15 Poly-ADP-Ribosylation in Cancer

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15.1 INTRODUCTION

Poly-ADP-ribosylation (PAR) is a posttranslational modification of proteins. The process was discovered and reported in early 1960s by Mandel and coworkers [1,2]. Since then it has been a subject of extensive research [reviewed in Refs. 3–5]. The interest has endured because of the uniqueness associated with PAR metabolism and its continuously expanding biological involvement, implications, and roles. The ubiquitous, enzyme catalyzed, fully reversible metabolic reaction involves transfer of an ADP-ribose moiety from a metabolic donor, nicotinamide adenine dinucleotide (NAD^+), to acceptor amino acid residues of a target protein. The primary biosynthesizing enzyme for PAR reaction is poly-ADP-ribose polymerase (PARP). The commonly modified amino acid residues in eukaryotes are glutamate and aspartate, though occasionally ADP-ribose moiety is found on residues such as arginine, cystine, asparagine, and diphthamide [3]. The target proteins for PAR are mostly nuclear and include, but are not limited to, such diverse array of proteins as histones, endonucleases, DNA pol α and β, DNA ligase I and II, topoisomerase I and II, RNA polymerases, reverse transcriptase, high mobility group (HMG) proteins, p53, Fos, AP endonuclease, Ku70, and the enzyme responsible for biosynthesis of PAR, that is, PARP itself. The metabolic reaction creates a complex, variably sized, and covalently attached homopolymeric, heterogeneous branched or unbranched ADP-ribose polymer adducts on the target protein thereby accomplishing the posttranslational modification of a protein (Figure 15.1). The complex homopolymeric ADP-ribose polymer adducts may contain up to 200 or, occasionally, more of monomers of ADP-ribose in linear or multiple branching architecture. Simultaneously, the main biodegrading enzyme of ADP-ribose polymer, the poly-ADP-ribose glycohydrolase (PARG), acts on the homopolymer attached to a modified protein and rapidly de-poly-ADP-ribosylates it by randomly and sequentially degrading the ADP-ribose monomers from the target protein (Figure 15.1). The two metabolic reactions occur simultaneously but in

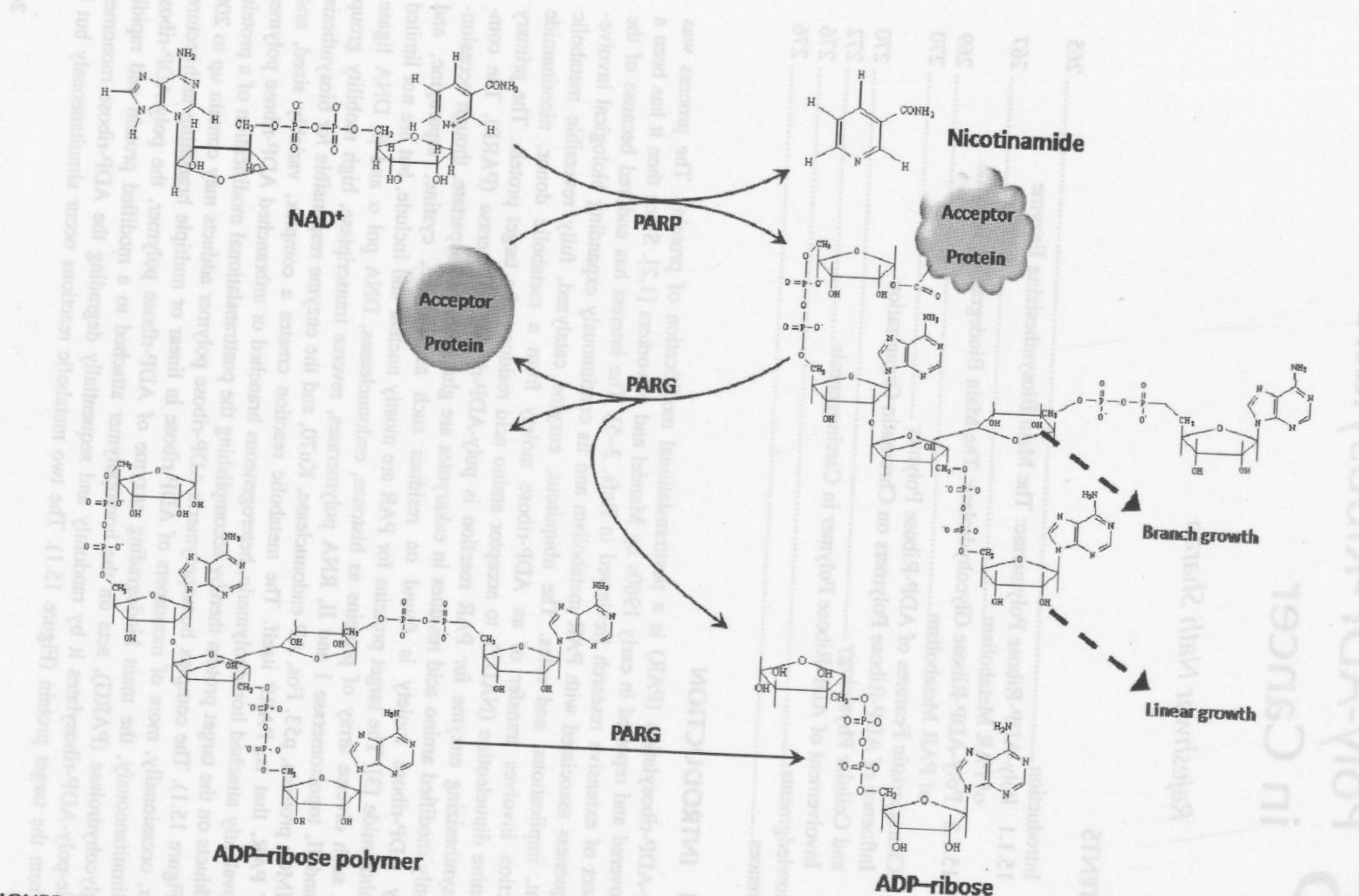

FIGURE 15.1 The biochemical pathway depicting the main features of the PAR metabolism. The ADP-ribose moiety from donor nicotinamide dinucleotide (NAD^+) is transferred onto an acceptor protein by PARP enzyme successively in a branched or unbranched complex architecture. The PARG enzyme breaks down the ADP-ribose polymers as ADP-ribose polymer or ADP-ribose monomer.

