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RNAse L and genome expression during early period of the rat liver regeneration

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Partial hepatectomy (PHE) stimulates an intact part of the liver to exchange the existing «quiescence» program for the proliferative one. Several changes between 0.5 and 3 h after PHE are considered as manifestations of the abolishment of «quiescence» program: a temporal decrease of either RNA synthesis or accumulation of the newly formed RNA; a restricted variability of RNA transcripts; a partial retention of newly synthesized RNA within the nuclei thereby providing less RNA for the cytoplasm; previously obtained data about transient dissociation of ribosomes from endoplasmic reticulum. An involvement of the 2',5'-oligo(A)synthetase—RNAse L system in the process is suggested by up- and down-regulation of 2',5'-oligo(A)synthetase activity in the cytoplasm and in the nucleus, respectively, and by up-regulation of RNAse L content in regenerating liver. The production of IFN α/β, an inducer of the 2',5'-oligo(A)synthetase—RNAse L system, is also increased during transition period. A specific role of sinusoidal cells, the main producers of IFN α/β in the liver, in the abolishment of the old program is strongly suggested.

Introduction. Extensive damage of liver parenchyma stimulates an intact part of the liver to exchange the existing «quiescence» program to the proliferative one with eventual restoration of liver mass and function. What kind of mechanisms is responsible for the abolishment of the old program is an intriguing question concerning not only regenerating liver but the transition periods of eukaryotic cell activity in general.

There are several evidences of partial restriction of genome expression during the transition period of liver regeneration e. g. temporal loss of ribosomes from endoplasmic reticulum [1], reciprocal alterations of 2',5'-oligo(A)synthetase (2',5'-OAS) activity in nuclei and cytoplasm [2] etc. The 2',5'-OAS is a limiting enzyme of 2',5'-OAS—RNAse L system [3] that is present in cells of higher vertebrates. The system is responsible for RNA degradation during viral infection and is induced by interferons α/β (IFN α/β) [3]. Its role in the absence of infection remains still obscure. This paper addresses general characteristics of genome expression in regenerating liver at the RNA level and the production of IFN α/β and RNAse L, the inducer and the target of 2',5'-OAS activity, respectively.

Materials and Methods. *Pretreatment of the animals.* Male Wistar rats (200—250 g) were used throughout. Partial (2/3) hepatectomy (PHE) and sham operation (ShO) were performed by standard procedures [4]. Livers were extirpated at the times indicated and processed as described below.

Investigation of intracellular distribution of newly synthesized RNA. ^{3}H-orotic acid (13.5 Ci/mmole) was injected intraperitoneally (3 μCi/g of body weight) at the times indicated. For each time point liver samples from five animals were fixed, processed for electron autography [5] and analyzed at the microscope JEM-100 B. Silver grains were counted over extranucleolar

part of the nucleus, nucleolus, mitochondria and endoplasmic reticulum of fifty hepatocytes from each sample.

Detection of kinetic complexity of nuclear RNA. Kinetic complexity of nuclear RNA was evaluated in hybridization reaction of the trace amount of unique DNA sequences with the excess of RNA according to [6, 7].

Evaluation of RNAse L and IFN α/β production. RNAse L was quantified by specific radiocovalent 2',5'-A affinity labelling. The reaction was performed with the liver extract with the subsequent PAGE/SDS [8]. Activity of interferon α/β was detected in S10 fraction of liver homogenate (1/10 w/v) by its ability to inhibit cytopathogenic action of vesicular stomatitis virus in the cultivated test cells [9].

Results. *Intracellular distribution of newly synthesized RNA.* The synthesis and accumulation of newly formed RNA were evaluated from the number of silver grains over corresponding cellular compartments after pulse (15 min) and more prolonged (^{3}H-orotic acid injected immediately after PHE) labeling, respectively. The RNA synthesis and transit into cytoplasm occur in two stages. Immediately after PHE the indices increase (Fig. 1, *A*). The subsequent changes are specific for each compartment. The gradual increase of silver grains over extranucleolar part of the nucleus coincides with the normalization of their amount over the nucleolus and mitochondria and transient decrease over ER (Fig. 1, *A*). Prolonged labeling reveals partial retention of newly synthesized RNA within the nuclei thereby temporally providing less RNA for the cytoplasm (Fig. 1, *B*).

