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Molecular cloning of a structural homolog of YY1AP, a coactivator of the multifunctional transcription factor YY1

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Summary. YY1 is a multifunctional transcription factor that activates or represses gene transcription depending on interactions with other regulatory proteins that include coactivator YY1AP. Here, we describe the cloning of a novel homolog of YY1AP, referred to as YARP, from the human neuroblastoma cell line SK–N–SH. The cloned cDNA encoded a 2240 amino acid protein that contained a domain which was 97% homologous to an entire YY1AP sequence of 739 amino acids. Two splice variants, YARP2 and YARP3, were also cloned. Northern blotting demonstrated the YARP mRNA (~10 kb), which was increased 1.7-fold after dibutyryl cAMP-induced neural differentiation of the cells. Presence of YARP mRNA was also confirmed in human tissues such as the heart, brain and placenta. Bioinformatic analysis predicted various functional motifs in the YARP structure, including nuclear localization signals and domains associated with protein–protein interactions (PAH2), DNA-binding (SANT), and chromatin assembly (nucleoplasmin-like), outside the YY1AP-homology domain. Thus, we propose that YARP is multifunctional and plays not only a role analogous to YY1AP, but also its own specific roles in DNA-utilizing processes such as transcription.

Keywords: YY1-associated protein – YARP – Dingo – GON4L – Gene expression – Human

Abbreviations: dbcAMP, dibutyryl cyclic AMP; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT, reverse transcription; YY1AP, YY1-associated protein; YARP, YY1AP-related protein (alias Dingo or GON4L)

Introduction

YY1 is a ubiquitously expressed, multifunctional transcription factor that can act as a repressor, activator or initiator element binding protein (Shi et al., 1997). The C-terminal domain of YY1 contains four C_2H_2-type zinc fingers that can bind to many different cellular and viral promoters in a sequence-specific manner at a consensus 5′-CGCCAT NTT-3 site (Shrivastava and Calame, 1994). It is estimated that more than 7% of all vertebrate gene promoters contain at least one YY1 consensus binding site (Hyde-DeRuyscher et al., 1995). Thus, YY1 potentially controls the expression of a vast array of genes. Indeed, the expression and function of several genes associated with basic cellular processes, such as cell-cycle control and programmed cell death, are regulated by YY1. It is therefore likely to be important in cancer biology, viral infections, immune responses and during development (Shi et al., 1997; Hyde-DeRuyscher et al., 1995; Thomas and Seto, 1999; Gordon et al., 2006). Consistent with this consideration, the targeted disruption of the YY1 gene in mice resulted in peri-implantation lethality (Donohoe et al., 1999). Heterozygotes can survive, but a subset display significant growth retardation and neurological defects. In humans, its inappropriate functioning is implicated in heart failure (Sucharov et al., 2003) and facioscapulohumeral muscular dystrophy (Gabellini et al., 2002).

The diverse functions of YY1 are due to its ubiquitous nature and ability to act both as a transcriptional activator and as a repressor. Whether YY1 acts as an activator or repressor on a gene depends on the promoter context and interactions with other regulatory proteins (Shi et al., 1997; Thomas and Seto, 1999; Gordon et al., 2006). Thus, a number of proteins have been investigated and demonstrated to physically interact with YY1. For example, YY1 interacts with p300 and the CREB-binding protein (CBP), coactivators with histone acetyltransferase activity (Lee et al., 1995; Austen et al., 1997; Yao et al., 2001), and with histone deacetylases HDACs 1–3 (Shi et al., 1997; Yang et al., 1996, 1997; Galasinski et al., 2002). This suggests that YY1 recruits these chromatin modifiers to activate or repress gene transcription upon its binding to specific promoters. Although several other models are also proposed regarding its mechanisms of action, YY1

can activate the transcription of many genes, including c-myc and p53, and also repress promoters such as interferon-β, c-fos and skeletal α-actin (Gordon et al., 2006). It has also been shown that YY1 is able to interact with Sp1 (Seto et al., 1993; Lee et al., 1993), ATF/CREB (Zhou et al., 1995), retinoblastoma protein (Rb) (Petkova et al., 2001), p53, Mdm2 (Gronroos et al., 2004; Sui et al., 2004) and Rel-B/NF-κB (Sepulveda et al., 2004).

