Title
Therapeutic Strategies for Restoring Tau Homeostasis

Journal
Cold Spring Harbor Perspectives in Medicine, 8(1)

ISSN
0079-1024

Authors
Young, Zapporah T
Mok, Sue Ann
Gestwicki, Jason E

Publication Date
2018

DOI
10.1101/cshperspect.a024612

Peer reviewed

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Therapeutic Strategies for Restoring Tau Homeostasis

Zapporah T. Young, Sue Ann Mok, and Jason E. Gestwicki

Institute for Neurodegenerative Disease, Department of Pharmaceutical Chemistry, Weill Institute for Neurosciences, University of California, San Francisco, San Francisco, California 94158

Correspondence: jason.gestwicki@ucsf.edu

Normal tau homeostasis is achieved when the synthesis, processing, and degradation of the protein is balanced. Together, the pathways that regulate tau homeostasis ensure that the protein is at the proper levels and that its posttranslational modifications and subcellular localization are appropriately controlled. These pathways include the enzymes responsible for posttranslational modifications, those systems that regulate mRNA splicing, and the molecular chaperones that control tau turnover and its binding to microtubules. In tauopathies, this delicate balance is disturbed. Tau becomes abnormally modified by posttranslational modification, it loses affinity for microtubules, and it accumulates in proteotoxic aggregates. How and why does this imbalance occur? In this review, we discuss how molecular chaperones and other components of the protein homeostasis (e.g., proteostasis) network normally govern tau quality control. We also discuss how aging might reduce the capacity of these systems and how tau mutations might further affect this balance. Finally, we discuss how small-molecule inhibitors are being used to probe and perturb the tau quality-control systems, playing a particularly prominent role in revealing the logic of tau homeostasis. As such, there is now interest in developing these chemical probes into therapeutics, with the goal of restoring normal tau homeostasis to treat disease.

THE ROLE OF TAU IN NEURODEGENERATIVE DISEASES

Tau is a microtubule-associated protein that is normally soluble but has a propensity to aggregate into oligomers, paired helical filaments, and neurofibrillary tangles (NFTs). Neurodegenerative diseases that are characterized by the appearance of NFTs are classified as tauopathies, including some forms of Alzheimer's disease (AD), frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), and progressive supranuclear palsy (PSP). A subset of FTDP-17 cases is caused by mutations in tau, providing a direct link between tau and disease (Ghetti et al. 2011). Moreover, the levels of NFT pathology closely correlate with AD progression (Braak and Braak 1991), indicating that normal, wild-type (WT) tau is readily corrupted by the cellular conditions that promote disease. There is also evidence from an AD mouse model indicating that tau is re-

Editor: Stanley B. Prusiner
Additional Perspectives on Prion Diseases available at www.perspectivesinmedicine.org

Cite this article as *Cold Spring Harb Perspect Med* doi: 10.1101/cshperspect.a024612

quired for some aspects of the observed pathologies (Roberson et al. 2007). Recent reviews further detail the links between tau and neurodegeneration (Spillantini and Goedert 2013; Frost et al. 2014) and outline the features of the animal models (see Noble et al. 2010; Clavaguera et al. 2016; Rauch et al. 2016). Here, we will focus on how tau protein levels are maintained, how disease-associated changes in tau disrupt this balance, and how this knowledge can be used to design new therapeutic strategies.

TAU STRUCTURE AND FUNCTION

Tau is a member of a family of microtubule-associated proteins that directly bind tubulin and are implicated in microtubule dynamics. Tau was originally identified as an important factor for microtubule assembly or stability (Weingarten et al. 1975); however, more recent studies argue that this particular function is not essential or is redundant with other microtubule-associated proteins (Qiang et al. 2006; Fanara et al. 2010). Tau expression is primarily restricted to the nervous system, where it is abundant in neurons and is present at much lower levels in glial cells, such as oligodendrocytes and astrocytes (Trojanowski et al. 1989; Shin et al. 1991; LoPresti et al. 1995). In neurons, tau is primarily localized to the axons where it co-localizes with microtubules (Binder et al. 1985). The tau protein is encoded by the *MAPT* gene, and alternative splicing of exons 2, 3, and 10 generates the six main isoforms that are expressed in the adult brain (Goedert et al. 1989). Nuclear magnetic resonance (NMR) studies have shown that tau proteins are disordered in solution, only transiently sampling secondary structures (Mukrasch et al. 2009). Nevertheless, tau sequence characteristics can be used to define major regions within the protein, including an N-terminal domain, a polyproline region, a microtubule-binding repeat (MTBR) domain, and a C-terminal segment (Fig. 1). The MTBR region is composed of imperfect repeat sequences (31 or 32 residues each) that, along with the polyproline region, mediate interactions with microtubules (Mukrasch et al. 2005; Sillen et al. 2007; Fauquant et al. 2011). Differential splicing of exon 10 generates either four or three microtubule-binding repeats (termed 4R or 3R); 4R forms have tighter affinity for microtubules (Goode et al. 2000) and nucleate microtubule assembly better than the 3R isoforms (Goedert and Jakes 1990).

NORMAL PROTEIN–PROTEIN INTERACTIONS WITH TAU

Like many intrinsically disordered proteins, tau interacts with multiple protein partners (Fig. 1) (for an overview, see Mandelkow and Mandelkow 2012). In addition to tubulin, tau also binds actin (Moraga et al. 1993), kinesin (Jancsik et al. 1996; Seitz et al. 2002), and dynactin (Magnani et al. 2007; Patterson et al. 2011). These interactions strongly link tau to trafficking and axonal transport. Indeed, overexpression of tau impairs both vesicle and mitochondrial transport (Ebneth et al. 1998; Stamer et al. 2002; Ittner et al. 2008). Tau also binds to a number of kinases, phosphatases, proteases, and acetylases that are involved in posttranslational modifications. For example, Fyn kinase binds proline-rich (PxxP) motifs in the polyproline region of tau (Reynolds et al. 2008) and phosphorylates its N terminus at Tyr18 (Sharma et al. 2007). Finally, as discussed in greater detail within this review, molecular chaperones are also prominent partners of tau. For example, heat-shock protein 70 (Hsp70) and heat-shock protein 90 (Hsp90) both bind tau within the MTBRs (Jinwal et al. 2013a; Karagoz et al. 2014) and regulate its turnover (Miyata et al. 2011). Together, these protein–protein interactions between tau and its many protein partners help regulate its trafficking, its posttranslational modification, and its turnover. Thus, to understand tau homeostasis, we must understand its interactions with itself (i.e., aggregation) and with its protein partners.

ABERRANT INTERACTIONS OF TAU

One of the hallmarks of tauopathies is that the protein–protein interactions of tau are altered (Mandelkow and Mandelkow 2012), meaning

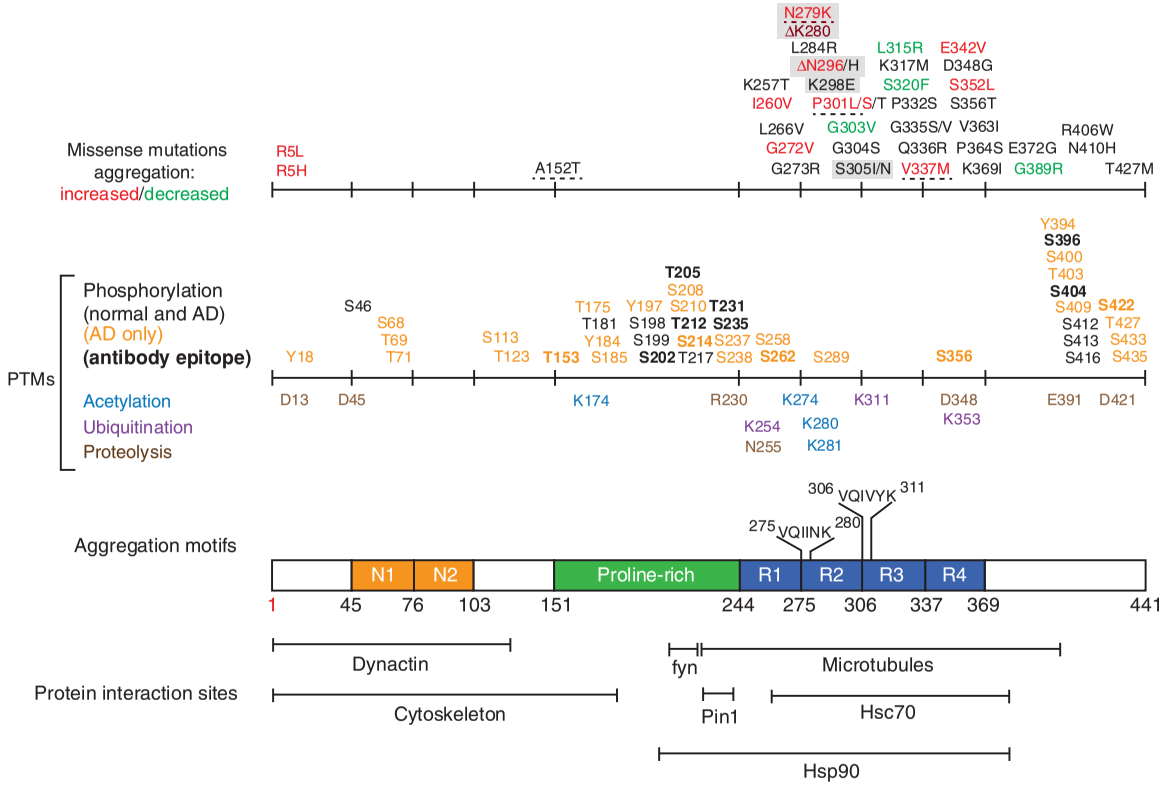

Figure 1. Map of the modifications and interaction sites on the tau sequence. A schematic of the longest adult isoform of tau (2N4R) is depicted. Several regions within the tau sequence are highlighted, including two N-terminal inserts (N1, N2), a polyproline region, and the microtubule-binding repeat region composed of four imperfect repeat sequences (R1–R4). Alternative splicing of N1, N2, or R2 leads to the generation of six tau isoforms expressed in adults. The sequences of the two known aggregation motifs are shown, located within R2 and R3 of tau. All of the known disease-associated missense mutations and the risk factor variant A152T (gray) are mapped. Intronic mutations linked to tauopathies are not shown. Missense mutations observed to kinetically favor increased or decreased aggregation in vitro are indicated by red or green, respectively. Gray boxes show mutations that alter splicing of the R2 repeat. Dashed underlines show mutations that display decreased affinity for microtubules. Posttranslational modifications associated with disease are mapped and color-coded as indicated in the figure. Regions of tau that interact with binding partners are charted *below* the tau sequence schematic.

that some of the interactions are favored and others are reduced. The most dramatic example of this change is the increase in tau–tau interactions, which lead to its aggregation. Two aggregation motifs within the MTBRs are required for this process, and several mutations in this region prevent self-assembly (von Bergen et al. 2000, 2001). However, this region is also required for binding to microtubules. When tau is not in contact with microtubules, these regions acquire an abnormal, β-sheet-rich structure that favors amyloid formation (von Bergen et al. 2001; Goux et al. 2004; Mukrasch et al. 2005). Furthermore, cysteines within the MTBRs can cross-link tau monomers, which also promotes aggregation (Barghorn and Mandelkow 2002). The importance of the MTBRs suggests that the loss of tau–microtubule interactions and the buildup of unbound cytosolic tau may be a step toward aggregation. However, tau is surprisingly soluble in vitro. Accelerants, particularly polyanionic molecules, such as heparin or fatty acid micelles, are required to initiate aggregation in vitro (Chirita et al. 2003). It is thought that a similar aggregation trigger, such as RNA or polyglutamates (Kampers et al. 1996; Friedhoff et al. 1998), might be similarly required in vivo.

