Bioresearch Communications
Volume 06, Issue 01, January 2020
Journal Homepage: www.bioresearchcommunications.com

Review Article

Molecular basis and functional diversity of TOUSLED kinase

Hashimul Ehsan*

Department of Biology, University of Houston-Victoria, 3007, N. Ben Wilson, Victoria, TX 77901

ABSTRACT: TOUSLED (TSL) gene initially identified in the model system *A. thaliana*, and later in numerous organisms, played diverse role in replication, chromatin remodeling, DNA repair for normal growth and development. In Arabidopsis and human, this kinase activity fluctuates during the cell cycle and interact with several substrates however the molecular mechanism of this gene regulation remains it its infancy. Although TOUSLED kinase and TOUSLED-like kinase (TLK1 & TLK2) research is increasingly advancing in particularly cell cycle studies, more research is needed to fully explore its functional role in cell division. This review will focus on a succinct description of this kinase during the cell cycle and as well as its interactors during the growth and development. Regardless of its progress, TOUSLED kinase has potential during the cell cycle but have yet to uncover furthermore using Arabidopsis cell suspension culture as a model system.

Keywords: TSL, TOUSLED-like kinase (TLKs), Asf1.

Article History
Received: 16 September 2019
Accepted: 19 December 2019

Scan the QR code to see the online version or,visit- www.bioresearchcommunications.com

Corresponding author
Dr. Hashimul Ehsan
Centre for Advanced Research in Department of Biology, University of Houston-Victoria, 3007
N. Ben Wilson Victoria, TX 77901
Email: ehsanh@uhv.edu
Phone: [361]570-4289

Citation: Hashimul Ehsan; Molecular basis and functional diversity. ***Biores Comm.*** 6(1), 840-843.

General information on TOUSLED and TOUSLED-like kinases

The TOUSLED (TSL) gene was first identified by mutagenesis studies in the *Arabidopsis* plant by Dr. Judy Roe (UC Berkeley) and continued by Dr. Hashimul Ehsan [1,2]. Several years later, it has been shown that these nuclear TOUSLED-like kinases (TLKs) are highly conserved in plants and animals. TOUSLED-like kinases (TLKs), have been found in mouse, human, tropical clawed frog, lizard, zebra fish, fruit fly, *Trypanosomes brucei* (African parasitic protist), Rotifers and worm except unicellular eukaryotes including bacterial cells and yeast[6]. Since then diverse range of TOUSLED kinase is responsible for susceptibility of such a wide range of cell cycle events in various organisms [3]. This kinase is composed of a C-terminal catalytic domain and an N-terminal regulatory domain **(Figure 1).**