Recently, a novel YY1-associated protein, referred to as YY1AP, was identified and characterized as a coactivator of YY1 (Wang et al., 2004). YY1AP is a 750 amino acid protein with a molecular mass of 90 kDa and capable of enhancing the transcriptional activation of a YY1 responsive promoter. Two YY1 binding domains and a transactivation domain are present in the YY1AP primary structure. However, typical DNA binding domains are not found. Therefore, it is suggested that YY1AP is recruited and tethered to the DNA by YY1 bound to target promoters, and in turn mediates or regulates the transcriptional activity of YY1. YY1AP is ubiquitously expressed in human tissues and encoded by a gene located on chromosome 1q21.3 (Wang et al., 2004). To date, its splice variants are known as a subtype of YY1AP (DDBJ accession nos. NM139118-139121). However, here we report that YY1AP constitutes a gene family in the human genome. We found a gene encoding a structural homolog of YY1AP, termed YARP for YY1AP-related protein, at a distance of only about 70 kb from the YY1AP gene on the same DNA strand (1q22). cDNA cloning revealed that YARP is a large protein in which the entire YY1AP structure is retained fairly intact as if either was generated by gene duplication occurring during genomic evolution. Moreover, several domains implicated in DNA-binding or molecular interactions were predicted on the YARP molecule. Thus, we propose that YARP also plays a role in transcriptional regulation of gene expression.

Materials and methods

Cell culture

The human neuroblastoma cell line SK–N–SH (RIKEN Cell Bank, Tsukuba, Japan) was maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin and streptomycin at 37 °C and 5% CO_2. Neural differentiation of the cell was induced by treatment with 2 mM dbcAMP for 72 h (Koshizawa et al., 1997). For microscopic examination, cells were stained with 0.2% crystal violet dissolved in 20% methanol. RNA was prepared using Isogen reagent and a Poly(A)$^+$ isolation kit (Nippon Gene, Tokyo, Japan).

Subcellular fractionation

SK–N–SH cells were homogenized in an SETP buffer (0.25 M sucrose, 1 mM EDTA, 10 mM Tris–HCl (pH 7.5) and protease inhibitor cocktail, (Roche Complete) using a Potter-Elvehjem glass homogenizer with a Teflon pestle. Homogenates were centrifuged at 400 *g* for 10 min and the precipitate washed once, resuspended in SETP buffer and used as the 400 *g* precipitate. The wash and the supernatant were combined and centrifuged at 22,000 *g* for 10 min, and the resultant supernatant used as the 22,000 *g* supernatant. The precipitate and the fraction left after the wash were also combined, resuspended and used as the 22,000 *g* precipitate. Nuclei were prepared by a published method (Greenberg and Ziff, 1984). Briefly, cells were homogenized in 10 mM Hepes-KOH (pH 8.0) containing 1 mM dithiothreitol (DTT), 1.5 mM $MgCl_2$, 10 mM KCl and 0.5 mM phenylmethylsulfonylfluoride (PMSF) and centrifuged at 1300 *g* for 5 min. The precipitate was suspended in 5 ml of TDM buffer (10 mM Tris–HCl (pH 8.0), 1 mM DTT, 5 mM $MgCl_2$) containing 0.25 M sucrose, 0.1% Triton X-100 and 0.5 mM PMSF and layered on 4 ml of TDM buffer containing 1.2 M sucrose then centrifuged at 10,000 *g* for 30 min. The precipitate under the 1.2 M sucrose was resuspended in the SETP buffer and used as purified nuclei. Protein concentration was determined using a Bio-Rad DC protein assay kit with bovine serum albumin as the standard.

cDNA cloning

In an experiment of cDNA subtraction on the brains of mice with or without ethanol treatment, we obtained a 249-bp cDNA fragment of YARP. The rapid amplification of cDNA ends (RACE system ver. 2.0, Invitrogen) was then performed and a 1018-bp sequence encompassing the 249 bp fragment was obtained. This sequence matched to the cDNA clones mouse mKIAA1606 (DDBJ accession no. AK122531) and human Dingo (DDBJ accession no. AY335490.1) in the NCBI BLAST search. To obtain a longer cDNA, 5′- and 3′-RACE was performed again, and finally a YARP cDNA was amplified by RT-PCR with 5′-TGGCGCATAGTGTTAGGCG CAT-3′ and 5′-GGAGCTGAACTCCACTCTCGATGC-3′ as PCR primers after oligo$(dT)_{18}$-primed RT of poly(A)$^+$ RNA prepared from SK–N–SH cells. The cDNA was cloned into pCR-XL-TOPO (Invitrogen), resulting in a plasmid pCR-TOPO-hYARP. RT reactions were incubated at 42 °C for 90 min with Superscript III RNase H$^-$ reverse transcriptase (Invitrogen). PCR was performed with LA Taq-GC Buffer (Takara Bio, Tokyo, Japan) and cycling conditions were 98 °C for 30 sec, followed by 35 cycles of 94 °C for 15 sec and 68 °C for 10 min. During the cDNA cloning, splice variants were found and similarly cloned using the PCR-primer pairs 5′-TGGCGCATAGTGTTAGGCGCAT-3′ and 5′-GGAGCTGAACTCCACT CTCGATGC-3′ for YARP2, and 5′-GTTTCCGTGTAATCAGGCCGGCT-3′ and 5′-CATCCTTTCCATCTCATTTCA-3′ for YARP3. The nucleotide sequences of the cDNAs have been submitted to the DDBJ (accession nos. AB195687, AB195688, and AB232668).