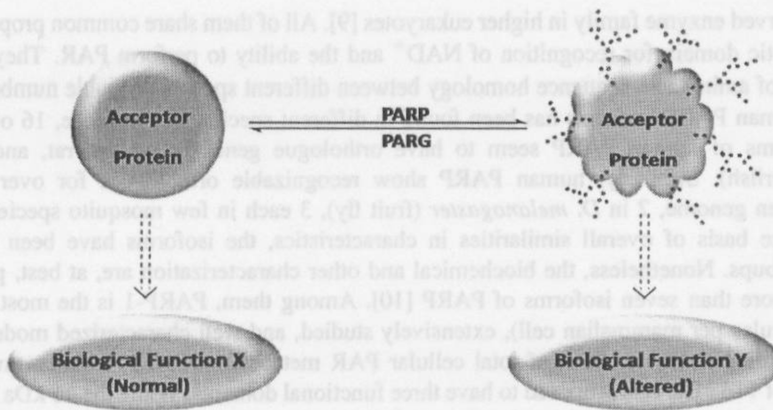

FIGURE 15.2 Schematic representation of the dynamism of the biosynthesizing and biodegrading pathways of PAR metabolism catalyzed by PARP and PARG. The normal biological functionality of an acceptor protein is altered after the modification.

opposite directions (Figure 15.2). Therefore, the net level of ADP-ribose polymer on cellular target proteins is the concerted outcome of these two opposing reactions.

PAR is reported to occur ubiquitously in eukaryotes, including some plants with a possible exception of yeast [6]. The addition of heterogeneous ADP-ribose polymers on a target protein influences the fundamental properties of the protein in several ways. Due to this, the cellular functioning of the modified protein is altered in various ways. Depending on the class of protein (e.g., structural, catalytic, regulatory, etc.) being modified, its biological consequences may vary widely (Figure 15.2). For example, if a structural protein was modified by PAR, structural organization and functions of the protein would be affected. On the other hand, if PAR of a catalytic protein took place the related metabolic pathway would manifest corresponding change. The biological expression of effect is not only dependent on the target proteins but also on the extent of its modification and the role of the modified protein in cellular metabolism. A logical consequence of this would be changes in cellular physiology and metabolism that are dependent on the target protein. This can be a very simple way of looking at the biological implication of PAR of cellular proteins. Therefore, in this review, I shall critically examine the role of PAR of cellular proteins and dwell upon its possible implication in cellular functioning, especially in carcinogenesis. However, before doing so it would be relevant to understand the metabolic process itself including the enzymes that are responsible for the metabolism and its effects on chromatin organization.

PAR is not to be confused with a different but closely related metabolic process called mono-ADP-ribosylation (MAR) in which a singular ADP-ribose moiety is attached to a target protein [reviewed in Ref. 7]. MAR is essentially a phenomenon of prokaryotic (bacterial) system and is associated with cellular signaling involving modification of signal transduction proteins, including G-protein, and expression of bacterial toxicity. The enzyme catalyzing MAR, mono-ADP-ribose transferase, is of considerable medical importance as it is associated with many infectious diseases of humans such as cholera, diphtheria, whooping cough, etc. [8].

15.1.1 Poly-ADP-Ribose Polymerase: The Main Biosynthesizing Enzyme of PAR Metabolism

The primary biosynthesizing enzyme for ADP-ribose polymer on a target protein is PARP [3–5]. Since its discovery about 40 years ago, it is now known to consist of over 17 isoforms in a highly

conserved enzyme family in higher eukaryotes [9]. All of them share common properties of having a catalytic domain for recognition of NAD^+ and the ability to perform PAR. They also show high level of amino acid sequence homology between different species. Variable number of orthologues of human PARP isoforms has been found in different species. For instance, 16 out of 17 reported isoforms of human PARP seem to have orthologue genes in mouse, rat, and *T. nigroviridis* (pufferfish). Similarly, human PARP show recognizable orthologues for over 12 isoforms in chicken genome, 2 in *D. melanogaster* (fruit fly), 3 each in few mosquito species, and so on [9]. On the basis of overall similarities in characteristics, the isoforms have been arranged in five subgroups. Nonetheless, the biochemical and other characterization are, at best, partially done for not more than seven isoforms of PARP [10]. Among them, PARP-1 is the most abundant ($\sim10^6$ molecules per mammalian cell), extensively studied, and well characterized model PARP enzyme accounting for about 90% of total cellular PAR metabolism. PARP-1 has an average molecular weight 113 kDa. It is proposed to have three functional domains. While the 46 kDa N-terminal DNA binding domain contains two zinc finger motifs supposedly mediating strand break recognition and a nuclear location signal, the largest, 54 kDa C-terminal catalytic domain binds to the substrate, NAD^+ [11]. The smallest, 22 kDa central automodification domain is involved with regulation of the catalytic property of the enzyme itself. It is known that PAR of PARP enzyme, appropriately named "automodification," causes loss of catalytic ability of the enzyme to further poly-ADP-ribosylate a target protein (heteromodification) and vice versa. To achieve it, the PARP enzyme functions as a dimer, such that one subunit of the holoenzyme can mutually catalyze addition of ADP-ribose polymer on the other subunit [8].

The significance of existence of multitude of PARP isoforms in eukaryotes is not yet clear and is a subject of extensive study [9,10]. Even though not all isoforms of PARP have been characterized [10], the information available from the best characterized isoforms support the contention that PARP is a conserved family of proteins with consensus amino acid sequences, motifs, intron positions, and domains. Existence of multitude of PARP isoforms in different species and their conservation through evolutionary tree point out to the criticality and importance of PARP enzyme in cellular metabolism. It could be speculated that existence of conserved and multiple isoforms of PARP in a genome is to just ensure continuance of PAR metabolism even under extreme conditions of cellular stress.

