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The Pur protein family: Genetic and structural features in development and disease

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Abstract

The Pur proteins are an ancient family of sequence-specific single-stranded nucleic acid-binding proteins. They bind a G-rich element in either single- or double-stranded nucleic acids and are capable of displacing the complementary C-rich strand. Recently several reports have described Pur family member knockouts, mutations and disease aberrations. Together with a recent crystal structure of Pura, these data reveal conserved structural features of these proteins that have been adapted to serve functions unique to higher eukaryotes. In humans Pur proteins are critical for myeloid cell development, muscle development, and brain development, including trafficking of mRNA to neuronal dendrites. Pur family members have been implicated in diseases as diverse as cancer, premature aging and fragile-X mental retardation syndrome.

Keywords

RNA dendritic transport; DNA replication and repair; PURA; transcription; translation

INTRODUCTION

The Pur family constitutes a unique structural class of nucleic acid-binding proteins, sequences of which are conserved in evolution from bacteria through humans. (6, 7) (40). Pura makes contact primarily with guanine bases, thus binding the G-rich single strand of the *PUR* element and displacing the complementary C-rich strand (6). Because Pura binds the guanine base, and not sugar or phosphate moieties, it is capable of binding both DNA and RNA (29, 31, 32) and disrupting G base pairing. By doing so Pura induces local DNA strand separation (17, 116). All vertebrate Pur proteins contain three repeats of an approximately 80 amino acid (aa) sequence termed the Pur domain. Bacterial Pur proteins possess one such domain. It was recognized upon sequencing that each Pur domain contains an aromatic-basic section and a leucine-acidic section (7). It is this unique, signature Pur domain that is so strongly conserved and that confers the ability to bind single-stranded nucleic acids. This review will consider all of the human Pur proteins in terms of their known functions. In light of recent structural data, we shall formulate a generalized principle of Pur protein function comprising its dual interaction with nucleic acids and multiple proteins.

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CONFLICT OF INTEREST DISCLOSURE

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Recent elucidation of the crystal structure of a segment of *Drosophila* Pura helps provide a structural basis for nucleic acid binding by the protein (38, 39). X-ray crystallography has revealed the structure of each Pur domain to be that of a so-called Whirly-like fold with three beta sheets and an alpha helix in the configuration N- $\beta\beta\beta\alpha$ -C (39). Wortman and colleagues (116) had earlier demonstrated that mutation of arginine 71 to glutamic acid of mouse Pura (R72 of human Pura) inhibits ssDNA binding and dsDNA strand separation. This R is part of a KR duo that corresponds to RK 102–103 of Pur repeat 2 (Fig. 1A) and KR 283–4 of repeat 3 (Fig. 1B). A basic aa in this duo, preceding aromatic aa's, is a defining feature of Pur repeats from bacteria through humans. Mutations by Graebisch et al. (39) have added further evidence that the β -sheets and connecting residues of Pur domains are the interaction surfaces for ss-nucleic acid binding. Pur domain II of hPura (Fig. 1A) contains sequences highly conserved in evolution. These observations strongly implicate the aromatic-basic motif of the β -sheet region of Pur domains in nucleic acid binding. It can be seen in Fig. 1B that the alpha helical region of human Pur repeat 3, containing the Psycho motif and amphipathic helix, is less conserved among repeats than is the beta sheet region. These helical motifs are involved in protein binding, and X-ray crystallography suggests that repeat 3 is specifically involved in external protein-protein interactions (39).

All human Pur proteins contain three repeats of a unique and signature-conferring amino acid sequence domain (Fig. 1C). Although these vary in length from 64 to 80 aa, they all contain sequence tracts that are immediately recognizable as Pur repeats from bacteria through humans. The four human protein family members each possess an N-terminal glycine-rich domain. This is most prominent in Pura, in which G comprises more than 50% of the first 58 aa. Pury possess the least N-terminal G residues, with 13 of the first 57 aa (Fig. 1C). The C-terminus is also a distinguishing feature among Pur family members. Pura has a span of 7 Q residues followed by an acid rich E–D region. Pur β lacks the glutamines but has the N-terminal E–D region. Pury lacks both of these regions, which have been implicated in binding of Pura to proteins (32, 47, 114). The Psycho motif (Psy, Fig. 1C) is a portion of the alpha helical region of repeat III. It is also present in several other known nucleic acid-binding proteins and has been implicated in binding the tumor suppressor, Rb (74). Note that Psy is absent in Pury-B, in which repeat III has been truncated (Fig. 1C).

