Life of SLBP, a major player in histone biosynthesis

Mark Kennedy 1*, Natasha Kovaleva2

1Department of Computer Science,
Suleyman Demirel University, Kaskelen, Kazakhstan, 040900
2Department of Molecular Biology and Medical Genetics,
Kazakh National Medicine University named after Asfendiyarov, Almaty, 035000
*Presenting author
e-mails: markkennedy@sdu.edu.kz, natasha@kaznmu.kz

ABSTRACT

Cells need to achieve very delicate balance between DNA synthesis and histone levels and ensure the synthesis of histone proteins just during the S phase where the DNA is being replicated. Restriction of histone biosynthesis to S phase is critical for maintenance of genomic stability and proper gene regulation. Many transcriptional and posttranscriptional factors contribute coordinately to regulate expression of histone proteins, including transcription of histone genes, efficiency of pre-mRNA processing (the percentage of mature mRNA that reach to cytoplasm), change in mRNA half- life and degradation of excess histone proteins. Stem-Loop Binding Protein (SLBP), which binds to 3' ends of histone mRNAs, is a key factor in histone biosynthesis. SLBP expression is cell cycle regulated without significant change in its mRNA level and this regulation is responsible for occurrence of bulk histone production during S phase. SLBP level is high during S phase and low in M and G1 phase until next S phase. It has been found that SLBP is degraded at the end of S phase due on double phosphorylation triggered by CyclinA/CDK2 and similarly in G1, the SLBP stability seems to be low. SLBP protein, which is not degraded at the end of S phase was found to be toxic for cells and effects the rate of DNA replication. These findings show that S phase limited expression of SLBP is very critical for histone mRNA biosynthesis.

Keywords: Stem Loop Binding Protein (SLBP), histone mRNA, cell cycle, protein degradation, histone mRNA processing.

SLBP is required in all aspects of histone mRNA regulation

Replication of eukaryotic chromosomes require synthesis of both DNA and sufficient histone proteins to package newly replicated DNA into chromatin structure. Cells adjust the level of histone mRNA to match the demand for histone protein which is dictated by the rate of DNA synthesis (reviewed in (Marzluff et al., 2008)). In mammalian cells there are four different regulatory mechanisms that contribute to proper histone protein level during the cell cycle: Transcription of histone genes, efficiency of pre-mRNA processing (the percentage of mature mRNA that reach to cytoplasm), change in mRNA half- life and degradation of excess histone proteins (Graves et al., 1987; Gunjan et al., 2006; Harris et al., 1991; Marzluff et al., 2008). Cells need to achieve very delicate balance between DNA synthesis and histone levels and ensure the synthesis of histone proteins occurs just during the S phase where the DNA is being replicated. Excess histones may alter the gene expression by nonspecifically binding to DNA (Au et al., 2008), cause mitotic chromosome loss (Meeks-Wagner and Hartwell, 1986) and mediate cytotoxicity by multiple mechanism (Singh et al., 2010). In contrary to this, scarcity of histone proteins results in cell death (Han et al., 1987). All five classes of histone proteins are encoded by so-called replication dependent histone mRNAs, which have unique and highly conserved stem loop at 3' end instead of poly-A tail (Busslinger et al., 1979; Connor et al., 1984). The 3'end of these mRNAs are required for histone stoichiometry in chromatin structure, regulation of the rate and timing of histone protein synthesis during cell cycle (Eckner et al., 1991; Marzluff and Duronio, 2002; Marzluff et al., 2008; Zanier et al., 2002). The level of histone mRNA increases dramatically as cells enter to S phase to cover the rapid need for new histone proteins.
At the beginning of S phase, the transcription of histone genes by RNA polymerase II, increases from three to five fold whereas processing efficiency increases eight to ten fold, overall accounting for almost 35 fold increase in histone mRNA level (Marzluff, 2005). As cells exist S phase, the half-life and processing efficiency of histone mRNA is reduced, which together lead to instantaneous decrease in histone mRNA level (Marzluff, 2010). In mammals, the cell cycle regulation of SLBP (Stem Loop Binding Protein) that binds to stem loop at 3' end of histone mRNA accounts for the regulation of histone pre-mRNA processing (DeJong et al., 2002; Martin et al., 1997; Whitfield et al., 2000; Zheng et al., 2003). SLBP expression is cell cycle regulated in parallel to histone mRNA. The level of SLBP is very low in G1 and increases around 10 fold at the end of G1 phase where cells get ready for replication and it is degraded rapidly at the end of S phase. The amount of SLBP mRNA is almost stable during cell cycle, indicating much of the regulation is at posttranscriptional level (Whitfield et al., 2000)

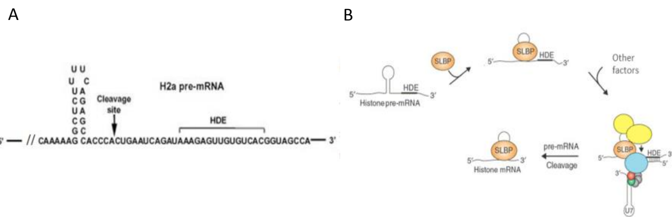

Figure1: Replication dependent histone mRNAs have unique 3' end. A) 3' end structure of histone mRNA**.** The only processing reaction for the mature histone mRNA is endonucleolytic cleavage after 3' stem-loop structure where Stem-Loop Binding Protein (SLBP) binds and mediates the reaction. **B)** SLBP binds to 3' end of histone mRNA and recruits other factors for endonucleatic cleavege (Marzluff *et al.*, 2008).

