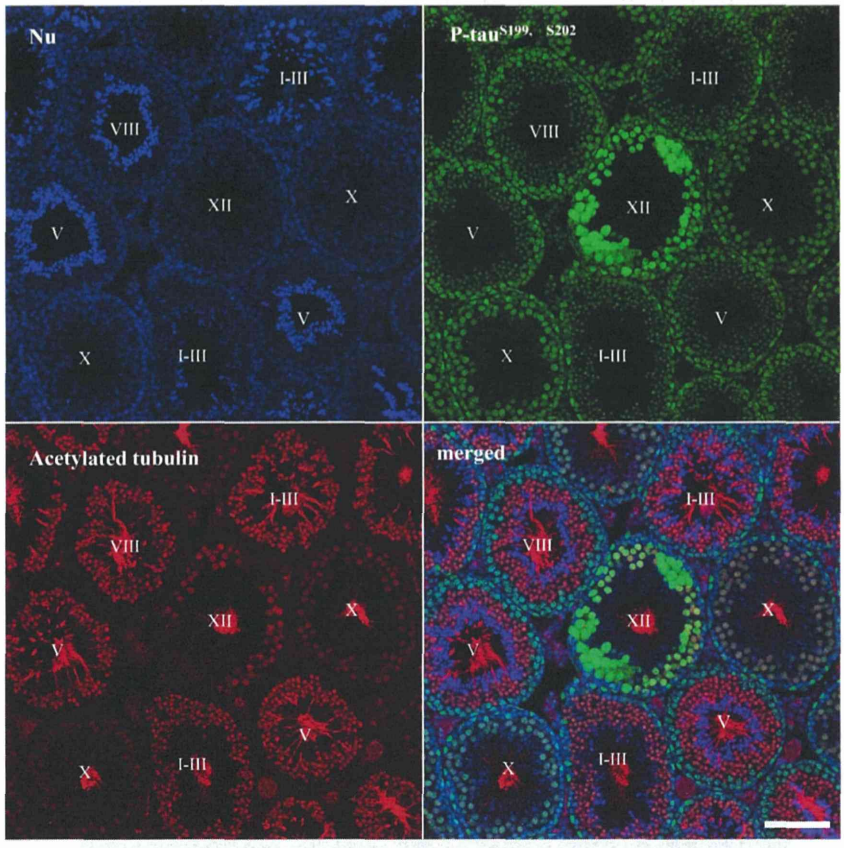

Fig. 3. Immunohistochemistry of adult mouse testis. Blue signals represent DNA counterstaining with Hoechst 33342 (diluted 1:5000). Green signals represent immunostaining for P-tau^{S199,S202} (diluted 1:1000). Red signals represent immunostaining for acetylated tubulin (diluted 1:500). Stages of seminiferous tubules were indicated. Scale bar = 100 μm.

mouse testis (Fig. 1A and B). To date, it has also been reported in the bull and rat [9,19]. In the adult rat brain, alternative splicing of the primary transcript of tau generates six isoforms with an apparent molecular weight between 48 and 67 kDa. However, Gu et al show tau protein was detected in two major bands in the testis with an apparent molecular mass of 34 and 37 kDa after alkaline phosphatase treatment. They suggest this different expression pattern indicates the presence of a testis-specific isoform [9]. In mouse, tau isoform-D (identifier: P10637-5) (uniprot) which molecular weight is about 39 kDa has been discovered [29]. Our sequencing of tau cDNA in the testes ensured that testis tau is corresponding with tau isoform-D. Our Western blot results also demonstrated that the presence of total tau protein with apparent molecular masses of 37 kDa in adult mice (Fig. 1A and B), consistent with previous reports. Furthermore, immunohistochemistry was performed to investigate the localization of phosphorylated tau.

