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CELL CYCLE REGULATION AND RNA POLYMERASE II

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1. ABSTRACT

The cell cycle and transcription by RNA polymerase II (RNAP II) are closely related. They utilize shared components. RNAP II transcriptional activity is modulated during the cell cycle. Cell cycle dependent changes in the phosphorylation status of the carboxyl-terminal domain (CTD) of the largest subunit of RNAP II (RNAP II-LS) alter transcription. Several CTD kinases are members of the cyclin-dependent kinase (cdk) superfamily, including p34^{cdc2} (cdk1), cdk7, cdk8, and cdk9. Each of these cdks, with their respective cyclin partners, have been linked to cell cycle regulatory events. Other CTD kinases such as casein kinase II (CKII) and c-abl have also been implicated in cell cycle dependent modifications of the CTD. In addition, the stalling of RNAP II complexes at DNA lesions helps stimulate p53 accumulation which largely determines the cell's DNA damage response, including cell cycle arrest. Alzheimer's disease pathology results partially from activation of mitotic cdks in postmitotic neurons which can phosphorylate RNAP II-LS and other targets.

2. INTRODUCTION

The normal mammalian cell cycle consists of transitions through several temporally distinct phases monitored by intracellular checkpoints (reviewed in ref. 1). These states include the initiation and completion of DNA replication (S) phase and of cell division or mitosis (M). Between these phases are gaps (G). In mammalian cells the restriction point in late G_1, also known as START in yeast, denotes a point at which the cell commits itself irrevocably to another round of DNA replication. In the presence of the appropriate environment, with adequate growth factors, passage through the restriction point is promoted by a group of cyclin-dependent kinase (cdk) holoenzymes (cyclin dependent catalytic subunit + cyclin). Phosphorylation of specific substrates by these holoenzymes promotes progression through the cell cycle. Phosphorylation and inactivation of the retinoblastoma tumor suppressor protein (pRB) for example leads to progression through the restriction point. Components of the basal transcription apparatus are also regulated by cyclin/cdk holoenzymes (2).

In eukaryotes, RNA polymerase II (RNAP II) is responsible for transcribing protein encoding genes. The RNAP II preinitiation complex includes both the RNAPII "catalytic core" (composed of 12 subunits) and a set of associated general transcription factors (GTFs) that include the TATA box binding protein (TBP), TBP associated

factors ($TAF_{II}s$), TFIIB, TFIIE, TFIIF and TFIIH (3,4). The complex consisting of the RNAPII catalytic core, GTFs and a coactivator complex known as SRB/MED are recruited to the promoter to mediate transcriptional regulation of target genes (4).

The mammalian RNA polymerase II large subunit (RNAP II-LS) consists of 1970 amino acids and contains an essential carboxy-terminal domain (CTD) composed of 52 tandem repeats of a heptapeptide with the consensus sequence, Tyr-Ser-Pro-Thr-Ser-Pro-Ser. (These heptapeptides may be either perfect or imperfect iterations of the consensus heptapeptide.) The CTD is phosphorylated on a portion of RNA polymerase II molecules *in vivo* and is phosphorylated by the general transcription factor TFIIH *in vitro*. TFIIH is composed of at least 9 subunits which include cdk7, cyclin H and MAT1 (p36). These three subunits (cdk7, cyclin H, and MAT1) form a ternary complex, cdk-activating kinase (CAK). The kinase activity of the holo-TFIIH complex is directed primarily towards the CTD (5,6), but cyclinH/cdk7 and CAK can also phosphorylate and activate cdk2, which is a key regulator of the cell cycle (7,8). The CTD is phosphorylated by several other kinases in response to mitogenic and cytostatic cues lending credence to a model in which the CTD functions as a sensor of the intracellular environment to coordinate transcription of specific target genes.

2.1. CTD phosphorylation and the Pol II transcription cycle

Five out of seven amino acids in the CTD's repetitive heptapeptide sequence have the potential to be modified by phosphorylation. Multiple CTD kinases, and at least one CTD phosphatase, have been identified thus far (see below). The deletion of more than approximately half of the CTD sequence leads to the loss of function at most promoters (9). In all likelihood, the coordinately regulated phosphorylation/dephosphorylation of the RNAP II CTD is responsible for not only the recruitment and assembly of active transcription/RNA processing complexes within the nucleus, but the temporal control of these processes during the cell cycle (see Figure 1). The phosphorylation status of the CTD also negatively regulates certain aspects of transcription (e.g., by cdk8). Finally, the action of multiple protein kinases and phosphatases has been shown to control the recruitment and assembly of transcription and splicing factors from specific sub-nuclear storage sites (e.g., intrachromatin granule clusters, IGCs) to active centers (Figure 2), a process that may also lend itself to cell cycle "control" (10).

The CTD serves to nucleate the formation of various protein complexes during different stages of transcription (reviewed in ref. 11). The form of RNAP II-LS competent to bind to the basal promoters of eukaryotic genes has a hypophosphorylated CTD (i.e., the IIa form). During the pre-initiation and initiation stages of transcription, proteins critical for basal as well as regulated transcription bind to the hypophosphorylated CTD (12,13). Subsequently, the CTD becomes hyperphosphorylated (i.e., the IIo form) whereupon promoter clearance and transcript elongation occur. At this time the other proteins that bind to the CTD include those involved in pre-mRNA processing (e.g., 5' capping, polyadenylation, and splicing) (14-18). After synthesis of a complete pre-mRNA transcript, the CTD must be de-phosphorylated to regenerate the initiation competent (IIa) form. During all phases of transcription, RNAP II-LS is complexed with the other core subunits of RNAP II as well as other transcription factors.

