Secrets of a double agent: CDK7 in cell-cycle control and transcription

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Summary

In metazoans, cyclin-dependent kinase 7 (CDK7) has essential roles in both the cell-division cycle and transcription, as a CDK-activating kinase (CAK) and as a component of the general transcription factor TFIIH, respectively. Controversy over its double duty has been resolved, but questions remain. First, how does CDK7 achieve the dual substrate specificity necessary to perform both roles? Second, is there a deeper connection implied by the dichotomy of CDK7 function, for example similar mechanisms controlling cell division and gene expression, and/or actual coordination of the two processes? Enzymological studies have revealed solutions to the unusual substrate recognition problem, and there is evidence that the distinct functions of CDK7 can be regulated independently. Finally, despite divergence in their wiring, the CAK-CDK networks of budding yeast, fission yeast and metazoans all link transcriptional regulation with operation of the cell-cycle machinery. This connection might help to ensure that mRNAs encoding effectors of cell division are expressed at the right time in the cycle.

Key words: Cell cycle, Transcription, Cyclin-dependent kinase (CDK), CDK-activating kinase (CAK), Transcription factor, TFIIH, C-terminal domain (CTD)

Introduction

Cyclin-dependent kinases (CDKs) first emerged as the controllers of two major transitions in the cell cycle: initiation of the DNA synthesis (S) phase and entry into mitosis (reviewed by Morgan, 1997). Subsequently, CDK-cyclin complexes were also identified as conserved components of the RNA polymerase (Pol) II transcriptional machinery (reviewed by Bregman et al., 2000). Two non-overlapping sets of CDKs have been defined in metazoans: CDK1, CDK2, CDK4 and CDK6, which are dedicated to cell-division control; and CDK8 and CDK9, which are primarily implicated in transcription. One CDK – the CDK7 complex – cannot be so easily classified. CDK7 is both a CDK-activating kinase (CAK), which phosphorylates cell-cycle CDKs within the activation segment (T-loop), and a component of the general transcription factor TFIIH, which phosphorylates the C-terminal domain (CTD) of the largest subunit of Pol II (reviewed by Harper and Elledge, 1998).

The direct linkage between CDK activation and transcription maintained by CDK7 raises many questions. How does one enzyme recognize two dissimilar classes of substrate? Does CDK7 act analogously to activate effector CDKs in both pathways? Why does *Saccharomyces cerevisiae* have two enzymes to perform these two functions separately, whereas *Schizosaccharomyces pombe* has two partially redundant CAKs, only one of which is a component of the transcriptional machinery?

Biochemical studies have yielded insight into the versatility of the CDK7 complex and have suggested mechanisms by which its two functions can be regulated independently. Meanwhile, examples of apparent specialization by the TFIIH-associated kinase to support specific programs of gene expression – including cell-cycle-regulated gene clusters in fission yeast – indicate the two functions might be coordinated. These studies hint at deeper connections between transcription and cell-cycle control. Here, I discuss recent investigations of CDK7, its regulators and its targets, and examine how these studies have begun to uncover similarity and crosstalk between the mechanisms regulating eukaryotic gene expression and cell division.

When CDKs met Pol II: the CDK7 story

Early studies of CDK regulation revealed phosphorylation within the T-loops of both vertebrate and fission yeast CDKs (Krek and Nigg, 1991; Norbury et al., 1991; Gould et al., 1991), and a genetic requirement for such phosphorylation in *S. pombe* cell-cycle progression (Gould et al., 1991). Studies of CDK activation in vitro indicated that both T-loop phosphorylation and cyclin binding is required to generate high levels of kinase activity and, moreover, that metazoan cell extracts contain CAK activity (Desai et al., 1992; Solomon et al., 1992). Biochemical purification of CAK from animal cell extracts led to the first identification of the CDK7 complex, and its reconstitution in vitro from recombinant CDK7 and its partner cyclin H (Fesquet et al., 1993; Poon et al., 1993; Solomon et al., 1993; Fisher and Morgan, 1994; Mäkelä et al., 1994).

Almost immediately, the identity of CAK and the function of CDK7 in vivo were called into question by two independent

discoveries. First, CDK7, cyclin H and a third subunit, the RING finger protein MAT1, were found to be associated with TFIIH (Roy et al., 1994; Fisher et al., 1995; Serizawa et al., 1995; Shiekhattar et al., 1995), as was the budding yeast ortholog of CDK7, Kin28 (Feaver et al., 1994). This discovery ended the search for the protein kinase associated with this general transcription factor, which was known to phosphorylate the CTD in vitro (Feaver et al., 1991; Lu et al., 1992; Serizawa et al., 1992). It also suggested a link between the seemingly unrelated events of CTD phosphorylation and CDK activation, which was an unexpected and to this day controversial notion. A second cause for doubt that CDK7 is a true CAK arose when the expectation that Kin28 would also have this function proved mistaken: *kin28* mutants have no specific defect in cell-cycle progression, and the enzyme has no CAK activity in vitro (Cismowski et al., 1995; Valay et al., 1995). Subsequently, three different laboratories identified the CAK of *S. cerevisiae* as Cak1, a single-subunit kinase related very distantly to the CDKs (Espinoza et al., 1996; Kaldis et al., 1996; Thuret et al., 1996). Although the identification of yeast Cak1, like that of the CDK7 complex, was made through biochemical purification of CAK activity from whole-cell extracts, it was quickly verified genetically (Espinoza et al., 1996; Kaldis et al., 1996; Thuret et al., 1996). Because no such confirmation had yet been obtained in a metazoan system, and because of the conservation of transcriptional function between CDK7 and Kin28, it was suggested that CDK7 might be a CAK only in vitro, and that a Cak1-like kinase remained to be found in higher eukaryotes (Kaldis et al., 1996; Thuret et al., 1996).