PHFs are composed of highly phosphorylated tau and accumulate in the brain of subjects with AD [30,31]. To elucidate the localization of site-specific phosphorylation status, several antibodies against p-tau were used. The anti-AT8, -AT100 and -AT270 antibodies recognize PHF-tau. Immunohistochemical studies indicated not only tau expression but also its phosphorylation patterns in the testis. Although total-tau expression, detected by anti-tau 1 and anti-AT8 and non-phosphorylated tau at S199, S202, detected by anti-tau 1, were constantly observed from spermatogonia to round spermatids (Fig. 1C–E, S1), p-tau^{S199,S202}, AT8, AT100 and AT270 were especially localized in spermatocytes during meiosis (Fig. 2–4). These results suggested that tau expression was not specific during meiosis but the phosphorylation is specific. In addition, because these antibodies specifically detected the phosphorylation in meiosis, we suggest that Tau might be phosphorylated at AD-specific sites in meiosis. Interestingly, the period of tubulin deacetylation was coincident with that of tau phosphorylation at the time of meiosis (Fig. 4A–D, U–X). The relationship between tau phosphorylation and tubulin acetylation is unidentified. However, North and Verdin reported that activity of the microtubule deacetylase, SIRT2, was elevated during mitotic division, suggesting its involvement in destabilization of the spindle microtubule for chromosome movements [32]. On the other hand, tau acts as HDAC6 inhibitor and PHFs exert the stronger inhibiting actions than tau [33]. During spermatogenesis, the expression level of HDAC6 was high in spermatogonia and low from pachytene spermatocytes to elongating spermatids [34]. Therefore, these site-specific phosphorylation localizations suggest involvement of spindle microtubule destabilization during meiotic division. Further studies are necessary to clarify the relation between tau and microtubule deacetylases.

Chromosomal segregation in both mitotic and meiotic division is controlled by spindle microtubule elongation and retraction.

