Nuclear condensates of p300 formed though the structured catalytic core can act as a storage pool of p300 with reduced HAT activity

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The transcriptional co-activator and acetyltransferase p300 is required for fundamental cellular processes, including differentiation and growth. Here, we report that p300 forms phase separated condensates in the cell nucleus. The phase separation ability of p300 is regulated by autoacetylation and relies on its catalytic core components, including the histone acetyltransferase (HAT) domain, the autoinhibition loop, and bromodomain. p300 condensates sequester chromatin components, such as histone H3 tail and DNA, and are amplified through binding of p300 to the nucleosome. The catalytic HAT activity of p300 is decreased due to occlusion of the active site in the phase separated droplets, a large portion of which co-localizes with chromatin regions enriched in H3K27me3. Our findings suggest a model in which p300 condensates can act as a storage pool of the protein with reduced HAT activity, allowing p300 to be compartmentalized and concentrated at poised or repressed chromatin regions.
Formation of condensates in the cell nucleus has been shown to drive the assembly of nuclear bodies, Cajal bodies, nucleoli, and speckles. These membraneless compartments are produced via the liquid–liquid phase separation (LLPS) mechanisms and depend on weak, multivalent interactions involving intrinsically disordered regions (IDRs) of biomolecules. Several nuclear proteins and chromatin regulators, including HP1, BRD4, CBX, and MORC3, have been shown to form condensates with their phase separation ability being necessary for biological functions and subnuclear localization of these proteins.

The transcriptional co-activator p300 is an acetyltransferase that is frequently dysregulated in disease, particularly cancer. p300 associates with over 400 binding partners, including transcription factors, DNA-binding proteins and subunits of the RNA polymerase II complex, to form activation complexes and facilitate transcription. p300 acetylates histones and a number of essential nonhistone proteins, notably p53, E2F1, and androgen and estrogen receptors. The highly selective acetylation of the H3K27 and H3K18 sites by p300 requires a cooperative action of its two functional domains: acetyllysine-binding bromodomain (BD) and the H3 tail-binding ZZ domain. p300 and its close homolog CBP share the same domain architecture, consisting of several conserved modules, including the catalytic histone acetyltransferase (HAT) domain. Located in the middle of the protein, the HAT domain is surrounded by BD, a RING finger, and a plant homeodomain finger (PHD) from one side and the ZZ domain from another side that together comprise the catalytic core of p300.

p300 catalytic activity is controlled by signal-dependent transcription factor that is frequently dysregulated in disease, particularly cancer. p300 but releases the inhibition upon hyper autoacetylation. The p300 catalytic activity is essential nonhistone proteins, notably p53, E2F1, and androgen and estrogen receptors. The highly selective acetylation of the H3K27 and H3K18 sites by p300 requires a cooperative action of its two functional domains: acetyllysine-binding bromodomain (BD) and the H3 tail-binding ZZ domain.

Results and discussion

p300 forms dynamic nuclear condensates in cells. p300 is highly abundant in the cell nucleus and can shuttle between the nucleoplasmic and cytoplasmic fractions. To characterize the distribution of p300, we transfected HeLa cells with YFP-tagged full-length p300 (YFP-p300FL) and monitored the localization of YFP-p300FL in live cells by fluorescence microscopy (Fig. 1b–d). In the majority of cells assayed, YFP-p300FL was somewhat diffusely distributed throughout the nucleus, but 15–30% of cells displayed nuclear speckles various in size (Fig. 1b). These data indicate the presence of discrete YFP-p300FL compartments with elevated protein concentration and are in line with previous reports showing that both endogenous and expressed p300 (and its homolog CBP) form dynamic nuclear bodies.

To determine whether p300 is mobile in the speckles, we measured the diffusion kinetics of selected p300 puncta by fluorescence recovery after photobleaching (FRAP) experiments. For each selected region, the laser beam was applied after two initial scans, and cell images were collected at 10 s intervals for a duration of 280 s (Fig. 1c, d). The experiments were performed on seven cells, and changes in the fluorescence signal of each bleached region were analyzed. The averaged signal intensity, which was normalized and plotted (Fig. 1c), showed a ~60% recovery of the bleached regions’ fluorescence with a half-life of 59 s. The fast signal recovery, and therefore the fast diffusion of p300, suggest that YFP-p300FL speckles are viscous liquid droplets characterized by rapid protein exchange with the surrounding environment. Collectively, these results demonstrate that full length p300 forms dynamic nuclear condensates with liquid/gel-like properties.

