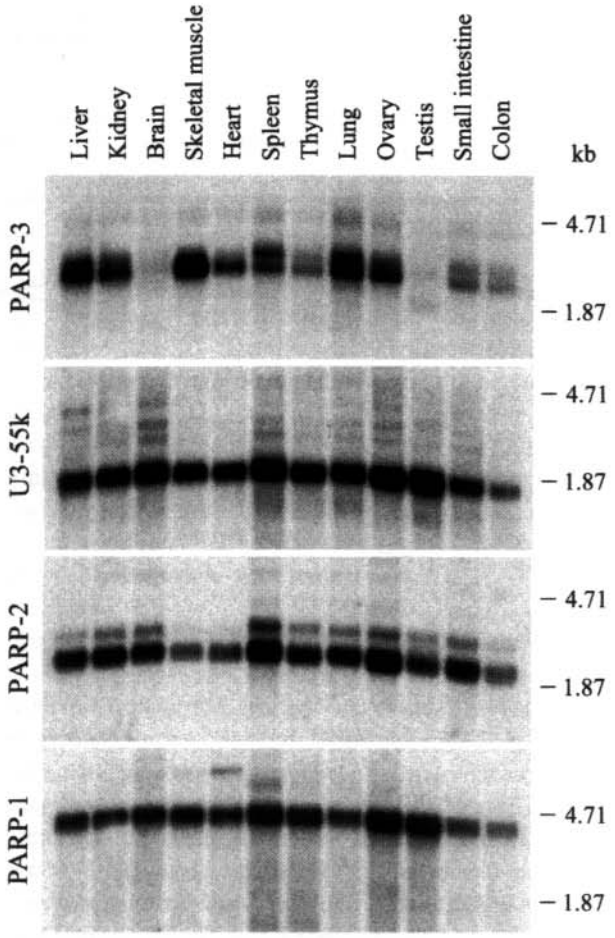

Fig. 2. Northern blot analysis of PARP-3, U3-55k, PARP-2 and PARP-1 mRNA in mouse tissues. The membrane was hybridized first with the PARP-3 probe, and then successively rehybridized with the U3-55k, PARP-2, and finally PARP-1 probes. The ubiquitously expressed U3-55k and PARP-1 mRNAs served as loading and transfer controls.

PARP-3/U3-55k genomic sequence showed that this gene organization is conserved between man and mouse (Fig. 1). Interestingly, the mouse and human *PARP-2* gene has recently been shown to be connected by a bidirectional promoter with the gene for the RNase P RNA subunit (Amé et al., 2001). Moreover, both the U3-55k protein and the RNase P RNA are involved in the processing of precursor RNAs of the protein-synthesizing machinery (pre-rRNA and pre-tRNA, respectively) (Lübben et al., 1993; Pluk et al., 1998; Lukowiak et al., 2000; Venema et al., 2000; for review on RNase P RNA see Gopalan et al., 2002). It is therefore tempting to hypothesize that there might be a functional reason for the above similarity in the promoter region organization between the *PARP* and the protein-synthesizing machinery RNA-processing genes. For example, the expression of the two groups of genes might be coordinately regulated under certain physiological or pathological conditions and/or in some cell types.