The prototypical Pur family member, Pura, has been best studied in relation to its roles in the initiation of DNA replication, cell cycle regulation, and in the regulation of transcription and mRNA translation. Indeed, Pura has been firmly established as a regulator of transcription for several cellular promoters through its direct interaction with viral promoters and with many transcription factors, both cellular and viral. Therefore, this review will not focus on these aspects. See Supplemental Table 1 and references therein as well as previous review articles (32, 47, 114). The Pura crystalized structure elucidates how Pur family members may interact with both their partner protein(s) along with target nucleic acids. The essential nature of Pura function in normal development has been highlighted by knockout of the *PURA* gene in mice and by mutations of the gene in human disease. These aspects will be reviewed here with special emphasis on those functions impaired in disease and defective development.

GENETIC INVOLVEMENT OF *PUR* FAMILY MEMBERS IN HUMAN DISEASE

PUR genes in neoplasia

There are four known members of the Pur protein family in humans, encoded by genes at three chromosome loci. The proteins are Pura, encoded at chromosome band 5q31 (75), Pur β , at 7p13 (65), and two isoforms of Pury, at 8p11 (71). The respective genes are termed *PURA*, *PURB*, and *PURG*. In this review protein names are not italicized, and the first letter is capitalized. Gene names are all upper case letters and italicized. In the case of Pury, the

two isoforms, Pury-A and Pury-B, are generated by differences in termination of transcription of *PURG* resulting in a splice variant, Pury-B, that has a distinct C-terminus (71). Aberrations in all three of the *PUR* gene loci are associated with disease, although evidence for involvement of the encoded proteins varies. At this time evidence is strongest for necessity of Pura expression in a developmentally regulated fashion, with aberrations leading to several defects, including neoplasias. The strength of this evidence is due primarily to statistical association of the *PURA* gene with human disease states and concurrence with genetic inactivation studies in mice.

All three *PUR* gene loci have been implicated in genetic aberrations related to multiple neoplasias. Chromosome band 5q31, the location of *PURA*, is one of the most frequently deleted chromosome regions in malignant myeloid disorders (63). Although the role of Pura in myeloid development will be discussed separately below, certain aspects of *PURA* chromosomal positioning are relevant to neoplasia in general. 5q31 contains genes encoding multiple cytokines, including several interleukins (63, 103) and the early growth response gene-1 (81). *PURA* is located approximately 1 Mb telomeric to the EGR-1 gene and segregates with it in several translocations in myelodysplastic syndrome (MDS), as determined by fluorescence in-situ hybridization (FISH) (65). MDS frequently progresses to acute myelogenous leukemia (AML). 5q- syndrome, i.e., the loss of the long arm of chromosome 5, is recognized as a common and separate category of MDS (100, 107, 108, 113). When 5q- occurs as the sole genotypic aberration, without accompanying chromosome changes, the clinical course of MDS is relatively mild, and the progression to AML less frequent (108). When, however, 5q31 deletion is accompanied by an additional 5q31 alteration or alterations involving other *PUR* gene loci, for example *PURB*, progression to AML is seen at increased frequency (65). In addition to deletions, translocations of the *PURA* locus are also seen in both MDS and AML (65). Although deletions or alterations of a single *PURA* allele are detected by FISH in MDS and AML, there have been no detected mutations in the Pura coding sequence, and there is no evidence at this time that *PURA* fits the profile of a classical tumor suppressor gene. If *PURA* is the primary target of chromosome rearrangements at 5q31, it is likely that haploinsufficiency is a factor in the resulting oncogenic phenotype.