Tau aggregation leads to the formation of an ensemble of aggregate structures, including oligomers and fibrils, that are found in tissue samples from AD and PSP (Maeda et al. 2007; Gerson et al. 2014). Although it is not yet clear which tau structures are most toxic, it is likely that oligomers are especially detrimental to cells, because they decrease cell viability more potently than tau fibrils (Flach et al. 2012), and antibodies against oligomers of tau are protective when administered to animal models of tauopathy (Castillo-Carranza et al. 2014, 2015). Furthermore, tau oligomers seem to spread in the brain and in cell culture, exhibiting prion-like characteristics (de Calignon et al. 2012; Lasagna-Reeves et al. 2012; Liu et al. 2012; Clavaguera et al. 2013). A cell-to-cell transmission of tau aggregates fits with the characteristic spreading of pathology from an epicenter to connected brain regions observed in disease (Clavaguera et al. 2016; Rauch et al. 2016).

TAU MUTATIONS AND DISEASE-ASSOCIATED POSTTRANSLATIONAL MODIFICATIONS

Thus far, 53 pathogenic mutations within the *MAPT* gene encoding tau have been reported (Fig. 1) (Ghetti et al. 2015). There are also reports of risk factors linked to *MAPT*, including the A152T variant within the protein coding region (Coppola et al. 2012) and the *MAPT* variation associated with the H1 haplotype (Baker et al. 1999). The pathogenic mutations can be broadly characterized as missense/deletion mutations or intronic mutations. The vast majority of the mutations cluster to the MTBRs, perhaps underscoring the role played by dynamic microtubule binding and self-assembly.

The mechanisms of toxicity that occur owing to *MAPT* mutations remain poorly understood. The intronic mutations, and even some coding mutations, promote inclusion of exon 10 and lead to increased expression of 4R tau (Niblock and Gallo 2012; Qian and Liu 2014). In vitro, 4R tau binds to microtubules with a higher affinity than the 3R isoforms (Goedert and Jakes 1990; Panda et al. 2003), but 4R tau also aggregates with faster kinetics than 3R tau (Barghorn and Mandelkow 2002), and the 3R isoform can inhibit 4R aggregation (Adams et al. 2010). Thus, an imbalance in 3R versus 4R tau seems to contribute to disease. Other coding mutations do not appear to alter splicing. Rather, they reduce microtubule affinity and/or increase aggregation compared with WT (Fig. 1) (Hong et al. 1998; Barghorn et al. 2000; Chang et al. 2008a; Combs and Gamblin 2012; Beharry et al. 2014). It is tempting to speculate that these biochemical features are the major determinants of tauopathy. However, this possibility is unlikely because the age of onset, duration, clinical symptoms, and neuropathology differ among individuals harboring related mutants and sometimes for patients with the same mutation (Ghetti et al. 2011). These data suggest that other factors contribute to tau pathology. One possibility is that environmental or other genetic factors tune the response to tau mutations (Bugiani et al. 1999; Reed et al. 2001; Saito et al. 2002). Another

possibility is that specific mutations alter the protein–protein interaction network of tau (Fig. 1), perhaps releasing proteins that protect against disease and/or favoring partners that are detrimental.

Although mechanistically informative, the vast majority of tauopathies are not associated with tau variants. Rather, abnormal posttranslational modifications appear to be the main culprit. Tau is potentially subject to more than 100 of these modifications, including phosphorylation, acetylation, proteolytic cleavage, glycosylation, ubiquitination, sumoylation, amination, nitration, oxidation, and methylation (Martin et al. 2011; Morris et al. 2015). Increases in tau phosphorylation have been most extensively linked to tau pathology. Tau in the healthy brain is phosphorylated at an average of two to three residues per molecule, whereas tau from the AD brain contains three- to fourfold more phosphorylated residues (Köpke et al. 1993). Indeed, antibodies recognizing phospho-epitopes, such as AT8, AT180, and PHF1, are commonly used to mark neuropathology in all tauopathies (Mercken et al. 1992; Augustinack et al. 2002). However, a clear cause-and-effect relationship between hyperphosphorylation and disease onset remains to be demonstrated, and tau hyperphosphorylation is associated with normal development (Kenessey and Yen 1993) and animal hibernation (Arendt et al. 2003). Despite this finding, there is some evidence that hyperphosphorylated tau has weakened activity in microtubule assembly assays and that it assembles into oligomers more readily (Tepper et al. 2014). Multiple kinases have been reported to phosphorylate tau, but the kinases and sites relevant to disease remain poorly defined (Martin et al. 2011). The kinases GSK3β, Fyn, and Cdk5 have all been implicated in abnormal tau phosphorylation, and inhibitors for these kinases are currently being developed as therapeutics (Höglinger et al. 2014; Kimura et al. 2014; Nygaard et al. 2014). One phosphatase, PP2A, has also been identified as the major tau dephosphatase in vivo, and there are small molecules that enhance the activity of PP2A and reduce tau phosphorylation (Liu et al. 2005; Kickstein et al. 2010; van Eersel et al. 2010). Ubiquitination of tau may serve to mark abnormal or free cytosolic tau for chaperone-mediated degradation (Sahara et al. 2005; Dickey et al. 2006). Multiple ubiquitination sites have been identified by mass spectroscopy analysis of PHF–tau preparations (Morishima-Kawashima et al. 1993; Cripps et al. 2006; Thomas et al. 2012); however, the requirement of these specific sites for the degradation of tau has not been confirmed. At least three acetylation sites on tau, at residues K174, K280, and K281, correlate with other markers of tau neuropathology (Irwin et al. 2012; Min et al. 2015). In vitro, acetylated tau has a decreased propensity to form fibrils (Grinberg et al. 2013; Cook et al. 2014a), perhaps suggesting that acetylation is protective. However, a recent study suggests that acetylation at K174 prevents tau clearance and promotes pathology in mice, and preventing acetylation with acetyltransferase inhibitors ameliorates these phenotypes (Min et al. 2015). Cleaved fragments of tau are released by proteolysis, and these fragments have been observed in NFTs isolated from AD patients (Horowitz et al. 2004; Basurto-Islas et al. 2008). The most well-characterized cleavage site (at D421) is generally attributed to proteolytic activity of caspase 3 (Fasulo et al. 2000), although multiple caspases can cleave the same site in vitro (Gamblin et al. 2003). Cellular assays provide evidence that truncation at D421 leads to defects in microtubule binding (Ding et al. 2006), preferential degradation by autophagy (Dolan and Johnson 2010), mitochondrial dysfunction (Quintanilla et al. 2009), and enhanced secretion from cells (Plouffe et al. 2012). Interestingly, D421 truncated tau has been detected in the cerebrospinal fluid from AD patients, and its levels correlate with cognitive decline (Ramcharitar et al. 2013). Finally, methylation is the main modification of tau lysines observed in the normal brain (Funk et al. 2014), and levels of methylated tau are decreased in AD samples (Thomas et al. 2012).

Similar to that discussed for disease-associated mutations, one can imagine how aberrant posttranslational modifications might weaken favorable protein–protein interactions and/or strengthen self-association. These events might even be synergistic, as shown by observations

that the R406W tau mutation leads to higher rates of tau phosphorylation in vitro (Alonso Adel et al. 2004). The emerging theme is that tau normally engages in a dynamic series of protein–protein interactions and posttranslational modifications. These events are important for the function of tau in trafficking and its normal turnover. However, disruption of this delicate balance, either triggered by aging, mutations, trauma, or another initiating event, leads to a remodeling of these tau-associated protein–protein interactions. The net effect of this abnormal state is that tau becomes aggregated and loses affinity for microtubules.

POINTS OF INTERVENTION FOR TREATING TAUOPATHIES

The path toward the treatment of neurodegenerative tauopathies will likely involve rebalancing tau interactions (Fig. 2). Perhaps the most straightforward approach is to directly inhibit tau aggregation (Bulic et al. 2010). This strategy is discussed in more detail in Rauch et al. (2016). Other initiatives have focused on small molecules that favor the normal protein–protein interactions of tau by stabilizing microtubules (Brunden et al. 2011). The idea behind this approach is that tau cannot aggregate if it

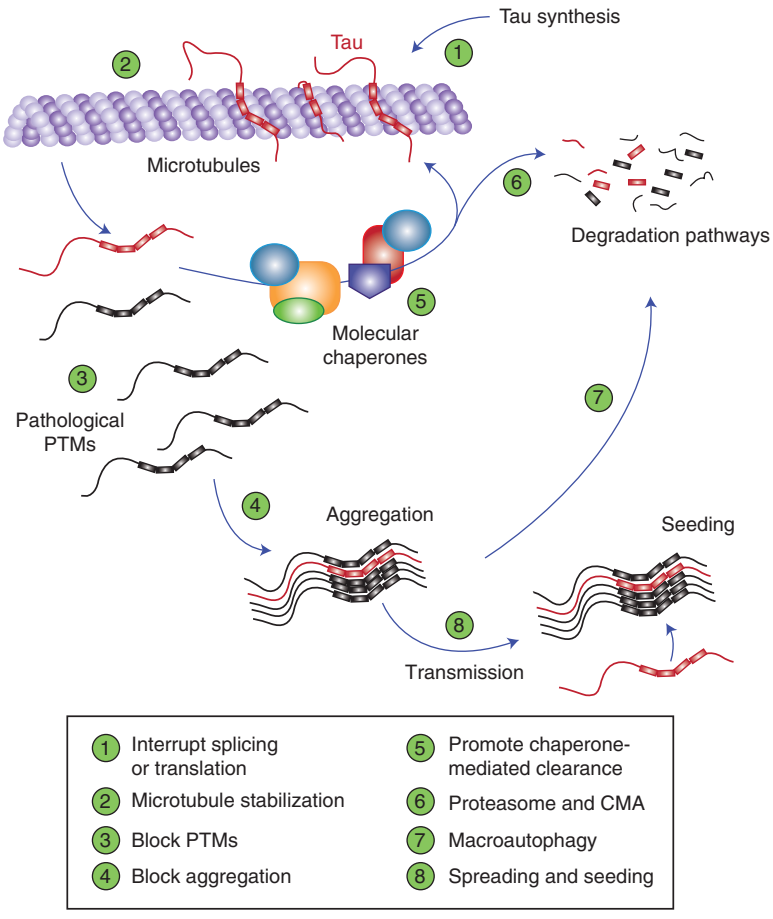

Figure 2. Possible mechanisms for restoring tau homeostasis. Tau homeostasis is defined as a product of its synthesis, normal cycling on microtubules, chaperone-mediated clearance, aggregation, and spreading from cell to cell. Each of these stages during the life cycle of tau is a potential site of pharmacological intervention. The goal is to normalize tau homeostasis and rebalance its levels. PTM, Posttranslational modification; CMA, chaperone-mediated autophagy.

is bound to microtubules, because the MTBRs contain the essential aggregation motif. As mentioned above, other approaches have targeted the enzymes involved in tau posttranslational modifications, such as kinases or phosphatases. The challenge with these approaches is that it is not clear whether the small molecules can be safely deployed in a way that normalizes tau protein–protein interactions and posttranslational modifications. However, ongoing preclinical programs are likely to answer this critical question.

Another promising approach to treating tauopathies is to accelerate the protein's turnover. Tau$^{-/-}$ knockout mice display only subtle phenotypes (Roberson et al. 2007; Morris et al. 2013), arguing that tau may not be critical in adult animals or that it can be compensated for by other microtubule-associated proteins. One way of clearing tau is to target it at the mRNA level with antisense oligonucleotides (DeVos et al. 2013). Another way is with therapeutic antibodies (Sigurdsson 2008; Chai et al. 2011) or molecules that block tau translation (Lee et al. 2011). As discussed above, these approaches are being explored in preclinical programs. In the following sections, we focus on another approach to normalizing tau homeostasis, by targeting proteins that maintain its quality control.

MOLECULAR CHAPERONES REGULATE TAU HOMEOSTASIS

The molecular chaperone network consists of several chaperone systems, which intersect and work cooperatively to maintain protein homeostasis. Chaperones are particularly vital in maintaining tau structure and function (Patury et al. 2009; Gestwicki and Garza 2012; Pratt et al. 2015). For example, Hsp90 and Hsp70 assist in proper tau assembly with the microtubule (Dou et al. 2003). In addition, Hsp90 and Hsp70 can facilitate the clearance of toxic forms of tau by acting as scaffolding proteins for degradation pathways, including both the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (Petrucelli et al. 2004; Dickey et al. 2008; Wong et al. 2008; Wang et al. 2009; Krüger et al. 2012).

