

Pharmacological inhibition of PARP may be a novel approach to treating diabetic cardiovascular complications.

PARP as a Drug Target for the Therapy of Diabetic Cardiovascular Dysfunction

by Csaba Szabó

Poly(ADP-ribose) polymerase-1 (PARP-1, EC 2.4.2.30), also known as poly(ADP-ribose) synthetase and poly(ADP-ribose) transferase, is a monomeric nuclear enzyme present in eukaryotes. PARP-1, one of the most abundant proteins in the nucleus, is a 116 kDa protein consisting of three main domains: the N-terminal DNA-binding domain containing two zinc fingers; the auto-modification domain; and the C-terminal catalytic domain. The primary structure of the enzyme is highly conserved in eukaryotes (human and mouse enzyme have 92% homology at the level of amino acid sequence) with the catalytic domain showing the highest degree of homology between different species; the catalytic domain contains the so-called PARP signature sequence, a 50-amino acid block showing 100% homology between vertebrates.

The physiological functions of PARP are multiple and are the subject

Summary

Poly(ADP-ribose) polymerase-1 (PARP-1) is a member of the PARP enzyme family consisting of PARP-1 and a growing family of additional, novel poly(ADP-ribosylating) enzymes. PARP-1 is one of the most abundant nuclear proteins, and it functions as a DNA nick sensor enzyme. Upon binding to DNA breaks, activated PARP cleaves NAD⁺ into nicotinamide and ADP-ribose and polymerizes the latter onto nuclear acceptor proteins including histones, transcription factors and PARP itself. Overactivation of PARP in response to oxidant- and free radical-mediated excessive DNA single strand breaks promotes cell dysfunction and necrotic-type cell death in a variety of pathophysiological conditions. Emerging data indicate that high circulating glucose in diabetes mellitus is able to induce free radical and oxidant generation in the cardiovascular system with the concomitant activation of PARP. This process results in acute loss of the ability of the endothelium to release nitric oxide (endothelial dysfunction) and leads to a severe functional impairment of the heart (diabetic cardiomyopathy). Accordingly, pharmacological inhibition of PARP protects against diabetic cardiovascular dysfunction. Surprisingly, PARP inhibition not only prevents the development of diabetic endothelial dysfunction, but also restores normal vascular function in established diabetes. In addition to the direct cytotoxic pathway regulated by DNA injury and PARP activation, PARP also modulates the course of cardiovascular inflammation and injury by regulating the activation of NF- κ B, and the expression of a number of proinflammatory genes. The research into the role of PARP in diabetic cardiovascular injury is now supported by novel tools, such as new classes of potent inhibitors of PARP, as well as genetically engineered animals lacking the gene for PARP. Inhibitors of PARP may become useful in the experimental therapy of diabetic vascular complications. © 2002 Prous Science. All rights reserved.

of several recent reviews and monographs.¹⁻⁵ PARP-1 functions as a DNA damage sensor and signaling molecule binding to both single- and double-stranded DNA breaks. Upon binding to damaged DNA, mainly through the

second zinc finger domain, PARP-1 forms homodimers and catalyzes the cleavage of NAD⁺ into nicotinamide and ADP-ribose. It then uses the nicotinamide to synthesize branched nucleic acid-like polymers poly(ADP-ribose)

covalently attached to nuclear acceptor proteins. The size of the branched polymer varies from a few to 200 ADP-ribose units. Because of its high negative charge, covalently attached ADP-ribose polymer dramatically affects the function of target proteins. *In vivo*, the most abundant poly(ADP-ribosylated) protein is PARP-1, and auto-poly(ADP-ribosylation) represents a major regulatory mechanism for PARP-1 resulting in the down-regulation of the enzyme activity. In addition to PARP-1, histones are also considered major acceptors of poly(ADP-ribose). Poly(ADP-ribosylation) confers negative charge to histones leading to electrostatic repulsion between DNA and histones. This process has been implicated in chromatin remodeling, DNA repair and transcriptional regulation. Several transcription factors, DNA replication factors and signaling molecules (NF- κ B, AP-2, Oct-1, YY1, TEF-1, DNA-PK, p53) have also been shown to become poly(ADP-ribosylated) by PARP-1. The effect of PARP-1 on the function of these proteins is carried out by noncovalent protein-protein interactions and by covalent poly(ADP-ribosylation). Poly(ADP-ribosylation) is a dynamic process, as indicated by the short half-life of the polymer. Two enzymes—poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribosyl protein lyase—are involved in the catabolism of poly(ADP-ribose). PARG cleaves the ribose-ribose bonds of both linear and branched portions of poly(ADP-ribose) and the lyase removes the protein proximal ADP-ribose monomer.⁶

The biological role of poly(ADP-ribose) is complex and involves the following functions:

- PARP-1 plays a role in DNA repair and maintenance of genomic integrity. This protective function is signified by delayed DNA base excision repair and a high frequency of sister chromatid exchange in PARP-1-deficient cells exposed to ionizing radiation or treated with alkylating agents.^{7,8}

- PARP-1 also regulates the expression of various proteins at the transcriptional level. Of special importance is the regulation by PARP-1 of the production of inflammatory mediators such as inducible nitric oxide synthase (iNOS), intercellular adhesion molecule-1 (ICAM-1) and major histocompatibility complex class II (MHC class II). NF- κ B is a key transcription factor in the regulation of this set of proteins, and PARP has been shown to act as a co-activator in NF- κ B-mediated transcription. Poly(ADP-ribosylation) can loosen up chromatin structure and thereby make genes more accessible for the transcriptional machinery.⁹⁻¹⁴
- PARP-1 activation has been proposed to represent a cell elimination pathway whereby severely damaged cells are removed from tissues. PARP-1-mediated cell death occurs in the form of necrosis, which is probably the least desirable form of cell death. During necrotic cell death, the cellular content is released into the tissue, posing neighboring cells to harmful attacks by proteases and various proinflammatory intracellular factors (see below in detail).
- Recently, poly(ADP-ribose) polymer can serve as an emergency source of energy used by the base excision machinery to synthesize ATP.¹⁵
- Poly(ADP-ribose) may also serve as a signal for protein degradation in oxidatively injured cells.¹⁶

In response to the observation that PARP-1-deficient cells have some residual PARP activity, intensive research began to identify the enzymes responsible for this activity. In the last two years several other enzymes possessing poly(ADP-ribosylation) activity have been described and named PARPs 2-6, and the founding member of the PARP enzyme family is now designated as PARP-1. Many differences between the various PARP isoenzymes in domain structure, subcellular localization, tissue distribution and ability to bind to DNA have already been established. The novel PARP isoforms have been subject to a recent review.¹⁷ For the purpose of the current

article, it is important to point out that PARP-1 is considered the major isoform of PARP in intact cells and remains commonly termed as "PARP." This first isoform of PARP appears to play the most crucial roles in the pathophysiological aspects of PARP, including the promotion of cell necrosis and the amplification of proinflammatory signaling.

