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Poly(ADP-ribose) polymerase-2: emerging transcriptional roles of a DNA repair protein --Manuscript Draft--

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Abstract:	Poly(ADP-ribose) polymerase (PARP)-2 is a nuclear enzyme that belongs to the PARP family and PARP-2 is responsible for 5-15% of total cellular PARP activity. PARP-2 was originally described in connection to DNA repair and in physiological and pathophysiological processes associated with genome maintenance (e.g. centromere and telomere protection, spermiogenesis, thymopoiesis, azoospermia and tumorigenesis). Recent reports identified important rearrangements in gene expression upon the knockout of PARP-2. Such rearrangements heavily impact on inflammation and metabolism. Metabolic effects are mediated through modifying PPAR γ and SIRT1 function. Altered gene expression gives rise to a complex phenotype characterized primarily by enhanced mitochondrial activity that results both in beneficial (loss of fat, enhanced insulin sensitivity) and in disadvantageous (pancreatic beta cell hypofunction upon high fat feeding) consequences. Enhanced mitochondrial biogenesis provides protection in oxidative stress related diseases. Hereby, we review the recent developments in PARP-2 research with special attention to the involvement of PARP-2 in transcriptional and metabolic regulation.
Response to Reviewers:	Response to Reviewers MS # CMLS-D-12-00064 Poly(ADP-ribose) polymerase-2: emerging transcriptional roles of a DNA repair protein Reviewer #1: We would like to thank the referee for his/her efforts to further improve our manuscript. 1. Page 2 - the modifications here are fairly minimalistic and the abbreviations ARTD2 and ARTD1 are not even defined.

and ARTD1 are not even defined.

In the corresponding section we incorporated the basic information on the newly proposed nomenclature in our previous version upon the suggestion of the Reviewer. We agree with the Reviewer that the appearance of the new nomenclature in our manuscript is important. Moreover, in the current version we added ARTD-2 as a keyword to help those future readers that follow the new nomenclature. The fact that ARTD1 is the equivalent of PARP-1 is defined in the chapter "PARP superfamily" second paragraph, first row. We added the abbreviation of ARTD-2 in the last sentence in the first paragraph of the chapter "The PARP superfamily". We think that these are sufficient for the understanding of the position of PARP-2 in the PARP/ARTD superfamily.

We would like to note here, that the proposed new nomenclature did not gain ground in the scientific community yet. There are only 3 relevant papers on Medline for the keyword ARTD1, ARTD2 or ARTD (since 2010, the publication of the paper by Hottiger et al. describing the new nomenclature). According to Medline there are already around 290 papers published only in 2012 that contain the keyword PARP. These data (290 vs. 3) clearly show that the PARP nomenclature is used mostly in the literature. Moreover, even the authors of the new proposal themselves still use the old nomenclature "PARP" (e.g. Erener S ... Hottiger MO: Inflammasome-Activated Caspase 7 Cleaves PARP1 to Enhance the Expression of a Subset of NF- κ B Target Genes. *Mol Cell*. 2012 Mar 28. [Epub ahead of print]), or Erener S ... Hottiger MO Poly(ADP-ribose)polymerase-1 (PARP1) controls adipogenic gene expression and adipocyte function. *Mol Endocrinol*. 2012 Jan;26(1):79-86.).

In summary, we are confident that our review sufficiently takes the new nomenclature into consideration despite of the fact that it is not yet accepted by the scientific community.

2. Page 3 line 12 - what is meant by "bear" mutations ? Do you mean that there is no sequence homology?

Sequence homology modeling was carried out by Ame and co-workers (Ame et al. *Bioessays*. 26:882-893. 2004). They classified sequences as PARPs on the basis of sequence homology, therefore sequence homology does exist. However, mutation(s) in a sequence does not necessarily mean that sequence homology is lost. In the incriminated text, mutations mean rather point mutations that affect usually catalytic amino acids. We had no intention to indicate the loss of sequence homology. To avoid such misunderstanding, we exchanged "bear" to "carry".

3. Page 3 line 34 - can more details of the alternative splicing be given and a reference?

We drew our conclusions from the sequences available sequences at NCBI. Now we stated the source of these data in the manuscript.

4. Page 6 line 27 - maybe "suggest" would be more appropriate than "indicate" . Is there any evidence that PARP-2 has a similar role in humans? If not then this sentence needs qualification to this effect (eg The results from these experimental models would suggest that ??).

In agreement with the Reviewer we changed "indicate" to "suggest". Indeed, there is no human data available in that regard. To fulfill the demand of the Reviewer we included the above proposed text.

5. Page 7 line 8 - what SNPs impact on PARP-2 function and how?

We thank for the Reviewer for this suggestion. We would like to point out that although the SNPs were described but their actual mode of action is unknown. Nonetheless, we included some possible explanations.

Sakugawa and colleagues have shown on a cohort of 18 Japanese men that such PARP-2 SNPs coincide with azoospermia in humans. Sakugawa and colleagues identified five SNPs. Three of them fall into the coding region, while two into the 3' UTR. The SNPs in the coding region are all in the catalytic domain. One is synonymous (1159C/T), while two others (1359G/A and 1469A/C) lead to an amino acid change: Arg/Gln and Asn/His, respectively. It is tempting to speculate that changes in the catalytic domain may affect the catalytic activity of PARP-2. SNPs in the 3'UTR (1789A/C and 1790 T/C) may interfere with mRNA stability leading to the reduction of PARP-2 mRNA copy number and consequently decreasing PARP-2 protein levels. Lower PARP-2 levels, or lower PARP-2 activity may interfere with spermiogenesis as described in the respective chapter. We have incorporated these data into the manuscript.

6. Page 7 line 51 in the revised sentence, should it read "A defect in PARP-2 in mouse models?".

We narrowed the focus of our affirmation by adding "in murine models".

7. Page 8 line 9 - please give model.

We specified the model in the given sentence.

8. Page 12 line 56 in the revised sentences: What evidence is there that PARP-1 and PARP-2 ribosylate different substrates?

At the indicated site there is no suggestion that PARP-1 and PARP-2 would PARylate different substrates.

9. Page 13 line 22 in the revised sentences: Would not the selective targeting of PARP-2 mediated repair run the risk of increasing the complications in normal tissue where radiation exposure doses might be lower?

The Reviewer is absolutely right, such phenomenon may take place. Based on the comment of the Reviewer we feel that we drew too distant conclusions, therefore we removed these sentences.

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6 **Poly(ADP-ribose) polymerase-2: emerging transcriptional roles of a**
7 **DNA repair protein**
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35 Running title: PARP-2 as a regulator of transcription
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42 Keywords: PARP-2, **ARTD2**, SIRT1, DNA repair, differentiation, metabolism, mitochondria
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Abstract

Poly(ADP-ribose) polymerase (PARP)-2 is a nuclear enzyme that belongs to the PARP family and PARP-2 is responsible for 5-15% of total cellular PARP activity. PARP-2 was originally described in connection to DNA repair and in physiological and pathophysiological processes associated with genome maintenance (e.g. centromere and telomere protection, spermiogenesis, thymopoiesis, azoospermia and tumorigenesis). Recent reports identified important rearrangements in gene expression upon the knockout of PARP-2. Such rearrangements heavily impact on inflammation and metabolism. Metabolic effects are mediated through modifying PPAR γ and SIRT1 function. Altered gene expression gives rise to a complex phenotype characterized primarily by enhanced mitochondrial activity that results both in beneficial (loss of fat, enhanced insulin sensitivity) and in disadvantageous (pancreatic beta cell hypofunction upon high fat feeding) consequences. Enhanced mitochondrial biogenesis provides protection in oxidative stress related diseases. Hereby, we review the recent developments in PARP-2 research with special attention to the involvement of PARP-2 in transcriptional and metabolic regulation.