In reality, however, no Cak1 ortholog appeared upon complete sequencing of human, fly or worm genomes (Murray and Marks, 2001). Genetics also provided conclusive proof that CDK7 is a CAK in vivo. In *Drosophila*, inactivation of a temperature-sensitive CDK7 mutant (*cdk7*^{P140S}) specifically prevents activation of CDK1 by T-loop phosphorylation and causes embryonic and larval lethality and a block to mitosis in the germline. Adult flies bearing the temperature-sensitive allele are viable and, apart from a cessation of egg laying, are phenotypically nearly normal at the restrictive temperature (Larochelle et al., 1998); this indicates that little or no impairment of transcription is caused by this particular mutation, cell division being non-essential for adult survival. In *Caenorhabditis*, the combination of a temperature-sensitive mutation in *cdk7* with RNA interference (RNAi)-mediated ablation of residual mRNA causes a block to cell division at the one-cell stage, which precedes any requirement for transcription in the embryo (Wallenfang and Seydoux, 2002). In flies, the cell-cycle phenotypes of *cdk7* mutants resemble those of *cdk1* mutants (Larochelle et al., 1998) and, in worms, Seydoux and colleagues demonstrated epistasis between severe *cdk1* and *cdk7* mutations (Wallenfang and Seydoux, 2002).

One CAK or two: the fission yeast variation

Another validation of the CDK7-as-CAK paradigm emerged in fission yeast, but with a potentially revealing twist. Genes encoding orthologs of both CDK7 (Mcs6) and cyclin H (Mcs2) were originally identified in a screen for positive regulators of the G2-M transition in *S. pombe* (Molz et al., 1989; Molz and Beach, 1993). The two proteins were later shown to form an active kinase complex (Buck et al., 1995; Damagnez et al., 1995) and to activate fission yeast CDK1–cyclin-B complexes in vitro (Lee et al., 1999). However, mutations in *mcs6*, *mcs2* or *pmh1* (which encodes the RING finger subunit) cause neither a defect in CDK activation nor cell-cycle arrest (Molz and Beach, 1993; Buck et al., 1995; Damagnez et al., 1995; Lee et al., 1999; Saiz and Fisher, 2002; Bamps et al., 2004; Lee et al., 2005).

The apparent explanation is that another kinase called Csk1, which was originally identified as a high-copy-number suppressor of *mcs2* (Molz and Beach, 1993), is a second CAK in *S. pombe* and can activate both CDK1–cyclin-B (Lee et al., 1999) and Mcs6-Mcs2 complexes (Hermand et al., 1998; Lee et al., 1999). Only upon inactivation of both Csk1 and Mcs6 has it been possible to observe cell-cycle arrest with impaired phosphorylation of the CDK1 T-loop owing to CAK deficiency (Saiz and Fisher, 2002). This suggests that the two CAKs have overlapping functions in vivo. Csk1 is most closely related evolutionarily to budding yeast Cak1 (Liu and Kipreos, 2000), and the two enzymes share distinctive enzymatic features (Tsakraklides and Solomon, 2002). Overexpression of Csk1, moreover, can rescue growth of a budding yeast *cak1* mutant (Hermand et al., 2001).

Curiously, the temperature-sensitive lethality caused by an *mcs6*$^{S165A/L238R}$ mutation – in which mutation of the site phosphorylated by Csk1 (S165A) is combined with the point mutation (L238R) in the original *mcs6-13* allele isolated by Beach and coworkers on the basis of a cell-cycle defect (Molz and Beach, 1993) – is suppressed by overexpression of Cak1 but not of Csk1 (Hermand et al., 2001). This led to the proposal that Csk1 cannot normally activate fission yeast CDK1 in vivo, despite its ability to do so both in vitro (Lee et al., 1999) and in vivo when the two proteins are coexpressed in insect cells (Saiz and Fisher, 2002). This assumes, incorrectly as it turns out, that, by mimicking the lack of Mcs6 T-loop phosphorylation in the absence of Csk1, the *mcs6*$^{S165A/L238R}$ mutation phenocopies the CAK defect of the *mcs6-13 csk1Δ* mutant. The *mcs6*$^{S165A/L238R}$ mutant is in fact still capable of CDK1 activation at the restrictive temperature, but shows transcriptional impairment (Saiz and Fisher, 2002; Lee et al., 2005). Moreover, deletion of *csk1*$^{+}$ is unconditionally lethal in the *mcs6*$^{S165A/L238R}$ background (Saiz and Fisher, 2002). Because the only essential target for CAK in fission yeast is CDK1, the simplest explanation of the synthetic lethality is loss of Csk1-mediated CDK1 activation. It remains possible, however, that Csk1 alone cannot provide all the CAK function the cell needs; testing this idea would require an allele of *mcs6* that completely inactivates its CAK function yet spares its essential function in gene expression – something of a tall order.