Northern blotting

RNA was separated on formaldehyde-agarose gels and transferred onto HybondN$^+$ nylon membranes (Amersham Biosciences) using Turboblotter rapid downward transfer system (Schleicher and Schuell) with 3 M NaCl containing 0.01 M NaOH. Approximately 1 kbp fragments of the YARP cDNA, which were amplified by PCR and designated P1–P8 (for the precise sequences, see the legend of Fig. 1), were labeled with [α-^{32}P] dCTP using a high prime kit (Roche). Hybridization with the radiolabeled probes was performed overnight in 6× SSC containing 1× Denhardt's, 0.5% SDS, 50% formamide, 0.1 mg/ml salmon testis DNA at 42 °C. Blots were washed with 2× SSC then 1× SSC at room temperature, further with 0.1× SSC at 55 °C, in which the SSC contained 0.1% SDS and 0.2% sodium pyrophosphate. The hybridization signal was detected with the bioimaging analyzer BAS 2000 II (Fuji Photo Film, Tokyo, Japan). The human tissue RNA blots (Human MTN blot) were purchased from Clontech.

Antibody preparation and western blotting

A polypeptide, CRAPDNIIKFYKKTKQLPVL, corresponding to human YARP amino acids 875–893 with an additional cysteine residue, was

synthesized and conjugated to keyhole limpet hemocyanin (Wako, Osaka, Japan) using *m*-maleimidobenzoyl N-hydroxysuccinimide ester (MP Biomedicals) as a cross linker, as described previously (Mochizuki et al., 2003; Liu et al., 1979). Rabbits were immunized with this conjugate and the antibody (designated as αYH875) affinity-purified from the serum using a column coupled with the antigen polypeptide as described previously (Kuramochi et al., 2002). For western blotting, proteins were resolved on 7.5% SDS-polyacrylamide gels and transferred onto the immun-blot PVDF membranes (Bio-Rad). Membranes were blocked with 1% ECL advance blocking agent (GE Healthcare) for 1 h and then incubated with the primary antibody followed by a horseradish peroxidase-conjugated secondary antibody. Enzyme activity was visualized using ECL western blotting detection reagents (GE Healthcare) and analyzed by an luminoimaging analyzer LAS1000-Plus (Fuji Photo Film, Tokyo, Japan). On western blots, the antibody αYH875 was confirmed to recognize a human YARP polypeptide (amino acids 601–1000) expressed in bacteria as a glutathione S-transferase fusion protein (data not shown).

Bioinformatic analysis

Functional domains and motifs in the YARP structure were analyzed by the following programs and databases: Pfam (http://www.sanger.ac uk/Software/Pfam/index.shtml), ProDom (http://prodes.toulouse.inra.fr/prodom/current/html/home.php), SMART (http://smart.embl-heidelberg.de/), PSORT II (http://psort.nibb.ac.jp/), InterProScan (http://www.ebi.ac.uk/interpro/) and PROSITE (http://kr.expasy.org/prosite).

Results and discussion

Cloning of human YARP cDNA

As schematically represented in Fig. 1, the cloned cDNA for human YARP was 7150 bp in length, not including the poly(A) tail, and contained a 175 bp 5′-untranslated region (UTR) and an open reading frame (ORF) encoding 2240 amino acids with a calculated molecular mass of 248 kDa, followed by the stop codon TGA. This was followed in turn by 252-bp 3′-UTR which contained a consensus polyadenylation signal (AGTAAA) located 13 bp upstream of the poly(A) tail. Since an in-frame stop codon (TGA) was located 147 bp upstream of the presumed start codon, the cDNA clone was found to cover the entire translated region. The coding sequence at nucleotides 1964–4199 of the YARP cDNA was 96% identical to that of human YY1AP cDNA (nucleotides 298–2531, DDBJ accession no. AF466401) (Wang et al., 2004) which contained the entire translated region of YY1AP, thus encoding the "YY1AP-homology" domain (see below, Figs. 5B and 6). The splice variants, YARP2 and YARP3, encoded a 970 amino acid (110 kDa) and 1529 amino acid (146 kDa) protein, respectively.

