The role of O-GlcNAcylation in RNA polymerase II transcription

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Eukaryotic RNA polymerase II (RNAPII) is responsible for the transcription of the protein-coding genes in the cell. Enormous progress has been made in discovering the protein activities that are required for transcription to occur, but the effects of post-translational modifications (PTMs) on RNAPII transcriptional regulation are much less understood. Most of our understanding relates to the cyclin-dependent kinases (CDKs), which appear to act relatively early in transcription. However, it is becoming apparent that other PTMs play a crucial role in the transcriptional cycle, and it is doubtful that any sort of complete understanding of this regulation is attainable without understanding the spectra of PTMs that occur on the transcriptional machinery. Among these is O-GlcNAcylation. Recent experiments have shown that the O-GlcNAc PTM likely has a prominent role in transcription. This review will cover the role of the O-GlcNAcylation in RNAPII transcription during initiation, pausing, and elongation, which will hopefully be of interest to both O-GlcNAc and RNAPII transcription researchers.

RNA polymerase II pausing

In the current model of RNAPII-dependent transcription, RNAPII is recruited to the promoter by other members of the preinitiation complex (PIC: generally defined as TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, RNAPII, Mediator (1), and presumably a DNA-binding transcriptional activator as well). TFIIH helicase activity and ribonucleotides begin initiation by melting the DNA in the transcriptional start site region (TSS) (1, 2) giving RNAPII access to the single-stranded DNA to begin RNA synthesis.

It is now thought that on most genes, instead of RNAPII residing in a PIC, initiation occurs and RNAPII enters a transcriptionally engaged, paused state approximately 50 nucleotides downstream of the TSS through interactions with DRB Sensitivity-Inducing Factor (DSIF) and Negative Elongation Factor (NELF), which misalign the RNAPII active site to prevent elongation (3–6). Paused RNAPII populations are apparent in chromatin immunoprecipitation-sequencing (ChIP-seq) assays directed against RNAPII and with various nascent RNA-seq approaches (7). These experiments show that 20 to 70% of active genes have a paused RNAPII (3). Release of RNAPII into productive elongation occurs via the phosphorylation of DSIF and NELF (by CDK9), releasing NELF while DSIF remains bound to RNAPII as a positive elongation factor (8) (Fig. 1). PARP-1 also stimulates pause release *via* NELF ADP-ribosylation (9). MYC (10), TRIM28 (11–13), PAF (14, 15), FACT (16, 17), CDK7 (18), SIRT6 (19), U2 snRNP (20), ARID1A (21), and Integrator (22–27) all have roles in pausing and/or pause release.

The function of a paused RNAPII is not clear. Some have suggested that it is a rate-limiting step in transcription, while others suggest that a paused RNAPII is necessary for the rapid induction of transcription. The former lacks evidence of being an actual biochemically defined rate-limiting step. Others have concluded that rapid induction of paused RNAPII is not experimentally supported either (28). RNAPII pausing is a regulated step, requiring P-TEFb activity to stimulate productive elongation. The entire process through the promoter is a dynamic one of continuous initiation, pausing, and termination of those paused polymerases (29–32), and regulation may target all of these steps, not just pause release.

Since the paused RNAPII is prevalent in so many genes, one might suggest that it represents the default ground state of a promoter. Why? Firstly, most promoters exist in this paused state. Secondly, because NTPs are freely diffusing, one might expect that as soon as a PIC forms, transcription is initiated and the RNAPII enters the paused, transcriptionally engaged state. It is not the PIC itself, but the paused RNAPII population that responds to tissue-specific and induced transcriptional activators, such as NF-κB, HIF-1α, and HSF. Additionally, Levens and colleagues suggested that pausing controls the level of stochastic noise (variance) in transcription by using multiple, biochemically distinct kinetic steps (33).

Cyclin-dependent kinases and RNA polymerase II transcription

In terms of post-translational modifications involved in transcription, research has largely focused on the role of various kinases and how they regulate initiation and elongation. These are the cyclin-dependent kinases: CDK7, CDK8, CDK9, and CDK12/13, all of which phosphorylate serine and threonine residues (34). CDK7 is part of the TFIIH complex and phosphorylates the C-terminal domain (CTD) of RNAPII RPB1 subunit. CDK7 activity is required for proper pausing (35). CDK9 phosphorylates the CTD, along with DSIF and

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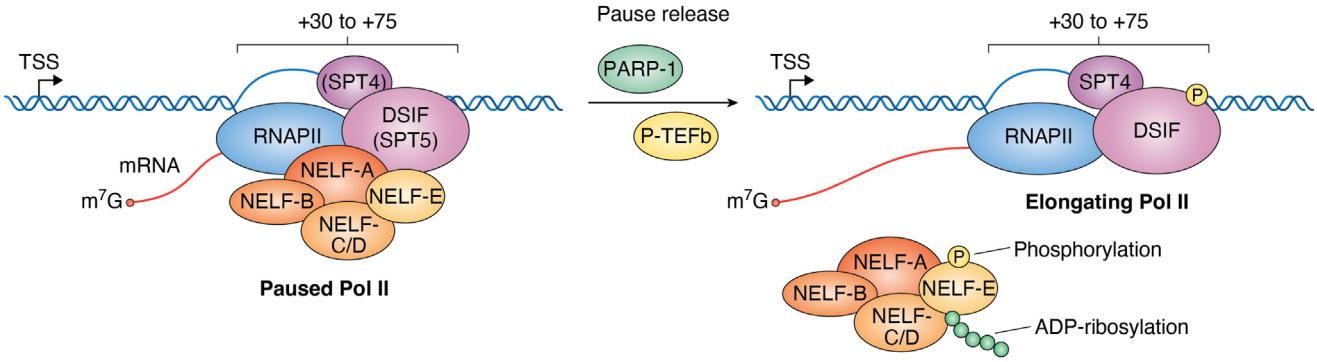

Figure 1. The current model of RNAPII pausing and pause release (3–5). Transcription from the PIC is initiated in the immediate vicinity of the TSS (not shown). The elongating RNAPII then pauses in the region between +30 and +75 nucleotides downstream of the TSS *via* interactions with the DSIF (containing SPT4 and 5) and NELF complexes (subunits A-E). Release of RNAPII into productive elongation is regulated by the P-TEFb complex (containing the CDK9/cyclin T pair), which phosphorylates both DSIF and NELF. NELF is released while the phospho-DSIF operates as a positive elongation factor. P-TEFb is thought to be recruited by various DNA binding transcriptional activators. More recently, PARP-1 was shown to play a role in RNAPII release by the ADP-ribosylation of NELF-E.

NELF mentioned above. CDK9 has more than 50 substrates, and it is likely that the other CDKs do as well (36). CDK8 is a module of the activator-dependent Mediator coactivator complex (37, 38). CDK12/13 seems to have positive roles in elongation and has some activity on a CTD substrate (39, 40).

Human RNAPII CTD consists of 52 repeats of the loose consensus heptad $Y_1S_2P_3T_4S_5P_6S_7$. The 52 repeats are specific to humans: the fruit fly (*D. melanogaster*) has 41, and budding yeast (*S. cerevisiae*) has 26 (41–44). Human RNAPII CTD is a remarkably complex intrinsically disordered domain. The first 26 repeats contain 21 repeats of the heptad $Y_1S_2P_3T_4S_5P_6S_7$ (*S. cerevisiae* has 19) and 5 less-mentioned repeats with asparagine (N) residues at position 7. Heptads 27 to 52 further deviate: eight lysine (K), six threonine (T), and one each of arginine (R), glutamic acid (E), valine (V), and glycine (G) (45). The 7-heptad repeat is reminiscent of an α-helix and K, R, and T residues are potentially substrates for methyltransferase (K, R), or phosphorylation and O-GlcNAcylation (see below) (S, T). These modifications may then greatly affect the conformational space occupied by the CTD.

