Dynamic networks observed in the nucleosome core particles couple the histone globular domains with DNA

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The dynamics of eukaryotic nucleosomes are essential in gene activity and well regulated by various factors. Here, we elucidated the internal dynamics at multiple timescales for the human histones hH3 and hH4 in the Widom 601 nucleosome core particles (NCP), suggesting that four dynamic networks are formed by the residues exhibiting larger-scale \(\mu\)-ms motions that extend from the NCP core to the histone tails and DNA. Furthermore, despite possessing highly conserved structural features, histones in the telomeric NCP exhibit enhanced \(\mu\)-ms dynamics in the globular sites residing at the identified dynamic networks and in a neighboring region. In addition, higher mobility was observed for the N-terminal tails of hH3 and hH4 in the telomeric NCP. The results demonstrate the existence of dynamic networks in nucleosomes, through which the center of the core regions could interactively communicate with histone tails and DNA to potentially propagate epigenetic changes.
enomic DNA in eukaryotic cells is organized into nucleosome core particles (NCP) that are further packed into chromatin fibers. The NCP is formed by ~147 bp DNA wrapped around a histone octamer (HO) consisting of two H2A–H2B dimers and one (H3–H4) tetramer. Atomic structures have been solved for a number of nucleosomes, of which the majority are NCPs sharing highly identical structural features regardless of the differences in DNA sequences and histone variants. To precisely govern gene activities, eukaryotes evolve various mechanisms, such as post-translational modifications (PTMs) and recruitment of histone variants to promote or suppress DNA accessibility. The PTM sites occur mainly on the histone N-terminal tails, however, novel PTMs recently discovered in the globular domains suggest that the nucleosome core also mediates gene activities. H3 modifications, such as K56Ac, K64Me3, K79Ac, and K122Ac modulate transcription by altering the mobility and stability of DNA. The underlying mechanisms of chromatin regulation by introducing PTMs are still limited. Furthermore, it was recently discovered that the potential correlation with DNA dynamics by characterizing a nucleosome with telomeric DNA that lacks positive information. We measured the internal dynamics of the hH3 in the Widom 601 NCP in the μs–ms and nanosecond to microsecond (ns–μs) timescales. Combined with our previous characterization of hH4 (ref. 23), this suggests the presence of four distinct networks that comprise the hH3 and hH4 core residues exhibiting larger-scale collective motions in the μs–ms timescale, which propagate through the NCP core to the histone tails and DNA. These unique networks may transfer long-range changes induced by epigenetic modifications.

Results

Nanosecond–microsecond dynamics of hH3 in Widom 601 NCP. The complete SSNMR 13C/15N assignments for the hH3 and hH4 core in the Widom 601 NCP have been obtained in our previous studies. The elucidated NMR structures agree well with previous XRD studies. In addition, our study suggested that the internal dynamics of hH4 contributes to modulating DNA accessibility and, therefore, potentially gene activity. In order to obtain a more complete picture of the dynamics in the H3/H4 tetramer, we now also characterized the internal dynamics of the hH3. We first conducted three-dimensional (3D) dipole chemical shift correlation (DIPSHIFT) SSNMR measurements for a NCP reconstituted from human histones and the 145 bp Widom 601 DNA to characterize the dynamics in the timescale of ns–μs in the hH3. Forty six residues in the hH3 globular domain are well resolved in the NCA dimension, of which the backbone H–Cα and H–Cα dipolar line shapes are extracted from the 3D experiments. Site-resolved dipolar order parameters, S(CH) and S(α), derived from the line shapes are shown in Fig. 1, which report the motional amplitudes in the ns–μs timescale. Large-order parameters (>0.8) are determined for a majority of the residues. Residues in the hH3 LN (the loop between αN and α1)
and L1 exhibit slightly smaller order parameters (compared to the rest of the globular domain) corresponding to larger-amplitude dynamics in the ns–μs timescale. The relatively looser packing of the coil structure determines the higher flexibility of LN and L1 in the ns–μs timescale. In comparison, the L2 has slightly larger-order parameters comparable to those of the helix regions, likely due to interaction with DNA through hydrogen bonds and salt bridges. It is noticed that the differences of the order parameters between the loop regions and the well-structured helices are rather small due to the compaction of the disc-like NCP core. Overall, the hH3 core in the Widom 601 NCP undergoes small-amplitude ns–μs motions and the absence of pronounced variations across the globular domain illustrates that NCP core is tightly packed, and provides overall structural stability and integrity for nucleosomes during genome activities.