PARP was originally recognized as an important DNA damage sensor protein mediating DNA repair, genome integrity, and cell survival on one hand, and cytotoxicity and cell death on the other [12]. It was thought that a damaged DNA, in particular a strand break or nick in DNA, was an essential molecular trigger for PARP activity. It appears that the free-floating cellular PARP responds to a molecular trigger (e.g., single-strand break or SSB), rapidly binds to the damaged or nicked DNA, acquires metabolically active status, and initiates biosynthesis of ADP-ribose polymers on target proteins (heteromodification) as well as itself (automodification). Depending on the rate of this reaction a sudden depletion of endogenous NAD^+ might occur. The automodified PARP correspondingly becomes inactive and the process of PAR of self and other nonself target proteins stops (Figure 15.3). On the other hand, evidences have accumulated over the recent years showing that PARP enzyme may also be metabolically activated by structural status of DNA [13] in absence of a damaged or nicked DNA [14]. These observations suggest that PARP might also participate in a variety of normal metabolic processes of a cell upon appropriate stimulation [15]. In elucidating this aspect, use of a host of inhibitors of PARP has been made thereby paving the way for deeper understanding of biological role of PARP, which appears to be more complicated than what we currently understand [reviewed in Ref. 16]. This line of investigation has opened up a new possibility of therapeutic use of PARP inhibitors after many unresolved issues are settled. That the PARP family of enzymes are involved with diverse cellular functions is also supported by the fact that the enzyme or its isoforms is not only localized in the nucleus but also in extranuclear region of a cell, e.g., in centrosomes, mitochondria, etc. [9,10,15].

other pharmacological properties indicate that it may not be a suitable biological inhibitor of PARG [23]. Oka et al. have recently reported existence of a 39 kDa poly-ADP-ribose hydrolase (ARH 3) enzyme in mammalian cells, which exhibited PARG activity despite being structurally different from it [24].

Nonetheless, the polymer of ADP-ribose biosynthesized on a target protein is very rapidly degraded by PARG in vivo; a rough biological half-life of PAR is estimated to be ≤1 min in mammalian cell [25]. It appears that the turnover of cellular ADP-ribose polymer is very important in maintenance of normal cellular metabolism, physiology, and development. In absence of a suitable biological inhibitor of PARG, some studies utilized an approach of disruption of PARG gene to understand its role in cellular physiology and metabolism. Partial and complete knockout mouse for PARG showed continued accumulation of ADP-ribose polymers on target proteins that lead to embryonic lethality pointing to strong influence of failure of degradation or turnover of ADP-ribose polymers from proteins on cellular well being [20,26].

15.2 CHARACTERISTIC FEATURES OF ADP-RIBOSE POLYMER

The heterogeneity and bulk of the ADP-ribose polymers as well as the resulting alteration in the net charge of the modified protein make this modification a unique posttranslational modification. There are three special features of this modification that separate it from other posttranslational modifications (e.g., phosphorylation, acetylation, methylation, etc.). In the first, it is to be noted that each moiety of ADP-ribose confers two negative charges emancipating from its two phosphate groups on the modified protein (Figure 15.1). Since this modification is in form of a polymer comprising up to 200 monomer units of ADP-ribose, each target protein being modified is likely to experience a highly significant change in its net charge. No other posttranslational modification of proteins can match the quantum of this change in the net charge of a modified protein. Dramatically altered charge status of a protein would strongly influence its conformational status and, consequently, its functionality. It has been shown that PAR of histone H1 protein lead to significant change in chromatin superstructure even when ≤5% of H1 was modified [27]. Secondly, the histone proteins, a preferred target protein family [3–5], are small-sized proteins with molecular weights in the range of approximately 11–21 kDa [28]. The molecular weight of a moiety of ADP-ribose is approximately 550 Da (Figure 15.1). Thus, an ADP-ribose polymer consisting of, for example, 200 monomers of ADP-ribose would be a very bulky adduct of molecular weight of ~110 kDa. Thus, in case of PAR of histone proteins, the size and bulk of the modification or ADP-ribose polymer adduct would, on an average, exceed the size and bulk of the target protein itself by 6- to 11-folds. Lastly, it is known that there exists a wide variation in the size, number, and pattern of branching of ADP-ribose polymers conferring a great heterogeneity on the modified cellular proteins [3–5] (Figures 15.1 and 15.3). In different organizational status and conformations, free or protein associated ADP-ribose polymers are likely to influence (1) protein–protein interaction that is very common and critical among histone proteins (e.g., in core histone organization) as well as (2) protein–DNA interaction that is the basis of chromosomal organization [26,29]. All these features are unique to PAR and are not found in other posttranslational modifications of proteins making PAR a unique metabolic process.

15.3 INFLUENCE OF ADP-RIBOSE POLYMERS ON CHROMATIN ORGANIZATION AND CELLULAR PHYSIOLOGY

It is estimated that over 3.2 giga base pairs (Gbp) of nucleotides (NT) constitute a human genomic DNA comprising an estimated 25 to 30 thousand genes [30]. The double helix of mammalian DNA should comprise approximately 25 million nucleosomes at the first level of chromatin organization. This level of structural organization of DNA essentially revolves around an octamer of core histone proteins consisting of two molecules each of H2A, H2B, H3, and H4. The histone protein H1 is

The PAR metabolism is known to have extensive involvement in the structural organization and functional status of chromatin as well as in diverse cellular physiology. PAR was initially considered a cellular or metabolic response to DNA damage induced by ionizing radiations and alkylating agents [3–5,12]. Upon induction of DNA damage, particularly SSB or nicks, the biosynthesizing enzyme PARP was rapidly stimulated several folds. Concomitantly ADP-ribose polymer was biosynthesized accompanied by depletion of cellular NAD^+ (Figure 15.1), which facilitated repair of the DNA strand breaks. Rapid NAD^+ depletion in extreme cases resulted in cell death. This suggests that PAR is not only a repair mediating process but also facilitates cell death in heavily damaged cells. Two aspects of the PAR metabolism need special attention. Firstly, stimulated PARP activity alone cannot give precise idea of status of ADP-ribose polymer on a target protein. The activity of the opposite reaction catalyzed by PARG has also to be known simultaneously besides, perhaps, status of the cellular pool of NAD^+, as the status of actual ADP-ribose polymer on a target protein is decided by the interplay of two opposing reactions (Figure 15.2). The interaction of a modified protein with other proteins or DNA, in turn, would be decided by the ADP-ribose polymer adducts rather than PARP or PARG enzyme activities. Secondly, rapid depletion of NAD^+ observed during PARP stimulation would also affect other NAD^+-dependent metabolic processes that might have its own influences on cell response.