The PARP superfamily

Poly(ADP-ribosyl)ation is a transient post-translational modification of proteins mediated by poly(ADP-ribose) polymerase (PARP) enzymes. This is a dynamic process during which the enzymes catalyze the formation of ADP-ribose polymers onto different acceptor proteins using NAD⁺ as a substrate. The half-life of the polymer is very short since it is quickly degraded by poly(ADP-ribose) glycohydrolase (PARG). PARPs constitute a family of 17 members, encoded by 17 different genes sharing a conserved sequence coding for the catalytic domain that contains the PARP signature motif, a highly conserved sequence that forms the active site [1]. Based on sequence and structural homologies and the similarity of the reactions catalyzed, Hottiger and colleagues recently proposed to unite all ADP-ribose transferases (PARPs and mono-ADP-ribosyl transferases) in one protein family [2]. The same study proposed a new nomenclature for these enzymes, where PARP-2 was renamed ARTD2 (ADP-ribosyltransferase Diphtheria toxin-like 2).

The prototypical enzyme of the PARP family is PARP-1 (ARTD1). PARP-1 cleaves NAD⁺ to ADP-ribose and nicotinamide followed by the attachment of the first ADP-ribose moiety to a glutamate or aspartate residue of target proteins. During the elongation of the polymer further ADP-ribose moieties are attached to these protein-bound monomers. In the absence of DNA damage the constitutive polymer levels are usually very low and appear as mono- or oligo(ADP-ribose). However, in response to DNA strand breaks the levels of poly(ADP-ribose) (PAR) polymers increase 10-500-fold and large [3,4], branched PAR polymers occur

1 on different acceptor proteins and PARP-1 itself (auto-PARylation) (Fig. 1.). Upon extensive
2 PARP-1 activation cellular NAD⁺ levels are markedly reduced [5]. Poly(ADP-ribose)ation, at
3 any level, is likely to have important effects on the acceptor's properties, hence PARylation
4 and PARPs are involved in the regulation of various cellular processes [6]. In cells polymers
5 can be detected as early as 2 to 3 minutes after the one-off induction of DNA damage, and
6 then PAR polymers are quickly degraded by PARG. In tissues, PAR levels can be detected
7 on a longer timeline as reflecting a steady state between synthesis and degradation. Some
8 PARP enzymes **carry** mutations in the catalytic domain and hence are either inactive or
9 perform only mono-, or oligo(ADP-ribose)ation [1,2,7].
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16 **The structure of the *PARP-2* gene and PARP-2 protein**

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18 PARP-2 was discovered when residual DNA-dependent PARP activity was detected in
19 *PARP-1*^{-/-} murine embryonic fibroblasts (MEFs) [8]. So far PARP-1, PARP-2 and PARP-3 are
20 the only PARP enzymes whose catalytic activity is stimulated by DNA strand breaks
21 suggesting that they function as crucial members in the cellular pathways responding to DNA
22 damage [8-11].
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27 *PARP-2* gene is located on chromosome 14 in humans. The gene is driven by a
28 bidirectional promoter that *PARP-2* shares with *RNase P* [12]. Such combination of RNA
29 polymerase II and RNA polymerase III genes is relatively rare. A functional TATA box and
30 DSE/Oct-1 expression control elements were identified in the promoter regulating *PARP-2*
31 expression [12]. **Due to alternative splicing, two isoforms of *PARP-2* exist with the longer
32 isoform containing an extra set of 13 amino acids on the border between the DNA binding
33 domain and domain E. The longer isoform has been identified, or predicted in humans [13],
34 common chimpanzee (*Pan troglodytes*) [14], northern white-cheeked gibbon (*Nomascus
35 leucogenys*) [15] and sumatran orangutan (*Pongo abelii*) [16] according to the NCBI
36 database.** The sequences of different mammalian *PARP-2* genes are highly homologous
37 (Fig. 2.). Although *PARP-2* is absent in birds, sequences similar to PARP-2 can be found in
38 lower vertebrates (*Danio rerio*, *Xenopus*), lower animals (e.g. sponges) and in *Arabidopsis
39 thaliana* [17].
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49 The tissue-specific expression of *PARP-2* was primarily characterized by *in situ*
50 hybridization. Liver expression of *PARP-2* was high at fetal age 12.5 days, decreased at 18.5
51 days fetal age and was even lower in newborn mice [8,18]. In adult mice the expression of
52 PARP-2 is low in the liver (the lowest among the metabolic tissues; Bai P, unpublished data).
53 It is tempting to speculate that the gradual decrease in *PARP-2* expression by age during
54 fetal and postnatal development points toward the possible involvement of PARP-2 in early
55 stage hemopoiesis that takes place in the liver.
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In the central nervous system PARP-2 content was high in the spinal ganglia and in certain parts of the brain. In the neocortical areas *PARP-2* expression is elevated as compared to lower brain regions. High *PARP-2* expression was detected in stratum granulosum of the dentate gyrus and the stratum pyramidale of the hippocampus and was even higher in the cortex and the olfactory bulb [18]. Apart from the previously mentioned tissues, *PARP-2* is highly expressed in the cortical region of the kidneys, the spleen, adrenal glands, stomach, thymus and intestinal epithelium [18]. The testis was also positive for *PARP-2* expression.

In humans a slightly different expression pattern was detected. *PARP-2* was very abundant in the skeletal muscle, brain, heart, testis, it was high in pancreas, kidney, placenta, ovary, spleen and low *PARP-2* expression was detected in the lungs, leukocytes, gastrointestinal tract (both colon and small intestine), thymus and liver [19].

Translation of the *PARP-2* mRNA yields a protein product of 62 kDa apparent molecular weight. PARP-2 protein can be divided into similar functional regions as PARP-1: The N-terminus of mouse PARP-2 contains the DNA binding domain (DBD), followed by domain E and the catalytic domain (domain F) [8]. The DBD is formed by a SAP domain that is responsible for DNA binding [20], and contains a functional nuclear localization signal (NLS) [21] and a nucleolar localization signal (NoLS) [22]. A caspase-3 cleavage site defines the border between the DBD and domain E, which is homologous to the caspase-3 site in the E domain of PARP-1 [23]. Domain E serves as a homodimerization interface, an automodification domain and a protein-protein interaction domain as well [24]. Auto-poly(ADP-ribosylation) of PARP-2 takes place on domain E [25] and on lysine 36 and 37 that are targets of simultaneous acetylation [26,27]. The PARP-2 interactome was mapped by Isabelle and co-workers [28], who identified a large number of proteins. These proteins covered a wide array of functions such as cell cycle, cell death, DNA repair, DNA replication, transcription, metabolism, energy homeostasis and RNA metabolism.

Domain F on the C-terminus of PARP-2 harbours the PARP signature motif carrying the essential amino acid residues for catalysis [8]. Domain F is separated from domain E by a caspase-8 cleavage site [29]. PARP-2 and PARP-1 share a catalytic domain of 69% similarity, with the exception that PARP-2 contains an additional three amino acid insertion in the loop connecting the β -strands *k* and *l* in PARP-1 [8,30,31] (Fig. 3.). The three dimensional structure of the catalytic domain also shows high similarity, however the catalytic domain of PARP-2 has a narrower catalytic cleft that likely explains the lower substrate affinity and turnover rate of PARP-2 as compared to PARP-1 (K_M for NAD^+ 50/130 μM ; k_{cat}/K_M 6000 $s^{-1} M^{-1}$ /323 $s^{-1} M^{-1}$ for PARP-1/-2 respectively) [8,30]. PARP-2 accounts for 5-15% of total PARP activity in cells depending on the model used [8,32,33]. PARP-2 performs auto [18] and hetero-PARylation of proteins. Troiani and co-workers have identified possible

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targets of PARP-2 activity that covered proteins involved in transcription, translation and mitochondrial organization [34].