Hsp70 CONTROLS TAU STABILITY

The 70-kDa molecular chaperone, Hsp70, has two domains: a nucleotide-binding domain responsible for binding and hydrolyzing ATP and a substrate-binding domain that interacts with clients (Fig. 3A) such as tau. The nucleotide state of the nucleotide-binding domain allosterically regulates substrate binding in the substrate-binding domain (Zhuravleva and Gierasch 2011). Specifically, ATP binding causes the substrate-binding domain to dock with the nucleotide-binding domain, allowing clients to bind. Then, hydrolysis of ATP releases the interaction between the domains, which causes a "lid" subdomain in the substrate-binding domain to close on top of its clients (Zhuravleva et al. 2012). The cycling of Hsp70 through these conformations is regulated by co-chaperones. J-proteins bind between the domains to accelerate ATP hydrolysis (Ahmad et al. 2011), whereas nucleotide exchange factors bind the nucleotide-binding domain and help release ADP (Brehmer et al. 2001). Other co-chaperones for Hsp70 include the C terminus of Hsc70 interacting protein (CHIP), which is an E3 ligase that adds ubiquitin chains to tau (Dickey et al. 2006). Overexpression of CHIP decreases tau levels (Sahara et al. 2005; Dickey et al. 2006, 2008), showing the dramatic effect of this system on tau homeostasis. Binding of tau to the Hsp70–CHIP complex leads to ubiquitin-mediated degradation of tau and subsequent prevention of tau aggregation (Petrucelli et al. 2004; Shimura et al. 2004b; Saidia et al. 2015). Other co-chaperone interactions also regulate tau levels. For example, Bag-1 is a nucleotide exchange factor for Hsp70 that, when overexpressed, increases total tau levels (Elliott et al. 2007). Knockdown of Bag-1 decreases total tau, but counterintuitively, increases the levels of hyperphosphorylated tau (Elliott et al. 2007). Bag-1 also links the Hsp70 chaperone network to the UPS (Lüders et al. 2000; Demand et al. 2001) and may associate with Hsp70 client proteins to target them for degradation. Another Hsp70 nucleotide exchange factor, Bag-2, promotes ubiquitin-independent degradation of phosphorylated tau (Carrettiero

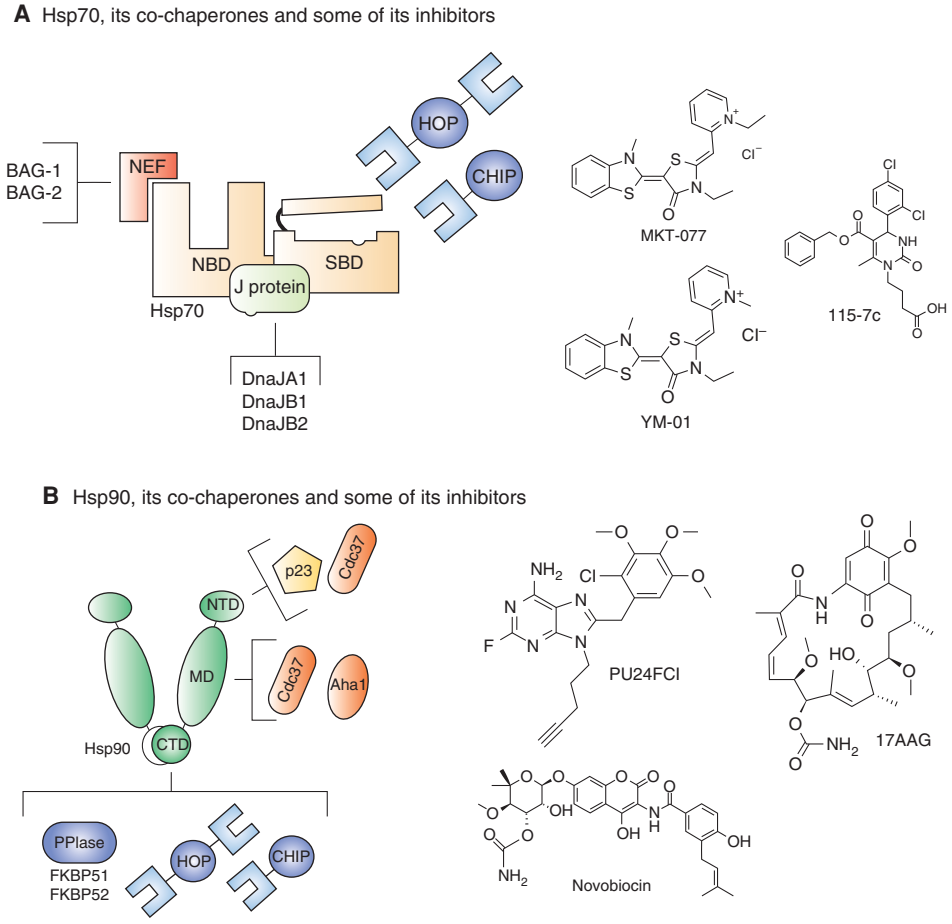

Figure 3. The molecular chaperones important for tau homeostasis. Hsp70 (*A*) and Hsp90 (*B*) are shown, along with a sampling of the co-chaperones and chemical inhibitors discussed. Together, these systems coordinate tau quality control. Chemicals that inhibit specific aspects of chaperone function have been shown to promote tau turnover.

et al. 2009). Thus, the fate of tau is partly determined by which nucleotide exchange factor (i.e., Bag-1 vs. Bag-2) is associated with the Hsp70–tau complex. The J-proteins are also linked to controlling tau homeostasis. DnaJA1 is a co-chaperone that facilitates degradation of tau through the UPS (Abisambra et al. 2012). Likewise, overexpression of DnaJB1 and DnaJB2 reduces tau levels in an Hsp70-dependent manner (Abisambra et al. 2013). Finally, Hsp70 isoforms can have distinct effects on tau stability (Jinwal et al. 2013a). Hsc70 (HSPA8), the cytosolic paralog, partitions tau onto microtubules (Jinwal et al. 2013a; Fontaine et al. 2015a). In contrast, Hsp72 (HSPA1A), the closely related and stress-inducible paralog, promotes tau dissociation from microtubules and its trafficking to the proteasome (Jinwal et al. 2013a). This result is surprising because Hsc70 and Hsp72 are nearly identical, yet subtle differences in their C-terminal domains appear to dictate diametrically opposed activities, as revealed by chimeric proteins (Jinwal et al. 2013a).

In many ways, Hsp70 acts as a scaffold, which binds tau and uses co-chaperone protein–protein interactions to shuttle tau to either the degradation or retention pathways. Ac-

cordingly, a key interaction for tau homeostasis is its direct binding to Hsp70s. There are multiple binding sites for Hsc70 and Hsp72 on tau. Peptide microarray experiments and mutations have shown that Hsp70s bind three sites in or near the MTBRs and an additional site in the N terminus (Sarkar et al. 2008; Thompson et al. 2012). The N-terminal tau binding site is specific for Hsp72 and may contribute to the selective prodegradation effects of this isoform on tau (Thompson et al. 2012). NMR results confirm that Hsp70 binds two short sequences of 0N4R tau in the second and third repeat regions, specifically the aggregation motifs ^{275}VQIINK^{280} and ^{306}VQIVYK^{311} (Jinwal et al. 2013a). Binding of Hsp70s to these hydrophobic repeat domains suggests that they may prevent tau aggregation by sequestering free tau that has been released from the microtubule. In other words, tau aggregation may be suppressed by being bound to either microtubules or Hsp70s. Indeed, Hsp70 can block tau aggregation in vitro (Voss et al. 2012).

MODULATORS OF Hsp70 AND THEIR EFFECTS ON TAU STABILITY

In part, the central role of Hsp70 in tau homeostasis has been clarified and revealed through the use of small-molecule agonists and antagonists. This "chemical biology" approach is based on the idea that perturbing the function of Hsp70 and its protein–protein interactions with co-chaperones might be a powerful way to find drug targets within the Hsp70 subnetwork. This concept is illustrated by recent studies using the rhodacyanine MKT-077 and its analogs (Fig. 3A). These compounds bind the nucleotide-binding domain of Hsp70 (Rousaki et al. 2011) and allosterically decouple nucleotide exchange factor interactions (Colvin et al. 2014; Li et al. 2015). Treatment with these molecules reduces the levels of phosphorylated tau in multiple cellular models of tauopathy (Abisambra et al. 2013; Miyata et al. 2013). Treatment with one analog, YM-01, also reduces tau levels in brain slices from rTg4510 tau transgenic (Tg) mice and restores normal long-term potentiation (Abisambra et al. 2013). The same molecule has no effect on WT tau from normal mice, and in cell models, YM-01 has preferential activity against hyperphosphorylated tau. However, if the microtubule network is chemically disrupted, YM-01 is able to effectively reduce even WT tau. Together, these results suggest that Hsp70 only acts on tau that is dissociated from microtubules, consistent with the known binding site of the chaperone. This is an important distinction because eventual clinical use of an Hsp70 agonist may depend on the safe and selective activity on only abnormal, and not functional, tau.

Beyond these compounds, other modulators of the Hsp70 multiprotein system have been described (Chang et al. 2008b; Jinwal et al. 2009). Of note, the flavonoid-based natural product myricetin reduces tau levels through inhibition of Hsp70 interactions with J-proteins (Jinwal et al. 2009; Chang et al. 2011). In contrast, the dihydropyrimidines SW-02 and 115-7c activate Hsp70 ATPase turnover, which results in an increase in total tau levels in vivo (Chang et al. 2008b; Jinwal et al. 2009). Interestingly, additional neurodegenerative markers such as α-synuclein and TDP-43 were not sensitive to treatment with Hsp70 modulators, indicating that chemical manipulation of Hsp70 can selectively target tau without necessarily interrupting homeostasis of other proteins (Jinwal et al. 2009).

Hsp90 AND ITS CO-CHAPERONES REGULATE TAU STABILITY

Hsp90 is another molecular chaperone that tightly controls the fate of tau (Salminen et al. 2011). Hsp90 is a homodimer, with each protomer consisting of three domains: an N-terminal ATP-binding domain, a middle domain for binding client proteins, and a C-terminal domain, which stabilizes Hsp90 dimer formation (Fig. 3B). Like Hsp70, Hsp90 has an ATPase cycle that is regulated by co-chaperones, which direct conformational changes between open and closed states of Hsp90 (Hessling et al. 2009). The co-chaperone Aha1 binds the middle domain of Hsp90 and increases ATP hydrolysis through stabilization of the closed ATP-bound

state of Hsp90 (Hessling et al. 2009; Retzlaff et al. 2010). The co-chaperone p23, which binds to the N-terminal ATP-binding domain, also stabilizes the closed state of Hsp90 and traps substrate clients while accelerating ATP hydrolysis (McLaughlin et al. 2006). In the closed ATP state, Hsp90 has a high affinity for its substrates. These trapped client proteins can then be modified by Hsp90 co-chaperones to initiate refolding or degradation, and upon ATP hydrolysis, substrates are released from the middle domain. For example, Hsp90 and CHIP coordinate to ubiquitinate phosphorylated tau, like the Hsp70–CHIP complex, leading to its degradation by the UPS (Dickey et al. 2007). Another co-chaperone, Cdc37, inhibits Hsp90 ATPase activity through binding to the N-terminal ATP-binding domain and the middle domain, and delaying the formation of the closed state of Hsp90, a rate-limiting step of the ATPase cycle (Eckl et al. 2013). Hsp90 and its co-chaperones have been linked to tau proteostasis through promotion of tau degradation, as well as tau re-binding to microtubules. Knockdown of Aha1, p23, or Cdc37 decreases both total and phospho-tau levels (Dickey et al. 2007; Jinwal et al. 2013b). Upregulation of Cdc37 preserves total tau and phospho-tau but has no effect on α-synuclein (Jinwal et al. 2011). This might occur because Cdc37 could localize kinases to the Hsp90–tau complex. Similarly, the Hsp90 co-chaperone, FKBP51, stabilizes tau and promotes its aggregation by preventing CHIP-mediated ubiquitination (Jinwal et al. 2010). FKBP52 is another Hsp90 co-chaperone similar in structure and function to FKBP51; however, this co-chaperone preferentially binds phospho-tau and prevents its accumulation in vivo (Chambraud et al. 2010). The differential effects of co-chaperones on Hsp90–tau complexes emphasize that the outcome of client interactions is dependent on which co-chaperones are bound (Röhl et al. 2013). This model further supports the idea that targeting specific chaperone protein–protein interactions may be the safest approach to removing distinct, abnormal pools of tau.