PARP as a mediator of oxidative cell dysfunction and cell necrosis

More than a decade after the identification of PARP-like enzymatic activities in mammalian cells, Dr. Nathan Berger and colleagues proposed a novel role for this enzyme, namely mediation of a suicidal mechanism triggered by DNA strand breakage.^{18,19} As summarized in 1995 by Berger, "...when DNA strand breaks are extensive or when breaks fail to be repaired, the stimulus for activation of poly(ADP-ribose) persists and the activated enzyme is capable of totally consuming cellular pools of NAD. Depletion of NAD and consequent lowering of cellular ATP pools, due to activation of poly(ADP-ribose) polymerase, may account for rapid cell death before DNA repair takes place and before the genetic effects of DNA damage become manifest."²⁰ This hypothesis has since become a controversial centerpiece of the PARP field. The validity of the PARP suicide theory has been confirmed and extended into many experimental systems and areas of biomedical research. For instance, in the mid 1980s, Cochrane and his group carried out a detailed characterization of the PARP suicide pathway in white blood cells stimulated with hydrogen peroxide.^{21,22} In the early 1990s, several groups provided evidence that nitric oxide, a labile free radical with multiple roles in physiology and pathophysiology, can activate the PARP pathway leading to cell death.^{23,24} Peroxynitrite, a reactive oxidant species, produced from the reaction of nitric oxide and superoxide free radicals, was subsequently established as a key endogenous trigger of DNA single strand breakage and PARP acti-

vation.^{25,26} Additional endogenous triggers of DNA single strand breakage and PARP activation include hydrogen peroxide, hydroxyl radical and nitroxyl anion, but not nitric oxide, superoxide or hypochlorous acid.²¹⁻²⁷ Recent investigations have clarified the mode of oxidant-induced cell death affected by PARP. Exposure to massive amounts of DNA-damaging mediators leads to a significant degree of DNA damage, with a consequent massive synthesis of poly(ADP-ribose) polymer and loss of cellular NAD⁺ and ATP. It is now clear that the PARP suicide pathway, the process of "DNA single strand breakage >> PARP activation >> energy depletion," triggers necrotic cell death characterized by rapid cell injury, changes in membrane integrity, a progressive development of mitochondrial dysfunction, and release of intracellular content evidenced by increases in the plasma levels of various necrotic markers, such as lactate dehydrogenase.²⁵⁻³¹ Prior to completing the process of full-fledged necrosis, cells are likely to reside in a distressed, dysfunctional state, which may be termed "pre-necrosis." Pharmacological inhibition or genetic inactivation of PARP restores the function of pre-necrotic cells and thereby can prevent the occurrence of cell necrosis. Examples of the restorative effect of PARP inhibition were found in *ex vivo* experiments in endothelial cells producing high levels of endogenous oxidants during diabetes³² and in intestinal epithelial cells from colitic guts.³³ The recognition that cell necrosis can be influenced by pharmacological means can be considered a key revelation, because prior to these observations necrotic cell death was not generally considered to be amenable to pharmacological or therapeutic interventions. Because cell necrosis is a key constituent of the organ injury in stroke, myocardial infarction, as well as a variety of other forms of reperfusion, shock and inflammatory and neurodegenerative conditions, it is not surprising that PARP inhibition or deficiency exerts marked protective effects in experimental models of many of these conditions.¹⁻⁵

It is noteworthy that while in conjunction with cell death the main function of PARP is now considered to be the promotion of cell necrosis, much of the published literature is concerned with PARP and apoptosis. In fact, over the last decade PARP has become widely known among cell biologists as the "death substrate." Historically PARP was one of the first identified substrates of caspases, the main executioners of apoptosis.^{34,35} Therefore, a role for PARP-1 in the regulation of apoptosis has been put forward, and cleaved PARP fragments are widely being used to demonstrate apoptotic processes. Although cleavage of PARP undoubtedly can occur during apoptosis, recent work demonstrated that the cleavage of PARP is neither a necessary nor a sufficient step for the actual process of apoptosis.^{30,36,37} The more important role of PARP lays in promoting cellular energetic dysfunction in response to oxidative injury, which ultimately culminates in necrotic type cell death (see above). In this respect, the cleavage of PARP during apoptosis is now considered a protective mechanism that serves to prevent the activation of PARP, thereby attempting to preserve cellular energy for certain ATP-sensitive steps of apoptosis.^{30,37} In fact, expression of a caspase-uncleavable, modified version of PARP leads to NAD⁺ depletion and cell necrosis, and inhibition of PARP activity in this experimental system inhibits NAD⁺ depletion and protects against cell death.³⁷

PARP as a regulator of gene expression and mononuclear cell recruitment

As briefly mentioned earlier, PARP plays an important role in the regulation of gene expression and cell differentiation. Under basal conditions, PARP is closely associated with DNA, with preference to regions of cruciform DNA and bent DNA, and in A-T rich regions. PARP also appears to be more frequently associated with transcriptionally active regions of chromatin. Basal PARP activity thereby regulates

histone shuttling and nucleosomal unfolding. Using pharmacological inhibitors of PARP, it has been demonstrated that the activity of PARP is required for the expression of the major histocompatibility complex class II gene, DNA methyltransferase gene, protein kinase C, collagenase, ICAM-1 and inducible nitric oxide synthase.³⁸⁻⁴¹ An oligonucleotide microarray analysis identified multiple genes that appear to be under the control of PARP-1.⁴² A distinct mode of inhibition of the expression of pro-inflammatory mediators by inhibition of PARP relates to the regulation of NF- κ B activation. It is unclear whether PARP catalytic activity versus PARP as a structural protein plays the most important role in its stimulatory role on NF- κ B activation;⁴³⁻⁴⁵ it may well be that both mechanisms can be involved under certain experimental conditions. Pharmacological evidence supports the view that PARP also regulates the c-fos-P-1 transcription system^{46,47} as well as the activation of MAP kinase.³⁹ From the above experimental data it appears that PARP, via a not yet fully characterized mechanism, regulates the expression of a variety of genes, with the net result that PARP inhibition or PARP genetic inactivation results in the down-regulation of a variety of important proinflammatory mediators and pathways.