The catalytic core of p300 phase separates into liquid droplets in cells and in vitro. The middle part of p300, consisting of the BD followed by the RING, PHD, HAT, and ZZ domains, comprises the catalytic core that we postulated might be involved in the phase separation process. To test this idea, we generated YFP-tagged truncated p300 constructs (aa 1024–1830 of p300), which in addition to the catalytic core contains a TAZ2 domain and is referred to as p300BRPHZ. We have previously shown that p300BRPHZ associates with chromatin in vitro and in vivo comparably to full-length p300. The YFP-p300BRPHZ protein was produced using a doxycycline (DOX) inducible t’RipZ vector, and expression of p300BRPHZ was visualized in live HeLa cells. Much like the full-length protein, YFP-p300BRPHZ localized primarily to the nucleus and formed foci of various sizes (Supplementary Fig. 1). Furthermore, FRAP experiments showed a ~80% fluorescence recovery with a half-life of 29 s, indicating that YFP-p300BRPHZ and full length YFP-p300 have similar capabilities to form dynamic condensates in cells.

The cell nucleus is a highly crowded and viscous compartment packed with DNA, histones, and a multitude of nuclear proteins. To mimic molecular crowding, polyethylene glycol (PEG) is frequently used in vitro. Addition of PEG (PEG3350) to a solution containing the purified catalytic core, p300BRPHZ (aa 1035–1720 of p300), rapidly induced cloudiness in the originally clear solution, indicative of the conversion to a heterogeneous suspension (described in detail below). Under a microscope, we observed spherical droplets of various sizes, suggesting the formation of liquid–liquid phase-separated condensates of the catalytic core of p300. About 35% of p300BRPHZ remained in the supernatant after removing the droplets by centrifugation (Supplementary Fig. 2).

Self-acetylation of p300 impairs its ability to phase separate. We found that different batches of purified p300BRPHZ showed variable ability to form droplets under otherwise identical conditions (Figs. 1f and 2a, b). Recombinantly expressed p300 is auto-acetylated to varying degrees. We, therefore, assessed
the self-acetylation levels of two different batches of purified p300BRPHZ by liquid chromatography–mass spectrometry (LC–MS). Acetylation levels of p300BRPHZ were highly heterogeneous, containing a series of species with discrete molecular mass. For example, purification 1 contained p300 BRPHZ with molecular mass ranging from ~81 to 81.7 kDa (Fig. 2a, c, and d). The increment of each mass peak was ~42 Da, the mass of one acetyl group. Compared with its theoretical mass of 80,548.5 Da, recombinantly expressed p300 BRPHZ from purification 1 was acetylated on 13–25 lysine residues, with a median of 16 acetylated lysines (Fig. 2c, d). The sequence of p300BRPHZ contains 60 lysine residues, indicating that ~20–40% of lysine residues were acetylated. The p300BRPHZ protein from purification 2 had on average 26 acetylated lysines.

**Fig. 1 p300 phase separates to condensates in living cells.** a Schematic of p300FL and p300BRPHZ. b Representative images of HeLa cells expressing YFP-p300FL. Scale bar, 5 µm. c FRAP curve of YFP-p300FL was obtained from averaging data from six HeLa cells. Error bars represent SEM. n = 6 cells examined over three independent experiments. d Representative FRAP images of YFP-p300FL expressed in HeLa cells. The images were taken before and after photobleaching at indicated time points. The bleached condensate is indicated by a white arrowhead. Scale bar, 5 µm. e FRAP curve of YFP-p300BRPHZT condensates in HeLa cells. The FRAP curve was obtained from averaging data from four cells. Error bars represent SEM. f A representative image of a sample (from at least three replicates) containing 13 µM p300BRPHZ and 12% PEG3350 (PEG) in a 100 µm × 100 µm square region under a microscope.
acetylation level (purification 1) visibly formed condensates in the presence of PEG (Fig. 2a), while p300BRPHZ with the higher acetylation level (purification 2) showed almost no droplet formation (Fig. 2b). Together, these results suggest a negative correlation between the acetylation level of p300 and its ability to form condensates in vitro.

We further examined whether acetylation levels modulate the condensation behavior of p300BRPHZ. The NAD\(^+\)-dependent histone deacetylase SIRT2 has been shown to selectively and uniquely deacetylate p300 in cells and in vitro\(^{42}\). We incubated p300BRPHZ with SIRT2 and NAD\(^+\) and then purified the protein by size-exclusion chromatography. This reduced the number of
acetylated lysines in p300BRPHZ (purification 1) from a median of 16 to a median of 4 (Fig. 2c, d, g, h). The same treatment of p300BRPHZ (purification 2) reduced the acetylation level from a median of 26 to 12 acetylated lysines. Generally, lower acetylation levels were correlated with an increased propensity to form droplets (Fig. 2i).