Microsecond-millisecond dynamics of hH3 in Widom 601 NCP. Our previous study of nucleosome internal dynamics revealed that several regions in the hH4 globular domain exhibit larger-scale dynamics in the μs–ms time window, potentially contributing to chromatin regulation; however, the underlying mechanism of such regulation is unclear. To further understand the nucleosome dynamics in the H3/H4 tetramer in the nucleosome core and its possible functional relevance, we investigated the μs–ms motions for hH3 in the Widom 601 NCP. The cross-peak intensities in the dipolar-based heteronuclear correlation experiments, such as CANCO and NCA are determined by the efficiencies of multiple dipolar heteronuclear transfers, and can be used to qualitatively track the μs–ms dynamics that interferes with relaxation rates (T1ρ and T2*). Here, the CANCO peak heights are extracted for 76 residues of hH3 in the NCP (Fig. 2). The aa P43–R52 in the αN helix, I62–L67 in LN and α1 helix, and L1 exhibit lower CANCO intensities in comparison with the rest core residues. L1 is the most dynamic region in the μs–ms timescale. In particular, the absence of CANCO correlations demonstrates the significant mobility of the F78–D81 region that resides at the DNA-free surface of the NCP and is absent from direct interaction with DNA. Recent studies showed that this stretch is involved in gene regulation and harbors several novel PTMs, including acetylation/methylation of K79 (ref. 34) and phosphorylation of T80 (ref. 35). The fact that this region does not interface directly with DNA leads to a possibility of remote signaling via dynamic networks. Indeed, this gene regulation mode is supported by the other dynamics properties of the hH3 in Widom 601 NCP. The red box marks F78–D81 absent in the NCA spectrum. The dotted lines are given to guide visualization. c, d Regions of hH3 (one copy is shown) exhibiting relatively smaller dipolar order parameters are highlighted in red (PDB: 3LZ0). The N-tail and C-terminus residues absent in those dipolar-based experiments are also highlighted in red as they are highly dynamic. The rest of the hH3 residues having relatively larger dipolar order parameters are in blue. Sites of other histones and DNA that are ≤10 Å away from these regions (in red) are in green. The rest of the histones and DNA are shown as light and dark gray ribbons with smaller thickness, respectively. 1H-15N and 1H-13C dipolar line shape fitting used to extract S(CH)α is provided in the Supplementary Fig. 2.
observed in the Widom 601 NCP and in the telomeric NCP, as discussed in the following. Relatively, larger-scale μ–ms dynamics is also observed in the majority of hH3 αN, including P43–R52 (Fig. 2). A previous study found altered nucleosome DNA sliding and unwrapping in H3 with site mutations in the αN and N-terminal tail35. The hH3 αN is located near the DNA entry–exit and is sandwiched between DNA, hH4 L1, and the hH2A C-terminus. A mutagenesis study suggested that the hH2A C-terminal loop participates in regulating nucleosome mobility37. In addition, we observed higher flexibility for the hH4 L1 (ref. 23). Consequently, these dynamic regions seem to overlap with the histone residues linked to the functional response of modifications and mutations. Another hH3 motif showing relatively enhanced μ–ms dynamics is the stretch I62–L67 located at LN and α1 (Fig. 2b). Overall, three hH3 regions, including the αN, L1, partial LN and the adjacent residues in α1 exhibit relatively larger-scale μ–ms dynamics compared to the rest of the histone H3.