PARP-2 in the maintenance of genomic integrity

PARP-2 in DNA repair and genomic integrity

PARP-1 is a well established DNA-repair protein [6], therefore the functional similarity with PARP-2 suggested a role for PARP-2 in the maintenance of DNA integrity. Upon the induction of DNA damage (ionizing irradiation, or laser irradiation), PARP-2 accumulates at the damage foci [35] with a slower kinetics than PARP-1, and PARP-2 persisted longer at DNA damage sites [36]. In murine embryonic fibroblasts (MEFs) the loss of PARP-2 leads to hypersensitivity to ionizing irradiation and cell cycle arrest in G1 [23], although PARP-2^{-/-} cells are less sensitive to ionizing radiation than PARP-1^{-/-} cells [23,24]. In line with these observations female lethality due to X chromosome instability was observed in *PARP-1*^{+/-} *PARP-2*^{-/-} mice [23].

PARP-2 preferentially binds to one nucleotide gaps [25] and it is involved in single strand repair processes. As shown in murine models, upon the loss of PARP-2, base excision repair (BER) slows down [18]. Moreover, PARP-2 interacts with numerous members of the BER machinery such as XRCC1, PARP-1, DNA pol β and DNA ligase III [18] that further signifies its importance in BER. It is tempting to hypothesize that the early embryonic lethality of the *PARP-1/PARP-2* double knockout mice [23] might be due to the strong impairment of DNA repair processes.

PARP-1 has been described to participate in double strand break repair [37]. Nicolás and co-workers have identified the accumulation of double strand breaks in *PARP-2*^{-/-} murine thymocytes [38]. This observation is in line with previous report by Yelamos and colleagues [24] who suggested that PARP-2 interacts with the Ku proteins, mediators of double strand break repair. Moreover, Robert and colleagues have identified PARP-2 as a suppressor of recombination during immunoglobulin class switch events in murine and human B cells [39], while Bryant et al. have suggested that both PARP-1 and -2 are essential in resolving blocked replication forks by homologous recombination in CHO and murine embryonic fibroblasts (MEFs) [37,40]. The fact that the ATM/PARP-2 double knockout genotype is embryonic lethal [20] further supports the involvement of PARP-2 in double strand break repair during replication. Moreover, the fact that mitomycin C treatment that leads to DNA double strand breakage provoked the induction of PARP-2 expression and other double strand break repair proteins in human cervical carcinoma cells also underlines the involvement of PARP-2 in double strand break repair [41].

1 Appropriate telomere and centromere maintenance requires PARP-2. PARP-2 binds to
2 and negatively regulates the DNA binding of telomere-binding protein, TRF-2 in different
3 rodent and human cell models. The loss of PARP-2 expression increased the frequency of
4 spontaneous chromosome and chromatid breaks and the number of DNA ends lacking
5 detectable telomere repeats [42].
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8 PARP-2 localizes to centromeres in human and murine cells in a cell-cycle dependent
9 manner and interacts with the kinetochore proteins centromere protein A (CENPA),
10 centromere protein B (CENPB) and mitotic spindle checkpoint protein BUB3 in
11 prometaphase and metaphase [43]. Interestingly, this centromeric accumulation of PARP-2
12 is increased when microtubule dynamics are disrupted suggesting a dominant role of PARP-
13 2 in accurate chromosome segregation [44].
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18 Incomplete or insufficient DNA repair may ultimately lead to either cell death, or cellular
19 transformation and tumorigenesis. PARP-1 has been associated with both cell death [45] and
20 tumorigenesis [46]. PARP-2 seems to be involved in cell death regulation similarly to PARP-1
21 [44,47]. However, Cohausz and colleagues have found differences in the expression of cell
22 death genes upon N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) treatment in PARP-1 and -2
23 knockdown cells. Furthermore, PARP-2 is also engaged in tumorigenesis. In mice the double
24 deletion of PARP-2 and p53 induced spontaneous lymphomas and certain sarcomas [38]
25 and decreased expression of PARP-2 correlated with increased susceptibility to alkylator-
26 induced acute myeloid leukemia (AML) [48]. **Results from these experimental models**
27 **suggest** that PARP-2 has a dominant role in suppressing leukemias [49].
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37 **Role of PARP-2 in chromatin remodeling and genome maintenance during** 38 **spermiogenesis** 39

40 *PARP-2* is expressed in the testis of mice [18] and rats [50], and is highly expressed in
41 human testis [19]. Moreover, PARP-2 is found in the ejaculated spermatozoa in both mice
42 and in humans [44,51]. However, PARP-2 seems to be responsible for a smaller portion of
43 PARP activity than PARP-1 in rat testis [52]. These observations prompted the study of the
44 possible testicular functions of PARP-2 in mice. Dantzer and co-workers have revealed that
45 upon crossing of PARP-2^{-/-} males and females litter size was lower than in colonies bred by
46 crossing wild type mice. A smaller testis size and high number of abnormal spermatids in the
47 distal epidymis have also been reported [44].
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54 Decreased spermatogenesis is likely to have multiple roots that all trace back to
55 insufficient maintenance of genomic integrity during spermatocyte differentiation. The
56 differentiation of spermatozoa was found to be hampered and large numbers of apoptotic
57 cells were detected in murine testis [44]. Cell death is probably linked to hampered meiotic
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1 sex chromosome inactivation, and the block of cell division in meiosis I, whereby
2 chromosome missegregation was detected [44]. Jha and colleagues, based on studies on 18
3 healthy and 12 infertile humans, also proposed a role for PARP-2 in the preservation of
4 genomic integrity by protecting DNA against oxidative stress [53]. Spermiogenesis involves
5 the compaction of DNA and the exchange of histones to different protamines [54]. In this
6 process PARP-2 (and PARP-1) regulate the activity of topoisomerase II β that is essential for
7 appropriate DNA organization (e.g. removal of histone 1) [55], transition protein 2 (TP2) and
8 the transition chaperone HSPA2 [56] as shown in mice.

13 Different SNPs impacting on PARP-2 functionality may also hamper human
14 spermiogenesis. Sakugawa and colleagues [51] have shown on a cohort of 18 Japanese
15 men that such *PARP-2* SNPs coincide with azoospermia in humans. Sakugawa and
16 colleagues identified five SNPs. Three of them fall into the coding region, while two into the 3'
17 UTR. The SNPs in the coding region are all in the catalytic domain. One is synonymous
18 (1159C/T), while two others (1359G/A and 1469A/C) lead to an amino acid change: Arg/Gln
19 and Asn/His, respectively. It is tempting to speculate that changes in the catalytic domain
20 may affect the catalytic activity of PARP-2. SNPs in the 3'UTR (1789A/C and 1790 T/C) may
21 interfere with mRNA stability leading to the reduction of PARP-2 mRNA copy number and
22 consequently decreasing PARP-2 protein levels. Lower PARP-2 levels, or lower PARP-2
23 activity may interfere with spermiogenesis as described above.