Chromosomal alteration is a characteristic uniting all three *PUR* genes in both myeloid diseases and aspects of CNS development and aging. In addition to those in MDS and AML, chromosome alterations at 5q31 have been reported in primitive neuroectodermal tumors and pineoblastomas (76) as well as intracranial ependymomas (78). At this point, however, specific involvement of the *PURA* locus in these brain tumors has been unexplored. The ability of enhanced levels of Pura protein to inhibit proliferation of tumor cells in culture (5, 96), including glioblastoma cells (18), has been established. Chromosome band 7p13, the location of *PURB*, is associated with abnormalities linked to myeloproliferative disorders, particularly when occurring in conjunction with alterations involving *PURA* at 5q31 (65). Monosomy 7 is a well-known aberration associated with MDS and AML (37). Although evidence is lacking for a mechanistic link, aberrations at 7p13 have been reported for brain tumors, including glioblastoma (73). A common involvement of *PURA* and *PURB* genes in specific disease states may be anticipated because of the reported functional interaction of the two encoded proteins. Pura forms multimeric complexes in the presence of nucleic acids (30). For example, Pura and Purβ, which are approximately 70% homologous, reportedly function as a heterodimer to regulate the vascular smooth muscle alpha-actin promoter (55).

The *PURG* locus at 8p11 is very intriguing with regard to neoplasia. In general the presence of increased levels of the Pura protein confer tumor suppressive (5, 18, 65, 66) and antiproliferative (5, 21, 96) activities. In contrast, increased expression of Pury, at 8p11, is associated with several tumor types and enhanced cell proliferation. Alterations at 8p11 are

the basis of a recognized myeloproliferative syndrome (8, 44). Although many of these cases involve translocations at various breakpoints, in many cases the *PUR* gene copy number is increased by trisomy of chromosome 8 (57, 65, 69, 86, 88). It is particularly interesting that reports have linked simultaneous occurrence of trisomy 8, monosomy 7 and del 5q to AML (86, 88), thereby potentially involving all three *PUR* gene members in specific cases of myeloid neoplasia. Although the convergence of these three chromosome alterations is undoubtedly a rare event, neither the copy numbers nor expression of each of the *PUR* genes has been determined, and there has been no statistical linkage of these events to leukemia. Selective amplification of 8p11 has been reported for several tumor types other than leukemia. Breast cancer amplification of 8p11-12 has been most thoroughly examined (1, 25, 34, 36, 85, 118). Amplification of 8p11-12 is one of the most commonly reported chromosome aberrations in breast carcinoma, occurring in approximately 15% of all cases and associated with a poor prognosis (118). Thus far there is no consensus on which gene(s) are responsible for transforming properties of such amplification, but several genes in close proximity to *PUR* have been implicated, and the phenomenon may be complex, involving more than one gene.

***PUR* genes in diseases of development and degeneration of the central nervous system: a role for Pur proteins in dendritic transport of mRNA**

A major category in which *PUR* genes have gathered recent attention comprises diseases involving abnormal development and degeneration of the central nervous system (CNS). This may reflect recent heightened attention investigators have paid to these diseases, but it also reflects the importance of Pur protein functions to genome maintenance and specialized aspects of gene expression in specific organs. A common characteristic of cells in the immune system and the CNS is that they both involve plasticity in response to a multitude of exquisitely differential extracellular signals. That is, in response to external stimuli, these organ systems form adaptable signal transduction pathways and entire pathways of cell differentiation to strengthen or dampen future responses. Neurons and glia of the CNS extend cell outgrowths that must respond very quickly to signals that act at great distances from the cell nucleus. In terms of protein synthesis it is thus efficient for such cells to develop specialized mechanisms of mRNA transport and translation that can respond at sites of signal transduction distal from the genetic apparatus of the nucleus. Several recent advances in elucidating such mechanisms have been derived from studies of fragile-X mental retardation syndrome and the fragile-X-related tremor/ataxia syndrome, in which Pura has been identified as a major contributor (35, 39, 45, 47, 49, 52, 68, 82).

