CBI Student Sabbatical Proposal:

Biophysical methods to investigate chromatin remodeling

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- Sabbatical host: Geeta Narlikar, Ph.D.
- Host location: University of California, San Francisco, Mission Bay Campus, Biochemistry and biophysics department
- Sabbatical duration: 10 weeks
- Time frame: Fall 2012
- Sabbatical summary: During my sabbatical in the Narlikar lab I will learn to reconstitute chromatin from recombinant components and to use a variety of biophysical approaches to study how chromatin structure is altered by chromatin remodeling enzymes. These assays and techniques will broaden my skill set and can be applied to future study of biological functions of deacetylases. This sabbatical will enhance my thesis project by enabling me to extend my current enzymological studies to chromatin templates.
Proposal Letter

Background

Lysine acetylation is a post-translational modification studied for its importance in transcriptional regulation, cytoskeleton dynamics, and signaling (reviewed in 1, 2, 3). The addition of an acetyl group to lysine side chain amines is enzymatically catalyzed by lysine/histone acetyltransferases (KATs or HATs) and deacetylation is catalyzed by lysine/histone deacetylases (KDACs or HDACs). Over 3,800 acetylation sites have been identified on mammalian proteins thus far (4). Acetylation modifies the electrostatic properties of the lysine residue (reviewed in 1, 2) and, in the case of histone acetylation, serves as an epigenetic marker for recruitment of specific binding proteins (reviewed in 5). Lysine acetylation and deacetylation is suggested to be important to transcriptional regulation via effects on chromatin structure as well as by modifying the interactions between DNA and transcription factors (reviewed in 3) and KDAC inhibitors are used to treat some lymphomas (reviewed in 6). Despite the importance of acetylation and deacetylation, the biological roles and modes of regulation for these processes are not well understood.

The pathways and mechanisms governing KDAC regulation in vivo are not known. These enzymes were first identified as histone modifying enzymes, but many acetylated non-histone proteins have since been observed (1). With over 3,800 mammalian acetylation sites (4) and 18 human lysine deacetylases, the identification of deacetylase substrates and the delineation of KDAC substrate specificity are especially important questions which our lab is addressing. Studying the substrate specificity of these enzymes will provide insight into how KDACs interact with their substrates and how protein deacetylation is regulated. Another important issue we are studying is the identity of the active site metal ion used in vivo by the
metal-dependent KDACs such as KDAC8, the substrate specificity of which changes with the metal ion in vitro (7).

The biological roles of acetylation are not well understood, so this continues to be an area of active research. Robinson et al. demonstrated that acetylation of the H4 histone tail is one regulator of folding for the 30 nm chromatin fiber, inhibiting compaction through a mechanism other than simple disruption of charge (8). Acetylation effects on chromatin remodeling enzymes are also being investigated. A recent study by the Narlikar lab presented evidence that acetylation of Remodels the Structure of Chromatin (RSC) in Saccharomyces cerevisiae is important for cellular resistance to DNA damage (9). Thus assays that analyze changes in chromatin conformation are useful for investigating the effects of histone or transcription factor acetylation on chromatin structure and transcription. This approach targets the biological role of acetylation in vivo.

**Preliminary Work**

The Fierke lab is well-established in the metal-dependent lysine deacetylase field. We are particularly interested in studying the substrate specificity of KDAC isoymes KDAC8 and KDAC11, but have begun to investigate KDAC4 and KDAC7 as well. Previous work in the lab has eludcitated the proposed catalytic mechanism of these metallohydrolases (10-12). Additionally, the identity of the catalytic metal ion was found to be an important determinant for substrate specificity in vitro (7, 11). Ongoing aims in the lab focus on further elucidating the factors governing KDAC substrate specificity, measuring kinetic data for deacetylation of full-length protein substrates, identifying in vivo substrates, and identifying the in vivo metal ions of these enzymes.

My work focuses on investigating factors that may regulate KDAC8 activity, such as phosphorylation of the enzyme, and investigating regulation of the enzyme in vivo. The effects
of phosphorylation will be analyzed by \textit{in vitro} kinetic assays. Using tissue culture cells, I am working to identify the metal ion(s) bound to KDAC8. The \textit{in vivo} metal ion will be identified using mass spectrometry. I am collaborating with Kicki Hakansson’s laboratory at the University of Michigan to develop a mass spectrometric assay for identifying the bound metal ion. Another \textit{in vivo} direction I want to pursue is to further investigate the biological roles of acetylation and deacetylation.

\textbf{Proposal}

I propose to work in the laboratory of Dr. Geeta Narlikar at the University of California San Francisco to learn their chromatin-based assays, which are being developed and utilized by this lab to investigate the biological roles of chromatin remodeling enzymes. The Narlikar lab is generally interested in the mechanisms by which chromatin structure and function is regulated (9). Towards these goals, the Narlikar lab has developed and adapted many quantitative methods to study the kinetics of conformational changes in nucleosomes. Learning these techniques will allow me to apply similar methods to investigate the action of KDACs on nucleosomes in the future.

During the sabbatical, I will learn and use three different types of methods to assay nucleosomal changes (Yang, et al. 2006): (i) a restriction enzyme accessibility assay: this method measures the accessibility of nucleosomal DNA to specific restriction enzymes as a function of remodeling enzyme activity; (ii) FRET based assay: this method measures the change in FRET between fluorescent probes attached to specific locations on the nucleosome and, (iii) native gel-based assay: this assay measures changes in the positions of nucleosomes based on their different mobilities on a native gel. Recombinant histones will be made in \textit{Escherichia coli} and chromatin remodeling complexes will be purified from yeast or made recombinantly in \textit{E. coli}. As part of the sabbatical, I will also learn to reconstitute the various
chromatin templates used in the kinetic assays from recombinant histones and specific DNA sequences.

**Relationship to thesis research**

The majority of deacetylase studies on histones have been carried out on histone tail peptides or isolated histones. There is a dearth of studies of KDAC activity on defined chromatin substrates. As a result it is not clear how specific features of nucleosomes contribute to KDAC specificity and catalytic efficiency. What I learn in the Narlikar lab will allow me to study KDAC action on chromatin in the future, and potentially seed a collaboration between the Narlikar and Fierke labs. The techniques that I learn in the sabbatical could be applied to more complex systems later as well, for example the study of the DNA replication licensing factor Cdt1. Cdt1 is a putative substrate of a deacetylase studied in our lab (13) and is involved in chromatin decondensation prior to DNA replication (14). Importantly, during my sabbatical experience I will be introduced to biophysical methods for studying chromatin that I have not previously learned. Learning these assays and methods will be useful to me in my thesis work and also in my scientific career.

**References**