To identify regions in p300 affected by SIRT2 treatment, we analyzed tryptic peptides derived from untreated and SIRT2-treated p300BRPHZ using liquid chromatography–tandem mass spectrometry (LC–MS/MS). 49 out of 60 lysine residues were acetylated in untreated p300BRPHZ. Comparison of the signal intensities of peptides revealed substantial differences in acetylation levels of untreated and SIRT2-treated p300BRPHZ (Fig. 2j). The most notable changes were observed for K1551, K1554, K1555, K1557, and K1560 located in the AIL, as acetylation of these lysine residues was not detectable in the SIRT2-treated p300BRPHZ sample. Another AIL residue, K1568, also displayed a considerable reduction in signal intensity upon SIRT2 treatment. In contrast, signal intensity for lysine residues located in other regions of p300BRPHZ was reduced to a substantially lesser degree.

These data corroborate the idea that p300 self-acetylates a wide range of lysine residues spanning the catalytic BD–RING–PHD–HAT–ZZ core, and that the auto-inhibitory loop undergoes fast deacetylation, whereas deacetylation of other regions of p300BRPHZ occurs much slower.

Because our data suggest that acetylation of p300 negatively regulates its ability to phase separate, we reasoned that acetylation should decrease or even eliminate the PEG-induced p300BRPHZ droplet formation. To test this, we first measured the HAT activity of untreated and SIRT2-treated p300BRPHZ in the presence of acetyl-CoA by monitoring the release of the CoA product over time. As expected, SIRT2-treated p300BRPHZ produced more CoA than untreated p300BRPHZ due to the higher number of unmodified lysine residues (substrates) present in SIRT2-treated p300BRPHZ (Fig. 3a). The acetylation reaction was fast and completed before 20 min, in keeping with the previous reported activity of the p300 HAT domain.41 Adding acetyl-CoA to a SIRT2-treated p300BRPHZ/PEG suspension led to a decrease in the cloudiness of the sample within 1 min (Fig. 3b), and liquid droplets were no longer visible under the microscope (Fig. 3c, d, and Supplementary Fig. 3a), indicating a disruption of LLPS due to the HAT reaction. Collectively, these data suggest that auto-acetylation of the AIL decreases the formation of p300BRPHZ condensates.

**Phase separation of p300 relies on both BD and AIL.** Weak and often nonspecific multivalent interactions are believed to drive phase separation.14,7,8. The p300 HAT domain has been associated to interact with the hypoacetylated AIL from another p300 molecule28 and BD of CBP was shown to bind the AIL peptide acetylated at K159645. Accordingly, we envisage two distinct mechanisms for the transition of the hyperacetylated p300 catalytic core (incapable of phase separation) to the hypoacetylated p300 catalytic core (capable of phase separation), which rely on intermolecular ‘in trans’ HAT–AIL or BD–acetyl–lysine (Kac) interactions (Fig. 3e). To test these possibilities, we examined the role of the BD and AIL in the formation of condensates. We used a deletion of the entire autoinhibitory loop (ΔAIL) in p300BRPHZ or a mutation N1132A, which was previously shown to abrogate acetylysine binding of the BD27.

Compared to the WT p300BRPHZ protein that contained on average ~12 Kac sites and readily underwent phase separation (Fig. 3f), the N1132A mutant did not form PEG-induced droplets, despite having a similar acetylation level (~14 Kac sites) (Fig. 3f–h). Likewise, the ΔAIL p300BRPHZ mutant with ~7 Kac sites formed less visible condensates than the WT protein with ~4 Kac sites (Fig. 3i–k). We note that although the entire AIL was deleted, the ΔAIL p300BRPHZ mutant was still acetylated at 7 lysine residues located outside this loop in the BD–RING–PHD–HAT–ZZ region. These data suggest that both HAT–AIL and BD–Kac-binding mechanisms contribute to p300BRPHZ phase separation. In a hyper-acetylated form where both the AIL and other regions of p300BRPHZ are acetylated, BD may favor intramolecular ‘in cis’ contacts with the acetylated AIL, limiting the probability of intermolecular interactions and leading to a diffused distribution of p300BRPHZ. Upon SIRT2-treatment of WT p300BRPHZ, deacetylation of the AIL results in the release of BD, allowing intermolecular ‘in trans’ BD–Kac interactions and both intra- and intermolecular HAT–AIL interactions (Fig. 3e).

