Identification of a Tankyrase-binding Motif Shared by IRAP, TAB182, and Human TRF1 but Not Mouse TRF1

NuMA CONTAINS THIS RXXPDG MOTIF AND IS A NOVEL TANKYRASE PARTNER*

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Tankyrase-1 and -2 are closely related poly(ADP-ribose) polymerases that use an ankyrin-repeat domain to bind diverse proteins, including TRF (telomere-repeat binding factor)-1, IRAP (insulin-responsive aminopeptidase), and TAB182 (182-kDa tankyrase-binding protein). TRF1 binding allows tankyrase to regulate telomere dynamics in human cells, whereas IRAP binding presumably allows tankyrase to regulate the targeting of IRAP. The mechanism by which tankyrase binds to diverse proteins has not been investigated. Herein we describe a novel RXXPDG motif shared by IRAP, TAB182, and human TRF1 that mediates their binding to tankyrases. Interestingly, mouse TRF1 lacks this motif and thus does not bind either tankyrase-1 or -2. Using the ankyrin domain of tankyrase as a bait in a yeast two-hybrid screen, we also found the R domain of tankyrase as a hetero-oligomerize and colocalize in vivo. Like tankyrase-1, tankyrase-2 is proposed to regulate protein targeting in response to insulin (3). This is because tankyrase-1 is phosphorylated by mitogen-activated protein kinases in insulin-stimulated cells, and this phosphorylation enhances the PARP activity of tankyrase-1 (3). A third protein known to bind the ANK domain of tankyrase-1 is a novel 182-kDa tankyrase-binding protein called TAB182, whose function is largely unknown except for its dual targeting to both the nucleus and the periphery of cytosol (5).

Functional characterization of tankyrase-1 has been confounded by its complex targeting pattern that varies throughout the cell cycle (6). During mitosis, tankyrase-1 is concentrated around the centrosomes at either end of the bipolar mitotic apparatus (6). Among several centrosomal markers tested, the spindle pole marker NuMA (nuclear/mitotic apparatus protein) colocalizes best with tankyrase-1 (6). This colocalization ceases after mitosis, when NuMA returns to the nucleus (7) while tankyrase-1 becomes associated with Golgi membranes that coalesce around the centrosomes (3). Although Golgi and spindle poles alternately contain the bulk of tankyrase-1, a small fraction of tankyrase-1 is targeted to human telomeres through TRF1 binding (1, 6). For yet-unclear reasons, mouse telomeres do not contain detectable amounts of tankyrase-1 by immunofluorescence analysis (8).

Tankyrase-1 is mirrored in many aspects by its closely related homologue, tankyrase-2 (9–11). In fact, both tankyrases hetero-oligomerize and colocalize in vivo (9). Like tankyrase-1, tankyrase-2 is proposed to regulate protein targeting in response to growth factor signaling (10), and tankyrase-2 overexpression (tagged with a nuclear localization sequence) in human cells has been shown to elongate telomeres (12). Moreover, the ANK domain of tankyrase-2 also binds to TRF1, IRAP, and TAB182 (5, 9, 11).

The specific binding of these three apparently unrelated proteins to tankyrases suggests two likely scenarios. First, a cryptic structural motif common to TRF1, IRAP, and TAB182 could enable them to bind tankyrase using the same mechanism. Alternatively, each partner might use a unique mechanism to bind distinct sites within the ANK domain of tankyrase. According to our sequence analysis, the ANK domain comprises 20 ANK repeats (19 full repeats plus two flanking half-repeats) that are evenly demarcated into five ANK subdomains by a recurrent insertion sequence (9) (Fig. 3, herein). Consistent with these subdomains arising through gene quintuplication, the full blown ANK domain contains redundant binding sites for IRAP (9). In an alternative sequence analysis that invokes multiple irregular insertions and deletions, the ANK domain is depicted as comprising 24, instead of 20, ANK repeats that are grouped into five ANK-repeat clusters or ARCs (1, 5). These ARCs generally correspond to the

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* The abbreviations used are: PARP, poly(ADP-ribose) polymerase; ANK, ankyrin repeat; TRF, telomeric-repeat binding factor; IRAP, insulin-responsive aminopeptidase; MAP, mitogen-activated protein; TAB182, 182-kDa tankyrase-binding protein; NuMA, nuclear/mitotic apparatus protein; ARC, ANK-repeat clusters; aa, amino acid(s); GST, glutathione S-transferase.

