

The cyclin-dependent kinase family in the social amoebozoan *Dictyostelium discoideum*

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Abstract Cyclin-dependent kinases (Cdk) are a family of serine/threonine protein kinases that regulate eukaryotic cell cycle progression. Their ability to modulate the cell cycle has made them an attractive target for anti-cancer therapies. Cdk protein function has been studied in a variety of Eukaryotes ranging from yeast to humans. In the social amoebozoan *Dictyostelium discoideum*, several homologues of mammalian Cdks have been identified and characterized. The life cycle of this model organism is comprised of a feeding stage where single cells grow and divide mitotically as they feed on their bacterial food source and a multicellular developmental stage that is induced by starvation. Thus it is a valuable system for studying a variety of cellular and developmental processes. In this review I summarize the current knowledge of the Cdk protein family in *Dictyostelium* by highlighting the research efforts focused on the characterization of Cdk1, Cdk5, and Cdk8 in this model Eukaryote. Accumulated evidence indicates that each protein performs distinct functions during the *Dictyostelium* life cycle with Cdk1 being required for growth and Cdk5 and Cdk8 being required for processes that occur during development. Recent studies have shown that *Dictyostelium* Cdk5 shares attributes with mammalian Cdk5 and that the mammalian Cdk inhibitor roscovitine can be used to inhibit Cdk5 activity in *Dictyostelium*. Together, these results show that *Dictyostelium* can be used as a model system for studying Cdk protein function.

Keywords Cyclin-dependent kinase · Mitosis · Growth · Development · Cell cycle · *Dictyostelium*

Abbreviations

AcaA	Adenylyl cyclase A
cAMP	3'-5'-cyclic adenosine monophosphate
CaM	Calmodulin
CaMBP	Calmodulin-binding protein
CaMBD	Calmodulin-binding domain
carA	3'-5'-cyclic adenosine monophosphate receptor A
Cdk	Cyclin-dependent kinase
CycB	Cyclin B
CycC	Cyclin C
Disc1	Discoidin 1
PKAc	Protein kinase A catalytic subunit
PsaA	Puromycin-sensitive aminopeptidase A

Cyclin-dependent kinases

The cyclin-dependent kinases (Cdk) belong to a family of serine/threonine protein kinases that were first discovered for their role in regulating eukaryotic cell cycle progression [1, 2]. Cdks are present in all eukaryotic cells and their kinase activity is regulated through their association with cyclins, which also function to recruit substrates [1, 2]. In mammals, the main cell cycle regulator is Cdk1, however additional Cdks (i.e., Cdk2, Cdk4, and Cdk6) are required for normal development and the proliferation of specialized tissues [3]. Due to their ability to regulate the cell cycle and the fact that tumor cells evade anti-growth signals, members of the Cdk protein family are potential targets for anti-cancer therapies [4, 5]. Cdk1 is the most well studied Cdk and its function is best understood in the model organism

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Saccharomyces cerevisiae where the protein controls the cell cycle by interacting with nine different cyclins (i.e., three G1 cyclins and six B-type cyclins) [3]. In yeast, Cdk1 also regulates DNA replication, chromosome segregation, transcription, and cell morphogenesis and polarization [3]. There are approximately 75 *bona fide* and over 300 potential Cdk1 targets [6].

Although most Cdks regulate the cell cycle, a subset of Cdks, namely Cdk7, Cdk8, and Cdk9, regulate gene expression through direct interactions with the transcription machinery. In mammals, Cdk8 represses transcription by phosphorylating the carboxy terminal domain of RNA polymerase II [7]. However, Cdk8 has also been reported to activate transcription through the phosphorylation of specific transcription factors [8]. Cdk8 associates with cyclin C to form part of the Mediator complex, which controls both basal and regulated transcription and is composed of at least 36 other proteins that are highly conserved from yeast to human [7, 9]. Like Cdk8, Cdk5 is another member of the Cdk protein family that possesses functions unrelated to the cell cycle. Unlike other members of the mammalian Cdk protein family, Cdk5 is not activated by cyclins. Instead, the protein is activated through its association with the neuron-specific activator molecules p35, p39, and p67 [10]. Cdk5 functions in a diversity of cellular processes including axon guidance, neurite outgrowth, insulin secretion, lens differentiation, membrane transport, myogenesis, and ubiquitin-dependent degradation [11–14]. In addition, unlike other Cdks whose functions have been implicated in tumor growth, Cdk5 dysregulation has been linked to neurodegenerative diseases including amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease [15–17].