The PARP-mediated pathway of cell necrosis and the PARP-mediated pathway of inflammatory signal transduction and gene expression may be interrelated in pathophysiological conditions. Oxidant stress can generate DNA single-strand breaks. DNA strand breaks then activate PARP, which in turn potentiates NF- κ B activation and AP-1 expression, resulting in greater expression of the AP-1- and NF- κ B-dependent genes, such as the gene for ICAM-1, as well as chemokines such as MIP-1 α and cytokines such as TNF- α . Chemokine generation, in combination with increased endothelial expression of ICAM-1, recruits more activated leukocytes to inflammatory foci, producing greater oxidant

stress. It is possible that, on a small scale, PARP-mediated necrosis and PARP-mediated proinflammatory gene expression are beneficial or protective processes. For example, in an inflammatory focus, NAD⁺ depletion and cell necrosis may help eliminate “innocent bystander” parenchymal cells with severely damaged DNA. It is also possible that a low-level, localized inflammatory response may be beneficial in recruiting mononuclear cells to an inflammatory site. However, in many pathophysiological states, the above-described feedback cycles amplify themselves beyond control.

Role of PARP in the pathogenesis of diabetic endothelial dysfunction

In established diabetics, the quality of life and life expectations are principally determined by the complications of diabetes rather than the primary disease. Among these complications, vasculopathies affecting both the microcirculation and macrocirculation (evidenced clinically by accelerated atherosclerosis, dysfunction of the eyes and kidneys, diminished blood flow to extremities and an increased risk of developing a variety of cardiovascular diseases) are probably the most dominant factors. The major cause of mortality in diabetes is macrovascular disease affecting the cardiac and cerebrovascular circulation.^{48–50} The processes involved in atherothrombotic disease are complex and include variation in lipid metabolism, vascular responses, cell/cell interactions and the fluid and cellular phases of coagulation and fibrinolysis. The interactions between all of these processes are crucially altered by the metabolic milieu that characterizes diabetes mellitus, tipping the delicate balance toward atheroma formation, platelet aggregation and thrombus formation. In contrast to macrovascular alterations, diabetes-associated microvascular disease has been strongly related to glycemic control. Hyperglycemic episodes occur even in the most balanced forms of diabetes mellitus and are closely associated with the

development of vascular failure. A significant portion of diabetic patients will eventually develop some degree of vascular failure.^{49–52}

The vascular tone is regulated by various neurohumoral mediators and mechanical forces acting upon the innermost layer of blood vessels, the endothelium. The main pathway of vasoregulation involves the activation of the constitutive, endothelial isoform of nitric oxide synthase (eNOS) resulting in nitric oxide production.⁵³ Endothelium-dependent vasodilatation is frequently used as a reproducible and accessible parameter to probe endothelial function in various pathophysiological conditions. It is well established that endothelial dysfunction, in many diseases, precedes and predicts as well as predisposes for the subsequent, more severe vascular alterations. Endothelial dysfunction has been documented in individuals with various forms of diabetes and even in prediabetic individuals.^{54,55} The pathogenesis of this endothelial dysfunction has many components including increased polyol pathway flux, altered cellular redox state, increased formation of diacylglycerol and the subsequent activation of specific protein kinase C isoforms, and accelerated nonenzymatic formation of advanced glycation end products.^{56,57} Many of these pathways, in concert, trigger the production of oxygen- and nitrogen-derived oxidants and free radicals, which play a significant role in the pathogenesis of the diabetes-associated endothelial dysfunction.^{56–58} The cellular sources of reactive oxygen are multiple and include advanced glycation end products NADH/NADPH oxidases, as well as the mitochondrial electron transport chain. We have recently found that high glucose-induced oxidative stress leads to DNA single strand breakage and PARP activation in murine and human endothelial cells.⁵⁹ Both the involvement of oxyradicals and nitric oxide-derived reactive species in PARP activation and the evidence for nitrated tyrosine residues suggest that peroxynitrite may be one

of the final mediators responsible for single strand breakage and subsequent PARP activation.⁵⁹ Pharmacological inhibition of PARP or genetic inactivation of PARP-1 protects against the development of the high glucose-induced endothelial dysfunction *in vitro*.⁵⁹

In order to elucidate the cellular mechanisms of this PARP-dependent, high glucose-induced cell dysfunction, we have decided to measure cellular pyridine nucleotide concentrations in the endothelial cells exposed to high glucose. There was a severe suppression of cellular high-energy phosphate levels, as well as a suppression of NAD⁺ and NADPH levels, in endothelial cells exposed to high glucose for 1–2 days. These effects were prevented by PARP inhibition or by PARP^{-/-} phenotype.⁵⁹ Since eNOS is an NADPH-dependent enzyme, we proposed that the cellular depletion of NADPH in endothelial cells exposed to high glucose is directly responsible for the suppression of eNOS activity and the reduction in the endothelium-dependent relaxant ability of diabetic vessels. In support of this hypothesis, we have subsequently demonstrated that there is a PARP-dependent suppression of vascular NADPH levels in diabetic blood vessels *in vivo*.³²

In a streptozotocin-model of diabetes in the mouse, the time course of endothelial dysfunction was compared with that of the activation of PARP in the blood vessels. Intravascular PARP activation was already apparent two weeks after the onset of diabetes, and, thus, it slightly preceded the occurrence of the endothelial dysfunction that developed between the second and fourth weeks of diabetes.⁵⁹ Delayed treatment with the PARP inhibitor—starting at one week after streptozotocin—ameliorated vascular poly-(ADP-ribose) accumulation and restored normal vascular function without altering systemic glucose levels, plasma glycated hemoglobin levels or pancreatic insulin content.^{32,59} Furthermore, delayed treatment of the animals

