Chapter 4
TIPs: Tankyrase Interacting Proteins

Susan Smith

Abstract Tankyrase 1 and tankyrase 2 are "card carrying" members of the poly(ADP-ribose) polymerase (PARP) family of enzymes. PARPs use NAD^+as a substrate to generate ADP-ribose polymers on protein acceptors. For over thirty years PARP-1 reigned supreme as the original and only known protein with this unusual enzymatic activity. Then, beginning in 1998 new functionally distinct PARPs, tankyrase 1 among them, were reported. Tankyrase 1 was found in a two-hybrid screen with the telomere-specific DNA binding protein TRF1. Subsequently in 2000, a closely related homolog tankyrase 2 was found in a two-hybrid screen with the insulin-responsive amino peptidase (IRAP). Tankyrases have a catalytic PARP domain in common with PARP-1, but are distinguished by a large ankyrin repeat domain that serves as a platform for numerous, diverse protein binding partners, resulting in a remarkable range of biological activities involved in telomere function, inherited disease, and cancer. With the recent discovery of potent tankyrase-specific small molecule inhibitors, understanding the diverse functions of tankyrases has become more than just a fascinating cell biological puzzle. Elucidation of tankyrase function will pave the way for future therapeutic strategies, while at the same time provide insights into potential deleterious side effects.

Keywords Tankyrase · PARP · Telomere · Cohesion · Mitosis

4.1 Introduction

Tankyrase 1 and 2 are closely related proteins encoded by distinct genes, TNKS and TNKS2, located on human chromosomes 8 and 10, respectively [1, 2]. The tankyrases have a similar primary structure comprised of the signature C-terminal catalytic PARP domain, a SAM (sterile alpha module) domain, and an ankyrin domain comprised of 24-ankyrin repeats ([3], Fig. 4.1). Tankyrase 1 has an amino-terminal domain comprised of homopolymeric tracts of His, Pro and Ser (HPS) that is missing in

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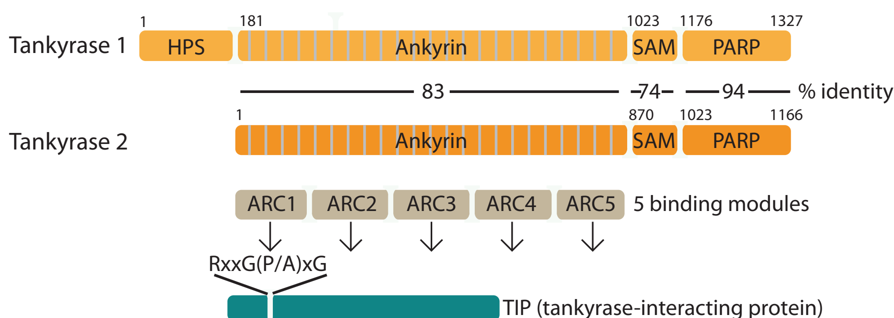

Fig. 4.1 Primary structure of human tankyrase 1 and tankyrase 2. HPS, his pro ser; ankyrin, ank repeat domain; SAM, sterile alpha module; PARP, poly(ADP-ribose) polymerase; ARC, ank repeat clusters

tankyrase 2. Like PARP-1 tankyrases undergo auto-PARsylation, but unlike PARP-1 the activity is not stimulated by DNA ends [3, 4]. Tankyrases can homo- and hetero-dimerize through the SAM domains, however little is known about the regulation or frequency of these interactions in vivo [5–7]. Finally, it is the ankyrin domain that endows the tankyrases with their remarkable diversity. The 24 ankyrin repeats cluster into five conserved domains termed ANK repeat clusters (ARCs), each capable of binding the interacting partner TRF1 ([8], Fig. 4.1). Tankyrase binding to TRF1 requires a six amino acid sequence RGCADG near the TRF1 N-terminus [9]. Subsequent studies have revealed a great number of tankyrase binding partners. All of them bind to tankyrase through the ARCs and they do so with a conserved six amino acid motif RXXG(P/A)XG [9, 10]. The high conservation (83 % identity) between the ankyrin domains of tankyrase 1 and 2 predict that they will bind the same binding partners and indeed, this is the case (where it has been addressed).

What then is the distinction (if any) between tankyrase 1 and 2? The genes are ubiquitously expressed in most human cell lines and tissues [4, 11–13]. Tankyrase 1 protein appears to be more abundant than tankyrase 2, but this has not been rigorously addressed; it may simply be the robustness of tankyrase 1 antibodies. Depletion of tankyrase 1 in human cells has led to striking phenotypes including, persistent sister telomere cohesion [14] mitotic arrest in HeLa cells [14, 15] and mitotic delay in other human cell lines [16]. Whether tankyrase 2 can substitute for tankyrase 1 (if expressed at the same level) or if it has distinct functions has not been determined. Mice lacking PARP-active tankyrase 2 are viable and fertile [17, 18] but have a short stature mouse phenotype. Mice deficient in tankyrase 1 appear to develop normally and have no defects in body size [19] but suffer from a metabolic disorder [20] The double knockout is embryonic lethal, indicating functional redundancy in mouse [19]. Whether a similar scenario holds in human cells, remains to be determined. Perhaps in the future we will be able to distinguish between tankyrase 1 and 2 using small molecule inhibitors. Indeed, one of the most exciting discoveries in the field over the last few years has been the identification of tankyrase specific small molecule inhibitors [21]. However, while these inhibitors can distinguish tankyrases from other PARPs, they do not distinguish between tankyrase 1 and 2.

