purified nuclei were made immediately from the control kidney and incubated in transport medium at 30°. Aliquots were removed at 0, 5, 10, 20, and 40 min and assayed for acid-precipitable radioactivity. (A) 4 hr 0° storage of kidney (– – –); control (——). The counts per minute for 100% transport would equal 6279 for the ischemic and 4176 for the control. (B) 24 hr 0° storage (– – –); control (——). The counts per minute for 100% transport would equal 11,955 for the ischemic and 1977 for the control. (C) 48 hr 0° storage (– – –); control (——). The counts per minute for 100% transport would equal 11,482 for the ischemic and 8342 for the control.

to the cytosol (Fig. 3C, Table I) to a greater degree than did the nuclei from the control kidneys.

The apparent increased transport of RNA at 48 hr after cold storage was evaluated further. If the RNA were actively transported, the system should be energy dependent as with the control kidney nuclei in Fig. 1. The results of energy dependence for nuclei from kidneys stored 48 hr at 0° are shown in Fig. 4. The amount of RNA released to the cytosol by nuclei from kidneys stored 48 hr at 0° in the absence of ATP was approximately equal to the amount of RNA released by the nuclei from the control kidney in the presence of energy

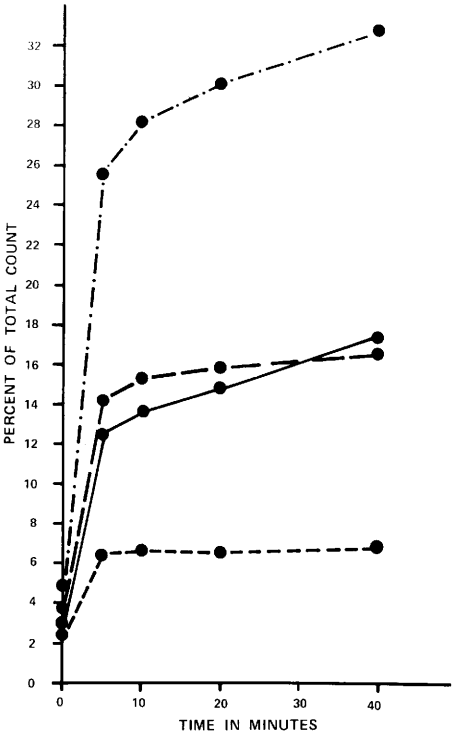

FIG. 4. Energy dependence of RNA transport after cold organ storage. After 30 min of *in vivo* labeling, test kidneys were stored at 0° for 48 hr before nuclei were made while control kidney nuclei were prepared immediately. The nuclei were incubated with and without ATP and phospho(enol)pyruvate in transport medium at 30° for 40 min. Assays for released RNA were made at 0, 5, 10, 20, and 40 min. Stored 48 hr assayed with energy (– · – · –), 100% transport would equal 3104 cpm. Stored 48 hr assayed without energy (——), 100% transport would equal 3025 cpm. Control assay with energy (– – –), 100% transport would equal 2363 cpm. Control assayed without energy (– – –), 100% transport would equal 1980 cpm.

(ATP). The addition of ATP caused an additional release of RNA. The mechanism for this high release of nuclear RNA after 48 hr of renal injury by the cold is unknown. After 24 hr of cold storage, the early decrease in active transport seen at 4 hr was offset by the second mechanism for nuclear release of RNA. These two factors probably caused the transport of nuclear RNA to the cytosol to appear equivalent to the controls after 24 hr of cold ischemia.

Discussion. Interest in post-transcriptional events has increased in recent years (13). The mechanism for how RNA exits from the nucleus is not established. However, an *in vitro* assay for studying some aspects of this phenomenon has been developed (3–6). Utilizing this system we have demonstrated that ischemic tissue injury markedly affects the ability of nuclei to participate in the transport of RNA to the cytosol. The amount of reduction in transport of RNA is similar for 1 hr of warm and 4 hr of cold storage. The lower temperature will cause nuclear envelope pore complexes to change from a relatively "open" to a relatively "closed" state. The altered pore opening will decrease RNA transport from the nucleus to the cytoplasm (14). The 4-hr period of cold storage does not result in a loss of tissue viability whereas the 1 hr of warm ischemia does (15). The changes at 1 and 2 hr of warm ischemia may be due to early autolysis, but autolysis would not be a factor at 4 hr of cold storage. Additionally, the changes that occurred in the transport of [^{3}H]RNA from the nuclei after extensive cold storage, 48 and 72 hr, resulted in an increased loss of [^{3}H]RNA to cytosol. This degree of storage would also result in a loss of viability. Therefore, the decrease in [^{3}H]RNA transfer from the nucleus to the cytosol is not related to viability or autolysis.