Thus, *S. pombe* appears to have two bona fide CAKs – orthologs of metazoan CDK7 and budding yeast Cak1. It therefore seems unique among metazoans and fungi [but see a review that describes a possibly similar situation in plants (Umeda et al., 2005)]. It appears rather perverse that the organism with the smallest genome and the most streamlined cell-cycle machinery should have the most CAKs. What it might reveal, however, is the fallacy of assuming that the numbers of CAKs and CAK targets in an organism are directly proportional – i.e. that complexity at one level of a biochemical pathway automatically begets complexity at another. Instead, fission yeast might compensate for its relative paucity

of distinct CDK-cyclin complexes by maintaining two CAKs, which can sustain two CDK activation pathways that are differentiated, perhaps, by how the two universally required steps – cyclin binding and phosphorylation of the T-loop – are ordered (Fig. 1).

Why might this matter? Even when phosphorylated on its T-loop at Thr160, monomeric CDK2 – the most extensively studied CDK both biochemically and structurally – has negligible kinase activity compared with the phosphorylated CDK2–cyclin-A complex (Fisher and Morgan, 1994; Brown et al., 1999) and is therefore unlikely to have a biological function. An interesting question for future experiments is whether there is any difference, enzymatically, between the same CDK phosphorylated prior to or after binding cyclin. Whether or not the two pathways shown schematically in Fig. 1 actually produce biochemically distinct outputs (i.e. CDK complexes with different activities or specificities), there may be biologically important differences between them. Inhibitory phosphorylation of CDKs at sites within the ATP-phosphate-binding site (Thr14 and Tyr15 in human CDK1 and CDK2) by Wee1 family kinases probably depends on conformational changes induced by cyclin binding that render these residues accessible to solvent (reviewed by Morgan, 1997). Similarly, CDK inhibitors (CKIs) of the CIP/KIP class, such as p21 and p27, bind preferentially to CDK-cyclin complexes, as opposed to CDK monomers (Polyak et al., 1994; Harper et al., 1995). Thus, the inactive intermediate in pathway 1 – the CDK-cyclin complex lacking T-loop phosphorylation – is a better target for at least two mechanisms of negative regulation than the one in pathway 2 – the phosphorylated CDK monomer. It should be pointed out that both inhibitory mechanisms could also operate on the fully active, phosphorylated CDK-cyclin complex, which is the common end product of both pathways.

The sharpness of the G2-M transition crucially depends on tight control of CDK activity during G2 (Dunphy, 1994), which in turn rests on the dominance of inhibitory phosphorylation events over activating ones on assembled CDK1–cyclin-B complexes formed through pathway 1, both in fission yeast and metazoan cell cycles. By contrast, CDK-dependent functions during interphase might be better served by maintaining a pool of phosphorylated CDK monomer, which becomes activated immediately (even if only transiently) upon binding to a cyclin. Operation of both pathways would be ensured in *S. pombe* by the distinct substrate preferences of Csk1 and Mcs6 for CDK monomers versus CDK-cyclin complexes (Tsakraklides and Solomon, 2002). In metazoans, similar flexibility can be achieved because of an expanded repertoire of CDKs; phosphorylation by CDK7 of CDK1, but not of CDK2, depends on prior binding to cyclin (Fisher and Morgan, 1994; Desai et al., 1995). Although purely speculative at this point, the model might help to explain certain phenotypic features of *csk1Δ* fission yeast strains, such as their hypersensitivity to DNA-damaging and replication-blocking agents (Bimbó et al., 2005; H. Gerber, Y. Pikman and R.P.F., unpublished). Similar derangements might also be found in mammalian cells surviving without CDK2 (Berthet et al., 2003; Ortega et al., 2003).

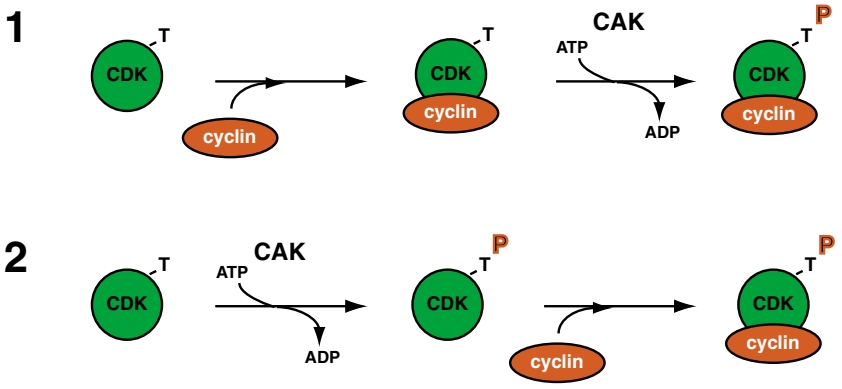

Fig. 1. The ordering of the two universal steps in CDK activation is variable. In pathway 1, binding of cyclin to form an inactive complex precedes phosphorylation within the activation loop by a CAK. This is the obligate pathway for activation of CDK1–cyclin-B complexes by CDK7 in metazoan cells (Fisher and Morgan, 1994; Desai et al., 1995). The steps are switched around in pathway 2, in which a CAK phosphorylates the T-loop of a monomeric CDK, which only becomes active upon subsequent binding of a cyclin. This is the preferred, but not obligate, pathway for activation of CDKs by the fungal CAKs: Cak1 (Ross et al., 2000) and Csk1 (Tsakraklides and Solomon, 2002). It is also a pathway available to metazoan CDK2, which can be efficiently phosphorylated by CDK7 in the absence of a cyclin partner (Fisher and Morgan, 1994; S. Larochelle and R.P.F., unpublished).