In the recently updated version of the NCBI DNA database, the sequences of the above-mentioned Dingo and related genes were integrated as the "reference sequence" and named GON4L (gon-4-like) due to its similarity to the nematode gon-4 gene product (Friedman et al., 2000). The nucleotide sequence of the YARP cDNA was almost identical to that of GON4L (DDBJ accession no. NM_001037533), although our clone was 126 bp longer at the 5′ end and 608 bp shorter at the 3′ end. The GON4L sequence has an in-frame CAG-triplet insert between nucleotides 6014 and 6015 of YARP cDNA, encoding a 2241 amino acid protein. However, we could not confirm this insertion using a genomic DNA database search. The functional significance of this difference, or whether it represents a polymorphism, is currently unclear.

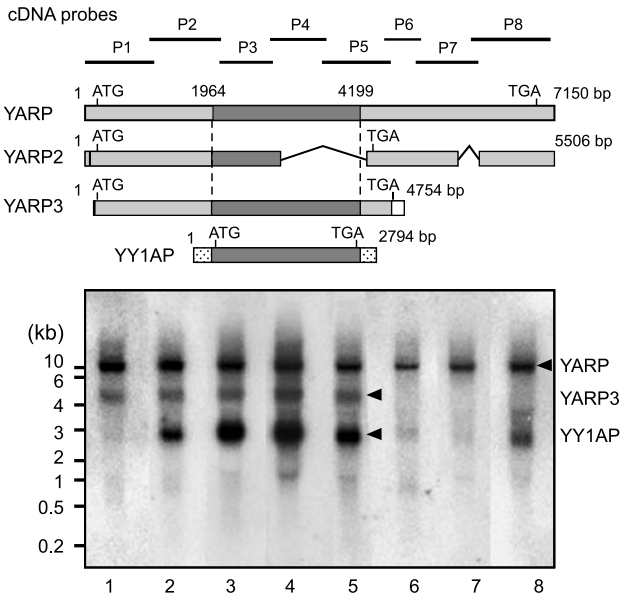

Fig. 1. Northern blots of YARP variants and YY1AP. Poly(A)$^+$ RNA (2 μg/lane) prepared from SK–N–SH cells was probed with YARP cDNA fragments corresponding to the nucleotides 1–1066 (*P1*), 986–2089 (*P2*), 2067–2902 (*P3*), 2855–3720 (*P4*), 3654–4687 (*P5*), 4562–5113 (*P6*), 5064–6050 (*P7*) and 5926–7150 (*P8*) in *1–8*, respectively. Arrowheads indicate positions of hybridized RNAs for YARP (∼10 kb), YARP3 (∼5 kb) and YY1AP (∼3 kb). The positions of size markers are indicated on the left. The cDNA sequences of YARP, its splice variants (YARP2 and YARP3) and YY1AP (Wang et al., 2004) are schematically represented above the blots, along with the cDNA probes used. The regions highly conserved between YARP variants and YY1AP are shown as dark gray bars

Analysis of YARP transcripts

In order to demonstrate the existence of YARP RNA, northern blotting was performed on poly(A)$^+$ RNA prepared from SK–N–SH cells and a series of YARP cDNA fragments (P1–P8), that covered the overall YARP sequence, as probes (Fig. 1). All of these probes hybridized to the same sized RNA of ∼10 kb. This result indicated that the YARP transcript exists in these cells. Compared

with the length of the mRNA (∼10 kb), the cloned cDNA (7150 bp) and registered GON4L sequence (7676 bp) are apparently short in length. This suggests that the full sequence of the UTR of the YARP or GON4L transcript remained to be cloned. When P1–P5 were used as probes, an RNA species of ∼5 kb was detected, and P2–P5 also hybridized to a ∼3 kb size RNA. The former transcript corresponded to YARP3 and the latter was YY1AP as it is consistent with the mRNA size estimated previously (Wang et al., 2004). YARP2 was not detected on these blots. Although RT-PCR clearly demonstrated the presence of this variant in SK–N–SH cells, the expression level of YARP2 mRNA seemed negligible (data not shown). A faint minor band at ∼1 kb was detected on these blots when using P4 and P5; we could not, however, identify this band (lanes 4 and 5). This band may represent a YY1AP splice variant. The P8 probe also hybridized to a ∼2.5-kb RNA, but further analysis was not performed in this study (lane 8).

