Free Rad. Res. Comms., Vol. 7, No. 3–6, pp. 301–305
Reprints available directly from the publisher
Photocopying permitted by license only

PATTERNS OF RNA AND PROTEIN SYNTHESIS IN POST-ISCHEMIC LIVERS

A. BERNELLI-ZAZZERA

Istituto di Patologia Generale, Centro di Studio sulla Patologia Cellulare del CNR, Via Mangiagalli n. 31 – 20133 Milano, Italy

The re-establishment of the blood supply to a formerly ischemic liver lobe, before the "point of no return" of the tissue is reached, induces a series of changes in protein and RNA metabolism that are functional to the repair of the damage suffered by the cells. Among these events there is the increase in synthesis of a group of proteins known as heat-shock (or stress) proteins, which are also induced in liver cells by different kinds of oxidative stress. The increase in synthesis of these proteins is largely due to the activation of their genes: some of these genes are also activated in cells stimulated to grow.

These observations suggest a link between oxidative stress, repair of cell damage and cell multiplication.

KEY WORDS: RNA, heat-shock protein, ischemia, reperfusion, oxidative stress.

Protein and RNA synthesis are no exception to the rule that most of the changes found in ischemic tissues are predictable on the basis of the concurrent energy shortage. Things are more interesting and, in a sense, surprising when we remove the clamp that is used to interrupt the circulation, and we study what happens during reperfusion after a time-period of ischemia before the "point of no return" of the tissue (see Table 1).

Work from this laboratory has shown that the amount of ribosomal RNA decreases in ischemic livers, in relation to the duration of ischemia, but the polysomal size-distribution pattern is essentially normal: 15 minutes after the re-establishment of the blood supply, however, polysomes disappear and most of the ribosome population is in monomeric form.[1] After this sudden monomerization the polysomal distribution pattern returns to normal within two hours if previous ischemia did not last beyond the point of no return to the tissue. The dynamic of change of the pattern is consistent with a metabolic degradation of the polysomes, in agreement with the "n – 1 model" rather than with "random fragmentation" by ribonucleases. This interpretation is supported by the fact that monosomes isolated from reperfused livers are responsive to synthetic messengers, such as polyuridylic acid: this means that they do not have any old mRNA associated with them that can prevent the binding of polyuridylate. Moreover, monosome formation can be prevented by treatment of the animals with cycloheximide.

This protection supports the idea that, upon reperfusion, elongation of the initiated polypeptide chains is resumed, but the initiation of new polypeptides is still hampered for some time.

The degradation of the polysomal distribution pattern is accompanied by detachment of bound ribosomes from their sites of attachment on the membranes of the endoplasmic reticulum: and the reconstitution of the pattern – with concurrent resumption of protein synthesis – is accompanied by reassociation of the ribosomes

TABLE I

Some events in RNA and protein synthesis occurring during reperfusion after a period of ischemia not exceeding the "point of no return" of the liver cells.

**Degradation of the polysomal pattern: elongation of nascent peptides is resumed quickly, initiation of new peptides still hampered for 60–90 minutes.
**Increase in activity of ornithine decarboxylase (but not of S-adenosylmethionine decarboxylase). Increase in concentration of putrescine (but not of spermine and spermidine).
**Reconstitution of the polysomal pattern 2 hours after reoxygenation
**Increase of RNA synthesis above the normal levels (both pre-mRNA and pre-rRNA):
a) sustained by enhanced activity of "engaged" polymerases
b) due chiefly to an increase in number of the polymerase molecules engaged in transcription, and to a more limited increase in elongation rate
c) not affected by adrenalectomy or fasting
d) inhibited by cycloheximide at doses that do not interfere with the background level of RNA synthesis.
**Increase in expression of heat-shock genes and c-fos oncogene (short-lived)
**Resumption of a normal level of protein synthesis.
**Changes in the pattern of protein synthesis
a) decrease in albumin synthesis
b) increase in synthesis of heat-shock proteins (heat-shock proteins induced in liver cells by lipoperoxidation or products derived from this process).