Over the years the PAR of cellular proteins has acquired a very broad meaning in the realm of cellular metabolism and is expanding. Its role and implications have penetrated a wide variety of normal cellular physiology and metabolism even in absence of DNA break [13,14,38,39]. At the same time, its original role as a DNA damage sensor remains intact. As a cellular response to DNA damage, over 500-fold induction of ADP-ribose polymers has been reported [40]. The notable inter- and intraspecies sequence/domain homology and widespread intracellular distribution of PARP and its isoforms [9,10] certainly suggest that it may have multifarious metabolic roles to play. Consequently, dysregulation of PAR metabolic reactions might be important in manifestation of disease conditions as well as in signaling pathways [15]. For example, involvement of PAR metabolism has been shown in carcinogenesis [41], maintenance of genome integrity [42] and cellular detoxification, cell division [43], cell cycle progression, strand break repair as a recruiter of associated factors [44], maintenance of long-term cellular memory [45], apoptosis [15] (especially for caspase-independent, apoptosis inducing factor mediated pathway) to name the obvious. To interlink these seemingly very diverse biological responses reported in the literature, I have attempted to at least partially map the metabolic processes and molecules associated with PAR metabolism using PathwayStudio (ver. 4.0) software in Figure 15.4. The map shows a very wide spectrum of possible involvement of PAR in cellular functions with PARP at the center stage (Figure 15.4). PathwayStudio is a bioinformatics software, which enables in depth analysis of interrelated biological data using pathway reconstruction algorithms [46]. The software finds common regulator molecules, biological entities, and associated pathways from a large collection of databases covering mammalian and other life forms. This analysis gives a new perspective to PAR metabolism in which PARP-1 enzyme exerts influences, either directly or indirectly, on diverse biological functions such as cancer, cell division, proliferation, differentiation, repair pathways, inflammation, senescence, necrosis through apoptosis (Figure 15.4). The pathway map also depicts a large number of metabolites, molecules, and processes that might be involved in expression and regulation of PAR metabolism as well as in manifestation of its biological influence. Even though not every aspect of cellular physiology mapped is clear at this time, it is obvious that a comprehensive and complete understanding of the PAR metabolism is presently far from our reach.

15.4 INVOLVEMENT OF ADP-RIBOSE POLYMER IN CARCINOGENESIS

Carcinogenesis is a complex, multistep process that can be categorized into three clearly defined stages, namely, initiation, promotion, and progression. Conceptually, irreversible molecular events of "initiation" stage lead to monoclonal expansion of the initiated cells during "promotion," which

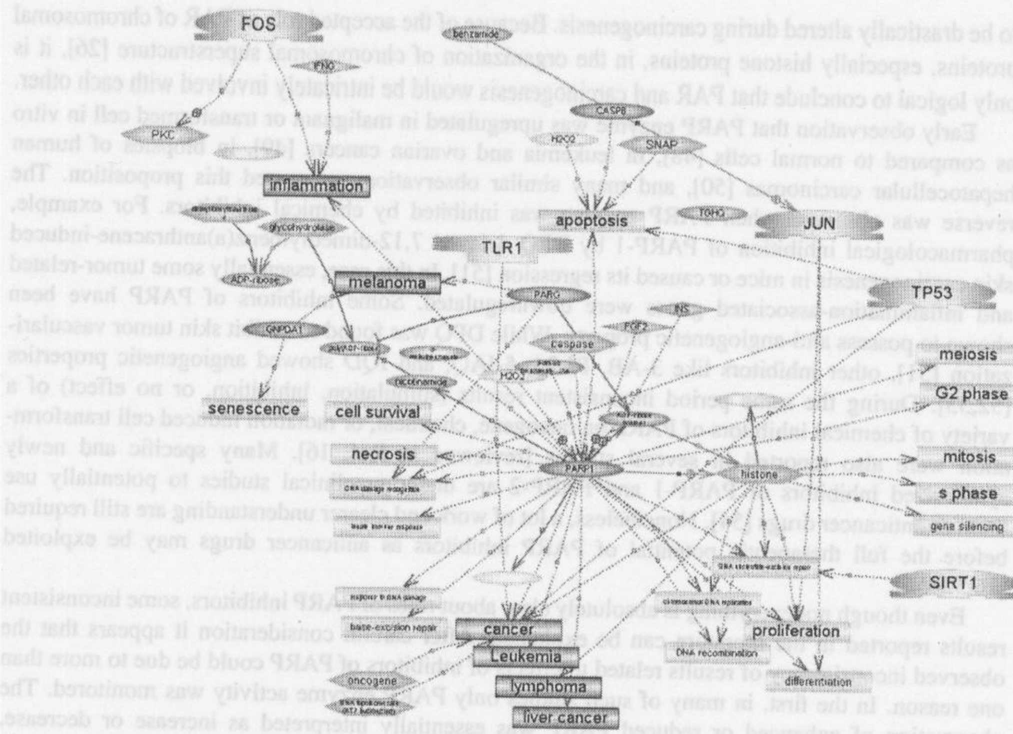

FIGURE 15.4 **(See color insert following page 272.)** Complex, hypothetical and partial map of metabolic pathways and cellular processes/functions that are influenced by PAR metabolism and associated molecules with PARP enzyme at the center. The map is based on data retrieval from various data banks using the PathwayStudio software.

is partially reversible. During the third and last "progression" stage, which is also irreversible, the promoted cells progressively turn malignant characterized by uncontrolled cell division, loss of contact inhibition, and dedifferentiation [reviewed in Ref. 39,47]. During the process multiple genetic alterations occur. This includes mutations of various kinds, chromosomal aberrations, gene rearrangements, gene silencing and shutdown, neogene expression, and gene amplification, to name the obvious. All these molecular events can, in principle, be seriously influenced by PAR metabolism as ADP-ribose polymers on chromosomal proteins potentially alter chromatin superstructure transiently and locally. In a relaxed, negatively supercoiled state of chromatin organization gene expression is permissible. The reverse would lead to shut down, silencing or downregulation of genes, and so on. In general, it is accepted that structural organization of chromatin strongly influences damage and repair of DNA, replication, gene expression, and other genome dependent functions. It essentially means that for the genome to perform its biological functions, the higher order of chromatin superstructure must collapse. Logistically, the entire chromatin superstructure cannot collapse in the confines of a nucleus. Therefore, the collapse has to be transient and local. Once the biological function has been performed, the reverse ought to happen. This dynamism seems to be the hallmark and essence of the highly regulated living process (Figure 15.4). Thus, in a relaxed organizational state, various agents, proteins, biomolecules, regulatory entities, etc., can easily access the genomic DNA. In its condensed state, on the contrary, access is prevented or, at least, not permitted to the same extent [reviewed in Ref. 39]. The genomic functions are known

to be drastically altered during carcinogenesis. Because of the accepted role of PAR of chromosomal proteins, especially histone proteins, in the organization of chromosomal superstructure [26], it is only logical to conclude that PAR and carcinogenesis would be intricately involved with each other.