The life cycle of *Dictyostelium discoideum*

Dictyostelium discoideum is a fascinating organism that has been chosen by the National Institutes of Health as part of its model organism initiative for biomedical and human disease research [18, 19]. This model Eukaryote undergoes an asexual life cycle comprised of a vegetative feeding stage where single cells grow and divide mitotically as they chemotactically respond to folic acid, which is secreted by their bacterial food source and a multicellular developmental stage that is induced by starvation [20]. The *Dictyostelium* cell cycle does not possess a discernible G1 phase during growth and almost all cells are in G2 during both growth and development [21]. Growing cells released from stationary phase enter a short S phase lasting less than 30 min. This is followed by a long G2 phase that lasts 6.5 h on average. Cells then enter M phase, which lasts about 12 min. Upon starvation, cells enter a developmental program that begins

with the secretion of 3'-5'-cyclic adenosine monophosphate (cAMP), which acts as a chemoattractant causing cells to aggregate into mounds [20]. Aggregated cells then develop into a motile, multicellular structure known as a pseudoplasmodium or slug. Cells destined to become spores (i.e., pre-spore cells, 80 % of the total cell population) sort to the middle and posterior regions of the slug, while cells destined to become stalk (i.e., pre-stalk cells, 20 % of the total cell population) sort to the anterior region. When conditions are suitable, the slug will develop into a fruiting body comprised of a mass of spores that is supported by a stalk of dead cells. Two periods of cell division occur during *Dictyostelium* development, one during the early stages of development and the other after multicellular structures have formed [22]. However, only the second period of cell division has been shown to be a result of mitosis [23]. The observed increase in cell number during early development is not due to mitosis, but instead is a result of multinucleated cells undergoing cytokinesis to complete their final growth-stage cell cycle [23].

In addition to its involvement in the regulation of growth, the cell cycle also influences cell type differentiation in *Dictyostelium* [24–29]. This is an interesting feature of the *Dictyostelium* life cycle, which has recently been reviewed [30, 31]. Cells within a growing population will be in different phases of the cell cycle (i.e., S, early G2, mid-G2, late G2, M). However, since the G2 phase in *Dictyostelium* is relatively long compared to the S and M phases, the majority of cells will be in G2 [25]. Upon starvation, cells in M, S, or early G2 tend to form pre-stalk cells, while cells in mid- or late G2 preferentially form pre-spore cells. Unlike growth, there is evidence for a G1 phase during *Dictyostelium* development [23, 32]. Pre-stalk cells undergo mitosis just before stalk formation (i.e., after 22 h) and arrest in G1 prior to terminal differentiation [23]. Pre-spore cells undergo mitosis between 12 and 20 h of development [23], however whether these cells arrest in G1 or G2 prior to terminal differentiation remains unclear. Chen et al. [23, 32] have reported that pre-spore cells arrest in G1 prior to encapsulation. Their studies, which were based upon cellular DNA content profiles obtained by flow cytometry and quantification of extra-chromosomal and chromosomal DNA, also showed that *Dictyostelium* cells can carry out the entire developmental sequence in G1 [32]. In contrast, MacWilliams et al. [33] used direct fluorescence measurements of DAPI-stained nuclei to show that the nuclear DNA content of cells and spores do not differ significantly. Since vegetative cells are largely in G2, it follows that the spores under their experimental conditions would also be in G2 [33]. These results were supported by Muramoto and Chubb [34] who introduced a live cell S-phase marker into developing cells (i.e., fluorescently tagged replication factor, proliferating cell nuclear