The function of the CTD in transcription is under some debate. CTD deletions in budding yeast and humans are lethal (46, 47) while severe deletions in humans prevent PIC formation and initiation (48, 49). More recent experiments indicate that the CTD is not required for post-initiation events such as pausing (50) although there is disagreement here (51). In various cell-free systems, the CTD is not required for transcription (52–54).

CTD phosphorylation occurs immediately after initiation, predominantly on serine five but more recent data suggests serine two phosphorylation too (41, 55). Serine five phosphorylation is located at the 5′ of a gene while serine two phosphorylation increases towards the 3′ end of the gene (56). These marks are often used to evaluate RNAPII elongation, but it is not clear whether these phosphorylation events have any role in pause release and elongation. Threonine four phosphorylation is perhaps the most interesting of the phosphorylated CTD residues, seeming to have gene- and signal-dependent effects on elongation efficacy (57). Post-transcriptionally, the CTD recruits capping enzymes and splicing factors (58, 59) and it is likely that the CTD is playing a functional role in these events (60–62). Thus, the CTD may be a scaffold for recruiting the splicing machinery as part of the coupling between elongation and splicing.

O-GlcNAcylation enzymes

O-GlcNAcylation is a common PTM, but its role in transcription has largely been overlooked. O-GlcNAc is essentially a modified glucose molecule with an acetylated amine added to the C2 carbon of glucose. Like the CDKs, the O-GlcNAc transferase (OGT) modifies serine and threonine residues via hydrolysis of the nucleotide sugar substrate UDP-GlcNAc. O-GlcNAcylation occurs via OH groups on serine or threonine residues to form an O-glycosidic linkage with N-acetylglucosamine. Mass spectrometry has identified approximately 4000 O-GlcNAcylated proteins, many of which are involved in gene regulation (63–66). There are interesting overlaps between the targets of OGT, CDK9, and PARP-1 (also required for pause release/elongation (9)) as well as the interactomes of OGT and OGA, suggesting that there is considerable crosstalk between these enzymes (see Table 1).

OGT consists of a globular domain containing the active site and a 13.5 helical tetratricopeptide repeat (TPR) domain. The TPR helical structure participates in substrate recognition via interactions with the helical channels (67–69). OGT also acts as a protease to cleave the Oct-1 coactivator Host Cell Factor 1 (HCF-1) into its mature form (70). More recently, noncatalytic functions of OGT were described (71).

The O-GlcNAc aminidase (OGA) removes GlcNAc from proteins. Its substrate specificity is under debate, and overall, the regulation of OGT and OGA activity is not well understood [see (72, 73) for an in-depth discussion of these enzymes].

The hexosamine biosynthetic pathway (HBP) diverts 2 to 5% of glucose to make UDP-GlcNAc. First, fructose-6-phosphate is converted to glucosamine by glutamine:fructose-amidotransferase (GFAT) and glutamine. The glucosamine is

Table 1
Shown in the left column are components of the RNAPII transcriptional machinery that are O-GlcNAcylated

O-GlcNAcylated Pausing & elongation factors	CDK9 substrates	PARP substrates	OGT interactome	OGA Interactome
NELFA/CD/E		Yes: NELF-E		Yes: NELF-B
TAT-SF1	Yes			Yes
SPT5	Yes		Yes	
PARP-1			Yes	Yes
SPT6			Yes	
FACT		Yes	Yes	Yes
Top1		Yes	Yes	
Top2B		Yes	Yes	
TRIM28	Yes	Yes	Yes	Yes
CDK9		Yes	Yes	Yes
BRD4	Yes		Yes	
CDC73		Yes	Yes	Yes
RTF1		Yes		
PAF1		Yes	Yes	
Leo1		Yes		
RPAP2				
HCF-1			Yes	
Capping				
RMNT		Yes		
DCP1B				
Termination				
XRN2	Yes		Yes	
INTS3/6			Yes	
PP2A p65			Yes	Yes
PP1				

The list is based on mass spectrometry data in (62), CDK9 substrates based on (35), OGT interactome based on (110), PARP substrates based on O-GlcNAcylated members of the PIC include TFIIE, RPB1, RPB2, TFIIA, p62/TFIIH, RBP7, TFIIB, TFIIF, MED1/4/9/30/15, TAF4/6/10/15, FCP1, CDK7, cyclin H, cyclin K. OGA interactome is from (88).

acetylated to become N-acetylglucosamine by GlcNAc-6-phosphate acetyltransferase and acetyl CoA, and then the GlcNAc-1-phosphate is converted to UDP-GlcNAc by UDP-GlcNAc pyrophosphorylase and UTP (74). The requirements for three major metabolites suggest that the intracellular [UDP-GlcNAc] is an indicator of the nutrient state of the cell (75). Presumably, then there are functional consequences of changing UDP-GlcNAc levels.

RNA polymerase II CTD O-GlcNAcylation

Since the 1990s, the formation of PICs has been predicated on the idea that the hypo-phosphorylated species of RNAPII ($RNAPII_A$) is incorporated into the PIC (76). The hyper-phosphorylated RNAPII ($RNAPII_O$) is widely considered to be the elongation-specific species. However, $RNAPII_A$ was shown to be O-GlcNAcylated as well and thus the ambiguity arises as to what form of RNAPII is required for PIC formation (77). Presumably, there are unknown factors establishing this CTD specificity in nuclear extracts, as this distinction is not made using highly purified reconstituted systems (54, 78–80). In any case, there is some justification for re-considering the identity of the PIC-specific RNAPII species. Is it unphosphorylated, hypo-phosphorylated, or O-GlcNAcylated?

OGT modifies the RNAPII CTD and RNAPII is O-GlcNAcylated across species (77, 81, 82). Recombinant OGT O-GlcNAcylated the CTD, and OGA removed the O-GlcNAc modification *in vitro* (82). Mass spectrometry showed that both serine two and five residues of the human CTD were O-GlcNAcylated (83). These were found on CTD repeats 34 and 44. However, Lu and colleagues found that CTD O-GlcNAcylation was much more extensive (84). Others have suggested that both O-GlcNAcylation and phosphorylation can affect secondary structure (85). Since no O-GlcNAc binding domains/proteins are known it appears more likely that the O-GlcNAcylation alters a protein's conformational space or regulates kinase access to relevant S and T residues/substrates.

In extracts, CTD O-GlcNAcylation is blocked with the OGT inhibitor ST045849 (82, 86). There is some direct evidence that CTD phosphorylation and O-GlcNAcylation oppose each other, as phosphorylation of the CTD with purified TFIIH and its resident CDK7 will block O-GlcNAcylation by OGT (82), leading to various models that OGT and TFIIH and other PIC-associated kinases are together regulating early transcription events (82). Lastly, biochemical experiments indicated that O-GlcNAcylated RNAPII ($RNAPII_Y$) resides in the PIC and that, upon the addition of ATP, the O-GlcNAc modification is removed (83). These data, especially that in (83), show that O-GlcNAcylated RNAPII is a PIC-specific species of RNAPII.

OGT and OGA functions in RNA polymerase II transcription

Early experiments determined that OGT and OGA inhibitors (ST045849 and PUGNAc, respectively) (86, 87), added to an *in vitro* transcription assay containing HeLa cell nuclear extracts, blocked transcription in primer extension assays (82). OGT inhibition with ST045849 added before PIC formation completely abrogates transcription (88). Overall, these data indicate that OGT activity is necessary for PIC formation and early events in transcription, although OGT's substrates in this context remain to be determined.