Early observation that PARP enzyme was upregulated in malignant or transformed cell in vitro as compared to normal cells [48], in leukemia and ovarian cancers [49], in biopsies of human hepatocellular carcinomas [50], and many similar observations supported this proposition. The reverse was observed when PARP enzyme was inhibited by chemical inhibitors. For example, pharmacological inhibition of PARP-1 by DPQ delayed 7,12-dimethylbenz(a)anthracene-induced skin carcinogenesis in mice or caused its regression [51]. In this case, essentially some tumor-related and inflammation-associated genes were downregulated. Some inhibitors of PARP have been shown to possess anti-angiogenetic property. While DPQ was found to inhibit skin tumor vascularization [51], other inhibitors like 3-AB, PJ-34, 5-IAQ, and IQD showed angiogenetic properties [52,53]. During the same period inconsistent results (stimulation, inhibition, or no effect) of a variety of chemical inhibitors of PARP on oncogene, chemical, or radiation induced cell transformation were also reported in several studies [reviewed in Ref. 16]. Many specific and newly synthesized inhibitors of PARP-1 and PARP-2 are under preclinical studies to potentially use them as anticancer drugs [54]. Nonetheless, a lot of work and clearer understanding are still required before the full therapeutic potential of PARP inhibitors as anticancer drugs may be exploited [16,52–54].

Even though not everything is absolutely clear about roles of PARP inhibitors, some inconsistent results reported in the literature can be explained. After careful consideration it appears that the observed inconsistency of results related to effects of inhibitors of PARP could be due to more than one reason. In the first, in many of such studies only PARP enzyme activity was monitored. The observation of enhanced or reduced PARP was essentially interpreted as increase or decrease, respectively, in the level of ADP-ribose polymers on chromosomal proteins and correlated to carcinogenesis. Monitoring only PARP activity is unlikely to reveal the metabolic consequence of addition of ADP-ribose polymer onto chromosomal proteins, which actually alters chromatin structural, and, consequently, its functional standing (Figure 15.2). Therefore, inconsistency might have crept into different studies as the activity of PARG, the biodegrading enzyme in PAR metabolism, might have been different under dissimilar experimental conditions. Secondly, it is now known that various isoforms of PARP respond differently to different inhibitors, thereby, exerting variable biological effects [9,10,16]. This may also be a source of inconsistency in observations by different researchers using different inhibitors of PARP. Thirdly, it is possible that cells at different stages of transformation and carcinogenesis responded differently to inhibitors of PARP as different characteristic molecular events occur in three stages of the process. Finally, the inhibitors of PARP, besides inhibiting the enzyme, might also exert some other nonspecific metabolic influence on other enzymes/biomolecules about which we do not know yet. Instead of monitoring PARP or PARG enzymes of PAR metabolism, in some studies the level of ADP-ribose polymers on cellular protein has been directly measured so that correlation between ADP-ribose polymer and DNA damage, repair, and carcinogenesis induced by various factors could be established. High levels of ADP-ribose polymer on cellular proteins, including a 113 kDa protein, was reported in rectal cancer biopsies [55]. In contrast, a series of investigations carried out in my laboratory show that cellular ADP-ribose polymers progressively and statistically significantly reduced during carcinogenesis induced by chemical carcinogens in mice [56–59]. In this series of investigations a novel, sensitive, and specific slot blot and Western blot immunoprobe assay of ADP-ribose polymer has been utilized, which directly assays the polymeric adducts on cellular proteins. The immunoprobe assay utilizes a polyclonal antibody (PAb) raised against natural, heterogeneous ADP-ribose polymers of mouse spleen cells as an antigen. The advantage of this approach is that the PAb directly detects both free as well as protein associated cellular ADP-ribose polymers in a given sample. Thus, the assay gave a true metabolic measure of cellular ADP-ribose polymers [49]. The PAb has been raised against the isolated ADP-ribose polymers, and not against ADP-ribose polymer bound to a particular protein.

Thus, the nature of target protein to which the ADP-ribose polymer is attached does not effect detection. In this way, one could conveniently measure total cellular level of ADP-ribose polymers using slot blot immunoprobe assay [49]. Likewise, when Western blot immunoprobe assay was used, individual poly-ADP-ribosylated proteins could be visualized and quantified. Thus, it became possible to quantify true metabolic levels of either total cellular ADP-ribose polymers of all proteins or of an individual cellular protein. The ADP-ribose polymers of total cellular as well as of histone proteins of mouse liver and spleen cells were found to progressively go down during initiation of carcinogenesis induced by dimethyl- or diethyl-nitrosamine [49,51,52,60,61]. Similarly, aqueous extract of betel nut or arecoline also progressively inhibited ADP-ribose polymer adducts on total, histone or HMG proteins of mice tissues during initiation period [31,53,62,63]. Using a Dalton's lymphoma ascites tumorigenesis model in mice, it was found that a similar negative correlation existed between ADP-ribose polymers on histone proteins and carcinogenesis during latter stages of cancer development [50]. This approach of ADP-ribose polymer assay [49,64] has recently been extended to quantification of total ADP-ribose polymer on peripheral blood lymphocyte proteins isolated from blood samples of patients in advance stages (III/IV) of cancers of breast, cervix, and head and neck. A significant reduction in ADP-ribose polymers on total cellular proteins has been observed in all three cancers [65]. Preliminary calculations convincingly show nearly 45% reduction in the level of total ADP-ribose polymers on blood lymphocyte proteins in these patients as compared to the controls samples obtained from young volunteers with no known history of cancer (R.O. Lakadong and R.N. Sharan, unpublished results). Though more preclinical studies involving early stages of human cancer would need to be done, one may take cue from the mouse model to hypothesize a direct link between ADP-ribose polymers on cellular proteins, particularly histone proteins, and carcinogenesis. In this progressive lowering of metabolic level of ADP-ribose polymer adducts on cellular proteins stands out as a hallmark of carcinogenesis.