This paper is available on line at http://www.jbc.org
fave ANK subdomains that we defined (9), and each ARC represents an independent binding site in tankyrase (5). Importantly, a yeast two-hybrid analysis showed that ARC-I and -V interact with TRF1 but not with TAB182, whereas the other three ARCs interact with both partners (5). Such a partner selectivity by ARCs (hereafter referred to as ANK subdomains) suggests a mechanistic distinction in how TRF1 and TAB182 bind to tankyrase. However, biochemical analyses herein indicate that ANK subdomain-V of tankyrase binds indiscriminately to TRF1, IRAP, and TAB182. Moreover, all three partners use a shared RXXPDG motif to bind tankyrase. This tankyrase-binding motif prompted us to uncover NuMA as an RXXPDG-containing partner of tankyrase. We also show that mouse TRF1, unlike human TRF1, lacks an RXXPDG motif in its acidic domain and thus does not bind to tankyrase. Therefore, the RXXPDG motif is an important determinant for diverse proteins to bind the ANK domain of tankyrase.

EXPERIMENTAL PROCEDURES

**Yeast Two-hybrid Screen**—A human skeletal muscle cDNA library (CLONTECH) was screened by yeast mating as described (3) using baits that encoded aa 153–599 (bait A, /H1101111 ANK repeats) and aa 436–1166 (bait B, /H110119 ANK repeats) of tankyrase-2 (GenBank accession no. AF309033). These baits were constructed by subcloning fragments A and B of tankyrase-2 cDNA previously isolated based on their interaction with IRAP (9) into the BglII site of pGBDu-C (2) and the SalI site of pGBDu-C (3) (13), respectively. Using these baits, we isolated 33 Ade′/H11001His′ clones whose prey cDNAs reproduced the Ade′/H11001His′ phenotype when introduced to a fresh tankyrase-2 bait but not to the control TPK-2 bait (9). Restriction analysis of these cDNA clones revealed only 10 distinct inserts. These inserts were sequenced from both ends for ~300 nucleotides, and their complete sequences were inferred from alignment with published sequences.

**Expression Vectors**—All constructs involved human cDNA sequences unless otherwise specified. For FLAG-SubV-TNKS-1 and -2, sequences encoding tankyrase-1 1,728–1,846 and tankyrase-2 628–826 (subdomain-V)

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**Fig. 1.** RXXPDG is the minimal tankyrase-binding motif in IRAP. Tankyrase-2 labeled in vitro with [35S]methionine was incubated with resins containing GST fusions of either wild-type RQSPDG sequence (lane 1, IRAP 96–101) or alanine-substituted mutants (lanes 2–7) as described under “Experimental Procedures.” Resin-bound 35S-labeled tankyrase-2 was washed and detected by autoradiography (A). GST fusions (5 μg/lane) are shown in Coomassie Blue-stained 10% gels (B).

**Fig. 2.** Tankyrase interacts with the RXXPDG motif of TAB182 and hTRF1. Extracts of cells overexpressing FLAG-tankyrase-1 (top panels) or -2 (middle panels) were incubated with resins containing GST fusions of various TAB182 (A) and hTRF1 sequences (B) as indicated. GST-TAB-G1513A (lane 5 in A) and GST-hTRF1-G18A (lane 4 in B) contain TAB1824,150-1,542 and hTRF11,65 sequences that harbor an alanine substitution at position 6 of the RXXPDG motif, respectively. Resin-bound proteins were immunoblotted in 6.5% gels with an anti-FLAG antibody as described under “Experimental Procedures.” GST fusions (5 μg/lane) are shown in Coomassie Blue-stained 10% gels (lower panels).
Extracts of cells overexpressing FLAG-tagged subdomain V of tankyrase-1 (A) or -2 (B) were incubated with resins coated with the indicated GST fusion proteins. Resin-bound proteins (lanes 1–4) and 15% of the input were immunoblotted in 10% gels using an anti-FLAG antibody as described under “Experimental Procedures.” GST fusions (5 µg/lane) are shown in the Coomassie Blue-stained 10% gel (C). The diagram at the top depicts subdomain V in the context of the entire ANK domain. The ANK repeats (rectangles) and insertions within the repeats (ovals) are numbered according to Ref. 9.