2.2. Scope of the review.

This review will summarize the evidence that global changes in transcription brought about by the action of the various CTD kinases may help regulate the cell proliferation cycle. The CTD protein kinases (cdk 7, 8, and 9) known to regulate the phosphorylation status of the CTD during the transcription cycle will be discussed first, emphasizing instances in which their activity has been shown to change in a cell cycle dependent manner. Several additional serine/threonine protein kinases, as well as two unique tyrosine kinases (c-abl and Arg) known to phosphorylate the CTD *in vitro* and/or *in vivo*, will each be described as well. The roles of the TFIIH and TFIID general transcription factors in the cell cycle will be discussed. Cell cycle dependent changes in the subcellular compartmentalization of RNAP II and its relationship to the phosphorylation status of the CTD will be also be discussed. The evidence that the stalling of RNAP II at DNA lesions represents a mechanism for inducing p53-dependent cell cycle arrest will be presented. In addition, modifications of RNAP II-LS induced by UV radiation will be described along with the argument that such modifications may contribute DNA damage-induced cell cycle regulation. Lastly, evidence will be presented that activation of mitotic cdks in post mitotic neurons may play a role in Alzheimer's disease pathology; one target of these cdks appears to be RNAP II-LS.

3. CDK 7

Cdk7, also known as $p40^{MO15}$, has an apparent dual role; it is involved in transcriptional control through CTD phosphorylation and control of the cell cycle through its ability to phosphorylate several cyclin dependent kinases (cdks). Cdk7 is a component of the general RNAP II transcription factor TFIIH, which is comprised of 9 subunits (reviewed in ref. 19). These include cdk 7 and cyclin H, as well as the helicases XPB and XPD which are essential for the initiation of RNAP II transcription as well as for nucleotide excision repair (NER). Regulated transcription of a gene requires recruitment of the RNAP II holoenzyme to the promoter. The assembled proteins include gene specific transcription factors and general transcription factors (e.g., TFII A, B, and D) as well as co-factors that can bind to the transcription factors as well as to the CTD. The form of the CTD that binds to the proteins involved in transcriptional initiation is the hypophosphorylated, or IIa form. Phosphorylation of IIa by TFIIH occurs shortly after the pre-initiation complex assembles at the promoter and in turn permits promoter clearance and the onset of transcript elongation (19). The XPB and XPD helicases are also important early in transcription in order to "open up" the double helix so that

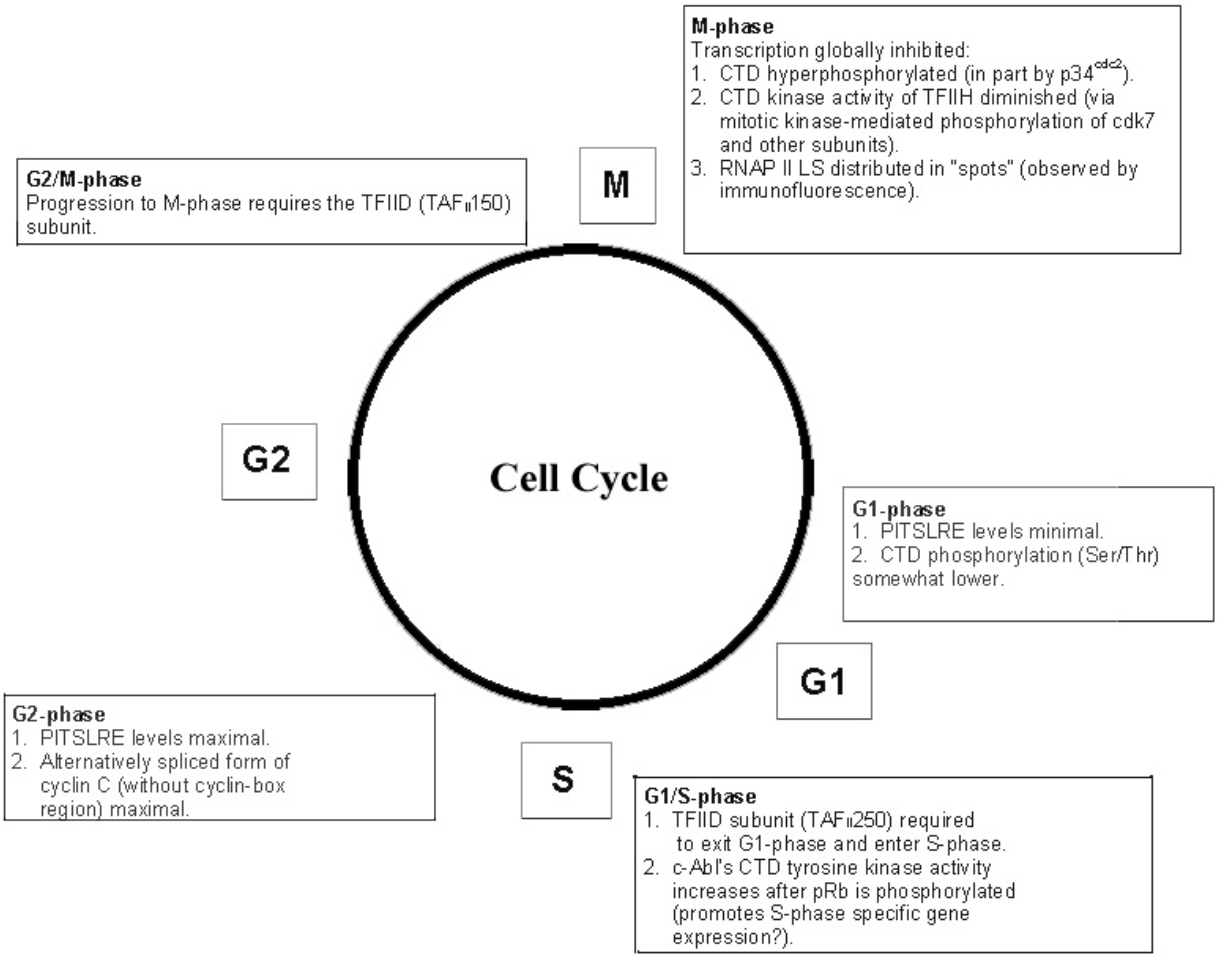

Figure 1. RNAP II transcriptional events and modifications of the CTD which have been associated with specific phases of the cell cycle.

the core polymerase can begin to transcribe the coding bases. Furthermore, the alteration in the phosphorylation status of the CTD changes the affinity of its binding proteins. While the general transcription factors involved in initiation (e.g., TFII D, E, and F) bind to the IIa form (12,13), proteins involved in pre-mRNA capping, polyadenylation, and splicing bind preferentially to the more highly phosphorylated, or IIo, form of the CTD (14-18). Using synthetic peptides, cdk7 has been repeatedly shown to target serine residues at the 5 position of the consensus heptapeptide, YS^2PTS^5PS, for phosphorylation (20,21). One study, utilizing monoclonal antibody CC3 that selectively recognizes a CTD phosphoepitope phosphorylated at Serine 2 (22), showed that TFIIH increased CC3 immunoreactivity at the C-terminal half of the CTD (23). This indicates that in addition to phosphorylating Serines at position 5, cdk7 may be able to phosphorylate Serines at position 2 (11).