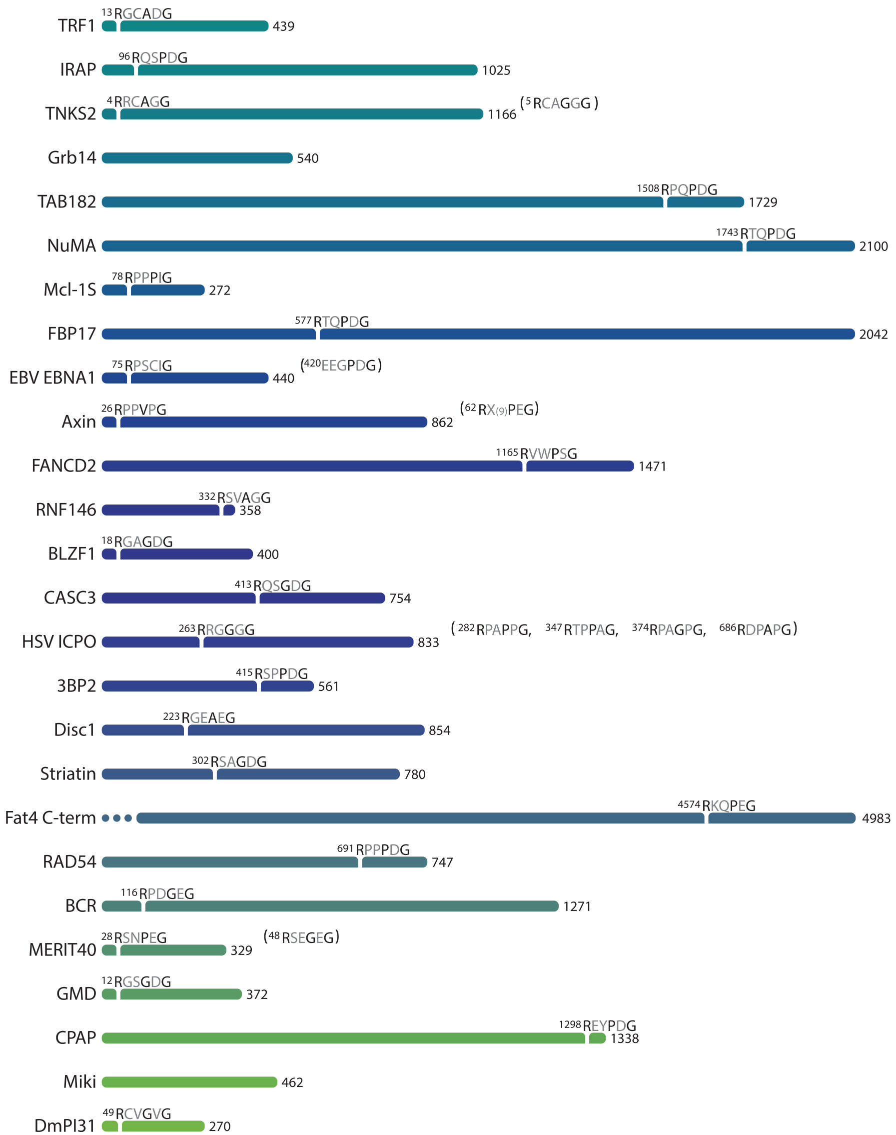

Fig. 4.2 TIPs (tankyrase interacting proteins) listed chronologically according to their report in Pubmed. All proteins listed are from human except for the viral proteins, EBV EBNA1 and HSV ICPO, and the Drosophila protein DmPI31

Hence, a complex picture emerges where we have two closely related homologs that may or may not be redundant in human cells and that have the capacity to bind the same partners. The list of validated tankyrase binding partners continues to grow, adding to the complexity. Here I will describe all the TIPs (Tankyrase Interacting Proteins) based upon their chronological report in the scientific literature (Fig. 4.2).

I will confine the list to those partners that have been validated, not just reported in a proteomic or genetic screen. The list, as I think you will see, is remarkable with respect to its range of subcellular localizations, functions, and impact on human disease.

