Hypothesis

Poly ADP-ribosylation – A cellular emergency reaction?

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We propose that the activation of poly(ADP-ribose) synthetase by DNA damage serves to decrease rapidly and transiently the cellular level of NAD (by production therefrom of poly ADP-ribose). The result is a slow-down of energy-requiring reactions, in particular of replicative DNA synthesis giving cells more time to repair the damage. We do not attribute any specific role to poly ADP-ribosylated proteins in this reaction beyond their action as acceptors for poly ADP-ribose

Poly ADP-ribose *DNA repair* *NAD* *Nucleotide pool* *DNA replication* *Cellular stress*

1. INTRODUCTION

Nuclei of most eukaryotic cells contain an enzyme, poly(ADP-ribose) synthetase, which catalyses the transfer of the ADP-ribosyl moiety of NAD to protein acceptors with a concomitant release of nicotinamide. The resulting homopolymer is a linear or branched sequence of repeating ADP-ribose units linked together by ribose to ribose bonds (for reviews see [1–3]). Histones, in particular histone H1 and histone H2B and various non-histone proteins can serve as acceptors for poly ADP-ribose chains, the predominant acceptor, however, is the poly(ADP-ribose) synthetase itself. Synthesis of poly ADP-ribose by the synthetase in vitro is strongly stimulated by DNA containing single-strand breaks. Fully double-stranded DNA proved inactive [4].

Much effort by a large number of investigators in the last 15 years has gone into elucidating the biological role of poly(ADP-ribose) synthetase. There is hardly any nuclear function (DNA replication and its regulation, DNA repair, differential gene expression and others) that was not at one time or another throught to be regulated by poly ADP-ribosylation [3]. Still, there is so far no firm evidence that any of these nuclear processes is in fact controlled by poly ADP-ribosylation. For our own consideration the following facts are especially remarkable: First, if the acceptor protein is an enzyme like the synthetase itself its activity usually does not change dramatically in the course of poly ADP-ribosylation. (This is in contrast to highly specific non-nuclear mono ADP-ribosylation reactions catalysed by bacterial toxins which strongly influence the adenylate cyclase system or protein synthesis [3].) Second, if the protein acceptor interacts directly with DNA like the histones or the synthetase itself the poly ADP-ribosylation obviously must influence the binding to DNA because of the large number of negative charges added to the protein. Hence, poly ADP-ribosylation might well loosen the chromatin structure. However, important questions, unanswered so far, are, for instance: Where does the specificity come from, i.e., how is a *localised* change in chromatin structure achieved? How can a transient reaction like the poly ADP-ribosylation of a protein (turnover of poly ADP-ribose in vivo is high) lead to a stable change of gene expression?

So far, the only cellular process for which a contribution of poly ADP-ribosylation is supported by solid experimental evidence, is DNA repair (reviewed in [5]). There is, to start with, the fact that poly(ADP-ribose) synthetase is strongly activated by DNA containing single-strand breaks. There is

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furthermore the long-standing observation that DNA-damaging agents cause an inhibition of glycolysis which is due to a decrease in the cellular level of NAD [6–8]. Concomitant with this decrease is an increase of the activity of poly(ADP-ribose) synthetase and of the amount of poly ADP-ribose in nuclei [9,10]. Inhibition of poly(ADP-ribose) synthetase following the action of DNA-damaging agents increases their lethal effect [9,11]. Hence, poly(ADP-ribose) synthetase seems to play a role in the cellular recovery from DNA damage. Despite all this indirect evidence it has not been possible so far to assign a specific role to poly ADP-ribosylation in a particular step of DNA repair, except that DNA incision and repair synthesis seem to occur in the absence of poly ADP-ribosylation [9]. Any more specific assignments of particular enzymatic reactions being regulated by poly ADP-ribosylation proved short-lived. (For a summary on the confusion in the field see also the relevant discussion in [12].)