Three subunits, cdk7, cyclin H and MAT 1 (menage-a-trois 1), comprise a distinct sub-component of TFIIH that is also known as CAK, for cdk activating kinase. In metazoans CAK is responsible for activating several cell cycle dependent kinases (including cdks 2, 4 and 6) through phosphorylation of a conserved threonine residue in their predicted T-loops (24,25). The T-loop normally blocks the catalytic site of the cdk when this threonine residue is not phosphorylated (26,27). It also prevents the stable association between the cdk and its cognate cyclin regulatory subunit. In the budding yeast *S cerevisiae* the CTD kinase, Kin28 (28), is distinct from the CAK otherwise known as CIV1/CAK1p (29-31). In metazoans, however, cdk7 appears to carry out both of these functions (24,25). The argument that cdk7 and cyclin H actually plays a role in the control of the cell cycle, despite its resemblance to other *bona fide* cyclin-cdk complexes, was weakened by the lack of any variation in its level or kinase activity during the cell cycle (32). It was subsequently shown, however, that CAK activity is diminished during mitosis (33,34) (discussed in Section 7, "$p34^{cdc2}$ and mitotic inhibition of transcription"). In budding yeast, the activity of Kin28 was recently shown to be activated by CAK1p; thus, even in S. cerevisiae the cdk and CTD phosphorylation events are functionally related

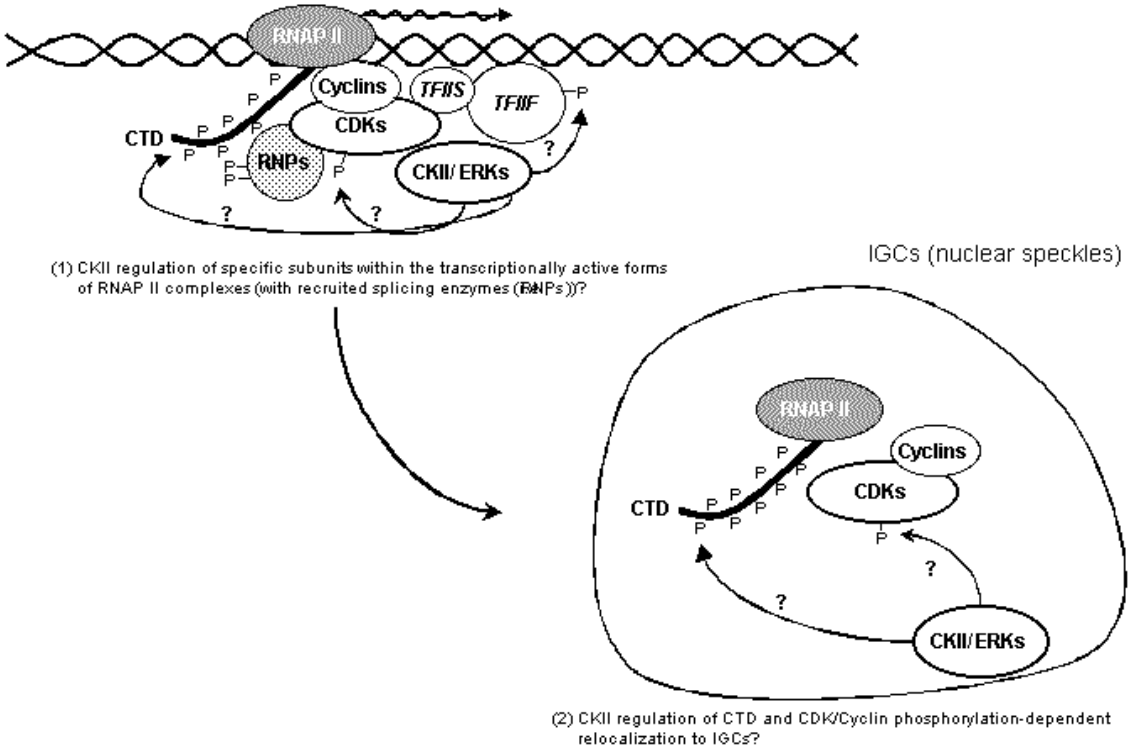

Figure 2. A hypothetical model of the possible effect(s) of changes in RNAP II CTD phosphorylation status on transcriptional/RNA processing events and the sub-nuclear localization of these factors. TFIIF and TFIIS are RNAP II-associated elongation factors. CKII and ERK are examples of kinases that can phosphorylate the C-terminal domain (CTD) of RNAP II. IGCs (interchromatin granule clusters) are structural components of the nucleus, also known as speckles (10). Additional details are provided in the text.

by their ability to cross-talk even though they are carried out by distinct enzymes (35).

The substrate specificity of the cdk7 protein kinase is also altered by MAT1 and the other subunits of TFIIH. Recombinant cdk7 and cyclin H phosphorylate cdk2 or the CTD with approximately equal efficiency, but the addition of recombinant MAT 1 to cdk7 and cyclin H results in an approximately 4:1 preference for phosphorylation of the CTD. CAK (i.e., cdk 7, cyclin H,

and MAT1) purified from HeLa cells has the same 4:1 preference for phosphorylation of the CTD (8). Holo TFIIH enzyme purified from HeLa cells demonstrates an even stronger preference for the CTD over cdk 2 (7,8). In addition, when incorporated into holo-TFIIH, cdk7 can also phosphorylate the transcription factors TFIIE (p56) and TFIIF (RAP74) (7). When cdk7 is complexed with MAT 1, its catalytic activity does not depend upon phosphorylation of the previously mentioned threonine residue found in its T-loop (36,37).

