


Spys Make Asymmetric Chromatin

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Asymmetry expands the signaling potential of chromatin. Applying a SpyCatcher/SpyTag approach makes asymmetric chromatin templates now widely accessible.

Molecular processes that occur on symmetric substrates can theoretically produce symmetric or asymmetric outcomes. When nonrandom but controlled and directed, symmetry vs asymmetry can establish complexity and signaling potential. Biological processes often take advantage of discriminating symmetry vs asymmetry, and studying such processes requires molecular and chemical methods that capture the complexity of the natural systems. In this issue of *ACS Central Science*, Muir and co-workers describe an advanced strategy for making asymmetric chromatin in the test tube.¹

Chromatin refers to the complex of DNA and histone proteins that serves as the substrate of all genomic (i.e., based on and affecting the genome) processes in a cell. It is a major signaling platform integrating various cellular functions. The fundamental, repeating unit of chromatin, the nucleosome, is composed of 146 base pairs of double-stranded DNA wrapped around an octameric protein complex made up of two copies of each of the core histones H2A, H2B, H3, and H4. Linear stretches of DNA packaged into nucleosomes are arranged into higher-order structures (e.g., the well-known appearance of mitotic chromosomes, Figure 1A) by additional proteins, RNA, and with the involvement of other molecules. Due to their composition and structure, nucleosomes bear pseudo-2-fold rotational symmetry with a so-called dyad symmetry axis (Figure 1B). All of the nucleosomes of a cell are essentially composed the same and have the same fundamental structure. A local difference between a given nucleosome and its neighbors besides the sequence and modification state of the wrapped

DNA is brought about by (i) a plethora of posttranslational modifications (PTMs) that can occur on the histone proteins, (ii) the incorporation of histone variants, i.e., histone proteins that differ in primary amino acid sequence instead of the canonical histones, (iii) several mutations (i.e., single amino acid changes) of the histone proteins that have been linked to different types of cancer, and (iv) the establishment of subnucleosomal complexes that do not contain the canonical histone octamer.

A major question in the field of chromatin research is whether these changes occur in a symmetric (i.e., simultaneously affecting both nucleosomal copies of a histone protein) or asymmetric (i.e., affecting only one of the two copies of a nucleosomal histone) fashion. Initially, the asymmetry of nucleosome codes was hypothesized based on test tube reactions analyzing histone modifying enzymes.² Later, a detailed cellular analysis defined various asymmetric modification states of nucleosomes.³ This has been further expanded in recent years to include histone sequence variation (histone variants and mutations) and nucleosomal composition,⁴ with the occurrence of asymmetric nucleosomes being linked to various cellular states in health and disease (Figure 1C). Together with the variability brought about by PTMs, sequence variation, and nucleosome composition, the concept of asymmetry in an otherwise symmetric context produces an immense signaling complexity with the potential to encode an enormous number of chromatin functional states.

To begin elucidating the molecular function of asymmetric nucleosomes, chromatin substrates that capture the

