Biotinylation of Yeast Histones and its Characteristics

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Abstract
Biotinylation is a posttranslational modification that has an important role in the covalent binding of biotin to lysine residues of protein aided by biotin protein ligase (Bpl1p). Biotin is important for fatty acid metabolism and cell growth. Biotin is used as a cofactor for carboxylase such as pyruvate carboxylase and acetyl-CoA carboxylase. The biotinylated lysine residue is almost invariably located in a consensus sequence, AMKM, within carboxylases. As a result, biotinylation can occur across widely divergent organisms. Additionally, the histones H2A, H2B and H4 of Candida albicans lack a consensus sequence, but can be biotinylated by native C. albicans Bpl1p in vivo which is biotinylated in non-site-specific proteins.

In this research, we used in vivo and in vitro experiments and analysis by SDS-PAGE and Western blotting. The results show that the biotinylation of H2B is temperature and biotin concentration dependent. Moreover, the Bpl1p of C. albicans failed to biotinylate other yeast histone proteins (Saccharomyces cerevisiae) in vivo and in vitro. Future studies are underway to analyze the specificity and mechanism of biotinylation by C. albicans Bpl1p.

Keywords: Bpl1p, *Candida albicans*, *Saccharomyces cerevisiae*, H2B, biotinylation, AMKM, Arc1p, *in vivo*, *in vitro*.

Introduction

Biotin and its Properties: Biotin, also known as vitamin B7 or H, has a key role as a protein-bound cofactor and is important for the synthesis of functional biotin carboxylases and decarboxylases. Biotin is also required by all organisms, including humans[4] and is correlated to a distinct set of carboxylases involved in the synthesis of fatty acid, gluconeogenesis and branched-chain amino acid catabolism.

This vitamin is mostly produced by plants and many prokaryotes, but not mammals. Carboxylase enzymes catalyze essential metabolic processes in organisms using the biotin prosthetic group as a mobile carboxyl carrier.

The attachment site of biotin to these enzymes in the posttranslational process via the ε-amino group of a specific lysine residue is catalyzed by specific protein called biotin protein ligase e.g. Bpl1p in yeast, BirA in *E. coli*[15] and holo-carboxylase synthetase (HCS) in mammals. This process is a two-step reaction[17]:

$$Biotin + ATP \leftrightarrow Biotinyl\text{-}5'\text{-}AMP + PPi$$
$$Biotinyl\text{-}5'\text{-}AMP + Apo\ protein \rightarrow Biotinylated\ protein + AMP$$

Biotinylation by Specific Protein and Specific Targets: Bpl1p (biotin protein ligase in yeast) is an essential enzyme that is responsible for the activation of biotin-dependent enzymes through its covalent attachment. In yeast, deletion of the *BPL1* gene affects important metabolic pathways such as gluconeogenesis and fatty acid biosynthesis[16]. Biotin accepting proteins have been identified and biotinylated by Bpl1p derived from various organisms. Specifically, the determinants of a functional protein-protein interaction have been highly conserved during evolution. Two widely used yeasts, *Saccharomyces cerevisiae* and *Candida albicans*, have been modeled. *C. albicans* is a fungus with dimorphic characteristic and one of the most important fungi in medicines because of its close association with diseases in humans[9].

Biotinylation also occurs in other organisms by enzymes with similar activity such as BirA in *E. coli* and holocarboxylase synthetases in humans[22] and mammals.

Biotin has an essential nutrient. In mammals, there are five biotin-dependent carboxylases: pyruvate carboxylase (PC), acetyl-CoA carboxylase 1 and 2 (ACC1 and ACC2), propionyl-CoA carboxylase (PCC) and β-methylcrotonyl-CoA carboxylase (MCC). These biotin-containing enzymes are responsible for the transfer of CO_2 from bicarbonate to metabolic intermediates in fatty acid, gluconeogenesis and amino acid metabolism[7]. Alternatively, yeasts lack β-MCC and PCC, but contain urea amydolase.

In each of these carboxylases, the biotin moiety is covalently bound to the Ɛ-amino group of a lysine residue in a domain (60 to 80) amino acids long. The biotin accepting domain is structurally similar among carboxylases from bacteria to mammals and is located at the center and is a short peptide (A/V)MKM which is the universal biotin acceptor sequence. Surprisingly, while no AMKM motif exists in yeast Arc1p, a tRNA-binding protein can be biotinylated by Bpl1p.