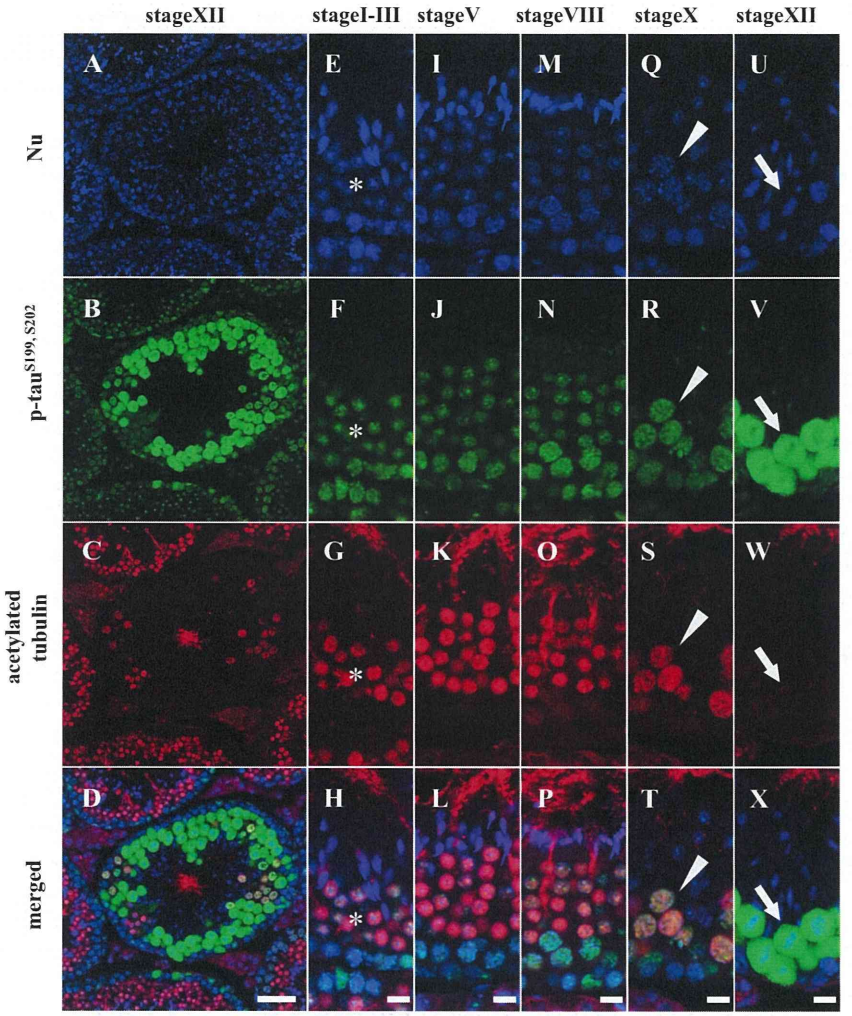

Fig. 4. Immunohistochemistry of adult mouse testis. (A, E, I, M, Q, U) Blue signals represent DNA counterstaining with Hoechst 33342 (diluted 1:5000). (B, F, J, N, R, V) Green signals represent immunostaining for P-tau^{S199,S202} (diluted 1:1000). (C, G, K, O, S, W) Red signals represent immunostaining for acetylated tubulin (diluted 1:500). (D, H, L, P, T, X) Merged Images. (A–D) Images of seminiferous tubules at stage XII. (E–X) Images of seminiferous epithelia. (E–H) Seminiferous epithelia at stages I–III. Early pachytene spermatocytes, step 1 round spermatids (asterisks) and step 13 elongated spermatids are present. (I–L) Seminiferous epithelium at stage V. Intermediate spermatogonia, pachytene spermatocytes, step 5 round spermatids and step 15 elongated spermatids are present. (M–P) Seminiferous epithelium at stage VIII. Preleptotene spermatocytes, pachytene spermatocytes, step 7 round spermatids and step 16 elongated spermatids are present. (Q–T) Seminiferous epithelium at stage X. Leptotene spermatocytes, late pachytene spermatocytes (indicated by arrowheads) and step 10 elongating spermatids are present. (U–X) Seminiferous epithelium at stage XII. Zygotene spermatocytes, spermatocytes during meiotic division (arrows) and step 12 elongating spermatids are present. (A–D) Scale bars in A–D = 50 μm; scale bars in E–X = 10 μm.

Segregation errors during meiotic division cause chromosomal aberrations, which if transferred to offspring cause malformation and miscarriage. Spindles, mainly composed of microtubules, are controlled by MAPs. It is known that spindle microtubules repeatedly extend and retract to catch the kinetochores of chromosomes during metaphase of mitotic and meiotic divisions [35,36]. Therefore, phosphorylation of the protein might contribute to this extension and retraction of microtubules during meiotic division.

Futhermore, the relationship between tau and DNA/chromosomes has recently reported. Tau has been reported to protect DNA from Oxidation and heat stresses [37,38]. In this study, P-tau^{S202,T205} and P-tau^{S212,T214} are localized all over spermatocytes except around the nucleus (Fig. 2A and B). This result agrees with the previous report [38]. In addition, P-tau^{T181} is localized at the nucleus (Fig. 2C). These results suggest that tau may interact with DNA/chromosomes also in the testis.

In conclusion, we have revealed that site-specific tau phosphorylation is localized specifically during spermatogenesis. Furthermore, there appears to be an interaction between tau phosphorylation and microtubule deacetylation, suggesting the possibility of involvement of both processes during meiosis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.04.021.

References

[1] Neely, M.D. and Boekelheide, K. (1988) Sertoli cell processes have axoplasmic features: an ordered microtubule distribution and an abundant high molecular weight microtubule-associated protein (cytoplasmic dynein). J. Cell Biol. 107, 1767–1776.

[2] Cleveland, D.W., Hwo, S.Y. and Kirschner, M.W. (1977) Physical and chemical properties of purified tau factor and the role of tau in microtubule assembly. J. Mol. Biol. 116, 227–247.

[3] Weingarten, M.D., Lockwood, A.H., Hwo, S.Y. and Kirschner, M.W. (1975) A protein factor essential for microtubule assembly. Proc. Natl. Acad. Sci. U.S.A. 72, 1858–1862.