Dynamic networks formed by the hH3 and hH4 in the nucleosome. The previous study of hH4 in the NCP revealed that regions exhibiting higher dynamics in the μ–ms timescales harbor many residues significantly contributing to nucleosome biological activities23. Here, we summarize the dynamic regions of hH3 and hH4 in the Widom 601 NCP, as presented in Fig. 3. Remarkably, the hH3 and hH4 domains that display significantly more pronounced dynamics compared to the rest of the H3/H4 tetramer can be clustered together to form four dynamic networks: (1) the hH3 N-terminal tail, part of αN and H4 L1 (Fig. 3c); (2) hH3 I62–F67 in α1 and LN, the H4 N-terminal tail and H4 LN (Fig. 3d); (3) H3 L1 and part of the H4 N-terminal tail (Fig. 3e); and (4) the H4 C-terminal residues L97–G102 and part of H4 α3 (Fig. 3b). The first three dynamic networks directly interact with DNA and their enhanced mobility likely couple to the flexibility of DNA. The fourth network is buried in the NCP core and likely extends to the H2A C-terminus at the DNA-free surface of the NCP contacting the first network. Given that many of these residues in these dynamic networks were previously found to be functionally relevant as discussed above, we hypothesize that these networks may couple epigenetic modifications in the nucleosome core with DNA, thus altering its mobility and accessibility. Furthermore, we hypothesize that differences in DNA sequences may reversibly induce changes in the NCP core via the same dynamic networks. To confirm this, we conducted SSNMR on a telomeric NCP reconstituted from a 145 bp telomeric DNA comprised of TTAGGG sequence repeats to probe the unique structural and dynamic features of hH3 and hH4.

Structure and dynamics of hH3 and hH4 in human telomeric NCP. The recent XRD structure of the telomeric nucleosome showed that the histones in the NCP reconstituted from the 145 bp human telomeric DNA fold into the same structures, as in other canonical NCPs14. Here, we first characterized the hH3 and hH4 structures in the precipitated telomeric NCP at more physiologically relevant conditions than in the XRD study. To investigate the globular core domains, we performed two-dimensional (2D) NCA and NCO, and dipolar assisted rotational resonance (DARR)38 experiments for the human telomeric NCP structures in the precipitated telomeric NCP at more physiological conditions than in the XRD study. To investigate the globular core domains, we performed two-dimensional (2D) NCA and NCO, and dipolar assisted rotational resonance (DARR)38 experiments for the human telomeric NCP structures in the precipitated telomeric NCP at more physiological conditions than in the XRD study. To investigate the globular core domains, we performed two-dimensional (2D) NCA and NCO, and dipolar assisted rotational resonance (DARR)38 experiments for the human telomeric NCP structures in the precipitated telomeric NCP at more physiological conditions than in the XRD study.
Fig. 3 hH3 and hH4 residues in the NCP exhibiting pronounced microsecond–millisecond mobility form dynamic networks. a Dynamic networks composed of H3 and H4 residues exhibiting pronounced μs–ms motions. Dynamics is highlighted on one copy of H3 and H4. Regions in red and pink are residues exhibiting negligible and relatively low CANCO peak intensities, respectively, that correspond to relatively larger-scale collective μs–ms motions. The rest of H3 and H4 are colored in blue. The rest histones and DNA are shown as light and dark gray ribbons with smaller thickness, respectively. b–e A zoomed-in view of these dynamic networks. Only one copy of each histones are displayed in b–e.