In support of this model, SIRT2 treatment of N1132A p300BRPHZ, which did not originally phase separate (Fig. 4a, left panel), led to the condensate formation (Fig. 4a, right panel, and Fig. 4b), suggesting that deacetylation of AIL promotes phase separation through the HAT–AIL interaction (Fig. 4c). Furthermore, both SIRT2-treated or untreated ΔAIL p300BRPHZ formed droplets, although to a lesser degree compared to the WT protein, reinforcing the role of the BD–Kac interaction in promoting phase separation (Fig. 4d–f). Moreover, the catalytically impaired mutant D1399A p300BRPHZ showed the formation of the droplets through the HAT–AIL interaction, since no self-acetylation occurred in this mutant (Fig. 4g, h, and Supplementary Fig. 2b, c). The SIRT2-dependent phase separation was also observed in the p300ΔHZ construct containing only the HAT and ZZ domains (Fig. 4i, j), whereas further deletion of AIL completely abolished droplet formation, regardless of the SIRT2 treatment (Fig. 4k, l). Lastly, both N1132A and ΔAIL mutants of YFP-p300FL formed droplets in HeLa cells, confirming that full-length p300 can phase separate through either HAT–AIL interaction in N1132A YFP-p300FL or BD–acetyl–lysine interaction in ΔAIL YFP-p300FL (Fig. 4m).

To further confirm our model, we performed small-angle X-ray scattering (SAXS) experiments on purified WT p300340–2094 and ΔAIL p300340–2094 proteins at various time points before and after incubating with acetyl-CoA (Fig. 5a). SAXS experiments provide
information about the protein's size and shape in solution. For WT p300<sub>340–2094</sub>, autoacetylation led to little changes in the SAXS profile: the Rg value remained unchanged at ~10 nm, in agreement with monomeric p300, possibly because the acetylated AIL engages the BD 'in cis' (Fig. 5b, top panel). In contrast, for ΔAIL p300<sub>340–2094</sub>, the Rg value increased over time (to ~35 nm) after autoacetylation for 1.5 h resulting in spherical droplets with a maximum dimension of ~125 nm (Fig. 5a, bottom panel). These droplets likely arise through 'in trans' engagement of the BD with acetylated lysine residues outside AIL (Fig. 5b, bottom panel).

**p300 condensates sequester nucleosomal substrates.** Besides auto-acetylation, p300 catalyzes acetylation of lysine residues of
Fig. 3 p300BRPHZ phase separation requires both AIL and BD. 

**a** HAT activity of untreated p300BRPHZ (blue) and SIRT2-treated p300BRPHZ (orange) measured by a fluorometric assay. Reactions were started by the addition of acetyl-CoA and quenched by flash-freeze at indicated time points. Data are presented as mean values ± SD; error bars represent SD from triplicate measurements.

**b** Phase separation of SIRT2-treated p300BRPHZ. The reaction mixture contained 10 μM p300BRPHZ and 12% PEG. Addition of Acetyl-CoA led to the disassembly of the p300BRPHZ droplets.

**c** Representative images of p300BRPHZ samples from (i) after incubation for 2 min under a microscope.

The number of droplets was counted in five non-overlapping 50 μm × 50 μm square regions and the mean value was plotted. Error bar represents SD.

**e** Schematics of the p300BRPHZ phase separation mechanisms that can occur simultaneously. Multivalent 'in trans' interactions between HAT and deacetylated AIL and/or BD and acetylated lysines outside AIL can promote the formation of condensates.

**f, g** Representative images of WT p300BRPHZ (f) and N1132A mutant (g) samples (from at least three replicates) containing 13 μM protein and 12% PEG in a 50 μm × 50 μm square region on cover slides under a microscope.

**h** LC–MS analysis of N1132A p300BRPHZ shown in (g).

**i, j** Representative images of WT p300BRPHZ (i) and ΔAIL mutant (j) samples (from at least three replicates) containing 13 μM protein and 12% PEG in a 50 μm × 50 μm square region on cover slides under a microscope.