## Affinity Precipitation of Tankyrase-1 and -2

### Experimental Procedures

A cDNA library was screened in a yeast two-hybrid system using two overlapping tankyrase-2 baits, A and B, as described under “Experimental Procedures.” Proteins encoded by cDNA clones that specifically interacted with either bait were listed. The coding interval of each cDNA and the location of any RXXPDG motif within these intervals are also shown.

### Table I: RXXPDG motif in candidate interactors of tankyrase

| Prey     | GenBank accession no. | # bits | aa encoded by insert | RXPDG |
|----------|-----------------------|--------|----------------------|--------|
| NuMA     | S23647                | 2B     | 1605–2016            | RTQP2D1746 |
| Hox-B2   | P14652                | 4A + 4B | 112–356              | REQP2DG1710 |
| L-type calcium channel | NP 00060 | 1A | 1555–1873            | REAPDG1064 |
| PP1 subunitc | AF312028               | 1A     | 1584–1873            | REAPDG1034 |
| TXB151   | AAA75595              | 5A + 3B | 311–747              | RKMEDG6055 |
| USP25    | AAFA52263             | 1A     | 542–747              | none |
| Fibulin-2| CAAG57876             | 4B     | 665–1087             | RFTP2DG1066 |

> Full names are given in the text.

> The frequency each cDNA was isolated using baits A and B.

> The PP1 cDNA isolated encodes a deletion at Ala<sup>109</sup>; a polymorphism also found in GenBank no. AK027086 and six additional ESTs.

For Figs. 1 and 4, 3T3-L1 fibroblasts cultured on 10-cm plates as described (3) were lysed in buffer A, and aliquots of extracts were incubated with resins coated with GST-hTRF1 or -mTRF1 (5 µg) at 4 °C for 3 h. For Figs. 2 and 3, BOSC cells were transfected with the indicated vectors and lysed 1–2 days later in buffer A as described (9). Equal aliquots of extracts were incubated in 200 µl of buffer A with glutathione resin (3 µl, Fig. 4, B and C; Amersham Biosciences) or resin-immobilized GST proteins (5 µg, Figs. 2 and 3) at 4 °C for 1–4 h. Resin-bound proteins were washed three to four times in buffer A and immunoblotted using anti-GST (Santa Cruz), anti-tankyrase (T12, 1 µg/ml; Ref. 3), or anti-FLAG M2 antibody (Sigma). Protein molecular weight markers were from Amersham Biosciences.

### RESULTS

**RXXPDG Is a Minimal Tankyrase-binding Motif**—We have shown that the IRAP hexapeptide RQSPDG<sup>101</sup> is entirely responsible for IRAP binding to tankyrase-1 (3). Not surprisingly, GST fusion of this hexapeptide also bound to tankyrase-2 that

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**Fig. 3.** Subdomain V of tankyrase binds to hTRF1, IRAP, and TAB182.

For Figs. 1 and 4, tankyrase-2 was <sup>35</sup>S-labeled in vitro by coupled transcription/translation of pT7-TNKS-2 as described (3) and was incubated in 100 µl of buffer A (3) with resin containing indicated GST fusions (10 µg) at 4 °C for 1 h. Resin-bound proteins were washed and autoradiographed as described (3). For Fig. 6, 3T3-L1 fibroblasts cultured on 10-cm plates as described (3) were lysed in buffer A, and aliquots of extracts were incubated with resin coated with GST-hTRF1 or -mTRF1 (5 µg) at 4 °C for 3 h. For Figs. 2 and 3, BOSC cells were transfected with the indicated vectors and lysed 1–2 days later in buffer A as described (9). Equal aliquots of extracts were incubated in 200 µl of buffer A with glutathione resin (3 µl, Fig. 4, B and C; Amersham Biosciences) or resin-immobilized GST proteins (5 µg, Figs. 2 and 3) at 4 °C for 1–4 h. Resin-bound proteins were washed three to four times in buffer A and immunoblotted using anti-GST (Santa Cruz), anti-tankyrase (T12, 1 µg/ml; Ref. 3), or anti-FLAG M2 antibody (Sigma). Protein molecular weight markers were from Amersham Biosciences.

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| TXB151   | AAA75595              | 5A + 3B | 311–747              | RKMEDG6055 |
| USP25    | AAFA52263             | 1A     | 542–747              | none |
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> The frequency each cDNA was isolated using baits A and B.