to the membranes. Experiments using techniques that measure the flow of labeled compounds along the membranes of the endoplasmic reticulum seem to suggest that slowing – down of this flow is not related to the occurrence of cell necrosis. On the contrary, synthesis of nascent peptide chains and of intrinsic membrane protein, which are resumed only if reperfusion occurs before the "point of no return" of the tissue, are better correlated with the final destiny of the cell: their resumption may be tentatively interpreted as the beginning and the necessary prerequisite for cell repair after injury.[2]

RNA synthesis by isolated nuclei also decreases during ischemia. But when the clamp is removed and blood flow is re-established there is full recovery and something more. Isolated post-ischemic nuclei show a rebound of RNA synthesis, which increases well over the normal, reaching a maximum between 8 and 16 hours from the re-establishment of the circulation. Both α-amanitin – resistant and – sensitive activities are increased, thus indicating a stimulation of the synthesis of the precursors of both m-RNA and r-RNA. The effects on r-RNA synthesis are best seen working with isolated nucleoli, which are the cellular site of synthesis of the ribosomes. With nucleoli, a sharp peak of activation is seen after 8 hours of recovery from ischemia.[3]

The post-ischemic increase in RNA synthesis is essentially due to activation of the engaged (endogenous template-dependent) polymerases; free polymerases do not change in activity.[4]

Adrenalectomy and fasting do not inhibit the post-ischemic response but cycloheximide, at a dose that severely inhibits protein synthesis without primary effects on RNA synthesis in the controls, suppresses the stimulation of RNA synthesis that occurs upon reperfusion.[5] To day, this response can be interpreted in terms of DNA-binding proteins implied in gene activation.

The unexpected overshooting above the normal values of RNA synthesis, which involves both RNA polymerase I and RNA polymerase II, is reminiscent of events occurring in regenerating livers, with the basic difference that it is not followed by thymidine incorporation into DNA. This indicates that the process of restoration in post-ischemic livers can be interpreted essentially as an "intracellular repair".[3]

Post-ischemic liver cells seem to follow, up to a certain point, the pathway of regeneration, but they stop before the final decision for DNA duplication is taken. Polyamine metabolism in reperfused liver is a case in point.[6] We decided to study polyamine metabolism because of the relationships between polyamines and RNA synthesis. In fact, ornithine decarboxylase is supposed to be involved in the transcription of nucleolar genes which seems to require a short-lived protein. In liver cells recovering from ischemia the increase in RNA synthesis is preceded by activation of ornithine decarboxylase, leading in turn to an increase in putrescine concentration. In contrast to regenerating liver, however, the changes in ODC activity and putrescine concentration in reperfused liver are not associated with changes in S-adenosylmethionine decarboxylase activity and in spermine and spermidine concentrations that seem to be charateristic of tissues where increases in RNA synthesis are followed by DNA synthesis and cell multiplication. The lack of activation of S-adenosylmethionine decarboxylase would differentiate reperfused liver, where only the first step of polyamine metabolism is stimulated and only for a short time-period, from regenerating liver, where the entire pathway is sequentially activated and leads to the accumulation of those polyamines, (such as spermine and spermidine), more directly associated to the start of DNA replication and to cell multiplication. The next question we addressed was: what kind of proteins are synthesized by post-ischemic cells. Overall synthesis returns to normal but how is the pattern of protein synthesis? We started with albumin, which is the product of a liver specific gene.[7]

Working with post-mitochondrial supernatants and isolated ribosomal fractions we demonstrated that absolute values of incorporation of labelled leucine are almost unchanged. However, the relative proportion of specific proteins is changed, since the incorporation of ^{3}H-leucine *in vivo* into liver albumin, relative to incorporation into total protein, as determined by precipitation of labelled albumin with the specific antibody, decreases by 40–50% in post-ischemic livers. Cell-free synthesis by membrane-bound polysomes and poly(A) – enriched RNA isolated from unfractionated liver homogenate shows that the decrease in albumin synthesis in liver of rats recovering from ischemia is due to relative decrease in translatable albumin mRNA. If the synthesis of one protein is reduced, are there any protein synthesized in excess in post-ischemic liver?