While this manuscript was in preparation, four cDNA sequences and one genomic sequence became available in the GenBank (Benson et al., 2002) and RefSeq (Pruitt and Maglott, 2001) (http://www.ncbi.nlm.nih.gov/LocusLink/refseq.html) databases of the National Center for Biotechnology Information (NCBI) under the following accession numbers and definitions: **gb:BC014703** [*Mus musculus*, similar to U3 snoRNP-associated 55-kDa protein, clone MGC:25949 IMAGE:4237895, mRNA, complete cds], **gb:BC014870** [*Mus musculus*, clone MGC:11997 IMAGE:3602116, mRNA, complete cds], **ref:XM_135106** [*Mus musculus* similar to U3 snoRNP-associated 55-kDa protein (LOC235588), mRNA], **ref:XM_135141** [*Mus musculus* similar to Poly [ADP-ribose] polymerase-3 (PARP-3) (NAD(+) ADP-ribosyltransferase-3) (Poly[ADP-ribose] synthetase-3) (pADPRT-3) (hPARP-3) (LOC235587), mRNA], **ref:NW_000356** [*Mus musculus* WGS supercontig Mm9_WIFeb01_200.]. The cDNA sequences gb:BC014870 and ref:XM_135141 correspond to our PARP-3 cDNA sequence (splice variant with the N-terminal exon 1c, i.e. gb:AY046317 – includes exon 1c, and gb:AF368233 – starting from nt 160; see also Fig. 1). The cDNA sequences gb:BC014703 and ref:XM_135106 correspond to our U3-55k cDNA sequence (gb:AF368232). The four cDNA sequences differ from the PARP-3 and U3-55k cDNAs presented in this work at several single-nucleotide positions (1 position in ref:XM_135141, 11 positions in gb:BC014870, 7 positions in gb:BC014703) and one three-nucleotide position in XM_135106. The differences may represent nucleotide polymorphisms of mouse strains. The genomic sequence ref:NW_000356 is a 7888514-bp NCBI RefSeq supercontig from mouse chromosome 9, which includes both the *PARP-3* (LocusLink LocusID: 235587) and *U3-55k* (LocusLink LocusID: 235588) genes (http://www.ncbi.nlm.nih.gov/LocusLink/) (Pruitt and Maglott, 2001) and the region corresponding to our promoter region sequence (gb:AF368234).

Mouse *PARP-3* and *U3-55k* genes are located on chromosome 9 (see above). Their chromosomal position is defined, for example, by the D9Wsu10e locus (DNA segment, Chr 9, Wayne State University 10, expressed) on chromosome 9 at 56.00 cM (Ko et al., 1998) (LocusLink LocusID: 27966, http://www.ncbi.nlm.nih.gov/LocusLink/; UniSTS: 142814, http://www.ncbi.nlm.nih.gov/genome/sts/) (Pruitt and Maglott, 2001). The D9Wsu10e marker, contained in EST gb:AA407505 (Ko et al., 1998) (LocusLink LocusID: 27966; UniSTS: 142814), is localized in the 3'-end region of the mouse U3-55k cDNA sequence (nt 1281 to 1458 in gb:AF368232 – this work). The above mouse chromosomal region is syntenic with the human chromosome 3p (Lyon and Kirby, 1996), where the human *ADPRTL3* (*PARP-3*) locus has previously been mapped to 3p21.1-p22.2 (Johansson, 1999). The syntenic chromosomal location,

together with a high degree of identity between mouse and human PARP-3 and U3-55k proteins and conserved gene arrangement in the two species (see above), identify the two genes as human and mouse orthologues.

There is less similarity between the human and mouse PARP-3 protein sequences than between the pairs of PARP-1 or PARP-2 orthologues (Table 1). Thus, in mammals, the PARP-3 proteins appear to evolve with a higher divergence rate than the PARP-1 or PARP-2 proteins. It would be interesting to see whether also the function(s) of PARP-3 tends to diverge more rapidly between man and mouse, compared to the cases of PARP-1 or PARP-2 proteins, where it could remain more conserved between the two species. This notion might be supported by the fact that the human *PARP-3* gene was shown to be ubiquitously expressed in a panel of human tissues, including brain and testis (Johansson, 1999).

Adult mouse *PARP-3* expression is regulated in a tissue-specific manner. In contrast to PARP-3, both the PARP-1 and PARP-2 mRNAs were expressed ubiquitously in the adult mouse tissues analysed, including brain and testis (Fig. 2, see also Ogura et al., 1990; Wang et al., 1995; Amé et al., 1999; Berghammer et al., 1999; Schreiber et al., 2002). We cannot, however, rule out the possibility that PARP-3 expression is restricted to a small region or few cells in these two organs.

We noted that some of the tissues (organs) where PARP-3 is not expressed or where its expression is low (i.e. brain, testis and thymus) are known to be protected by physiological barriers, entirely or in part, against the entry of some molecules from the blood (e.g. blood-brain or blood-thymus barrier) (Junqueira et al., 1995). Conversely, some other tissues that lack this barrier (e.g. liver, kidney, lung, muscle, spleen) show high levels of PARP-3 expression. In view of the role of PARP-1 in DNA repair and in the maintenance of genome integrity, we speculate that the level of the *PARP-3* gene product might in some tissues correlate with the amount of the physiological genotoxic stress the particular tissue is exposed to during organism ontogeny.