Pura in fragile X mental retardation syndrome and fragile X-associated tremor/ataxia syndrome

Fragile X syndrome (FXS) is the most common cause of inherited mental disability, manifest by cognitive defects, hyperactivity, attention deficits and diverse behavioral characteristics with aspects of autism. Expansion of a trinucleotide CGG repeat in the 5'-untranslated region of the Fragile X Mental Retardation gene (FMR1) has been identified as a primary cause of FXS. This expansion leads to chromosomal instability and loss of expression of the encoded protein, FMRP (22, 23, 93-95, 109, 115). FMRP is an RNA-binding protein potentially involved in mRNA transport in neurons (26, 49, 52, 64, 82). FMRP has been detected in large intraneuronal, dendritic mRNA-containing complexes together with Pura and non-coding RNA molecules implicated in RNA transport (49, 52, 59, 60, 82). Pura binds to mouse BC1 RNA (49, 59) and to its human counterpart, BC200 (49). BC1 RNA has been implicated in targeting mRNA to sites of translation in neurites (49, 80, 101, 102), as has BC200 (27). Pura is specifically localized in dendrites, not axons, of rat hippocampal neurons, and it colocalizes with FMRP at specific dendritic junction sites associated with translation (49). Although both FMRP and BC1 are implicated in mRNA

binding at sites of translation, the association of FMRP with its target mRNAs is reportedly independent of the presence of BC1 in vivo (43), suggesting that FMRP and BC1 act separately to exert effects on translation. Because Pura interacts with both FMRP and BC1 RNA, Pura may be involved in separate translational activities of each factor. On the other hand, because Pura is capable of interacting independently with both FMRP and BC1, the presence of Pura may allow formation of a complex including all three factors. Double RNA immunoprecipitation experiments have indicated that FMRP and Pura interact simultaneously with mRNA encoding Map2, a dendrite-specific protein (49).

The evidence that Pura is present with FMRP in complexes regulating transport of mRNA to sites of translation in dendrites of CNS neurons raises the question: are changes in Pura structure or function associated with FXS? The evidence thus far is derived from observations of patients with fragile X-associated tremor/ataxia syndrome (FXTAS). FXTAS is considered a premutation stage of FXS, in which the number of rCGG repeats in the FMRP mRNA is intermediate between that of normal individuals (<60 repeats) and FXS patients (>200 repeats). It has been reported that a total of 90 rCGG repeats alone is sufficient to cause neurodegeneration in *Drosophila* (46). It has not escaped notice of investigators that CGG repeats are ideal Pura-binding elements (7, 116), and the interaction of Pura and associated RNA-binding proteins with the FMRP mRNA repeat sequence has been documented (45, 84, 97, 99). Binding of Pura to rCGG repeats has been observed to modulate the repeat-mediated neurodegeneration in a *Drosophila* model of FXTAS (45). It was found that expression of the Pura-binding protein Rm62, an RNA helicase, is influenced by the number of rCGG repeats (84). The role of Pura in binding to rCGG repeats may extend to mRNA transport systems outside of neurons. It has recently been reported that Pura binds to rCGG repeats in mRNA of *Drosophila* oocytes and mediates nuclear export of the mRNP complex in a manner analogous to the role of Pura in neurons (3). The mechanism of involvement of rCGG repeats in neurodegeneration has yet to be elucidated. Nonetheless, an intriguing multilayered feedback pathway has been revealed in which Pura directly interacts with the mRNA encoding a protein, FMRP, which itself interacts with Pura to regulate the transport and translation of mRNAs in neuronal dendrites.