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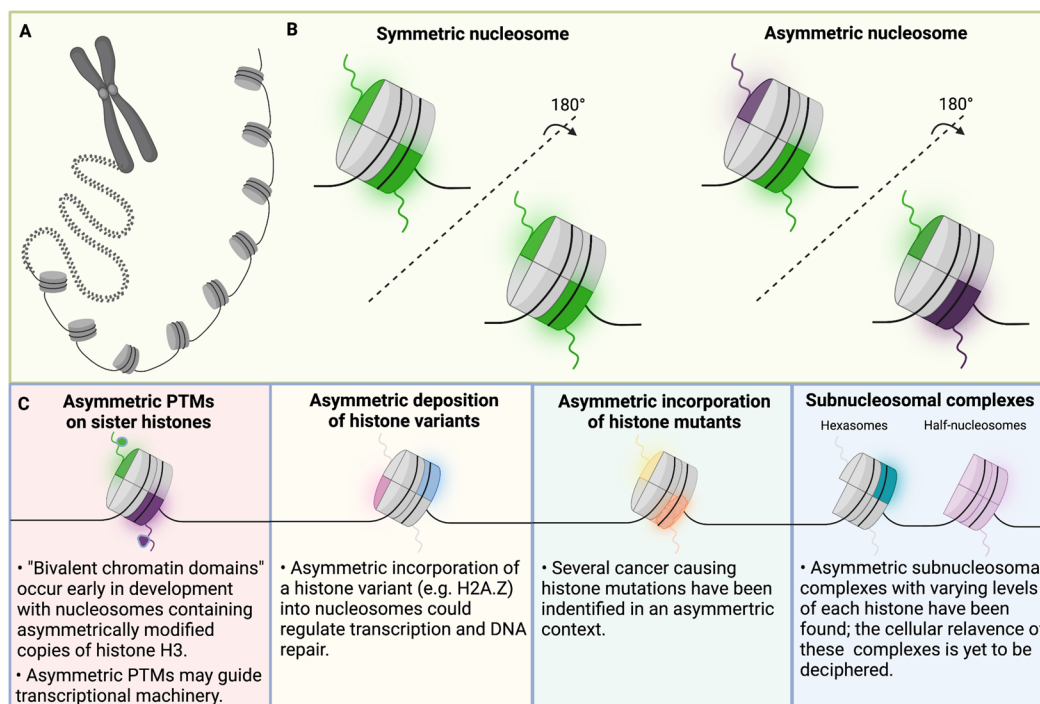


Figure 1. Asymmetry in chromatin. (A) Scheme of a chromosome constituted of linearly arrayed nucleosomes with DNA wrapped around a core made of histone proteins. (B) Nucleosomes have a pseudo-2-fold symmetry that can be broken by replacing one of the two copies of the contained histone protein by a varied form. (C) Different modes of asymmetry in nucleosomes and their (putative) biological relevance.

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associated complexity are required. Nucleosomes and stretches of nucleosomes (aka oligonucleosomes) can be reconstituted in the test tube in a two-step process that assembles and purifies histone octamers and then complexes these with DNA. However, how can asymmetry be introduced to this procedure that itself is not selective? Early approaches made use of purification tags on histone proteins together with elaborate dilution and purification schemes combining different histone species (unmodified or carrying a specific PTM). At the cost of major sacrifices in yield, tolerating large losses of the input material, this strategy allowed isolating asymmetrically modified nucleosomes for enzymatic and protein interaction studies (Figure 2A).⁵ A major advancement

in the establishment of asymmetric nucleosomes was put forward by the Fierz group which chemically engineered covalent bridges between the two asymmetric copies of a histone to be incorporated into a nucleosome before octamer assembly. This kept the nonidentical histone copies adjoined and nonmixed. The bridges could be removed by simple reactions after nucleosome reconstitution (Figure 2B).⁶ While this is a high-yield process, the chemistry for making the bridged histone pairs is not accessible to and cannot be handled by most chromatin research laboratories. Another approach made use of mutagenesis of histone contact interfaces paired with selection strategies to establish heterologous histone protein pairs that preferentially interact with each other. In contrast to the chemical approaches, this method can also be applied in cells (Figure 2C).⁷ Lastly, specific substoichiometric reconstitution schemes enabled accessing subnucleosomal complexes. By adding varied histone dimers containing different PTMs, histone variants, or mutations, these complexes can theoretically be reconstituted to asymmetric nucleosomes (Figure 2D).⁸ In the absence of purification schemes for the subnucleosomal complexes, the procedure also produces symmetric nucleosomes. By introducing affinity purification tags to the varied histone dimers that are added to the subnucleosomal complexes, direct purification of the asymmetric nucleosomes could be accomplished (Figure 2E).⁹