Therefore, Arc1p possesses a noncanonical biotinylation site, SSKD. HCS catalyzes the transfer of biotin to all of the apocarboxylase substrates in mammals[7].

Diversity of Biotinylated Proteins: The lysine residue that is biotinylated is almost invariably positioned in a consensus sequence, AMKM, within carboxylases. As a result, biotinylation can widely occur across organisms.[3] Biotin binds strongly to avidin (with a K_d of ~10^{-15} M). This feature

is often exploited in biotechnological applications such as immobilization, localization and protein purification.

Five carboxylases have been identified in *S. cerevisiae*: cytosolic and mitochondrial forms of PC, cytosolic and mitochondrial forms of acetyl-CoA carboxylase and urea amidolyase[8,21]. These enzymes are highly biotinylated *in vivo*, even under conditions with low levels of biotin suggestive of the indispensable nature of this cofactor for carboxylases. Even though Arc1p is not involved in any carboxylation reaction and lacks the canonical consensus sequence of biotin-binding domains, it is a natural target of biotinylation[10]. Recent studies further showed that human histones H3 and H4 are biotinylated, albeit to a much lesser extent[13,23].

Histone Biotinylation and its Function: Biotinylation of histones is a relatively new research field and little is known about its role in chromatin remodeling or DNA repair. However, biotinylation of histones participates in several biological processes. For example, biotinylation of histones increases lymphocyte cells proliferation in humans[20]. Biotinylation levels of histones increase the early phase of the cell cycle (G1 phase) and remain increased during the later phases (S, G2 and M phase) with quiescent controls. The increased level is more than four times that of a control. Deficient histone biotinylation can be initiated because of a holocarboxylase synthetase deficiency[14]. Recent evidence suggests that biotinylation of distinct lysine residues in histone H4 changes at the specific phases of the cell cycle[12].

Research in chicken erythrocytes has provided important evidence that biotinylated histones are enriched in transcriptionally silent chromatin. Additionally, biotinylation of histones might play a role in cellular responses to DNA damage[11].

It is still unclear whether biotinylation of histones is a mechanism leading to DNA repair or apoptosis. With this consideration, research on histone biotinylation becomes important. In this research, yeast was used as model organisms to elucidate more about histone biotinylation.

Previously, Hasim et al[5] had proof; biotinylation also occurs in histones H2A, H2B and H4 of *C. albicans*, but not in *S. cerevisiae*[5]. The specific aims of this research are to determine whether biotin protein ligase (Bpl1p) from *S. cerevisiae* may biotinylate *C. albicans* H2B, if Bpl1p from *C. albicans* can biotinylate *S. cerevisiae* H2B and to elucidate the characteristics of histone biotinylation using *in vivo* and *in vitro* assays.

Material and Methods

Strains and Plasmids Carrier: DH10B (*E. coli* strain) (Gibco) (F^- *mcrA Δ* (*mrr-hsd*RMS-*mcr*BC) φ80d*lac*ZM15 Δ*lac*X74 *deo*R *rec*A1 *end*A1 *ara*Δ139 D (*ara, leu*) 7697 *gal*U *gal*K λ^- *rps*L *nup*G.), INVSc1 (*S. cerevisiae* strain) (*MATa, his3D1, leu2, trp1-289, ura3-52.*), YAO1 *(S. cerevisiae BPL1* knock out strain), SC 5314 (*C. albcans* wild type strain) strains were used.

The plasmids carrier used in the research were pRS315: (Ap^r, LEU2)[19], pRS425: (Ap^r, LEU2)[19], pADH: (Ap^r, LEU 2)[1], pBluescript KS II (+): (Ap^r) (Stratagene). Luria-Bertani (LB) medium with 100 µg/ml ampicillin antibiotic (Sigma, Darmstadt) was used to maintain the plasmids.

Preparation and Transformation of *E. coli* Competent Cells

Preparation of *E. coli* competent cells: After overnight incubation in 4 ml LB broth at 37^0C, a single colony of the DH10B strain was isolated. 4 ml of the overnight broth culture was then placed in 50 ml TB broth incubated at 37^0C. The culture was incubated until the 600 nm absorbance value was greater than 0.8 OD (Optical Density) (about 4 hours). The bacteria was transferred to a centrifuge tube in an ice bath for 30 minutes, then centrifuged at 4^0C for 10 minutes at 4,500 xg. The supernatant was removed and 30 ml sterile water was added to wash the ice-cold cell pellet followed by another centrifuge step at 4^0C for 10 minutes at 4,500 xg.