34 35 **The role of PARP-2 in thymopoiesis and inflammatory regulation**

36 The earliest reports on PARP-2 described high *PARP-2* expression in the subcapsular
37 zone of the thymus where lymphocyte proliferation is the most intense. *PARP-2* expression
38 gradually decreases towards the center of the thymus as lymphocytes differentiate and
39 mature [18,47]. *PARP-2* transcripts were detected in the white pulp of the spleen and Peyer
40 patches in mice which also points toward the involvement of PARP-2 in the proliferation of
41 lymphocytes [18].

42 In line with these observations, the deletion of *PARP-2* in mice led to decreases in the
43 weight of thymus and in the total cell numbers and number of CD4⁺, CD8⁺ thymocytes in
44 thymus [47]. The loss of the double positive thymocytes was due to enhanced p53-mediated
45 apoptosis [47]. Increased expression of a pro-apoptotic, bcl-2 homolog NOXA showed
46 correlation with the enhanced apoptosis [47]. Apoptosis can be reversed by the removal of
47 p53 [38] suggesting that cell death is induced by unresolved DNA damage. In line with this
48 observation, when *PARP-2*^{-/-} mice were bred on a *p53*^{-/-} background, spontaneous
49 lymphomas and to a smaller extent other sarcomas developed in the double knockout mice
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[38] indicating a functional interplay between these two proteins in protecting genome integrity.

It remains to be seen whether atrophy of thymus and the higher rate of thymocyte apoptosis in *PARP-2*^{-/-} mice results in a restricted T cell repertoire and altered T cell responses. In fact, PARP inhibition or *PARP-1* depletion has provided marked protection in most animal models of inflammation with many of them being dependent on T cell functions [45,57]. **The defect of PARP-2 seems to be associated with a narrower spectrum of diseases in murine models.** The lack of PARP-2 impairs astrocyte activation [58] and provides protection against colitis [59], while it has no effect in models of contact hypersensitivity [60], irritative dermatitis [60] or pancreatitis [61]. Interestingly, a common set of genes (iNOS, IL-1 β , TNF α) has been shown to be regulated by both PARP-1 and PARP-2 suggesting similar or overlapping mechanisms in inflammatory regulation by the two PARP isoforms. However, yet the exact mechanism of protection by genetic PARP-2 deletion is unknown [58,59].

PARP-2 in the regulation of gene expression

Recent reports revealed that the depletion of PARP-2 modifies the activity of multiple transcription factors [62-64]. **In HepG2 cells depleted of PARP-2 by shRNA, we have found the dysregulation of more than 600 genes in microarray experiments (Szántó and Bai, unpublished data) indicating an important role for PARP-2 in the regulation of gene expression.**

PARP-2 acts at multiple levels on gene transcription. PARP-2 might be capable of modifying chromatin through regulating transcriptional intermediary factor (TIF)-1 β and heterochromatin protein (HP)-1 α [65]: depletion of PARP-2 modified the expression of two genes (*Mest* and *HNF4*) that are dependent on the TIF1 β -HP1 α complex [65]. Poly(ADP-ribosylation) and PARP-1 have eminent roles in epigenetic control [66-69]. Based on the similarities of PARP- and PARP-2-catalyzed reactions, and partially overlapping interactome and acceptor protein profile, it is tempting to assume that similar epigenetic roles may also be assigned to PARP-2.

PARP-2 can influence gene expression through more direct interactions. PARP-2 interacts with topoisomerase I and topoisomerase II β [40,55] and may thus regulate the rearrangement of DNA structure in conjunction with RNA transcription. Moreover, PARP-2 has been shown to interact with nucleophosmin/B23 [22] that is involved in rRNA transcription [70]. RNA polymerase I inhibition removes PARP-2 from the nucleolus, however the deletion of PARP-2 does not change rRNA expression. Thus the exact mechanism whereby PARP-2 regulates rRNA expression requires further investigation. On the course of mRNA expression, also known as RNA polymerase II-mediated transcription, PARP-2 can

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act as either a positive co-factor, or a repressor of gene expression. Transcription factors regulated by PARP-2 are summarized in Table 1.

Nuclear receptor signaling

PARP-2 has been shown to interact with several members of the nuclear receptor superfamily such as the peroxisome proliferator activated receptors (PPARs) and estrogen receptor (ER) α .

The group of PPARs has three members, PPAR α , PPAR δ and PPAR γ [71] that heterodimerize with the retinoid X receptor (RXR) and thus bind to DNA [72,73]. PPARs bind different lipophylic ligands [74] that regulate their transcriptional activity. PPARs control the expression of a large set of genes involved in the regulation of energy, lipid and glucose homeostasis [75]. The binding of ligands to the receptors leads to receptor activation and the release of corepressor proteins and the subsequent binding of activators [76]. PARP-1 has been suggested to be involved in nuclear receptor function. Ju and colleagues [77] have shown that upon estrogen receptor activation, topoisomerase II β creates DNA strand breaks that are resolved through the action of PARP-1. Moreover, inhibition of topoisomerase II β or PARP-1 hampered efficient gene expression [77].

PARP-2 serves as a cofactor for the members of the PPAR transcription factor family. The absence of PARP-2 impairs PPAR γ activation but enhances PPAR α and PPAR δ activation [63]. PARP-2 binds to PPAR γ -driven promoters and its absence decreases the expression of genes such as *adipocytes protein 2 (aP2)*, *CD36*, *lipoprotein lipase (LPL)* and *fatty acid synthase (FAS)* [63]. Since PARP-2 is a DNA repair protein and can interact with topoisomerase II β [55], it is possible that PARP-2 may also play a role in resealing transcription-related DNA breaks. The effects of PARP-2 depletion on PPAR α and PPAR δ activation were demonstrated only in reporter assays [63], therefore further molecular and *in vivo* verification of these interactions is necessary.

Estrogen receptor (ER) α activation is repressed by the depletion of PARP-2 in luciferase reporter assays (P. Bai, unpublished data). The effect of PARP-2 on PPAR γ and ER α may share similar molecular characteristics. Further investigation is required to reveal possible physiological consequences of the reduced ER α activity upon PARP-2 ablation. It is important to note that PARP-2 does not interfere with the activation of ER β , therefore ER β and its target genes (e.g. *keratin 19*) are ideal negative controls in studies addressing the role of PARP-2 in nuclear receptor-mediated gene expression [63].

Interaction with SIRT1

1 SIRT1 belongs to the family of sirtuins. Sirtuins have seven homologs in humans and
2 mice (SIRT1-7) [78,79]. SIRT1 is considered to be a nuclear enzyme [80], although it may
3 also appear in the cytosol [81]. SIRT1 is an NAD⁺-dependent protein deacetylase [82] that
4 enables SIRT1 to sense the energetic status of cells (e.g. changes in NAD⁺/NADH ratio) [83].
5 SIRT1 is activated by increases in NAD⁺ levels, or indirectly by different small molecule
6 activators such as resveratrol [84], SIRT1720 [85], AMPK activators [86] or PARP inhibitors
7 [87]. SIRT1 activation leads to the deacetylation and activation of numerous metabolic
8 transcription factors such as PPAR gamma coactivator (PGC)-1 α [88], FOXOs [89] and p53
9 [90]. Their activation leads to increased mitochondrial biogenesis and oxidative metabolism
10 through enhancing the expression of key mitochondrial enzymes involved in terminal
11 oxidation, fatty acid degradation, and mitochondrial uncoupling in several target tissues
12 [88,91].