OGA also functions in the transcription process, acting after OGT (89). The OGA inhibitors PUGNAc and Thiamet G blocked elongation but had little effect on transcription events that occurred before +75 (the region that harbors paused RNAPII) (89). Interestingly, the effects of OGA inhibition are

identical to CDK9 inhibition: a complete block in elongation, suggesting that there are co-dependent regulatory steps in pause release (89). The addition of recombinant OGA affected elongation but the effect was the opposite of what occurred with PUGNAc (44). This is not necessarily a discrepancy, as the PUGNAc experiment was done using crude nuclear extracts, while the addbacks of recombinant OGA or a natively purified OGA complex were assessed using immobilized promoter templates that were isolated after the pulse-labeling step, washed in high salt, OGA was added back, and transcription was resumed with NTPs. Here, OGA appears to be acting after approximately +200 in the elongation process (89). Thus, there is an O-GlcNAcylation requirement during elongation at approximately +200 in the cell-free assays, as both rOGA (89) and the OGT inhibitor ST045849 arrest RNAPII at that point (88). OGA was further linked to elongation functions as it was purified as part of a large, 700 kDa complex, containing several pausing/elongation factors including SPT5 and TRIM28 (89).

Finally, a note describing the differences in the transcription assays used. The primer extension assay used in (82) is less informative in that it simply detects a predetermined length of transcribed RNA but gives no information on the entire population of transcribed RNAs. Since the primer extension assay does not detect shorter RNAs, it is of little use given that most active promoters harbor a paused RNAPII. The pulse-chase transcription assay used in (89, 90) was a powerful improvement over the primer extension assay. In a pulse-chase assay, PICs are formed as usual, simply by mixing the nuclear extract with promoter DNA and the necessary buffers. Transcription is initiated with a short pulse of a small amount (~1 μM) of ^{32}P-CTP and non-radioactive rGUA (0.5 mM) for 30 s. Then an excess of non-radioactive CTP (3 mM) is added as the chase step (90, 91) (Fig. 2*A*). The short pulse allows one to follow those RNAs that initiated during that time and the chase shows the behavior of those labeled RNAs without having to consider later initiation events that might occur (because the ^{32}P-CTP is diluted with nonradioactive CTP). The researcher can then discriminate between initiation, pausing, termination, and productive elongation events, all physiologically relevant transcription events. In general, cell-free transcription systems are very useful, as the indirect effects in experiments *in vivo* (perturbations of splicing or translation, for example) are not part of the transcription assay. Thus, various secondary effects are excluded.

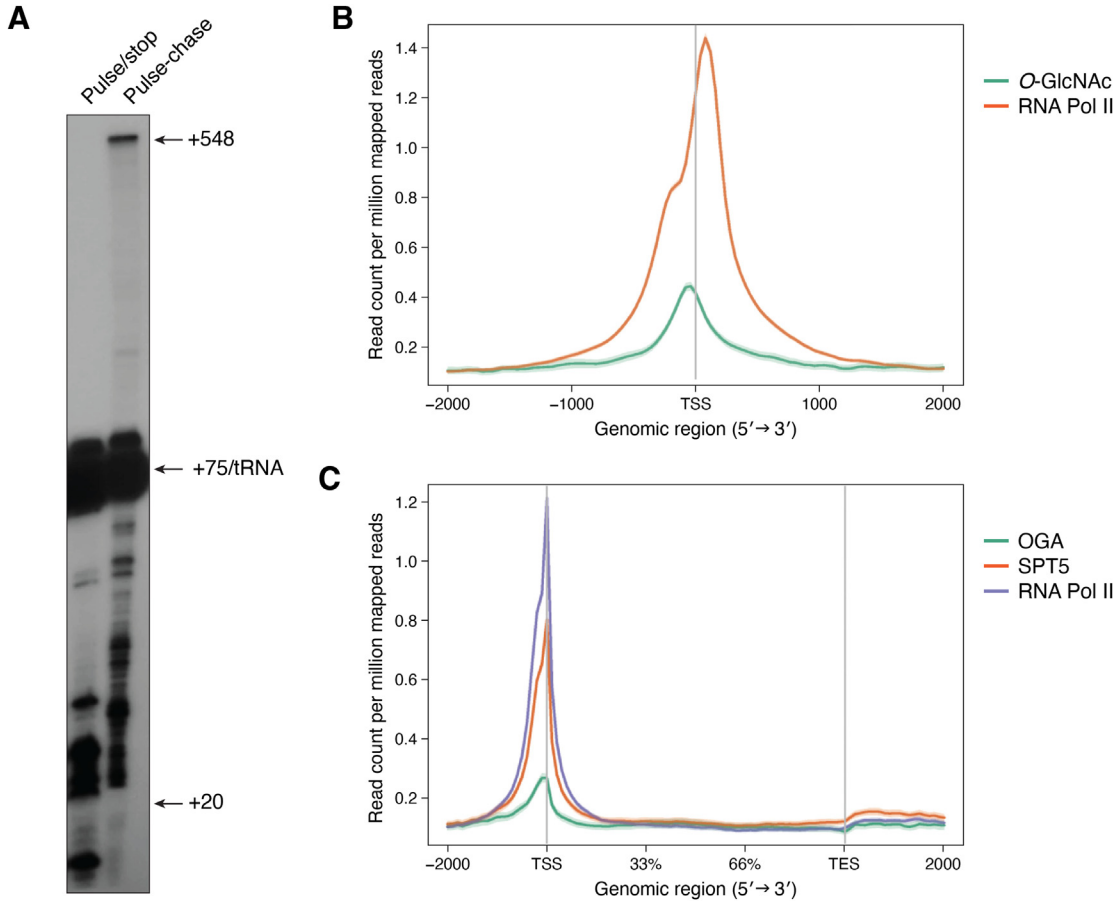

Figure 2. Example pulse-chase assay and O-GlcNAc ChIP-seq data. *A*, the representative pulse-chase assay. The *left lane* shows a 30 s pulse using ^{32}P-CTP and rGUA. The *right lane* shows the chase step, where excess unlabeled CTP was added for 5 min. One can clearly see the increasing RNA lengths originating from the pulsed RNAs. *B* and *C*, ChIP-seq experiments show a large concentration of O-GlcNAc colocalizing with paused RNA pol II (*B*) in the proximal promoter region and colocalization of OGA and the SPT5 elongation factor with paused RNA pol II (*C*). *A*, was reproduced from (88) and (*B* and *C*) from (89) under CC-BY licenses.

Distribution of O-GlcNAc, OGT, and OGA on promoters in vivo

Numerous studies have shown that the O-GlcNAcylation machinery localized to the TSS *in vivo* and significantly overlapped the paused RNAPII population. This is true for O-GlcNAc itself, OGT, and OGA in humans, mice, *Drosophila*, and *Caenorhabditis elegans* (81, 89, 92–94). The location of the O-GlcNAcylation machinery is no different than for known pausing and elongation factors such as DSIF, NELF, PAF, Integrator, SPT5, and P-TEFb. Thus, the colocalization of OGT, OGA, and O-GlcNAc along with paused RNAPII at the 5′ end of genes suggests O-GlcNAcylation requirements at that time (Fig. 2*B*).

Effects of genetic ablation of OGT and OGA on RNA polymerase II transcription

OGT and OGA are found in higher metazoans, such as *Drosophila*, *C. elegans*, mice, and humans. In mice, the OGT gene resides on the X-chromosome, and an OGT knockout was embryonic lethal (95). OGA deletions show perinatal lethality (96). Thus, both OGT and OGA play essential roles in metazoan biology.