Kinetic complexity of nuclear RNA. The nuclear RNA from the liver in 3 h after PHE hybridizes with lower percent of unique DNA sequences than nuclear RNA from intact animals (7.9 % versus 11.4 %) (Fig. 2). As kinetic complexity of unique sequences of rat DNA is equal to $1.9 \cdot 10^9$ base pairs [8] the kinetic complexity of nuclear RNA from intact and partially hepatectomized rats comprises $4.3 \cdot 10^8$ and $3.0 \cdot 10^8$ nucleotides correspondingly.

RNAse L and IFN α/β production. PHE induces an increase of 2',5'-oligo(A) affinity labelling (Fig. 3) and therefore content of enzyme RNAse L. Antiviral activity of liver cytosol from partially hepatectomized rats also increases with the maximal value in 0.5 h after operation. An active S10 fraction dilution from intact rats and rats in 0.5 h after PHE is equal to 1/80 and 1/320 correspondingly.

Discussion. Several findings of this work are interpreted to result from partial restriction of genome expression and loss of the previous phenotype: a temporal decrease of either RNA synthesis or accumulation of the newly formed RNA; a restricted variability of RNA transcripts; a partial retention of newly synthesized RNA within the nuclei thereby providing less RNA for the cytoplasm. Previously obtained data about transient dissociation of ribosomes from endoplasmic reticulum [1] add the picture.

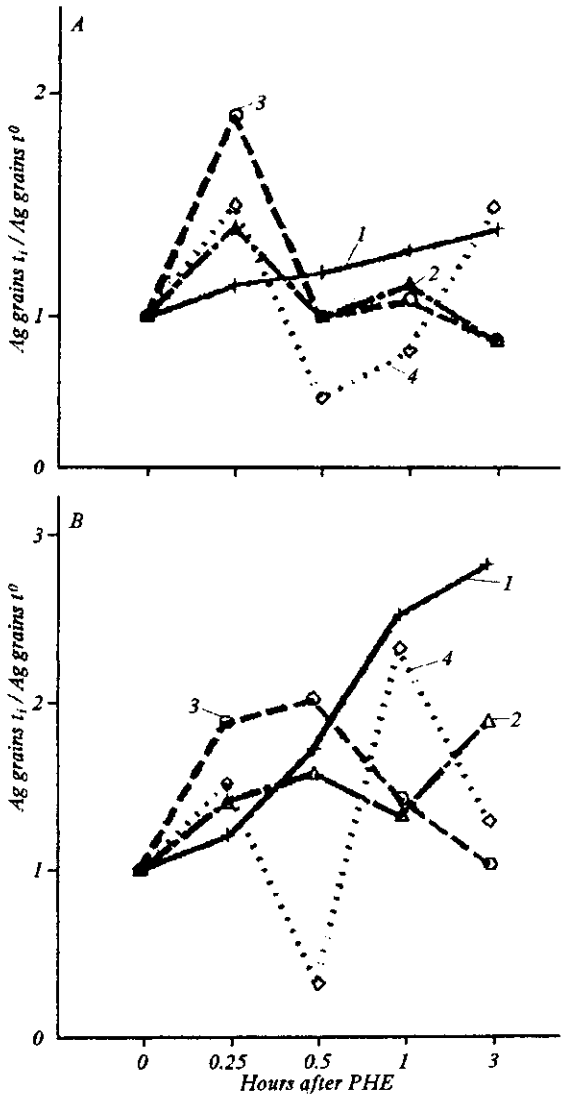

Fig. 1. The average number of silver grains over compartments of singular hepatocyte: *1* — extranucleolar part of nucleus; *2* — nucleolus; *3* — mitochondria; *4* — endoplasmic reticulum; t_i and t_0 are indicated and zero time, respectively; *A* and *B* — ^{3}H-orotic acid was injected 0.25 h before slaughter and immediately after PHE, respectively