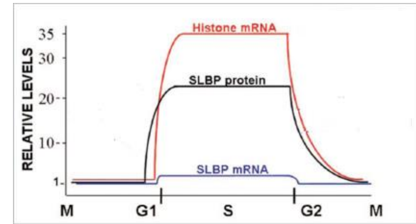

Figure2: Cell cycle regulated expression of SLBP, SLBP mRNA and histone mRNA levels. The level of SLBP and histone mRNA peaks during S phase, whereas SLBP mRNA level does not change significantly throughout the cell cycle.

SLBP is required for all aspects of histone mRNA biosynthesis: proper processing of pre-mRNAs, nuclear export, translation and regulation of stability of mRNA (Dominski and Marzluff, 1999; Erkmann, Sanchez, et al., 2005; Gorgoni et al., 2005; Kaygun and Marzluff, 2005b; Sanchez and Marzluff, 2002; K. D. Sullivan et al., 2009; Whitfield et al., 2004). Mutation in stem loop results in no expression of processed histone mRNA in vivo (Ingledue et al., 1995; Levine et al., 1987, Pandey et al., 1994; Yang et al., 2009) most probably via abolishing SLBP binding. To date, there is no evidence for functions of SLBP outside those directly involved in histone biosynthesis (Townley-Tilson et al., 2006). Analysis of genetic mutations or knockdown of SLBP in Drosophila melanogaster (E. Sullivan et al., 2001), mouse (Arnold et al., 2008) and human cultured cells (Zhao et al., 2004) revealed that SLBP is required for cell cycle coupled histone mRNA production and indicate that the role of SLBP in histone production is highly conserved among different species. Other than in eukaryotes, SLBP was shown to be present in several types of protozoa (Davila Lopez and Samuelsson, 2008). Depletion of SLBP level in continuously cycling cells causes reduction in the rate of cell division, accumulation of cells in S phase probably via preventing sufficient histone production (K. D. Sullivan et al., 2009; Wagner et al., 2005; Zhao et al., 2004). One of the major defects caused in absence of SLBP is increase in the rate of formation of polyadenylated histone mRNAs, supporting the notion that SLBP is required for proper pre-mRNA processing (E. Sullivan et al., 2001). Expression of polyadenylated histone mRNAs have been revealed by microarray analyses in various subtypes of breast cancer (Zhao et al., 2004) and oropharyngeal squamous-cell carcinoma (Beggs et al., 2012; Feng et al., 2005; Kari et al., 2013). It is likely that, polyadenylated histone mRNAs might provide advantage to rapidly dividing cells by supplying additional source of histones outside of S phase, as they own cell cycle independent stability and translation (Kirsh et al., 1989).

SLBP expression is limited to S phase by several mechanisms

One should expect that, restriction of SLBP expression just to S phase might require involvement of multiple regulatory mechanisms, as it is the core player of cell cycle regulation of histone biosynthesis. It was shown that rapid degradation of SLBP at S/G2 border is triggered due on phosphorylation by Cyclin A/CDK1 on Thr 61 which subsequently stimulates phosphorylation of adjacent Thr 60 by CK2 (M. M. Koseoglu et al., 2008). In our previous article (Djakbarova et al., 2016), we have identified the E3 Ligase, the CRL4DCAF11, which mediates SLBP degradation at S/G2 border, depending on double phosphorylation of SLBP. In order to identify the E3 Ligase, we have used 55 amino acid long SLBP piece, which is sufficient to mimic S/G2 regulation of SLBP expression (M. M. Koseoglu et al., 2008). By using this piece as a bait, we have found that CRL4DCAF11 binds to SLBP in phosphorylation dependent manner and mediates its degradation.
Followed by the rapid decline at the end of S phase, SLBP expression is repressed until next onset of S phase. Zheng et al, suggested that low translation efficiency was responsible for low SLBP level in G1 (Zheng et al., 2003), however it was shown that the low translation rate is limited to early G1 phase and it reaches to S phase level somewhere in between early to mid G1 ((Mehmet Murat Koseoglu, 2007) ; (Djakbarova et al., 2014). Unexpectedly, even after translation rate was enhanced, the level of SLBP does not reach to S phase level for couple of more hours, indicating the presence of additional mechanism with contrary effect. Previously proteasome mediated degradation was proposed that regulated proteasome mediated degradation is responsible for suppressed SLBP expression during G1 (Mehmet Murat Koseoglu, 2007). In our work, we have confirmed the proteasome mediated degradation suppresses SLBP expression low in G1 and showed that this degradation is independent from previously identified degradation at the end of S phase (Djakbarova et al., 2013).

CONCLUSION

S phase restricted expression of SLBP is crucial for cell cycle regulated expression of histone mRNA, thus histone proteins. Expression of SLBP outside of S phase impairs the histone biosynthesis and effects the rate and fate of DNA replication, genomic stability and gene expression

REFERENCES

1. Koseoglu MM *et al*., MCB. 2008; 28:4469–4479.
2. Marzluff WF *et al*. Nature reviews Genetics. 2008; 9:843–854
3. Djakbarova *et al*., J Cell Biochem. 2014; 115(3): 523–530
4. Whitfield ML *et al*., MCB. 2000; 20:4188–4198.
5. Dominski Z *et al*., MCB. 1999; 19:3561–3570.
6. Marzluff WF and Duronio RJ. Current opinion in cell bio. 2002; 14:692–699.

ACKNOWLEDGEMENT:

- This work is supported by TUBITAK GRANT #110T987 (to MMK)