13 It has been shown that PARP-2 can directly regulate the expression of SIRT1 [62].
14 PARP-2 serves as a negative regulator of SIRT1 expression, as the absence of PARP-2
15 induces SIRT1 expression and results in higher SIRT1 activity [33,62]. PARP-2 binds to the
16 murine SIRT1 promoter in a region between -1 - -91, which is a highly conserved region
17 among mammals, showing homology even in *Xenopus* [62]. It must be noted that the
18 ablation, or pharmacological inhibition of PARP-1 also induces SIRT1 activity. However
19 SIRT1 activation in the absence of PARP-1 depends on enhanced NAD⁺ availability and the
20 ablation of PARP-1 does not alter the activity of the SIRT1 promoter [87].

21 SIRT1 induction upon PARP-2 ablation causes the deacetylation of PGC-1 α and FOXO1,
22 which in turn boost mitochondrial biogenesis by enhancing the expression of *PGC-1 α ,*
23 *uncoupling protein (UCP)-2,* muscle isoform of *carnitine O-palmitoyltransferase 1 (mCPT1b),*
24 *acyl coenzyme A oxidase I (ACOX1), medium-chain specific acyl-CoA dehydrogenase*
25 *(MCAD), malonyl-CoA decarboxylase (MCD), Ndufa2, cytochrome c (cyt c)* and *COX IV* [62].
26 The action of PARP-2 has been shown in multiple organs and tissues such as skeletal
27 muscle, liver, smooth muscle [33,62] and an unexpected diadvantageous effect has been
28 shown in the pancreas [62]. The depletion of PARP-2 was found not to interfere with SIRT2
29 or SIRT3 activation [62].

30 Similar to PARP-2 gene inactivation, SIRT1 activation has been shown to inhibit the
31 production of inflammatory mediators and suppress certain forms of inflammation [92-94]. It
32 is therefore plausible that the induction of SIRT1 may be responsible for the antiinflammatory
33 effect of PARP-2 depletion in colitis [59] and in astrocyte activation [58].

34 *Thyroid transcription factor-1*

35 Nkx-2 transcription factors constitute a family of homeodomain-containing transcription
36 factors. Thyroid transcription factor (TTF)-1 belongs to the Nkx-2 family and TTF-1 plays a

1 dominant role in lung morphogenesis, respiratory epithelial cell morphogenesis and
2 differentiation [95,96]. In cultured lung epithelial cells PARP-2 interacts with TTF1 [64]. By
3 affecting TTF1 activity PARP-2 may regulate the expression of *surfactant protein-B*.
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6 **The role of PARP-2 in metabolic regulation**

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8 Alterations in gene expression accompany various biological phenomena ranging from
9 inflammatory responses (NOXA, TNF α , IL-1 β , etc.) to metabolic regulation. The
10 transcriptional regulatory role of PARP-2 has been linked to cellular metabolism. *PARP-2*^{-/-}
11 mice are smaller and leaner as they have less body fat than their wild type littermates
12 [62,63]. At the same time *PARP-2*^{-/-} mice showed higher oxygen consumption rates and
13 lower respiratory quotient during the active (dark) phase which points toward higher fatty acid
14 oxidation [62].
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17 When examining the skeletal muscle of *PARP-2*^{-/-} mice, increased mitochondrial content
18 was observed in line with higher expression of genes related to oxidative metabolism and
19 fatty acid oxidation which is in line with the above described phenotype [62]. The increase in
20 oxidative metabolism can be explained by higher SIRT1 expression due the loss of the
21 transcriptional repressor activity of PARP-2. It is the increase in SIRT1 expression that
22 induces mitochondrial biogenesis through PGC-1 α and FOXO1 deacetylation [62].
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25 The liver of the *PARP-2*^{-/-} mice displayed characteristics similar to the ones in skeletal
26 muscle: SIRT1 induction and consequently enhanced mitochondrial biogenesis and oxidative
27 metabolism [62]. Interestingly, the brown adipose tissue was not involved in the development
28 of the energy expenditure phenotype in contrast to *PARP-1*^{-/-} mice [62,87]
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31 The increased energy expenditure fuelled by enhanced mitochondrial biogenesis in
32 skeletal muscle and liver had beneficial effects on the metabolism of *PARP-2*^{-/-} mice. *PARP-*
33 *2*^{-/-} mice are protected against diet-induced obesity, and insulin resistance of the animals was
34 retained even after high fat feeding [62]. Interestingly, *PARP-2*^{-/-} mice proved to be glucose
35 intolerant after high fat feeding [62]. The pancreas in *PARP-2*^{-/-} mice failed to appropriately
36 respond to diet-induced insulin resistance as it showed no signs of hyperproliferation or
37 reduction in pancreas weight, islet size and pancreatic insulin content [62]. Reduced
38 expression of pancreatic and duodenal homeobox 1 (pdx-1) is likely to be responsible for the
39 pancreatic hypofunction in *PARP-2*^{-/-} mice [62].
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42 Functions of the white adipose tissue (WAT) are orchestrated by the RXR/PPAR γ
43 receptor [72]. We have shown that PARP-2 acts a cofactor of the RXR/PPAR γ dimer [63,97].
44 The loss of PARP-2 hampers RXR/PPAR γ receptor activation and decreases the expression
45 of certain PPAR γ -driven genes (e.g. *LPL*, *CD36*, etc.). Due to that alterations in gene
46 expression, the WAT of *PARP-2*^{-/-} mice turned hypomorphic and hypofunctional [63].
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Moreover, in cellular models of adipocyte differentiation, the lack of PARP-2 resulted in decreased adipocytic differentiation [63]. It is of note that SIRT1 induction may inhibit PPAR γ [98] that may provide an auxiliary mechanism underlying WAT hypofunction in the absence of PARP-2.

PARP-2 in oxidative stress-related diseases

PARP-1 depletion, or pharmacological PARP inhibition is protective against numerous oxidative stress-related diseases [45]. Depletion of PARP-2 also resulted in a protective phenotype against diseases associated with increased oxidative stress. Genetic deletion or silencing of PARP-2 has provided protection in models of focal and global cerebral ischaemia [99,100], colitis [59] and doxorubicin-induced vascular smooth muscle damage [33]. Since PARP-2 accounts for a small fraction of total cellular PARP activity [8,32,33], it is unlikely that the ablation of PARP-2 could protect against the loss of cellular NAD⁺ and ATP suggesting different mechanisms of cell death as compared to the case of PARP-1 ablation.

Cerebral ischaemia and doxorubicin-induced vascular impairment involve mitochondrial damage [101,102] and preventing mitochondrial damage proved to be a successful novel treatment in these pathologies [103-108]. Since SIRT1 has been demonstrated to enhance or restore mitochondrial activity in various tissues [62,87,91,109-111] it is logical to assume that the interference between PARP-2 and SIRT1 expression [62] could be key for the protective phenotype. Indeed, in the case of doxorubicin-induced vascular damage, enhanced SIRT1 expression and consequent stabilization of the mitochondrial membrane potential was proposed to be responsible for the protection provided by the *PARP-2*^{-/-} phenotype [33] which might be a prototypical mechanism by which PARP-2 mediates oxidative stress-related pathologies. Moreover, ablation of PARP-2 led to the mitochondrial retention of apoptosis inducing factor (AIF) [100]. AIF is a mitochondrial protein that shuttles to the nucleus upon oxidative stress-evoked cell death in a PARP-1-dependent manner [112]. In a model of focal cerebral ischemia, ablation of PARP-2 only slightly reduced PAR formation but markedly inhibited the nuclear translocation of AIF [100]. This interesting finding may also be linked to the stabilization of mitochondrial membrane upon SIRT1 induction.