The wash step was repeated and 30 ml of pre-cooled sterilized 10% glycerol was added and mixed. The mixture was centrifuged again under the same conditions. Finally, the supernatant was removed and 1 ml of sterile 10% glycerol was added to break up the cell pellet, aliquoted into 50 µl tubes and stored at -80^0C.

Transformation of *E. coli* competent cells: 1 µl plasmid DNA was recovered from 50 µl competent *E. coli* cells. The cells were mixed and placed in a cell electroporation small tube and subject to a current of 2.5 KV. The bacteria was removed by a glass pipette dropper and 1 ml SOC medium was injected. The solution was mixed at 37^0C for 40 minutes and centrifuged for 1 minute at 16,000 xg to remove the supernatant. The solution was added to a mixed broth LB / amp plate and incubated at 37^0C overnight.

Preparation and Transformation of Yeast Competent Cells

Yeast competent cell: A single yeast strain, cultured in 4 ml YPD broth at 30^0C overnight was isolated. 4 ml of the overnight broth culture was mixed with 50 ml YPD broth at 30^0C for 4 hours. The yeast was transferred to a centrifuge tube and centrifuged for 10 minutes at 4,500 xg at room temperature. The supernatant was removed and 30 ml sterile water was added to mix the cell pellet. The solution was centrifuged for 10 minutes at 4,500 xg and a 1.5 ml TE / LiAc / H_2O mixture was added to break up the cell pellet. The mixtures were stored at -80^0C (glycerol was added for a final concentration of 10 %).

Yeast competent cell transformation: 1 µl plasmid DNA, 1 µl salmon sperm DNA and 100 µl yeast competent cells were placed in a 1.5 ml microcentrifuge tube. 600 µl of a TE / LiAc / PEG mixture, mixed and incubated at 30^0C, under

shaking for 30 to 60 minutes. The solution was mixed briefly with 70 µl DMSO and heated over a 45^{0}C water bath for 10 minutes. The solution was then placed in an ice bath to cool for 3 minutes, followed by pelleting by centrifuging forward at 3000 xg and reverse for 10 seconds. The supernatant was aspirated and 80 µl water was used to mixed the cells. Finally, the bacteria was coated on selective medium at 30^{0}C for 3 days.

Cloning of Genes Encoding *BPL1* and *H2B*: *BPL1* of *Saccharomyces cerevisiae* and *Candida albicans* wild type gene were amplified using a PCR using genomic DNA as the template with a pair of gene-specific primers clipped from the ends of a DNA fragment containing the NdeI-XhoI enzyme with a length of 2,073 bp (*S. cerevisiae*) and 1,998 bp (*C. albicans*). After cutting with the enzyme, it was cloned to pADH plasmid (a high copy number plasmid, with a constitutive *ADH* promoter). *H2B* (Histone 2B) of *S. cerevisiae* and *C. albicans* was cloned into a vector to be used as substrate for histone biotinylation by BPL1.

Protein Preparation: This preparation is specific to LEU2 gene screening for the transformation yeast INVsc1 and culture in SD /Leu$^-$ solid medium. One colony was inoculated in 2 ml SD /Leu$^-$ liquid medium at 30^{0}C for (12 to 18) hours, the cells were re-suspended in 200 µl and cultured in 4 ml SD / Leu$^-$ liquid medium at 30^{0}C until the OD 600 nm reached about 1.0. The solution was centrifuged at 16,000 xg for 1 minute to collect the cells and the cells were re-suspended (4^{0}C) by 1 ml pre-cooled sterile water twice. The solution was centrifuged at 16,000 xg, 4^{0}C for 1 minute to remove the supernatant and 100 µl lysis buffer was used to resuspend the cells.

100 µl volume was added to 0.5 mm glass beads. The cells were cooled and vortexed every 30 seconds for 5 minutes. The solution was centrifuged at 16,000 xg for 10 minutes and the supernatant transferred to a clean 1.5 ml centrifuge tube.

SDS-PAGE: An equal amount of protein was extracted by 4x protein dye, boiled for 5 minutes, placed on ice, centrifuged for 5 minutes and electrophoresed by SDS-PAGE at current 25 mA. The product was then stained by Coomassie blue or used for Western blotting.