4.2 Chronological list of TIPs

4.2.1 TRF1

Tankyrase 1 was identified in a two-hybrid screen [3] with TRF1, a TTAGGG repeat binding protein that coats the telomere repeats and regulates telomere elongation by telomerase [22]. Based on its TRF1 interaction, 24-ankyrin repeats, and PARP domain it was named tankyrase (*T*RF1-interacting, *ankyr*in-related poly(ADP-ribose polymer*ase*). Tankyrase was renamed tankyrase 1 upon identification of its homolog, tankyrase 2. Tankyrase 1 coimmunoprecipitated with TRF1 from human cells [4]. In vitro analysis with recombinant proteins showed that tankyrase 1 PARsylated itself and TRF1 in vitro and this modification inhibited TRF1 binding to telomeric DNA [3]. Tankyrase binds to TRF1 through an RGCADG motif at amino acid position 13; a G18 A mutation in TRF1 abrogates binding to tankyrase [9]. Each of the five ARCs in the tankyrase ankyrin domain can bind TRF1 [8]. Immunofluorescence analysis of endogenous tankyrase 1 showed that it localized to telomeres in prometaphase spreads [3, 4] to spindle poles during mitosis [23] and throughout the cytoplasm during interphase [23]. Insights to the function of tankyrase 1 came initially from overexpression studies. Transfected tankyrase 1, which lacks a nuclear localization signal (NLS), localizes to the cytoplasm. However, if artificially endowed with a NLS, transfected tankyrase 1 localizes to the nucleus where it PARsylates TRF1 leading to its eviction from telomeres [24]. Telomere unbound TRF1 is then subject to ubiquitination and degradation by the proteasome [25]. Loss of TRF1 permits telomere elongation by telomerase. Long-term overexpression of tankyrase 1 leads to telomere elongation, dependent on tankyrase 1's catalytic PARP activity and on telomerase [4, 24, 25]. Conversely, long-term partial knockdown of tankyrase 1 in telomerase positive cells leads to telomere shortening [26]. Together these data suggest a model where tankyrase 1 functions as a positive regulator of telomere length. According to this model, telomeres are normally held in a repressed state by the negative regulator TRF1. PARsylation of TRF1 by tankyrase 1 releases the repression allowing access to telomerase.

Can this same function be ascribed to tankyrase 2? Tankyrase 2 binds and PARsylates TRF1 and (when overexpressed in the nucleus) it can evict TRF1 from telomeres and induce telomere elongation [4]. However, endogenous tankyrase 2 has not been detected at human telomeres and knockdown studies in human cells have not been reported, hence the role of tankyrase 2 at telomeres remains to be determined. Is the tankyrase -TRF1 interaction conserved in mouse? Remarkably,

despite the high conservation of tankyrases and TRF1 between mouse and human [27] the RGCADG site is deleted in mouse TRF1 and mouse TRF1 does not bind tankyrase [9]. Mouse TRF1 cannot recruit tankyrase to telomeres [27] overexpression of tankyrase I in mouse cells does not evict TRF1 from telomeres [26], and tankyrase 1 (while robust at human telomeres in spermatocytes) could not be detected at telomeres in mouse spermatocytes [28]. Thus, the telomeric function of tankyrase 1 may not be conserved in mouse.

RNAi depletion analysis revealed an unexpected activity for tankyrase 1 in the resolution of sister chromatid cohesion, specifically at telomeres [14]. Sister chromatids are held together from the time of their replication in S phase until their separation at mitosis by protein complexes termed cohesins [29]. In vertebrates cohesins are removed in two stages, first in prophase from chromosome arms and second in metaphase from centromeres, reviewed by [30]. Depletion of tankyrase 1 in HeLa cells by siRNA resulted in mitotic arrest [14, 15]. Live image analysis of tankyrase 1 siRNA cells showed that chromosomes aligned normally on the metaphase plate, but then struggled and were unable to segregate to daughter cells [14, 16]. Fluorescent in situ hybridization with chromosome specific probes showed that in tankyrase 1 siRNA cells sister arms and centromeres separated, but sister telomeres remained cohered [14]. Subsequent studies found that TRF1 (along with its binding partner TIN2 and the cohesion subunit SA1) mediate telomere cohesion [31–33]. Cell cycle analysis indicated that sister telomere cohesion was resolved in late G2/early mitosis, dependent on tankyrase 1 [31]. Chromatin immunoprecipitation (ChIP) analysis showed that endogenous tankyrase 1 localized to telomeres during precisely the same window of the cell cycle [34]. Persistent telomere cohesion could be rescued by tankyrase 1 dependent on its catalytic activity [31]. Together these data indicate that tankyrase 1 localizes to telomeres (most likely upon nuclear envelope breakdown) to resolve telomere cohesion prior to mitosis.

Is tankyrase 1 required for mitotic progression? While RNAi-mediated depletion of tankyrase 1 in HeLa cells led to a robust mitotic arrest, most other human cell lines tested showed robust persistent telomere cohesion, but no apparent mitotic arrest [35]. Careful examination by live cell imaging revealed a transient anaphase delay in all other tankyrase 1 depleted cell lines; chromosomes aligned on the metaphase plate, struggled to separate, but ultimately exited mitosis [16]. Hence the requirement for tankyrase 1 in sister telomere resolution and the subsequent anaphase delay caused by unresolved telomere cohesion appears to be a general phenotype, where as the mitotic arrest is specific to HeLa cells.