The CAK-CDK network: a phylogenetic comparison

A comparison of the CAK-CDK networks of metazoans, fission and budding yeast (Fig. 2A) reveals an underlying conservation of components, with the notable exception of the Cak1/Csk1 ortholog that is absent in higher eukaryotes (see below). It also reveals a divergence in connectivity: either the gain by CDK7 and Mcs6, or loss by Kin28, of CAK function during evolution. That the Mcs6-Mcs2 complex can activate *S. pombe* CDK1 but not mammalian CDKs in vitro supports the former possibility (Lee et al., 1999), perhaps suggesting that CAK activity arose independently in both Mcs6 and CDK7 after their respective CDK targets had diverged.

Supposedly Cak1-like mammalian enzymes have been isolated, but either have remained unidentified (Nagahara et al., 1999; Kaldis and Solomon, 2000) or have weak activity in vitro relative to CDK7 (Liu et al., 2004), and so are unlikely to represent the true missing link between fungal and metazoan CDK activation. In budding yeast, when the essential function of CAK is bypassed by mutations within the *CDC28* gene encoding CDK1 (Cross and Levine, 1998), *cak1* mutants display defects during vegetative growth as a result of diminished function of other CAK targets involved in transcription, such as Bur1 (Yao and Prelich, 2002) and Ctk1 (Ostapenko and Solomon, 2005), and meiotic defects associated with impaired activation of the sporulation-specific kinase Ime2 (Schindler et al., 2003). *S. pombe* strains lacking *csk1*$^{+}$ are viable (Molz and Beach, 1993) but display cold sensitivity, poor growth on minimal media and sensitivity to DNA-damaging agents (Bimbó et al., 2005). Metazoans might

have adapted to life with a single CAK by replacing the regulatory and stress-responsive functions of Cak1/Csk1 at other points in the network.

Multi-task or die: how CDK7 leads a double life and gets away with it

The two enzymatic functions of metazoan CDK7 seem very different. In its cell-cycle role, CDK7 is an upstream regulator, causing a conformational change that boosts CDK activity ~300-fold in the case of the human CDK2–cyclin-A complex (Russo et al., 1996). In transcription, CDK7 apparently behaves more like one of its targets – i.e. as a downstream effector kinase. This would perhaps not be so surprising, if the two types of substrate – CDK activation loops and the CTD – looked alike, but they do not (Poon and Hunter, 1995). The heptad repeat units of the CTD consist of the sequence YSPTSPS, within which CDK7 is selective for the serine residue at position 5 (Roy et al., 1994; Trigon et al., 1998; Rickert et al., 1999; Ramanathan et al., 2001; Pinhero et al., 2004). CDK7 phosphorylates this residue selectively within a single heptad repeat and efficiently within synthetic peptides containing as few as two repeats (Trigon et al., 1998). The sequence of the heptad thus appears to be all that is required to direct phosphorylation by CDK7.

CDK7 probably adopts a different mechanism to target its CDK substrates, because recognition of a consensus sequence around the phosphorylation site (shown in italics) cannot account for why human CDK7 phosphorylates CDK1 (Arg-Val-Tyr-*Thr*-His-Glu-Val), CDK4 (Met-Ala-Leu-*Thr*-Pro-Val-Val) and CDK6 (Met-Ala-Leu-*Thr*-Ser-Val-Val), but not the sequence Arg-Ala-Tyr-*Thr*-His-Gln-Val at the same position within its own activation loop. Another property not easily explained by direct recognition of the T-loop is the ability to phosphorylate the sequence Arg-Thr-Tyr-*Thr*-His-Glu-Val in CDK2 monomers, but to phosphorylate the nearly identical sequence in CDK1 only in the context of a CDK1-cyclin complex (Fisher and Morgan, 1994). Finally, short peptides containing the T-loop sequence of human CDK2 are poor substrates for CDK7 complexes in vitro (S. Larochelle and R.P.F., unpublished), which is also consistent with the absence of a consensus recognition sequence.