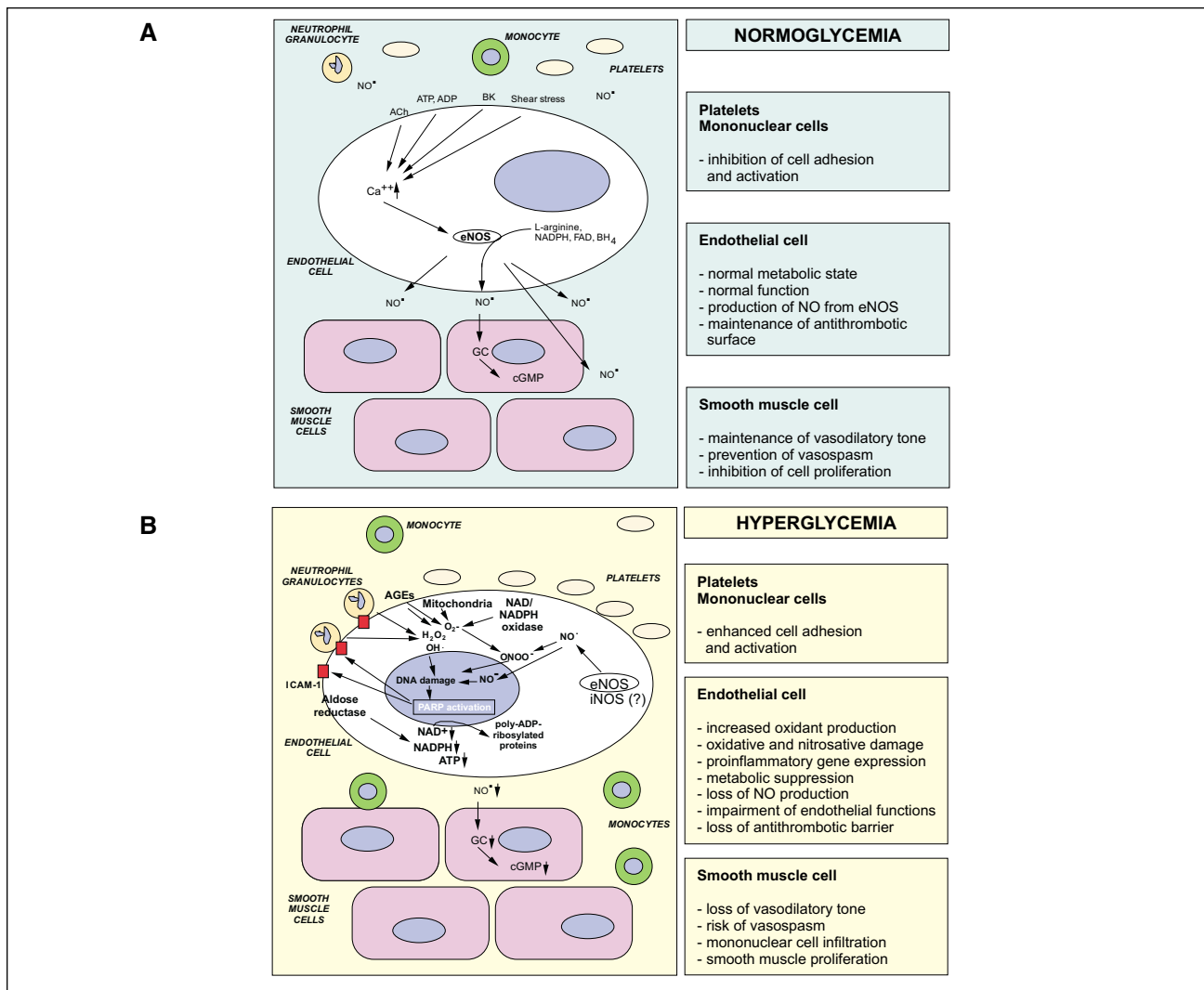


Fig. 1. A. Normal endothelial function in normoglycemia. The vascular tone is regulated by various neurohumoral mediators such as acetylcholine (ACh), ATP, ADP and bradykinin (BK) and mechanical forces (e.g., pulsatile flow and shear stress) physiologically activating the innermost layer of blood vessels, the endothelium. The main pathway of vasoregulation involves the mobilization of intracellular calcium in endothelial cells, followed by activation of eNOS resulting in nitric oxide production. The enzyme utilizes the substrates L-arginine and molecular oxygen to produce nitric oxide and is dependent on a variety of substrates including NADPH and tetrahydrobiopterin (BH₄). Nitric oxide produced by endothelial nitric oxide synthase (eNOS) then diffuses to the smooth muscles, activates soluble guanylyl cyclase (GC) and thereby triggers a cyclic GMP (cGMP) mediated relaxation of the vascular smooth muscle. The nitric oxide released from the endothelium also maintains an antiatherogenic luminal surface and inhibits the adhesion and activation of neutrophils, monocytes and platelets. **B.** Endothelial dysfunction in diabetes. PARP-dependent and PARP-independent cytotoxic pathways involving nitric oxide (NO^{*}), hydroxyl radical (OH^{*}) and peroxynitrite (ONOO⁻) in the context of diabetic endothelial dysfunction. Transiently or chronically elevated high circulating glucose in diabetes interrupts the normal homeostatic functions of the vascular endothelium. First, hyperglycemia triggers the release of oxidant mediators from the mitochondrial electron transport chain, from NADH/NADPH oxidase and other sources. High glucose may also up-regulate endothelial nitric oxide synthase (eNOS) expression or may trigger the expression of the inducible nitric oxide synthase (iNOS) in the endothelium. Nitric oxide, in turn, combines with superoxide to yield peroxynitrite. Hydroxyl radical (produced from superoxide via the iron-catalyzed Haber-Weiss reaction) and peroxynitrite or peroxynitrous acid induce the development of DNA single strand breakage, with consequent activation of PARP. Depletion of the cellular NAD⁺ leads to inhibition of cellular ATP-generating pathways leading to a global cellular dysfunction. This energetic failure may culminate in endothelial cell necrosis. The PARP-triggered depletion of cellular NADPH directly impairs the endothelium-dependent relaxations. The effects of elevated glucose are also exacerbated by increased aldose reductase activity leading to depletion of NADPH and generation of reactive oxidants. Nitric oxide alone does not induce DNA single strand breakage, but may combine with superoxide (produced from the mitochondrial chain or from other cellular sources) to yield peroxynitrite. PARP activation, via a not yet characterized fashion, promotes the activation of nuclear factor-κB, AP-1, MAP kinases and the expression of proinflammatory mediators, adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and iNOS. Poly(ADP-ribose) polymerase- (PARP) independent, parallel pathways of cellular metabolic inhibition can be activated by nitric oxide, hydroxyl radical, superoxide and peroxynitrite. By promoting neutrophil recruitment and oxidant generation, positive feedback cycles are triggered. PARP activation and mitochondrial oxidant production form another positive feedback cycle. Ultimately, the reduced nitric oxide output from the endothelial cells reduces the antithrombotic properties of the endothelial surface and trigger the adhesion and activation of platelets. The reduced endothelial nitric oxide output reduces the basal vasodilatory tone of the vascular smooth muscle, leading to transient or chronic vasospasm, end-organ ischemia and increased incidence of cardiovascular events such as coronary vasospasm, myocardial infarction or stroke. In addition, activation and intravascular migration of neutrophils and monocytes may also promote atherogenesis.