3.1. Cdk7 and control of the cell cycle

The apparent dual role of cdk7 protein kinase function could be interpreted as meaning that in organisms more complex than the budding yeast *S. cerevisiae*, the same protein kinase is employed for two independent functions (i.e., transcriptional initiation and cdk activation). It is also possible that transcriptional control through CTD phosphorylation and cdk activation, through its phosphorylation, could represent two complimentary cell cycle control mechanisms that are conveniently coordinated by the same enzyme. The tumor suppressor $p16^{INK4a}$ negatively regulates cell cycle progression by binding to cdk4 and cdk6 and thereby inhibiting their catalytic activity (38). It has recently been shown that $p16^{INK4a}$ can also bind to TFIIH and inhibit its ability to phosphorylate the CTD (39). Interestingly, cdk2 phosphorylation by cdk7 was not inhibited by $p16^{INK4a}$.

Cell cycle arrest is an important consequence of cellular insults, such as viral infection or DNA damage, which occur in part due to the upregulation of the cellular guardian p53 (Figure 3). p53 is a transcription factor that can induce the expression of proteins such as $p21^{cip1}$, which in turn inhibits the activity of cdks (e.g., cdk2) involved in cell cycle progression (40). Cdk7-cyclinH-MAT1 has been shown to phosphorylate p53 *in vitro* at Ser 33 as well as C-terminal residues known to increase sequence specific binding of p53 to DNA (41,42). It has also been shown that p53 can inhibit the ability of baculovirus expressed

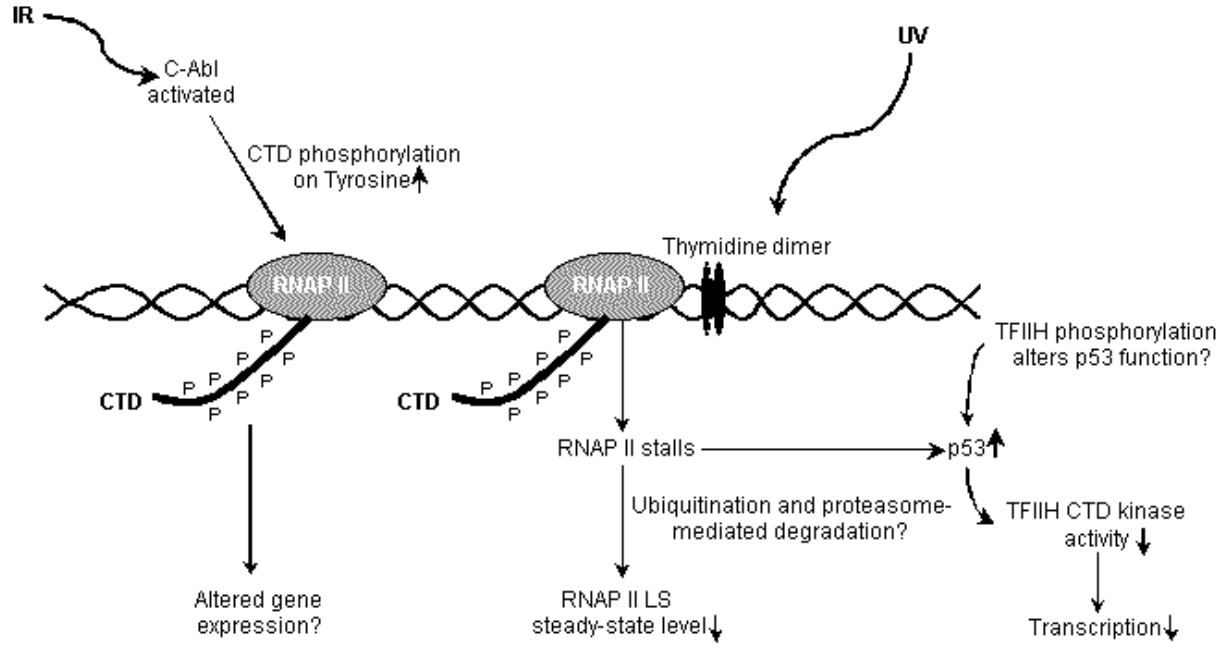

Figure 3. DNA damage and RNAP II. Ionizing radiation (IR) can activate the c-abl tyrosine kinase. Altered tyrosine phosphorylation of the CTD may lead to changes in gene expression that help the cell to respond to the DNA damage. UV radiation (as well as cisplatin and other chemical agents) induce bulky, helix distorting lesions that cause RNAP II to stall. Stalling induces accumulation of p53 that in turn orchestrates the cellular response to DNA damage (by induction of genes involved in DNA repair, cell cycle arrest, and apoptosis). In addition, TFIIH and p53 may influence each other's function. Lastly, the intracellular level of RNAP II-LS decreases after DNA damage, and the subsequent impediment to global transcription may inhibit cell cycle progression. This, like other checkpoints, may provide time for the cell to repair DNA damage before the next S or M phase.

CAK to phosphorylate either the CTD or cdk2 *in vitro* (43). A mutated and dysfunctional form of p53 (p53His175) failed to inhibit CAK activity under similar conditions. *In vivo*, UV radiation (40 J/m^2) inhibited the CTD kinase activity of CAK in a p53 dependent manner. When CTD kinase activity was immunoprecipitated (with an anti-cyclin H antibody) from extracts of UV-irradiated, p21 -/-, mouse embryonic fibroblasts (MEFs) the activity was diminished compared to that of unirradiated MEFs. UV radiation did not inhibit CTD kinase activity immunoprecipitated from p53$^{-/-}$ MEFs, indicating that p53 (but not p21) is required for UV-induced inhibition of CTD kinase activity (43).

An earlier study showed that the same dose of UV radiation inhibited the CTD kinase activity of CAK only when it was associated with the other components of TFIIH. The role of p53 was not assessed in this study in which either anti-cdk7 or anti XPD was used to immunoprecipitate CTD kinase activity associated with cdk7-cyclin H-MAT1, or holo-TFIIH, respectively (44). Heat shock, another cellular insult that can result in cell cycle arrest (45,46), also inactivates TFIIH-associated CTD kinase activity (23). Activation of the Ras oncogene is known to promote cell cycle progression (47). A recent study showed that increased *Ras* activity promoted increased transcription by RNAP II, increased CTD phosphorylation and increased cdk7 expression (48). Experimental manipulations known to promote cardiac hypertrophy (e.g., α_1-adrenergic stimulation and aortic banding) also promoted increased CTD phosphorylation. Taken together, these studies suggest that cell cycle arrest by stressful stimuli (UV and heat shock) as well as cell cycle stimulation by *Ras* may be transduced via altering TFIIH's CTD kinase activity.