> The PP1 cDNA isolated encodes a deletion at Ala<sup>109</sup>; a polymorphism also found in GenBank no. AK027086 and six additional ESTs.

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**Fig. 3.** Subdomain V of tankyrase binds to hTRF1, IRAP, and TAB182.

Extracts of cells overexpressing FLAG-tagged subdomain V of tankyrase-1 (A) or -2 (B) were incubated with resins coated with the indicated GST fusion proteins. Resin-bound proteins (lanes 1–4) and 15% of the input were immunoblotted in 10% gels using an anti-FLAG antibody as described under “Experimental Procedures.” GST fusions (5 µg/lane) are shown in the Coomassie Blue-stained 10% gel (C). The diagram at the top depicts subdomain V in the context of the entire ANK domain. The ANK repeats (rectangles) and insertions within the repeats (ovals) are numbered according to Ref. 9.
had been $^{35}$S-labeled in vitro (Fig. 1, lane 1). To pinpoint residues critical for binding tankyrase-2, we introduced alanine substitutions across this hexapeptide. Fig. 1 shows that substitutions at position 2 or 3 of this hexapeptide did not affect tankyrase-2 binding (lanes 3–4). By contrast, the binding was attenuated by substitution at position 4 (lane 5) and was abolished by those at positions 1, 5, and 6 (lanes 2, 6, and 7). Therefore, the minimal tankyrase-binding sequence as defined in IRAP is $R_{XX}PDG$, and the proline requirement at position 4 is not absolute. To validate this motif as a predictor of tankyrase-2 binding, we found that tankyrase-2 bound to GST fused with other $R_{XX}PDG$ sequences such as $R_{D}DM PDG$, $R_{D}TDG$, and $R_{Q}QPDG$ (residues matching the motif are in boldface), but not a $KGKPDG$ hexapeptide that deviated from the motif (data not shown). Therefore, the $R_{XX}PDG$ motif is a useful predictor for tankyrase-2 binding in vitro.

An $RXXPDG$ Motif in TAB182 Also Binds to Tankyrase—The $RXXPDG$ motif is expected based on its complexity to occur once every 204 aa; its probability of chance occurrence within a given 100-aa region is therefore 0.06%. Interestingly, TAB182 harbors this motif as $R_{PQ}PDG$ in the 93-aa region (aa 1450–1542) that binds tankyrase (5). To verify that TAB182 uses this $R_{XX}PDG$ sequence to bind tankyrase, we expressed FLAG-tagged tankyrase-1 and -2 in transfected cells and incubated cell extracts with GST fusions of various TAB182 sequences. Using GST as a control, Fig. 2 shows that GST-TAB1821450–1542 bound to FLAG-tankyrase-1 and -2 as efficiently as did GST-IRAP2–109 (lanes 2–3), and this binding was abolished by a Gly 1513 $\rightarrow$ Ala substitution at position 6 of the $RXXPDG$ motif in TAB1821450–1542 (lanes 3 versus 5).

**Fig. 4.** Tankyrase interacts with NuMA in transfected cells and in vitro. A–C, BOSC cells were cotransfected with the indicated combinations of FLAG-tagged full-length tankyrase-1 and -2, GST-NuMA1605–2016, and control GST vector (pEBG) as described under “Experimental Procedures.” Cell extracts were resolved in a 10% SDS-gel and immunoblotted with an anti-FLAG antibody (A). Extracts were also affinity-purified using glutathione resins, and resin-bound proteins were immunoblotted in 10% SDS-gels with either anti-GST (B) or anti-FLAG antibodies (C). D, tankyrase-2 labeled in vitro with $[35]$S-methionine was incubated with resins containing GST-RTQPDG (lane 1) or GST-RDTADG (lane 2) as described under “Experimental Procedures.” Resin-bound $[35]$S-tankyrase-2 and 20% of the input tankyrase-2 were resolved in a 6.5% SDS gel and autoradiographed.