with the PARP inhibitor restored the established diabetic endothelial dysfunction; even *in vitro* incubation of diabetic dysfunctional blood vessels with PARP inhibitors of various structural classes (a benzamide, an isoquinolinone and a phenanthridinone derivative) significantly enhanced the endothelium-dependent relaxant responsiveness.³² The development of the endothelial dysfunction and its reversibility by pharmacological inhibition of PARP has also recently been demonstrated in an autoimmune model of diabetes, a nonobese diabetic mouse model.⁶⁵

Although most of the studies on the role of PARP in the pathogenesis of diabetic endothelial dysfunction, as discussed above, originated in macrovessels, there is circumstantial evidence that similar processes are operative for the pathogenesis of diabetic microvascular injury (retinopathy, nephropathy). In fact, there is now evidence for PARP activation in the microvessels of the diabetic retina.⁶⁰ In addition, a study performed more than a decade ago demonstrated that the presence of glomerular deposits (mesangial distribution) of IgG was significantly reduced in streptozotocin-diabetic rats treated with the PARP inhibitor nicotinamide for six months.⁶¹ Additional studies utilizing potent and specific inhibitors of PARP are needed to further delineate the role of PARP in the pathogenesis of diabetic retinopathy, neuropathy and nephropathy.

Role of PARP in the pathogenesis of diabetic myocardial dysfunction

The development of myocardial dysfunction independent of coronary artery disease in diabetes mellitus has been well documented, both in humans and experimental studies in animals.⁶²⁻⁶⁴ This diabetic cardiomyopathy is characterized by an early diastolic dysfunction and a late systolic dysfunction, with intracellular retention of calcium and sodium and loss of

potassium. The mechanism of diastolic dysfunction remains unknown, but it does not appear to be because of changes in blood pressure, microvascular complications or elevated circulating glycosylated hemoglobin levels. Recent data demonstrate that the PARP pathway also plays a role in the pathogenesis of diabetic cardiomyopathy.⁶⁵ Cardiac dysfunction was noted both in the streptozotocin-induced and genetic (nonobese diabetic) models of diabetes mellitus in rats and mice.⁶⁵ Treatment with the phenanthridinone-based PARP inhibitor PJ34 starting one week after the onset of diabetes, restored normal vascular responsiveness and significantly improved cardiac dysfunction, despite the persistence of severe hyperglycemia.⁶⁵ The beneficial effect of PARP inhibition persisted even after several weeks of discontinuation of the treatment.⁶⁵

It is possible that the diabetic endothelial PARP pathway and the diabetic cardiomyopathy are interrelated: The impairment of the endothelial function may lead to global or regional myocardial ischemia, which may secondarily impair cardiac performance. The beneficial effect of PARP inhibition on myocardial function, however, is not related to an anabolic effect, since PJ34 treatment did not influence the body and heart weight loss in diabetic animals, while it dramatically improved cardiac function. It is noteworthy that the protective effect of PARP inhibition against diabetic cardiac dysfunction extended several weeks beyond the discontinuation of treatment; this observation may have important implications for the design of future clinical trials with PARP inhibitors.⁶⁵ The prolonged protective effect may be related to the permanent interruption by the PARP inhibitor of positive feedback cycles of cardiac injury. Indeed, previous studies in various pathophysiological conditions have demonstrated that PARP inhibitors suppress positive feedback cycles of adhesion receptor expression and mononuclear cell infiltration, as well as cellular oxidant generation.^{41,66}

Conclusions and implications

The role of PARP activation in diabetes is not limited to the development of various forms of cardiovascular dysfunction. A vast body of evidence supports the role of PARP activation in the process of autoimmune islet cell death⁶⁷⁻⁷⁰ as well as in the process of islet regeneration.⁷¹⁻⁷³ Taken together, multiple lines of evidence support the view that PARP activation plays a crucial role in multiple interrelated aspects of diabetes and its complications. We expect that potent, bioavailable and nontoxic PARP inhibitors will exert beneficial effects against the development of both the primary diabetes as well as its cardiovascular complications.

PARP plays an important role in a variety of physiological processes and in DNA repair. Nevertheless, PARP-1-deficient mice are viable and disease-free, and PARP-deficient mice have not been shown to be prone to spontaneous malignancies—although they do develop an increased incidence of chemically-induced tumors.⁷⁴ Interestingly, and somewhat in contrast with the latter report, a recent study shows that PARP deficiency reduces the incidence of malignancies in the p53-deficient mice.⁷⁵ Since PARP has catalytic as well as scaffolding functions in the nucleus, in many cases PARP deficiency and pharmacological PARP inhibition yields discordant results.^{76,77} Thus, only direct investigations (such as chronic treatment of animals with potent PARP inhibitors) will be able to directly address the various potential side effects of PARP inhibitors for chronic indications such as diabetes mellitus and its cardiovascular complications. It is possible that a partial degree of pharmacological inhibition of PARP *in vivo* will be able to reduce the development of oxidant-induced cell dysfunction, while at the same time maintain the protective function of PARP it elicits on the level of genetic integrity. As with all novel therapeutic agents, the risk of potential side effects of PARP inhibition will

have to be carefully balanced with the degree of expected therapeutic benefit.