The involvement of PARP-2 in oxidative stress-related pathologies points towards the applicability and hence the development of PARP-2 specific inhibitors. However, all known PARP inhibitors are capable of inhibiting both PARP-1 and -2, which is not surprising since the catalytic domain of the enzymes are very similar and most PARP inhibitors bind there [30,31]. In the quest for synthesizing PARP-2 specific inhibitors, the laboratory of Gilbert de Murcia suggested the targeting of a loop that is unique in PARP-2, [25,30]. Efforts to develop

1 highly PARP-2-selective compounds have given rise to inhibitors that have 10-60 fold higher
2 affinity for PARP-2 as compared to PARP-1 [113-117]. One of these inhibitors, UPF-1069
3 that has 60 fold higher affinity towards PARP-2 than PARP-1, was shown to provide
4 protection against cerebral ischemia [117]. Although at the moment such selectivity is the
5 highest achievable, it is possible that in cellular models or in *in vivo* settings these inhibitors
6 may partially inhibit PARP-1 too. Nevertheless, the development of highly PARP-2 specific
7 inhibitors is of current interest. Since PARP-2 is a minor PARP isoform, its inhibition is an
8 attractive way to counteract certain drawbacks of pan-PARP inhibition or PARP-1 specific
9 inhibitors. Since PARP-2 accounts for only 5-15% of PARP activity [8,18,32,33] therefore it is
10 tempting to speculate that its loss probably would not drastically hamper PARylation-
11 dependent DNA repair. Thus highly PARP-2 specific inhibitors may provide a preferable
12 alternative for the treatment of metabolic diseases, whereas pan-PARP inhibitors may be
13 superior in severe oxidative injury. However DNA damage assessment in such cases is an
14 absolute necessity.

25 **Conclusions and perspectives**

26 PARP-2 has been shown to participate in multiple cellular processes such as DNA repair,
27 maintenance of genomic integrity, spermiogenesis and thymopoiesis. On the other hand,
28 PARP-2 is involved in transcriptional regulation of metabolism and oxidative stress response.
29 In fact, this plethora of functions partly overlaps with the functions of PARP-1 [24]. A better
30 understanding of the similarities and differences between the actions of PARP-1 and -2 is of
31 utmost importance. On the one hand PARP-1 and -2 can act synergistically, while on the
32 other hand isoform specific functions of the PARP enzymes also exist. Specific targeting of
33 PARP-2 may help overcome unwanted side effects of pan-PARP inhibition.

34 **Understanding the role of PARP-2 in DNA repair may hold importance in tumor biology.
35 The better understanding of the role of PARP-2 in DNA repair may provide new knowledge
36 on tumorigenesis, and can be capitalized in inducing synthetic lethality by joint inhibition of
37 parallel DNA repair pathways [49].**

38 The metabolic effects of PARP-2 can be exploited in multiple different manners.
39 Obviously, the depletion of PARP-2 can be utilized in combating metabolic diseases and
40 mitochondrial stabilization may overcome oxidative stress-evoked damage. Better
41 understanding the properties of PARP-2 may in turn facilitate the development of PARP-2
42 specific inhibitors that may have advantages over pan-PARP inhibitors.

56 **Conflict of interest**

57 The authors declare no conflict of interest.

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Figure legends

Figure 1. The poly(ADP-ribosyl)ation cycle.

NAM- nicotinamide, ADPR- ADP-ribose, PAR- poly(ADP-ribose), all other abbreviations are in the text.

Figure 2. The structure of *PARP-2* gene and *PARP-2* protein.

The *PARP-2* gene is driven by a bidirectional promoter and consists of 16 exons. The protein product of the gene can be divided into three domains: DBD, domain E, domain F. Numbers bellow the protein product indicate amino acids on the border between domains. The arrows point at caspases-3 and caspases-8 cleavage sites. The highlighted amino acid sequence is the conserved 13 amino acid sequence of the longer *PARP-2* isoform.

18 mammalian *PARP-2* sequences of the shorter isoform (*Ailuropoda melanoleuca* [118], *Bos taurus* [119], *Callithrix jacchus* [120], *Canis lupus familiaris* [121], *Cavia porcellus* [122], *Cricetulus griseus* [123], *Equus caballus* [124], *Homo sapiens* [13], *Loxodonta africana* [125], *Macaca mulatta* [126], *Monodelphis domestica* [127], *Mus musculus* [128], *Nomascus leucogenys* [15], *Oryctolagus cuniculus* [129], *Pan troglodytes* [14], *Pongo abelii* [16], *Rattus norvegicus* [130], *Sus scrofa* [131]) were compared in the Clustal W2 software (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) and relative conservation of the amino acids were plotted. Higher values indicate higher levels of conservation.

DBD- DNA binding domain, AM- automodification, NLS- nuclear localization signal, NoLS- nucleolar localization signal, SAP- SAP domain

Figure 3. The three dimensional structure of the catalytic domain of *PARP-1* and *PARP-2*.

Crystal structure of *PARP-1* (3GN7) and *PARP-2* (3KJD) were retrieved from the protein data bank (PDB, www.rcsb.org). Both structures contain an inhibitor (in color), the *PARP-1* catalytic domain is in complex with A861696, while the *PARP-2* catalytic domain is in complex with ABT-888 [31]. The catalytic cleft and the *PARP-2* specific loop is indicated.

Figure 4. Tissue specific functions of *PARP-2*.