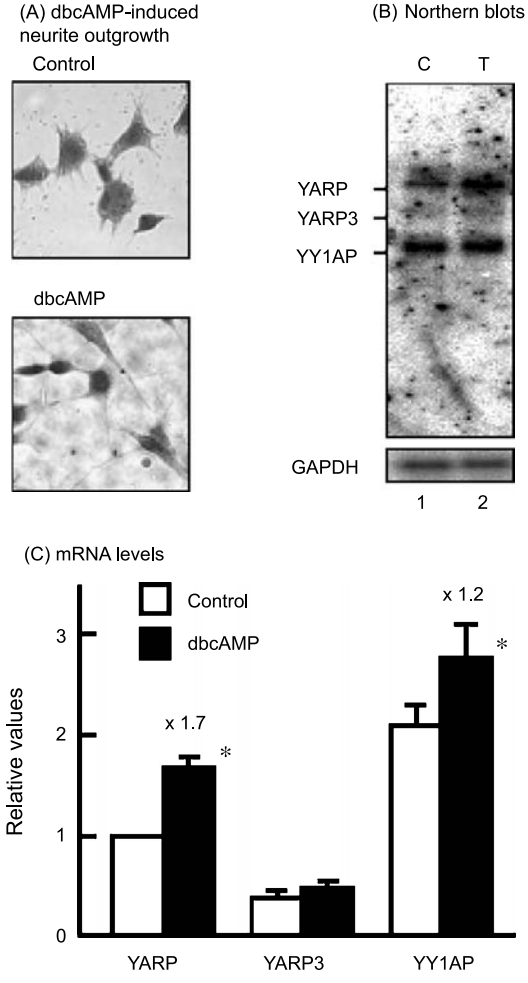

Fig. 2. Changes in mRNA levels of the YARP variants and YY1AP after neural differentiation of SK–N–SH cells induced by dbcAMP. The cell cultures were treated with 2 mM dbcAMP for 72 h. The poly(A)$^+$ RNA (2 μg/lane) was prepared and subjected to northern blotting using the YARP cDNA probe P4 described in the legend for Fig. 1. **A** Micrographic images of dbcAMP-induced neurite outgrowth. Upper and lower panels, control and treated cells, respectively. **B** Northern blots. The positions of hybridized RNAs for YARP, YARP3 and YY1AP are indicated. Blots probed by GAPDH cDNA as loading control are also shown. Lanes 1 and 2, control and treated cells, respectively. **C** mRNA levels are normalized with those of GAPDH and expressed as relative values (mean ± SEM, n = 3), taking the value for YARP in the control as 1. Open and closed columns, control and treated cells, respectively. *Significantly different from the control, $P < 0.05$ (Student's *t*-test)

Next, we examined whether, in addition to YARP2 and YARP3, other variants, especially those having the ''YY1AP-homology'' domain conserved in their structures, exist in SK–N–SH cells. This cell line is known to differentiate into neuron-like cells and gain several features specific to nerve cells upon treatment with appropriate inducers, such as dibutyryl cAMP (dbcAMP) (Koshizawa et al., 1997). Whether the expression levels of YARP variants are affected by such conditions was also examined. As shown in Fig. 2A, treatment of SK–N–SH cells with 2 mM dbcAMP for 72 h induced cell differentiation and elicited neurite outgrowth characteristic to nerve cells. Under these conditions, no apparent changes in the composition of YARP variants were observed, as determined by northern blotting using the cDNA fragment P4 as a probe (Fig. 2B). However, mRNA levels of YARP and YY1AP increased 1.7- and 1.2-fold respectively, whereas that of YARP3 did not change significantly (Fig. 2C). Therefore, YY1AP, YARP and YARP3 were the major homologs containing the ''YY1AP-homology'' domain found to be expressed in SK–N–SH cells, whether these were differentiated or not. Although the balance between their expression levels changed slightly, the relative order in their expression levels, of YY1AP > YARP ≫ YARP3, remained unchanged. When other human cell lines including MKN28, HEK293 and U251 were examined, the mRNA levels of the three were similar to those found in SK–N–SH cells (data not shown).