References

1. Szabó, C. and Dawson, V.L. *Role of poly(ADP-ribose) synthetase activation in inflammation and reperfusion injury*. Trends Pharmacol Sci 1998, 19: 287–98.
2. De Murcia, G., Schreiber, V., Molinete, M. et al. *Structure and function of poly(ADP-ribose) polymerase*. Mol Cell Biochem 1994, 138: 15–24.
3. Le Rhun, Y., Kirkland, J.B. and Shah, G.M. *Cellular responses to DNA damage in the absence of poly(ADP-ribose) polymerase*. Biochem Biophys Res Commun 1998, 245: 1–10.
4. C. Szabó (Ed.) *Cell Death: The Role of PARP*. CRC Press, Boca Raton, FL, USA, 2000.
5. G. De Murcia and S. Shall (Eds.) *From DNA Damage and Stress Signaling to Cell Death; Poly ADP-ribosylation Reactions*. Oxford University Press, Oxford, 2000.
6. Davidovic, L., Vodenicharov, M., Affar, E.B. and Poirier, G.G. *Importance of poly(ADP-ribose) glycohydrolase in the control of poly(ADP-ribose) metabolism*. Exp Cell Res 2001, 268: 7–13.
7. Rudat, V., Kupper, J.H. and Weber, K.J. *Trans-dominant inhibition of poly(ADP-ribose) polymerase leads to decreased recovery from ionizing radiation-induced cell killing*. Int J Radiat Biol 1998, 73: 325–30.
8. Menissier-de Murcia, J., Niedergang, C., Trucco, C. et al. *Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells*. Proc Natl Acad Sci USA 1997, 94: 7303.
9. Satoh, M.S. and Lindahl, T. *Role of poly(ADP-ribose) formation in DNA repair*. Nature 1992, 356: 356–8.
10. Oikawa, A., Tohda, H., Kanai, M., Miwa, M. and Sugimura, T. *Inhibitors of poly(adenosine diphosphate ribose) polymerase induce sister chromatid exchanges*. Biochem Biophys Res Commun 1980, 97: 1311–6.
11. Park, S.D., Kim, C.G. and Kim, M.G. *Inhibitors of poly(ADP-ribose) polymerase enhance DNA strand breaks, excision repair, and sister chromatid exchanges induced by alkylating agents*. Environ Mutagen 1983, 5: 515–25.
12. Herceg, Z. and Wang, Z.Q. *Functions of poly(ADP-ribose) polymerase (PARP) in DNA repair, genomic integrity and cell death*. Mutat Res 2001, 477: 97–110.
13. Poirier, G.G., de Murcia, G., Jongstra-Bilen, J., Niedergang, C. and Mandel, P. *Poly(ADP-ribose) polymerase inhibition causes relaxation of chromatin structure*. Proc Natl Acad Sci USA 1982, 79: 3423–7.
14. Lautier, D., Lageux, J., Thibodeau, J., Ménard, L. and Poirier, G.G. *Molecular and biochemical features of poly(ADP-ribose) metabolism*. Mol Cell Biochem 1993, 122: 171–93.
15. Oei, S.L. and Ziegler, M. *ATP for the DNA ligation step in base excision repair is generated from poly(ADP-ribose)*. J Biol Chem 2000, 275: 23234–9.
16. Ullrich, O., Ciftci, O. and Hass, R. *Proteasome activation by poly-ADP-ribose-polymerase in human myelomonocytic cells after oxidative stress*. Free Radic Biol Med 2000, 29: 995–1004.
17. Smith, S. *The world according to PARP*. Trends Biochem Sci 2001, 26: 174–9.
18. Berger, N.A., Catino, D.M. and Vietti, T.J. *Synergistic antileukemic effect of 6-aminonicotinamide and 1,3-bis(2-chloroethyl)-1-nitrosourea on L1210 cells in vitro and in vivo*. Cancer Res 1982, 42: 4382–6.
19. Sims, J.L., Berger, S.J. and Berger, N.A. *Poly(ADP-ribose) polymerase inhibitors preserve nicotinamide adenine dinucleotide and adenosine 5'-triphosphate pools in DNA-damaged cells: Mechanism of stimulation of unscheduled DNA synthesis*. Biochemistry 1983, 22: 5188–94.
20. Berger, N.A. *Poly(ADP-ribose) in the cellular response to DNA damage*. Radiat Res 1985, 101: 4–15.
21. Schraufstatter, I.U., Hinshaw, D.B., Hyslop, P.A., Spragg, R.G. and Cochrane, C.G. *Oxidant injury of cells. DNA strand-breaks activate polyadenosine diphosphate-ribose polymerase and lead to depletion of nicotinamide adenine dinucleotide*. J Clin Invest 1986, 77: 1312–20.
