論文の内容の要旨

論文題目: Development and Improvement of Artificial Catalyst Systems to Achieve Histone-selective Acylations toward Regulation of Cellular Functions

(細胞機能制御を指向したヒストン選択的アシル化触媒システムの開発と改良)

氏　　名: 劉　家安

Introduction

Post-translational modification (PTM) of a protein, which is a covalent modification of amino acid residues in a protein, is one of the most efficient biological mechanisms for expanding the genetic code and for regulating cellular physiology. Among proteins undergoing PTMs, histones play fundamental roles. Histones are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural unit called nucleosome. It contains 147 base pairs of DNA, which are wrapped around a histone octamer that consists of two copies of the four core histones H2A, H2B, H3 and H4. Each core histone is composed of two structurally distinct regions, an *N*-terminal tail region, which is rich in positively-charged lysine and arginine residues and flexibly protrudes through the DNA superhelices, and a globular region, which forms nucleosome's scaffold[1]. Among PTMs on histones, acetylation of lysine residues is a fundamental regulatory element for activating gene expression and epigenetics[2]. In cells, the acetylation level of histones is controlled by the balance of two enzymes, histone acetyltransferase (HAT) and histone deacetylase (HDAC). Small molecule inhibitors of HDAC (e.g., suberoylanilide hydroxamic acid, also known as vorinostat or Zolinza) are potential anticancer agents because promoting histone acetylation likely activates the transcription of a series of genes, including tumor suppressor genes (p53), which are often inactivated in cancer cells. HDAC inhibitors rely on HAT's activity for their promotion of lysine acetylation, so an alternative strategy to bypass these enzymes is essential for treating cancer cells with low or no HAT activity. To date, however, there are no synthetic systems enabling HAT-independent and selective acetylation of histones.

We previously developed two types of artificial catalyst systems named SynCAc (Figure 1) and DSH (Figure 2). SynCAc system is composed of an acetylation mediator (8DMAP) and an acetyl donor (3NMD-8R), both bind with DNA in the nucleosomes, enabling tail region-selective lysine acetylation of recombinant nucleosomes (rNuc). DSH promotes lysine residue-

selective acetylation of histones in rNuc with acetyl-CoA, endogenous acetyl donor, when conjugated with different histone-binding ligands.

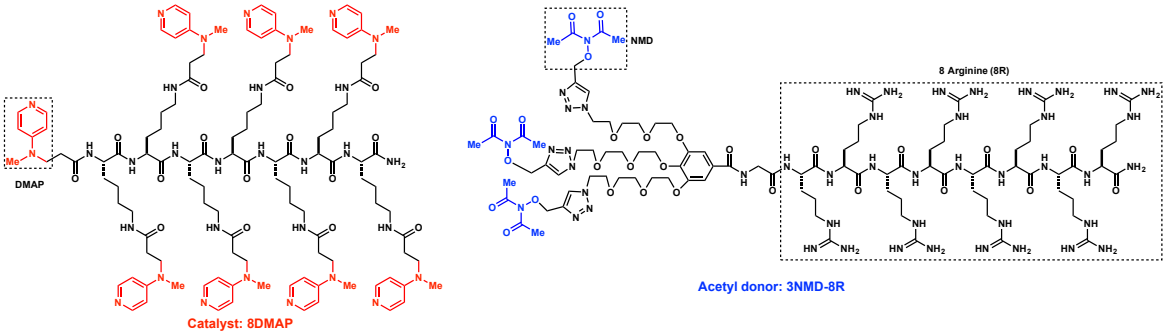

Figure 1. The components of SynCAc system.

It was, however, unknown whether recently identified lysine acylations other than acetylation, such as butyrylation and malonylation, are possible by our system. And it was necessary to develop a more powerful catalyst moiety in order to achieve in-cell acetylation reactions. Here I report tail-region selective acylations systems based on SynCAc, leading to induction of biochemical phenotypes by lysine malonylation, and development of a glutathione-resistant acetylation catalyst based on DSH for realization of in-cell acetylation reactions.