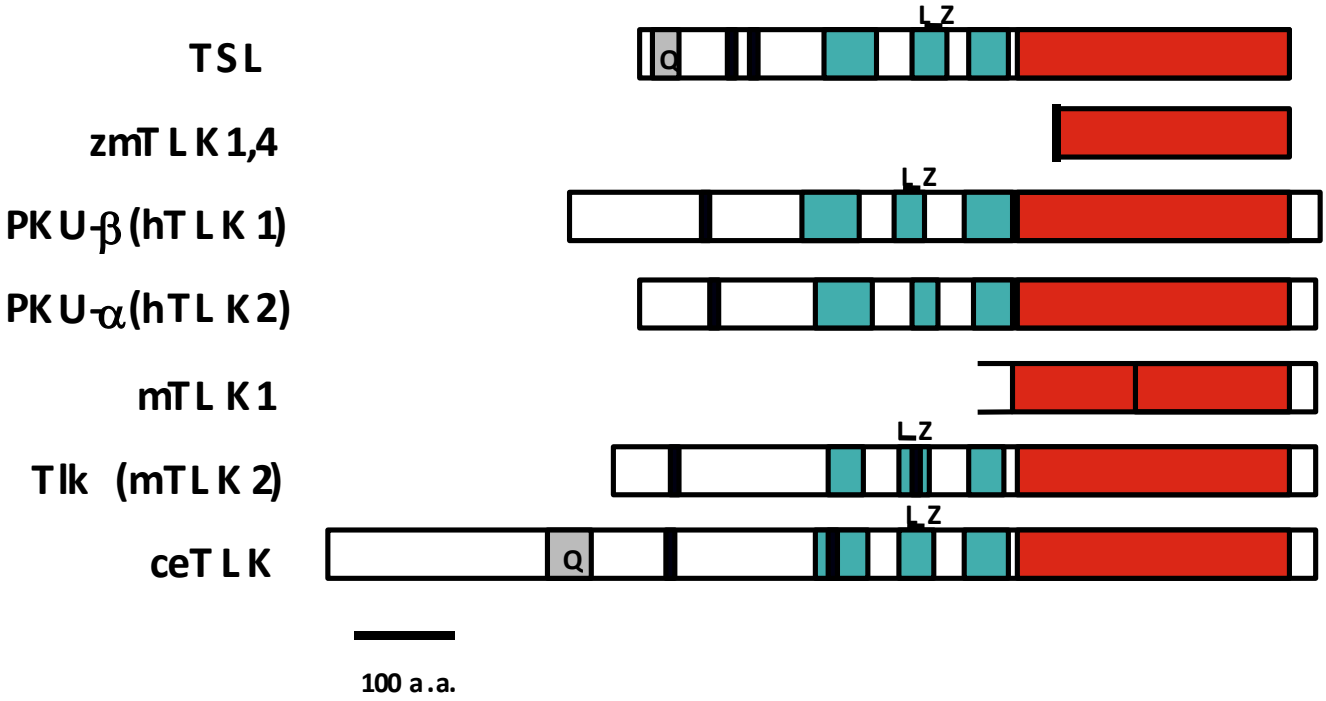

Figure 1. Schematic depiction of deduced protein products of TOUSLED-like kinase family member genes showing conserved features, The serine/threonine C-terminal catalytic domains are red, glutamine-rich stretches are shown in grey, nuclear localization signals (NLS) in solid bars. Arabidopsis TSL protein is 78kDa and has an N-terminal and regulatory and a C-terminal catalytic domain. MTK1 and MTK4 from *Z. mays* are almost identical to each other .Human TLK1is an 82 kDa protein kinase which contains an NLS and three coiled-coil segments in positions similar to TSL. A TLK1 from *C. Elegans*, ceTLK, is derived from the sequence of cosmid CO7A9 and its expression is confirmed by the number of ESTs. The human Tlks and mouse Tlk2 have also been cloned with names PKU-α and PKU-ß.

The regulatory domain contains a predicted coiled-coil region, including a leucine zipper segment that is required for catalytic activity and oligomerization *in vitro* [4]. This domain also includes three nuclear localization signals (NLS), at least one of which is functional, and a glutamine rich motif [4]. Cloned TLKs from human and mouse and have shown involvement in cell cycle regulation and that they are sensitive to DNA-damaging agents and inhibitors of DNA replication [5]. The two-known human TLKs, TLK1 and TLK2, are 84% similar at the amino acid sequence level, ubiquitously expressed and act as dimers/ oligomers [5]. TLKs contain similar functional motifs as TSL, and these motifs are arranged in a comparable manner **(Figure 1**).

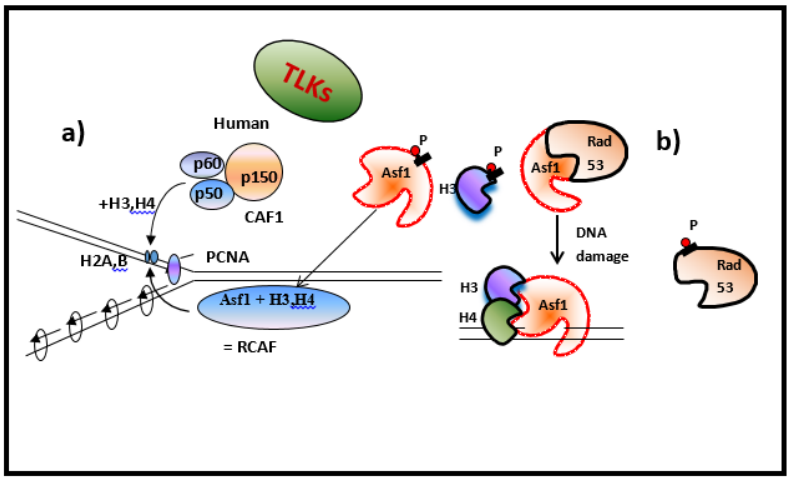

Figure 2. a) Asf1 phosphorylates by TLKs and stimulate chromatin assembly. CAF-1 mediates H3 deposition through its interaction with PCNA during replication and DNA repair. ASF1 recruits H3 and H4 histones to RACF together with CAF1 to assist the assembly of nucleosomes onto newly synthesized DNA during replication. b) Asf1 in DNA Repair: Asf1 dissociates from Rad53 protein kinase upon DNA damage and promotes nucleosome assembly on damaged DNA.