22. Schraufstatter, I.U., Hyslop, P.A., Hinshaw, D.B., Spragg, R.G., Sklar, L.A. and Cochrane, C.G. *Hydrogen peroxide-induced injury of cells and its prevention by inhibitors of poly(ADP-ribose) polymerase*. Proc Natl Acad Sci USA 1986, 83: 4908–12.
23. Zhang, J., Dawson, V.L., Dawson, T.M. and Snyder, S.H. *Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity*. Science 1994, 263: 687–9.
24. Radons, J., Heller, B., Burkle, A. et al. *Nitric oxide toxicity in islet cells involves poly(ADP-ribose) polymerase activation and concomitant NAD depletion*. Biochem Biophys Res Commun 1994, 199: 1270–7.
25. Szabó, C., Zingarelli, B., O'Connor, M. and Salzman, A.L. *DNA strand breakage, activation of poly-ADP-ribose synthetase, and cellular energy depletion are involved in the cytotoxicity in macrophages and smooth muscle cells exposed to peroxynitrite*. Proc Natl Acad Sci USA 1996, 93: 1753–8.
26. Szabó, C., Virág, L., Cuzzocrea, S. et al. *Protection against peroxynitrite-induced fibroblast injury and arthritis development by inhibition of poly(ADP-ribose) synthetase*. Proc Natl Acad Sci USA 1998, 95: 3867–72.
27. Bai, P., Bakondi, E., Szabo, E.E., Gergely, P., Szabo, C. and Virág, L. *Partial protection by poly(ADP-ribose) polymerase inhibitors from nitroxyl-induced cytotoxicity in thymocytes*. Free Radic Biol Med 2001, 31: 1616–23.
28. Heller, B., Wang, Z.Q., Wagner, E.F. et al. *Inactivation of the poly(ADP-ribose) polymerase gene affects oxygen radical and nitric oxide toxicity in islet cells*. J Biol Chem 1985, 270: 11176–80.
29. Watson, A.J., Askew, J.N. and Benson, R.S. *Poly(adenosine diphosphate ribose) polymerase inhibition prevents necrosis induced by H₂O₂ but not apoptosis*. Gastroenterology 1995, 109: 472–82.
30. Virág, L., Scott, G.S., Salzman, A.L. and Szabó, C. *Peroxynitrite-induced thymocyte apoptosis: The role of caspases and poly(ADP-ribose) synthetase (PARS) activation*. Immunology 1998, 94: 345–55.
31. Ha, H.C. and Snyder, S.H. *Poly(ADP-ribose) polymerase is a mediator of necrotic cell death by ATP depletion*. Proc Natl Acad Sci USA 1999, 96: 13978–82.
32. Soriano, F.G., Mabley, J.G., Pacher, P., Liaudet, L. and Szabó, C. *Rapid reversal of the diabetic endothelial dysfunction by pharmacological inhibition of poly(ADP-ribose) polymerase*. Circ Res 2001, 89: 684–91.
33. Jijon, H.B., Churchill, T., Malfair, D. et al. *Inhibition of poly(ADP-ribose) polymerase attenuates inflammation in a model of chronic colitis*. Am J Physiol 2000, 279: G641–51.
34. Casiano, C.A., Ochs, R.L. and Tan, E.M. *Distinct cleavage products of nuclear proteins in apoptosis and necrosis revealed by autoantibody probes*. Cell Death Differ 1998, 5: 183–90.
35. Smulson, M.E., Simbulan-Rosenthal, C.M., Boulares, A.H. et al. *Roles of poly(ADP-ribose) polymerase and PARP in apoptosis, DNA repair, genomic stability and functions of p53 and E2F-1*. Adv Enzyme Regul 2000, 40: 183–215.
36. Wang, Z.Q., Stingl, L., Morrison, C. et al. *PARP is important for genomic stability but dispensable in apoptosis*. Genes Dev 1997, 11: 2347–58.
37. Herceg, Z. and Wang, Z.Q. *Failure of poly(ADP-ribose) polymerase cleavage by caspases leads to induction of necrosis and enhanced apoptosis*. Mol Cell Biol 1999, 19: 5124–33.
38. Hiromatsu, Y., Sato, M., Yamada, K. et al. *Nicotinamide and 3-aminobenzamide inhibit recombinant human interferon-gamma-induced HLA-DR antigen expression, but not HLA-A, B, C antigen expression, on cultured human thyroid cells*. Clin Endocrinol 1992, 36: 91.
39. Szabó, C., Wong, H., Bauer, P.I. et al. *Regulation of components of the inflammatory response by 5-iodo-6-amino-1,2-benzopyrone, an inhibitor of poly(ADP-ribose) synthetase and pleiotropic modifier of cellular signal pathways*. Int J Oncol 1997, 10: 1093–104.