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References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., Lipman, D. J. (1990) Basic local alignment search tool. *J. Mol. Biol.* **215**, 403-410.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389-3402.

Amé, J.-C., Rolli, V., Schreiber, V., Niedergang, C., Apiou, F., Decker, P., Muller, S., Höger, T., Ménissier-de Murcia, J., de Murcia, G. (1999) PARP-2, a novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase. *J. Biol. Chem.* **274**, 17860-17868.

Amé, J.-C., Schreiber, V., Fraulob, V., Dollé, P., de Murcia, G., Niedergang, C. P. (2001) A bidirectional promoter connects the poly(ADP-ribose) polymerase 2 (PARP-2) gene to the gene for RNase P RNA. Structure and expression of the mouse PARP-2 gene. *J. Biol. Chem.* **276**, 11092-11099.

Babiychuk, E., Cottrill, P. B., Storozhenko, S., Fuangthong, M., Chen, Y., O'Farrell, M. K., Van Montagu, M., Inzé, D., Kushnir, S. (1998) Higher plants possess two structurally different poly(ADP-ribose) polymerases. *Plant J.* **15**, 635-645.

Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., Rapp, B. A., Wheeler, D. L. (2002) GenBank. *Nucleic Acids Res.* **30**, 17-20.

Berghammer, H., Ebner, M., Marksteiner, R., Auer, B. (1999) pADPRT-2: a novel mammalian polymerizing(ADP-ribosyl)transferase gene related to truncated pADPRT homologues in plants and *Caenorhabditis elegans*. *FEBS Lett.* **449**, 259-263.

Bürkle, A. (2001) Physiology and pathophysiology of poly(ADP-ribosyl)ation. *BioEssays* **23**, 795-806.

Cherney, B. W., McBride, O. W., Chen, D., Alkhatib, H., Bhatia, K., Hensley, P., Smulson, M. E. (1987) cDNA sequence, protein structure, and chromosomal location of the human gene for poly(ADP-ribose) polymerase. *Proc. Natl. Acad. Sci. USA* **84**, 8370-8374.

D'Amours, D., Desnoyers, S., D'Silva, I., Poirier, G. G. (1999) Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. *Biochem. J.* **342**, 249-268.

de Murcia, G., Ménissier-de Murcia, J. (1994) Poly(ADP-ribose) polymerase: a molecular nick-sensor. *Trends Biochem. Sci.* **19**, 172-176.

Frohman, M. A., Dush, M. K., Martin, G. R. (1988) Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc. Natl. Acad. Sci. USA* **85**, 8998-9002.

Gopalan, V., Vioque, A., Altman, S. (2002) RNase P: variations and uses. *J. Biol. Chem.* **277**, 6759-6762.

Herceg, Z., Wang, Z.-Q. (2001) Functions of poly(ADP-ribose) polymerase (PARP) in DNA repair, genomic integrity and cell death. *Mutat. Res.* **477**, 97-110.

Huppi, K., Bhatia, K., Siwarski, D., Klinman, D., Cherney, B., Smulson, M. (1989) Sequence and organization of the mouse poly(ADP-ribose) polymerase gene. *Nucleic Acids Res.* **17**, 3387-3401.

Jacobson, M. K., Jacobson, E. L. (1999) Discovering new ADP-ribose polymer cycles: protecting the genome and more. *Trends Biochem. Sci.* **24**, 415-417.

Jeggo, P. A. (1998) DNA repair: PARP – another guardian angel? *Curr. Biol.* **8**, R49-R51.

Johansson, M. (1999) A human poly(ADP-ribose) polymerase gene family (*ADPRTL*): cDNA cloning of two novel poly(ADP-ribose) polymerase homologues. *Genomics* **57**, 442-445.

Junqueira, L. C., Carneiro, J., Kelley, R. O. (1995) *Basic Histology*. 8th edition, a LANGE medical book, Prentice-Hall International, Inc.

Kaminker, P. G., Kim, S.-H., Taylor, R. D., Zebarjadian, Y., Funk, W. D., Morin, G. B., Yaswen, P., Campisi, J. (2001) TANK2, a new TRF1-associated poly(ADP-ribose) polymerase, causes rapid induction of cell death upon overexpression. *J. Biol. Chem.* **276**, 35891-35899.