2. HYPOTHESIS

In view of several controversial ideas about the possible biological role of poly ADP-ribosylation we should like to propose an alternative hypothesis, some aspects of which may not be new but forgotten. We suggest that ADP-ribosylation serves to reduce rapidly and transiently the NAD level and to increase the level of nicotinamide in the cell. *According to this view no regulatory mechanism involving a change in the activity or function of a particular protein by poly ADP-ribosylation exists.* The role of the various poly ADP-ribosylated proteins is only to serve as acceptors which is in line with the fact that the primary acceptor is the synthetase itself. Therefore, we do not attribute specificity to this process beyond accessibility of the acceptor protein to the synthetase and this may vary depending on the state of chromatin condensation. What effect could the reduction of the NAD level and the concomitant increase in the nicotinamide level have on the cell? First, the rate of glycolysis is reduced and herewith of one of the important energy-producing pathways in the cell with the possible consequence of a slow-down of energy-requiring processes. A general slow-down of metabolism would be a useful reaction of a cell to DNA damage, it would decrease its proliferative activity and thereby provide time to finish the repair of damaged DNA before the next cell division. Every ADP-ribose moiety generated from NAD by the synthetase on the other hand will leave behind one molecule of nicotinamide. This probably will be used to resynthesize NAD whereby the first reaction is the condensation of nicotinamide with 5-phosphoribosyl-1-pyrophosphate (PRPP) to produce nicotinamide mononucleotide. Many DNA-damaging agents quickly lead to a dramatic decrease of the cellular NAD level (to about 10–30% of the control) which recovers in the following hours [9,10]. Resynthesis of NAD will consume a large amount of PRPP which is also required for the production of ribo- and deoxyribonucleotides. We propose that the necessity to resynthesize NAD requires much PRPP which might result in a transient imbalance in the pool of nucleotides. In fact, it has already been reported that the ATP level decreases concomitantly with the NAD level in cells treated with DNA-damaging agents [13,14]. This effect may be caused by reduction of both glycolysis and purine biosynthesis. Since the cellular pool of deoxyribonucleotides has a regulatory role in DNA replication during S-phase [15] this process in particular should be slowed down by the action of poly(ADP-ribose) synthetase (although RNA and protein synthesis will also be affected by the energy and nucleotide shortage). DNA repair synthesis, on the other hand, should hardly be impeded even by a 10-fold reduction of the precursor pool because the K_m values for deoxyribonucleoside triphosphates measured in vivo in permeable cells were found to be one order of magnitude lower for repair synthesis than for replicative DNA synthesis [16]. The various steps of NAD synthesis are distributed in the cell between the cytoplasm and nucleus [5]. The nuclear location of poly(ADP-ribose) synthetase and its activation by single-strand breaks in DNA would ensure an immediate response to DNA damage. Poly ADP-ribose is rapidly turning over. It is degraded by one of two enzymes, a glycohydrolase and a phosphodiesterase (reviewed in [2]). This high rate of turnover ensures that proteins serving as acceptors of poly ADP-ribose are modified only during a short period of time which may not have dramatic or long-lasting effects on the protein.

Since poly(ADP-ribose) synthetase is activated

by single-strand breaks to which it binds, it will also reduce the number of unprotected DNA breaks which may otherwise give rise to unspecific initiation of transcription [17] and of recombination events. Poly(ADP-ribose) synthetase, hence, might prevent such deleterious reactions until the reduced energy and precursor levels make their occurrence less likely. When ADP-ribosylated, the synthetase molecules will dissociate from the breaks which then can be repaired.

It has been observed that a nutritional deprivation of cells for NAD (by reducing the amount of nicotinamide in the medium) has an effect similar to that of inhibition of poly(ADP-ribose) synthetase [5,9]. One could argue then, that there is a situation where NAD is low to start with (a condition which we propose to be achieved by the production of poly ADP-ribose from NAD) and there is still no improvement of the repair reaction. However, there is a major difference between a condition in which poly ADP-ribosylation reduces the NAD level and a nutritional deprivation of NAD by reduced supplementation of nicotinamide: in the former reaction the amounts of nicotinamide in the cell are not low, contrary to the case of nutritional deprivation. Hence, competition of nicotinamide with the nucleotide synthesis for PRPP and imbalance in the level of deoxynucleoside triphosphates can only be achieved by transient NAD consumption via poly ADP-ribosylation. Nutritional deprivation for nicotinamide will in contrast leave normal levels of PRPP for nucleotide synthesis and DNA replication should not be impaired by this condition as observed [9].

In summary, therefore, we propose that poly ADP-ribosylation serves as a trap for NAD to reduce transiently the energy supply and the level of deoxyribonucleoside triphosphates to slow down replicative DNA synthesis and may thus represent another example of a reaction of cells to a stress situation. The extent of this reaction may greatly depend on the physiological state of the cell (stage in the cell cycle, rapidly dividing or not, etc.). Furthermore, the different types of DNA breaks or gaps produced by various DNA damaging agents may explain the great variability in the degree of activation of poly(ADP-ribose) synthetase observed in different cell systems. Several consequences of this hypothesis are experimentally testable.

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