The Hsp90 network is linked to the Hsp70 network through the shared co-chaperone, HOP. Hsp90 and Hsp70 form well-characterized complexes with HOP, which allow for the exchange of substrate clients between these protein networks (Alvira et al. 2014; Röhl et al. 2015). In the case of tau, it is reported that the protein can be transferred from Hsp70 to Hsp90 before its degradation (Thompson et al. 2012; Fontaine et al. 2015b). The Hsp90 binding site on 0N4R tau consists of the entire MTBR, which overlaps with the two Hsp70 binding sites in the 3R and 4R repeat domains, suggesting that these chaperones likely do not bind tau simultaneously (Thompson et al. 2012; Karagoz et al. 2014).

INHIBITORS OF Hsp90 CLEAR TAU

Inhibitors of Hsp90, such as PU24FCl, bind to the N-terminal ATP-binding domain and compete with nucleotide (Vilenchik et al. 2004). Treatment with PU24FCl reduces hyperphosphorylated tau and insoluble tau in cellular models (Dickey et al. 2007; Luo et al. 2007), whereas no effect is seen on normal tau (Luo et al. 2007), suggesting that Hsp90 and its co-chaperones preferentially act on abnormal tau. Another competitive inhibitor of Hsp90, 17-(allylamino)-17-demethoxygeldanamycin (17AAG), also reduces P301L tau levels (Luo et al. 2007); therefore, this effect appears to occur independently of chemotype (Fig. 3B). Hsp90 inhibitors are being explored in numerous clinical trials for cancer (Kummar et al. 2010; Do et al. 2015), so there is some possibility that the compounds might be suitable for eventual exploration in tauopathies. However, such an approach would likely require a more chronic dosing schedule, and the safety of long-term chaperone manipulation is uncertain.

Another distinct class of Hsp90 inhibitors, including novobiocin, binds to the C-terminal domain instead of the N-terminal ATP-binding domain (Marcu et al. 2000). Upon binding to Hsp90, novobiocin prevents dimerization of C-terminal domains and stalls the Hsp90 cycle by a unique mechanism (Matts et al. 2011). Novobiocin analogs and other C-terminal domain-binding small molecules have been developed (Moroni et al. 2014) and shown to reduce tau levels in cellular models (Ansar et al. 2007; Kha-

lid and Paul 2014). It is worth making the distinction between N- and C-terminal Hsp90 inhibitors, because the C-terminal inhibitors do not seem to activate a stress response (Wang and McAlpine 2015). It is not yet clear how a stress response might contribute to the activity of Hsp90 inhibitors, but the pharmacological tools to answer this question are becoming available.

OTHER PATHWAYS FOR REDUCING TAU LEVELS

Although Hsp70 and Hsp90 may be the best-studied tau-binding partners, there are many other possible targets in the proteostasis network. In the following section, we introduce some of the most likely, emerging targets, which (in most cases) have more speculative links to tau quality control.

Hsp27

Hsp27 is a small heat-shock protein that interacts with client proteins, including tau, as part of a multiprotein oligomer (Jakob et al. 1993). Upon stress-induced phosphorylation, Hsp27 disassembles from larger oligomers into smaller complexes, which seem to be potent chaperones for intrinsically disordered proteins (Jovcevski et al. 2015). Indeed, Hsp27 preferentially binds phosphorylated tau in AD brain homogenates and neuronal cell models and is correlated with dephosphorylation and degradation of tau (Shimura et al. 2004a). Overexpression of Hsp27 reduces tau fibril formation and restores long-term potentiation in a Tg mouse model expressing mutant P301L tau (Abisambra et al. 2010, 2011). However, a gap in our understanding of this system is how phosphorylation regulates Hsp27 client binding. In addition, small-molecule activators of this system have not been described. Nevertheless, this goal is worthwhile because Hsp27 levels are increased 20% in AD brains (Björkdahl et al. 2008).

Peptidyl-Prolyl Isomerases

Peptidyl-prolyl isomerases are another class of co-chaperones known to effect tau homeostasis by catalyzing the isomerization of proline residues (Blair et al. 2015). One major protein in this class, Pin1, is highly expressed in neurons and is implicated in Aβ pathology (Pastorino et al. 2006) and tauopathies like FTDP-17 and AD (Yotsumoto et al. 2009; Kimura et al. 2013). Pin1 binds tau phosphorylated at several major phosphorylation sites and localizes with NFTs in AD brains (Lu et al. 1999; Kimura et al. 2013). Upon binding to phospho-tau, specifically pT231, Pin1 converts *cis*-prolines preceding phosphorylated serine and threonine residues into *trans*-prolines (Nakamura et al. 2012). *Cis*-tau accumulates and aggregates in AD brains and is unable to polymerize microtubules (Nakamura et al. 2012). As a result, Pin1 conversion of *cis*-tau to *trans*-tau restores microtubule assembly (Lu et al. 1999; Liou et al. 2003). Pin1 expression is reduced in AD brains (Lu et al. 1999); therefore, normalizing expression of Pin1 may be one therapeutic strategy to prevent the accumulation of hyperphosphorylated tau.

Clusterin

Genome-wide association studies have implicated the molecular chaperone clusterin in AD (Harold et al. 2009; Lambert et al. 2009). This chaperone, which is highly expressed in neurons, especially in AD and PD patients, is reported to promote amyloid-β pathology and is correlated with the deposition of Aβ fibrils (Desikan et al. 2014). Clusterin has also been linked to tau pathology attributable to elevated levels of intracellular clusterin in tau Tg mouse models (Zhou et al. 2014). Although there is clinical evidence to suggest that clusterin plays a critical role in AD, the molecular biology of this protein and its effects on tau remain unknown. One possible explanation for clusterin's role in Aβ and tau pathology is that, in addition to modulating apoptosis and cell signaling pathways, clusterin is also a member of the autophagy/beclin-1 interactome (Salminen et al. 2013). It will be interesting to see whether chemical probes targeting clusterin can be developed to aid in understanding how this protein regulates tau pathology.

HDAC6

Histone deacetylase 6 (HDAC6) is another key partner for tau that directly impacts its homeostasis, especially after inhibition of the proteasome (Ding et al. 2008). HDAC6 plays an important role in autophagic clearance of tau, and HDAC6 inhibitors lead to increased tau acetylation and aggregation (Noack et al. 2014; Leyk et al. 2015). However, to illustrate the delicate balance required for proper tau homeostasis, inhibition of HDAC6 actually reduced phospho-tau in some models (Cook et al. 2012, 2014a,b), perhaps because of competition between acetylation and phosphorylation (Cook et al. 2014a).

Autophagy and UPS Targets

Chaperone-mediated autophagy and the UPS are the major clearance pathways for removing tau (Wong et al. 2008; Wang et al. 2009; Ozcelik et al. 2013). Consistent with this idea, activation of autophagy by rapamycin reduces tau phosphorylation and tau tangle formation in cellular models of tauopathy and in P301S tau Tg mice (Ozcelik et al. 2013; Jiang et al. 2014). Rapamycin treatment also reduces phosphorylation of tau by the kinase GSK3β (glycogen synthase kinase 3β), highlighting the possibility that combinations of autophagy activators with kinase inhibitors may be effective at reducing the levels of hyperphosphorylated tau (Jiang et al. 2014). To accelerate clearance through the UPS, one enticing approach is to activate the proteasome with inhibitors of USP14, a deubiquitinating enzyme that dampens turnover of proteasome substrates, including tau (Lee et al. 2010).

THE FUTURE OF TAU-REDUCTION THERAPIES

Tauopathies result from the failure of neurons to maintain proper tau homeostasis. Therefore, one approach to therapy may be to modulate targets that control the flux of tau (Fig. 2). As mentioned above, this goal might be achieved by blocking tau transcription or translation, preventing its aggregation, or by accelerating its clearance using antibodies. An alternative approach is to target the host factors that normally regulate tau homeostasis to accelerate turnover of abnormal tau. However, the path forward for tau-based therapies remains at the preclinical stage. In our view, antibodies that bind to tau, especially phospho-tau and oligomeric tau, seem to offer the most immediate pathway to clinically testing the tau-reduction hypothesis (Selenica et al. 2014; Funk et al. 2015; Ittner et al. 2015). Nevertheless, to overcome limitations associated with the cost and delivery of antibodies to the central nervous system (CNS), second-generation approaches might be focused on small-molecule tau aggregation inhibitors and activators of chaperone-mediated tau turnover (Calcul et al. 2012; Blair et al. 2013; Paranjape et al. 2015). The major concern with these approaches will be safety because it is unclear whether any of them will be able to selectively reduce abnormal tau without disturbing proteostasis.

Aging-associated collapse of proteostasis is likely to contribute to tau accumulation. For example, the total activity of the autophagy and proteasome deteriorate during aging. By activating factors important in tau turnover, might we be able to reverse aging-associated tau imbalance? There are many obstacles to achieving this goal, but the concept is compelling because these systems appear to be already poised to selectively identify abnormal tau. Thus, stimulating these pathways may present an opportunity to take advantage of this intrinsic discriminating potential.

Finally, a hallmark of tauopathy is the seeding and spreading of tau aggregation and pathology through the uptake of extracellular tau fibrils (Frost and Diamond 2009). The mechanisms of cell-to-cell transmission remain unclear, but cell and animal models are in development (Frost et al. 2009; Kfoury et al. 2012; Yanamandra et al. 2013; Holmes et al. 2014), which will provide, for the first time, an opportunity to target proteins important in this process. We predict that a better understanding of these mechanisms will reveal unexpected new drug targets.

ACKNOWLEDGMENTS

Our work on tau homeostasis is funded by the National Institutes of Health (Grant NS059690), the Brightfocus Foundation, and the Tau Consortium.

REFERENCES

*Reference is also in this collection.

Abisambra JF, Blair LJ, Hill SE, Jones JR, Kraft C, Rogers J, Koren J, Jinwal UK, Lawson L, Johnson AG, et al. 2010. Phosphorylation dynamics regulate Hsp27-mediated rescue of neuronal plasticity deficits in tau transgenic mice. *J Neurosci* **30:** 15374–15382.

Abisambra JF, Jinwal UK, Jones JR, Blair LJ, Koren J III, Dickey CA. 2011. Exploiting the diversity of the heat-shock protein family for primary and secondary tauopathy therapeutics. *Curr Neuropharmacol* **9:** 623–631.

Abisambra JF, Jinwal UK, Suntharalingam A, Arulselvam K, Brady S, Cockman M, Jin Y, Zhang B, Dickey CA. 2012. DnaJA1 antagonizes constitutive Hsp70-mediated stabilization of tau. *J Mol Biol* **421:** 653–661.

Abisambra J, Jinwal UK, Miyata Y, Rogers J, Blair L, Li X, Seguin SP, Wang L, Jin Y, Bacon J, et al. 2013. Allosteric heat shock protein 70 inhibitors rapidly rescue synaptic plasticity deficits by reducing aberrant tau. *Biol Psychiatry* **74:** 367–374.

Adams SJ, DeTure MA, McBride M, Dickson DW, Petrucelli L. 2010. Three repeat isoforms of tau inhibit assembly of four repeat tau filaments. *PLoS ONE* **5:** e10810.

Ahmad A, Bhattacharya A, McDonald RA, Cordes M, Ellington B, Bertelsen EB, Zuiderweg ERP. 2011. Heat shock protein 70 kDa chaperone/DnaJ cochaperone complex employs an unusual dynamic interface. *Proc Natl Acad Sci* **108:** 18966–18971.

Alonso Adel C, Mederlyova A, Novak M, Grundke-Iqbal I, Iqbal K. 2004. Promotion of hyperphosphorylation by frontotemporal dementia tau mutations. *J Biol Chem* **279:** 34873–34881.