**k** LC–MS analysis of ΔAIL p300BRPHZ shown in (j). Source data are provided in the Source Data file.
histone proteins in nucleosomes, particularly at H3K18 and H3K27 sites. We, therefore, tested whether p300 droplets could concentrate nucleosomal components, such as DNA and histone tails. We monitored the recruitment of fluorescein (FAM)-labeled histone H3 tail (FAM-H3, residues 1–12 of H3) and FAM-labeled 37 bp double-stranded DNA (FAM-DNA) to p300 BRPHZ droplets using confocal microscopy (Fig. 6a and Supplementary Fig. 3b). The p300 condensates exhibited bright fluorescence when incubated with FAM-DNA or FAM-H3, but not when incubated with the control FAM. The recruitment of histone H3 tail to condensates was due to binding of the ZZ domain of p300 to the unmodified H3 tail, however, p300 and CBP were thought to not contact DNA themselves. We noticed lower background fluorescence when p300 condensates were incubated with FAM-DNA compared to FAM-H3, which suggests that p300 BRPHZ concentrates better and therefore binds stronger to DNA than to the histone H31–12 peptide. Overall, these results demonstrate that p300 BRPHZ condensates can sequester nucleosomal components like histone H3 tail and DNA from the surrounding environment.

Can p300 bind DNA and is this reaction mediated by p300 self-acetylation? We tested the association of untreated and SIRT2-treated p300 BRPHZ with 601 DNA (147 bp) by electrophoretic mobility shift assay (EMSA). The SIRT2-treated p300 BRPHZ, containing on average 4 Kac outside the AIL, strongly bound to 601 DNA with an apparent Kd of ~0.3 μM (Fig. 6b). In contrast, untreated p300 BRPHZ, which contains on average ~16 Kac including in the AIL, showed much weaker binding to 601 DNA in the same condition (Fig. 6c). DNA binding of p300 BRPHZ, therefore, is directly regulated by its acetylation level, as acetylation neutralizes the positive charge of the unmodified lysine side chain, leading to a decrease in binding to the negatively charged DNA. We also found that auto-acetylation regulates p300 BRPHZ association with nucleosomes. While SIRT2-treated p300 BRPHZ bound to the reconstituted nucleosome core particle (NCP) with a low micromolar Kd in EMSA assays (Fig. 6d), the association of untreated p300 BRPHZ with NCP was not significantly compromised (Fig. 6e). Collectively, these data suggest that the phase transition and nucleosome binding functions of p300 BRPHZ are linked to and regulated by its auto-acetylation.

The results described above may suggest that DNA and the HAT domain compete for the same hypoacetylated AIL, which could lead to a decrease in phase separation ability of p300 BRPHZ. However, the addition of an equimolar amount of NCP to p300 BRPHZ stimulated the formation of more and larger droplets, whereas NCPs alone did not phase separate under the same condition (Fig. 6f). These data imply that even when the AIL binds to DNA and is, therefore, less available to contribute to the droplet formation through the HAT–AIL mechanism, the intranucleosomal association through the BD–Kac mechanism is sufficient to maintain phase separation.

Catalytic HAT activity of p300 is decreased in condensates. We examined the effect of phase separation on enzymatic activity and histone substrate selectivity of p300 BRPHZ. Because phase separation conditions can promote enzymatic reactions owing to higher local concentrations of both the enzyme and substrate, we initially thought that p300 HAT activity would also be elevated due to increased local concentration of p300 BRPHZ and the substrate, either p300 BRPHZ and/or NCP inside the droplets. On the other hand, the intermolecular AIL–HAT interaction, which leads to the formation of p300 condensates, physically blocks the active site of the HAT domain and thus would lead to a reduction in the enzymatic activity, similar to what was observed for the TOR kinase. In addition to sterically occluding the substrate–enzyme complex formation, the crowded viscous environment in condensates slows diffusion of substrates and products, which results in the reduced catalytic activity of enzymes.

To compare the HAT activity in solution and phase-separated droplets, we first monitored the self-acetylation of p300 BRPHZ. As shown in Fig. 6g, the addition of PEG to the SIRT2-treated p300 BRPHZ, which does not phase separate because of low protein concentration (0.2 μM), had essentially no effect on the change in the fluorescence signal (due to CoA release) over time, suggesting that the presence of PEG did not alter the catalytic activity of p300 BRPHZ (Fig. 6g). In contrast, the change in fluorescence was slower in the PEG-induced phase-separated suspension (Fig. 6h, red line) or in the supernatant after the droplets were spun down (Fig. 6i) compared to the change in fluorescence in the solution without droplets (Fig. 6h, blue line). These data indicate that autoacetylation is inhibited when p300 BRPHZ forms condensates.