4. CDK8

Another CTD protein kinase resembling the cdk superfamily was discovered through its association with a novel yeast cyclin capable of rescuing a compound yeast cyclin mutant (49,50). This cdk was named cdk8, and one of its cyclin binding partners was denoted cyclin C. The yeast counterparts, *SRB10* (cdk8) and *SRB11* (cyclin C) were shown to interact with a dephosphorylated form of the CTD as a component of the RNAP II holoenzyme (51). This cyclin C/cdk8 complex associated with the RNAP II holoenzyme in mammalian cells; however, neither the level of this cyclin/cdk complex or its associated CTD kinase activity changed during the cell cycle (52). Both cdk7/cyclin H and ckd8/cyclin C are associated with RNAP II early in the transcription cycle (at or near pre-initiation complex formation) and appear to phosphorylate consensus YSPTSPS heptapeptides at the serine 5 position (53,54) as well as at the serine 2 position (21,23). It has recently been

shown that these two holoenzymes are biochemically distinct (54). Cdk7/cyclin H is much more able to phosphorylates non-consensus heptapeptides that diverge from the YSPTSPS sequence (which are preferentially located in the carboxyl-terminal half of metazoan CTDs), than is cdk8/cyclin C. In addition, these two protein kinases are differentially sensitive to various CTD kinase inhibitors. Whereas cdk7/cyclinH may be a general transcriptional stimulator due to its ability to phosphorylate the CTD of preinitiation complexes at most genes, cdk8/cyclinC inhibits a subset of genes involved in control of cell type specificity, meiosis, and sugar metabolism (55-57). Cdk8/cyclin C inhibits transcription by phosphorylating the CTD before the RNAP II holoenzyme has bound to the promoter to form the initiation complex (21,53). This function appears to be promoter specific and subject to inhibition by cellular factors that remain to be identified.

4.1. Cyclin C and the cell cycle

Levels of cyclin C mRNA can be induced in response to serum or cytokine stimulation (50,58). An alternatively spliced avian mRNA for cyclin C has been identified which lacks part of the conserved, helix rich cyclin box region. The smaller mRNA and a smaller cyclin C protein probably encoded by the smaller transcript were shown to demonstrate a pattern of cell cycle variation similar to that of cyclin B2 (59). The human gene for cyclin C is localized to chromosome 6q21, a region frequently deleted in acute lymphoblastic leukemia, ALL (60).

5. CDK 9 AND MITOGEN ACTIVATION OF LYMPHOID CELLS

Cdk9/cyclin T is a CTD kinase/cyclin pair that comprises a positive RNAP II elongation factor called pTEFb (61). pTEFb promotes CTD phosphorylation after promoter clearance at a step that occurs later in transcription than either of those regulated by cdk7 or cdk8. pTEFb counteracts the function of negative transcriptional elongation factors, such as DRB sensitivity inducing factor (DSIF), which confers sensitivity to the transcriptional inhibitor 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole (DRB) and Factor 2, a protein which can promote the release of Pol II from the DNA template (62,63). pTEFb remains associated with elongating RNAP II (64). Thus far, two cyclin T genes, T1 and T2 and a separate and unique cyclin K gene product, all of which are related to cyclin C, have been found in association with cdk9 (61,65). Furthermore, alternative splicing of the cyclin T2 gene locus yields two T2 transcripts, T2a and T2b (61). The cdk9/cyclin T protein kinase is extremely sensitive to the transcriptional inhibitor, DRB.

In most eukaryotic cells examined, the level of cdk9 and cyclin T proteins as well as their associated CTD kinase activity do not vary appreciably during the cell cycle (66). However, activation of human peripheral blood mononuclear cells (PBMCs) with the mitogens PMA and PHA led to a general increase in S phase, greatly increased cyclin T1 levels, slightly increased cdk9 levels, and greatly increased cdk9/cyclinT1 mediated CTD kinase activity (66). Cyclin T2a and T2b levels did not change. Although the combination of PHA and PMA induced cyclin A in PBMCs, cyclin T1 was believed to be the primary target (66). In addition, whereas cyclins A or E can be induced by these same mitogens in T cells, increased cdk2/cyclin A or cdk2/cyclin E activity requires additional mitogenic signals to counteract the negative regulation of these complexes provided by the $p27^{Kip1}$ gene product (67). Thus, PHA- and PMA-induction of cyclin T1/cdk9 represents an example of a cell type specific, exogenous activation of a CTD kinase by signaling molecules that can promote progression of the cell cycle. Additionally, activation of T cells by PHA and PMA promotes HIV replication; it has been shown that cdk9/cyclin T are directly responsible for the increased transcription of HIV genes induced by binding to an HIV-encoded transcriptional processivity factor called TAT (66,68).

6. THE BUDDING YEAST CTD KINASE, CTDK-I

The *S. cerevisiae* CTD kinase, CTDK-I, is closely related to metazoan pTEFb. CTDK-I is comprised of three subunits, Ctk1, Ctk2, and Ctk3 (69). Ctk1 resembles cdk9 and Ctk2 resembles cyclin T (69,70). Although no direct cell cycle regulation of any of the CTDK-I components has been demonstrated, deletion of the *CTK1* gene leads to altered patterns of CTD phosphorylation and altered growth properties (22,71). *Ctk1* deficient yeast lack the transcriptional burst of certain genes (e.g., those encoding glycogen synthase and cytosolic catalase) that normally occurs during the diauxic shift. The diauxic shift refers to a metabolic transition that occurs late in the exponential growth phase of yeast cultures growing in a glucose-based medium; they shift from fermentation to respiratory metabolism (72,73). When CTD phosphorylation patterns are analyzed using two highly specific monoclonal antibodies (mAbs) that bind to CTD epitopes with either phosphorylated Serine 2 (mAb H5) or Serine 5 (mAb H14) of the consensus heptapeptide YSPTSPS (22,74), *Ctk1*-deficient yeast fail to demonstrate the accumulation of phosphorylation on Serine 2 observed in wild type yeast as they undergo diauxic shift (71). These results provide another example in which environmentally induced changes in CTD phosphorylation may result in altered RNAP II transcription (which may be gene specific) and altered cellular growth properties.