Alvira S, Cuéllar J, Röhl A, Yamamoto S, Itoh H, Alfonso C, Rivas G, Buchner J, Valpuesta JM. 2014. Structural characterization of the substrate transfer mechanism in Hsp70/Hsp90 folding machinery mediated by Hop. *Nat Commun* **5:** 5484.

Ansar S, Burlison JA, Hadden MK, Yu XM, Desino KE, Bean J, Neckers L, Audus KL, Michaelis ML, Blagg BS. 2007. A non-toxic Hsp90 inhibitor protects neurons from Aβ-induced toxicity. *Bioorg Med Chem Lett* **17:** 1984–1990.

Arendt T, Stieler J, Strijkstra AM, Hut RA, Rüdiger J, Van der Zee EA, Harkany T, Holzer M, Härtig W. 2003. Reversible paired helical filament-like phosphorylation of tau is an adaptive process associated with neuronal plasticity in hibernating animals. *J Neurosci* **23:** 6972–6981.

Augustinack JC, Schneider A, Mandelkow EM, Hyman BT. 2002. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol* **103:** 26–35.

Baker M, Litvan I, Houlden H, Adamson J, Dickson D, Perez-Tur J, Hardy J, Lynch T, Bigio E, Hutton M. 1999. Association of an extended haplotype in the *tau* gene with progressive supranuclear palsy. *Hum Mol Genet* **8:** 711–715.

Barghorn S, Mandelkow E. 2002. Toward a unified scheme for the aggregation of tau into Alzheimer paired helical filaments. *Biochemistry* **41:** 14885–14896.

Barghorn S, Zheng-Fischhöfer Q, Ackmann M, Biernat J, von Bergen M, Mandelkow EM, Mandelkow E. 2000. Structure, microtubule interactions, and paired helical filament aggregation by tau mutants of frontotemporal dementias. *Biochemistry* **39:** 11714–11721.

Basurto-Islas G, Luna-Muñoz J, Guillozet-Bongaarts AL, Binder LI, Mena R, García-Sierra F. 2008. Accumulation of aspartic acid^{421}- and glutamic acid^{391}-cleaved tau in neurofibrillary tangles correlates with progression in Alzheimer disease. *J Neuropathol Exp Neurol* **67:** 470–483.

Beharry C, Cohen LS, Di J, Ibrahim K, Briffa-Mirabella S, Alonso Adel C. 2014. Tau-induced neurodegeneration: Mechanisms and targets. *Neurosci Bull* **30:** 346–358.

Binder LI, Frankfurter A, Rebhun LI. 1985. The distribution of tau in the mammalian central nervous system. *J Cell Biol* **101:** 1371–1378.

Björkdahl C, Sjögren MJ, Zhou X, Concha H, Avila J, Winblad B, Pei JJ. 2008. Small heat shock proteins Hsp27 or αB-crystallin and the protein components of neurofibrillary tangles: Tau and neurofilaments. *J Neurosci Res* **86:** 1343–1352.

Blair LJ, Zhang B, Dickey CA. 2013. Potential synergy between tau aggregation inhibitors and tau chaperone modulators. *Alzheimers Res Ther* **5:** 41.

Blair LJ, Baker JD, Sabbagh JJ, Dickey CA. 2015. The emerging role of peptidyl-prolyl isomerase chaperones in tau oligomerization, amyloid processing, and Alzheimer's disease. *J Neurochem* **133:** 1–13.

Braak H, Braak E. 1991. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* **82:** 239–259.

Brehmer D, Rüdiger S, Gässler CS, Klostermeier D, Packschies L, Reinstein J, Mayer MP, Bukau B. 2001. Tuning of chaperone activity of Hsp70 proteins by modulation of nucleotide exchange. *Nat Struct Biol* **8:** 427–432.

Brunden KR, Yao Y, Potuzak JS, Ferrer NI, Ballatore C, James MJ, Hogan AM, Trojanowski JQ, Smith AB III, Lee VM. 2011. The characterization of microtubule-stabilizing drugs as possible therapeutic agents for Alzheimer's disease and related tauopathies. *Pharmacol Res* **63:** 341–351.

Bugiani O, Murrell JR, Giaccone G, Hasegawa M, Ghigo G, Tabaton M, Morbin M, Primavera A, Carella F, Solaro C, et al. 1999. Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in tau. *J Neuropathol Exp Neurol* **58:** 667–677.

Bulic B, Pickhardt M, Mandelkow EM, Mandelkow E. 2010. Tau protein and tau aggregation inhibitors. *Neuropharmacology* **59:** 276–289.

Calcul L, Zhang B, Jinwal UK, Dickey CA, Baker BJ. 2012. Natural products as a rich source of tau-targeting drugs for Alzheimer's disease. *Future Med Chem* **4:** 1751–1761.

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Carrettiero DC, Hernandez I, Neveu P, Papagiannakopoulos T, Kosik KS. 2009. The cochaperone BAG2 sweeps paired helical filament-insoluble tau from the microtubule. *J Neurosci* **29:** 2151–2161.

Castillo-Carranza DL, Sengupta U, Guerrero-Muñoz MJ, Lasagna-Reeves CA, Gerson JE, Singh G, Estes DM, Barrett AD, Dineley KT, Jackson GR, et al. 2014. Passive immunization with Tau oligomer monoclonal antibody reverses tauopathy phenotypes without affecting hyperphosphorylated neurofibrillary tangles. *J Neurosci* **34:** 4260–4272.

Castillo-Carranza DL, Guerrero-Muñoz MJ, Sengupta U, Hernandez C, Barrett AD, Dineley K, Kayed R. 2015. Tau immunotherapy modulates both pathological tau and upstream amyloid pathology in an Alzheimer's disease mouse model. *J Neurosci* **35:** 4857–4868.

Chai X, Wu S, Murray TK, Kinley R, Cella CV, Sims H, Buckner N, Hanmer J, Davies P, O'Neill MJ, et al. 2011. Passive immunization with anti-Tau antibodies in two transgenic models: Reduction of Tau pathology and delay of disease progression. *J Biol Chem* **286:** 34457–34467.

Chambraud B, Sardin E, Giustiniani J, Dounane O, Schumacher M, Goedert M, Baulieu EE. 2010. A role for FKBP52 in Tau protein function. *Proc Natl Acad Sci* **107:** 2658–2663.

Chang E, Kim S, Yin H, Nagaraja HN, Kuret J. 2008a. Pathogenic missense MAPT mutations differentially modulate tau aggregation propensity at nucleation and extension steps. *J Neurochem* **107:** 1113–1123.

Chang L, Bertelsen EB, Wisén S, Larsen EM, Zuiderweg ER, Gestwicki JE. 2008b. High-throughput screen for small molecules that modulate the ATPase activity of the molecular chaperone DnaK. *Anal Biochem* **372:** 167–176.

Chang L, Miyata Y, Ung PM, Bertelsen EB, McQuade TJ, Carlson Ha, Zuiderweg ER, Gestwicki JE. 2011. Chemical screens against a reconstituted multiprotein complex: Myricetin blocks DnaJ regulation of DnaK through an allosteric mechanism. *Chem Biol* **18:** 210–221.

Chirita CN, Necula M, Kuret J. 2003. Anionic micelles and vesicles induce tau fibrillization in vitro. *J Biol Chem* **278:** 25644–25650.

Clavaguera F, Akatsu H, Fraser G, Crowther RA, Frank S, Hench J, Probst A, Winkler DT, Reichwald J, Staufenbiel M, et al. 2013. Brain homogenates from human tauopathies induce tau inclusions in mouse brain. *Proc Natl Acad Sci* **110:** 9535–9540.

* Clavaguera F, Tolnay M, Goedert M. 2016. The prion-like behavior of assembled tau in transgenic mice. *Cold Spring Harb Perspect Med.* doi: 10.1101/cshperspect.a024372.

Colvin TA, Gabai VL, Gong J, Calderwood SK, Li H, Gummuluru S, Matchuk ON, Smirnova SG, Orlova NV, Zamulaeva IA, et al. 2014. Hsp70–Bag3 interactions regulate cancer-related signaling networks. *Cancer Res* **74:** 4731–4740.

Combs B, Gamblin TC. 2012. FTDP-17 tau mutations induce distinct effects on aggregation and microtubule interactions. *Biochemistry* **51:** 8597–8607.

Cook C, Gendron TF, Scheffel K, Carlomagno Y, Dunmore J, DeTure M, Petrucelli L. 2012. Loss of HDAC6, a novel CHIP substrate, alleviates abnormal tau accumulation. *Hum Mol Genet* **21:** 2936–2945.

Cook C, Carlomagno Y, Gendron TF, Dunmore J, Scheffel K, Stetler C, Davis M, Dickson D, Jarpe M, DeTure M, et al. 2014a. Acetylation of the KXGS motifs in tau is a critical determinant in modulation of tau aggregation and clearance. *Hum Mol Genet* **23:** 104–116.

Cook C, Stankowski J, Carlomagno Y, Stetler C, Petrucelli L. 2014b. Acetylation: A new key to unlock tau's role in neurodegeneration. *Alzheimers Res Ther* **6:** 29.

Coppola G, Chinnathambi S, Lee JJ, Dombroski BA, Baker MC, Soto-Ortolaza AI, Lee SE, Klein E, Huang AY, Sears R, et al. 2012. Evidence for a role of the rare p.A152T variant in *MAPT* in increasing the risk for FTD-spectrum and Alzheimer's diseases. *Hum Mol Genet* **21:** 3500–3512.

Cripps D, Thomas SN, Jeng Y, Yang F, Davies P, Yang AJ. 2006. Alzheimer disease-specific conformation of hyperphosphorylated paired helical filament-Tau is polyubiquitinated through Lys-48, Lys-11, and Lys-6 ubiquitin conjugation. *J Biol Chem* **281:** 10825–10838.

de Calignon A, Polydoro M, Suarez-Calvet M, William C, Adamowicz DH, Kopeikina KJ, Pitstick R, Sahara N, Ashe KH, Carlson GA, et al. 2012. Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron* **73:** 685–697.

Demand J, Alberti S, Patterson C, Höhfeld J. 2001. Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. *Curr Biol* **11:** 1569–1577.

Desikan RS, Thompson WK, Holland D, Hess CP, Brewer JB, Zetterberg H, Blennow K, Andreassen OA, McEvoy LK, Hyman BT, et al. 2014. The role of clusterin in amyloid-β–associated neurodegeneration. *JAMA Neurol* **71:** 180–187.

DeVos SL, Goncharoff DK, Chen G, Kebodeaux CS, Yamada K, Stewart FR, Schuler DR, Maloney SE, Wozniak DF, Rigo F, et al. 2013. Antisense reduction of tau in adult mice protects against seizures. *J Neurosci* **33:** 12887–12897.

Dickey CA, Yue M, Lin WL, Dickson DW, Dunmore JH, Lee WC, Zehr C, West G, Cao S, Clark AM, et al. 2006. Deletion of the ubiquitin ligase CHIP leads to the accumulation, but not the aggregation, of both endogenous phospho- and caspase-3-cleaved tau species. *J Neurosci* **26:** 6985–6996.

Dickey CA, Kamal A, Lundgren K, Klosak N, Bailey RM, Dunmore J, Ash P, Shoraka S, Zlatkovic J, Eckman CB, et al. 2007. The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. *J Clin Invest* **117:** 648–658.

Dickey CA, Koren J, Zhang YJ, Xu YF, Jinwal UK, Birnbaum MJ, Monks B, Sun M, Cheng AQ, Patterson C, et al. 2008. Akt and CHIP coregulate tau degradation through coordinated interactions. *Proc Natl Acad Sci* **105:** 3622–3627.

Ding H, Matthews TA, Johnson GV. 2006. Site-specific phosphorylation and caspase cleavage differentially impact tau-microtubule interactions and tau aggregation. *J Biol Chem* **281:** 19107–19114.

Ding H, Dolan PJ, Johnson GV. 2008. Histone deacetylase 6 interacts with the microtubule-associated protein tau. *J Neurochem* **106:** 2119–2130.