Finally, studies have shown that normal human fibroblasts accumulate persistent telomere cohesion in mitosis at late population doublings prior to senescence [36, 37]. Live cell imaging revealed an anaphase delay in aging fibroblasts similar to tankyrase 1 depleted cells [16]. As human cells age, telomeres shorten and accumulate DNA damage [38, 39]. The accompanying persistent telomere cohesion could be a response to this damage and the ensuing anaphase delay may provide an opportunity for repair. Interestingly, despite the fact that tankyrase 1 is expressed in aging cells, overexpression of tankyrase was able to partially rescue the persistent

cohesion in aging fibroblast [16] suggesting that tankyrase 1 function (regarding resolution of cohesion) may be impaired in aging cells. It will be interesting to determine if tankyrase 1 localization or activity is influenced during senescence.

4.2.2 IRAP

IRAP (insulin responsive amino peptidase) is an integral membrane protein that localizes to GLUT4 storage vesicles, specialized endocytic vesicles that are sequestered in the trans Golgi-reticulum in insulin responsive tissues, reviewed by [40]. In response to insulin, these vesicles translocate to the cell surface to facilitate glucose uptake. A two-hybrid screen with IRAP as bait identified tankyrase as a binding partner [9]. Tankyrase 2 (not 1) was identified in the screen, but all subsequent analysis on the tankyrase-IRAP interaction was performed with tankyrase 1. Tankyrase 1 binds to IRAP through a RQSPDG motif in the cytoplasmic tail of IRAP at amino acid position 96; a G106A mutation in IRAP abrogates binding to tankyrase [41]. Tankyrase 1 coimmunoprecipitates with IRAP and PARsylates IRAP in vitro. Immunofluorescence analysis indicates that endogenous tankyrase 1 localized to the vesicles with GLUT4 in 3T3-L1 adipocytes and subcellular fractionation indicated that tankyrase 1 partially fractionated with the low density microsomal fraction [41]. More recent functional studies showed that knockdown of IRAP or tankyrase 1 (using siRNA) attenuated insulin stimulated GLUT4 vesicle translocation, suggesting that tankyrase 1, via its association with IRAP, may influence GLUT4 vesicle trafficking [42]. TNKS1 deficient mice show an increase in insulin-stimulated glucose utilization. Although TNKS1 deficiency did not compromise insulin-stimulated GLUT4 translocation in primary adipocytes, it led to the post-transcriptional upregulation of GLUT4 [20].

4.2.3 Tankyrase 2

Tankyrase 2 was identified in two-hybrid screens using three distinct baits: IRAP [41] Grb14, an endosomal adapter protein [12] and the telomeric protein, TRF1 [1]. In addition, two serological screens of expression libraries yielded tankyrase 2 [11, 13]. Tankyrase 2 recognizes the same RXXG(P/A)XG motif-containing partners as tankyrase 1. Coimmunoprecipitation analysis indicated that full-length tankyrase 1 and 2 associate in vivo [7]. Subsequent analysis indicated the SAM domains were capable of interacting [6], however whether the SAM domain is required for interaction of the full length proteins in vivo was not determined. Interestingly, while tankyrase 1 lacks TNKS-binding motifs, tankyrase 2 contains two overlapping motifs at its N-terminus. Whether these motifs can mediate interaction between tankyrase 2 and itself or tankyrase 1 remains to be determined.

4.2.4 Grb14

Grb14 is a SH2-domain containing adapter protein that regulates insulin and type 1 insulin-like growth factor signaling, for review see [45]. Grb14 identified tankyrase 2 in a yeast two-hybrid screen [12]. Transfected Grb14 coimmunoprecipitated transfected tankyrase 2. The interacting domains were mapped by two-hybrid analysis to the N-terminal 110 amino acids of Grb14 and an internal ankyrin domain fragment of tankyrase 2 harboring about 10 Ankyrin repeats [12]. Surprisingly the N-terminal domain of Grb14 does not contain even the most degenerate TNKS binding motif RXXXXG. Future experiments will be required to determine if the Grb14-tankyrase 2 binding reflects a novel mode of interaction that differs from the canonical RXXXXG motif binding to ARCs.

4.2.5 TAB182

TAB182 (tankyrase binding protein of 182 kDa) is a ubiquitously expressed acidic protein with a complex pattern of subcellular localization to the nucleus and cytoplasm that was identified in a two-hybrid screen with tankyrase 1 [8]. The tankyrase 1 ankyrin domain binds to TAB182 through a carboxy terminal RPQPDG motif at amino acid position 1508; a G1513 A mutation in TAB182 abrogates binding [9]. Tankyrase 1 binds directly to TAB182 and PARsylates it in vitro and the endogenous proteins can be coimmunoprecipitated from cells [8]. More recently TAB182 was identified as a novel core complex component of the Ccr4-Not complex, a conserved multifunctional complex important for regulation of mRNA synthesis and decay [46]. Whether tankyrase associates with TAB182 in the context of this complex and how it impacts TAB182 function remains to be determined.