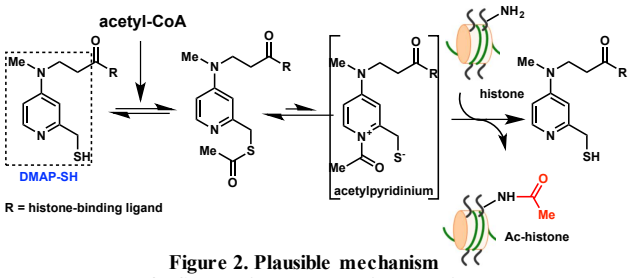

Figure 2. Plausible mechanism of DSH-mediated acetylation reaction.

Results and Discussion

1. Development of histone tail-selective acylation systems based on SynCAc

Given a distinct chemical nature of malonyl-lysine with a negatively charged carboxylate group, it has been proposed that lysine malonylaiton may result in significant changes in histone structure and functions. I, therefore, started with challenging this anionic modification. However, due to high reactivity of the original donor core, it was impossible to obtain this kind of malonyl donor. After intensive structural modifications, I found that a phenyl-ester type acyl donor has sufficient stability and enough reactivity under the physiological conditions. I successfully developed a variety of acyl donors (malonyl, glutaryl, butyryl and biotinyl) and realized histone-selective acylations of rNuc in the presence of HeLa cell cytoplasmic extracts (Figure 3). Preliminary LC-MS/MS quantification of the malonylation suggested that the acylation preferentially occurred in tail-regions.

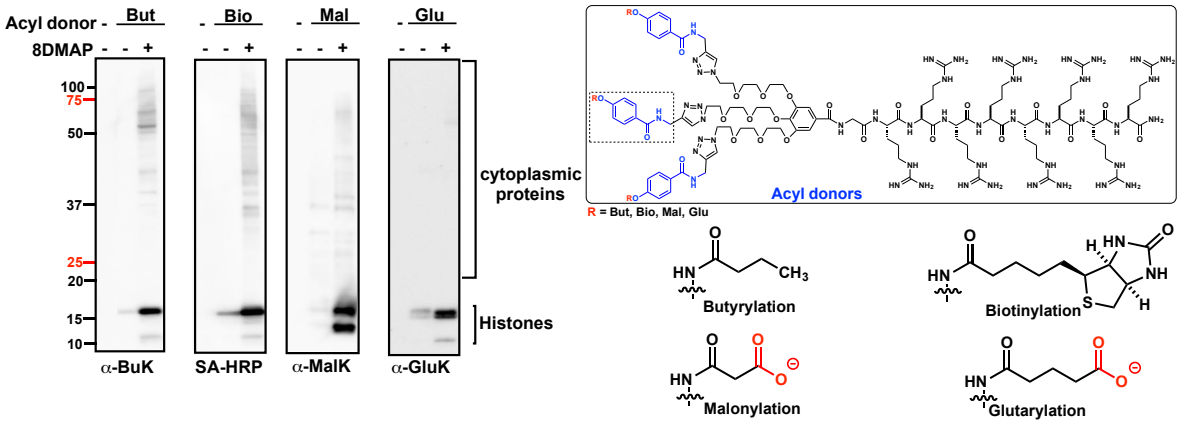

Figure 3. **Selective introduction of acylations to recombinant nucleosome in the presence of cytoplasmic extracts.**
But: Butyryl, Bio: Biotinyl, Mal: Malonyl, Glu: Glutaryl.

Next, I examined biophysical properties of the malonylated nucleosomes. To evaluate internucleosomal interactions and the stability of nucleosomes, we performed a Mg^{2+}-dependent nucleosome self-association assay and a thermal shift assay, respectively[3]. As a result, malonylation by our system suppressed internucleosomal interactions significantly, and reduced the stability of the nucleosomes as well (Figure 4). To the best of knowledge, this is firstly-reported molecular functions of lysine malonylation of histone, and indicates a possibility that malonylation of histones promotes RNA transcription. This investigation is now ongoing.