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Do K, Speranza G, Chang LC, Polley E, Bishop R, Zhu W, Trepel J, Lee S, Lee MJ, Kinders R, et al. 2015. Phase I study of the heat shock protein 90 (Hsp90) inhibitor onalespib (AT13387) administered on a daily for 2 consecutive days per week dosing schedule in patients with advanced solid tumors. *Invest New Drugs* **33:** 921–930.

Dolan PJ, Johnson GV. 2010. A caspase cleaved form of tau is preferentially degraded through the autophagy pathway. *J Biol Chem* **285:** 21978–21987.

Dou F, Netzer WJ, Tanemura K, Li F, Hartl FU, Takashima A, Gouras GK, Greengard P, Xu H. 2003. Chaperones increase association of tau protein with microtubules. *Proc Natl Acad Sci* **100:** 721–726.

Ebneth A, Godemann R, Stamer K, Illenberger S, Trinczek B, Mandelkow E. 1998. Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: Implications for Alzheimer's disease. *J Cell Biol* **143:** 777–794.

Eckl JM, Rutz DA, Haslbeck V, Zierer BK, Reinstein J, Richter K. 2013. Cdc37 (cell division cycle 37) restricts Hsp90 (heat shock protein 90) motility by interaction with N-terminal and middle domain binding sites. *J Biol Chem* **288:** 16032–16042.

Elliott E, Tsvetkov P, Ginzburg I. 2007. BAG-1 associates with Hsc70.Tau complex and regulates the proteasomal degradation of Tau protein. *J Biol Chem* **282:** 37276–37284.

Fanara P, Husted KH, Selle K, Wong PY, Banerjee J, Brandt R, Hellerstein MK. 2010. Changes in microtubule turnover accompany synaptic plasticity and memory formation in response to contextual fear conditioning in mice. *Neuroscience* **168:** 167–178.

Fasulo L, Ugolini G, Visintin M, Bradbury A, Brancolini C, Verzillo V, Novak M, Cattaneo A. 2000. The neuronal microtubule-associated protein tau is a substrate for caspase-3 and an effector of apoptosis. *J Neurochem* **75:** 624–633.

Fauquant C, Redeker V, Landrieu I, Wieruszeski JM, Verdegem D, Laprevote O, Lippens G, Gigant B, Knossow M. 2011. Systematic identification of tubulin-interacting fragments of the microtubule-associated protein Tau leads to a highly efficient promoter of microtubule assembly. *J Biol Chem* **286:** 33358–33368.

Flach K, Hilbrich I, Schiffmann A, Gärtner U, Krüger M, Leonhardt M, Waschipky H, Wick L, Arendt T, Holzer M. 2012. Tau oligomers impair artificial membrane integrity and cellular viability. *J Biol Chem* **287:** 43223–43233.

Fontaine SN, Martin MD, Akoury E, Assimon VA, Borysov S, Nordhues BA, Sabbagh JJ, Cockman M, Gestwicki JE, Zweckstetter M, et al. 2015a. The active Hsc70/tau complex can be exploited to enhance tau turnover without damaging microtubule dynamics. *Hum Mol Genet* **24:** 3971–3981.

Fontaine SN, Rauch JN, Nordhues BA, Assimon VA, Stothert AR, Jinwal UK, Sabbagh JJ, Chang L, Stevens SM Jr, Zuiderweg ER, et al. 2015b. Isoform-selective genetic inhibition of constitutive cytosolic Hsp70 activity promotes client tau degradation using an altered co-chaperone complement. *J Biol Chem* **290:** 13115–13127.

Friedhoff P, Schneider A, Mandelkow EM, Mandelkow E. 1998. Rapid assembly of Alzheimer-like paired helical filaments from microtubule-associated protein tau monitored by fluorescence in solution. *Biochemistry* **37:** 10223–10230.

Frost B, Diamond MI. 2009. The expanding realm of prion phenomena in neurodegenerative disease. *Prion* **3:** 74–77.

Frost B, Jacks RL, Diamond MI. 2009. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem* **284:** 12845–12852.

Frost B, Hemberg M, Lewis J, Feany MB. 2014. Tau promotes neurodegeneration through global chromatin relaxation. *Nat Neurosci* **17:** 357–366.

Funk KE, Thomas SN, Schafer KN, Cooper GL, Liao Z, Clark DJ, Yang AJ, Kuret J. 2014. Lysine methylation is an endogenous post-translational modification of tau protein in human brain and a modulator of aggregation propensity. *Biochem J* **462:** 77–88.

Funk KE, Mirbaha H, Jiang H, Holtzman DM, Diamond MI. 2015. Distinct therapeutic mechanisms of tau antibodies: Promoting microglial clearance versus blocking neuronal uptake. *J Biol Chem* **290:** 21652–21662.

Gamblin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, Lu M, Fu Y, García-Sierra F, LaPointe N, et al. 2003. Caspase cleavage of tau: Linking amyloid and neurofibrillary tangles in Alzheimer's disease. *Proc Natl Acad Sci* **100:** 10032–10037.

Gerson JE, Sengupta U, Lasagna-Reeves CA, Guerrero-Muñoz MJ, Troncoso J, Kayed R. 2014. Characterization of tau oligomeric seeds in progressive supranuclear palsy. *Acta Neuropathol Commun* **2:** 73.

Gestwicki JE, Garza D. 2012. Protein quality control in neurodegenerative disease. *Prog Mol Biol Transl Sci* **107:** 327–353.

Ghetti B, Wszolek ZK, Boeve BF, Spina S, Goedert M. 2011. Frontotemporal dementia and parkinsonism linked to chromosome 17. In *Neurodegeneration: The molecular pathology of dementia and movement disorders*, 2nd ed. (ed. Dickson DW, Weller RO), pp. 110–134. Wiley, Oxford.

Ghetti B, Oblak AL, Boeve BF, Johnson KA, Dickerson BC, Goedert M. 2015. Invited review: Frontotemporal dementia caused by *microtubule-associated protein tau* gene (*MAPT*) mutations: A chameleon for neuropathology and neuroimaging. *Neuropathol Appl Neurobiol* **41:** 24–46.

Goedert M, Jakes R. 1990. Expression of separate isoforms of human tau protein: Correlation with the tau pattern in brain and effects on tubulin polymerization. *EMBO J* **9:** 4225–4230.

Goedert M, Spillantini MG, Jakes R, Rutherford D, Crowther RA. 1989. Multiple isoforms of human microtubule-associated protein tau: Sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron* **3:** 519–526.

Goode BL, Chau M, Denis PE, Feinstein SC. 2000. Structural and functional differences between 3-repeat and 4-repeat tau isoforms. Implications for normal tau function and the onset of neurodegenerative disease. *J Biol Chem* **275:** 38182–38189.

Goux WJ, Kopplin L, Nguyen AD, Leak K, Rutkofsky M, Shanmuganandam VD, Sharma D, Inouye H, Kirschner DA. 2004. The formation of straight and twisted filaments from short tau peptides. *J Biol Chem* **279:** 26868–26875.

Grinberg LT, Wang X, Wang C, Sohn PD, Theofilas P, Sidhu M, Arevalo JB, Heinsen H, Huang EJ, Rosen H, et al. 2013. Argyrophilic grain disease differs from other tauopathies by lacking tau acetylation. *Acta Neuropathol* **125:** 581–593.

Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A, et al. 2009. Genome-wide association study identifies variants at *CLU* and *PICALM* associated with Alzheimer's disease. *Nat Genet* **41:** 1088–1093.

Hessling M, Richter K, Buchner J. 2009. Dissection of the ATP-induced conformational cycle of the molecular chaperone Hsp90. *Nat Struct Mol Biol* **16:** 287–293.

Höglinger GU, Huppertz HJ, Wagenpfeil S, Andrés MV, Belloch V, León T, Del Ser T, Investigators TM. 2014. Tideglusib reduces progression of brain atrophy in progressive supranuclear palsy in a randomized trial. *Mov Disord* **29:** 479–487.

Holmes BB, Furman JL, Mahan TE, Yamasaki TR, Mirbaha H, Eades WC, Belaygorod L, Cairns NJ, Holtzman DM, Diamond MI. 2014. Proteopathic tau seeding predicts tauopathy in vivo. *Proc Natl Acad Sci* **111:** E4376–E4385.

Hong M, Zhukareva V, Vogelsberg-Ragaglia V, Wszolek Z, Reed L, Miller BI, Geschwind DH, Bird TD, McKeel D, Goate A, et al. 1998. Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17. *Science* **282:** 1914–1917.

Horowitz PM, Patterson KR, Guillozet-Bongaarts AL, Reynolds MR, Carroll CA, Weintraub ST, Bennett DA, Cryns VL, Berry RW, Binder LI. 2004. Early N-terminal changes and caspase-6 cleavage of tau in Alzheimer's disease. *J Neurosci* **24:** 7895–7902.

Irwin DJ, Cohen TJ, Grossman M, Arnold SE, Xie SX, Lee VM, Trojanowski JQ. 2012. Acetylated tau, a novel pathological signature in Alzheimer's disease and other tauopathies. *Brain* **135:** 807–818.

Ittner LM, Fath T, Ke YD, Bi M, van Eersel J, Li KM, Gunning P, Götz J. 2008. Parkinsonism and impaired axonal transport in a mouse model of frontotemporal dementia. *Proc Natl Acad Sci* **105:** 15997–16002.

Ittner A, Bertz J, Suh LS, Stevens CH, Götz J, Ittner LM. 2015. Tau-targeting passive immunization modulates aspects of pathology in tau transgenic mice. *J Neurochem* **132:** 135–145.

Jakob U, Gaestel M, Engel K, Buchner J. 1993. Small heat shock proteins are molecular chaperones. *J Biol Chem* **268:** 1517–1520.

Jancsik V, Filliol D, Rendon A. 1996. Tau proteins bind to kinesin and modulate its activation by microtubules. *Neurobiology (Bp)* **4:** 417–429.

Jiang T, Yu JT, Zhu XC, Zhang QQ, Cao L, Wang HF, Tan MS, Gao Q, Qin H, Zhang YD, et al. 2014. Temsirolimus attenuates tauopathy in vitro and in vivo by targeting tau hyperphosphorylation and autophagic clearance. *Neuropharmacology* **85:** 121–130.

Jinwal UK, Miyata Y, Koren J, Jones JR, Trotter JH, Chang L, O'Leary J, Morgan D, Lee DC, Shults CL, et al. 2009. Chemical manipulation of Hsp70 ATPase activity regulates tau stability. *J Neurosci* **29:** 12079–12088.

Jinwal UK, Koren J, Borysov SI, Schmid AB, Abisambra JF, Blair LJ, Johnson AG, Jones JR, Shults CL, O'Leary JC, et al. 2010. The Hsp90 cochaperone, FKBP51, increases tau stability and polymerizes microtubules. *J Neurosci* **30:** 591–599.

Jinwal UK, Trotter JH, Abisambra JF, Koren J, Lawson LY, Vestal GD, O'Leary JC, Johnson AG, Jin Y, Jones JR, et al. 2011. The Hsp90 kinase co-chaperone Cdc37 regulates tau stability and phosphorylation dynamics. *J Biol Chem* **286:** 16976–16983.

Jinwal UK, Akoury E, Abisambra JF, O'Leary JC III, Thompson AD, Blair LJ, Jin Y, Bacon J, Nordhues BA, Cockman M, et al. 2013a. Imbalance of Hsp70 family variants fosters tau accumulation. *FASEB J* **27:** 1450–1459.

Jinwal UK, Koren J, Dickey CA. 2013b. Reconstructing the Hsp90/Tau machine. *Curr Enzym Inhib* **9:** 41–45.

Jovcevski B, Kelly MMA, Rote AAP, Berg T, Gastall HHY, Benesch JL, Aquilina JA, Ecroyd H. 2015. Phosphomimics destabilize Hsp27 oligomeric assemblies and enhance chaperone activity. *Chem Biol* **22:** 186–195.

Kampers T, Friedhoff P, Biernat J, Mandelkow EM, Mandelkow E. 1996. RNA stimulates aggregation of microtubule-associated protein tau into Alzheimer-like paired helical filaments. *FEBS Lett* **399:** 344–349.

Karagoz GE, Duarte AM, Akoury E, Ippel H, Biernat J, Moran Luengo T, Radli M, Didenko T, Nordhues BA, Veprintsev DB, et al. 2014. Hsp90-Tau complex reveals molecular basis for specificity in chaperone action. *Cell* **156:** 963–974.