GENETIC INACTIVATION OF PUR α IN THE MOUSE: CORRELATIONS WITH HUMAN DISEASE

The mouse *PUR* family genes are remarkably syntenic with their human counterparts. For example, non-coding sequences adjacent to the *PURA* gene in humans, including promoter sequences, 5'-UTR and 5' intron sequences, are all highly conserved in the mouse, as are genes in the vicinity of *PURA* (unpublished observations). Similarly, *PURG* is located head-to-head with *WRN* in both human and mouse chromosomes with intergenic bidirectional control sequences highly conserved (D. Daniel, unpublished observations). There are presently two recorded homozygous knockouts of the *PURA* gene in mice and no homozygous knockouts of the other *PUR* family genes. In the first of the reported *PURA* knockouts it was found that mice lacking *PURA*, and consequently expression of the Pura protein, proceed through embryogenesis to produce live births, but that the mice so born do not survive beyond 21 to 25 days post-birth (56). The mice are born with near normal appearance, but by day 10 they begin to exhibit uncoordinated movement, tremor, lack of activity and wasting, all despite eating and sleeping nearly normally. At death the mice are abnormally small, all measured organs so affected while the brain upon gross examination displays some evidence of sparing compared to the other organs. Blood cells were carefully assessed in the knockout mice because of the prominent myeloid defects in humans with *PURA* defects. While every major differentiated hematopoietic cell was represented in the knockout mice at time of death, the relative numbers of different cell types were skewed

toward reduction of monocyte development vs. lymphoid cell development, a feature particularly notable in histology of the spleen, in which the percentage of replicating myeloid cells was $<1/10$ that recorded in wild type mice of the same age. Defects were also histologically apparent in the developing brains of knockout mice, consistent with a critical role proposed for *Pura* in the CNS (49). There was mislamination of the cerebellum and cerebrum in the knockout mice, and the numbers of differentiated Purkinje cell neurons, cerebral, and hippocampal neurons were distinctly low. The number of synaptic connections, as measured by number of PSD95 foci in the hippocampus, was low in the *PURA* $-/-$ mice (56). *Pura* levels are low in the embryonic brains of developing wild-type mice but increase to peak at approximately 20 days after birth (56), at about the time when the *PURA* $-/-$ mice die. *PURA* $+/-$ heterozygous mice display a near normal phenotype that still includes myeloid and neurological defects. *Pura* levels in measured tissues of the heterozygotes are intermediate between those of the homozygotes and the wild type mice. The numbers of myeloid cells undergoing DNA replication in the spleens of heterozygotes are accordingly intermediate between homozygotes and wild types (56). The heterozygote mice survive normally but are subject to sudden death with seizure-like symptoms. Cross-species comparisons indicate that disruption of *PURA* gene expression may be correlated with defects in aspects of myeloid and neural development in both mice and humans.

A *PURA* mouse knockout construct has recently been reported in which defects in postnatal brain development have also been found (41). The homozygous mice in this model displayed a persistent tremor, although the level of quantification of this symptom was descriptive. A minor but significant megalencephaly, in terms of overall brain weight, was reported. The Purkinje cell density in the cerebellum was, however, significantly reduced. In the more recent *PURA* knockout model there was a marked reduction in the expression of dendritic protein, Map2 (41), the translation of which was observed to be *Pura*-dependent in the earlier *PURA* $-/-$ mouse model (49). Details of the extent to which *Pura* amino acid sequences have been deleted in the more recent *PURA* knockout system (41) have not been disclosed, making it difficult to correlate *Pura* protein expression levels with symptomatic quantification. It is notable that the homozygous knockout mice in the recent model did not die prematurely. Because there are four *Pur* family proteins, including two isoforms of *Pury*, expressed in both humans and mice, the possibility of selection of revertant mice based on gene redundancy must be carefully evaluated in all *PUR* knockout models to prevent dilution of phenotypes. It is conceivable that another *Pur* family member, or a combination of them, given altered expression, could provide compensatory effects to revert a knockout *Pura* phenotype. Recent data from mouse brain support a role for *Pura* in dendritic protein translation and dendrite maturation. *Pura* regulates the neuronal levels of RhoA, a GTPase critical for mRNA translation and dendritic maturation, at several points, including subcellular compartmentalization and turnover. In *PURA* knockout mice RhoA levels are reduced and dendritic maturation impaired (77).