After some wrong steps, in the direction of acute-phase proteins that we were studying at the time,[8] we tested the hypothesis that reperfused liver could synthesize heat-shock proteins, and the experiment confirmed this idea.[9] Polysomes were isolated from reperfused livers and incubated with ^{35}S-methionine and the products where run on a gel and subjected to fluorography. Comparisons were made with normal rats and rats made hyperthermic (42°C) by physical means.

Post-ischemic livers synthesize at least two heat-shock proteins (HSP) i.e. HSP 70 and HSP 89, which are the two most important HSP of high molecular range. There is an enormous literature on HSP which is well covered in some recent review articles.[10–12] It would be more appropriate to speak of "Stress proteins" since heat-shock gene transcription can be induced by a variety of changes in the cellular state: blood reperfusion after ischemia now adds to the list. I will not go into further details

on "stress proteins" but I will only say that the most conserved during evolution, and the best known from the point of view of molecular biology is HSP 70. We concentrated on the m-RNA of this proteins. Total m-RNA were extracted from different types of liver and tested in a mRNA-dependent reticulocyte lysate system. The products were then analysed by 2D-electroforesis. The m-RNA is barely expressed in normal liver, but it codes for a protein that appears in several isoforms in liver subjected to heat shock. Nothing happens during ischemia, but the spots of the different isoforms of HSP 70 appear in the 2D-fluorographs of reperfused livers.

Many similarities have been described, from the metabolic point of view, between heat exposure and post-ischemic reperfusion: in both cases a common trigger for HSP synthesis could be oxidative stress. It had been recognized that HSP could be induced by a number of oxidants, such as hydrogen peroxide or quinones, and by depletion of intracellular thiols induced by diamide, iodoacetamide or cadmium.[13–16] The links between heat-shock and oxidative stress have been further emphasized by the observation that heat-shock induces superoxide dismutase in mammalian cells as well as in *E. coli*.[17] Following this line of thought, we have demonstrated that exposure of liver cells or hepatoma cell lines to ADP-iron or to a product of lipoperoxidation such as 4-hydroxynonenal can induce a subset of HSP. The proteins induced by these two agencies are always a subset of the proteins induced in each type of cells upon heat-shock. For 4-hydroxynonenal, the increase is dose-dependent and the effect of heat and the chemical seems to be additive. However, we can only say that lipoperoxidation may be implicated in the induction of some of the heat-shock proteins but, under the conditions of our experiments, it reproduces only a portion of the response of protein synthesis typical of heat-shock.

What is the possible role of HSP in post-ischemic livers? The idea of a protective role is rather vague, and originates from correlations occurring sometimes between synthesis of hsp and establishment of thermotolerance.

Recent observations that hsp and some related proteins, such as GRP 78, which is also expressed in reperfused liver, can bind to hydrophobic regions of proteins, either normally exposed or revealed as a result of denaturation, suggest that HSP have something to do with the correct sorting of some proteins inside the cell.[19] But there might be more general perspectives. The analysis of genes newly expressed or overexpressed in reperfused livers confirms our previous idea of a similarity between reperfused and proliferating liver cells. We have seen that one gene of the HSP 70 family, HSC 73 which is expressed constitutively, becomes overexpressed in growing tissue, such as regenerating liver and fast-growing tumors.[20] The expression of this gene increases also upon reperfusion. Expression of HSP 70 and HSC 73 genes peaks at 4 hours after the re-establishment of the blood flow and is preceded by expression of one oncogene, c-fos, that gives a clear signal already 1 Hr after reperfusion. The expression of this oncogene, which is typical of growing cells, is more prompt in reperfused than in regenerating liver but is extremely short-lived. Depending on other modulatory and regulatory factors, oxidative stress could start the reperfusion reaction or stimulate cell growth.