Mutations in the founding member of the TOUSLED family, *Arabidopsis thaliana* TOUSLED, resulted in numerous developmental defects that led to a "TOUSLED" appearance of various plant tissues. Since then diverse range of TOUSLED kinase is responsible for susceptibility of such a wide range of cellular events in various organisms. In higher plants, the mechanisms by which TOUSLED kinase contributes to an enhanced response include the phosphorylation during the growth and development and studies by several groups indicate a good starting point for further functional role of this kinase. *tsl* loss of function mutations cause pleiotropic defects in both leaf and flower development, and growth and initiation of floral organ primordia is abnormal, suggesting that basic cellular processes are affected.

Role of TOUSLED and TOUSLED-like kinases (TLKs) in cell cycle

Human TOUSLED-like kinase (TLK) activity oscillates during the cell cycle. TLK1 and TLK2 are involved in chromatin assembly, DNA repair, transcription and chromosome segregation [6]. TLK1 interacts specifically with the chromatin assembly factors Asf1 and Rad 9 [7-9]. Asf1 is a histone H3-H4 chaperone that is essential in mammals and other organisms [10-13]. Asf1, in conjugation with another chaperone called CAF1, promotes the assembly of nucleosomes onto newly replicated DNA strands [14]. In mammals, Asf1 has been identified as a phosphorylation target of TLKs which stimulate chromatin assembly [5,15]. Human TLK activity increases in S phase (DNA replication) of the cycle and interacts redundantly with chromatin assembly factor. Phosphorylation of histone H3 at ser-10 by TLK 1B has been shown but the significance of this phosphorylation remains unclear. Asf1 recruits H3 and H4 histones to form replication-coupling assembly factor (RCAF) together with chromatin assembly factor 1 (CAF1) to assist the assembly of nucleosomes onto newly synthesized DNA during replication [12, 16] **(Figure: 3).**

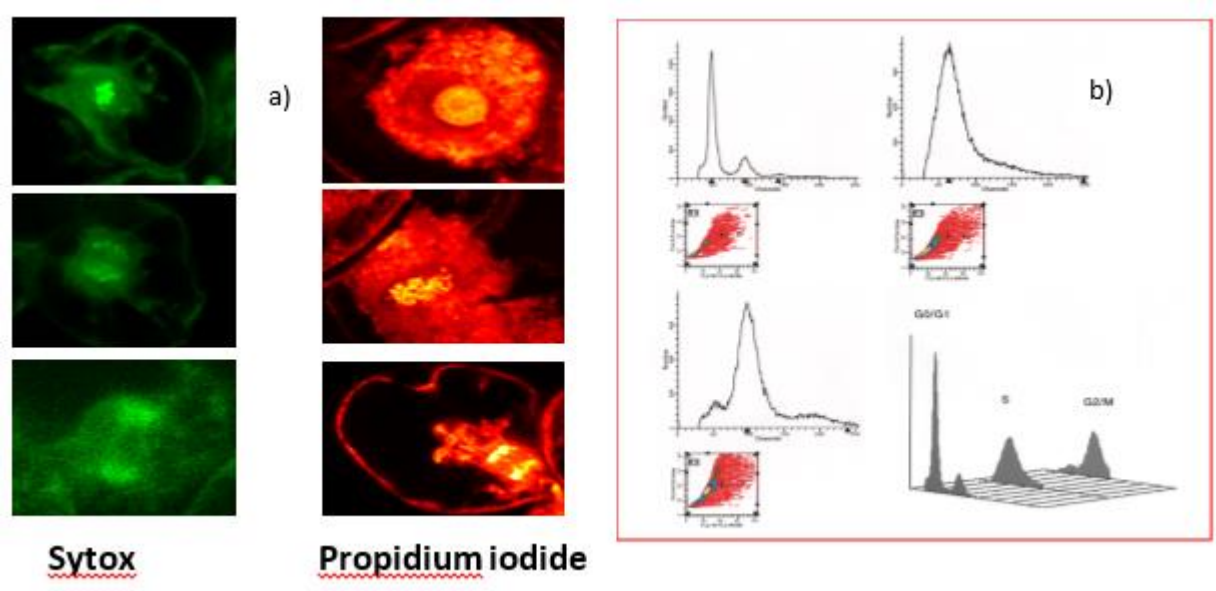

Figure 3. Arabidopsis suspension culture cells were synchronized by aphidicolin treatment followed by washing. a) (left) Cells from 13 hours after release were stained with propidium iodide and viewed by confocal microscope. Three examples are shown, the lower two are in mitosis. b) Flow cytometry was performed on nuclei isolated and stained with propidium iodide from stationary (upper left), S-phase (upper right), and G2/M (lower left) cells. The same data is shown in the bottom right combined into a three-dimensional histogram. The stationary phase cells are in G 0/G1 with a 2C DNA content. The S-phase sample contains nuclei which show an increase in DNA content as cells are replicating their DNA. The G2/M cells show a peak at 4C DNA content. Approximately 30,000 nuclei were measured at each time point.