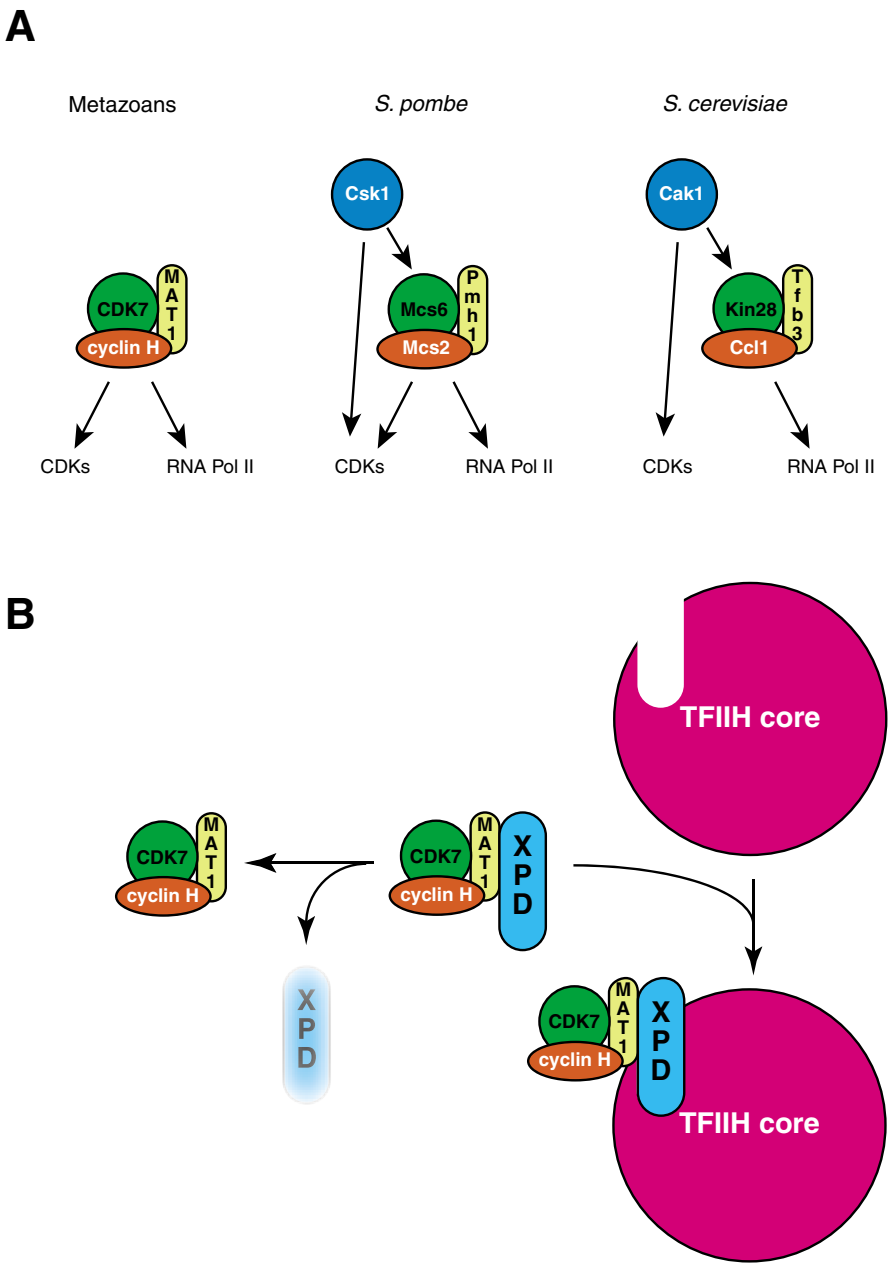

Fig. 2. Alternative strategies to link CDK activation to RNA polymerase II (Pol II) phosphorylation. (A) The TFIIH-associated CDK-cyclin-RING protein complex has been conserved throughout eukaryotic evolution but is incapable of activating cell-cycle CDKs in budding yeast. The monomeric kinase responsible for all known CDK-activating phosphorylation in *S. cerevisiae* (Cak1) has a non-essential ortholog (Csk1) with similar functions in *S. pombe*, but there is no such enzyme in metazoan cells. Thus, the CAK-CDK networks controlling cell division and gene expression are connected at different levels depending on the organism: directly by a common enzyme that is both a CAK and a CTD kinase in most eukaryotes, and by the upstream CAK common to both transcriptional and cell-cycle CDKs in budding yeast. Fission yeast has both connections. (B) The XPD helicase tethers, and may actively recruit, the CDK7 complex to the TFIIH core. XPD has also been proposed to sequester CAK in the cytoplasm during interphase in *Drosophila* embryos; its degradation by an unknown mechanism might release CDK7 to sustain the high levels of CDK activation needed during mitosis.

Surprisingly, CDK7 activity towards a CTD substrate can nevertheless be inhibited in vitro by a peptide derived from the T-loop of CDK2, in which Ala substitutes for the activating Thr residue (Cujec et al., 1997; Okamoto et al., 2000). Perhaps this peptide does not prevent CTD phosphorylation by binding to the active site of CDK7 as a pseudosubstrate (as was supposed). Alternatively, it could compete directly for binding to the CTD substrate with the T-loop of CDK7, which has a similar sequence, and which probably plays an important role in positioning the CTD for the phosphorylation reaction.

Direct evidence that CDK7 is insensitive to the sequence surrounding the sites it phosphorylates in CDKs came from a T-loop swap. When the sequence of the CDK2 activation loop is replaced with one derived from CDK7, CDK7 can still phosphorylate and activate the chimeric CDK (Garrett et al., 2001), despite the fact that the CDK7 T-loop is not normally a substrate for auto-phosphorylation.

CDK7 phosphorylates CDK2 and a fusion protein containing the Pol II CTD with different apparent kinetics (Larochelle et al., 2001): it exhibits high affinity for the CDK2 substrate (K_m ~0.2 μM) with slow turnover (k_{cat} ~0.03 s^{-1}), but exhibits lower affinity for the CTD substrate (K_m ~4 μM) and more rapid turnover (k_{cat} ~4 s^{-1}). The slow turnover of the CDK substrate probably reflects slow release of the phosphorylated CDK product, as indicated by the formation in vitro of a stable quaternary complex containing CDK7–cyclin-H complexes and CDK2–cyclin-A complexes phosphorylated at Thr160 (Russo, 1996). An interesting question for future investigations is whether such a persistent association with CDK7 complexes targets activated CDKs to their sites of action in vivo.

Wanted: a CDK9-activating kinase

To date, metazoan CDK7 has only been shown to activate the CDKs dedicated to cell-cycle control, leaving open the possibility that the CDKs primarily involved in transcription have their own activators, or depend on other mechanisms to achieve full activity. The latter is clearly the case for CDK8 and its budding yeast ortholog Srb10, in which the position in the T-loop normally taken by a phosphoacceptor is instead occupied by an aspartate residue (Liao et al., 1995; Tassan et al., 1995b). CDK7 itself is an interesting case because it can be activated by its targets CDK1 and CDK2 in vitro (Garrett et al., 2001). This suggests a feedback mechanism to allow the cell-cycle CDKs to influence gene expression.