7. $P34^{CDC2}$ AND MITOTIC INHIBITION OF TRANSCRIPTION

Cellular mitosis is associated with distinct changes in CTD function and activity as transcriptional activity of RNA polymerase I, II and III is lost and the nucleus disassembles during mitosis chromatin condenses. It has been shown by immunofluorescence and immunoblotting studies with phosphorylation dependent anti-CTD antibodies that during mitosis hypophosphorylated (IIa) form of RNAP II virtually disappears and the hyperphosphorylated (IIo) predominates. During mitosis, the RNAP II is distributed in large spots distributed throughout the mitotic cell (75,76). Mitotic RNAP II is incompetent to initiate transcription because it is hyperphosphorylated, and

therefore unable to bind stably to promoter complexes (34,77,78). In yeast phosphorylation of the CTD by $p34^{cdc2}$, the cyclin dependent kinase that triggers entry into mitosis, was shown to inhibit transcription *in vitro* (78). Furthermore, cdk7 is phosphorylated in mitotic HeLa cells by mitotic promoting factor (MPF), which inhibits its CTD kinase activity and its ability to stimulate transcription as a component of TFIIH (34). The mitotic cdc2/cyclin B was also shown to inhibit the ability of TFIIH to stimulate transcription by phosphorylating p62 and p36, two additional subunits of TFIIH (33). Lastly, the transcription factor TFIID isolated from mitotic cells was found to be incompetent to initiate transcription (79). Most TFIID was found to be dissociated from mitotic chromatin; the fraction that remained associated with chromatin was heavily phosphorylated. Dephosphorylation restored its transcriptional activity.

8. PITSLRE AND CASEIN KINASE II (CKII)

PITSLRE, yet another member of the cdk superfamily, binds to a hyperphosphorylated form of the CTD as well as to casein kinase II (CKII), a novel CTD kinase required for cell cycle progression (80). It was recently been found that PITSLRE binds to the RNAP II elongation factor ELL2 using a yeast two-hybrid assay (81). In this study it was also found that PITSLRE bound to the CTD when it was hyperphosphorylated and co-purified with a novel CTD kinase activity that was shown to be casein kinase II (CKII). CKII is a heterotetramer composed of two regulatory (β) and two catalytic (α) subunits (82); the regulatory β subunits stimulate the catalytic activity of CKII. The consensus sequence for CKII phosphorylation is S/TXXD/E, and this sequence is found in three non-consensus heptapeptide repeats toward the carboxyl-terminus of the human CTD. CKII failed to phosphorylate an amino-terminal CTD fragment containing only consensus, or perfect, heptapeptide repeats (81). It appears therefore that CKII may be the first CTD kinase that preferentially targets specific domains on the complex and heterogeneous CTD. Nuclear CKII activity is required for cell cycle progression in the G1 phase of the cell cycle (83). In addition, PITSLRE protein levels change during the cell cycle, with maximal levels in G2/M and minimal levels during mid-to-late G1 phase. CTD phosphorylation status also changes somewhat, being higher in S, G2 and M phases than during G1 phase (J.H. Trembley and V.J. Kidd, *unpublished data*). This could correlate with the mitotic transcriptional arrest induced by $p34^{cdc2}$ -mediated CTD phosphorylation (see section 7).

9. ERK 1/2 (MAP KINASE)

Several *in vivo* and *in vitro* studies have indicated that the p42, p44 ERK1/2 kinases can phosphorylate the CTD. Serum stimulation of quiescent cells, which can cause Go cells to re-enter the cell cycle, leads to ERK 1/2 induced phosphorylation of the CTD (84,85). *In vitro* studies indicate a predilection for Serine 5 of the YSPTSPS consensus sequence with no particular preference for consensus vs. nonconsensus heptapeptides (20,86). Heat shock, a model form of stress shown to also inhibit cell cycle progression, activates ERK1/2 and induces extensive CTD phosphorylation (87). The hyperphosphorylated form of CTD is resistant to DRB, an inhibitor that targets cdk7, cdk8 and cdk9 (54,88). It is possible that this heat shock-induced CTD hyperphosphorylation contributes to altered gene expression patterns that are characteristic of heat shock. Other stressful stimuli, including ionizing radiation, osmotic and oxidative stress, were shown to promote ERK 1/2-induced phosphorylation of RNAP II-LS, causing a novel form, called IIm, to accumulate (85). This novel IIm form of RNAP II-LS migrated between IIo and IIa.

9.1.. ERK 1/2 induced phosphorylation and cell cycle control.

Mature Xenopus oocytes are characterized by cell cycle arrest (in meiotic metaphase II) and by a large stockpile of transcriptionally silent RNAP II. At the time that transcription and meiosis arrest, a large amount of hyperphosphorylated RNAP II-LS appears (89). This form of hyperphosphorylated RNAP II-LS has been shown to be due to ERK 1/2 mediated phosphorylation of the CTD. At the time of fertilization, transcription remains inactive for the first few cell divisions (90). Resumption of transcription during early post-fertilization development in both mouse and rabbit zygotes is characterized by reappearance of mostly IIa forms of RNAP II, inactivation of ERK 1/2, and the nuclear reaccumulation of RNAP II-LS, which at fertilization is largely outside the pronucleus (90). Thus fertilization of mammalian zygotes, like mitosis, provides another example in which RNAP II-LS compartmentalization (possibly regulated by CTD phosphorylation status) provides a mechanism for global transcriptional control.