Kenessey A, Yen SH. 1993. The extent of phosphorylation of fetal tau is comparable to that of PHF-tau from Alzheimer paired helical filaments. *Brain Res* **629:** 40–46.

Kfoury N, Holmes BB, Jiang H, Holtzman DM, Diamond MI. 2012. Trans-cellular propagation of Tau aggregation by fibrillar species. *J Biol Chem* **287:** 19440–19451.

Khalid S, Paul S. 2014. Identifying a C-terminal ATP binding sites-based novel Hsp90-Inhibitor in silico: A plausible therapeutic approach in Alzheimer's disease. *Med Hypotheses* **83:** 39–46.

Kickstein E, Krauss S, Thornhill P, Rutschow D, Zeller R, Sharkey J, Williamson R, Fuchs M, Köhler A, Glossmann H, et al. 2010. Biguanide metformin acts on tau phosphorylation via mTOR/protein phosphatase 2A (PP2A) signaling. *Proc Natl Acad Sci* **107:** 21830–21835.

Kimura T, Tsutsumi K, Taoka M, Saito T, Masuda-Suzukake M, Ishiguro K, Plattner F, Uchida T, Isobe T, Hasegawa M, et al. 2013. Isomerase Pin1 stimulates dephosphorylation of tau protein at cyclin-dependent kinase (Cdk5)-dependent Alzheimer phosphorylation sites. *J Biol Chem* **288:** 7968–7977.

Kimura T, Ishiguro K, Hisanaga S. 2014. Physiological and pathological phosphorylation of tau by Cdk5. *Front Mol Neurosci* **7:** 65.

Köpke E, Tung YC, Shaikh S, Alonso AC, Iqbal K, Grundke-Iqbal I. 1993. Microtubule-associated protein tau. Abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. *J Biol Chem* **268:** 24374–24384.

Krüger U, Wang Y, Kumar S, Mandelkow EM. 2012. Autophagic degradation of tau in primary neurons and its enhancement by trehalose. *Neurobiol Aging* **33:** 2291–2305.

Kummar S, Gutierrez ME, Gardner ER, Chen X, Figg WD, Zajac-Kaye M, Chen M, Steinberg SM, Muir CA, Yancey MA, et al. 2010. Phase I trial of 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), a heat shock protein inhibitor, administered twice weekly in patients with advanced malignancies. *Eur J Cancer* **46:** 340–347.

Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, et al. 2009. Genome-wide association study identifies variants at *CLU* and *CR1* associated with Alzheimer's disease. *Nat Genet* **41:** 1094–1099.

Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Guerrero-Muñoz MJ, Kiritoshi T, Neugebauer V, Jackson GR, Kayed R. 2012. Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. *Sci Rep* **2:** 700.

Lee BH, Lee MJ, Park S, Oh DC, Elsasser S, Chen PC, Gartner C, Dimova N, Hanna J, Gygi SP, et al. 2010. Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* **467:** 179–184.

Lee VM, Brunden KR, Hutton M, Trojanowski JQ. 2011. Developing therapeutic approaches to tau, selected kinases, and related neuronal protein targets. *Cold Spring Harb Perspect Med* **1:** a006437.

Leyk J, Goldbaum O, Noack M, Richter-Landsberg C. 2015. Inhibition of HDAC6 modifies tau inclusion body formation and impairs autophagic clearance. *J Mol Neurosci* **55:** 1031–1046.

Li X, Colvin T, Rauch JN, Acosta-Alvear D, Kampmann M, Dunyak B, Hann B, Aftab BT, Murnane M, Cho M, et al. 2015. Validation of the Hsp70–Bag3 protein–protein interaction as a potential therapeutic target in cancer. *Mol Cancer Ther* **14:** 642–648.

Liou YC, Sun A, Ryo A, Zhou XZ, Yu ZX, Huang HK, Uchida T, Bronson R, Bing G, Li X, et al. 2003. Role of the prolyl isomerase Pin1 in protecting against age-dependent neurodegeneration. *Nature* **424:** 556–561.

Liu F, Grundke-Iqbal I, Iqbal K, Gong CX. 2005. Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. *Eur J Neurosci* **22:** 1942–1950.

Liu L, Drouet V, Wu JW, Witter MP, Small SA, Clelland C, Duff K. 2012. Trans-synaptic spread of tau pathology in vivo. *PLoS ONE* **7:** e31302.

LoPresti P, Szuchet S, Papasozomenos SC, Zinkowski RP, Binder LI. 1995. Functional implications for the microtubule-associated protein tau: Localization in oligodendrocytes. *Proc Natl Acad Sci* **92:** 10369–10373.

Lu PJ, Wulf G, Zhou XZ, Davies P, Lu KP. 1999. The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. *Nature* **399:** 784–788.

Lüders J, Demand J, Höhfeld J. 2000. The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. *J Biol Chem* **275:** 4613–4617.

Luo W, Dou F, Rodina A, Chip S, Kim J, Zhao Q, Moulick K, Aguirre J, Wu N, Greengard P, et al. 2007. Roles of heat-shock protein 90 in maintaining and facilitating the neurodegenerative phenotype in tauopathies. *Proc Natl Acad Sci* **104:** 9511–9516.

Maeda S, Sahara N, Saito Y, Murayama M, Yoshiike Y, Kim H, Miyasaka T, Murayama S, Ikai A, Takashima A. 2007. Granular tau oligomers as intermediates of tau filaments. *Biochemistry* **46:** 3856–3861.

Magnani E, Fan J, Gasparini L, Golding M, Williams M, Schiavo G, Goedert M, Amos LA, Spillantini MG. 2007. Interaction of tau protein with the dynactin complex. *EMBO J* **26:** 4546–4554.

Mandelkow EM, Mandelkow E. 2012. Biochemistry and cell biology of tau protein in neurofibrillary degeneration. *Cold Spring Harb Perspect Med* **2:** a006247.

Marcu MG, Schulte TW, Neckers L. 2000. Novobiocin and related coumarins and depletion of heat shock protein 90-dependent signaling proteins. *J Natl Cancer Inst* **92:** 242–248.

Martin L, Latypova X, Terro F. 2011. Post-translational modifications of tau protein: Implications for Alzheimer's disease. *Neurochem Int* **58:** 458–471.

Matts RL, Dixit A, Peterson LB, Sun L, Voruganti S, Kalyanaraman P, Hartson SD, Verkhivker GM, Blagg BSJ. 2011. Elucidation of the Hsp90 C-terminal inhibitor binding site. *ACS Chem Biol* **6:** 800–807.

McLaughlin SH, Sobott F, Yao ZP, Zhang W, Nielsen PR, Grossmann JG, Laue ED, Robinson CV, Jackson SE. 2006. The co-chaperone p23 arrests the Hsp90 ATPase cycle to trap client proteins. *J Mol Biol* **356:** 746–758.

Mercken M, Vandermeeren M, Lübke U, Six J, Boons J, Van de Voorde A, Martin JJ, Gheuens J. 1992. Monoclonal antibodies with selective specificity for Alzheimer Tau are directed against phosphatase-sensitive epitopes. *Acta Neuropathol* **84:** 265–272.

Min SW, Chen X, Tracy TE, Li Y, Zhou Y, Wang C, Shirakawa K, Minami SS, Defensor E, Mok SA, et al. 2015. Critical role of acetylation in tau-mediated neurodegeneration and cognitive deficits. *Nat Med* **21:** 1154–1162.

Miyata Y, Koren J, Kiray J, Dickey CA, Gestwicki JE. 2011. Molecular chaperones and regulation of tau quality control: Strategies for drug discovery in tauopathies. *Future Med Chem* **3:** 1523–1537.

Miyata Y, Li X, Lee HF, Jinwal U, Srinivasan SR, Seguin SP, Young ZT, Brodsky JL, Dickey CA, Sun D, et al. 2013. Synthesis and initial evaluation of YM-08, a blood–brain barrier permeable derivative of the heat shock protein 70 (Hsp70) inhibitor MKT-077, which reduces tau levels. *ACS Chem Neurosci* **4:** 930–939.

Moraga DM, Nunez P, Garrido J, Maccioni RB. 1993. A tau fragment containing a repetitive sequence induces bundling of actin filaments. *J Neurochem* **61:** 979–986.

Morishima-Kawashima M, Hasegawa M, Takio K, Suzuki M, Titani K, Ihara Y. 1993. Ubiquitin is conjugated with amino-terminally processed tau in paired helical filaments. *Neuron* **10:** 1151–1160.

Moroni E, Zhao H, Blagg BS, Colombo G. 2014. Exploiting conformational dynamics in drug discovery: Design of C-terminal inhibitors of Hsp90 with improved activities. *J Chem Inf Model* **54:** 195–208.

Morris M, Hamto P, Adame A, Devidze N, Masliah E, Mucke L. 2013. Age-appropriate cognition and subtle dopa-

mine-independent motor deficits in aged tau knockout mice. *Neurobiol Aging* **34:** 1523–1529.

Morris M, Knudsen GM, Maeda S, Trinidad JC, Ioanoviciu A, Burlingame AL, Mucke L. 2015. Tau post-translational modifications in wild-type and human amyloid precursor protein transgenic mice. *Nat Neurosci* **18:** 1183–1189.

Mukrasch MD, Biernat J, von Bergen M, Griesinger C, Mandelkow E, Zweckstetter M. 2005. Sites of tau important for aggregation populate β-structure and bind to microtubules and polyanions. *J Biol Chem* **280:** 24978–24986.

Mukrasch MD, Bibow S, Korukottu J, Jeganathan S, Biernat J, Griesinger C, Mandelkow E, Zweckstetter M. 2009. Structural polymorphism of 441-residue tau at single residue resolution. *PLoS Biol* **7:** e34.

Nakamura K, Greenwood A, Binder L, Bigio Eileen H, Denial S, Nicholson L, Zhou Xiao Z, Lu Kun P. 2012. Proline isomer-specific antibodies reveal the early pathogenic tau conformation in Alzheimer's disease. *Cell* **149:** 232–244.

Niblock M, Gallo JM. 2012. Tau alternative splicing in familial and sporadic tauopathies. *Biochem Soc Trans* **40:** 677–680.

Noack M, Leyk J, Richter-Landsberg C. 2014. HDAC6 inhibition results in tau acetylation and modulates tau phosphorylation and degradation in oligodendrocytes. *Glia* **62:** 535–547.

Noble W, Hanger DP, Gallo JM. 2010. Transgenic mouse models of tauopathy in drug discovery. *CNS Neurol Disord Drug Targets* **9:** 403–428.

Nygaard HB, van Dyck CH, Strittmatter SM. 2014. Fyn kinase inhibition as a novel therapy for Alzheimer's disease. *Alzheimers Res Ther* **6:** 8.

Ozcelik S, Fraser G, Castets P, Schaeffer V, Skachokova Z, Breu K, Clavaguera F, Sinnreich M, Kappos L, Goedert M, et al. 2013. Rapamycin attenuates the progression of tau pathology in P301S tau transgenic mice. *PLoS One* **8:** e62459.

Panda D, Samuel JC, Massie M, Feinstein SC, Wilson L. 2003. Differential regulation of microtubule dynamics by three- and four-repeat tau: Implications for the onset of neurodegenerative disease. *Proc Natl Acad Sci* **100:** 9548–9553.

Paranjape SR, Riley AP, Somoza AD, Oakley CE, Wang CC, Prisinzano TE, Oakley BR, Gamblin TC. 2015. Azaphilones inhibit tau aggregation and dissolve tau aggregates in vitro. *ACS Chem Neurosci* **6:** 751–760.

Pastorino L, Sun A, Lu PJ, Zhou XZ, Balastik M, Finn G, Wulf G, Lim J, Li SH, Li X, et al. 2006. The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-β production. *Nature* **440:** 528–534.

Patterson KR, Ward SM, Combs B, Voss K, Kanaan NM, Morfini G, Brady ST, Gamblin TC, Binder LI. 2011. Heat shock protein 70 prevents both tau aggregation and the inhibitory effects of preexisting tau aggregates on fast axonal transport. *Biochemistry* **50:** 10300–10310.