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**Fig. 5.** Conservation of the $R_{XX}PDG$ motif. For each tankyrase partner, sequences of various species available in the expressed sequence tag and SwissProt databases are aligned around the $RXXPDG$ motif shown in boldface. GenBank™ accession numbers are shown to the right. TRF1 was aligned according to Refs. 21 and 28; dashes denote a gap.

| **IRAP**       | Human  | NSATGYRQSPDGACSVPS | CAB61646 |
|----------------|--------|---------------------|-----------|
|                 | Mouse  | NSAARYRQSPDGTCSLPS  | BB617405  |
|                 | Rat    | NSATGYRQSPDGTCSVPS  | AAB19066  |
|                 | Chicken| NNMSVRQSPDGCSSVPS   | B1065249  |

| **TAB182**     | Human  | DSPSWRPQPDGGEASQTE | AF441771 |
|----------------|--------|---------------------|-----------|
|                 | Mouse  | APLPSSRPQPDGGEASQVE| AAH25943 |
|                 | Cow    | DPLPSPRPQPDGEASWTD | B1847826 |
|                 | Pig    | DLPSPSPRPQPDGEASRTE| BB612961 |

| **NuMA**       | Human  | ITSSLKPRTPDGVSTGVE | S23647   |
|----------------|--------|-------------------|----------|
|                 | Mouse  | VASKLPRTPDGVSTGVE | AAH06631 |
|                 | Cow    | VTSKLPRTPDGVSTGVE | BM031539 |

| **TRF1**       | Human  | MAEDVSSAAPSPRGCADGRADPTEEQMAE | XP_005140 |
|----------------|--------|--------------------------------|-----------|
|                 | Mouse  | MAETVSSAAP-------------------------RAAPSREGWTS | P70371   |
|                 | Chinese| MAEDVSSATAPSPRGCADGRADPTEEQMAQ | AAC02531 |

**TAB182** Also Binds to Tankyrase—The RXXPDG motif is expected based on its complexity to occur once every 204 aa; its probability of chance occurrence within a given 100-aa region is therefore 0.06%. Interestingly, TAB182 harbors this motif as $RPQPDG^{218}$ in the 93-aa region (aa 1450–1542) that binds tankyrase (5). To verify that TAB182 uses this RXXPDG sequence to bind tankyrase, we expressed FLAG-tagged tankyrase-1 and -2 in transfected cells and incubated cell extracts with GST fusions of various TAB182 sequences. Using GST as a control, Fig. 2A shows that GST-TAB1821450–1542 bound to FLAG-tankyrase-1 and -2 as efficiently as did GST-IRAP2–109 (lanes 2–3), and this binding was abolished by a Gly{sup 1513} $\rightarrow$ Ala substitution at position 6 of the RXXPDG motif in TAB1821450–1542 (lanes 3 versus 5). The
TAB182 RXXPDG hexapeptide by itself was comparable with TAB182 R150–154 in binding to tankyrase (Lanes 3–4). Therefore, the RXXPDG motif in TAB182 is both necessary and sufficient for tankyrase binding.

**An RXXPDG Variant in Human TRF1 Binds to Tankyrase—** Human TRF1 does not match the RXXPDG motif despite its acidic domain (aa 1–68) binding to both tankyrase-1 and -2 (1, 9). However, this hTRF1 domain does contain an RGCADG18 hexapeptide that matches the motif except at position 4, where the proline requirement is not absolute (Fig. 1). To establish RGCADG18 as an RXXPDG variant that enables hTRF1 to bind tankyrase, GST fusions containing various hTRF1 sequences were incubated with transfected FLAG-tankyrase-1 and -2. Fig. 2B shows that tankyrase bound to hTRF1 acidic domain, and this binding was abolished by a Gly18 → Ala substitution at position 6 of its RXXPDG motif (lanes 2 versus 4). Compared with the acidic domain, the hTRF1 hexapeptide RGCADG by itself was less efficient in binding tankyrase-1 and -2 (lanes 2 versus 3). Therefore, hTRF1 uses a variant RXXPDG motif to bind tankyrase, and this binding is strengthened by sequences flanking the motif.

**ANK Subdomain of Tankyrase Does Not Distinguish among Binding Partners—** The binding of tankyrase to a motif shared by hTRF1 and TAB182 was unexpected because a yeast two-hybrid study showed that ANK subdomains I and V of tankyrase-1 interacted with hTRF1 but not with TAB182 (5), which implies a mechanistic distinction in how hTRF1 and TAB182 bind tankyrase. To investigate the binding selectivity of ANK subdomains in tankyrase, FLAG-tagged subdomain V of tankyrase-1 and -2 was expressed and incubated in vitro with GST fusion proteins containing the tankyrase-binding domain of IRAP, TAB182, or hTRF1. Using GST as a control, Fig. 3 shows that all three GST fusions affinity-precipitated subdomain V of tankyrase-1 and -2 with comparable efficiency. We therefore conclude that ANK subdomain V lacks partner selectivity.