10. ADDITIONAL (SER/THR) CTD KINASES AND A CTD PHOSPHATASE

Human cyclin K was recently identified utilizing a screen to rescue yeast cyclin mutants. This cyclin resembles both cyclins C and H (91). It has also been shown to be part of RNAP II complexes (65) and, like cyclin H, to be associated with both CTD kinase and CAK activity in vitro. As mentioned earlier, one cdk associated with cyclin K corresponds to cdk 9 (91). DNA dependent protein kinase (DNA-PK), which is involved in double strand break repair and V(D)J recombination, can also phosphorylate the CTD. DNA-PK may stimulate RNAP II transcription at specific promoters by interacting with both promoter-specific DNA sequences and promoter-specific activator proteins (92). Alternatively, it may facilitate RNAP II's ability to engage in multiple rounds of transcription at a specific gene (93). A single CTD phosphatase, FCP1, has also been identified and cloned (94-96). Activity is stimulated by the RAP74 component of TFIIF as well as by RNAP II itself. The phosphatase FCP1 can also be induced by induced by heat shock (97).

11. TYROSINE PHOSPHORYLATION OF THE CTD AND CELL CYCLE REGULATION

The CTD heptapeptide repeat (YSPTSPS) includes a tyrosine residue. The proto-oncogene c-abl has been shown to phosphorylate this residue in vitro and in vivo (98,99). It

appears that tyrosine phosphorylation of the CTD can increase the processivity of RNAP II and that the CTD is an important physiologic substrate of c-abl (100,101). The function of c-abl depends on a DNA binding domain as well as its tyrosine kinase domain, both of which are regulated during the cell cycle (102,103). The DNA binding domain is activated by cdc2 in a cell cycle dependent manner. An ATP-binding site in c-abl's catalytic site is blocked by the retinoblastoma tumor suppressor protein, pRb, until pRb is phosphorylated at the G1/S transition. In addition, ionizing radiation can activate c-abl by inducing the ability of the protein kinase Atm to phosphorylate a regulatory Serine at position 465 on c-abl (99). Ionizing radiation can stimulate the amount of tyrosine phosphorylation of the CTD about 4-fold (99). Another DNA damaging agent, methyl methane sulfonate (MMS), also induces the tyrosine phosphorylation of RNAP II-LS by c-abl (104). c-abl is not required for genotoxin-induced cell cycle arrest; c-abl-/- cells maintain S-phase arrest after ionizing radiation (99). It is believed that increased CTD phosphorylation on tyrosine residues after DNA damage or upon entry into S phase may contribute to altered expression of specific genes (Figure 3)(99,101). A second tyrosine kinase, Arg (for abl-related gene) can also phosphorylate the CTD at tyrosines (105).

12. RNA POLYMERASE II AND THE INDUCTION OF CELL CYCLE ARREST

Cell cycle arrest in response to DNA damage is an important cell cycle checkpoint; it blocks further progress of DNA replication or cell division until DNA damage can be repaired (106). Irreparable DNA damage will lead to apoptosis. DNA damage-induced cell cycle arrest, as well as apoptosis, are both p53-dependent (106). DNA damaging agents such as ultraviolet (UV) radiation and cisplatin induce lesions such as intra-strand DNA cross-links that cause elongating RNAP II to stall (107,108). The stalling of RNAP II efficiently recruits the repair apparatus to such lesions, and it also signals the intracellular accumulation of p53 (Figure 3). The importance of RNAP II stalling was established by comparing the UV doses required in cells with various deficiencies in nucleotide excision repair (NER), the repair pathway that removes lesions induced by UV radiation or cisplatin (108,109). UV-induced lesions located on the transcribed strand of active genes are repaired by transcription-coupled NER, which requires functional RNAP II-LS as well as functional CSA and CSB proteins. The *CSA* or *CSB* gene products are mutated in patients with either of the two forms (A or B) of Cockayne syndrome (CS) (110). Repair of lesions located elsewhere in the genome requires the XPC protein, which is deficient in the type C form of Xeroderma pigmentosum (110). Transcription coupled repair can still take place in XP-C cells. The dose of UV required to induce p53 accumulation is far lower in CS-A or CS-B cells than WT or XP-C cells even though low doses of UV are still lethal to XP-C cells (109). The doses of ionizing radiation, which does not induce RNAP II stalling, required to induce p53 accumulation are similar in WT, XP-C, CS-A and CS-B cells. The most plausible explanation is that persistently stalled Pol II in CS-A and CS-B cells leads to a stronger signal promoting the accumulation of p53 than in WT or XP-C cells in which transcription coupled repair is able to take place. Ionizing radiation induces p53 through the induction of double stranded DNA breaks (111,112). It has been suggested that p53 induction through UV radiation occurs via the DNA strand breaks induced during the NER process; however since p53 is robustly induced in XP cell lines in which no NER related strand breaks occur, it appears more likely that RNAP II stalling is the stimulatory event (108). In addition, other agents (such as α-amanitin and DRB) that inhibited RNAP II transcription also led to p53 induction without generating any detectable DNA strand breaks (108). Persistently stalled RNAP II can also signal UV-induced apoptosis (108,113).

12.1. UV-induced modifications of RNA polymerase II

We and others have shown that UV radiation and cisplatin induce the ubiquitination and proteasomal degradation of RNAP II-LS (114-118). Evidence exists that a hyperphosphorylated fraction of RNAP II-LS is preferentially ubiquitinated in mammalian cells (114,115). We have demonstrated that proteasomal degradation causes the steady state level of RNAP II-LS to drop dramatically after UV radiation. 1-8 hours after UV irradiation the IIa form of RNAP II-LS is virtually absent and the hyperphosphorylated forms are also diminished. RNAP II-LS levels of all phosphorylation states (IIo and IIa) will return to baseline about 16-24 hours after irradiation if the cells can repair the damage and if new protein synthesis occurs (118). The down-regulation of RNAP II-LS may represent a component of a DNA damage induced cell cycle arrest mechanism (Figure 3). Other models posit that stalled RNAPII LS can completes the transcript (119,120); these models must be reconciled with the ubiquitination/proteasomal degradation model.

In addition to permitting transcription-coupled NER, the CSA and CSB proteins also play a distinct role in permitting RNAP II transcription to recover after the lesions have been repaired (121). This has been demonstrated by showing that certain chemical-induced lesions (such as those induced by NA-AAF), which are not repaired by transcription-coupled NER, still require functional CS proteins for transcription to recover (122,123). It appears that the CS gene products are necessary for permitting basal transcription to resume after cells successfully repair DNA damage. Recent studies have provided evidence that the *CSB* gene product is required to permit the re-accumulation of the initiation-competent IIa form of RNAP II-LS after transient DNA damage-induced loss of this form of RNAP II (118 and D. Rockx, A. van Hoffen, M. Barton, D.B. Bregman, L. Mullenders, and A. van Zeeland. *manuscript in preparation*).