4.2.6 NuMA

NuMA (nuclear mitotic apparatus) is a large coiled-coil protein that shuttles between the nuclear matrix in interphase and the spindle poles in mitosis, for review [43]. Functional studies indicate an essential role for NuMA in mitotic spindle assembly, where it is required to organize and stabilize a focused array of microtubules [44]. NuMA was identified as a tankyrase binding partner by a yeast two-hybrid screen with the tankyrase 1 ankyrin domain as bait [9]. Several other candidates containing tankyrase-binding motifs were identified in this screen, including HoxB2 205-REPPDG, L-type calcium channel 1836-REAPDG, PP1 subunit 609-REAPDG, and USP25 1081-RTPADG [9]. Only NuMA has been further investigated. Tankyrase 1 binds to the RTQPDG motif at amino acid position 1743 in the carboxy terminal domain of NuMA; a P1746A mutation abrogates binding [9]. Endogenous tankyrase

1 colocalizes with NuMA to spindle poles in mitosis, from prophase to anaphase and localization of tankyrase 1 to spindle poles is dependent on NuMA; knockdown of NuMA results in loss of tankyrase 1 from the poles [47]. Tankyrase 1 PARsylates NuMA in vitro and in vivo in mitosis [15, 47]. The endogenous proteins can be coimmunoprecipitated from mitotic cells [47]. In addition to the sister telomere cohesion defects described above, tankyrase 1 knockdown cells have spindle defects [15, 47]. Indeed, tankyrase 1 knockdown cells show defects in assembly of bipolar spindles as well as supernumerary spindles and microtubule defects [15], suggesting a role for tankyrase 1 in mitotic spindle function. Future experiments will be required to determine if PARsylation of NuMA by tankyrase 1 is required for normal spindle function.

4.2.7 Mcl-1

Mcl-1 (myeloid cell leukemia-1) is an anti-apoptotic Bcl-2 family protein discovered as an early induction gene during leukemia cell differentiation, reviewed in [48]. Tankyrase 1 was identified in a yeast two-hybrid screen with Mcl-1 [49]. Transfected tankyrase 1 coimmunoprecipitated Mcl-1. The interaction was mapped to the ankyrin repeat domain of tankyrase 1 and to amino acids 76–100 of Mcl-1, which contains a RPPPIG motif [49]. Tankyrase 1 bound Mcl-1 in vitro, but did not PARsylate it. In fact, Mcl-1 inhibited tankyrase 1 PARsylation of itself and of TRF1. Overexpression of tankyrase 1 lead to decreased Mcl-1 protein levels, independent of its catalytic PARP domain. Hence, tankyrase 1 may influence the apoptosis pathway through Mcl-1, but the mechanism remains to be determined.

4.2.8 FBP17

FBP17 (formin-binding protein 17) was identified as a mixed lineage leukemia (MLL) fusion partner found in acute myelogenous and lymphoid leukemias [50]. Tankyrase 1 was identified in a yeast two-hybrid screen with FBP17 [51]. The interaction was demonstrated by immunoprecipitation of transfected (as well as endogenous) proteins and by immunofluorescence analysis showing colocalization of the transfected proteins to the cytoplasm. The interacting domains were mapped to the ankyrin domain of tankyrase 1 and to a RESPDG motif at amino acid position 577 in FBP17; an R577 A mutation abrogates binding [51]. More recent studies indicate a role for FBF17 in membrane deformation and actin polymerization [52]. The physiological relevance of the interaction between FPB17 and tankyrase 1 remains to be determined.

4.2.9 EBV EBNA1

EBNA1 (Epstein-Barr virus nuclear antigen 1) is required for maintenance of the Epstein Bar virus (EBV) genome. The possibility of an interaction between tankyrase 1 and EBNA1 was investigated following studies that suggested a role for tankyrase 1 in EBV replication [53]. Transfected full length tankyrase 1 or the ankyrin domain of tankyrase 2 coimmunoprecipitated EBNA1 [54]. Binding was also demonstrated in vitro using purified proteins. EBNA1 was found to contain two domains that were each sufficient for binding dependent on a tankyrase-binding motif: RPSCIG at amino acid 75 and second site and EEGPDG at amino acid 420. Neither site fits the consensus sequence RXXG(P/A)XG. Nonetheless, mutations of G to A in the sixth position of each site eliminated binding and both sites contributed to binding in the context of the whole protein. EBNA1 was PARsylated by recombinant tankyrase 1 in vitro. Tankyrase binding to EBNA1 down regulated replication and plasmid maintenance and this inhibition correlated with PARsylation of EBNA1, suggesting a role for tankyrase in EBV viral replication [54].

4.2.10 Axin

Axin is a key component in the Wnt signaling pathway that has been implicated in many cancers, reviewed in [55]. A chemical genetic screen for inhibitors of the Wnt pathway identified the small molecule XAV939, which was found to inhibit Wnt signaling by increasing axin levels [56]. To identify the targets though which XAV939 increased axin protein levels, affinity capture with immobilized XAV939 was used and resulted in identification of tankyrase 1 and 2. XAV939 inhibits the catalytic PARP activity of tankyrase. Depletion of tankyrases phenocopied XAV939 by increasing axin levels. A small amino terminal region from amino acids 19–30 (containing a RPPVPG at position 26) in axin was necessary and sufficient for tankyrase binding. The study showed that tankyrase (1 or 2) PARsylated axin (1 or 2), leading to ubiquitylation and degradation of axin by the proteasome. It was suggested that tankyrase-mediated PARsylation might directly promote ubiquitylation and degradation, and further that this could be a general regulatory mechanism.

