Linker histones as liquid-like glue for chromatin

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Linker histones play essential roles in chromatin structure and function by binding to nucleosomes and modulating the accessibility of DNA for biological processes such as gene transcription and DNA replication (reviewed in ref. 1). For example, DNA promoters of actively transcribed genes and DNA undergoing replication both exhibit reduced linker histone binding (2–4). However, although the structure of the nucleosome core particle was reported in 1997 (5), insights into the structure and function of linker histones within chromatin have emerged only recently (1). Linker histones are small proteins with an ~75-residue globular domain flanked by N- and C-terminal intrinsically disordered regions (IDRs; between ~20 and ~100 residues and ~100 residues, respectively). The folded domain binds core and linker DNA on (6) or near (7) the nucleosome dyad symmetry axis (6). While the N-terminal IDR plays only a minor role, the C-terminal IDR [termed C-terminal domain (CTD) hereafter], composed of ~40% lysine residues, contributes significantly to nucleosome condensation by linker histones (8, 9) and dramatically enhances nucleosome binding (>1,000-fold increased affinity) (10). Due to their intrinsic disorder, the CTDs are usually not resolved in X-ray crystallography and cryo-electron microscopy (cryo-EM) data for nucleosomes containing linker histones. A recent cryo-EM study of histone H1 bound to nucleosomes (11) revealed that density for the CTD preferentially associated with one of the two linker DNA segments; however, relatively low resolution (~10 Å) precluded detailed structural analysis of this density. Thus, while the structure and nucleosome-positioning role of the globular domain of linker histones are known, the structural details of CTD–linker DNA interactions have remained elusive. In PNAS, Turner et al. (12) report that the CTD of chicken histone H1.11L (termed CH1) remains disordered and dynamic upon binding to short double-stranded DNA (dsDNA) oligonucleotides and that, upon reaching a critical stoichiometry that depends on DNA length, promotes liquid–liquid phase separation with DNA through “complex

coacervation”. These results provide intriguing insights into the nature of CTD–dsDNA interactions and inspire new ideas on the molecular mechanisms of linker histone–nucleosome interactions.

Turner et al. (12) used NMR spectroscopy to show that CH1 is disordered and moderately dynamic, as reported previously (13). Remarkably, these disordered features persisted upon binding of CH1 to 36-bp dsDNA in twofold molar excess. Analysis using isothermal titration calorimetry (ITC) revealed a K_d value of ~300 nM for CH1 binding to 36-bp dsDNA under physiological ionic conditions. Reducing the DNA length to 20 bp and lowering the ionic strength to 10 mM yielded higher affinity and biphasic binding as measured by ITC, with the first binding phase (at a CH1 to 20-bp dsDNA ratio of ~0.5:1; $K_d \approx 43$ nM) associated with the formation of soluble CH1–20-bp dsDNA complexes, and the second binding phase (at a CH1 to 20-bp dsDNA ratio of ~1:1; $K_d \approx 19$ nM) associated with phase separation through complex coacervation to form micrometer-sized, liquid-like droplets. Interestingly, with the 20-bp dsDNA at low ionic strength, complex coacervation was accompanied by formation of a parallel twisted helical DNA conformation termed psi (ψ)-DNA. However, this DNA conformation was not observed upon complex coacervation at physiological ionic strength, which reduced CH1–DNA binding affinity through electrostatic screening. Posttranslational modifications are known to weaken interactions of linker histones with nucleosomes (1), and Turner et al. (12) show that phosphorylation of three serine-proline motifs by CDK2/cyclin A, which promotes cell cycle progression and DNA replication, reduced the affinity of CH1 for 20-bp dsDNA, reduced phase separation, and abrogated the ψ -DNA conformation at low ionic strength. The observation of reduced phase separation with dsDNA upon phosphorylation of CH1 is significant because the interaction of linker histones with chromatin is reduced as cells transition into S phase of the division cycle. Moreover, it has been shown that CTDs contribute significantly to inhibition of DNA replication by linker

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Fig. 1. Recent studies suggest that in the nuclei of cells (A), chromatin is organized into domains in which nucleosomes form clusters (B) and exhibit diffusive dynamics. Within these clusters, pairs of nucleosomes interact to form parallel stacks, termed tetranucleosomes, connected by crisscrossing linker DNA segments (straight red lines between blue cylinders) (C). While the core histones comprise the structure of the nucleosome core (blue cylinders), linker histones (blue ellipses with wavy lines) bind nucleosomal and linker DNA near the dyad symmetry axis and occupy the interior space of tetranucleosomes. The results of Turner et al. (12) present the possibility that the CTDs of linker histones (long blue wavy lines) phase-separate with linker DNA segments within the interior of nucleosome clusters, serving as liquid-like glue to promote chromatin compaction.

histones (14). Therefore, the findings of Turner et al. (12) suggest a relationship between the phosphorylation-dependent reduction in phase separation of CH1 with dsDNA and phosphorylation-dependent reduction of linker histone-mediated chromatin compaction and inhibition of DNA replication.