We next measured the acetyltransferase activity of p300 BRPHZ on reconstituted nucleosomes in homogeneous solutions and under the phase separation condition. SIRT2-treated (containing ~12 Kac) p300 BRPHZ and untreated (containing ~26 Kac) p300 BRPHZ were mixed with NCP at a 1:1 ratio, and after the addition of acetyl-CoA, the CoA release was monitored at indicated time points. The reactions with NCP as a substrate (Fig. 6), blue and orange bars, and Supplementary Fig. 4a) were completed within 2 min for both SIRT2-treated and untreated p300 BRPHZ and were much faster than p300 BRPHZ autoacetylation.
(Fig. 3a). The HAT reaction was also carried out in a suspension of PEG-induced droplets of SIRT2-treated p300BRPHZ and NCP. Again, as in the case of autoacetylation (Supplementary Fig. 4b, gray bars), the apparent HAT activity of p300BRPHZ in the phase-separated condensates was decreased compared to the HAT activity of p300BRPHZ in solution (Fig. 6j, gray bars).

To assess the histone substrate selectivity of p300BRPHZ, we monitored acetylation of NCP by western blot using antibodies against H3K27ac, H3K9ac, and H3K4ac (Fig. 7a–d and Supplementary Fig. 5). We found that either SIRT2-treated or untreated p300BRPHZ robustly acetylates H3K27 in NCP but produces H3K9ac and H3K4ac to a lesser degree, which is in agreement with our previous findings and with the data shown in Fig. 6j—the reaction was fast and completed in 2 min. The selectivity of p300BRPHZ toward the H3K27 site was conserved in the suspension of SIRT2-treated p300BRPHZ droplets, however, again, the rate of NCP acetylation was decreased (Fig. 7b, d, yellow line). These data further substantiate a reduction in the catalytic activity of p300BRPHZ upon formation of the condensates.

p300 condensates preferably localize to chromatin regions marked by H3K27me3. p300 often binds to enhancers and promoters enriched in active mono- and trimethylated H3K4 (H3K4me1 and H3K4me3, respectively) marks, acetylating histones, and stimulating gene transcription. However, a pool of p300/CBP with a suppressed HAT activity has been shown to associate with poised and silent genomic regions marked by the repressive modification H3K27me325,29–31,48,49. Indeed, ChIP-seq analysis of the H1299 cells expressing Flag-tagged WT p300BRPHZT identified 679 p300BRPHZT binding sites, and of these,
117 p300BRPHZT binding sites were also enriched in H3K27ac and H3K18ac—the primary products of acetylation by p300 (Fig. 7e). 76 p300BRPHZ binding sites however were enriched in H3K27me3. The notable absence of acetylation of H3K18 at these sites suggests that the HAT activity of p300BRPHZT is decreased when p300 colocalizes with H3K27me3.

Because the HAT activity of p300 is decreased in the phase-separated condensates, we examined whether the condensates could select for chromatin modifications in HeLa cells using immunofluorescence. As shown in Fig. 8, the YFP-p300ζL condensates co-localize to a higher degree with the regions enriched in H3K27me3 with a Pearson’s correlation coefficient (Pearson’s R) of 0.45 ± 0.09, but co-localization with H3K4me3 was less pronounced (Pearson’s R of 0.37 ± 0.09), and even lower level of co-localization was observed with H3K9me3 (Pearson’s R of 0.25 ± 0.05). Together, these data suggest that a pool of p300 condensates has a preference to localize to the chromatin regions containing transcriptionally repressive H3K27me3 modification as compared to the regions containing transcriptionally active modification H3K4me3 or the heterochromatin mark H3K9me3.

It is becoming increasingly clear that the LLPS phenomenon and the assembly of membraneless condensates by biological macromolecules in the nucleus play a crucial role in numerous cellular processes. Formation of condensates allows for efficient separation of the nuclear compartments in a spatiotemporal manner and/or concentration of the macromolecules to regulate, activate or reduce their functions. Although the phase separation mechanisms in the nucleus are currently a subject of intense studies, multivalent weak contacts involving IDRs of macromolecules have been widely acknowledged as a driving force for the formation of biomolecular condensates. In this work, we show...
that the major human acetyltransferase p300 forms liquid condensates in the nucleus and this function depends rather on the structured catalytic core of p300, including the HAT domain and its AIL, and BD. We demonstrate that hyperacetylation of the p300 catalytic core, particularly of AIL, decreases the phase separation ability, and that p300 utilizes two distinct molecular mechanisms to assemble the condensates, which rely on intermolecular ‘in trans’ HAT–AIL and BD–(Kac outside AIL) interactions. Furthermore, we found that the catalytic HAT activity of p300 is decreased in the phase-separated droplets, which is likely due to steric blocking of the HAT active site.