Fig. 4 hH3 and hH4 in the telomeric NCP fold into the same structures as in the Widom 601 NCP with a few minor local conformational differences. a Overlay of the 2D NCA, b NCO, and c $^{13}$C–$^{13}$C DARR SSNMR spectra of the Widom 601 NCP (red) and telomeric NCP (black) containing uniformly $^{13}$C, $^{15}$N-labeled hH3. d Overlay of the 2D NCA, e NCO, and f $^{13}$C–$^{13}$C DARR SSNMR spectra of the Widom 601 NCP (red) and telomeric NCP (black) containing uniformly $^{13}$C, $^{15}$N-labeled hH4. DARR spectra were collected with 20 ms mixing time.
we plot the nonoverlapping NCA and NCO cross-peak intensities of hH3 and hH4 as shown in Fig. 5. Figure 6 shows the clusters of residues exhibiting lower relative NCA and NCO intensities, which correspond to larger-scale dynamics relative to other regions in the telomeric NCP in comparison with the Widom 601 NCP. The majority of residues with enhanced dynamics are found in the four networks (Fig. 6a–d) with the rest in spatial proximity to them (Fig. 6a, e). Previous SAXS and biochemical assay studies suggested that the telomeric NCP exhibits overall higher mobility in comparison with other canonical NCPs22. In addition, as discussed in the below section, our SSNMR data illustrates that the hH3 and hH4 N-terminal tails possess higher degree of flexibility in the telomeric NCP compared with the Widom 601 NCP. In summary, replacing the Widom 601 DNA with the telomeric DNA results in enhanced mobility in the identified dynamic networks and nearby motifs, leading to an overall more dynamic structure of the NCP formed by the unique repetitive G-rich telomeric DNA, which lacks positioning information. Thus, this provides another strong indication that the DNA in the nucleosomes can couple with the residues in those dynamic networks.

**hH3 and hH4 tails in Widom 601 and human telomeric NCP.**

In the cell nucleus, histone N-terminal tails must remain high degree of mobility even in the condensed gene regions, such as heterochromatin, to have the conformational flexibility to enable the interaction with various factors for gene regulation, which is indeed demonstrated by several studies. For example, the hH3 tails were shown to possess distinct conformations and are highly dynamic within NCP in solution39, and the histones hH3 and hH4 N-terminal tails were previously demonstrated to exhibit significant mobility in the condensed nucleosome arrays40. Here, we first explore whether the hH3 and hH4 N-terminal tails remain mobile in the NCP of the most compacted state. We then assay the molecular differences at an detailed level, introduced within the histone tails by altering the DNA sequence in the nucleosomes. As discussed in the above sections and the previous studies23,31, the R42–G132 of hH3 and the I26–G101 of hH4 were observed in the dipolar-based SSNMR experiments in well-hydrated and highly compacted Widom 601 NCP (50–65% water content by weight corresponding to 500–350 mg/mL nucleosomes). In contrast, residues in or close to the N-terminal tails are absent in those spectra due to the significantly higher mobility. To probe the conformation and dynamics of the histone N-terminal tails in the tightly compacted NCP, \(^{1}H-^{13}C/^{15}N\) correlation SSNMR spectra were collected using 2D refocused J-based INEPT pulse schemes, which can detect components with significant mobility in protein samples. The \(^{1}H-^{13}C\) and \(^{1}H-^{15}N\) spectra are shown in Fig. 7b, d for hH3 and hH4, respectively, in the NCP reconstituted from the 145 bp Widom 601 DNA and precipitated with 20 mM Mg\(^{2+}\), which is known to form ordered columnar structures.
hexagonal stacking NCP assemblies. The peaks are assigned by comparing with the spectra and assignments obtained from multidimensional liquid-state NMR experiments of the NCP dissolved in solution without Mg²⁺. As displayed in Fig. 7b, d, A1–V35 of hH3 and S1–V21 of hH4 are observed in the precipitated Widom 601 NCP. This suggests that the histone N-terminal tails stay mobile in the most condensed nucleosome state, which enables the participation in the regulation of heterochromatin. The well-overlaid state, which enables the participation in the regulation of heterochromatin.

Interestingly, two distinct S1 CB and HA show significant mobility in hH3 N-terminal tail extends further toward the globular domain in the telomeric NCP.

Similarly, the incorporation of telomeric DNA introduces differences to hH4 in the NCP; however, fewer residues are affected in comparison with the case of hH3. The hH4 tail forms a contact with the acidic patch of the H2A globular domains, some degree of conformational heterogeneity is expected for K36–Y41 of hH3 and L22–N25 of hH4 as evidenced by their absence in these 1H–13C/15N correlation spectra and in our previously reported SSNMR study detecting the rigid core components.