Western Blot Method: A methanol treated PVDF membrane was immersed in a transfer buffer solution followed by a colloid and PVDF membrane sequentially stacked on paper for electrophoresis. A transfer bath at 300 mA power-transfer was used for 60 minutes. The transferred PVDF membrane was immersed in a blocking buffer: 5% non-fat milk, 1 % BSA / TBST, 12 hours at 4^{0}C.

The PVDF membrane was removed and washed with TBST for 10 minutes. This step was repeated three times and diluted antiserum (anti-Strept-HRP antibody or anti-His antibody was dissolved blocking buffer) was added for 1 hour at 4^{0}C, for 10 minutes at 3 TBST. The solution was analyzed by a colorimetric method using chemiluminescence.

Purification of 6xHis-tagged Proteins: The protein was corrected using a French press cell and then filtered or centrifuged to remove the cell debris. A protease inhibitor cocktail was used to stabilize the proteins before the French press application. Protein purification should be performed quickly to reduce degradation of the protein. After protein collection, Ni-NTA was added to catch specific proteins that contain a 6x-His tag.

***In Vitro* Biotinylation:** Bpl1p and H2b from *S. cerevisiae* and *C. albicans* were purified and used for the biotinylation reactions. The process was performed at 30^{0}C for three hours. The following compositions were used in the in vitro biotinylation reaction: 30 nM enzyme (Bpl1) (7.2 µg/l), 20 µM substrate (4.8 µg/l), 2 µM 5x buffer reaction, 10 µM Biotin, 3 mM ATP and 0.1 mM DTT. The biotinylation level of H2B was measured under various conditions of different temperatures, pH values and biotin concentration. The relative biotinylation levels were determined using HRP-streptavidin as a probe.

Results

***Candida albicans* Histone Biotinylation - Concentration Dependent:** Studies of the biotin supply effect on biotinylation of histones in various human-derived cell lines have been investigated. The common processes involve the culture of cell lines in media containing deficient concentrations of biotin up to several weeks. Biotin concentrations in culture media have a moderate impact on biotinylation of histones. Alternatively, biotinylation of carboxylases correlates strongly with biotin concentrations in culture media.

Notably, even small changes in biotinylation of histones might be physiologically insightful as they could influence other modifications of histones e.g. acetylation and methylation. In the case of yeasts *in vivo*, the influence of the biotin concentration on biotinylation is noticeable. The additional amount of biotin yields prominent changes in the histone protein (Figure 1), notably at concentration of 0.2 µg/l to 20 µg/l. Such influence is not noticeable when the concentration exceeds 20 µg/l (20 µg/l to 2000 µg/l).

Temperature Sensitive Histone Biotinylation in *Candida albicans:* Another point investigated in this research is whether proteins favor the biotinylated form in vivo at high or low temperature. To investigate, a yeast was grown, cultured and divided into groups. The temperature was maintained either at 30–37^{0}C or 20^{0}C for 12 hours. The relative biotinylation levels of Arc1p (control) and histone were determined by Western blotting.

Unlike carboxylases that exhibit independency under all tested conditions and retain near normal level of

biotinylation, temperature dependent biotinylation levels of Arc1p and histones were significant. Changes in the temperature from 30^0C to 37^0C affected the protein expression level of Arc1p and histones (probed by HRP-conjugated streptavidin).

Figure 2 shows the discrepancies of the histone biotinylation level at low and high temperature wherein the level remained normal only between 20^0C and 30^0C. The biotinylation level dropped dramatically once the temperature reached 37^0C, which has inferred with the protein instability, leading to a decline in the biotinylation level. Based on this observation, the biotinylation properties in histone may have the same characteristic as biotinylation in Arc1p regarding temperature resistant properties.

Biotinylation of Overexpressed ScH2B: *S. cerevisiae* and *C. albicans* are species with the ability for biotinylation using their own biotin protein ligase agent. These species are also known to produce their own biotinylated carboxylase protein group. However, since *S. cerevisiae BPL1* does not generate their own biotinylated histones, an overexpression of biotin protein ligase from *C. albicans* (*CaBPL1*) and *S. cerevisiae* (*ScBPL1*) was performed prior to transformation into the yeast *BPL1* knockout strain. The vigorous activity of *ScBPL1* and *CaBPL1* observed using Western blot (probed by HRP-conjugated streptavidin) may indicate the influence of the biotinylation of native histones on *S. cerevisiae*.