STRUCTURAL FEATURES OF PUR PROTEINS AND COOPERATIVE FUNCTIONS OF PUR FAMILY MEMBERS DURING DEVELOPMENT

Pur protein interaction with non-coding RNAs in nucleic acid transport

Pura binds not only to BC1 and BC200 RNAs, but to several non-coding RNA sequences with regulatory properties influencing mRNA synthesis and translation (47, 49). These non-coding RNAs include 7SL RNA, a component of the signal recognition particle derived from a segment of the human Alu sequence element (106, 110). 7SL RNA participates in cotranslational translocation of proteins, whereby a protein traverses a membrane during translation in order to enter a separate cellular or extracellular compartment (90). The association of *Pura* with these non-coding RNAs reveals a striking theme in *Pur*-nucleic

acid functional interaction: these RNA molecules have similar hairpin structures containing *PUR* elements, and they all participate in the transport or kinetic processing of nucleic acids, particularly mRNA. Furthermore, all of these molecules possess sequences and a hairpin structure in common with the HIV-1 TAR RNA element (49), responsible for the processive transactivation of HIV-1 transcription. HIV-1 has in a sense co-opted these mRNA regulatory activities through binding of the viral protein, Tat, to Pura, which cooperates with Tat to stimulate HIV-1 transcription (14) (Wright, C.A. and Johnson, E.M., 2012, unpublished) and DNA replication (15, 16). The co-opting of Pur activities by HIV-1 may extend to more than the Tat-Pura interaction. It has recently been reported that HIV-1 packages 7SL RNA into virions as determined by *cis*-acting elements in the RNA (53). These observations emphasize the importance to HIV-1 infection of subverting the cellular mRNA transport mechanism and highlight the critical role of Pura in this process.

Cooperation between Pur family members, Pura and Purβ: examples in muscle development

The single-stranded DNA binding properties of Purβ have been implicated in transcriptional repression of genes encoding muscle-specific isoforms of actin and myosin in heart, skeletal muscle and vascular smooth muscle (58, 87, 120). Studies on the mouse smooth muscle alpha actin gene have revealed an interaction between Pura, Purβ and the Y-box protein, MSY1 (10, 55). Recent observations implicate interactions of the Pur proteins with another Y-box binding protein, YB-1 (24). Pura and Purβ bind to each other through specific protein-protein interactions, bind to the purine-rich strand of their regulatory DNA element as dimers and multimers. As does YB-1 (12), MSY1 has an affinity for the complementary pyrimidine-rich strand of the *PUR* element. This complex interaction is linked to the ability of Pura to participate in mRNA transport and compartmentalization through the interaction of the Pur proteins with MSY1. MSY1 is present in an mRNP complex implicated in the storage, transport and translation of mRNAs in mouse germ cells (98). The studies on muscle development thus suggest a cooperative interaction between Pura and Purβ. This familial interaction may extend to mRNA transport in neuronal processes. Both Pura and Purβ have been reported as present in the mRNP complex transported along microtubules by a kinesin motor in neurites (52).

Distinct gene regulatory mechanisms govern Pura and Pury

At this time, Pury is the least studied of the Pur family members. Pury is notable for certain very unusual gene expression features. Humans possess a single *PURG* gene that encodes two isoforms of the Pury protein, as determined by differential termination/polyadenylation (71). Pury-A is expressed as a single, intronless coding sequence, as are family members Pura and Purβ. Pury-B, however, is expressed as a very long transcript that reads through the Pury-A transcriptional termination site. Instead, an intron of >30 kb is spliced out of the Pury-B transcript, removing the Pury-A translational stop codon and resulting in a different Pury-B C-terminus (71). This highly unusual switching mechanism is utilized in human development and is characteristic of certain neoplasias. *PURG-B* is the predominant *PURG* transcript found in human testis, but it was undetectable in members of a non-cancerous adult tissue cDNA panel. *PURG-A* levels were low or undetectable in the non-tumor tissue panel, but they were increased in members of a tumor tissue panel. *PURG-B* was detected in several of the tumor tissues (71).