It was previously shown that hH3 N-terminal tails robustly form intermolecular contacts with the DNA in NCP. Furthermore, the hH4 N-terminal tail can interact with DNA in addition with the H2A–H2B acidic patch of the neighboring nucleosomes. Herein, we examine whether varying the DNA sequence will lead to the conformational and/or dynamical changes of hH3 and hH4 tails in the condensed NCP. The 1H–13C spectra of the precipitated telomeric NCP are displayed in Fig. 7c, e and are compared with those of the Widom 601 NCP. The majority of the peaks overlay well, suggesting that the hH3 and hH4 N-terminal tail residues exhibit high mobility in the condensed NCP as well. In the 1H–13C spectrum of hH3, a few peaks, including T22, A24, A25, and S28 CA–HA show perturbations in the telomeric NCP. In addition, those residues, as well as Q5 and R8 possess 1H–15N chemical shift differences between the two NCPs. These observations suggest that altering the DNA sequence in the NCP changes the chemical environment for the hH3 N-terminal tails in the nucleosomes, illustrating that the hH3 conformations tightly correlate to the DNA sequences, likely due to the direct interaction between the two. Furthermore, a few extra CA–HA peaks are present in the 1H–13C spectrum of hH3 in the telomeric NCP. These peaks possibly belong to the residues between K36–R42. This is also evidenced by the extra Pro CD–HD peak, which can only be assigned to P38 since this is the only extra Pro within the highly flexible tail region or close to the globular domain.

In addition, a Gly 1H–15N peak at 8.82–109.7 p.p.m. is observed in the telomeric NCP, but not in the Widom 601 NCP (Fig. 7e). As there is no Gly in the L22–N25 stretch, we presume that this extra Gly peak belongs to one of the Gly observed in the Widom 601 NCP that possesses small chemical shift perturbations in the telomeric NCP, although we cannot pinpoint the exact residue due to peak overlapping.

Replacing the Widom 601 DNA with the telomeric DNA results in some conformational changes within the hH4 N-terminal tails; however, the affected residues are not as many as in the hH3 tails. This agrees with the participation of direct interaction with DNA. Interestingly, two distinct S1 CB–HB peaks are observed for hH4 in the telomeric NCP, suggesting two local conformations. Furthermore, the L10 CA–HA and CB–HB peaks are absent in the Widom 601 NCP, but present in the telomeric NCP, illustrating that the hH4 tails exhibit higher degree of flexibility in the later sample. Overall, we observed the first 35 residues of hH3 and the first 21 residues of hH4 in the Widom 601 NCP, confirming that these regions of the histone N-terminal tails possess significant mobility in the nucleosomes in their most compacted states. In the telomeric NCP, these tails become more dynamic in comparison with the Widom 601 NCP. It is worth noting that although hH3 and hH4 likely interact with DNA in these condensed NCPs, they possess a high degree of conformational
flexibility as distinct peaks are observed for the residues in these experiments detecting dynamic components.

Discussion
The plasticity of the nucleosome core has been demonstrated to associate with DNA translocation and contributes to the gene regulation by chromatin factors\textsuperscript{15–18}. The current study determined the dynamics of the hH3 and hH4 globular domains at two different timescales. The fact that no significant variation was observed for the ns–μs mobility across the hH3 in the Widom 601 NCP further conforms that the nucleosome core is compact. On the other hand, the μs–ms dynamics exhibits considerable variation among different regions of the histones in the NCP. The observations of relatively lower CANCO peak intensities that are localized in distinct regions spanning both hH3 and hH4 histones clearly show the clustering of more dynamic aa residues. This strongly suggests that the μs–ms dynamics are correlated although the motional timescales, and amplitudes cannot be quantified by the present experiments. The presence of such networks immediately opens up the possibility of coupling of the nucleosome core with DNA and histone tails. This furthermore suggests that perturbations induced by epigenetic modifications in the core might be transmitted to DNA, thus, potentially altering its accessibility and function in an allosteric manner.