With cold storage for 24 hr, the nuclear transport of RNA increased to seemingly normal levels and reached supranormal values at 48 and 72 hr. Nuclei from kidneys stored cold for 48 hr, in the absence of ATP, transported to the cytosol an amount of [^{3}H]RNA equivalent to that of nuclei from control kidneys in a complete transport system containing ATP. Therefore, part of the elevated level of [^{3}H]RNA release was not ATP dependent. The release of informosomes from tumor nuclei is also partially or completely energy independent (4). The transport after cold tissue injury without ATP is similar to the loss of normal transport shown for the system in the absence of cytosol. In the absence of cytosol, nuclei from the control kidneys released greater than 40% of the [^{3}H]RNA in a nonenergy-dependent manner. Most of this

transfer of the [^{3}H]RNA occurs within 5 min. Therefore, the increased release of nuclear RNA after prolonged cold storage of the kidney is presumably not representative of the in vivo mechanism.

Not all biochemical measurements within the nucleus have shown changes with 1 hr of warm ischemia; e.g., RNA/DNA and NAD pyrophosporylase (14). Previously the changes in nuclear nucleic acid metabolism that occur with tissue injury (i.e., the stability of HnRNA and ribosomal precursor RNA (1), the nuclear ability to synthesize RNA (16), the molecular weight of DNA (17), and chromatin template (11)) have been similarly affected by warm storage for 1 hr or cold storage for 48 or 72 hr. Tissue viability is lost after 60 min of warm ischemia and 48 hr of cold storage in saline. Therefore, previously altered nuclear parameters changed with the conditions and times that corresponded with the loss of viability. The increased transport of RNA at 48 and 72 hr implies that a different mechanism has been altered in this complex assay system. Also, it indicates that the biochemical changes that occur when viability is lost with warm or cold storage are not equivalent. To understand properly the effects of ischemia on the biochemical status of the kidney prior to organ transplantation, we have to understand the effects of warm and cold ischemia separately. Also, clinical storage methods have to be altered after warm ischemia versus pure cold storage (18). The biochemical changes induced by warm ischemic tissue injury are not equal to the changes caused by cold ischemic injury, even for prolonged periods. Ischemic death may occur through different biochemical pathways that are dependent on different storage temperatures. To protect nuclear function during cold storage will require the blockage of pathways that are different from those necessary for protection during warm ischemia.

The nucleus has less rapidly labeled RNA after organ storage (1), even though the total amount of cellular and nuclear RNA was unchanged (16). This present study reveals that the nucleus is also not able to transport its RNA to the cytoplasm normally after organ storage. With this altered transport, nuclear RNA may have more difficulty getting to its place of function in the cytoplasm to direct protein synthesis. The altered transport of informational molecules to the cytoplasm may compound the cellular difficulty of returning to normal protein synthesis after organ storage. It is not possible to say which kidney cells the isolated nuclei represent. The kidney contains many different types of cells and future plans include an effort to identify the cell types involved.

The effects of cold storage imply that the normal mechanism for the transport of RNA from the nuclei is no longer operational. The integrity of the nucleus to process RNA for movement to the cytoplasm in an orderly manner is gone. This rapid dispersement of RNA to the cytoplasm may be a positive protective response, or a sign of cellular dissolution. The significance has yet to be determined.

We have previously studied several areas of change in the nucleus which follow kidney storage. We have shown nuclear RNA, DNA, and chromatin to be altered by organ storage (1, 11, 17). The ability of the nuclei after organ storage to synthesize RNA was imparied (14, 19). In this study we have examined the impact of organ storage on an aspect of RNA processing, namely, the transport of RNA from the nucleus to the cytosol. The RNA processing of a cell has been described as a major mechanism to the expression of the gene (20). In the future, we need to understand the biological significance of these molecular changes, to elucidate the mechanism for these alterations, and to be able to prevent these changes in order to enhance viability after storage and ischemia.

Summary. The transport of kidney nuclear RNA from the nucleus to the cytoplasm in an *in vitro* system was determined after ischemic injury to the tissue. The transport of the [^{3}H]RNA was decreased to approximately half that of the control level by 1 hr of warm ischemia or 4 hr of cold ischemia. The transport control is energy dependent. After prolonged cold storage, 24 to 72 hr, the release of [^{3}H]RNA from the nucleus to

the cytoplasm increases. This increased release of RNA that occurs with ischemia in the cold is less energy dependent and presumably not representative of *in vivo* mechanisms.

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