Alternatively, the experimental result (Figure 3) suggests repudiated results; histones in *S. cerevisiae* could not be biotinylated regardless of the amount of available biotin protein ligases. This suggests the species could not govern the substrate (histones) for biotinylation, even with abundant expression for biotin protein ligase protein. Similarly, the overexpression of *CaBPL1* into the *ScBPL1* knockout strain did not biotinylate its histones. Another strategy may be to use domain swapping of the biotin protein ligase from *S. cerevisiae* and *C. albicans*[18].

Lane	Strain	Concentration biotin added (µg/l)
1	*S. cereviseae*	2
2	*C. albicans*	0.2
3	*C. albicans*	2
4	*C. albicans*	20
5	*C. albicans*	200
6	*C. albicans*	2000

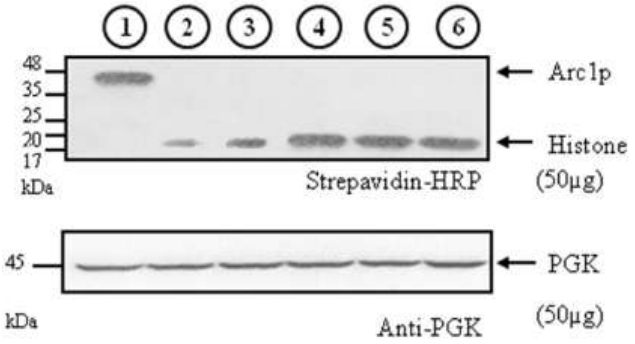


Figure 1: The effect of biotin concentration on histone biotinylation in vivo. The yeast *C. albicans* was grown at 30°C for 12 hour in medium containing different concentrations of biotin (0.2 ~ 2000 µg/l). Arc1p of *S. cereviseae* was used as control (lane 1). Western blotting was carried out using HRP-streptavidin as a probe

Lane	Strain	Temperature (°C)
1	*S. cereviseae*	30
2	*C. albicans*	20
3	*C. albicans*	30
4	*C. albicans*	37

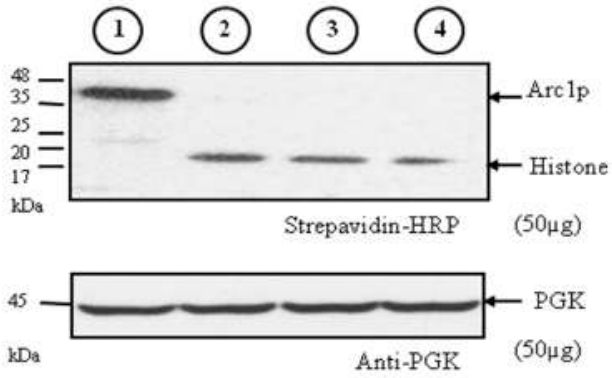


Figure 2: The effect of temperature variation on histone biotinylation in vivo. The yeast *C. albicans* was grown in medium containing of 2 µg/l of biotin, at different temperatures (20 °C, 30 °C and 37 °C) for 12 hour. Arc1p of *S. cereviseae* was used as a control (lane 1). Western blotting was carried out using HRP-streptavidin as a probe

No.	Construct	Target Gene	Vector
1	pADH1	None	pADH1
2	WT *C. albicans*	None	None
3	pYAO104	ScBPL1	pADH1
4	pYAO121	CaBPL1	pADH1

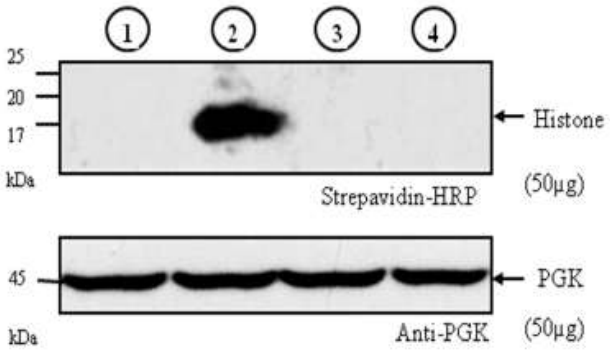


Figure 3: Biotinylation of ScH2B by overexpression ScBpl1p and CaBpl1p *in vivo*. The cells were cultured in 12hr in 30ºC. Western blotting was carried out using HRP-streptavidin as a probe. Phosphoglycerate Kinase (PGK) served as a loading control and was probed by anti-PGK antibody