Though in these reports ADP-ribose polymer has been directly measured, it has to be noted that the reports of Yalcintepe et al. [48] and that from my laboratory [40–45] essentially show contradictory results. This needs an explanation. I believe, the explanation lies in the methods employed in these studies to assay and quantify ADP-ribose polymers on cellular proteins. ADP-ribose polymer has been assayed in a variety of ways [reviewed in Ref. 66]. Radioisotopic method, a widely used assay of ADP-ribose polymers utilizing radiolabeled-NAD^+ [67], was employed in the investigation of Yalcintepe et al. [48]. We have earlier shown that different methodological interventions associated with the radioisotopic assay per se induced unphysiologically high levels of cellular ADP-ribose polymers, which does not necessarily reflect the in vivo or metabolic level of cellular ADP-ribose polymers [49,57,68]. It is possible that the high ADP-ribose polymer reported by Yalcintepe et al. [48] could be due to such methodological problems. Its assumption derives strength from the same study wherein the authors also report high level of ADP-ribose polymer on a 113 kDa protein. On the basis of molecular weight similarity and the fact the PARP is a preferred target for PAR (automodification) [8], it is likely that this 113 kDa protein is actually PARP enzyme. Since automodified PARP loses its catalytic ability to carry on PAR of other target proteins (heteromodification) [3–5,8] (Figure 15.3), it seems unlikely that metabolic level of ADP-ribose polymers on cellular proteins would actually increase under this condition. The immunoprobe assay employed in the study being carried out in my laboratory [40–45] actually measured the true metabolic level of ADP-ribose polymer adducts on cellular proteins [see Refs. 49,57]. As has been emphasized earlier, downregulation of PARP does not necessarily result in lowering of PAR of histone or other cellular proteins. It would be decided by the level of PARG enzyme activity. With downregulated PARP activity, the level of ADP-ribose polymers on histone or other proteins could still be high provided simultaneously the PARG enzyme was also downregulated and vice versa. Indirect support for this proposition comes from the observation that 3-aminobenzamide (3-AB), a widely used physiological inhibitor of PARP-1, did not completely inhibit PAR signal as revealed by slot- and Western blot immunoprobe assay in some of our studies in mice to quantify ADP-ribose

polymers [51,52]. The ADP-ribose polymer signals in both slot blot and Western blots were only subdued at a concentration of 3-AB that is known to fully inhibit PARP-1. This indicates two possibilities. Firstly, all isoforms of PARP may not be inhibited by 3-AB as efficiently as PARP-1. Secondly, it is possible that PARG was either fully or partially inhibited due to which existing ADP-ribose polymer adducts on target proteins were not removed. Much more investigation and deeper insight are required before these and other issues get fully resolved in the realm of PAR metabolism.

In conclusion, it appears that a global understanding of PAR metabolism still eludes us despite the tremendous wealth of scientific information available. The deep involvement of PAR metabolism or its components in diverse normal and pathophysiological processes are yet to be fully uncovered. Till such time no truly conclusive understanding can be arrived at. Full understanding of significance of existence of isoforms of PARP is urgently required. Similarly, importance of PARG enzyme in PAR metabolism needs to be elucidated further. In this, more research will be required to clearly understand contributions of these two enzymes and their isoforms in PAR metabolism and their significance in carcinogenesis. One approach to this could be use of different chemical inhibitors of enzymes PARP and PARG. Particular emphasis needs to be paid to differential influences of different inhibitors on various isoforms of PARP. Another, more direct approach to this could be further elucidation of influence of the heterogeneous ADP-ribose polymer adducts on target proteins itself, and consequent alterations in protein functionality and cellular metabolism. The known heterogeneity of ADP-ribose polymer adducts in terms of their size, branching pattern, and location on target protein suggests differential influence of the polymer on various proteins. This needs to be understood clearly. On the basis of present understanding, it is obvious that PAR metabolism is highly relevant to carcinogenesis and could be potentially exploited in cancer therapeutics and diagnostics. Some of our recent results do suggest potential use of ADP-ribose polymer immunoprobe assay in cancer screening programme [49–52,54,57,59]. More work will be required before the therapeutic as well as diagnostic potential of PAR metabolism in cancer can be fully exploited.

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REFERENCES

1. Chambon, P., Weill, J.D., and Mandel, P., Nicotinamide mononucleotide activation of new DNA-dependent polyadenylic acid synthesizing nuclear enzyme, *Biochem. Biophys. Res. Commun.*, 11, 39, 1963.
2. Chambon, P. et al., On the formation of a novel adenylytic compound by enzymatic extracts of liver nuclei, *Biochem. Biophys. Res. Commun.*, 25, 638, 1966.
3. Althaus, F.R., Poly-ADP-ribosylation reactions. In: *ADP-Ribosylation of Proteins: Enzymology and Biological Significance*, Althaus, F.R. and Richter, C. (Eds.), Part I, Springer Verlag, Berlin, 1987.
4. Jacobson, M.K. and Jacobson, E.L., *ADP-Ribose Transfer Reactions: Mechanisms and Biological Significance*, Springer, New York, 1989.
5. Poirier, G.G. and Moreau, P., *ADP-Ribosylation Reactions*, Springer, New York, 1992.
6. Park, J.K. et al., Inhibition of topoisomerase I by NAD and enhancement of cytotoxicity of MMS by inhibitors of poly(ADP-ribose) polymerase in *Saccharomyces cerevisiae*, *Cell. Mol. Biol.*, 37, 739, 1991.
7. Richter, C., Mono-ADP-ribosylation reactions. In: *ADP-Ribosylation of Proteins: Enzymology and Biological Significance*, Althaus, F.R. and Richter, C. (Eds.), Part II, Springer Verlag, Berlin, 1987.
8. De Murcia, G., Jacobson, M., and Shall, S., Regulation by ADP-ribosylation, *Trends Cell Biol.*, 5, 78, 1995.
9. Otto, H. et al., In silico characterization of the family PARP-like poly(ADP-ribosyl)transferases (pARTs), *BMC Genomics*, 6, 139, 2005.