Fig. 7 hH3 and hH4 tails are more dynamic in the telomeric NCP in comparison with the Widom 601 NCP. a Schematic representations of hH3 and hH4 tail sequences and secondary structures. b $^{1}H$–$^{13}C$ (upper panel) and $^{1}H$–$^{15}N$ (lower panel) correlation SSNMR spectra of the Widom 601 NCP containing uniformly $^{13}C$,$^{15}N$-labeled hH3. c The overlaid $^{1}H$–$^{13}C$ (upper panel) and $^{1}H$–$^{15}N$ (lower panel) correlation SSNMR spectra of the Widom 601 NCP (red) and telomeric NCP (black) containing uniformly $^{13}C$,$^{15}N$-labeled hH3. d The $^{1}H$–$^{13}C$ (upper panel) and $^{1}H$–$^{15}N$ (lower panel) correlation SSNMR spectra of the Widom 601 NCP containing uniformly $^{13}C$,$^{15}N$-labeled hH4. e The overlaid $^{1}H$–$^{13}C$ (upper panel) and $^{1}H$–$^{15}N$ (lower panel) correlation SSNMR spectra of the Widom 601 NCP (red) and telomeric NCP (black) containing uniformly $^{13}C$,$^{15}N$-labeled hH4. These spectra were obtained with 2D refocused $J$-based INEPT pulse schemes. In c, e, arrows point the peaks shifting between the Widom 601 NCP and the telomeric NCP, circles highlight peaks observed in the telomeric NCP, but not in the Widom 601 NCP.
Compared with the Widom 601 NCP, the histone structures in the telomeric NCP are highly conserved; however, a significant increase in dynamics is observed for residues in the observed dynamic networks and neighboring motifs, further confirming coupling between the nucleosome core and DNA. It was recently proposed that nucleosome can be treated as an allosteric scaffold, and that binding/modifications can regulate gene activity by changing DNA mobility and accessibility at distal sites\textsuperscript{14}. In addition, it has been studied from the sites of chromatin-remodeling systems that the modulations of gene activities are achieved in an allosteric manner. Functional allosteric behavior has been increasingly extended to include long-range correlations of structural and/or dynamical changes within a subdomain of a system\textsuperscript{15,16}. The allosteric response can be induced by perturbations of the internal correlated motions without structural changes\textsuperscript{17,18}, often occur through the pre-existing dynamic networks\textsuperscript{19–23}. The currently revealed distinct dynamic clusters in nucleosomes extend from the center of the nucleosome core to DNA, which may serve as a pathway to propogate long-range changes to achieve DNA regulation by modifications in an allosteric fashion. This study points to the possibility of a novel mechanism of regulating gene activity by PTMs in the nucleosome core and the incorporation of variants. Experiments extending this study to the histones hH2A and hH2B, as well as the investigation of the effects of selective mutations at critical positions in the networks will be needed to shed further light on the details of the present findings. Additional quantititative characterization on the nature of these dynamic features can also be obtained to further understand the correlation of the motions and the functional relevance.

**Methods**

**Preparation of nucleosome core particles.** Two Widom 601 NCP samples were reconstituted from 145 bp Widom 601 DNA and human histones containing uniformly \(^{13}C,^{15}N\)-labeled hH3 or \(^{13}C,^{15}N\)-labeled hH4. The human histones (sequences were harbored in pET-3a plasmids) were overexpressed using Escherichia coli BL21 (DE3) pLysS S. The plasmids harboring one repeat of 145 bp telomeric DNA was indirectly calculated\textsuperscript{17}.

**Liquid-state NMR experiments.** Liquid-state experiments were performed on a 18.8 T Bruker Advance III HD spectrometer equipped with either a 3.2 mm HCN EFree MAS probe (for Widom 601 NCP containing isotopically labeled hH3 or hH4) or a 1.9 mm HCN MAS probe (for telomeric NCP containing isotopically labeled hH3 or hH4). The actual sample temperature was controlled at 11–13 °C (calibrated externally with ethylene-glycol\textsuperscript{74}). \(^{1}H\) chemical shifts were referenced with adamantane using DSS scale (downfield signal at 40.48 ppm.) and \(^{15}N\) chemical shift was indirectly calculated\textsuperscript{52}. The typical 90° pulse lengths of \(^{1}H\), \(^{13}C\), and \(^{15}N\) were fitted using SIMPSON\textsuperscript{56}.