In *C. elegans*, approximately 40% of genes had both O-GlcNAc and RNAPII peaks at the promoters. An OGT catalytic mutant showed a complete absence of O-GlcNAc promoter peaks, while an OGA catalytic mutant did not show any changes (92). The authors also noted a lack of complete concordance of the O-GlcNAc peak (∼100 bp upstream of the TSS) and RNAPII (centered on the TSS). However, RNAPII density at the TSS increased with the OGA mutant. Approximately equal numbers of genes were up- and down-regulated in both mutants (at the RNA level). Interestingly, the OGA mutant showed increased levels of O-GlcNAc at promoters, indicating that OGT and OGA were working in concert to stabilize O-GlcNAc levels (81). However, under either starved or fed conditions, O-GlcNAc promoter levels did not change, suggesting that promoter O-GlcNAcylation was buffered. Furthermore, the OGT mutant showed an increase in the phosphoserine two RNAPII CTD levels, which is associated with elongation, but here the increase was near the TSS. This is consistent with the idea that CTD O-GlcNAcylation hinders phosphorylation of those same residues (82). As such these might be aberrant phosphorylation events. In a human B-cell line, shRNA targeted ablation of OGT showed a 20 to 60% reduction in expression of several B-cell-specific genes, along with reductions in RNAPII, O-GlcNAc, and phosphoserine five CTD (82, 83). This is the same result seen with the OGT inhibitor *in vitro*: loss of RNAPII in the PIC (83).

Possible role of O-GlcNAc in phase transitions and biomolecular condensates

Biochemistry typically uses relatively dilute solutions of proteins, nucleic acids, and other relevant molecules. Furthermore, the classic approaches of transcriptional biochemistry and the fractionation of nuclear extracts to identify the relevant transcriptional machinery are implicitly focused on higher-affinity interactions and identifying a set of factors that transcribe efficiently and synthesize high levels of RNA.

In contrast, *in vivo* the cytoplasm and nucleus are very concentrated, dense environments of macromolecules (∼200 mg/ml). These crowded environments display anomalous diffusion and kinetics, and weak protein-protein interactions become quite significant (97). Weakly interacting proteins likely have important roles to play *in vivo*. It is these weaker interactions that are thought to promote phase transitions of proteins, DNA, and RNA, and contribute to the dense nuclear environment.

In vitro, the resulting phase condensates exist as a concentrated, dense phase, along with a second dilute phase (98–100). *In vivo*, condensates are thought to establish functionally demarcated areas, such as paraspeckles, nucleoli, and Cajal bodies (101). However, it has been difficult to positively identify transcriptionally relevant condensates *in vivo* in other cases, while *in vitro*, although high concentrations of recombinant proteins can be forced to undergo a phase transition, their functional relevance is not clear. A physiologically relevant biochemical system would be very useful in bridging this gap.

A recent report found quite unexpectedly that HeLa cell nuclear extracts underwent a phase transition when mixed with promoter DNA, forming transcriptionally active biomolecular condensates (88). These condensates were very dense, approximately 100 mg/ml, and contained many factors necessary for transcription. Furthermore, the addition of the OGT inhibitor ST045849 to the nuclear extract before promoter DNA blocked condensate formation and transcription. These experiments and others showed that proper transcription in the nuclear extract was physically connected to the condensate: disruption of the condensate meant the disruption of transcription (88). Unexpectedly, these condensates contained proteins such as FUS, NONO, and SFPQ as well as OGT, which are not normally considered functionally relevant in the classic description of a PIC but which are required for membrane-less organelle formation *in vivo* (102). These data suggest that the definition of a "PIC" needs revision to one that is more physiologically relevant. Lastly, these data raise the interesting possibility that O-GlcNAcylation contributes to a phase transition/condensate formation (103), perhaps by blocking the ionic effects of phosphorylation, creating a less hydrophilic local environment between proteins, or inducing allosteric conformational changes.

In vitro *and* In vivo *caveats*

The difficulty in understanding O-GlcNAc function is that many proteins are phosphorylated on the same serine/threonine residues that are O-GlcNAcylated such that phosphorylation and O-GlcNAcylation of a residue are mutually exclusive. Thus, mutation of a serine/threonine residue eliminates the possibility of both modifications and makes the discerning function of either modification nearly impossible. Biochemical systems can get around this somewhat by focusing on specific functions and using catalytic inhibitors to assess blocks in these modifications. The drawback of these systems is that inhibitors block the modification of all targets.

Second, in general, it is difficult to interpret *in vivo* ablation experiments since the window of depletion is many hours. In other words, as with most *in vivo* perturbations, one observes the sum of direct and indirect effects on any cellular or organismal system. Such *in vivo* perturbations generate a phenotype. As such, a targeted ablation or inhibition cannot be causally assigned directly to a particular outcome. Even the more recent degron-based ablations do not eliminate indirect effects, especially those of catalytic activities or those that affect catalytic activities.

Thirdly, OGT and OGA protein levels are co-regulated *in vivo*: a decrease in one causes a decrease in the other (104–106) so it is impossible to comfortably interpret any sort of ablation or inhibitor experiments *in vivo*. Note that this is not an issue in the cell-free transcription system.

Lastly, catalytic inhibition is not the same as targeted ablation. A small molecule catalytic inhibitor is not expected to disrupt protein-protein interactions or disassemble a large (OGT-containing) complex. An ablation (by shRNA for example) likely perturbs any protein-protein interactions involving the target as well as any catalytic and non-catalytic functions. An ablation is far more deleterious than a catalytic inhibitor.

Conclusions

The data thus far point to a role for O-GlcNAcylation in the regulation of RNAPII-dependent transcription, specifically during PIC formation and elongation. O-GlcNAcylated RNAPII is the PIC-specific species. OGT inhibition with ST045849 blocked PIC formation and formation of transcriptionally active (pre-initiation) condensates, while OGA inhibition with PUGNAc and Thiamet G blocks elongation *in vitro*. shRNA ablation of OGT reduced RNAPII and O-GlcNAc levels at promoters *in vivo*. The colocalization *in vivo* of O-GlcNAc, OGT, and OGA at promoters with a paused RNAPII also suggests that these enzymes play a role in early transcription events. Consistent with this idea is that many of the pausing and elongation factors are O-GlcNAcylated (Table 1). These data suggest that OGT and OGA are dynamically regulating initiation, pausing, and elongation, perhaps acting as an ultrasensitive switch (107–109) (Fig. 3). Lastly, OGT hydrolysis of UDP-GlcNAc releases 8.8 kcal/mol of energy (110), which may be used to drive other reactions in the PIC. A model of the function of OGT and OGA is discussed in Figure 3.

The number of O-GlcNAcylated substrates likely will not permit any simple answers to the role of OGT and OGA in RNAPII transcription. Serine/threonine residues are often phosphorylated or O-GlcNAcylated, compounding the problem. However, the discovery of an O-GlcNAc-dependent *in vitro* transcription system offers unique inroads into examining OGT and OGA functions and other PTMs in transcription. It is likely that other approaches to the problems imposed by OGT and OGA will be needed to achieve a more complete understanding of O-GlcNAcylation in transcriptional regulation.