40. Ehrlich, W., Huser, H., and Kroger, H. *Inhibition of the induction of collagenase by interleukin-1 beta in cultured rabbit synovial fibroblasts after treatment with the poly(ADP-ribose)-polymerase inhibitor 3-aminobenzamide*. *Rheumatol Int* 1995, 15: 171.
41. Zingarelli, B., Salzman, A.L. and Szabó, C. *Genetic disruption of poly(ADP-ribose) synthetase inhibits the expression of P-selectin and intercellular adhesion molecule-1 in myocardial ischemia-reperfusion injury*. *Circ Res* 1998, 83: 85–94.
42. Simbulan-Rosenthal, C.M., Ly, D.H., Rosenthal, D.S. et al. *Misregulation of gene expression in primary fibroblasts lacking poly(ADP-ribose) polymerase*. *Proc Natl Acad Sci USA* 2000, 97: 11274–9.
43. Le Page, C., Sanceau, J., Drapier, J.C. and Wietzerbin, J. *Inhibitors of ADP-ribosylation impair inducible nitric oxide synthase gene transcription through inhibition of NF kappa B activation*. *Biochem Biophys Res Comm* 1998, 243: 451–7.
44. Hassa, P.O. and Hottiger, M.O. *A role of poly(ADP-ribose) polymerase in NF-kappaB transcriptional activation*. *Biol Chem* 1999, 380: 953.
45. Oliver, F.J., Menissier-de Murcia, J., Nacci, C. et al. *Resistance to endotoxic shock as a consequence of defective NF-kappaB activation in poly(ADP-ribose) polymerase-1 deficient mice*. *EMBO J* 1999, 18: 4446–54.
46. Amstad, P.A., Krupitza, G. and Cerutti, P.A. *Mechanism of c-fos induction by active oxygen*. *Cancer Res* 1992, 52: 3952–60.
47. Roebuck, K.A., Rahman, A., Lakshminarayanan, V., Janakidevi, K. and Malik, A.B. *H₂O₂ and tumor necrosis factor-alpha activate intercellular adhesion molecule 1 (ICAM-1) gene transcription through distinct cis-regulatory elements within the ICAM-1 promoter*. *J Biol Chem* 1995, 270: 18966–74.
48. Tooke, J.E. and Goh, K.L. *Vascular function in type 2 diabetes mellitus and pre-diabetes: The case for intrinsic endothelial pathology*. *Diabet Med* 1999, 16: 710–5.
49. King, G.L. *The role of hyperglycaemia and hyperinsulinaemia in causing vascular dysfunction in diabetes*. *Ann Med* 1996, 28: 427–32.
50. Giugliano, D., Ceriello, A. and Paolisso, G. *Oxidative stress and diabetic vascular complications*. *Diabetes Care* 1996, 19: 257–67.
51. Taylor A.A. *Pathophysiology of hypertension and endothelial dysfunction in patients with diabetes mellitus*. *Endocrinol Metab Clin North Am* 2001, 30: 983–97.
52. Schaper, N.C., Nabuurs-Franssen, M.H. and Huijberts, M.S. *Peripheral vascular disease and type 2 diabetes mellitus*. *Diabetes Metab Res Rev* 2000, 1: S11–5.
53. Furchgott, R.F. *Endothelium-derived relaxing factor: Discovery, early studies, and identification as nitric oxide*. *Biosci Rep* 1999, 19: 235–51.
54. Cai, H. and Harrison, D.G. *Endothelial dysfunction in cardiovascular diseases: The role of oxidant stress*. *Circ Res* 2000, 87: 840–4.
55. Caballero, A.E., Arora, S., Saouaf, R. et al. *Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes*. *Diabetes* 1999, 48: 1856–62.
56. Guzik, T.J., West, N.E., Black, E. et al. *Vascular superoxide production by NAD(P)H oxidase: Association with endothelial dysfunction and clinical risk factors*. *Circ Res* 2000, 86: E85–90.
57. De Vriese, A.S., Verbeuren, T.J., Van de Voorde, J., Lameire, N.H. and Vanhoutte, P.M. *Endothelial dysfunction in diabetes*. *Br J Pharmacol* 2000, 130: 963–74.
58. Nishikawa, T., Edelstein, D., Du, X.L. et al. *Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage*. *Nature* 2000, 404: 787–90.
59. Soriano, F.G., Virág, L., Jagtap, P. et al. *Diabetic endothelial dysfunction: The role of poly(ADP-ribose) polymerase activation*. *Nature Med* 2001, 7: 108–13.
60. Szabó, E., Kern, T.S., Virag, L., Mabley, J. and Szabó, C. *Evidence for poly(ADP-ribose) polymerase activation in the diabetic retina*. *FASEB J* 2001, 15: A942.
61. Wahlberg, G., Carlson, L.A., Wasserman, J. and Ljungqvist, A. *Protective effect of nicotinamide against nephropathy in diabetic rats*. *Diabetes Res* 1985, 2: 307–11.
62. Standl, E. and Schnell, O. *A new look at the heart in diabetes mellitus: From ailing to failing*. *Diabetologia* 2000, 43: 1455–69.
63. Fein, F.S. *Diabetic cardiomyopathy*. *Diabetes Care* 1990, 13: 1169–79.
64. Bell, D.S. *Diabetic cardiomyopathy. A unique entity or a complication of coronary artery disease?* *Diabetes Care* 1995, 18: 708–14.
65. Pacher, P., Liaudet, L., Soriano, F.G., Mabley, J.G., Szabó, E. and Szabó, C. *The role of poly(ADP-ribose) polymerase in the development of myocardial and endothelial dysfunction in diabetes mellitus*. *Diabetes* 2002, in press.
66. Szabo, C., Lim, L.H., Cuzzocrea, S. et al. *Inhibition of poly(ADP-ribose) synthetase attenuates neutrophil recruitment and exerts antiinflammatory effects*. *J Exp Med* 1997, 186: 1041–9.
67. Burkart, V., Wang, Z.Q., Radons, J. et al. *Mice lacking the poly(ADP-ribose) polymerase gene are resistant to pancreatic beta-cell destruction and diabetes development induced by streptozocin*. *Nature Med* 1999, 5: 314–9.
68. Masutani, M., Suzuki, H., Kamada, N. et al. *Poly(ADP-ribose) polymerase gene disruption conferred mice resistant to streptozocin-induced diabetes*. *Proc Natl Acad Sci USA* 1999, 96: 2301–4.
69. Pieper, A.A., Brat, D.J., Krug, D.K. et al. *Poly(ADP-ribose) polymerase-deficient mice are protected from streptozotocin-induced diabetes*. *Proc Natl Acad Sci USA* 1999, 96: 3059–64.
70. Mabley, J.G., Suarez-Pinzon, W.L., Hasko, G. et al. *Inhibition of poly(ADP-ribose) synthetase by gene disruption or inhibition with 5-iodo-6-amino-1,2-benzopyrone protects mice from multiple-low-dose-streptozotocin-induced diabetes*. *Br J Pharmacol* 2001, 133: 909–19.
71. Yonemura, Y., Takashima, T., Miwa, K., Miyazaki, I., Yamamoto, H. and Okamoto, H. *Amelioration of diabetes mellitus in partially depancreatized rats by poly(ADP-ribose) synthetase inhibitors. Evidence of islet B-cell regeneration*. *Diabetes* 1984, 33: 401.
72. Sugiyama, K., Yonemura, Y. and Okamoto, H. *Effects of poly(ADP-ribose) synthetase inhibitor on B-cells of a canine pancreas after massive pancreatectomy*. *Int J Pancreatol* 1991, 8: 85.
73. Akiyama, T., Takasawa, S., Nata, K. et al. *Activation of Reg gene, a gene for insulin-producing beta-cell regeneration: Poly(ADP-ribose) polymerase binds Reg promoter and regulates the transcription by autopoly(ADP-ribosylation)*. *Proc Natl Acad Sci USA* 2001, 98: 48–52.
74. Tsutsumi, M., Masutani, M., Nozaki, T. et al. *Increased susceptibility of poly(ADP-ribose) polymerase-1 knockout mice to nitrosamine carcinogenicity*. *Carcinogenesis* 2001, 22: 1–3.
75. Conde, C., Mark, M., Oliver, F.J., Huber, A., de Murcia, G. and Menissier-de Murcia, J. *Loss of poly(ADP-ribose) polymerase-1 causes increased tumour latency in p53-deficient mice*. *EMBO J* 2001, 20: 3535–43.
76. Simbulan-Rosenthal, C.M., Ly, D.H., Rosenthal, D.S. et al. *Misregulation of gene expression in primary fibroblasts lacking poly(ADP-ribose) polymerase*. *Proc Natl Acad Sci USA* 2000, 97: 11274–9.
77. Simbulan-Rosenthal, C.M., Rosenthal, D.S., Luo, R., Li, J.H., Zhang, J. and Smulson, M.E. *Inhibition of poly(ADP-ribose) polymerase activity is insufficient to induce tetraploidy*. *Nucleic Acids Res* 2001, 29: 841–9.

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