CDK9, the catalytic subunit of positive transcription elongation factor P-TEFb (reviewed by Price, 2000; Garriga and Grana, 2004), is a candidate for activation by a CAK, but attempts to detect phosphorylation of human CDK9 by CDK7 complexes in vitro have been unsuccessful (Chen and Zhou, 1999; Kim and Sharp, 2001; Chen et al., 2004). In budding yeast, the two putative orthologs of CDK9 – Bur1 and Ctk1 – are targets of Cak1 (Yao and Prelich, 2002; Ostapenko and Solomon, 2005), whereas fission yeast CDK9 is a target for activation by Csk1 but not by Mcs6 (Y. Pei, H. Du, J. Singer, S. Shuman and R.P.F., unpublished). An important question for the future is whether a relative of the monomeric CAKs of fungi will finally emerge as the CDK9-activating kinase of metazoans or whether P-TEFb phosphorylation will turn out to be in CDK7's job description.

Keeping separate functions separated: substrate-specific regulation of CDK7 activity

Early reports suggested that CDK7 changes its substrate specificity to favor the CTD over CDKs upon binding to MAT1 or incorporation into the TFIIH holoenzyme (Adamczewski et al., 1996; Rossignol et al., 1997; Yankulov and Bentley, 1997). Subsequent studies revealed that CTD kinase activity is selectively stimulated, with no loss of CAK activity, and that this requires both the presence of MAT1 and phosphorylation of CDK7 itself at its activating residue, Thr170 (Larochelle et al., 2001). Although CDK7 exists in both TFIIH-bound and free forms in cell-free extracts derived from several organisms (Fisher et al., 1995; Drapkin et al., 1996; Reardon et al., 1996; Larochelle et al., 2001) and can undergo fluctuations in the phosphorylation state of the T-loop (S. Larochelle and R.P.F., unpublished), evidence for a dimeric complex that lacks MAT1 in vivo is scant. It is therefore unlikely that the activity of the CDK7 complex is influenced by cycles of MAT1 association and dissociation/degradation in vivo, but its regulation by T-loop phosphorylation is a real possibility. Mutation of Thr170 of *Drosophila* CDK7 to alanine causes anomalies in gene expression during embryogenesis and morphogenetic defects in the wing when the mutant protein is overexpressed (Leclerc et al., 2000). When the same allele is expressed at physiological levels, in place of the wild-type version, it causes temperature-sensitive lethality at all stages of development, including adult (Larochelle et al., 2001), in contrast to the specifically CAK-deficient $cdk7^{P140S}$ allele, which is not lethal in adults (Larochelle et al., 1998). Another defect seen in the $cdk7^{T170A}$ mutant is impaired CTD hyperphosphorylation in response to heat shock in larval salivary glands (Larochelle et al., 2001). Thr170 phosphorylation could therefore be a crucial transcriptional switch in vivo.

Other phosphorylations have been reported to modulate the kinase activity of TFIIH-associated CDK7 in vivo. Phosphorylation at Ser164, a CDK consensus site within the CDK7 T-loop, apparently increases in cells treated with nocodazole, and has been proposed to account for the inhibition of TFIIH-associated CDK7 activity implicated in the global repression of transcription that occurs when mammalian cells enter mitosis (Akoulitchev and Reinberg, 1998). This model is attractive because TFIIH, but not free CAK, appears to be a target for inhibition by CDKs active in mitotic extracts (Long et al., 1998), and Ser164 is a proven site of phosphorylation by CDK1 and CDK2 in vitro (Garrett et al., 2001). However, the model is difficult to reconcile with genetic and biochemical evidence that phosphorylation at Ser164 is not inhibitory but instead stabilizes trimeric CDK7 complexes (Fisher et al., 1995; Martinez et al., 1997; Larochelle et al., 2001).

Another proposed mode of CDK7 inhibition is the phosphorylation of cyclin H by CDK7 itself (Lolli et al., 2004) or by another kinase (Akoulitchev et al., 2000). The CDK8–cyclin-C pair, associated with a subset of Pol II mediator complexes, was reported to phosphorylate cyclin H in vitro and thus inhibit the kinase activity of the CDK7–cyclin-H pair towards both CTD and CDK substrates and repress basal transcription in a reconstituted system (Akoulitchev et al., 2000). Whether this regulatory mechanism operates in vivo has not been established, in part because it has not been tested in a genetically tractable system; the suspected

sites of CDK8-mediated phosphorylation on human cyclin H are not conserved in lower eukaryotes (Akoulitchev et al., 2000).

CDK7 in cell-cycle control: constitutive or regulatory?

Whereas phosphorylation and dephosphorylation of the T-loop can modulate the CTD kinase activity of CDK7 over a ~20-fold range, no similarly potent effector of CDK7-associated CAK activity has been described. The abundance of all three CAK subunits – CDK7, cyclin H and MAT1 – appears constant throughout the cell cycle, as does the ability of mammalian cell extracts to activate exogenous CDKs in vitro (Brown et al., 1994; Poon et al., 1994; Tassan et al., 1994; Tassan et al., 1995a). Phosphorylation of the CDK7 T-loop likewise does not fluctuate during the cell cycle in human (Garrett et al., 2001) or *Drosophila* (Larochelle et al., 2001) cell extracts. A picture therefore emerged of CDK7 (and of CAK in general) as a constitutive activator – rather than a true regulator – of the CDKs that control cell division (Harper and Elledge, 1998; Murray and Marks, 2001).