Figure 1

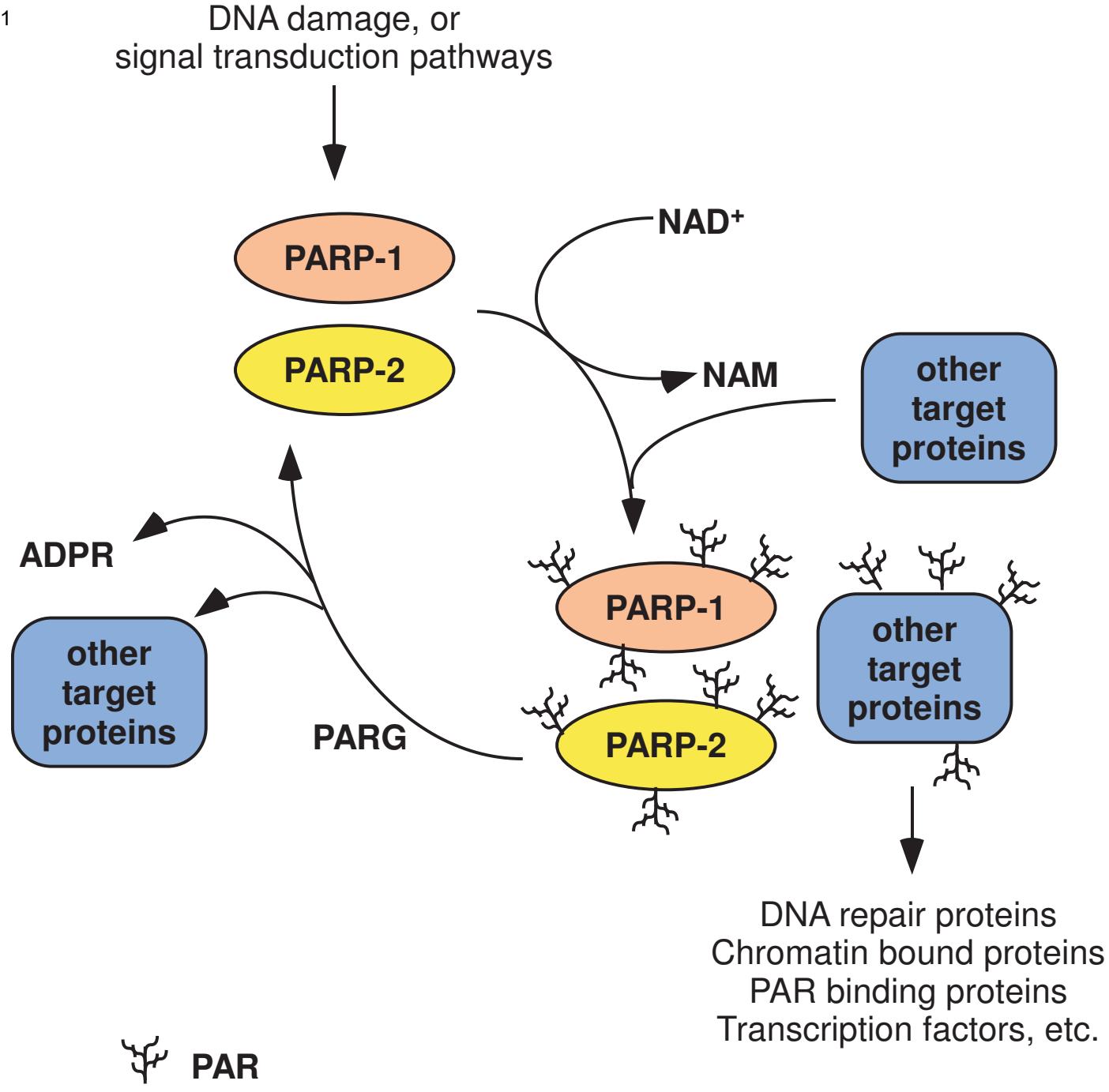


Fig.1.