Regarding the mechanism for binding of axin to tankyrase, the crystal structure of the complex confirmed the RPPVPG binding site at position 26 in axin, and additionally identified a non-canonical binding site at position 62, $R(X)_9PEG$ [57].

4.2.11 FANCD2

FANCD2 is a protein mutated in Fanconia Anemia, a recessive human disease associated with DNA damage and repair, reviewed in [58]. Tankyrase 1 was identified in a candidate screen for telomere associated proteins that coimmunoprecipitated

with FANCD2 [59]. The recombinant purified proteins bound in vitro and PARsylation reactions showed that FANCD2 inhibited tankyrase 1 autoPARsylation and TRF1 PARsylation. FANCD2 contains a potential TNKS-binding motif (RVWPSG at position 1165), but it's requirement for binding to tankyrase was not determined.

4.2.12 RNF146

RNF146 (Ring finger protein 146) was identified in a search (siRNA screen) for the E3 ligase that mediates the tankyrase-dependent axin degradation described above [60, 61]. RNF146, also known as Iduna [62], has a Ring finger (E2-binding) domain and a WWE domain that binds poly(ADP)ribose (PAR). RNF146 directly interacts with PAR through its WWE domain and promotes degradation of PARsylated proteins, including axin and tankyrase. Interestingly, RNF146 contains a potential tankyrase binding motif (RSVAGG) in its C terminus at amino acid position 332, raising the possibility that it might interact with tankyrase independent of PARsylation. It has not been determined if this motif can mediate binding to tankyrase 1 independent of PAR.

4.2.13 BLZF1

BLZF1 (basic leucine zipper nuclear factor 1) was identified in a proteomics screen for proteins targeted for degradation by PARsylation. PARsylated proteins were stabilized by depletion of RNF146, isolated using the PAR-binding domain WWE of RNF146, and detected by mass spectrometry [61]. Candidates were then screened for stabilization upon XAV939 treatment. Transfected BLZF1 and tankyrase 1 coimmunoprecipitated dependent on the BLZF1 tankyrase-binding motif RGAGDG at amino acid position 18; deletion of the motif abrogated binding. Deletion of the RGAGDG motif, treatment with XAV939, and depletion of tankyrase 1 and 2, all resulted in stabilization of BLZF1, consistent with the idea that BLZF1 is a target for degradation by tankyrase 1 and RNF146 in a PARsylation-dependent manner. BLZF1 (also known as Golgin-45) plays a role in maintenance of Golgi complex structure [63]. How tankyrase and RNF146-mediated degradation influence BLZF1 function remains to be determined.

4.2.14 CASC3

CASC3 (cancer susceptibility candidate 3) was identified by mining a yeast two-hybrid database for tankyrase interactors and then screening the candidates for stabilization upon treatment with XAV939 [61]. Transfected CASC3 and tankyrase 1 coimmunoprecipitated dependent on the CASC3 tankyrase-binding motif RQSGDG

at amino acid position 413; deletion of the motif abrogated binding. Deletion of the RQSGDG motif, treatment with XAV939, and depletion of tankyrase 1 and 2, all resulted in stabilization of CASC3, consistent with the idea that CASC3 is a target for degradation by tankyrase 1 and RNF146 in a PARsylation-dependent manner. CASC3 is a component of the exon junction complex that functions in post splicing events [64]. How tankyrase and RNF146-mediated degradation influence CASC3 function remains to be determined.

4.2.15 HSV-1 ICP0

HSV-1 ICP0 (herpes simplex virus type 1 immediate-early protein ICP0) is a RING finger protein important for the regulation of viral infection, reviewed in [65]. The role of tankyrase 1 and HSV ICP0 in Herpes Simplex Viral (HSV) infection was investigated [66]. Following infection tankyrase 1 was recruited to foci in the nucleus where it colocalized with HSV ICP0 to the HSV replication compartment. Transfected ICP0 coimmunoprecipitated endogenous tankyrase 1. Treatment of cells with tankyrase inhibitor XAV939 decreased viral titers suggesting a role for tankyrase 1 in HSV replication. ICP0 contains five potential tankyrase binding motifs (RRGGG, RPAPPG, RTPPAG, RPAGPG, and RDPAPG at amino acid positions 263, 282, 347, 374, and 686, respectively) but it was not determined if they are required for tankyrase binding.