Patury S, Miyata Y, Gestwicki JE. 2009. Pharmacological targeting of the Hsp70 chaperone. *Curr Top Med Chem* **9:** 1337–1351.

Petrucelli L, Dickson D, Kehoe K, Taylor J, Snyder H, Grover A, De Lucia M, McGowan E, Lewis J, Prihar G, et al. 2004. CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. *Hum Mol Genet* **13:** 703–714.

Plouffe V, Mohamed NV, Rivest-McGraw J, Bertrand J, Lauzon M, Leclerc N. 2012. Hyperphosphorylation and cleavage at D421 enhance tau secretion. *PLoS ONE* **7:** e36873.

Pratt WB, Gestwicki JE, Osawa Y, Lieberman AP. 2015. Targeting Hsp90/Hsp70-based protein quality control for treatment of adult onset neurodegenerative diseases. *Annu Rev Pharmacol Toxicol* **55:** 353–371.

Qian W, Liu F. 2014. Regulation of alternative splicing of tau exon 10. *Neurosci Bull* **30:** 367–377.

Qiang L, Yu W, Andreadis A, Luo M, Baas PW. 2006. Tau protects microtubules in the axon from severing by katanin. *J Neurosci* **26:** 3120–3129.

Quintanilla RA, Matthews-Roberson TA, Dolan PJ, Johnson GV. 2009. Caspase-cleaved tau expression induces mitochondrial dysfunction in immortalized cortical neurons: Implications for the pathogenesis of Alzheimer disease. *J Biol Chem* **284:** 18754–18766.

Ramcharitar J, Albrecht S, Afonso VM, Kaushal V, Bennett DA, Leblanc AC. 2013. Cerebrospinal fluid tau cleaved by caspase-6 reflects brain levels and cognition in aging and Alzheimer disease. *J Neuropathol Exp Neurol* **72:** 824–832.

* Rauch JN, Olson SH, Gestwicki JE. 2016. Interactions between microtubule-associated protein tau (MAPT) and small molecules. *Cold Spring Harb Perspect Med.* doi: 10.1101/cshperspect.a024034.

Reed LA, Wszolek ZK, Hutton M. 2001. Phenotypic correlations in FTDP-17. *Neurobiol Aging* **22:** 89–107.

Retzlaff M, Hagn F, Mitschke L, Hessling M, Gugel F, Kessler H, Richter K, Buchner J. 2010. Asymmetric activation of the hsp90 dimer by its cochaperone aha1. *Mol Cell* **37:** 344–354.

Reynolds CH, Garwood CJ, Wray S, Price C, Kellie S, Perera T, Zvelebil M, Yang A, Sheppard PW, Varndell IM, et al. 2008. Phosphorylation regulates tau interactions with Src homology 3 domains of phosphatidylinositol 3-kinase, phospholipase Cγ1, Grb2, and Src family kinases. *J Biol Chem* **283:** 18177–18186.

Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu GQ, Mucke L. 2007. Reducing endogenous tau ameliorates amyloid β-induced deficits in an Alzheimer's disease mouse model. *Science* **316:** 750–754.

Röhl A, Rohrberg J, Buchner J. 2013. The chaperone Hsp90: Changing partners for demanding clients. *Trends Biochem Sci* **38:** 253–262.

Röhl A, Wengler D, Madl T, Lagleder S, Tippel F, Herrmann M, Hendrix J, Richter K, Hack G, Schmid AB, et al. 2015. Hsp90 regulates the dynamics of its cochaperone Sti1 and the transfer of Hsp70 between modules. *Nat Commun* **6:** 6655.

Rousaki A, Miyata Y, Jinwal UK, Dickey Ca, Gestwicki JE, Zuiderweg ERP. 2011. Allosteric drugs: The interaction of antitumor compound MKT-077 with human Hsp70 chaperones. *J Mol Biol* **411:** 614–632.

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Sahara N, Murayama M, Mizoroki T, Urushitani M, Imai Y, Takahashi R, Murata S, Tanaka K, Takashima A. 2005. In vivo evidence of CHIP up-regulation attenuating tau aggregation. *J Neurochem* **94:** 1254–1263.

Saidi LJ, Polydoro M, Kay KR, Sanchez L, Mandelkow EM, Hyman BT, Spires-Jones TL. 2015. Carboxy terminus heat shock protein 70 interacting protein reduces tau-associated degenerative changes. *J Alzheimers Dis* **44:** 937–947.

Saito Y, Geyer A, Sasaki R, Kuzuhara S, Nanba E, Miyasaka T, Suzuki K, Murayama S. 2002. Early-onset, rapidly progressive familial tauopathy with R406W mutation. *Neurology* **58:** 811–813.

Salminen A, Ojala J, Kaarniranta K, Hiltunen M, Soininen H. 2011. Hsp90 regulates tau pathology through co-chaperone complexes in Alzheimer's disease. *Prog Neurobiol* **93:** 99–110.

Salminen A, Kaarniranta K, Kauppinen A, Ojala J, Haapasalo A, Soininen H, Hiltunen M. 2013. Impaired autophagy and APP processing in Alzheimer's disease: The potential role of Beclin 1 interactome. *Prog Neurobiol* **106–107:** 33–54.

Sarkar M, Kuret J, Lee G. 2008. Two motifs within the tau microtubule-binding domain mediate its association with the hsc70 molecular chaperone. *J Neurosci Res* **86:** 2763–2773.

Seitz A, Kojima H, Oiwa K, Mandelkow EM, Song YH, Mandelkow E. 2002. Single-molecule investigation of the interference between kinesin, tau and MAP2c. *EMBO J* **21:** 4896–4905.

Selenica ML, Davtyan H, Housley SB, Blair LJ, Gillies A, Nordhues BA, Zhang B, Liu J, Gestwicki JE, Lee DC, et al. 2014. Epitope analysis following active immunization with tau proteins reveals immunogens implicated in tau pathogenesis. *J Neuroinflammation* **11:** 152.

Sharma VM, Litersky JM, Bhaskar K, Lee G. 2007. Tau impacts on growth-factor-stimulated actin remodeling. *J Cell Sci* **120:** 748–757.

Shimura H, Miura-Shimura Y, Kosik KS. 2004a. Binding of tau to heat shock protein 27 leads to decreased concentration of hyperphosphorylated tau and enhanced cell survival. *J Biol Chem* **279:** 17957–17962.

Shimura H, Schwartz D, Gygi SP, Kosik KS. 2004b. CHIP-Hsc70 complex ubiquitinates phosphorylated tau and enhances cell survival. *J Biol Chem* **279:** 4869–4876.

Shin RW, Iwaki T, Kitamoto T, Tateishi J. 1991. Hydrated autoclave pretreatment enhances tau immunoreactivity in formalin-fixed normal and Alzheimer's disease brain tissues. *Lab Invest* **64:** 693–702.

Sigurdsson EM. 2008. Immunotherapy targeting pathological tau protein in Alzheimer's disease and related tauopathies. *J Alzheimers Dis* **15:** 157–168.

Sillen A, Barbier P, Landrieu I, Lefebvre S, Wieruszeski JM, Leroy A, Peyrot V, Lippens G. 2007. NMR investigation of the interaction between the neuronal protein tau and the microtubules. *Biochemistry* **46:** 3055–3064.

Spillantini MG, Goedert M. 2013. Tau pathology and neurodegeneration. *Lancet Neurol* **12:** 609–622.

Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkow EM. 2002. Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. *J Cell Biol* **156:** 1051–1063.

Tepper K, Biernat J, Kumar S, Wegmann S, Timm T, Hübschmann S, Redecke L, Mandelkow EM, Müller DJ, Mandelkow E. 2014. Oligomer formation of tau protein hyperphosphorylated in cells. *J Biol Chem* **289:** 34389–34407.

Thomas SN, Funk KE, Wan Y, Liao Z, Davies P, Kuret J, Yang AJ. 2012. Dual modification of Alzheimer's disease PHF-tau protein by lysine methylation and ubiquitylation: A mass spectrometry approach. *Acta Neuropathol* **123:** 105–117.

Thompson AD, Scaglione KM, Prensner J, Gillies AT, Chinnaiyan A, Paulson HL, Jinwal UK, Dickey CA, Gestwicki JE. 2012. Analysis of the tau-associated proteome reveals that exchange of Hsp70 for Hsp90 is involved in tau degradation. *ACS Chem Biol* **7:** 1677–1686.

Trojanowski JQ, Schuck T, Schmidt ML, Lee VM. 1989. Distribution of tau proteins in the normal human central and peripheral nervous system. *J Histochem Cytochem* **37:** 209–215.

van Eersel J, Ke YD, Liu X, Delerue F, Kril JJ, Götz J, Ittner LM. 2010. Sodium selenate mitigates tau pathology, neurodegeneration, and functional deficits in Alzheimer's disease models. *Proc Natl Acad Sci* **107:** 13888–13893.

Vilenchik M, Solit D, Basso A, Huezo H, Lucas B, He H, Rosen N, Spampinato C, Modrich P, Chiosis G. 2004. Targeting wide-range oncogenic transformation via PU24FCl, a specific inhibitor of tumor Hsp90. *Chem Biol* **11:** 787–797.

von Bergen M, Friedhoff P, Biernat J, Heberle J, Mandelkow EM, Mandelkow E. 2000. Assembly of τ protein into Alzheimer paired helical filaments depends on a local sequence motif (^{306}VQIVYK^{311}) forming β structure. *Proc Natl Acad Sci* **97:** 5129–5134.

von Bergen M, Barghorn S, Li L, Marx A, Biernat J, Mandelkow EM, Mandelkow E. 2001. Mutations of tau protein in frontotemporal dementia promote aggregation of paired helical filaments by enhancing local β-structure. *J Biol Chem* **276:** 48165–48174.

Voss K, Combs B, Patterson K, Binder LI, Gamblin TC. 2012. Hsp70 alters tau function and aggregation in an isoform specific manner. *Biochemistry* **51:** 888–898.

Wang Y, Martinez-Vicente M, Krüger U, Kaushik S, Wong E, Mandelkow EM, Cuervo AM, Mandelkow E. 2009. Tau fragmentation, aggregation and clearance: The dual role of lysosomal processing. *Hum Mol Genet* **18:** 4153–4170.

Wang Y, McAlpine SR. 2015. C-terminal heat shock protein 90 modulators produce desirable oncogenic properties. *Org Biomol Chem* **13:** 4627–4631.

Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. 1975. A protein factor essential for microtubule assembly. *Proc Natl Acad Sci* **72:** 1858–1862.

Wong ES, Tan JM, Soong WE, Hussein K, Nukina N, Dawson VL, Dawson TM, Cuervo AM, Lim KL. 2008. Autophagy-mediated clearance of aggresomes is not a universal phenomenon. *Hum Mol Genet* **17:** 2570–2582.

Yanamandra K, Kfoury N, Jiang H, Mahan TE, Ma S, Maloney SE, Wozniak DF, Diamond MI, Holtzman DM. 2013. Anti-tau antibodies that block tau aggregate seed-

ing in vitro markedly decrease pathology and improve cognition in vivo. *Neuron* **80:** 402–414.

Yotsumoto K, Saito T, Asada A, Oikawa T, Kimura T, Uchida C, Ishiguro K, Uchida T, Hasegawa M, Hisanaga Si. 2009. Effect of Pin1 or microtubule binding on dephosphorylation of FTDP-17 mutant tau. *J Biol Chem* **284:** 16840–16847.

Zhou Y, Hayashi I, Wong J, Tugusheva K, Renger JJ, Zerbinatti C. 2014. Intracellular clusterin interacts with brain isoforms of the bridging integrator 1 and with the microtubule-associated protein tau in Alzheimer's disease. *PLoS ONE* **9:** e103187.

Zhuravleva A, Gierasch LM. 2011. Allosteric signal transmission in the nucleotide-binding domain of 70-kDa heat shock protein (Hsp70) molecular chaperones. *Proc Natl Acad Sci* **108:** 6987–6992.

Zhuravleva A, Clerico EM, Gierasch LM. 2012. An interdomain energetic tug-of-war creates the allosterically active state in Hsp70 molecular chaperones. *Cell* **151:** 1296–1307.