Such a view presumes regulation is always due to relatively large changes in the intrinsic ability of effectors to act on their targets from one point in the cell cycle to another. This is a test that a general CAK such as CDK7 might fail, because it is needed to activate CDKs throughout the cell cycle. This does not necessarily mean, however, that the activation of CDKs by CAK is constitutive. Recently, studies of *Drosophila* CDK7 have revealed how CAKs might be regulated, and in turn perform regulation, without experiencing large fluctuations in their levels or activities.

As noted above, the temperature-sensitive $cdk7^{P140S}$ allele in *Drosophila* blocks cell division in the adult germline, as well as in proliferating cells of embryos and larvae, without critically impairing transcription at the restrictive temperature (Larochelle et al., 1998). The embryonic lethal cell-cycle defect can be suppressed by deletion of a single copy of *xpd*, which encodes one of two DNA helicases associated with TFIIH (Chen et al., 2003). This suggested that the cellular levels of XPD (also known as ERCC2) regulate the CAK function of CDK7. Overexpression of XPD in wild-type *Drosophila* causes only modest reductions in CAK activity measured in vitro and in CDK1 T-loop phosphorylation in vivo. However, it has dramatic effects on viability and produces pleiotropic defects in mitosis in early embryos. XPD appears to be degraded in wild-type embryos between prophase and metaphase of the first cell division after the onset of zygotic gene expression, which coincides with a redistribution of CDK7 from the cytoplasm to the nucleus. Significantly, CDK7 is mislocalized in mitotic cells overexpressing XPD (Chen et al., 2003).

XPD might thus sequester CAK in the cytoplasm during interphase and then be degraded, ensuring both adequate levels and proper localization of CDK7 as cells enter and progress through mitosis (Fig. 2B). How generally applicable such a model is, and how the relevant signals (e.g. the determinants of XPD proteolysis or CAK targeting) are generated in a cell-cycle-regulated manner remain unclear. Nevertheless, the case for true regulation of CDK7 by XPD is firmly grounded in genetics, as shown by the allele-specific suppression of a *cdk7* point mutant by a 50% reduction in *xpd* gene dosage (Chen et al., 2003). The identification of a discrete quaternary complex consisting of the CAK trimer (CDK7–cyclin-H–MAT1) and XPD in both mammalian (Drapkin et al., 1996; Reardon et al., 1996) and *Drosophila* (Larochelle et al., 2001) cell extracts also supports this idea.

The essential role of CDK7 in transcription: just how general is it?

As the CTD kinase associated with the general transcription factor TFIIH, CDK7 has long been suspected of playing an indispensable catalytic role in transcribing most, if not all, class II genes. The belief that CDK7 is globally required for transcription by Pol II in vivo is based largely on microarray hybridization studies in budding yeast, in which the large majority of Pol II transcripts are repressed upon thermal inactivation of a temperature-sensitive Kin28 (Holstege et al., 1998). Exceptions to this behavior (e.g. promoters refractory to inactivation of the TFIIH-associated kinase) exist in budding yeast (Lee and Lis, 1998; McNeil et al., 1998) and in *Drosophila* (Schwartz et al., 2003). Transcription of some mammalian genes in vitro is likewise independent of CDK7 catalytic activity (Mäkelä et al., 1995; Tirode et al., 1999), and $Mat1^{-/-}$ mouse cells are not devoid of Pol-II-dependent transcription in vivo (Rossi et al., 2001; Korsisaari et al., 2002). Recently, a second genome-wide expression analysis, in fission yeast, revealed a more restricted set of genes crucially affected by inactivation of the TFIIH-associated kinase. Only ~5% of all fission yeast transcripts, but most or all of the transcripts in a cell-cycle-regulated cluster of genes important for cell division (Rustici et al., 2004), were repressed more than twofold in severe *mcs6* or *pmh1* mutants (Lee et al., 2005).

The relative insensitivity of most Pol-II-mediated transcription to inactivation of the Mcs6 complex might reflect compensation by other CTD kinases, such as the recently identified ortholog of metazoan CDK9 (Pei et al., 2003; Pei and Shuman, 2003). Synthetic genetic interactions between orthologs of CDK7 and CDK9 in both budding and fission yeast (Lindstrom and Hartzog, 2001; H. Du, C. St. Amour and R.P.F., unpublished) indicate a degree of functional overlap. Moreover, both pharmacological inhibition of CDK9 and genetic inactivation of CDK7 are required to abolish Ser5 phosphorylation of Pol II engaged in transcription of heat shock genes in *Drosophila* larval salivary glands (Ni et al., 2004). Functional overlap among the CDKs that phosphorylate the Pol II CTD to influence transcription is still hypothetical but would be reminiscent of the apparent redundancy among the cyclins and CDKs that control cell-cycle progression (reviewed by Morgan, 1997; Murray, 2004).