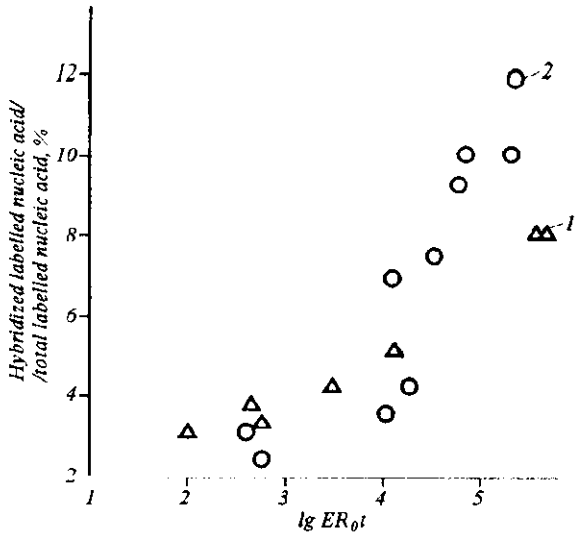

Fig. 2. Hybridization of unique DNA sequences with excess of liver nuclear RNA. ER_0t — equivalent values of RNA (moles nucleotides $\cdot l^{-1}$) $\cdot t$ (sec) reduced to hybridization in 0.12 M Na-phosphate buffer: *1* — PHE, 3 h; *2* — ShO, 3 h

The potential role of 2′,5′-OAS—RNAse L system in this process is supported by the first indications that production of RNAse L and IFN α/β is up-regulated. We hypothesize the following sequence of events after PHE providing the abolishment of «quiescence» program at the RNA level. The biological effect of the 2′,5′-OAS—RNAse L system is stimulated by interferon α/β that induces transcription of genes encoding 2′,5′-OAS and RNAse L [3]. The activated cytoplasmic synthetase [2] converts more ATP to PP_i and to oligoadenylates. The latter bind to RNAse L and activate its catalytic functions. The preferential ability of RNAse L to destroy the polysomes by the degradation of messenger RNA [10] is compatible with the reorganization of ribosomes above-mentioned [1] and with the general idea about the mechanisms of «quiescence» program exchange for proliferative one. In the nuclei, on the contrary, the decreased catalytic activity of 2′,5′-A-OAS may protect the «new» messages from degradation.