[4] Drubin, D.G. and Kirschner, M.W. (1986) Tau protein function in living cells. J. Cell Biol. 103, 2739–2746.

[5] Kanai, Y., Takemura, R., Oshima, T., Mori, H., Ihara, Y., Yanagisawa, M., Masaki, T. and Hirokawa, N. (1989) Expression of multiple tau isoforms and microtubule bundle formation in fibroblasts transfected with a single tau cDNA. J. Cell Biol. 109, 1173–1184.

[6] Yoshida, H. and Ihara, Y. (1993) Tau in paired helical filaments is functionally distinct from fetal tau: assembly incompetence of paired helical filament-tau. J. Neurochem. 61, 1183–1186.

[7] Goedert, M., Spillantini, M.G., Jakes, R., Rutherford, D. and Crowther, R.A. (1989) Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. Neuron 3, 519–526.

[8] Goedert, M., Spillantini, M.G., Potier, M.C., Ulrich, J. and Crowther, R.A. (1989) Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. EMBO J. 8, 393–399.

[9] Gu, Y., Oyama, F. and Ihara, Y. (1996) Tau is widely expressed in rat tissues. J. Neurochem. 67, 1235–1244.

[10] Buee, L., Bussiere, T., Buee-Scherrer, V., Delacourte, A. and Hof, P.R. (2000) Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res. Brain Res. Rev. 33, 95–130.

[11] Hanger, D.P., Betts, J.C., Loviny, T.L., Blackstock, W.P. and Anderton, B.H. (1998) New phosphorylation sites identified in hyperphosphorylated tau (paired helical filament-tau) from Alzheimer's disease brain using nanoelectrospray mass spectrometry. J. Neurochem. 71, 2465–2476.

[12] Sergeant, N. et al. (2008) Biochemistry of Tau in Alzheimer's disease and related neurological disorders. Expert Rev. Proteomics 5, 207–224.

[13] Dickey, C.A. et al. (2007) The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. J. Clin. Invest. 117, 648–658.

[14] Drewes, G., Trinczek, B., Illenberger, S., Biernat, J., Schmitt-Ulms, G., Meyer, H.E., Mandelkow, E.M. and Mandelkow, E. (1995) Microtubule-associated protein/microtubule affinity-regulating kinase (p110mark). A novel protein kinase that regulates tau-microtubule interactions and dynamic instability by phosphorylation at the Alzheimer-specific site serine 262. J. Biol. Chem. 270, 7679–7688.

[15] Sengupta, A., Kabat, J., Novak, M., Wu, Q., Grundke-Iqbal, I. and Iqbal, K. (1998) Phosphorylation of tau at both Thr 231 and Ser 262 is required for maximal inhibition of its binding to microtubules. Arch. Biochem. Biophys. 357, 299–309.

[16] Ishiguro, K. et al. (1999) Phosphorylated tau in human cerebrospinal fluid is a diagnostic marker for Alzheimer's disease. Neurosci. Lett. 270, 91–94.

[17] Itoh, N. et al. (2001) Large-scale, multicenter study of cerebrospinal fluid tau protein phosphorylated at serine 199 for the antemortem diagnosis of Alzheimer's disease. Ann. Neurol. 50, 150–156.

[18] Hampel, H. et al. (2004) Measurement of phosphorylated tau epitopes in the differential diagnosis of Alzheimer disease: a comparative cerebrospinal fluid study. Arch. Gen. Psychiatry 61, 95–102.

[19] Ashman, J.B., Hall, E.S., Eveleth, J. and Boekelheide, K. (1992) Tau, the neuronal heat-stable microtubule-associated protein, is also present in the cross-linked microtubule network of the testicular spermatid manchette. Biol. Reprod. 46, 120–129.

[20] Kishi, M., Pan, Y.A., Crump, J.G. and Sanes, J.R. (2005) Mammalian SAD kinases are required for neuronal polarization. Science 307, 929–932.