4.2.16 3BP2

3BP2 (c-ABL SH3 domain binding protein 2) is an adaptor protein that is part of a signaling complex containing multiple proteins including SRC family kinases. Cherubism, an autosomal dominant syndrome characterized by inflammatory destructive boney lesions, maps to single missence mutations that cluster within an RSPPDG at amino acid position 413 in 3BP2, reviewed in [67], but the disease mechanism was not understood. A yeast two hybrid screen with 3BP2 as bait identified tankyrase 2 [68]. Tankyrase 1 and 2 bind to 3BP2 and binding is disrupted by cherubism mutations in the RSSPDG motif. 3BP2 cherubism mutant proteins are stabilized compared to wild type. Tankyrase 2 PARsylates 3BP2 in vitro and in vivo. Tankyrase 2 promotes ubiquitylation and degradation of 3BP2 dependent on its PARP activity and on the E3 ligase RNF146. Using a mouse model of cherubism, it was demonstrated that cherubism mutations uncouple 3BP2 from tankyrase-mediated protein destruction leading to 3BP2 stabilization and subsequent hyperactivation of key signaling pathways that promote disease.

4.2.17 Disc1, Striatin, Fat4, RAD54, BCR, MERIT40

The structural basis by which tankyrase recognizes its substrates was determined by solving the crystal structure of the tankyrase 2 ankyrin subdomain ARC4 with the 3BP2 binding site [10]. Investigation of additional tankyrase binding partners was undertaken by a combined approach utilizing crystal structures, mutagenesis, and an extensive peptide library. The 6-amino acid tankyrase-binding motif was extending to an 8-residue consensus that indicated a preference for acidic residues at position 8. An in silico prediction yielded 257 candidate tankyrase binding proteins. A subset of six proteins Disc (disrupted in schizophrenia 1), Striatin (a calmodulin binding protein), Fat4 (a protein involved in tumor suppression), RAD54 (a helicase involved in homologous recombination), BCR (breakpoint cluster region protein of the BCR-ABL complex), and MERIT (mediator of Rap80 interactions and targeting 40 kD) validated by fluorescence polarization of the candidate peptide bound to ARC4, were then tested. Each of the six proteins coimmunoprecipitated with cotranfected tankyrase 2, dependent on its tankyrase binding motif. Five of the proteins were PARsylated by tankyrase 2: four (Disc1, Striatin, Fat4, and BCR) robustly and one (MERIT40) weakly. Rad54 was not PARsylated. The role of tankyrase in the function of these six partners remains to be determined.

4.2.18 GMD

GDP-mannose 4,6-dehydratase (GMD) is a cytosolic protein required for the first step of fucose synthesis, reviewed by [69]. GMD was identified in tankyrase 1 immunoprecipitates by mass spectrometry [34]. Endogenous and transfected GMD and tankyrase 1 coimmunoprecipitated. Tankyrase 1 binding to GMD was dependent on an RGSGDG motif at amino acid position 12 in GMD; a DG16AA double point mutation abrogated binding. GMD is complexed to tankyrase 1 in the cytosol throughout interphase. Upon entry into mitosis, tankyrase 1 is released from GMD and associates with its other binding partners TRF1 (at telomeres) and NuMA (at spindle poles). In contrast to most other binding partners, GMD is not PARsylated by tankyrase 1. GMD inhibits tankyrase 1 PARP activity in vitro, dependent on its tankyrase 1 binding motif. Association of tankyrase 1 with GMD in the cytosol may sequester tankyrase 1 in an inactive stable form that can be tapped by other target proteins as needed.

4.2.19 CPAP

Centrosomal P4.1-associated protein (CPAP) is required for procentriole formation and the gene is mutated in primary microcephaly [70, 71]. Transfected CPAP coimmunoprecipitated tankyrase 1, dependent on a C-terminal REYPDG motif at

position 1298; a DG1302AA double point mutation abrogated binding [72]. CPAP was PARsylated by tankyrase 1 in vitro and in vivo. Overexpression of tankyrase 1 led to ubiquitylation and proteasomal degradation of CPAP and prevented centriole duplication. Depletion of tankyrase 1 led to stabilization of CPAP in G1 and elongated procentrioles and multipolarity. Tankyrase 1 localized to centrosomes exclusively in G1, coinciding precisely with the timing of CPAP degradation. Hence, tankyrase 1-mediated PARsylation regulates CPAP levels during the cell cycle to limit centriole elongation and ensure normal centrosome function.

4.2.20 Miki

Miki (mitotic kinetics regulator) is the product of the LOC253012 gene, which is frequently deleted in myelodysplastic syndrome (MDS) [73]. Down regulation of Miki in HeLa cells leads to abnormal mitosis as in MDS cells [21]. Since the phenotype was reminiscent of tankyrase 1-depletion in HeLa cells [14, 25], a connection between Miki and tankyrase 1 was investigated [74]. Immunoprecipitation of extracts with anti-PAR antibody followed by blotting with anti-PAR yielded a 125 kDa band in G2/M phase of the cell cycle. Anti-Miki antibody detected the same band and it was reduced by Miki or tankyrase 1 siRNA. To determine directly if Miki was PARsylated by tankyrase 1, in vitro synthesized Miki (~50 kD) was incubated with immunoprecipitated tankyrase 1 and resulted in a smear at a 125 kDa. No evidence was presented for coimmunoprecipitation/PARsylation of transfected or endogenous tankyrase and Miki or for direct PARsylation of Miki by purified proteins in vitro. Miki does not contain any RXXXXG motifs. Hence, how Miki interacts with tankyrase 1 remains to be determined.