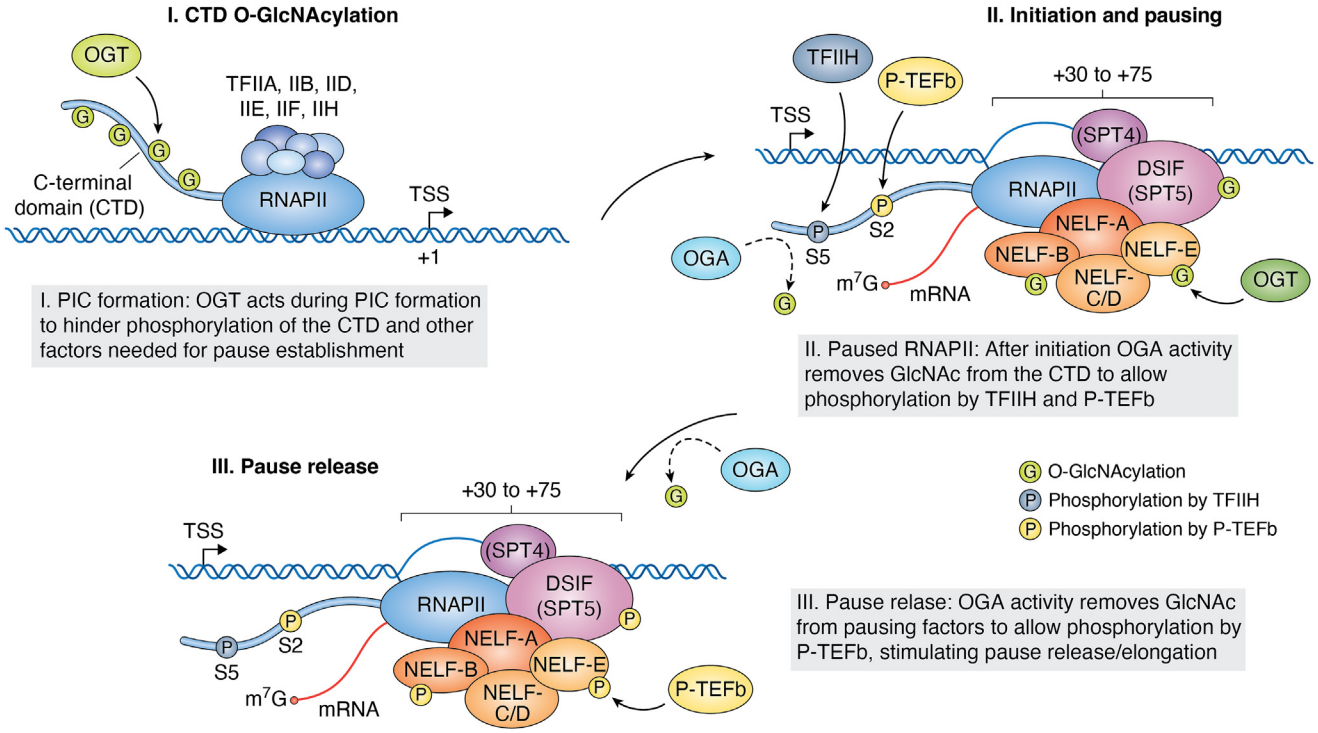

Figure 3. A hypothetical model of OGT and OGA function in transcription initiation and pausing. I. OGT acts during PIC formation to O-GlcNAcylate RNAPII CTD and other factors to prevent their phosphorylation. (Note that the PIC components shown here are based on highly purified reconstituted systems and likely do not represent the *in vivo* or nuclear extract complexity of a promoter.) II. OGA acts on the O-GlcNAcylated CTD to permit its phosphorylation early in the initiation process. III. The pausing machinery (such as DSIF and NELF) is likely O-GlcNAcylated. OGA removes the O-GlcNAc for phosphorylation by P-TEFb (CDK9) to promote RNAPII pause release and elongation.

Data availability

All data are contained within this paper.

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Abbreviations—The abbreviations used are: ARID1A, AT-rich interaction domain 1A; ATP, adenosine triphosphate; CDKs, cyclin-dependent kinases; ChIP-seq, chromatin immunoprecipitation-sequencing; CTD, C-terminal domain; CTP, cytosine triphosphate; DRB, 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole; DSIF, DRB sensitivity inducing factor; FACT, facilitator of chromatin transcription; FUS, fused in sarcoma; HIF-1α, hypoxia induced factor-1; HSF, heat shock factor; kDa, kilodaltons; MYC, myelocytosis gene; NELF, negative elongation factor; NF-κB, nuclear factor-kappa B; NONO, non-POU domain containing octamer binding; NTPs, nucleotide triphosphates; OGA, O-GlcNAc aminidase; O-GlcNAc, N-acetylglucosamine; OGT, O-GlcNAc transferase; PIC, preinitiation complex; PTM, post-translational modification; PAF, RNA polymerase II associated factor; PARP-1, poly-ADP ribose polymerase; P-TEFb, positive transcription elongation factor b; PUGNAc, O-(2-Acetamido-2-deoxy-D-glucopyranosylidenamino) N-phenylcarbamate; RNAPII, RNA polymerase II; SFPQ, splicing factor proline and glutamine rich; shRNA, short hairpin RNA; SIRT6, sirtuin 6; snRNP, small nuclear ribonucleoprotein; SPT5, suppressor of Ty 5; TRIM28, tripartite motif containing 28; TPR, tetratricopeptide repeat; TSS, transcriptional start site; UDP-GlcNAc, uridine diphosphate-N-acetylglucosamine.