References

1. Cajone, F., Ragnotti, G., Bernelli-Zazzera, A. and Bassi, M. State and function of liver polysomes during recovery from ischemia. *Exp. Mol. Pathol.*, **14**, 392–403, (1971).
2. Ferrero, M.E., Orsi, R. and Bernelli-Zazzera, A. Cell repair after liver injury. Membranes of the

endoplasmic reticulum, ribosomes and amino acids in post ischemic livers. *Exp. Mol. Pathol.*, **32**, 32–42, (1980).
3. Schiaffonati, L., Cairo, G. and Bernelli-Zazzera, A. RNA synthesis by nuclei and nucleoli from ischemic liver cells. *J. Cell. Physiol.*, **97**, 487–496, (1978).
4. Cairo, G., Schiaffonati, L. and Bernelli-Zazzera, A. Cell repair after liver injury. Stimulation of RNA synthesis, number of RNA transcribing molecules and elongation rate in post-ischemic liver nuclei. *Exp. Mol. Pathol.*, **35**, 231–243, (1981).
5. Piccoletti, R. and Bernelli-Zazzera, A. Cell repair after liver injury: an analysis of some metabolic conditions required for the stimulation of RNA synthesis in post-ischemic liver nuclei. *J. Cell. Physiol.*, **115**, 93–98, (1983).
6. Ferioli, M.E., Schiaffonati, L. Scalabrino, G., Cairo, G. and Bernelli-Zazzera, A. Relationships between polyamine metabolism and RNA synthesis in post-ischemic liver cell repair. *J. Cell. Phys.*, **103**, 121–128, (1980).
7. Cairo, G., Schiaffonati, L., Aletti, M.G. and Bernelli-Zazzera, A. Effect of post-ischemic recovery on albumin synthesis and relative amount of translatable albumin messenger RNA in rat liver. *Biochem. J.*, **204**, 197–202, (1-982).
8. Bardella, L., Cairo, G., Schiaffonati, L. and Bernelli-Zazzera, A. Post-ischemic recovery and acute-phase reaction in liver cells. *Front. Gastrontest. Res.*, **8**, 63–74, (1984).
9. Cairo, G., Bardella L., Schiaffonati, L. and Bernelli-Zazzera A. Synthesis of heat-shock proteins in rat liver after ischemia and hyperthemia. *Hepatol.*, **5**, 357–361, (1985).
10. Lindquist, S. The heat shock response. *Ann. Rev. Biochem.*, **55**, 1151–1191, (1986).
11. Burdon, R.H. Heat shock and the heat-shock proteins. *Biochem. J.*, **240**, 313–324, (1986).
12. Subjeck, J.R. and Shyy, T.-T. Stress protein system of mammalian cells. *Am. J. Physiol.*, **250**, C–C17, (1988).
13. Lee, P.C., Bochner B.R. and Ames, B.N. AppppA, heat shock stress and cell oxidation. *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 7486–7500, (1983).
14. Compton, J.L. and McCarthy, B.J. Induction of the drosophila heat shock response in isolated polytene nuclei. *Cell*, **14**, 191–201, (1978).
15. Bochner, B.R., Lee, P.C., Wilson, S.lW., Cutler, C.W. and Ames, B.N. AppppA and related adenylated nucleotides are synthesized as a consequence of oxidation stress. *Cell*, **37**, 225–232, (1984).
16. Levinson, W., Opperman, H. and Jackson, J. Transition series metals and sulphydryl reagents induce the synthesis of four proteins in eukaryotic cells. *Biochim. Biophys. Acta*, **606**, 170–180, (1980).
17. Privalle, C.T. and Fridovich, I. Induction of superoxide dismutase in *E. coli* by heat-shock. *Proc. Natl. Acad. Sci. USA*, **84**, 2723–2726, (1987).
18. Cajone, F. and Bernelli-Zazzera, A. Oxidative stress induces a subset of heat-shock proteins in rat hepatocytes and MH1C1 Cells. *Chem-Biol. Inter.*, **65**, 235–246, (1988).
19. Deshaies, R.J., Kock, B.D. and Schekman, R. The role of stress proteins in membrane biogenesis. *TIBS*, **13**, 384–388, (1988).
20. Cairo, G., Schiaffonati, L., Rappocciolo, E., Tacchini, L. and Bernelli-Zazzera, A. Expression of different members of hsp 70 gene family in liver and hepatomas. *Hepatology* **9**, 740–746, (1989).

Accepted by Prof. T.F. Slater