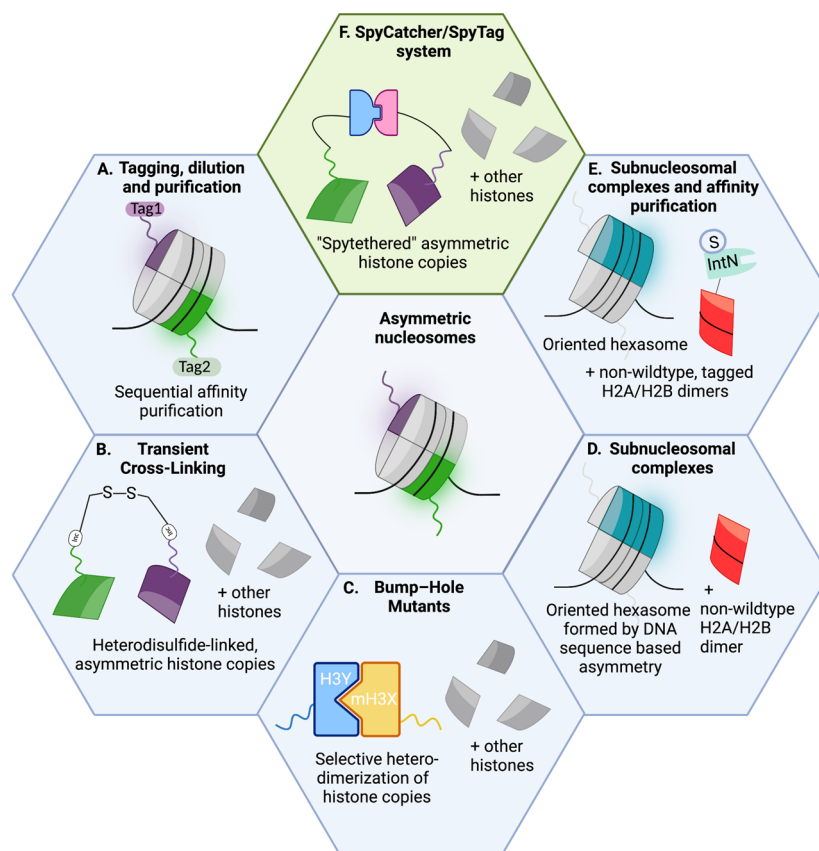


Figure 2. Methods for the reconstitution of asymmetric nucleosomes.

The complex schemes for making asymmetric nucleosomes have now been much simplified by the work of Muir and colleagues.¹ The new study expands on the idea of the Fierz group to physically link the varied copies of a histone to be incorporated into an asymmetric nucleosome. However, instead of a chemically engineered bridge, the SpyCatcher/SpyTag protein heterodimerization system is introduced (Figure 2F). SpyCatcher/SpyTag had already been developed and used to establish unconventional protein complexes.¹⁰ Being proteins, the system is genetically encoded and can be directly engineered to the histone of interest (e.g., fusion proteins with the variants or different mutants of a histone protein). Traceless removal after nucleosome reconstitution can be afforded by linking SpyCatcher/SpyTag to the histone of interest via short linkers that can be cleaved by specific proteases. Lastly, combining SpyCatcher/SpyTag with chemical biology methods of native protein ligation (NPL) that enable an incorporation of specific PTMs into the histones opens the horizon to the immense space of asymmetric nucleosomes. Not only do Muir and colleagues demonstrate the feasibility and yield of their approach to the reconstitution of asymmetric mononucleosomes including variation in PTMs, histone variants, and mutants, but they also establish dinucleosomes with asymmetry as well as nucleosomes that contain more than one asymmetry parameter (i.e., asymmetric

in respect to not only one but two histones). In analogy to and as an expansion of earlier studies, the new work also determines that symmetric and asymmetric nucleosome substrates are seen differently by distinct simple enzyme systems.

So, where do we stand now in the analysis of asymmetric chromatin? While the SpyCatcher/SpyTag approach makes asymmetric nucleosomes—at least when looking at histone variants and histone mutations—accessible to any research group capable of nucleosome reconstitution (the NPL methods for introducing PTMs still require a solid chemistry background), there is demand for more elaborate, complex biochemical schemes to determine the exact molecular functions of these substrates and further for experimental avenues that allow establishing asymmetric chromatin at will in a cellular context. In addition, the detection methods for symmetry and asymmetry in nucleosomes that currently rely on complex enrichment approaches, highly sophisticated mass spectrometry, or the use of elaborate single nucleosome microscopy methods need to be expanded. Lastly, it will need to be dissected how asymmetry in a symmetric context is established by cellular machinery in the first place. The newly introduced chemical biology methods will without doubt further fuel the interest in the study of asymmetric chromatin.

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