References

1. Schier, A. C., and Taatjes, D. J. (2020) Structure and mechanism of the RNA polymerase II transcription machinery. *Genes Development* **34**, 465–488
2. Thomas, M. C., and Chiang, C.-M. (2006) The general transcription machinery and general cofactors. *Crit. Rev. Biochem. Mol. Biol.* **41**, 105–178
3. Adelman, K., and Lis, J. T. (2012) Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nat. Rev. Genet.* **13**, 720–731
4. Core, L., and Adelman, K. (2019) Promoter-proximal pausing of RNA polymerase II: a nexus of gene regulation. *Genes Dev.* **33**, 960–982
5. Guo, J., and Price, D. H. (2013) RNA polymerase II transcription elongation control. *Chem. Rev.* **113**, 8583–8603
6. Vos, S. M., Farnung, L., Urlaub, H., and Cramer, P. (2018) Structure of paused transcription complex Pol II–DSIF–NELF. *Nature* **560**, 601–606
7. Wissink, E. M., Vihervaara, A., Tippens, N. D., and Lis, J. T. (2019) Nascent RNA analyses: tracking transcription and its regulation. *Nat. Rev. Genet.* **20**, 705–723
8. Dollinger, R., and Gilmour, D. S. (2021) Regulation of promoter proximal pausing of RNA polymerase II in metazoans. *J. Mol. Biol.* **433**, 166897
9. Gibson, B. A., Zhang, Y., Jiang, H., Hussey, K. M., Shrimp, J. H., Lin, H., *et al.* (2016) Chemical genetic discovery of PARP targets reveals a role for PARP-1 in transcription elongation. *Science* **353**, 45–50
10. Rahl, P. B., Lin, C. Y., Seila, A. C., Flynn, R. A., McCuine, S., Burge, C. B., *et al.* (2010) c-Myc regulates transcriptional pause release. *Cell* **141**, 432–445
11. Yang, Y., Lu, H., Chen, C., Lyu, Y., Cole, R. N., and Semenza, G. L. (2022) HIF-1 Interacts with TRIM28 and DNA-PK to release paused RNA polymerase II and activate target gene transcription in response to hypoxia. *Nat. Commun.* **13**, 316
12. Bunch, H., Zheng, X., Burkholder, A., Dillon, S. T., Motola, S., Birrane, G., *et al.* (2014) TRIM28 regulates RNA polymerase II promoter-proximal pausing and pause release. *Nat. Struct. Mol. Biol.* **21**, 876–883
13. McNamara, R. P., Reeder, J. E., McMillan, E. A., Bacon, C. W., McCann, J. L., and D'Orso, I. (2015) KAP1 recruitment of the 7SK snRNP complex to promoters enables transcription elongation by RNA polymerase II. *Mol. Cell* **61**, 39–53
14. Chen, F. X., Woodfin, A. R., Gardini, A., Rickels, R. A., Marshall, S. A., Smith, E. R., *et al.* (2015) PAF1, a molecular regulator of promoter-proximal pausing by RNA polymerase II. *Cell* **162**, 1003–1015
15. Yu, M., Yang, W., Ni, T., Tang, Z., Nakadai, T., Zhu, J., *et al.* (2015) RNA polymerase II-associated factor 1 regulates the release and phosphorylation of paused RNA polymerase II. *Science* **350**, 1383–1386
16. Tettey, T. T., Gao, X., Shao, W., Li, H., Story, B. A., Chitsazan, A. D., *et al.* (2019) A role for FACT in RNA polymerase II promoter-proximal pausing. *Cell Rep.* **27**, 3770–3779.e7
17. Wada, T., Orphanides, G., Hasegawa, J., Kim, D. K., Shima, D., Yamaguchi, Y., *et al.* (2000) FACT relieves DSIF/NELF-mediated inhibition of transcriptional elongation and reveals functional differences between P-TEFb and TFIIH. *Mol. Cell* **5**, 1067–1072
18. Glover-Cutter, K., Larochelle, S. S., Erickson, B., Zhang, C., Shokat, K., Fisher, R. P., *et al.* (2009) TFIIH-Associated Cdk7 kinase functions in phosphorylation of C-terminal domain Ser7 residues, promoter-proximal pausing, and termination by RNA polymerase II. *Mol. Cell Biol.* **29**, 5455–5464
19. Etchegaray, J. P., Zhong, L., Li, C., Henriques, T., Ablondi, E., Nakadai, T., *et al.* (2019) The histone deacetylase SIRT6 restrains transcription elongation via promoter-proximal pausing. *Mol. Cell* **75**, 683–699
20. Caizzi, L., Monteiro-Martins, S., Schwalb, B., Lysakovskaia, K., Schmitzova, J., Sawicka, A., *et al.* (2021) Efficient RNA polymerase II pause release requires U2 snRNP function. *Mol. Cell* **81**, 1920–1934
21. Trizzino, M., Barbieri, E., Petracovici, A., Wu, S., Welsh, S. A., Owens, T. A., *et al.* (2018) The tumor suppressor ARID1A controls global transcription via pausing of RNA polymerase II. *Cell Rep.* **23**, 3933–3945
22. Wagner, E. J., Tong, L., and Adelman, K. (2023) Integrator is a global promoter-proximal termination complex. *Mol. Cell* **83**, 416–427
23. Huang, K. L., Jee, D., Stein, C. B., Elrod, N. D., Henriques, T., Mascibroda, L. G., *et al.* (2020) Integrator recruits protein phosphatase 2A to prevent pause release and facilitate transcription termination. *Mol. Cell* **80**, 345–358
24. Beckedorff, F., Blumenthal, E., Dasilva, L. F., Aoi, Y., Cingaram, P. R., Yue, J., *et al.* (2020) The human integrator complex facilitates transcriptional elongation by endonucleolytic cleavage of nascent transcripts. *Cell Rep.* **32**, 107917
25. Elrod, N. D., Henriques, T., Huang, K. L., Tatomer, D. C., Wilusz, J. E., Wagner, E. J., *et al.* (2019) The integrator complex attenuates promoter-proximal transcription at protein-coding genes. *Mol. Cell* **76**, 738–752
26. Gardini, A., Baillat, D., Cesaroni, M., Hu, D., Marinis, J. M., Wagner, E. J., *et al.* (2014) Integrator regulates transcriptional initiation and pause release following activation. *Mol. Cell* **56**, 128–139
27. Stadelmayer, B., Micas, G., Gamot, A., Martin, P., Malirat, N., Koval, S., *et al.* (2014) Integrator complex regulates NELF-mediated RNA polymerase II pause/release and processivity at coding genes. *Nat. Commun.* **5**, 5531
28. Gilchrist, D. A., Fromm, G., dos Santos, G., Pham, L. N., McDaniel, I. E., Burkholder, A., *et al.* (2012) Regulating the regulators: the pervasive effects of Pol II pausing on stimulus-responsive gene networks. *Genes Dev.* **26**, 933–944
29. Erickson, B., Sheridan, R. M., Cortazar, M., and Bentley, D. L. (2018) Dynamic turnover of paused Pol II complexes at human promoters. *Genes Dev.* **32**, 1215–1225
30. Krebs, A. R., Imanci, D., Hoerner, L., Gaidatzis, D., Burger, L., and Schübeler, D. (2017) Genome-wide single-molecule footprinting reveals high RNA polymerase II turnover at paused promoters. *Mol. Cell* **67**, 411–422.e4