There is at least a temporal separation in the expression of Pur family members, Pura and Pury, if not also a functional separation. Pury protein levels are high in the developing mouse embryo brain from day e14 through day p1, whereas Pura levels in the brain are nearly undetectable at this stage (49). Pury protein levels in the brain decline to reach a nadir at 18–25 days after birth while Pura levels increase to a near maximum during this

time. These results have suggested that *Pury* may be an embryonic or fetal *Pur* family member supplanted by *Pura* as development ensues. This notion is complicated in humans, however, by the finding that *Pury* is highly expressed in certain tumor cells. For example, *Pury* and *Pura* levels are both significantly expressed in KG-1C oligodendroglioma cells (Daniel, D.C. and Johnson, E.M., unpublished observations). There is a single mouse *PURG* gene, but it is not known whether two isoforms of *Pury* can be expressed in the mouse as they can in human cells.

Data regarding the *PURG* gene suggests a link between *Pury* and prevention of aging. The *PURG* gene is located head-to-head with *WRN*, the gene encoding the WRN helicase (71). The transcription units of each gene initiate at multiple points, the major clusters of which are approximately 90 bp apart. Mutations in the *WRN* gene have been linked to several phenotypic changes characteristic of premature aging (28, 72, 89, 119). The WRN protein possesses both helicase and nuclease activities (9, 42, 79, 83, 117) and has been implicated in repair of DNA double-strand breaks (2, 9, 72, 91, 117), a process also involving *Pura* (50, 111, 112). WRN has also been implicated in telomere maintenance (4, 62, 67). Thus the known functions of WRN suggest that it plays a role in prevention of genomic degeneration due to accumulating damage during aging. The promoter region is shared between *PURG* and *WRN*. Both promoters lack TATA and CAAT elements, and both are positively regulated by Sp1 elements. Although promoter elements for the two genes overlap in contrapodal directions, various elements important for transcription of each gene are distinct (71). Due to the near identity of the DNA-binding β -sheet elements in *Pury* and *Pura* repeats (Fig. 1), *Pury* is likely to be a DNA strand-separating protein, as is *Pura* (17, 116). As a helicase, WRN would processively unwind regions of single-stranded DNA. It will be of great interest to determine whether there is any functional association between these two proteins and whether their shared promoter regions may affect this.

SUMMARY: STRUCTURES OF PUR FAMILY MEMBERS AFFECT FUNCTION AND DISEASE PREVENTION

Biochemical analyses indicate that *Pura* (30, 116) and *Pur β* (55) interact with nucleic acids as dimers or multimers. X-ray crystallographic analyses provide a structural basis for such a dimeric interaction (39). Each *Pur* amino acid repeat consists of a β -sheet domain and an α -helical domain (Fig. 1) arranged in a structure, termed a “Whirly fold,” in which convex β -sheets form a surface for interaction with nucleic acids (39). The remaining portions of the Whirly structure are engaged in protein-protein interactions. In a model proposed by Graebisch et al., (39) *Pur* repeats I and II form intramolecular protein-protein bonds while repeat III forms bonds with a second *Pura* molecule. Given the similarity in amino acid sequences of repeat III among the *Pur* protein family members, we propose (Fig. 2) that repeat III is capable of forming dimers involving any combination of two of the three *Pur* family members. Furthermore, because there are important differences in aa sequence in the α -helical regions of repeats III of the different *Pur* family members, (Fig. 2), these domains may be capable of forming bonds with proteins other than *Pur* family members. Evidence summarized in Fig. 2 has already implicated motifs within *Pur* repeat III in interaction with several cellular and viral regulatory proteins. The C-terminal, α -helical region of *Pur* repeat III may be important in targeting protein interactions that disrupt the normal cellular functions of *Pur* protein family members.

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Figure 1. Amino acid repeat structures in the human Pur family

A. Comparison of aa's of repeat II, a typical, evolutionarily-conserved Pur repeat among all human Pur family members. Repeat II is identical in Pur γ -A and Pur γ -B. In panels A and B red letters denote identity, blue denotes strong conservation, and green denotes partial conservation. Lines above the sequence refer to beta sheet and alpha helical portions of the Whirly fold described (39). **B.** All Pur repeats can bind both nucleic acids and proteins. Repeat III is known to bind to a variety of different regulatory proteins. The amino-terminal portion of the repeat, constituting primarily beta sheets, is involved in nucleic acid binding. Certain C-terminal sequences implicated in protein binding are underlined. **C.** Positions of the Pur repeats (white boxes) in all four known human Pur family members. The repeat homology used for this drawing extends from R at aa 149 in repeat II (panel A) to V, G or E at aa 248. Drawings in panel C are to scale. Note that in Pur γ -B Repeat III is truncated so that only beta sheet sequences remain and the alpha helical portions are eliminated. The N-terminal glycine (C)-rich region and the C-terminal glutamine (Q), glutamic acid (E) and aspartic acid (D) regions are indicated.