10. Ame, J.C., Spenjehauer, C., and De Murcia, G., The PARP superfamily, *BioEssays*, 26, 882, 2004.
11. Scovassi, A.I. and Diedrich, M., Modulation of poly(ADP-ribosylation) in apoptotic cells, *Biochem. Pharmacol.*, 68, 1041, 2004.
12. Virag, L., The expanding universe of poly(ADP-ribosyl)ation, *Cell. Mol. Life Sci.*, 62, 719, 2005.
13. Lonskaya, I. et al., Regulation of poly(ADP-ribose) polymerase-1 by DNA structure-specific binding, *J. Biol. Chem.*, 280, 17076, 2005.
14. Hassa, P.O. et al., The enzymatic and DNA binding activity of PARP-1 are not required for NF-kB coactivator function, *J. Biol. Chem.*, 276, 45588, 2001.
15. Gange, J.-P. et al., The expanding role of poly(ADP-ribose) metabolism: Current challenges and new perspectives, *Curr. Opinion Cell Biol.*, 18, 145, 2006.
16. Plummer, E.R., Inhibitors of poly(ADP-ribose) polymerase in cancer, *Curr. Opin. Pharmacol.*, 6, 364, 2006.
17. Bonicalzi, M.E. et al., Regulation of poly(ADP-ribose) metabolism by poly(ADP-ribose) glycohydrolase: Where and when? *Cell. Mol. Life Sci.*, 62, 739, 2005.
18. Meyer-Ficca, M.L. et al., Human poly(ADP-ribose) glycohydrolase is expressed in alternative splice variants yielding isoforms that localizes to different cell compartments, *Exp. Cell Res.*, 297, 521, 2004.
19. Haince, J.-F. et al., Dynamic relocation of poly(ADP-ribose) glycohydrolase isoforms during radiation-induced DNA damage, *Biochim. Biophys. Acta*, 1763, 226, 2006.
20. Ohashi, S. et al., Subcellular localization of poly(ADP-ribose) glycohydrolase in mammalian cells, *Biochem. Biophys. Res. Commun.*, 307, 915, 2003.
21. Cortes, U. et al., Depletion of the 110 kDa isoform of poly(ADP-ribose) glycohydrolase increases sensitivity to genotoxic and endotoxic stress to mice, *Mol. Cell. Biol.*, 24, 7163, 2004.
22. Lin, W. et al., Isolation and characterization of the cDNA encoding bovine poly(ADP-ribose) glycohydrolase, *J. Biol. Chem.*, 272, 11895, 1997.
23. Falsig, J. et al., Poly(ADP-ribose) glycohydrolase as a target for neuroprotective intervention: Assessment of currently available pharmacological tools, *Eur. J. Pharmacol.*, 497, 7, 2004.
24. Oka, S., Kato, J., and Moss, J., Identification and characterization of a mammalian 39 kDa poly(ADP-ribose) glycohydrolase, *J. Biol. Chem.*, 281, 705, 2006.
25. Herceg, Z. and Wang, Z.-Q., Functions of poly(ADP-ribose) polymerase (PARP) in DNA repair, genomic integrity and cell death, *Mutat. Res.*, 477, 97, 2001.
26. Koh, W. et al., Failure to degrade poly(ADP-ribose) causes increased sensitivity to cytotoxicity and early embryonic lethality, *Proc. Natl. Acad. Sci. U S A*, 101, 17699, 2004.
27. Aubin, R.J. et al., Correlation between endogenous nucleosomal hyper(ADP-ribosyl)ation of histone H1 and the induction of chromatin relaxation, *EMBO J.*, 2, 1685, 1983.
28. De Robertis, E.D.P. and De Robertis, E.M.F. Jr., *Cell and Molecular Biology*, Chapter 15, Saunders College, Philadelphia, 1980.
29. Peterman, E., Keil, C., and Oei, L., Importance of poly(ADP-ribose) polymerases in regulation of DNA-dependent processes, *Cell. Mol. Life Sci.*, 62, 731, 2005.
30. Baltimore, D., Our genome unveiled, *Nature*, 409, 814, 2001.
31. Boulikas, T., Nuclear envelope and chromatin structure, *Int. Rev. Cytol. Suppl.*, 17, 493, 1987.
32. Tulin, A. and Spradling, A., Chromatin loosening by poly(ADP)-ribose polymerase (PARP) in *Drosophila* puff loci, *Science*, 299, 560, 2003.
33. Saikia, J.R., Schneeweiss, F.H.A., and Sharan, R.N., Arecoline induced changes of poly-ADP-ribosylation of cellular proteins and its influence on chromatin organization, *Cancer Letts.*, 139, 59, 1999.
34. Plescheke, J.M. et al., Poly(ADP-ribose) binds to specific domains in DNA damage check points, *J. Biol. Chem.*, 275, 40974, 2000.
35. Realini, C.A. and Althaus, F.R., Histone shuttling by poly(ADP-ribosylation), *J. Biol. Chem.*, 267, 18858, 1992.
36. Sharan, R.N., Schneeweiss, F.H.A., and Feinendegen, L.E., Neutrons affect ADP-ribosylation of proteins in human kidney T1-Cells in vitro, *Indian J. Biochem. Biophys.*, 33, 281, 1996.
37. Davidovic, L. et al., Importance of poly(ADP-ribose) glycohydrolase in the control of poly(ADP-ribose) metabolism, *Exp. Cell Res.*, 268, 7, 2001.
38. Althaus, F.R., Poly-ADP ribosylation: A histone shuttle mechanism in DNA excision repair, *J. Cell Sci.*, 102, 663, 1992.