TLK1 inhibition in response to DNA damage is dependent on checkpoint protein CHK1, which phosphorylates S695 leading to transient inactivation [5]. This inactivation of TLK1 reduced its phosphorylation capabilities towards ASF1a which prevents DNA replication/repair [5].

TSL was found to be more highly expressed in exponentially growing *Arabidopsis thaliana* culture cells than in stationary, non-dividing cells. TSL kinase activity increases during M phase and G1 in synchronized *Arabidopsis* suspension culture cells and *tsl* mutants also display an aberrant pattern and increased expression levels of the mitotic cyclin gene CycB1;1, suggesting that TSL may repress *CycB1;1* expression at certain times during development or the cell cycle [2]. These above-mentioned findings will led to the assumption that TLKs are present only in multi-cellular eukaryotes and likely play the fundamental aspects of development during the cell cycle.

Future directions

Cell cycle research has been aided tremendously by the ability to synchronize a population of cells in culture [17,18]. These cell cultures lines are typically undifferentiated cells that are maintained in the presence of one or more plant "growth" hormones. These cell cultures have been tremendously useful for both plant cell cycle research, and for production of anti-cancer drugs, such as taxol, vinblastine, and vincristine, and other chemicals and herbal compounds. The readily synchronizable tobacco BY-2 cell line has been the workhouse in plant cell cycle research [17], whereas Arabidopsis cell cultures have been used to a lesser extent. The BY-2 cells are maintained in the presence of auxin, whereas the Arabidopsis cells require both auxin and cytokinin for proliferation. Two methods can be used for synchronization of cell cultures. First, hormone starvation causes the population to stall G 0, which is then followed by hormone addition to initiate synchronous entry into G1. The second method utilized cell cycle blockers. Cells are incubated in the drug, released from the block by washing, whereupon they enter the subsequent cell cycle phase synchronously. Depending on which drug(s) is used, cells can be blocked initially within a chosen stage, and the cells then progress synchronously from that stage after release [18-20].

The TSL proteins are located primarily in the nucleus and even less is known of their function and of biochemical pathway, chromatin remodeling and assembly, and organogenesis in higher plants. An open question needs to be elucidated: what coordinates all these cellular activities during higher plant development? The significance in higher plants is not yet fully understood, but so far suggests that to involve in cell division cycle perhaps similar to mammalian counterpart. One possible way similar to the human TOUSLED (TLKs) is to investigate the functional role during the plant cell cycle of this kinase. Using Arabidopsis cell suspension culture as a model system, it is interesting to find functional role of this kinase by applying molecular-genetic and biochemical approaches. The significance outcome on TSL and its interaction with other cell cycle regulatory protein will illuminate the mechanisms involved in the control of the plant cell cycle and in the regulation of nuclear events including transcription, DNA repair, and DNA replication.

ACKNOWLEDGEMENTS

I extend the deepest appreciation to the Arts and Sciences Department at the University of Houston-Victoria for encouraging me to reach new horizons in scientific knowledge on TOUSLED like kinase.

REFERENCES

1. Roe, J.L., Rivin, C.J., Sessions, R.A., Feldmann, K.A. and Zambryski, P.C. 1993. The TOUSLED gene in *A. thaliana* encodes a protein kinase homolog that is required for leaf and flower development. *Cell* **75**, 939–950.

2. Ehsan, H., Reichheld, J.P., Durfee, T., Roe, J.L. 2004. TOUSLED kinase activity oscillates during the cell cycle and interacts with chromatin regulators. *Plant Physiology*, **134(4)**, 1488-99.

3. Nurse, P. (2000) A long twentieth century of the cell cycle and beyond. *Cell* **100**,71-78.

4. Roe, J.L., Durfee. T., Zupan, J.R., Repetti, P.P., McLean, B.G., Zambryski, P.C. 1997.TOUSLED is a nuclear serine/threonine protein kinase that requires a coiled-coil region for oligomerization and catalytic activity. *J. Biol. Chem.* **272**:5838–5845.

5. Sillje, H.H., Takahashi, K., Tanaka, K., Van Houwe, G. and Nigg, E.A. 1999. Mammalian homologues of the plant *TOUSLED* gene code for cell-cycle-related kinases with maximal activities linked to ongoing DNA replication. *EMBO J.* **18**, 5691–5702.