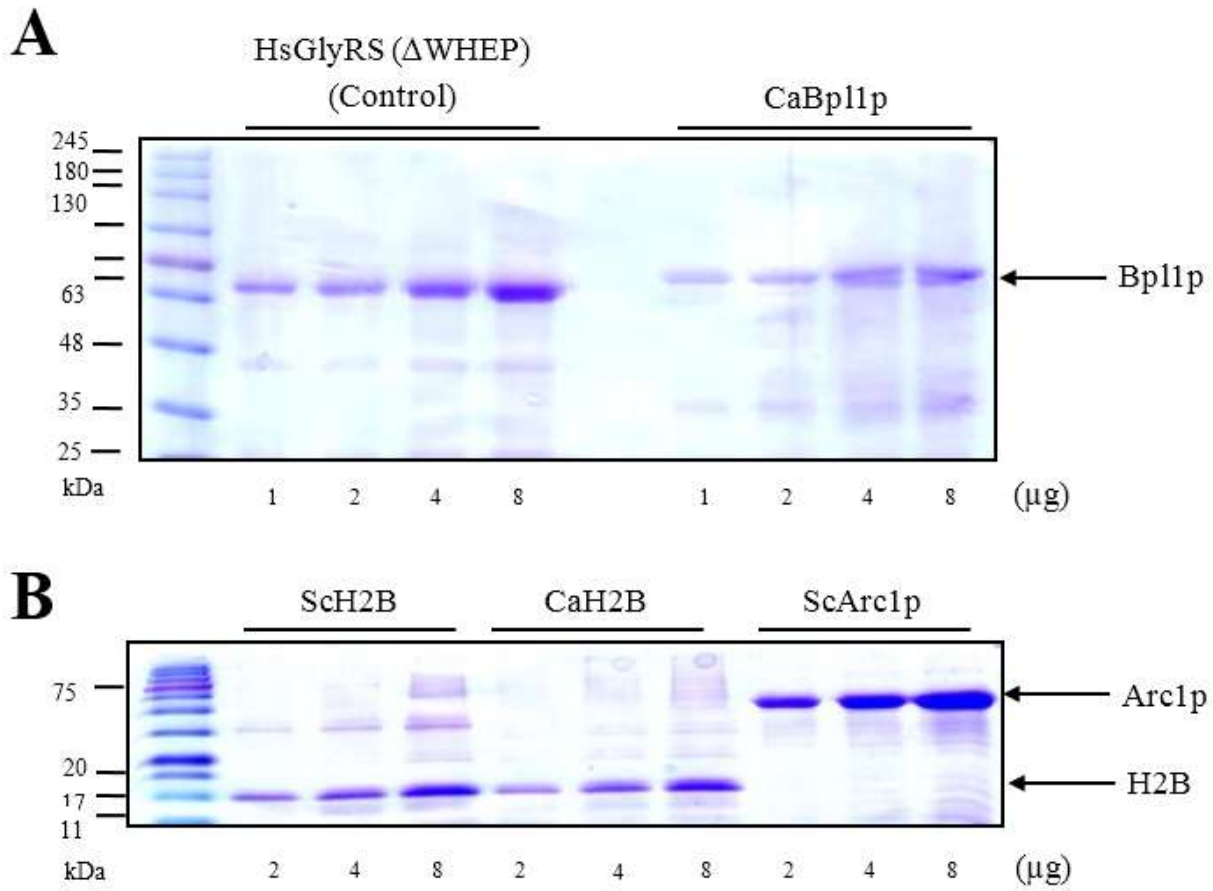


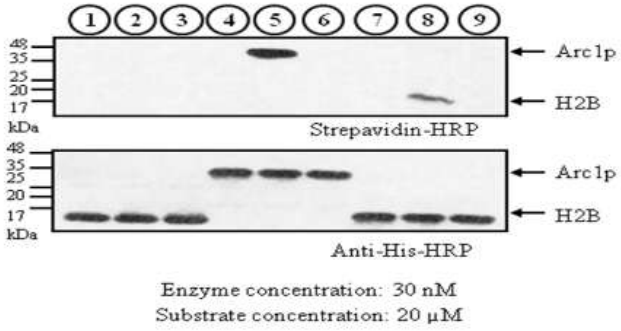
Figure 4: Purification of Bpl1p and H2B. Bpl1p and H2B were purified through Ni-NTA column chromatography to homogenity. ScBpl1p and CaBpl1p were purified from an *E. coli* transformant and a yeast transformant, respectively. Protein was loading into 10% (Bpl1p) abd 15% (H2B) SDS PAGE and stainied using Comassie Brilliant Blue. *A*, Purified Bpl1p. *B*, Purified H2B