[21] Lee, D.C. et al. (2010) LPS-induced inflammation exacerbates phospho-tau pathology in rTg4510 mice. J. Neuroinflammation 7, 56.

[22] Goedert, M., Jakes, R., Crowther, R.A., Cohen, P., Vanmechelen, E., Vandermeeren, M. and Cras, P. (1994) Epitope mapping of monoclonal antibodies to the paired helical filaments of Alzheimer's disease: identification of phosphorylation sites in tau protein. Biochem. J. 301 (Pt 3), 871–877.

[23] Mailliot, C., Bussiere, T., Caillet-Boudin, M.L., Delacourte, A. and Buee, L. (1998) Alzheimer-specific epitope of AT100 in transfected cell lines with tau: toward an efficient cell model of tau abnormal phosphorylation. Neurosci. Lett. 255, 13–16.

[24] Ho, Y.S. et al. (2012) Endoplasmic reticulum stress induces tau pathology and forms a vicious cycle: implication in Alzheimer's disease pathogenesis. J. Alzheimers Dis. 28, 839–854.

[25] Brecht, W.J. et al. (2004) Neuron-specific apolipoprotein e4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. J. Neurosci. 24, 2527–2534.

[26] Wilson, C.H. and Christie, A.E. (2010) Distribution of C-type allatostatin (C-AST)-like immunoreactivity in the central nervous system of the copepod *Calanus finmarchicus*. Gen. Comp. Endocrinol. 167, 252–260.

[27] Russell, L.D. et al. (1990) Histological and Histopathological Evaluation of the Testis, Cache River Press. Chapter 4 Staging for Laboratory Species, pp. 119–21.

[28] Grundke-Iqbal, I., Iqbal, K., Tung, Y.C., Quinlan, M., Wisniewski, H.M. and Binder, L.I. (1986) Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc. Natl. Acad. Sci. U.S.A. 83, 4913–4917.

[29] Kenner, L. et al. (1994) Expression of three- and four-repeat tau isoforms in mouse liver. Hepatology 20, 1086–1089.

[30] Lee, V.M., Balin, B.J., Otvos Jr., L. and Trojanowski, J.Q. (1991) A68: a major subunit of paired helical filaments and derivatized forms of normal Tau. Science 251, 675–678.

[31] Goedert, M. (1993) Tau protein and the neurofibrillary pathology of Alzheimer's disease. Trends Neurosci. 16, 460–465.

[32] North, B.J. and Verdin, E. (2007) Interphase nucleo-cytoplasmic shuttling and localization of SIRT2 during mitosis. PLoS ONE 2, e784.

[33] Perez, M. et al. (2009) Tau – an inhibitor of deacetylase HDAC6 function. J. Neurochem. 109, 1756–1766.

[34] Hazzouri, M., Pivot-Pajot, C., Faure, A.K., Usson, Y., Pelletier, R., Sele, B., Khochbin, S. and Rousseaux, S. (2000) Regulated hyperacetylation of core histones during mouse spermatogenesis: involvement of histone deacetylases. Eur. J. Cell Biol. 79, 950–960.

[35] Walczak, C.E. and Heald, R. (2008) Mechanisms of mitotic spindle assembly and function. Int. Rev. Cytol. 265, 111–158.

[36] Kitajima, T.S., Ohsugi, M. and Ellenberg, J. (2011) Complete kinetochore tracking reveals error-prone homologous chromosome biorientation in mammalian oocytes. Cell 146, 568–581.

[37] Wei, Y., Qu, M.H., Wang, X.S., Chen, L., Wang, D.L., Liu, Y., Hua, Q. and He, R.Q. (2008) Binding to the minor groove of the double-strand, tau protein prevents DNA from damage by peroxidation. PLoS ONE 3, e2600.

[38] Sultan, A. et al. (2011) Nuclear tau, a key player in neuronal DNA protection. J. Biol. Chem. 286, 4566–4575.