Figure 2. Diagrammatic representation of Pur multimerization, nucleic acid-binding and protein-binding domains

Nucleic acid beta sheet domains believed to be involved in nucleic acid binding are shown as curled lines. Pur multimerization and protein-binding domains are shown as filled ovals. Refer to Table 1, presented as supplemental data, for a list of nucleic acids and proteins reported as bound by human Pur α .

Table 1

Sequence- and structure-specific binding of Pura to nucleic acids and to nucleic acid-binding proteins

PROTEINS, DNA ELEMENTS, AND RNAs BOUND TO PURα		
<u>Protein</u>	<u>Reference</u>	
HIV-1 Tat	Krachmarov et al. (61), Gallia et al. (33)	
Rb	Johnson et al. (48)	
YB-1	Chen et al. (12, 13)	
JCV large T-antigen	Gallia et al. (31)	
Purβ	Kelm et al. (55)	
Cyclin A	Barr and Johnson (5)	
Cdk2	Liu et al. (70)	
Cdk1	Liu et al. (70)	
Cdk4	Liu et al. (70)	
Cyclin E/Cdk2	Barr and Johnson (5)	
Cyclin B/Cdk1	Barr and Johnson (5)	
Cyclin T1/Cdk9	Darbinian et al. (20)	
E2F-1	Darbinian et al. (19)	
SP-1	Tretiakov et al. (105)	
RARα	Chen et al. (11)	
<u>DNA element</u>	<u>Reference</u>	
(GGN) _n (ssDNA>>dsDNA)	Bergemann et al. (6, 7)	
c-MYC zone of replication initiation	Bergemann et al. (6, 7)	
JC virus non-coding control region	Chen and Khalili (13), Krachmarov (61)	
HIV-1 5′-LTR promoter	Chepenik et al. (14)	
Myelin basic protein promoter	Haas et al. (40)	
Smooth muscle alpha-actin promoter	Kelm et al. (54, 55)	
CD11c beta 2 integrin promoter	Shelley et al. (92)	
TNF-alpha promoter	Darbinian et al. (20)	
<u>Non-coding RNA</u>	<u>Reference</u>	
HIV-1 TAR	Chepenik et al. (14)	
Ribosomal RNA	Gallia et al. (29)	
7SL RNA	Tretiakova et al. (104)	
Mouse BC1 RNA	Johnson et al. (49)	
Human BC200 RNA	Johnson et al. (49)	
HIV-1 Rev response element RRE	Kaminsky et al. (51)	
<u>mRNA</u>	<u>Reference</u>	
Map2	Johnson et al. (49)	
Map1B	Johnson et al. (49)	
HIV-1 transcript	Kaminsky et al. (51)	

NUCLEIC ACID-PROTEIN COMPLEXES FORMED WITH PURα		
<u>Nucleic acids</u>	<u>Proteins</u>	<u>Reference</u>
General mRNA	Kinesin, FMRP, Staufen	Ohashi et al. (82)

NUCLEIC ACID-PROTEIN COMPLEXES FORMED WITH PUR α

<u>Nucleic acids</u>	<u>Proteins</u>	<u>Reference</u>
CaMKIIalpha mRNA, Arc mRNA	Kinesin (KIF5), Pur β , hnRNP-U, SYNCRIP FMRP, Staufen	Kanai, et al. (52)
Map2 mRNA, BC1 RNA	FMRP, Staufen	Johnson, et al. (49)
Map1B mRNA, BC1 RNA	FMRP, Staufen	Johnson, et al. (49)