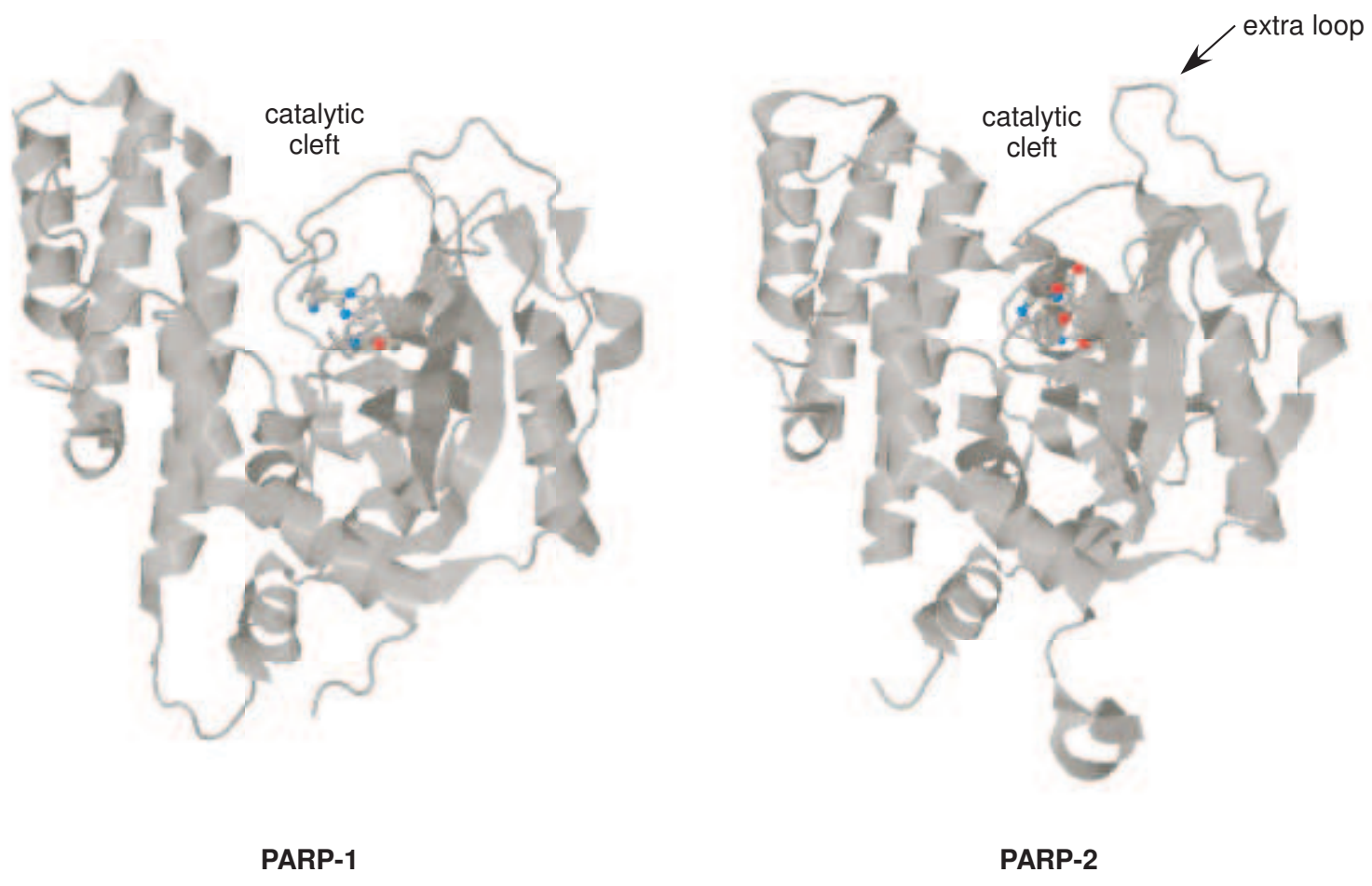


Figure2

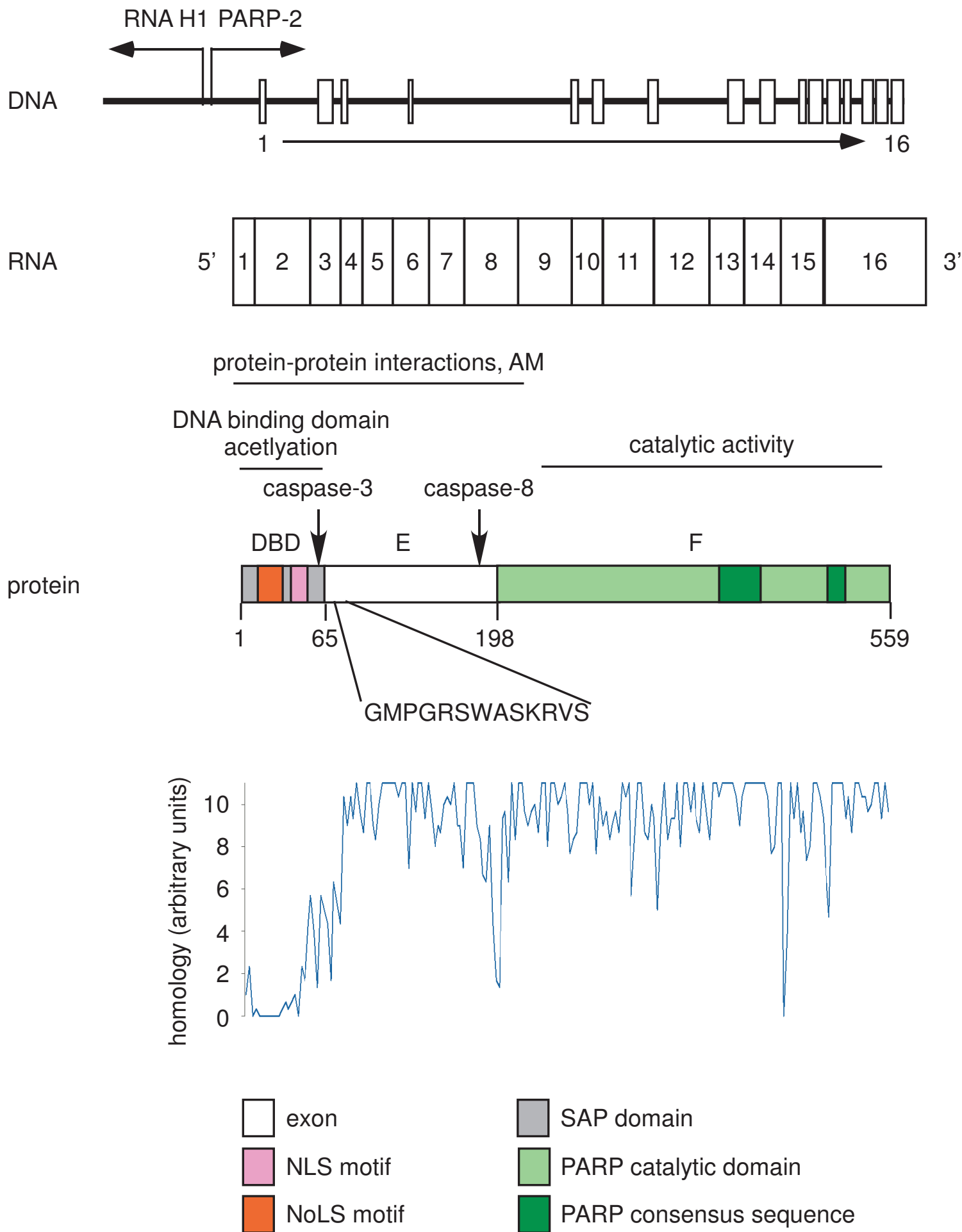


Fig.2.

Figure 4

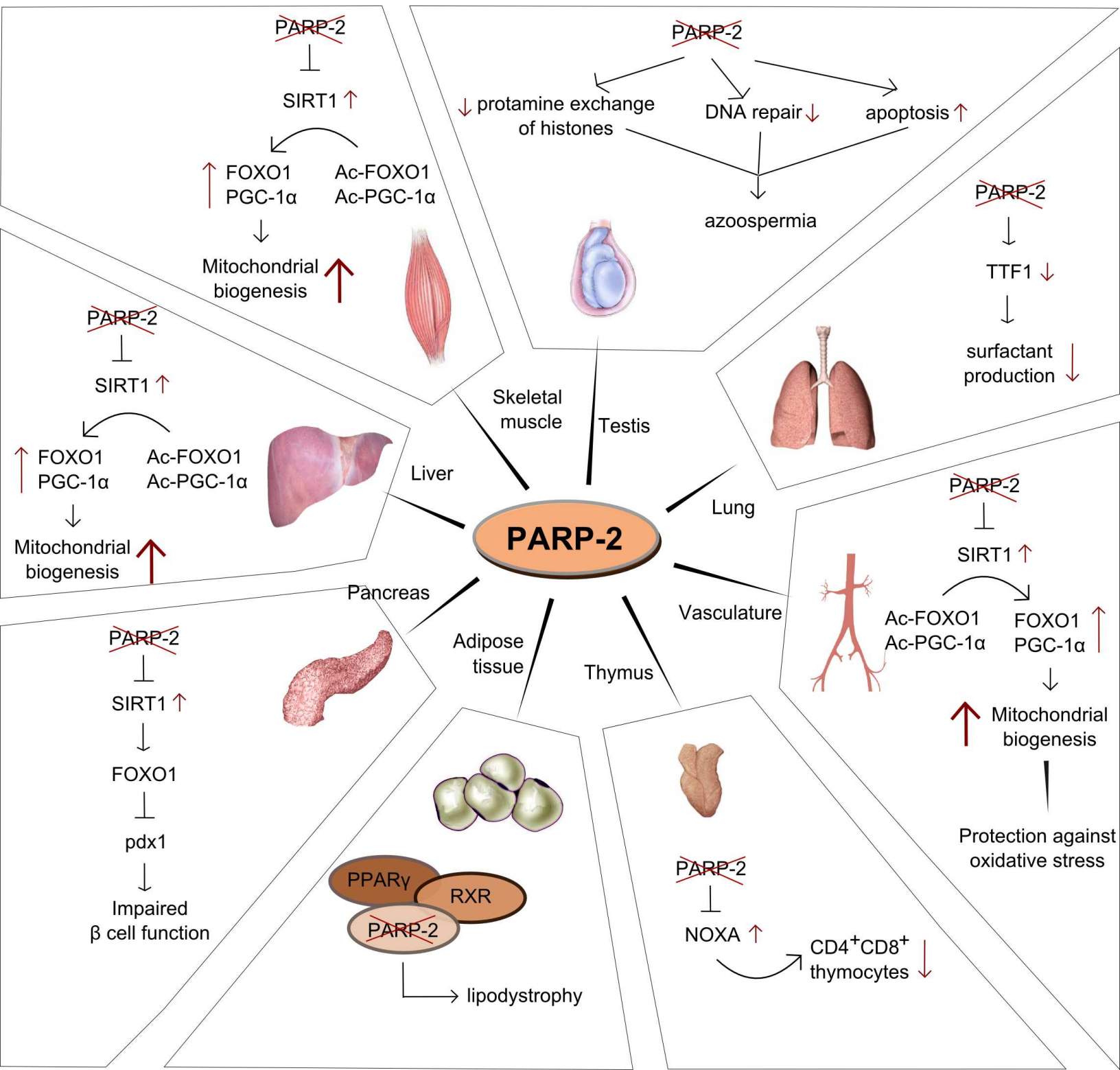


Table 1. Transcription factors directly regulated by PARP-2.

Name	Mode of action	Effects	Model system	Known tissue specificity	Ref
ER α	Unknown	Depletion of PARP-2 suppress ER α activation.	Luciferase reporter system in PARP-2 specific shRNS treated HEK293T cells.	unknown	-
RXR/PPAR α	Unknown	Depletion of PARP-2 enhance PPAR α activation.	Luciferase reporter system in PARP-2 specific shRNS treated HEK293T cells.	Unknown	[59]
RXR/PPAR δ	Unknown	Depletion of PARP-2 enhance PPAR δ activation.	Luciferase reporter HEK293T in PARP-2 specific shRNS treated HEK293T cells.	Unknown	[59]
RXR/PPAR γ	Cofactor of receptor	Modulates transcription of PPAR γ target genes, Depletion of PARP-2 leads to WAT hypofunction.	Luciferase reporter system in PARP-2 specific shRNS treated HEK293T cells; PARP-2 knockout mice; embryonic fibroblasts from PARP-2 knockout mice.	White adipose tissue	[59]
SIRT1	Transcriptional repressor of the SIRT1 promoter.	PARP-2 depletion induces SIRT1 and consequently enhance mitochondrial biogenesis in skeletal muscle and liver.	PARP-2 knockout mice; Luciferase reporter system in PARP-2 specific shRNS treated HEK293T cells; PARP-2 knockdown C2C12 cells.	Skeletal muscle, liver	[58, 83]
TTF1	Transcriptional cofactor	Regulates the expression of surfactant protein B.	Luciferase reporter system in PARP-2 specific shRNS treated HeLa/MLE15 cells, interaction mapping in mice and in cells.	Lungs	[60]