31. Steurer, B., Janssens, R. C., Geverts, B., Geijer, M. E., Wienholz, F., Theil, A. F., *et al.* (2018) Live-cell analysis of endogenous GFP-RPB1 uncovers rapid turnover of initiating and promoter-paused RNA Polymerase II. *Proc. Natl. Acad. Sci. U. S. A.* **115**, E4368–E4376
32. Darzacq, X., Shav-Tal, Y., de Turris, V., Brody, Y., Shenoy, S. M., Phair, R. D., *et al.* (2007) *In vivo* dynamics of RNA polymerase II transcription. *Nat. Struct. Mol. Biol.* **14**, 796–806
33. Weber, A., Liu, J., Collins, I., and Levens, D. (2005) TFIIH operates through an expanded proximal promoter to fine-tune c-myc expression. *Mol. Cell Biol.* **25**, 147–161
34. Fisher, R. P. (2017) CDK regulation of transcription by RNAP II: not over 'til it's over? *Transcription* **8**, 81–90
35. Larochelle, S., Amat, R., Glover-Cutter, K., Sanso, M., Zhang, C., Allen, J. J., *et al.* (2012) Cyclin-dependent kinase control of the initiation-to-elongation switch of RNA polymerase II. *Nat. Struct. Mol. Biol.* **19**, 1108–1115
36. Sansó, M., Levin, R. S., Lipp, J. J., Wang, V. Y.-f. F., Greifenberg, A. K., Quezada, E. M., *et al.* (2016) P-TEFb regulation of transcription termination factor Xrn2 revealed by a chemical genetic screen for Cdk9 substrates. *Genes Development* **30**, 117–131
37. Poss, Z. C., Ebmeier, C. C., and Taatjes, D. J. (2013) The Mediator complex and transcription regulation. *Crit. Rev. Biochem. Mol. Biol.* **48**, 575–608
38. Allen, B. L., and Taatjes, D. J. (2015) The Mediator complex: a central integrator of transcription. *Nat. Rev. Mol. Cell Biol.* **16**, 155–166
39. Greenleaf, A. L. (2019) Human CDK12 and CDK13, multi-tasking CTD kinases for the new millenium. *Transcription* **10**, 91–110
40. Liang, K., Gao, X., Gilmore, J. M., Florens, L., Washburn, M. P., Smith, E., *et al.* (2015) Characterization of human CDK12 and CDK13 complexes in CTD phosphorylation, gene transcription and RNA processing. *Mol. Cell Biol.* **35**, 928–938
41. Eick, D., and Geyer, M. (2013) The RNA polymerase II carboxy-terminal domain (CTD) code. *Chem. Rev.* **113**, 8456–8490
42. Jasnovidova, O., and Stefl, R. (2013) The CTD code of RNA polymerase II: A structural view. *Wiley Inter. Rev. RNA* **4**, 1–16
43. Jeronimo, C.l. C., Bataille, A. R., and Robert, F.o. F. (2013) The writers, readers, and functions of the RNA polymerase II C-terminal domain code. *Chem. Rev.* **113**, 8491–8522
44. Harlen, K. M., and Churchman, L. S. (2017) The code and beyond: transcription regulation by the RNA polymerase II carboxy-terminal domain. *Nat. Rev. Mol. Cell Biol.* **18**, 263–273
45. Hsin, J. P., and Manley, J. L. (2012) The RNA polymerase II CTD coordinates transcription and RNA processing. *Genes Dev.* **26**, 2119–2137
46. Bartolomei, M. S., Halden, N. F., Cullen, C. R., and Corden, J. L. (1988) Genetic analysis of the repetitive carboxyl-terminal domain of the largest subunit of mouse RNA polymerase II. *Mol. Cell Biol.* **8**, 330–339
47. Nonet, M., Sweetser, D., and Young, R. A. (1987) Functional redundancy and structural polymorphism in the large subunit of RNA polymerase II. *Cell* **50**, 909–915
48. Chapman, R. D., Conrad, M., and Eick, D. (2005) Role of the mammalian RNA polymerase II C-terminal domain (CTD) nonconsensus repeats in CTD stability and cell proliferation. *Mol. Cell Biol.* **25**, 7665–7674
49. Meininghaus, M., Chapman, R. D., Horndasch, M., and Eick, D. (2000) Conditional expression of RNA polymerase II in mammalian cells. Deletion of the carboxyl-terminal domain of the large subunit affects early steps in transcription. *J. Biol. Chem.* **275**, 24375–24382
50. Gerber, A., and Roeder, R. G. (2020) The CTD is not essential for the post-initiation control of RNA polymerase II activity. *J. Mol. Biol.* **432**, 5489–5498
51. Lux, C., Albiez, H., Chapman, R. D., Heidinger, M., Meininghaus, M., Brack-Werner, R., *et al.* (2005) Transition from initiation to promoter proximal pausing requires the CTD of RNA polymerase II. *Nucleic Acids Res.* **33**, 5139–5144
52. Zehring, W. A., and Greenleaf, A. L. (1990) The carboxyl-terminal repeat domain of RNA polymerase II is not required for transcription factor Sp1 to function *in vitro*. *J. Biol. Chem.* **265**, 8351–8353
53. Zehring, W. A., Lee, J. M., Weeks, J. R., Jokerst, R. S., and Greenleaf, A. L. (1988) The C-terminal repeat domain of RNA polymerase II largest subunit is essential *in vivo* but is not required for accurate transcription initiation *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* **85**, 3698–3702
54. Serizawa, H., Conaway, J. W., and Conaway, R. C. (1993) Phosphorylation of C-terminal domain of RNA polymerase II is not required in basal transcription. *Nature* **363**, 371–374
55. Vihervaara, A., Versluis, P., Himanen, S. V., and Lis, J. T. (2023) PRO-IP-seq tracks molecular modifications of engaged Pol II complexes at nucleotide resolution. *Nat. Commun.* **14**, 7039
56. Jeronimo, C., Collin, P., and Robert, F. (2016) The RNA polymerase II CTD: the increasing complexity of a low-complexity protein domain. *J. Mol. Biol.* **428**, 2607–2622
57. Kempen, R. P., Dabas, P., and Ansari, A. Z. (2023) The Phantom Mark: enigmatic roles of phospho-Threonine 4 modification of the C-terminal domain of RNA polymerase II. *WIREs RNA* **14**, e771
58. Saldi, T., Cortazar, M. A., Sheridan, R. M., and Bentley, D. L. (2016) Coupling of RNA polymerase II transcription elongation with pre-mRNA splicing. *J. Mol. Biol.* **428**, 2623–2635
59. Tellier, M., Zaborowska, J., Neve, J., Nojima, T., Hester, S., Fournier, M., *et al.* (2022) CDK9 and PP2A regulate RNA polymerase II transcription termination and coupled RNA maturation. *EMBO Rep.* **23**, e54520
60. David, C. J., Boyne, A. R., Millhouse, S. R., and Manley, J. L. (2011) The RNA polymerase II C-terminal domain promotes splicing activation through recruitment of a U2AF65-Prp19 complex. *Genes Dev.* **25**, 972–983
61. Ryan, K., Murthy, K. G. K., Kaneko, S., and Manley, J. L. (2002) Requirements of the RNA polymerase II C-terminal domain for reconstituting pre-mRNA 3′ cleavage. *Mol. Cell Biol.* **22**, 1684–1692
62. Hirose, Y., Tacke, R., and Manley, J. L. (1999) Phosphorylated RNA polymerase II stimulates pre-mRNA splicing. *Genes Dev.* **13**, 1234–1239
63. Hahne, H., Sobotzki, N., Nyberg, T., Helm, D., Borodkin, V. S., van Aalten, D. M. F., *et al.* (2013) Proteome wide purification and identification of O-GlcNAc-modified proteins using click chemistry and mass spectrometry. *J. Proteome Res.* **12**, 927–936
64. Liu, Y., Chen, Q., Zhang, N., Zhang, K., Dou, T., Cao, Y., *et al.* (2020) Proteomic profiling and genome-wide mapping of O-GlcNAc chromatin-associated proteins reveal an O-GlcNAc-regulated genotoxic stress response. *Nat. Commun.* **11**, 5898
65. Ma, J., Li, Y., Hou, C., and Wu, C. (2021) O-GlcNAcAtlas: a database of experimentally identified O-GlcNAc sites and proteins. *Glycobiology* **31**, 719–723
66. Ma, J., and Hart, G. W. (2014) O-GlcNAc profiling: from proteins to proteomes. *Clin. Proteomics* **11**, 8
67. Kenneth Allan, R., and Ratajczak, T. (2011) Versatile TPR domains accommodate different modes of target protein recognition and function. *Cell Stress Chaperones* **16**, 353–367
68. Janetzko, J., and Walker, S. (2014) The making of a sweet modification: structure and function of O-GlcNAc transferase. *J. Biol. Chem.* **289**, 34424–34432
69. Joiner, C. M., Hammel, F. A., Janetzko, J., and Walker, S. (2021) Protein substrates engage the lumen of O-GlcNAc transferase's tetratricopeptide repeat domain in different ways. *Biochemistry* **60**, 847–853
70. Lazarus, M. B., Jiang, J., Kapuria, V., Bhuiyan, T., Janetzko, J., Zandberg, W. F., *et al.* (2013) HCF-1 is cleaved in the active site of O-GlcNAc transferase. *Science* **342**, 1235–1239
71. Levine, Z. G., Potter, S. C., Joiner, C. M., Fei, G. Q., Nabet, B., Sonnett, M., *et al.* (2021) Mammalian cell proliferation requires noncatalytic functions of O-GlcNAc transferase. *Proc. Natl. Acad. Sci. U. S. A.* **118**, e2016778118
72. Stephen, H. M., Adams, T. M., and Wells, L. (2021) Regulating the regulators: mechanisms of substrate selection of the O-GlcNAc cycling enzymes OGT and OGA. *Glycobiology* **31**, 724–733
73. Joiner, C. M., Li, H., Jiang, J., and Walker, S. (2019) Structural characterization of the O-GlcNAc cycling enzymes: insights into substrate recognition and catalytic mechanisms. *Curr. Opin. Struct. Biol.* **56**, 97–106
74. Bond, M. R., and Hanover, J. A. (2015) A little sugar goes a long way: the cell biology of O-GlcNAc. *J. Cell Biol.* **208**, 869–880