The stark difference in results between similar experiments in budding and fission yeast raises an obvious question: which paradigm (if either) applies in metazoans? Is the requirement for CDK7 activity in transcription a general one, as in *S. cerevisiae*, or a more selective one, as appears to be in *S. pombe*? The preferential involvement of the TFIIH-associated kinase in specific programs of gene expression could be due to differential requirements for CTD phosphorylation at different promoters and/or the phosphorylation by CDK7 of other targets. In metazoans, CDK7 phosphorylates several sequence-specific DNA-binding transcription factors, including retinoic

acid receptor α [RAR-α (Rochette-Egly et al., 1997)], the tumor suppressor p53 (Ko et al., 1997; Lu et al., 1997), octamer transcription factors (Inamoto et al., 1997), the cell-cycle regulator E2F-1 (Vandel and Kouzarides, 1999), estrogen receptor [ER (Chen et al., 2000)], Ets1 (Drane et al., 2004) and the peroxisome-proliferator-activated receptors [PPARs (Compe et al., 2005)].

Of the sequence-specific factors reported to be targets of CDK7, the most extensively studied are the nuclear receptors – RAR-α, ER and the PPARs – which contain sites for phosphorylation by CDK7 (and perhaps other proline-directed kinases) within the conserved activation function 1 (AF1) domain (Rochette-Egly et al., 1997; Chen et al., 2000; Compe et al., 2005), mutation of which to alanine diminishes ligand-dependent transcription. The evidence that phosphorylation, specifically by CDK7, might be important for regulation of nuclear receptor function once again hinges on XPD, which plays an important structural role in tethering the CDK7–cyclin-H–MAT1 trimer to the core subunits of TFIIH (Coin et al., 1999) (Fig. 2B). Specific mutations in the gene encoding XPD, associated with the human diseases xeroderma pigmentosum and trichothiodystrophy, lead to dysregulation of nuclear-receptor-dependent gene expression and apparently decreased phosphorylation of RAR-α (Keriel et al., 2002) and PPARs α and γ (Compe et al., 2005). In an interesting variation on this theme, expression of a gene dependent on vitamin D_3 receptor (VDR) – a nuclear receptor that lacks an AF-1 region – is also defective in *xpd* mutant cell lines, apparently because of impaired TFIIH-dependent phosphorylation of another DNA-binding transcription factor, Ets1, which is required in turn for efficient recruitment of VDR to the promoter (Drane et al., 2004).

CAKs as drug targets: sledgehammer, magic bullet or double-edged sword?

There is no Cak1- or Csk1-like enzyme encoded in the human genome (Murray and Marks, 2001). Cak1 from the pathogenic fungus *Candida albicans*, like its counterparts in *S. cerevisiae* and *S. pombe*, is resistant both to drugs and substitutions of conserved residues that inactivate nearly all other serine/threonine kinases (Tsakraklides and Solomon, 2002). These features make the monomeric CAKs attractive targets for the development of new antifungal drugs.

Can the CAK-CDK pathway of metazoans be similarly targeted for pharmacological inhibition, in attempts to stop or slow division of tumor cells? Any consideration of CDK7 as a potential drug target must take into account its dual functions in cell-cycle control and transcription. Were CDK7 performing a general function in gene expression, as has long been supposed (and might still be the case), inhibiting it might be prohibitively toxic to normal cells. However, such initially pessimistic assessments should be re-evaluated, for two reasons.

First, as discussed above, it is possible to separate the two functions of the TFIIH-associated CAK genetically, by specific mutations that selectively impair only one process (Larochelle et al., 1998; Lee et al., 1999; Saiz and Fisher, 2002); temporally, on the basis of changing requirements for cell division and transcription at different times during development (Larochelle et al., 1998; Wallenfang and Seydoux, 2002); and biochemically, by manipulating subunit composition and modification state of the CDK7 complex (Adamczewski et al., 1996; Rossignol et al., 1997; Yankulov and Bentley, 1997; Larochelle et al., 2001). Selective inhibition of CAK or CTD kinase activity now seems at least possible, in light of the apparently distinct modes – and presumably different surfaces of the CDK7–cyclin-H–MAT1 complex – with which the enzyme recognizes its two classes of substrate. The recent X-ray crystal structure of monomeric, inactive CDK7 (Lolli et al., 2004) should pave the way to additional structures of the kinase in its various active conformations, which could illuminate those crucial substrate-interaction motifs. For a recent review of CDK7 from a structural perspective, see (Lolli and Johnson, 2005).

The second cause for renewed optimism is the recent work, also described above, challenging the notion that all (or even most) transcription by Pol II depends on the catalytic activity of CDK7. If the fission yeast paradigm applies to mammals – i.e. if CDK7 activity preferentially affects transcripts needed by dividing cells – targeting this kinase for anti-tumor therapy would be doubly attractive. Inhibition of CDK7 could simultaneously deprive tumor cells of the high CAK activity they might need to carry out faithful mitoses (Chen et al., 2003), and crucially limit the synthesis of mRNAs required for other steps in cell division (Lee et al., 2005), without shutting down transcription globally in nondividing cells. That said, the nuclear receptors identified as putative targets for positive regulation by mammalian CDK7 have pleiotropic functions and important roles in differentiation of various tissues. Interference with these functions will need to be carefully calibrated; further systematic analysis of the role of CDK7 in mammalian gene expression is therefore needed.

I have tried here to review the literature on metazoan CDK7 comprehensively but concisely, while introducing relevant observations concerning the fungal orthologs. To my colleagues whose work on those topics I have omitted, and to those studying CAK in plants, I apologize. I thank M. Gamble, H. Gerber, S. Larochelle and the two anonymous referees for critical review and comments, and all the members of my laboratory, past and present, for their contributions. Work in the lab is supported by NIH grants GM56985 and DK45460 and by American Cancer Society Grant RSG-99-043-044-CCG.

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