13. ROLE OF TFIID IN CELL CYCLE CONTROL

The RNA polymerase II general transcription factor TFIID, which is comprised of the TATA box binding protein and several associated factors called TAF_{II}s has also been implicated in cell cycle regulation. $TAF_{II}250$ was

originally identified as a cell cycle control gene, CCG1, in a temperature-sensitive Chinese hamster ovary cell line (ts13) which failed to exit G1 when grown at the non-permissive temperature of 39.5º C (124). It was later shown that the expression of the cyclin D1 gene is severely diminished in ts13 cells and that $TAF_{II}250$ (CCG1) binds to core promoter and enhancer elements of the cyclin D1 gene in order to induce its transcription (125). The yeast homolog of $TAF_{II}250$ ($yTAF_{II}145$) is similarly required for expression of G1 cyclins (126). Other studies in ts13 cells have shown that cyclin A expression is also decreased and p21 (the cdk inhibitor) is decreased in the presence of mutated $TAF_{II}250$ (127,128). In addition, $TAF_{II}250$ binds to pRb (129,130). Another mammalian TAF, $TAF_{II}150$, also appears to be essential for the normal progression of the cell cycle at the G2/M transition by virtue of its binding to specific Pol II core promoter elements essential for the expression of cyclins B1 and cyclin A (131).

14. MCM PROTEINS AND POL II HOLOENZYME

It has recently been shown that several MCM (*maintenance of minichromosomes*) proteins are components of the RNAP II holoenzyme (132). MCMs are a component of "licensing factor" that ensure that each part of the chromosome replicates exactly once during S phase. The MCMs are a group of six ATP-dependent helicases that bind to the "origin recognition complex" proteins that are in turn associated with chromosomal origins of replication (ORIs) (133). Three different antibodies to MCM2 were found to inhibit transcription by RNAP II but not RNAPs I or III. MCMs 2,3,5, and 7 were shown to interact with the RNAP II holoenzyme through interactions with the CTD (132). The function of MCMs with RNAP II holoenzyme remains to be determined but may function as a checkpoint to maintain DNA replication integrity.

15. PHOSPHOEPITOPES SHARED BY THE CTD, MITOTIC AND ALZHEIMER'S DISEASE ANTIGENS

Some CTD epitopes are shared by mitotic proteins. The monoclonal antibody MPM-2 recognizes a phosphorylated epitope present on several antigens present on key mitotic structures such as centrosomes, kinetochores and spindle fibers (134). The major interphase MPM-2 antigen is a hyperphosphorylated form of RNAP II-LS (135). MPM-2 also recognizes RNAP II-LS in mitotic cells (135). In addition, Pin1, a peptidyl proline isomerase essential for progression through mitosis (136) and able to bind the MPM-2 phosphoepitope (137), also binds to the CTD (135). Pin1 binds to prolines preceded by phosphorylated Ser or phosphorylated Thr; each YSPTSPS consensus heptapteptide could potentially include two such sites (137). It is therefore possible that Pin1 binding influences CTD function in some way.

It has also been found that MPM-2 binds to neuronal antigens destined to become parts of neurofibrillary tangles, a hallmark lesion of Alzheimer's Disease (138,139). These antigens get phosphorylated by kinases that normally act during mitosis, such as cdc2, despite the fact that mature neurons are no longer cycling and thus are post-mitotic (140). Recently, RNAP II-LS has been identified as one of these antigens; changes in RNAP II-LS phosphorylation status and subcellular localization have been found in biopsy specimens from Alzheimer's Disease Brains (J. Husseman, D.B. Bregman, D. Mann, T. Kavanaugh, D. Nochlin, and I. Vincent, *manuscript in preparation*).

16. CONCLUSIONS

RNAP II modifications occur during the course of the cell cycle as well as in response to extracellular stimuli (including growth factors, mitogens, and DNA damaging agents). Changes in the phosphorylation status of the C-terminal domain (CTD) of RNAP II's large subunit can alter transcription and pre-mRNA processing. Several CTD kinases are members of the cyclin-dependent kinase (cdk) superfamily including $p34^{cdc2}$ (cdk1), cdk7, cdk8, and cdk9. Cdk1-mediated phosphorylation of the CTD promotes transcriptional arrest during mitosis. Cdk 7 associates with MAT1 and cyclin H to constitute the metazoan CTD kinase activating kinase (CAK) that is essential for normal cell cycle progression. CAK is a component of TFIIH, one of the general transcription factors of RNA polymerase II. The cell cycle regulators p53 and p16INK4a can each inhibit cdk 7 when it is associated with TFIIH. The $TAF_{II}250$ and $TAF_{II}150$ subunits of TFIID, another basal transcription factor, are necessary for normal cell cycle progression. Casein Kinase II, the activity of which changes during the cell cycle, can phosphorylate the CTD and has recently been shown to bind PITSLRE, another member of the cdk superfamily that in turn binds to the CTD. Mitogens induce cdk9-mediated CTD phosphorylation in peripheral blood mononuclear cells. c-abl phosphorylates the CTD on tyrosines: cell cycle and DNA damage induced activation of c-abl may modify patterns of gene expression. Changes in CTD phosphorylation status also occur after fertilization, during oocyte development, and after serum stimulation. The stalling of RNA polymerase II at DNA lesions induced by UV radiation and cisplatin can stimulate p53 dependent cell cycle arrest. Mitotic phosphoepitopes have also been identified in postmitotic neurons and may play an etiologic role in neurodegenerative disorders such as Alzheimer's Disease.

Additional CTD kinases, phosphatases, and other CTD-modifying enzymes may be awaiting characterization, and some may function in a cell cycle dependent manner. CTD modifying enzymes may also be shown to be components of macromolecular complexes that perform cell cycle-related functions. Because changes in either global transcription or the transcription of particular regulatory proteins can each have profound effects on cell cycle progression, investigators will undoubtedly continue to recognize novel relationships between the cell cycle and transcription by RNAP II.

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