The participation of sinusoidal cells in the pro-

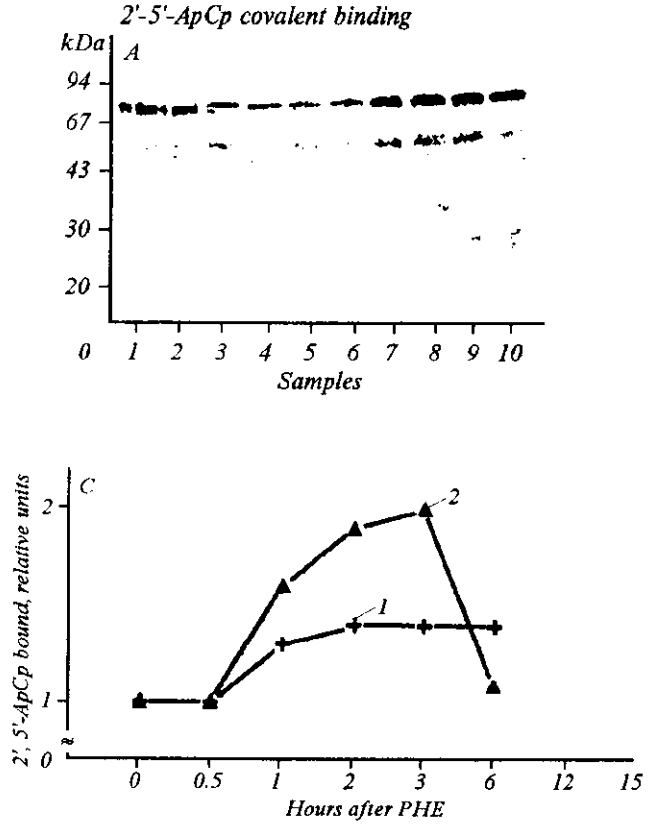

Fig. 3. Radiocovalent affinity labeling of RNAse L by 2′,5′-oligo(A). The liver specimen from intact and regenerating liver were taken from the same rats during PHE and after it at indicated time. In the following couples the first and the second numbers indicate the samples from «intact» and regenerating liver, respectively. The time after PHE is given in parentheses: *5*, *6* — 0.5 h; *4*, *7* — 1 h; *3*, *8* — 3 h; *2*, *9* — 6 h; *1*, *10* — 12 h. *A* — electrophoregramme autograph of cellular extracts after reaction with labeled oligoadenylates; *B* — autograph of cellular extracts Western-blot after its incubation with labeled oligoadenylates; *C* — RNAse L content in the liver after PHE in relation to its content in corresponding intact organ from results of densitometry of autographs *A* (*1*) and *B* (*2*)

cess, the main producers of IFN α/β in the liver [11], is in line with their regulatory role during hepatocytes transition from quiescence to proliferation [12].

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РНКаза L та експресія геному на ранньому етапі регенераційного процесу в печінці щурів

Резюме

Внаслідок часткової гепатектомії (ЧГЕ) в клітинах печінки відбувається зміна програми їхньої життєдіяльності, а саме: перехід від проліферативно неактивного до проліферативно активного стану. Декілька змін, що відбуваються між 0,5 і 3 год після ЧГЕ, розглядаються як прояв активного усунення існуючої до операції програми: тимчасове зниження синтезу та накопичення новоутвореної РНК; обмежена варіабельність РНК-транскриптів; затримка частини новоутвореної РНК в ядрі і відповідно менше надходження її в цитоплазму; раніше отримані нами відомості про тимчасову дисоціацію рибосом з ендоплазматичного ретикулуму. Зроблено припущення стосовно участі в цих процесах системи 2'5'-оліго(А)синтетаза—РНКаза L. Підставою для цього послужили дані щодо підвищення і зниження активності 2'5'-оліго(А)синтетази відповідно в цитоплазмі і ядрі, а також щодо зростання концентрації РНКази L у регенеруючій печінці. Продукування IFN α/β, який індукує систему 2'5'-оліго(А)синтетаза—РНКаза L, також підвищується протягом перехідного періоду. Наведені дані узгоджуються з гіпотезою щодо специфічної ролі синусоїдальних клітин печінки в переході від стану спокою до поділу, оскільки саме синусоїдальні клітини є основними продуцентами IFN α/β.

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РНКаза L и экспрессия генома на раннем этапе регенерационного процесса в печени крыс

Резюме

В результате частичной гепатэктомии (ЧГЭ) в клетках печени происходит изменение их жизнедеятельности, а именно: переход от пролиферативно неактивного к пролиферативно активному состоянию. Несколько изменений, происходящих между 0,5 и 3 ч после ЧГЭ, рассматриваются как проявление активного устранения существующей до операции программы: временное снижение синтеза и накопления новообразованной РНК; ограниченная вариабельность РНК-транскриптов; задержка части новообразованной РНК в ядре и соответственно меньшее ее поступление в цитоплазму; ранее полученные нами данные о временной диссоциации робосом из эндоплазматического ретикулума. Выдвинуто предположение об участии в этих процессах системы 2'5'-олиго(А)синтетаза—РНКаза L. Основанием для этого послужили данные о повышении и снижении активности 2'5'-олиго(А)синтетазы соответственно в цитоплазме и ядре, а также о возрастании концентрации РНКазы L в регенерирующей печени. Образование IFN α/β, индуцирующего систему 2'5'-олиго(А)синтетаза—РНКаза L, также повышается на протяжении переходного периода. Приведенные данные согласуются с гипотезой о специфической роли синусоидальных клеток печени в ее переходе от состояния покоя к делению, поскольку именно синусоидальные клетки являются основными продуцентами IFN α/β.

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