4.2.21 PI31

PI31 (proline-rich inhibitor of 31 kDa) is a conserved proteasome inhibitor. A Drosophila cDNA library screen for direct binding partners of Drosophila PI31 (DmPI31) identified DmTNKS1 [75]. Endogenous or transfected DmTNKS1 and DmP131 coimmunoprecipitated as a complex. Interaction depended on the ankyrin domain of tankyrase and on an RCVGVG motif at position 49 of DmPI31. In addition, point mutations at positions 210 and 241 and deletion of the C-terminal HbYX (hydrophobic residue-tyrosine-any amino acid) motif, which is commonly found in modulators of proteasome activity, abrogated binding to tankyrase 1. DmPI31 was PARsylated in vivo and could be PARsylated in vitro by recombinant tankyrase 1 dependent on it PARP domain. PARsylation of PI31promotes 26 S proteasome assembly and facilitates protein degradation. Inhibition of tankyrase with XAV939 severely affected proteasome assembly, raising the possibility of new therapeutic strategies for proteasome inhibitors in cancer.

Demonstration of the interaction between human PI31 and tankyrase was not shown. Inspection of the human PI31 sequence revealed FGLGVD (instead of RCVGVG), which does not fit the tankyrase consensus binding site. There is however, a potential tankyrase binding site at amino acid position 260 RFDPFG in human PI31. The other residues that were deemed important for DmPI31 binding to DmTNKS1 (L240, F241, and HbYX) are conserved between Drosophila and human PI31, but how these residues contribute to binding between PI31 and tankyrase is not known. Future studies will be required to determine if the interaction between PI31 and tankyrase is conserved in human cells.

4.3 Conclusions

The TIPs described above encompass a broad range of cellular activities. Some of these partners point to new therapeutic strategies for tankyrase inhibitors. Pharmacological inhibition of tankyrases by the specific small molecule inhibitor XAV939 results in dramatic consequences (via stabilization of axins) ranging from targeted killing of tumor cells [56] to enhanced myelin regeneration in nerve cells [76]. The identification of the proteasome regulator PI31 as a target of tankyrase PARsylation [75] raises the possibility of targeting the proteasome, a validated drug target for cancer therapy [77] with tankyrase inhibitors. On the other hand, the essential role of some TIPs predicts that sustained pharmacological inhibition of tankyrase could be detrimental in certain settings. For example, the demonstration that inhibition of tankyrase stabilizes its target 3BP2 leading to activation of the SRC family kinases, predicts that sustained inhibition of tankyrase could lead to the unfavorable effects of osteoporosis, myelomonocytic-mediated inflammation, or tumor promotion [68]. Nonetheless, initial results show that tankyrase inhibitors hold promise for new therapeutic strategies to treat human disease [78].

The identification of RNF146 as the E3 ligase that targets PARsylated proteins for ubiquitylation and degradation by the proteasome adds another level of complexity to the tankyrase story. How does RNF146 it select its targets? Does it physically associate with tankyrases so that it can bind tankyrase target proteins the moment they are PARsylated? Are all of the tankyrase target proteins that are destined for degradation targeted by the E3 ligase RNF146? Axin and 3BP2 are targeted by RNF146 [56, 68]. TRF1 degradation can be promoted by the E3 ligases RLIM [79] and Fbx4 [80] whether RNF146 plays a role has not been determined. Do all proteins that are PARsylated by tankyrase get ubiquitylated and degraded by the proteasome? In one study when candidates of a proteomics screen identifying tankyrase partners were tested for stabilization by XAV939 only 1 in 5 were stabilized [61], suggesting it may only be a small subset. Finally some tankyrase binding partners may not be targets for PARsylation. GMD, a robust binding partner of tankyrase 1 is not PARsylated. In fact it inhibits tankyrase PARP activity [34]. Understanding how GMD inhibits tankyrase could provide insights for designing novel tankyrase inhibitors.

Our list comprises 26 bona fide TIPs. Based on published studies using proteomics, in silico predictions, and two-hybrid screens, there are many more candidates in the running. How should we go forward? One approach might be to glean the common TIPs from all the lists. The best candidates will then have to be investigated the old fashioned way, one by one. It will be important to demonstrate that the proteins interact directly in vitro and that the endogenous proteins interact in vivo. Finally, it will be important to determine how tankyrase influences the function of each of its partners, as was clearly demonstrated with the naturally occurring mutations in 3BP2 in cherubism [68]. Going forward, with new technologies such as CRISPR [81], it should be possible to generate a point mutation in the endogenous TIP's tankyrase binding site to determine the importance of its interaction with tankyrase. All things considered, I think we can look forward to elucidation of the tankyrase proteome and its impact on human health and disease.

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