6. De Benedetti, A. 2012. The TOUSLED-Like Kinases as Guardians of Genome Integrity. *ISRN Mol Biol.*:**627596**. doi: 10.5402/2012/627596.

7. Sunavala-Dossabhoy, G. and De Benedetti, A. 2009. TOUSLED homolog, TLK1, binds and phosphorylates Rad9; TLK1 acts as a molecular chaperone in DNA repair. *DNA Repair (Amst).* **8(1)**, 87-102. doi: 10.1016/j.dnarep.2008.09.005.

8. Canfield, C., Rains, J. and De Benedetti, A. 2009. TLK1B promotes repair of DSBs via its interaction with Rad9 and Asf1. *BMC Mol Biol*. **10**:110. doi: 10.1186/1471-2199-10-110.

9. Sillje, H.H. and Nigg, E.A. 2001. Identification of human Asf1 chromatin assembly factors as substrates of TOUSLED-like kinases. *Curr Biol.* **11(13)**, 1068-73.

10. Munakata, T., Adachi, N., Yokoyama, N., Kuzuhara, T. and Horikoshi, M. 2000. A human homologue of yeast anti-silencing factor has histone chaperone activity. *Genes Cells*. **5(3)**, 221-33.

11. Sanematsu, F., Takami, Y., Barman, H.K., Fukagawa, T., Ono, T., Shibahara, K. and Nakayama, T. 2006. Asf1 is required for viability and chromatin assembly during DNA replication in vertebrate cells. *J Biol Chem.* **281(19)**, 13817-27. doi: 10.1074/jbc.M511590200.

12. Grigsby, I.F,. Rutledge, E.M., Morton, C.A. and Finger, F.P. 2009. Functional redundancy of two C. elegans homologs of the histone chaperone Asf1 in germline DNA replication. *Dev Biol.***329(1)**, 64-79.

13. Umehara, T., Chimura, T., Ichikawa, N. and Horikoshi, M. 2002. Polyanionic stretch-deleted histone chaperone cia1/Asf1p is functional both in vivo and in vitro. *Genes Cells*. **7(1)**:59-73.

14. Carrera, P., Moshkin, Y.M., Gronke, S., Sillje, H.H., Nigg, E.A., Jackle. H. and Karch, F. 2003. TOUSLED-like kinase functions with the chromatin assembly pathway regulating nuclear divisions. Genes Dev. **17(20)**, 2578-90.

15. De Benedetti, A. 2010. TOUSLED kinase TLK1B mediates chromatin assembly in conjunction with Asf1 regardless of its kinase activity. *BMC Res Notes.***3:68**. doi: 10.1186/1756-0500-3-68.

16. Mello, J.A. and Almouzni, G. 2001. The ins and outs of nucleosome assembly. *Curr Opin Genet Dev*. **11(2)**, 136-41.

17. Nagata T. and Kumagai F. 1999. Plant cell biology through the window of the highly synchronized tobacco BY-2 cell line. *Methods Cell Sci.* **21**,123–127. 10.1023/A:1009832822096.

18. Planchais S, Glab N, Inze D, Bergounioux C 2000. Chemical inhibitors: a tool for plant cell cycle studies. FEBS Lett. **476**: 78–83.

19. Ehsan, H., Reichheld, J-P., Luc, R., Witters, E., Lardon, F., Van Bockstaele, D., Van Montagu, M., Inze, D., and Van Onckelen, H. 1998. Effect of indomethacin on cell cycle dependent cyclic AMP fluxes in tobacco BY-2 cells. *FEBS Lett.* **422**:165-169.

20. Ehsan, H., Luc, R., Witters, E., Reichheld, J-P., Inze, D., and Van Onckelen, H. 1999. Indomethacin induced G1/S phase arrest of the plant cell cycle. *FEBS Lett.* **458**:349-353.

21. Fourquin, Chloé & Vinauger-Douard, Marion & Fogliani, B & Dumas, Christian & Scutt, Charlie. (2005). Evidence that CRABS CLAW and TOUSLED have conserved their roles in carpel development since the ancestor of the extant angiosperms. Proc. Natl. Acad. Sci. USA. **102(12)**: 4649–4654.

22. Wang Y., Liu J., Xia R., Wang J., Shen J., Cao R., Hong X., Zhu J.-K. and Gong Z. (2007). The protein kinase TOUSLED is required for maintenance of transcriptional gene silencing in Arabidopsis. *EMBO Rep*. **8**, 77-83. 10.1038/sj.embor.740085