***In Vitro* Biotinylation:** Purified Bpl1p and H2b from a yeast transformant were prepared to implement in vitro biotinylation assays (Figure 4). The result (Figure 5) indicate minimum discrepancies compared to *in vivo* condition. The variation was only apparent for histone purified from biotinylation of *C. albicans* with CaBpl1p as the biotin ligase agent. Histone from *S. cerevisiae* could not be biotinylated using purified Bpl1p from itself or purified Bpl1p from *C. albican*s.

In conjunction with the *in vivo* observations, purified Bpl1p and H2b using an *in vitro* assay exhibited distinctive outcomes under the various condition of temperature and biotin concentration.

A

Lane	Substrate (20 µM)	Enzyme (30 nM)	Substrate Biotinylated
1	ScH2b	-	-
2	ScH2b	ScBpl1	-
3	ScH2b	CaBpl1	-
4	ScArc1p	-	-
5	ScArc1p	ScBpl1	+
6	ScArc1p	CaBpl1	-
7	CaH2b	-	-
8	CaH2b	CaBpl1	+
9	CaH2b	ScBpl1	-



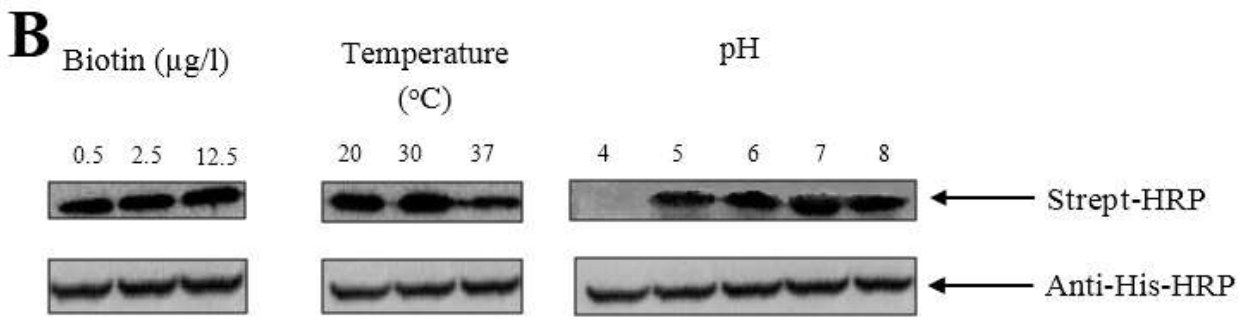


Figure 5: In vitro biotinylation of H2B. *A*, In vitro biotinylation of H2B. Amount of enzyme that loaded into reaction was 30 nM and the substrate was 20 µM. *B*, In vitro biotinylation under various conditions. Reaction time was 3 hour and the amount of enzyme that loaded into reaction was 30 nM and the substrate was 20 µM. Relative biotinylation levels oh H2B were probed with HRP-streptavidin and relative protein amounts loaded into each well were probed with HRP-Anti-His6 antibody

The results indicate the steady level of biotinylation between the in vitro condition of 20^0C and 30^0C which decreases after 37^0C. In the biotin concentration dependent scenario, additional biotin supply drives signal responses on histone proteins under the *in vivo* biotinylation assay. Alternatively, such event did not occur under the *in vitro* condition.

The amount of biotin with respect to the biotinylation level under the *in vitro* condition remained normal despite the different amounts of biotin (2 µM, 10 µM and 50 µM). The acidity test was performed under an *in vitro* assay at pH 4 to pH 8 to test the influence of acidity on the biotinylation. The histone of *C. albicans* could not be biotinylated unless the pH is greater than 4.

Based on these results, we deduced that histone (H2B) could only be biotinylated in *C. albicans*, not in *S. cerevisiae* and the biotinylation mechanisms on H2B are concentration dependent (*in vivo*, not *in vitro*), pH dependent and temperature dependent.