2. Development of glutathione-resistant acetylation catalyst

Our previous investigations indicated that glutathione (GSH), a tripeptide comprised of L-glutamic acid, L-cysteine and glycine, which is present in millimolar (1~10 mM) concentrations in animal cells, suppressed reactivity of catalyst DMAP-SH, and it was considered as one of the major reasons that DSH system could not promote acetylation in living cells. A thiol group in GSH competitively attacks acetylpyridinium cation intermediate, preventing amide formation of lysine amine. I

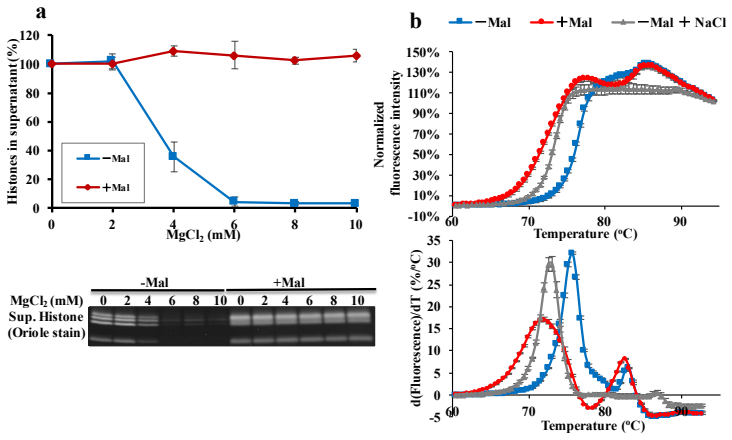

Figure 4. Biophysical nature of the malonylated recombinant mononucleosome.
(a) Mg^{2+}-dependent self-association assay of the malonylated recombinant mononucleosome. (b) Thermal stability assay of the malonylated recombinant mononucleosome. (error bar = SD, n = 3).

envisioned that by introduction of a formyl group to the catalyst, lysine amine was captured to form hemiaminal intermediate, leading to facile transfer of the activated acetyl group on the acetylpyridinium cation to the lysine in an intramolecular manner[4]. I investigated this possibility in a model study using a carbonic anhydrase (CA). In the absence of GSH, new catalyst PAQ-SH showed slightly lower reactivity than the original catalyst core (PDP-SH, Figure 5). In contrast, the new catalyst exhibited higher reactivity in the presence of 5 mM GSH than the original one. Quantification analysis by LC-MS/MS showed that around twice reactivity compared to PDP-SH was observed. This results suggest that PAQ-SH could be a superior catalyst motif than DMAP-SH for in-cell acetylation reactions.

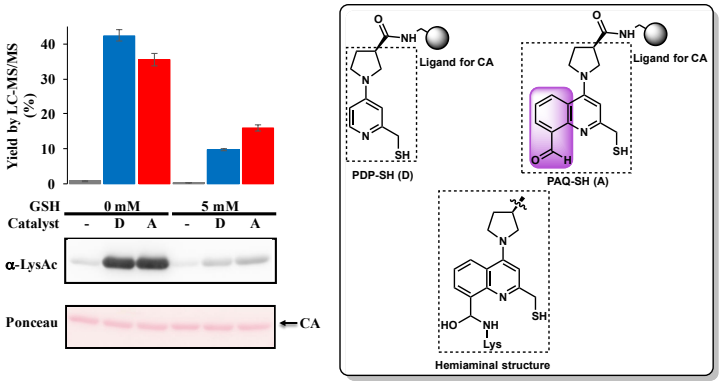

Figure 5. Quantitative comparison between PDP-SH and PAQ-SH in the absence or presence of GSH. (error bar = SD, n = 3).

Conclusion

I developed histone-selective acylation systems by redesigning the acyl donors. Histone-selective malonylation by the system efficiently suppressed internucleosomal interactions and reduced stability of nucleosome, which has a potential to promote transcription. A novel GSH-resistant acetylation catalyst was also developed, which is an important step to achieve acetylation in living cells for regulation of cellular functions.

References

(1) McGinty, R. K.; Tan, S. *Chem. Rev.* **2015**, *115*, 2255. (2) Bowman, G. D.; Poirier, M. G. *Chem. Rev.* **2015**, *115*, 2274. (3) Wakamori, M.; Fujii, Y.; Suka, N.; Shirouzu, M.; Sakamoto, K.; Umehara, T.; Yokoyama, S. *Sci. Rep.* **2015**, *5*, 7204. (4). Raj, M.; Wu, H. B.; Blosser, S. L.; Vittoria, M. A.; Arora, P. S. *J. Am. Chem. Soc.* **2015**, *137*, 6932.