The expression of these three homologs in normal human tissues was then confirmed by northern blotting (Fig. 3). A YY1AP mRNA of ∼3 kb was detected in all tissues examined, of which the heart and skeletal muscle showed the highest expression. These results are compatible with the previous study (Wang et al., 2004), although a higher level of expression was observed in the heart rather than muscle and the expression in the placenta and pancreas was more evident in this study. These differences are possibly due to differences between subjects or tissue portions employed to extract RNA from in

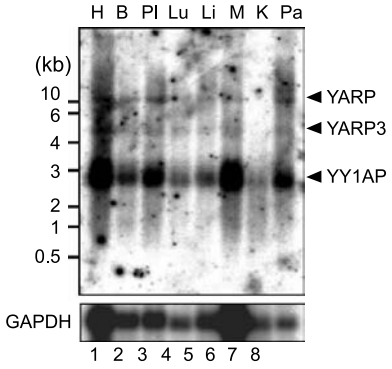

Fig. 3. YARP mRNA levels in human tissues. RNA blots (Human MTN blot, Clontech) were hybridized with a YARP cDNA probe P4 described in the legend for Fig. 1. *1–8* The heart (*H*), brain (*B*), placenta (*Pl*), lung (*Lu*), liver (*Li*), skeletal muscle (*M*), kidney (*K*) and pancreas (*Pa*), respectively. Arrowheads indicate the positions of hybridized RNAs. Positions of size markers are indicated on the left. Blots probed with GAPDH cDNA as loading control are also shown

these commercially available blots. YARP (∼10 kb) and YARP3 (∼3 kb) mRNAs were also detectable in the heart, brain, placenta, muscle and pancreas, albeit at much lower levels compared with YY1AP.

Detection of YARP protein

To detect YARP protein as the gene product, western blotting was performed with SK–N–SH cell homogenates (Fig. 4, lane 2). An antibody raised against a synthetic polypeptide corresponding to YARP amino acids 875–893 recognized a protein with a molecular mass of ∼250 kDa, which is consistent with the molecular weight of 248,000 calculated from the amino acid sequence deduced from the YARP cDNA. When cell homogenates were fractionated by centrifugation, a similar band was detected on the blots with crude nuclear fraction (lane 3), but not with particulate fraction (lane 4). Although the ∼250 kDa protein was also found in the 22,000 *g* supernatant (lane 5), it was demonstrated in purified nuclei (lane 1). These results suggested that YARP protein is expressed in SK–N–SH cells and, at least in part, located in the nucleus.

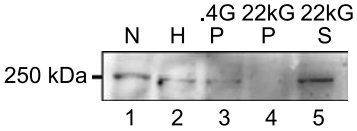

Fig. 4. Western blots of YARP protein. SK–N–SH cell homogenates were fractionated by sequential centrifugation and probed with an anti-YARP antibody (αYH875) on the blots (15 μg/lane). *1* Purified nuclei (*N*); *2* cell homogenate (H); *3* 400 *g* precipitate (.4GP); *4* 22,000 *g* precipitate (22kGP); *5* 22,000 *g* supernatant (22kGS)

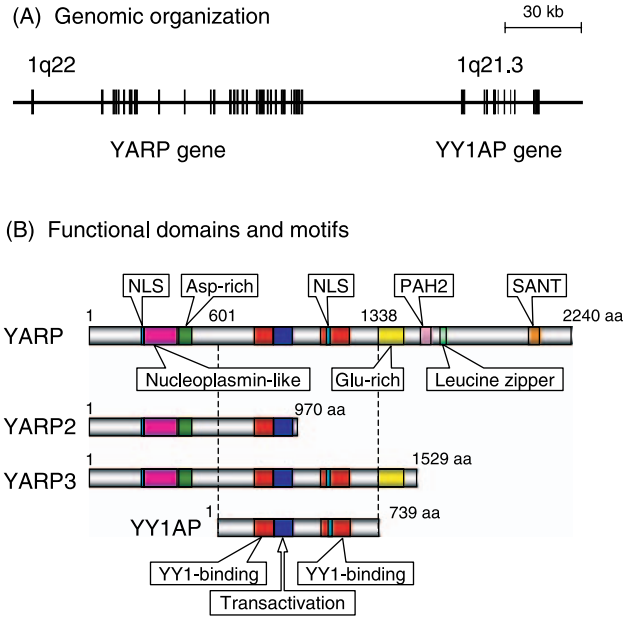

Fig. 5. Structural organization of the YARP and YY1AP genes (**A**) and the functional domains and motifs in the YARP variant and YY1AP proteins (**B**). In **A**, exons are depicted as closed boxes, and introns and flanking regions by solid lines. In **B**, the YY1AP-homology domains are indicated by a pair of broken lines. NLS, nuclear localization signal; PAH2, paired amphipathic helix repeat 2; SANT, SWI3/ADA2/N-CoR/TFIIIB DNA-binding domains

