Histone clipping: the punctuation in the histone code

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Histone clipping was first discovered in the 1960s and still is a lingering mystery. Considering the essential roles of histones in regulating eukaryotic transcription through the histone code, clipping is a post-translational modification that appeals to the imagination. In this issue of *EMBO Reports*, Marruecos and colleagues investigate histone H4 clipping during intestinal development (Marruecos *et al*, 2021), and are providing crucial clues to finally elucidate the intricacies of this elusive modification.

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See also: **L Marruecos *et al*** (August 2021)

The eukaryotic lineage arose from bacterial and archaeal cells that underwent a symbiotic merger. The bacterial partner contributed genes, metabolic energy, and the building blocks of the endomembrane system. The archaeal partner provided the potential for complex information processing by adding histones to the eukaryotic experiment (Brunk & Martin, 2019). In fact, histones enabled eukaryotes to evolve amounts of DNA so vastly greater than those of prokaryotes and archaea that there is barely any overlap of the distributions. However, a dramatic increase in DNA content requires not only improved mechanisms for replication and maintenance but also enhanced control of gene expression. The development of a nucleosome-based chromatin structure that emerged from archaeal histones fulfilled all these requirements (Brunk & Martin, 2019). In addition, histone modification provides an epigenetic control mechanism that can persist over many cell generations, allowing the differentiation of stable gene expression patterns in

various cell types. This is essential in multicellular organisms in which different portions of the genome are expressed in different tissues. Therefore, histones are at the heart of this complex and precisely timed eukaryotic information management system.

Because of their key role in information management, the primary structure of histones practically maintained constant over the entire eukaryotic lineage. Conversely, histone post-translational modifications (hPTMs) that mediate their function have evolved into an alphabet that has more letters than there are amino acids. More specifically, hPTMs are considered to be an ancient mechanism to directly sense the energetic state of the eukaryotic cell by translating metabolic information—derived from the prokaryote partner—into gene regulation via histones—the archaeal contribution. Indeed, many chemical reactions involve the energy-rich donors acyl-CoA (acylation), ATP (phosphorylation), and S-adenosylmethionine (methylation). Still, as the number of metabolites that can modify histones keeps on growing, e.g., with the recent addition of lactylation (Zhang *et al*, 2019), an even more complex language starts to surface. For example, histones can also sense cellular metal content (Attar *et al*, 2020) or become dopaminylated and serotinylation in the brain (Lepack *et al*, 2020), mediating drug addiction and depression. Yet, while trying to understand the function of so many different hPTMs is challenging in its own right, trying to grasp the emerging complexity of their interplay, i.e., the histone code, is simply overwhelming.

Therefore, studying the histone code is like studying the grammar of a language, focusing only on individual letters. Indeed, the field coined the terms “writers, readers

and erasers” to describe the families of proteins that have evolved to functionally mediate and interpret the histone code in a cell. In this analogy, histone clipping is the punctuation, with its own rules and intricacies. It is at least thought-provoking that the most abundant and essential proteins in a eukaryotic cell conserved their primary amino acid sequence while all around them, (proteolytical) enzymes—the most abundant functional family of proteins—continuously changed their structure, function, and interactions. It should therefore not come as a surprise that a lot of proteins have been associated with histone proteolysis, a list that has been growing ever since the 1960s (Dhaenens *et al*, 2015). Intuitively, there must be a very complex regulatory system in place to control unwanted interactions between the two. However, difficulties of specifying the *in vivo* versus *in vitro* origin of this hPTM, the fact that the same enzymes mediate both histone degradation and clipping, and the complexity and redundancy of the histone code, all have contributed to the surprising shortage of reports on the biology of this potentially far-reaching hPTM. Most people that have taken up the challenge of investigating histone clipping have in fact turned away again after publishing their initial findings, a recurring pattern ever since its first discovery (Dhaenens *et al*, 2015). More often than not, a promising and exciting result was followed by one that re-introduced uncertainty. We too have fallen to this pattern and have abandoned clipping research five years ago. Therefore, it is so exciting to witness the swelling third wave of histone clipping studies, which for the first time seems to provide a more coherent picture.

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Figure 1. Trypsin-mediated histone H4 clipping during enterocyte differentiation.

The interaction of IκBα with histone H4 regulates the differentiation of intestinal cells while they move out of the crypt stem cell niche (indicated by the black arrow). IκBα repression of targets depends on its interaction with the H4 tail. Removal of the tail (H4 clipping) by trypsin reduces IκB interactions with H4 and allows differentiation. This cleavage of the histone H4 tail at non-acetylated lysines is a very dramatic PTM, which seems to play a pivotal role in intestinal epithelium differentiation.

In this issue, Marruecos *et al* (2021), investigate histone H4 clipping during intestinal development. Their findings (see Fig 1) are exciting for four complementary reasons. First, to date histone H4 was the most stable and least affected histone in the context of clipping. Second, it is extremely encouraging that a very recent corroborating report describes how clipping of histone H3 too is essential during intestinal differentiation and is also mediated in part by trypsin (Ferrari *et al*, 2021). Third, Marruecos *et al* investigate a direct interaction with another essential cell signaling molecule, i.e., IκBα. Finally, the findings reported here are coherent with the few common notions about histone clipping (Dhaenens *et al*, 2015): (i) Histone clipping is strongly associated with developmental transitions (Duncan *et al*, 2008; De Clerck *et al*, 2019; Ferrari *et al*, 2021); (ii) proteins that can cleave histones have all been known for very different functions and are most often not associated with nuclear localization; (iii) hPTMs might regulate the clipping (Duncan *et al*, 2008; Ferrari *et al*, 2021); and (iv) very

plausibly, clipping is part of a complex regulatory system that has a lot of redundancy (Duncan *et al*, 2008; Dhaenens *et al*, 2015; Ferrari *et al*, 2021). This redundancy is now also confirmed in intestinal differentiation, through either cathepsin L on H3 (Ferrari *et al*, 2021) or chymotrypsin on H4 (Marruecos *et al*, 2021). However, one aspect of clipping is very different this time: Despite its dramatic nature, inhibition of clipping rarely inhibits cell transition (Duncan *et al*, 2008), yet in the current work (Marruecos *et al*, 2021) and in Ferrari *et al*, (2021), it is shown for the first time that trypsin inhibition can block intestinal differentiation effectively.

In conclusion, no biological phenomenon can escape scientific curiosity and persistence. Histone clipping too will reveal its secrets in time. Marruecos *et al*. showed that a digestive enzyme regulates the creation of the very intestinal cells it is secreted from (Marruecos *et al*, 2021). In this way, histone clipping is becoming unsurmountable and will prove to be manifold more complex and regulated than what we currently can

anticipate. In fact, yet another protease able to clip histones, metalloproteinase 2 (MMP-2), was just found bound to chromatin within the promoter of the ribosomal RNA (rRNA) gene and appears to be able to clip the N-terminal tail of histone H3 within the nucleolus, the cell's ribosomal factory (Ali *et al*, 2021). This molecular switch can initiate ribosomal synthesis in the preparation of large cellular transitions. Looking back at our own findings, we too found H3 clipping to precede ribosomal synthesis during human embryonic stem cell differentiation (De Clerck *et al*, 2019). My main concern is now that some viruses might have figured out a way how to hijack this molecular switch.

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