**Solid-state NMR experiments.** SSNMR experiments were performed on an 18.8 T Bruker Advance III HD spectrometer equipped with either a 3.2 mm HCN EFree MAS probe (for Widom 601 NCP containing isotopically labeled hH3 or hH4) or a 1.9 mm HCN MAS probe (for telomeric NCP containing isotopically labeled hH3 or hH4). The actual sample temperature was controlled at 11–13 °C (calibrated externally with ethylene-glycol\textsuperscript{74}). \(^{1}H\) chemical shifts were referenced with adamantane using DSS scale (downfield signal at 40.48 ppm.) and \(^{15}N\) chemical shift was indirectly calculated\textsuperscript{52}. The typical 90° pulse lengths of \(^{1}H\), \(^{13}C\), and \(^{15}N\) were 2.5, 3.6, and 5.0 μs, respectively, with the 3.2 mm probe, and were 2.2, 3.2, and 4.15 μs, respectively, with the 1.9 mm probe. 2D CC DARR\textsuperscript{48}, NCA, NCO, and 3D CANO were collected and the detailed experimental parameters are summarized in Supplementary Table 1.

For the Widom 601 NCP containing \(^{13}C\), \(^{15}N\)-labeled hH3, the 3D DIPSHIFT\textsuperscript{32} experiments were conducted at 15.151 KHz with the 3.2 mm probe, and the NCA transfer was achieved by 4 ms SPECIFIC-CP, and the \(^{1}H–^{2}N\) and \(^{1}H–^{15}C\) dipolar coupling were reintroduced by applying R12\textsuperscript{6}, (ref.) symmetry sequences\textsuperscript{41} on \(^{1}H\) channel in a constant-time manner. Data were processed using nmrPipe\textsuperscript{55} and analyzed with Sparky (T, D. Goddard and D. G. Kneller, University of California, San Francisco). The \(^{1}H–^{15}N\) and \(^{1}H–^{1}C\) dipolar coupling line shapes were extracted from the 3D DIPSHIFT experiment, and are fitted using SIMPSON\textsuperscript{56}. The line shapes in the frequency domain were obtained via Fourier transform of the experimental dipolar coupling trajectories with zero-filling to 256 points. The regions of –2 to 2 kHz of the \(^{1}H–^{15}N\) dipolar line shapes and –4 to 4 kHz of the \(^{1}H–^{1}C\) dipolar line shapes were considered in the fitting. The error bars plotted in Fig. 1 were calculated 95% confidence intervals.

**Statistics and reproducibility.** The 3D experiments used to extract dynamics information were performed eight to nine times and were co-added together. The error bars (calculated 95% confidence intervals) of the SSNMR dipolar line shape fitting and the root-mean-square deviation values of peak intensities are presented in the figures in the main text.

**Reporting summary.** Further information on research development is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** The data supporting the findings of this study are available within the paper and the Supplementary Information. All relevant data is readily available upon request from corresponding authors.
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Acknowledgements

We thank Dr. Nikolay Koselev for comments on the manuscript. This work was supported by the Singapore Ministry of Education Academic Research Fund (AcRF) Tier 2 (MOE2018-T2-1-112) and Tier 3 (MOE2012-T3-1-001). All SSNMR experiments were performed at the Nanyang Technological University (NTU) Center of High Field NMR.
Spectroscopy and Imaging. We also acknowledge the NTU Institute of Structural Biology (NISB) for supporting this research.

Author contributions
X.S. performed the NMR experiments and data analysis, and contributed to the sample preparation. C.P. prepared the samples and contributed to the NMR experiments and data analysis. A.S. contributed to the preparation of the telomeric NCP samples. X.S. and L.N. conceived the study and wrote the manuscript with input and comments from all the co-authors. K.P. contributed to conceiving the study. L.N. acquired funding for the project.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s42003-020-01369-3.

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