Genomic organization of human YARP gene

As determined by a human genome BLAST analysis using the NCBI Map Viewer program, the human YARP gene is comprised of at least 32 exons and spans ∼120 kb, mapped to 1q22 of the chromosome 1 (Fig. 5A). The gene may give rise to various transcripts via the alternative use of exons; indeed, we cloned two splice variants, YARP2 and YARP3, in addition to YARP itself. Of interest, the YY1AP gene spanning ∼30 kb is located on 1q21.3 at a distance of only ∼70 kb from the YARP gene. Moreover, these two genes encode highly conserved amino acid sequences, a YY1AP molecule itself (amino acids 1–739) and the "YY1AP-homology" domain in YARP (amino acids 601–1338), with 93% identity and 97% homology (Figs. 5B and 6), reflected by 96% identity of the corresponding nucleotide sequences (Fig. 1). These findings suggest that YARP and YY1AP genes may be products of gene duplication occurring during evolution of the human genome.

Structural properties of YARP

The function of YARP is currently not known. However, the presence of a YY1AP-homology domain in the struc-

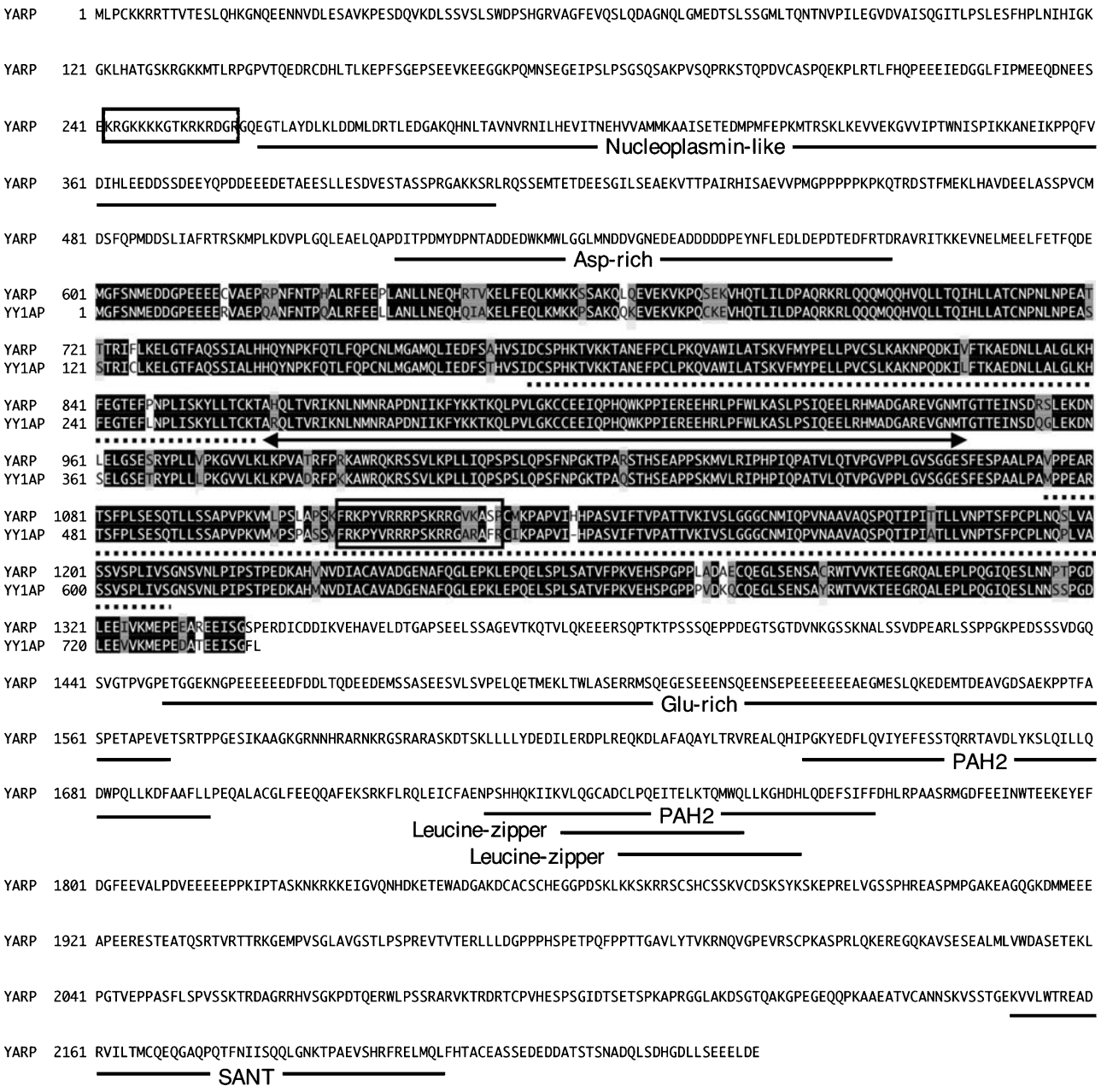

Fig. 6. Comparison of amino acid sequences between YARP and YY1AP. Identical amino acids are highlighted and conservative substitutions are shaded. The YY1-binding and transactivation domains are indicated by dotted and arrowhead underlines respectively. The nuclear localization signal is boxed and other functional domains and motifs are underlined

ture strongly suggests that YARP is involved in the transcriptional regulation of gene expression through an interaction with YY1. Within the YY1AP-homology domain of YARP, the YY1-binding and transactivation domains that were experimentally demonstrated in a previous study (Wang et al., 2004) are almost completely conserved. In addition, bioinformatic database searches predicted various functional domains and motifs in the primary and secondary structures of YARP (Figs. 5B and 6). For example, the paired amphipathic helix 2 (PAH2) is a domain involved in protein–protein interactions. The corepressor Sin3 interacts with the Mad family of repressors via its PAH2 domain and is recruited to the DNA (Spronk et al., 2000; Cowley et al., 2004). The SANT domain is a DNA-binding domain related to a Myb repeat, originally identified in a number of proteins, including the SWI3, ADA2,