**Tankyrase Interacts with Additional RXXPDG-containing Proteins—** The RXXPDG motif resides in nearly 0.5% of the proteins in the Swiss-Prot Database (www.expasy.ch/prosite), some of which might be novel partners of tankyrase. We therefore screened a cDNA library in a yeast two-hybrid system using two tankyrase-2 baits that contained overlapping sets of ANK repeats. Ten distinct cDNA clones isolated a total of 33 times were found to interact with either or both tankyrase-2 baits but not with the control bait TPK-2 (Table I). These clones encoded fragments of seven proteins (overlapping fragments in three cases), each fused in-frame to the GAL4 activation domain. Interestingly, the RXXPDG motif was found in four of these proteins: NuMA, Hox-B2 (a homeobox protein) (15), a regulatory subunit of the protein phosphatase PP1β (16), and the t-type calcium channel (in the cytosolic tail of its α1 subunit) (17). Two additional proteins matched the motif except at position 4 (as in hTRF1): the deubiquitinating enzyme USP25 (18) and the Tax1-binding protein TXBP151 (19). Therefore, the RXXPDG motif or its position 4 variant is present in all tankyrase interactors in Table I, with the exception of fibulin-2 (20). Serendipitously, fibulin-2 is also the only secreted protein, which precludes a meaningful interaction with tankyrase.

**Tankyrase Interacts with NuMA in Cultured Cells and in Vitro—** NuMA was chosen from Table I for further study based on its known colocalization with tankyrase at spindle poles (6). This 240-kDa protein shuttles between mitotic spindle poles and interphase nuclei, and its cell cycle-regulated targeting is specified by the globular tail domain (NuMA aa 1700–2115) (reviewed in Ref. 7). Interestingly, this NuMA-targeting domain harbors an RXXPDG motif (RTQPDG1748) and largely coincides with the tankyrase-binding region (aa 1605–2016) as described by our yeast two-hybrid screen (Table I). To establish NuMA as a tankyrase interactor, cultured cells were co-transfected with GST-NuMA1605–2016 and either FLAG-tagged tankyrase-1 or tankyrase-2. Fig. 4 (panels A and B) confirms the expression of transfected proteins; Fig. 4C shows that GST-NuMA1605–2016 but not the GST control co-precipitated tankyrase-1 and -2 (lanes 1–2 versus 3–4). To implicate the NuMA RXXPDG motif in tankyrase binding, we incubated a GST fusion of the NuMA hexapeptide RTQPDG1748 in vitro with 35S-labeled tankyrase-2. Fig. 4D shows that this NuMA sequence but not the control sequence RDTAGD affinity-precipitated tankyrase-2. Therefore, NuMA is indeed a novel RXXPDG-based partner of tankyrase.

**Mouse TRF1 Lacks the RXXPDG Motif, and Its Acidic Domain Does Not Bind Tankyrase—** This study has thus far examined proteins of human origin only. However, we expect mouse tankyrases to also recognize the RXXPDG motif because the RXXPDG hexapeptide RQSPDG binds to mouse tankyrase-1 in fibroblast extracts (9) and because mouse tankyrase-2 is 98.4% identical to human tankyrase-2 in the ANK domain (data not shown). Thus, mammalian tankyrases are likely conserved in their binding to the RXXPDG motif. This RXXPDG motif is strictly conserved by all available species of IRAP, TAB182, and NuMA (Fig. 5), suggesting that their tankyrase binding is conserved by diverse species. In contrast, the RXXPDG hexapeptide of human TRF1 is conserved by hamster, but is missing from mouse TRF1 along with a contiguous tripeptide (Fig. 5). In fact, this divergence involving the RXXPDG motif accounts for the largest gap in the published hTRF1-mTRF1 alignment (21). This gap does not reflect a splicing variant as it affects all mTRF1 expressed sequence tag clones sequenced to date (alignment not shown).