75. Zhu, Y., and Hart, G. W. (2021) Nutrient regulation of the flow of genetic information by O-GlcNAcylation. *Biochem. Soc. Trans.* **49**, 867–880
76. Cadena, D. L., and Dahmus, M. E. (1987) Messenger RNA synthesis in mammalian cells is catalyzed by the phosphorylated form of RNA polymerase II. *J. Biol. Chem.* **262**, 12468–12474
77. Kelly, W. G., Dahmus, M. E., and Hart, G. W. (1993) RNA polymerase II is a glycoprotein. Modification of the COOH-terminal domain by O-GlcNAc. *J. Biol. Chem.* **268**, 10416–10424
78. Buratowski, S., and Sharp, P. A. (1990) Transcription initiation complexes and upstream activation with RNA polymerase II lacking the C-terminal domain of the largest subunit. *Mol. Cell Biol.* **10**, 5562–5564
79. Kim, W. Y., and Dahmus, M. E. (1989) The major late promoter of adenovirus-2 is accurately transcribed by RNA polymerases IIO, IIA, and IIB. *J. Biol. Chem.* **264**, 3169–3176
80. Mäkelä, T., Parvin, J., Kim, J., Huber, J., Sharp, P., and Weinberg, R. (1995) A kinase-deficient transcription factor TFIIH is functional in basal and activated transcription. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 5174–5178
81. Krause, M. W., Love, D. C., Ghosh, S. K., Wang, P., Yun, S., Fukushige, T., *et al.* (2018) Nutrient-Driven O-GlcNAcylation at promoters impacts genome-wide RNA pol II distribution. *Front. Endocrinol.* **9**. https://doi.org/10.3389/fendo.2018.00521
82. Ranuncolo, S. M., Ghosh, S., Hanover, J. A., Hart, G. W., and Lewis, B. A. (2012) Evidence of the involvement of O-GlcNAc-modified human RNA polymerase II CTD in transcription *in vitro* and *in vivo*. *J. Biol. Chem.* **287**, 23549–23561
83. Lewis, B. A., Burlingame, A. L., and Myers, S. A. (2016) Human RNA polymerase II promoter recruitment *in Vitro* is regulated by O-linked N-acetylglucosaminyltransferase (OGT). *J. Biol. Chem.* **291**, 14056–14061
84. Lu, L., Fan, D., Hu, C. W., Worth, M., Ma, Z. X., and Jiang, J. (2016) Distributive O-GlcNAcylation on the highly repetitive C-terminal domain of RNA polymerase II. *Biochemistry* **55**, 1149–1158
85. Elbaum, M. B., and Zondlo, N. J. (2014) OGlcNAcylation and phosphorylation have similar structural effects in alpha-helices: post-translational modifications as inducible start and stop signals in alpha-helices, with greater structural effects on threonine modification. *Biochemistry* **53**, 2242–2260
86. Gross, B. J., Kraybill, B. C., and Walker, S. (2005) Discovery of O-GlcNAc transferase inhibitors. *J. Am. Chem. Soc.* **127**, 14588–14589
87. Haltiwanger, R. S., Grove, K., and Philipsberg, G. A. (1998) Modulation of O-linked N-acetylglucosamine levels on nuclear and cytoplasmic proteins *in vivo* using the peptide O-GlcNAc-beta-N-acetylglucosaminidase inhibitor O-(2-acetamido-2-deoxy-D-glucopyranosylidene) amino-N-phenylcarbamate. *J. Biol. Chem.* **273**, 3611–3617
88. Lewis, B. A., Das, S. K., Jha, R. K., and Levens, D. (2023) Self-assembly of promoter DNA and RNA Pol II machinery into transcriptionally active biomolecular condensates. *Sci. Adv.* **9**, eadi4565
89. Resto, M., Kim, B. H., Fernandez, A. G., Abraham, B. J., Zhao, K., and Lewis, B. A. (2016) O-GlcNAcase is an RNA polymerase II elongation factor coupled to pausing factors SPT5 and TIF1beta. *J. Biol. Chem.* **291**, 22703–22713
90. Adamson, T. E., Shore, S. M., and Price, D. H. (2003) Analysis of RNA polymerase II elongation *in vitro*. *Methods Enzymol.* **371**, 264–275
91. Cheng, B., and Price, D. H. (2009) Isolation and functional analysis of RNA polymerase II elongation complexes. *Methods* **48**, 346–352
92. Love, D. C., Ghosh, S., Mondoux, M. A., Fukushige, T., Wang, P., Wilson, M. A., *et al.* (2010) Dynamic O-GlcNAc cycling at promoters of Caenorhabditis elegans genes regulating longevity, stress, and immunity. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 7413–7418
93. Akan, I., Love, D. C., Harwood, K., Bond, M. R., and Hanover, J. A. (2016) Drosophila O-GlcNAcase deletion globally perturbs chromatin O-GlcNAcylation. *J. Biol. Chem.* **291**, 9906–9919
94. Deplus, R., Delatte, B., Schwinn, M. K., Defrance, M., Méndez, J., Murphy, N., *et al.* (2013) TET2 and TET3 regulate GlcNAcylation and H3K4 methylation through OGT and SET1/COMPASS. *EMBO J.* **32**, 645–655
95. Shafi, R., Iyer, S. P., Ellies, L. G., O'Donnell, N., Marek, K. W., Chui, D., *et al.* (2000) The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 5735–5739
96. Yang, Y. R., Song, M., Lee, H., Jeon, Y., Choi, E.-J., Jang, H.-J., *et al.* (2012) O-GlcNAcase is essential for embryonic development and maintenance of genomic stability. *Aging Cell* **11**, 439–448
97. Woringer, M., and Darzacq, X. (2018) Protein motion in the nucleus: from anomalous diffusion to weak interactions. *Biochem. Soc. Trans.* **46**, 945–956
98. Lyon, A. S., Peeples, W. B., and Rosen, M. K. (2021) A framework for understanding the functions of biomolecular condensates across scales. *Nat. Rev. Mol. Cell Biol.* **22**, 215–235
99. Sabari, B. R., Dall'Agnese, A., and Young, R. A. (2020) Biomolecular condensates in the nucleus. *Trends Biochem. Sci.* **45**, 961–977
100. Pappu, R. V., Cohen, S. R., Dar, F., Farag, M., and Kar, M. (2023) Phase transitions of associative biomacromolecules. *Chem. Rev.* **123**, 8945–8987
101. Banani, S. F., Lee, H. O., Hyman, A. A., and Rosen, M. K. (2017) Biomolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Biol.* **18**, 285–298
102. Fox, A. H., Nakagawa, S., Hirose, T., and Bond, C. S. (2018) Paraspeckles: where long noncoding RNA meets phase separation. *Trends Biochem. Sci.* **43**, 124–135
103. Li, X., Pinou, l., Du, Y., Chen, X., and Liu, C. (2023) Emerging roles of O-glycosylation in regulating protein aggregation, phase separation, and functions. *Curr. Opin. Chem. Biol.* **75**, 102314
104. Tan, Z.-W., Fei, G., Paulo, J. A., Bellaousov, S., Martin, S. E. S., Duveau, D. Y., *et al.* (2020) O-GlcNAc regulates gene expression by controlling detained intron splicing. *Nucleic Acids Res.* **48**, 5656–5669
105. Park, S. K., Zhou, X., Pendleton, K. E., Hunter, O. V., Kohler, J. J., O'Donnell, K. A., *et al.* (2017) A conserved splicing silencer dynamically regulates O-GlcNAc transferase intron retention and O-GlcNAc homeostasis. *Cell Rep.* **20**, 1088–1099
106. Itkonen, H. M., Urbanucci, A., Martin, S. E. S., Khan, A., Mathelier, A., Thiede, B., *et al.* (2019) High OGT activity is essential for MYC-driven proliferation of prostate cancer cells. *Theranostics* **9**, 2183–2197
107. Ferrell, J. E., Jr., and Ha, S. H. (2014) Ultrasensitivity part I: michaelian responses and zero-order ultrasensitivity. *Trends Biochem. Sci.* **39**, 496–503
108. Ferrell, J. E., Jr., and Ha, S. H. (2014) Ultrasensitivity part III: cascades, bistable switches, and oscillators. *Trends Biochem. Sci.* **39**, 612–618
109. Ferrell, J. E., Jr., and Ha, S. H. (2014) Ultrasensitivity part II: multisite phosphorylation, stoichiometric inhibitors, and positive feedback. *Trends Biochem. Sci.* **39**, 556–569
110. Okada, T., Ihara, H., Ito, R., Taniguchi, N., and Ikeda, Y. (2009) Bidirectional N-acetylglucosamine transfer mediated by beta-1,4-N-acetylglucosaminyltransferase III. *Glycobiology* **19**, 368–374