Our data suggest a model for compartmentalization and concentration of p300 with reduced catalytic activity. p300/CBP is often associated with transcriptional activation and occupies gene promoters and enhancer elements but has also been reported to localize to the repressive sites, particularly those enriched in H3K27me3. These sites are characterized by overall low acetylation of histones, and since H3K27me3 does not preclude binding of p300/CBP to the H3 tail, it was proposed that the HAT activity is blocked at such sites. The mechanism of this blockage remains unclear. A few concepts have been put forth to explain the decrease in p300/CBP catalytic activity, especially on histone lysine residues other than H3K27, as methylation of H3K27 obviously prevents its acetylation. These include regulation through phosphorylation or SUMOylation of p300/CBP and rapid degradation of p300.

Fig. 7 A pool of p300 co-localizes with H3K27me3. a–d H3K27ac and H3K9ac western blot analysis of the reaction mixtures containing p300BRPHZ and an equimolar amount of NCP as a substrate. Reactions were quenched by rapid freezing and the addition of SDS-loading buffer at indicated time points. The intensity of bands is quantified, normalized to the +SIRT2/PEG band at 2 min, and shown in (b) and (d). e Heatmaps of H3K4me3, H3K27me3, H3K27ac, H3K18ac, and Flag-p300BRPHZT. ChIP-seq signals centered on p300-H3K4me3 and p300-H3K27me3 binding sites in a ±20 kb window in H1299 cells stably expressing FLAG-p300BRPHZT. Heatmaps are ranked by H3K4me3 (upper panel) or H3K27me3 (lower panel). The color keys represent ChIP-seq densities normalized to total reads.
p300 condensates co-localizes with chromatin regions enriched in H3K27me3 suggests an alternative mechanism. The p300 condensates can act as a storage pool of the protein with reduced HAT activity, allowing p300 and possibly other elements of the transcriptional machinery to be compartmentalized and concentrated at the repressed chromatin sites. The formation of p300 condensates through blocking the catalytic site of the HAT domain provides a mechanism by which the enzymatic activity of p300 can be downregulated. Similar downregulation of the enzymatic activity through phase transition has been reported for the target of rapamycin (TOR) serine/threonine protein kinase in the TORC1 complex. The formation of the TORC1 foci results in steric occlusion of the TOR active site, subsequently leading to the inhibition of the catalytic activity. To better understand the p300-dependent activation of gene transcription, it will be essential in future studies to delineate and visualize by single-molecule imaging the precise contacts between p300 and the repressed chromatin regions.

**Fig. 8** p300 condensates show preference for co-localization with H3K27me3. a-f Nuclear localization of the YFP-p300FL condensates with indicated posttranslational histone modifications (PTMs) in HeLa cells were examined by immunofluorescence. The cells were fixed and probed with anti-H3K27me3 (a), anti-H3K4me3 (c), and anti-H3K9me3 (e) antibodies. Representative images are shown in the left panels: green, YFP-p300FL; red, PTMs; yellow, overlap of fluorescence signals of YFP-p300FL and PTMs. The ImageJ software was used to plot fluorescence signal intensities of YFP-p300FL and PTMs in the nuclei along the line (from left to right) indicated by white arrowheads (b, d, f). Scale bar, 5 µm. g A table of Pearson’s R values calculated using the entire cell nuclei (n = 3 cells examined for each mark). Data are presented as mean values ± SD. Pearson’s R values range between +1 (perfect positive correlation) and −1 (inverse correlation).
Methods
Cell culture, transfection, and imaging. WT full-length YFP-p300 plasmids were transfected into HeLa cells in a 3.5-cm diameter tissue culture dish by Lipofecta-
mine 3000 (Life Technology, L3000-075) using manufacturer instructions. The cells were cultured in DMEM (company) supplemented with 10% fetal bovine serum (company, need double-check) for 36 h. The cell culture medium was replaced with the live-cell imaging medium and maintained at 37 °C as a heater control. The 200 mM Boc-PhD–HAT–Z Z domain (BRPhZ, aa 1024–1830) was cloned into a pTrp-Z-YFP vector. To establish a stable cell line, cell
titer harvested from HEK293T cells was used to infect HeLa cells with fusion
gene. HEK293T cells were seeded in a 10-cm dish to reach 90–100% confluence the
day before. The following day the cells were transfected using calcium phosphate
capsulation (210 µg pPAX2, 10.5, 21.0 µg of pTrp-Z-YFP, p380, 250 mM CaCl2, and 1× Hank’s balanced salt solution) and incubated for 12 h at 37 °C and 5% CO2. Cells were washed twice with culture medium and then incubated in culture medium for 48 h. Viral
titer was harvested from HEK293T cell culture medium and was spun at 1000 × g for 5 min to remove any cell debris. This was added to a single cell
culture and cells were plated evenly into a 10-cm dish and incubated for 12 h. Culture medium of infected cells was then replaced, and cells were maintained.