Because tankyrase binds to hTRF1 only in the acidic domain (1) and this binding depends on the RXXPDG motif therein (Fig. 2B), the absence of this motif in mTRF1 suggests that mTRF1 might not bind to tankyrase. To verify this species specificity, we incubated mouse fibroblast extracts with GST fusions of either human or mouse TRF1 acidic domain. Fig. 6 shows that hTRF1 bound to both mouse tankyrase-1 and -2, whereas mTRF1 bound to neither. The same result was ob-
tained using three independent GST-mTRF1 constructs, one of which was verified by DNA sequencing. Therefore, the lack of RXXPDG in mTRF1 acidic domain correctly predicts its inability to bind tankyrase.

**DISCUSSION**

This study describes an RXXPDG motif in IRAP, TAB182, and human TRF1 that binds the ANK domain of tankyrase. Our yeast two-hybrid screen also revealed this RXXPDG motif in additional candidate interactors of tankyrase. Moreover, we established NuMA as an RXXPDG-containing partner of tankyrase.

ANK repeats form the protein-protein interacting domain of numerous molecules; at least four repeats are required to form an ANK domain (22). The ANK domains in the p53-binding protein 53BP2 and in the cytoskeletal protein ankyrin are known to bind to diverse partners. More specifically, 53BP2 binds to p53, Bcl2, and the catalytic subunit of the protein phosphatase PP1 (23, 24), whereas ankyrin binds to at least seven distinct membrane proteins (referenced in Ref. 25). However, neither 53BP2 nor ankyrin has a known binding motif that unifies its diverse partners. Therefore, tankyrase is unique in having a defined binding motif that is critical for binding IRAP, TAB182, and hTRF1 (3) (Fig. 2). In IRAP and TAB182, a hexapeptide sequence matching the motif is sufficient by itself for efficient tankyrase binding (3) (Fig. 2A). By contrast, hTRF1 contains a variant RXXPDG hexapeptide that requires flanking sequences for efficient tankyrase binding (Fig. 2B).

Our biochemical analysis showed that IRAP, TAB182, and hTRF1 all can interact with a minimal binding site (subdomain V) in tankyrase, consistent with their using the same RXXPDG-dependent binding mechanism (Fig. 3). This finding contradicts a previous yeast two-hybrid assay where subdomain V of tankyrase interacts with hTRF1 but not with TAB182 (5). Of note, when assayed in yeasts for the interaction with various tankyrase regions, hTRF1 systematically scores higher than TAB182 (up to 16-fold), but its interaction with subdomain V is relatively weak (5). It is therefore not surprising that the yeast system fails to detect the TAB182 interaction of subdomain V.

The specificity of human tankyrases for RXXPDG is apparently conserved by mouse tankyrases, as evidenced by the inability of the latter to bind the RXXPDG-less mTRF1 (Fig. 6). Because telomeric targeting of human tankyrase requires TRF1 binding (6), lack of this binding explains the absence of detectable tankyrase at mouse telomeres (8) and implies that tankyrase does not regulate telomerases in mice as it does in humans.

The RXXPDG motif is not always an accurate predictor for tankyrase binding, because its residues 2 and 3 are not entirely neutral, as evidenced by the failure of the RXXPDG hexapeptide REYPDG to bind to tankyrase (data not shown). Moreover, the context of a motif may preclude its interaction with tankyrase. For example, the hexapeptide RDTPDG by itself can bind tankyrase, but an SH2 domain containing this hexapeptide cannot bind (GenBank™ CAA56868; data not shown). As another caveat, Grb14 (an adapter for growth factor receptors) lacks a recognizable RXXPDG motif but nevertheless binds to the ANK domain of tankyrase-2 (10). In this unusual case, it remains to be shown if Grb14 uses a degenerative RXXPDG sequence (such as its LPLPDG hexapeptide) or an unrelated mechanism to bind tankyrase. Despite these caveats, RXXPDG is clearly important in allowing tankyrase to bind many partners, including NuMA (Fig. 4).

The significance of tankyrase binding to NuMA remains unknown. Given their mitotic colocalization at spindle poles (6), we propose that tankyrase acts as a spindle-pole scaffold that recruits NuMA upon its mitotic release from the nucleus. This notion is supported by tankyrase binding to the polar targeting domain of NuMA (Fig. 4) (7). Moreover, the RXXPDG sequence of NuMA (RTQPDG1748, Fig. 4D) resides in a small region (an 1667–1766) that, when deleted, impairs the polar targeting of NuMA (Ref. 26 but also see Ref. 27).

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