Importantly, the terminal domain of a linker histone from humans (isoform H1.0; termed H1.0-CTD) was recently shown by Borgia et al. (13) to remain highly disordered upon binding with very high affinity (K_d value <1 nM at physiological ionic strength) to the linker histone chaperone prothymosin α (ProT α), which is a polyanionic intrinsically disordered protein. Interestingly, in contrast to the results of Turner et al. (12), H1.0-CTD and ProT α did not undergo phase separation under a wide range of conditions. H1.0-CTD and ProT α have complementary chain lengths, and Borgia et al. (13) speculated that this limits formation of noncovalent intermolecular cross-links that underlie phase separation. H1.0-CTD and ProT α exhibit ultrafast association, which, despite binding with extremely high affinity, allows for biologically relevant dissociation rates, possibly enabling transfer of ProT α -bound linker histone molecules to nucleosomes within chromatin. Another

possibility is that disordered polyelectrolytes within high-affinity complexes can “slide” across one another and occasionally “invade” other complexes (13), facilitating molecular handoffs between like complexes and possibly to other polyelectrolytes (e.g., a linker histone from ProT α to nucleosomes). The results from Turner et al. (12) substantiate the model that emerged from Borgia et al. (13), in which disorder within high-affinity complexes of protein polyelectrolytes enables biological functions that rely upon dynamic molecular handoffs.

What are the implications of Turner et al.’s (12) findings regarding the role of linker histones in chromatin structure, dynamics, and function? Linker histones are essential for compaction of DNA within nucleosome arrays that comprise 30-nm chromatin fibers (15). However, these fibers, characterized *in vitro*, have not been observed in living cells. Instead, recent reports suggest that multiple nucleosomes are clustered within domains that lack long-range order (16, 17) but diffuse collectively in cells (16) (Fig. 1 A and B). Cryo-electron tomography analysis of cellular chromatin *in situ* (17) recently revealed that these clusters resemble features of nucleosome arrays observed *in vitro* using cryo-EM (18), in which nucleosomes form two parallel but twisted stacks connected by linker DNA (Fig. 1C). Within these stacks, the basic building blocks are assemblies of two pairs of stacked nucleosomes connected by crisscrossing linker DNAs termed tetranucleosomes. Importantly, these nucleosome arrays contain linker histones at 1:1 stoichiometry relative to the core histones. Although the globular domains of the linker histones are fully resolved in the cryo-EM structure noted above (18), only small regions of the CTD are represented. While the nucleosome cores within the tetranucleosome building blocks are densely packed, and these building blocks are further packed atop each other within nucleosome arrays composed of three tetranucleosomes, the volume between the two twisted, parallel stacks between which the linker DNAs crisscross back and forth is not fully occupied by electron density. As alluded to by Turner et al. (12), their results present the possibility that the CTDs of the linker histones fill this volume by serving as liquid-like “glue” through dynamic interactions with the crisscrossing linker DNA segments (Fig. 1C). The CTDs may dynamically sample myriad conformations that could reach from the linker DNA segments of their own nucleosome to those of other nucleosomes within an array. As noted above, only very small portions of the density of the CTDs have been visualized in cryo-EM studies (11, 18), consistent with them experiencing constantly fluctuating conformations and positioning. Further, linker histones are known to be highly dynamic within the nucleus (19). This is consistent with the moderate affinity of the globular domains of linker histones for their binding sites on nucleosomes, which is augmented by nonspecific interactions of the CTDs with linker DNA segments. Strikingly, the results of Borgia et al. (13) and Turner et al. (12) each indicate that linker histone CTDs remain disordered and highly dynamic while binding polyanions, either ProT α or dsDNA, consistent with the role proposed here as fluid glue within the core of nucleosome arrays. Extending this idea further and considering the nucleosome clustering models discussed above (16, 17), the CTDs of linker histones within different nucleosome arrays, each composed of variable numbers of tetranucleosome building blocks, may reach between these arrays, providing a molecular basis for adherence between nucleosomes within clusters (Fig. 1C). Importantly, the results from Turner et al. (12) illustrate that the disordered CTDs of linker histones can bind and condense dsDNA while remaining highly dynamic, which may enable the hypothetical fluid glue roles discussed above.

Proteins with phase separation-prone IDRs and DNA were recently shown to form condensed, liquid-like structures in the nuclei of cells, in association with either gene silencing (20, 21) or activation of gene expression (22–24). The results of Turner et al. (12) possibly extend the role of liquid–liquid phase separation as a mechanism for condensing biopolymers to the organization of nucleosomes within condensed domains in chromatin. This model—whereby different types of chromatin domains within cell nuclei form through phase separation of different sets of proteins and different regions of DNA, which are aligned with different biological functions (e.g., gene silencing, gene expression, and DNA replication)—allows for rapid regulation of the assembly and disassembly of these domains due to the ability of biomolecules

to rapidly diffuse into, out of, and within liquid-like condensates (25). The observation by Turner et al. (12) that phosphorylation of CH1 reduces its propensity for phase separation raises the possibility that its phosphorylation in cells “loosens” the organization of chromatin by modulating linker histone–linker DNA phase separation to facilitate gene expression and DNA replication. The structural roles of the CTDs of linker histones have been enigmatic for decades, and the results of Turner et al. (12) provide intriguing insights into these roles and will certainly inspire further investigation in the future. More broadly, their results strengthen the paradigm that disordered proteins can remain disordered when binding to their biological targets and that persistent disorder enables molecular dynamics directly related to biological function.

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