N-CoR and TFIIIB (Aasland et al., 1996; Boyer et al., 2004). The myb DNA-binding proteins are known to specifically recognize the sequence YAAC(G/T)G (Aasland et al., 1996). The nucleoplasmin-like domain is a structure associated with chromatin assembly and remodeling (Ito et al., 1996). Moreover, acidic amino acid-rich regions associated with transcriptional activation (Triezenberg, 1995), leucine zippers involved in protein–protein interaction (Ransone et al., 1989) and the nuclear localization signal (Hicks and Raikhel, 1995) were also predicted. Furthermore, a GON4-like region is present. The nematode C. elegans gon-4 gene encodes a nuclear acidic protein of 1338 amino acids which plays a key role in gonadogenesis by regulating cell lineage (Friedman et al., 2000). The amino acids 228–534 and 683–842 of YARP are similar to amino acids 7–301 and 382–530 of GON4 with a homology of 70 and 73%, respectively. Thus, YARP has this GON4-like region in the N-terminal structure, the YY1AP-homology domain in the middle, and its own characteristic region containing several domains and motifs in the C-terminal structure. Therefore, YARP may be multifunctional, acting not only as an analog of YY1AP or GON4, but also having its own specific roles in DNA-utilizing processes such as transcription within the nucleus.

Conclusion

In this study, we cloned a cDNA for human YARP gene and for the first time, report on its expression and the structural properties of its translation product. The most striking feature of the structure is the YY1AP-homology domain, and YARP is therefore characterized as a novel YY1AP-homolog. Currently, the respective roles assigned to these two homologs in the cell are unclear. They may antagonize or synergize each other's functions. However, it is also likely that YARP and YY1AP do not always operate in common cellular mechanisms that involve YY1, because YARP is predicted to have various functional structures not found in YY1AP. Their induction in differentiated SK–N–SH cells possibly implies a role in basic cellular processes such as cell-cycle control. A role of YARP in growing cells or tumor cells is also suggested, as the expression is relatively high in established cell lines compared with normal human tissues. Although much remains to be investigated, the discovery of YARP provides an opportunity to explore the complexity of YY1-mediated transcriptional mechanisms in terms of YY1AP functioning, and suggests further functional flexibility mediated by this class of coactivators.

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