FRAP experiments. FRAP imaging was performed using a Zeiss LSM 700
Observer as described previously. Briefly, two images were taken before photo-
bleaching and 15–30 images were taken with 10 s intervals immediately after
tophotobleaching. The images were analyzed using ImageJ. Fluorescence intensities
were normalized to the signal before photobleaching to obtain the fluorescence recovery.

Protein expression and purification. The construct of human full-length p300
protein-containing BD–RING–PHD–HAT–Z Z region (BRPhZ, amino acids 1035–1720) was cloned in the pGEX-6P-1 vector and expressed in BL21 (RIL) cells
and was mixed. Solution of virus and HeLa cells was plated evenly into a 10-cm
dish and incubated for 12 h. Culture medium of infected cells was then replaced, and cells were maintained.

Liquid chromatography and tandem mass spectrometry (LC
– MS/MS) analysis. For estimations of intact protein masses, untreated and SIRT2-treated WT and
mutant proteins were further purified by size-exclusion chromatography and concentrated in Millipore concentrators.

Trypsin digestion of acetylated p300 proteins. Purified p300WT and mutant proteins with SIRT2 treatment overnight in the cold room in a buffer containing 20–50 mM Tris (pH 7.5), 300 mM NaCl, 5 mM MgCl2, 2 mM NAD, and 2 mM DTT. Both WT and mutant p300 proteins were further purified by size-exclusion
chromatography and concentrated in Millipore concentrators.

Acetyltransferase assays. Purified untreated and SIRT2-treated p300WT and mutant proteins were buffer exchanged into reaction buffer containing 10 mM Tris–HCl (pH 7.5) and 50 mM NaCl for auto-acetylation experiments in Fig. 3a–c, 0.5 µM p300 was incubated in HAT reaction buffer (10 mM Tris pH 7.5 and 60 mM NaCl) in the absence or presence of 12% (v/v) PEG 3350. For histone acetyl-
transferase assays in Fig. 5, 6.4 µM p300 protein, and 6.4 µM reconstituted NCP were incubated in HAT reaction buffer (10 mM Tris pH 7.5 and 60 mM NaCl). Reactions were started by adding 0.5 mM acetyl-CoA at room temperature and
quenched by flash-freeze at indicated time points. For each time point, 8 µL reaction mixture was diluted to 200 µL assay buffer and immediately heated to 95 °C for 5 min to inactivate p300. The samples were then applied to an ultrafiltration device (10k cut-off) to collect flow-through for CoA quantification by the fluorometric assay kit (Ambac, 138889). The kit utilizes a fluorogenic green indicator that became strongly fluorescent upon reacting with the –SH group in CoA. The fluorescence signals in sample were measured using a fluorogenic reader according to the product manual using a 96-well microplate reader. For measuring the HAT activity of p300 (Fig. 6g–i) in the diluted solution, droplet mixture, and supernatant, reactions were quenched by mixing with a denaturing buffer conten-
taining 10 mM Tris–HCl (pH 7.5) 50 mM NaCl and 6 M guanidine–HCl at indicated points. All experiments were performed in triplicates.

To detect histone H3 acetylation at specific lysine sites, reactions were quenched by flash-freeze in liquid nitrogen and then analyzed by SDS–PAGE and western blot analysis. Western blot results were quantified by LI-COR Odyssey System using the following antibodies: anti-H3K4ac (ab176799, 1:1000), anti-H3K9ac (ab4492, 1:1000), and anti-H3K27ac (ab17777, 1:1000) from Abcam, and anti-
H3K18ac (39755, 1:1000) from Active Motif.

SAXS analysis. X-ray scattering data were collected at the Bio-SAXS beamline (BM29) of the European Synchrotron Radiation Facility. Data were collected with a
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**Competing interests**

The authors declare no competing interests.

**Additional information**

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