Discussion

Biotinylation is a relatively rare modification in cells with only one to five biotinylated proteins found in each organism. Previous work shows that biotin-binding domains and biotin protein ligases are highly conserved throughout biology[2]. Biotin protein ligases can biotinylate biotin acceptor proteins across widely divergent species. For example, the biotin protein ligases from *Homo sapiens*, *S. cerevisiae* and *Arabidopsis thaliana* can complement an *E. coli* birA mutant. While the biotinylation site (SSKD) of Arc1p bears little resemblance to the consensus sequence (AMKM) of biotin-binding domains, biotinylation of Arc1p is catalyzed by the only biotin protein ligase Bpl1p in yeast.[10] Additionally, only histones in *C. albicans* can be biotinylated by Bpl1p from several yeasts which suggests that histone biotinylation may represent a secondary biotinylation target for biotin ligase.

Histone tails carry many amino acids such as lysine, arginine and serine residues which are potential targets of

modifications such as acetylation, methylation, phosphorylation, ubiquitination, poly (ADP-ribosylation) and sumoylation. These post-translation modifications play an important key in mediating the interaction of DNA with histones and in maintaining chromatin structure[6]. Previous studies suggest that biotinylation may also occur naturally in human histones.

Biotinylated histone proteins are enriched in repeat regions and repressed loci, participating in the maintenance of genome stability and chromatin structures. While biotinylation does not occur in Arc1p of *C. albicans*, it occurs in histones H2A, H2B and H4 of the yeast species[5], the biological significance, of which remains to be elucidated. To date, only histones from *C. albicans* can be biotinylated in yeasts. This research focuses on the use of *C. albicans* and another common yeast *S. cerevisiae* to decode the histone biotinylation and its characteristics under various treatments (disparate influences of temperature, biotin concentration and acidity effects).

Several experimental strategies have been implemented in this research. One of the attempts was to express biotin protein ligase from *C. albicans* (*CaBPL1*) in the Sc *BPL1* knock out strain and examine whether it could produce histone of biotinylated *S. cerevisiae* or not. The result hinted at histones in *S. cerevisiae* could not be biotinylated under such circumstances. In spite of the use of native Bpl1p protein and the strong promotor, the abundant expression for biotin protein ligase protein could not promote the biotinylation of the substrate (histones).

Similarly, albeit using a homologous gene strategy, the overexpression of *CaBPL1* into the *ScBPL1* knockout strain did not biotinylate its histones. Based on these results, the ability of CaBpl1p to biotinylate their own histones (in *C. albicans*) could not be replicated in biotinylated cross species (*S. cerevisiae*). One possible reason could be the specific enzyme within its substrate[3].

In vitro and *in vivo* biotinylation of histone were significantly affected by temperature variation. Temperature changes from 30 °C to 37 °C had a significant effect on the protein expression level of histones. Similarly, the same occurred for Arc1p protein where the biotinylation level decreased as the temperature increased.

Previous research in various human-derived cell lines indicated that biotin concentrations in culture media had a moderate impact on the biotinylation of histones. The results show minor reliant of biotin concentration to biotinylation level on *in vivo* condition.

In contrast, various biotin concentration did not change the biotinylation level in *in vitro* condition. Although the concentration of biotin had little impact on the biotinylation level of histones, biotin is a compulsory compound needed to produce biotinylated histone proteins. This result agrees with previous studies that histones in *C. albicans* will not be biotinylated when the culture medium contains no biotin[5].

Another parameter highlighted during the *in vitro* biotinylation of *C. albicans* histone was pH. pH had a significant effect on the biotinylaton of CaH2b by CaBpl1p. The enzyme was biotinylated optimally only in a normal pH range (5 to 7). The biotinylation level increased from pH 5 until a maximum condition in pH 7. The biotinylation level slightly decreased when the pH was beyond pH 8. An overly acidic condition (pH 4) somewhat hinders the histone band inferring the inability of histone to be biotinylated.

Conclusion

Biotin protein ligase (Bpl1p) from *C. albicans* cannot biotinylate *S. cerevisiae* H2B. CaBpl1p only can biotinylate their own histones (H2A, H2B and H4). *S. cerevisiae* also cannot biotinylate *C. albicans* H2B, similar to ScBpl1p not biotinylating their own histones, except carboxylases and Arc1p. The characteristics of histone biotinylation are temperature dependent and concentration dependent *in vivo* and also temperature dependent and pH dependent *in vitro*.

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