39. Faraone-Mennella, M.R., Chromatin architecture and function: The role(s) of poly(ADP-ribose) polymerase and poly(ADP-ribosyl)ation of nuclear proteins, *Biochem. Cell Biol.*, 83, 396, 2005.
40. D'Amours, D. et al., Poly(ADP-ribosyl)ation reaction in the regulation of nuclear functions, *Biochem. J.*, 342, 249, 1999.
41. Boulikas, T., Relationship between carcinogenesis, chromatin structure and poly(ADP-ribosylation), *Anticancer Res.*, 11, 489, 1991.
42. Menissier de Murcia, J. et al., Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse, *EMBO J.*, 22, 2255, 2003.
43. Chang, P., Coughlin, M., and Mitchison, T.J., Tankyrase-1 polymerization of poly(ADP-ribose) is required for spindle structure and function, *Natl. Cell Biol.*, 7, 1133, 2005.
44. Leppard, J.B. et al., Physical and functional interaction between DNA ligase IIIα and poly(ADP-ribose) polymerase 1 in DNA single strand break repair, *Mol. Cell. Biol.*, 23, 5919, 2003.
45. Cohen-Armon, M., et al., Long-term memory requires poly-ADP-ribosylation, *Science*, 304, 1820, 2004.
46. www.ariadnegenomics.com/products/pathway-studio/
47. Miwa, M. and Sugimura, T., ADP ribosylation and carcinogenesis. In: *ADP-Ribosylating Toxins and G Proteins: Insights into Signal Transduction*, Moss, J. and Vaughan, M., (Eds.), American Society for Microbiology, Washington DC, pp. 543–560, 1990.
48. Miwa, M. et al., Cell density-dependent increase in chromatin-associated ADP-ribosyltransferase activity in simian virus 40-transformed cells, *Arch. Biochem. Biophys.*, 181, 313, 1977.
49. Singh, N., Enhanced poly ADP-ribosylation in human leukemia lymphocytes and ovarian cancers, *Cancer Letts.*, 58, 131, 1991.
50. Shimizu, S. et al., Expression of poly(ADP-ribose) polymerase in human hepatocellular carcinoma and analysis of biopsy specimens obtained under sonographic guidance, *Oncol. Rep.*, 12, 821, 2004.
51. Martin-Olivia, D. et al., Inhibition of poly(ADP-ribose) polymerase modulates tumor-related gene expression, including hypoxia-inducible factor-1 activation, during skin carcinogenesis, *Cancer Res.*, 66, 5744, 2006.
52. Rajesh, M. et al., Poly(ADP-ribose)polymerase inhibition decreases angiogenesis, *Biochem. Biophys. Res. Commun.*, 350, 1056, 2006.
53. Rajesh, M. et al., Pharmacological inhibition of poly(ADP-ribose) polymerase inhibits angiogenesis, *Biochem. Biophys. Res. Commun.*, 350, 352, 2006.
54. Tentori, L. and Graziani, G., Chemopotentiation by PARP 1 & 2 inhibitors in cancer therapy, *Pharmacol. Res.*, 52, 25, 2005.
55. Yalcintepe, L. et al., Change in NAD/ADP-ribose metabolism in rectal cancer, *Brazilian J. Med. Biol. Res.*, 38, 361, 2005.
56. Sharan, R.N. et al., Detection and quantification of poly-ADP-ribosylated cellular proteins of spleen and liver tissues of mice in vivo by slot and Western blot immunoprobing using polyclonal antibody against mouse ADP-ribose polymer, *Mol. Cell. Biochem.*, 278, 213, 2005.
57. Devi, B.J. and Sharan, R.N., Progressive reduction of poly-ADP-ribosylation of histone proteins during Dalton's lymphoma induced ascites tumorigenesis in mice, *Cancer Letts.*, 238, 135, 2006.
58. Devi, B.J., Schneeweiss, F.H.A., and Sharan, R.N., Negative correlation between poly-ADP-ribosylation of spleen cell histone proteins and initial duration of dimethylnitrosamine exposure to mice in vivo measured by Western blot immunoprobe assay: A possible biomarker for cancer detection, *Cancer Detect. Prev.*, 29, 66, 2005.
59. Kma, L. and Sharan, R.N., In vivo exposure of Swiss Albino mice to chronic low doses of dimethylnitrosamine (DMN) lowers poly-ADP-ribosylation of bone marrow cells and blood lymphocytes, *Mol. Cell. Biochem.*, 288, 143, 2006.
60. Pariat, T. and Sharan, R.N., Qualitative change in mice liver HMG proteins after low dose chronic administration of aqueous extract of betel but and diethylnitrosamine, *Hepatol. Res.*, 12, 177, 1998.
61. Kma, L. and Sharan, R.N., Negative correlation between poly-ADP-ribosylation of mouse blood lymphocyte proteins and dimethylnitrosamine induced initiation of carcinogenesis as revealed by slot- and Western blot immunoassay, *Proc. Natl. Acad. Sci. India*, 73B, 43, 2003.
62. Pariat, T. and Sharan, R.N., Role of mouse spleen cell HMG proteins and their poly-ADP-ribosylation in betel nut induced carcinogenesis, *Indian J. Biochem. Biophys.*, 39, 130, 2002.
63. Sharan, R.N., Association of betel nut with carcinogenesis (review), *Cancer J.*, 9, 13, 1996.

64. Sharan, R.N. et al., Immunodetection of cellular poly-ADP-ribosylation. In: *Trends in Radiation and Cancer Biology*, Sharan, R.N., (Ed.), Forschungszentrum Juelich GmbH, Juelich, Int. Coop. Bilateral Sem. Series Vol. 29, pp. 240–243, 1998.
65. Lakadong, R.O., Kma, L., and Sharan, R.N., Poly-ADP-ribosylation of blood lymphocyte proteins: A potential biomarker of cancer, presented at Natl. Sem. on Adaptation Biochemistry, Shillong, March 22–23, 2007.
66. Shah, G.M. et al., Methods for chemical study of poly(ADP-ribose) metabolism in vitro and in vivo, *Anal. Biochem.*, 227, 1, 1995.
67. Surowy, C.S. and Berger, N.A., Unique acceptors for poly(ADP-ribose) in resting, proliferating and DNA-damaged human lymphocytes, *Biochim. Biophys. Acta*, 740, 8, 1983.
68. Schneeweiss, F.H.A., Sharan, R.N., and Feinendegen, L.E., Change of ADP-ribosylation in human kidney T1 cells by various external stimuli, *Indian J. Biochem. Biophys.*, 32, 119, 1995.