Table 1 List of Cdk homologues in *Dictyostelium discoideum*

Gene	Identified in <i>Dictyostelium</i> ?	dictyBase gene ID	Function(s)
<i>cdk1</i>	Yes	DDB_G0272813	Growth [36, 39] Early development? [36, 39]
<i>cdk2</i>	No ^a	N/A	N/A
<i>cdk3</i>	No	N/A	N/A
<i>cdk4</i>	No	N/A	N/A
<i>cdk5</i>	Yes	DDB_G0288677	Growth [42–44] Mid development [42, 44] Late development [42, 44]
<i>cdk6</i>	No	N/A	N/A
<i>cdk7</i>	Yes	DDB_G0285417	Uncharacterized
<i>cdk8</i>	Yes	DDB_G0267442	Growth [84, 86] Early development [84–86] Late development [84–86]
<i>cdk9</i>	Yes	DDB_G0273207	Uncharacterized
<i>cdk10</i>	Yes	DDB_G0268480	Uncharacterized
<i>cdk11</i>	Yes	DDB_G0283279	Uncharacterized

^a *Dictyostelium* Cdk1 shares strong sequence similarity with both mammalian Cdk1 and Cdk2, but the genome does not contain separate genes for Cdk1 and Cdk2

antigen). They reported that germinating spores entered S-phase only after their first round of mitosis, indicating that the spores were in G2 [34]. In addition, while it is generally accepted that DNA replication does occur during development, the source of the DNA synthesis (i.e., nuclear or mitochondrial) is controversial with contradictory results reported in the literature [24, 32–35]. Zimmerman and Weijer [24] labeled developing cells with the thymidine analog 5'-bromodeoxyuridine and reported that 75 % of cells from axenically grown cultures go through S-phase. A later study by Shaulsky and Loomis [35] reported that while DNA synthesis does occur in pre-spore cells, the source of the DNA synthesis is mitochondrial, not nuclear. Unfortunately, more recent studies have yet to clarify this feature of the *Dictyostelium* life cycle. Chen and colleagues [32] reported that although substantial mitochondrial DNA synthesis does occur in pre-spore cells, chromosomal DNA synthesis is not observed. Using their imaging approach Muramoto and Chubb [34] reported that a large proportion of cells undergo nuclear DNA synthesis during development. As discussed previously, their study also showed that spores arrest in G2 [34]. Since the majority of cells upon starvation are in G2, and it is known that at least one round of mitosis occurs during the later stages of development, their results indicate that S-phase does occur during development [34]. Taken together, it has become increasingly evident that this aspect of the *Dictyostelium* life cycle requires further study to resolve and clarify the conflicting reports presented in the literature. In addition, although it is well established that the cell cycle influences cell type differentiation in *Dictyostelium* the function(s) (if any) of Cdk1 and cyclins during this process is unknown.

The *Dictyostelium* genome has been sequenced and several homologues of mammalian Cdk1 have been identified (Table 1). The most well studied of these homologues include Cdk1, Cdk5, and Cdk8, which will be discussed in detail below and will be the focus of this review. The *Dictyostelium* genome also encodes putative Cdk1s that have not yet been characterized (e.g., Cdk7, Cdk9, Cdk10, and Cdk11) as well as a number of cyclins and Cdk1-binding partners (Tables 1, 2). Some of these binding partners have been verified as *bona fide* Cdk1-binding proteins and will also be discussed in the review. The genome, however, does not contain obvious homologues of mammalian Cdk2, Cdk3, Cdk4, or Cdk6 (Table 1).

Cyclin-dependent kinase 1

Cdk1 functions during *Dictyostelium* growth

In *Dictyostelium*, Cdk1 functions primarily during vegetative growth (Fig. 1) [36]. *cdk1* mRNA and protein is expressed at a constant level during all phases of axenic growth (i.e., exponential and stationary phases) [37]. During development, the level of *cdk1* mRNA increases during aggregation and then decreases to low levels by the slug stage, eventually disappearing during the terminal stages of differentiation [36–38] (Fig. 2). The kinase activity of Cdk1 has been verified and the protein level remains constant during development up to 16 h, after which time the amount of Cdk1 protein decreases significantly [36]. Despite the fact that *cdk1* mRNA and protein are expressed during development, there is little evidence to suggest that

Table 2 List of identified and putative Cdk-binding partners in *Dictyostelium discoideum*

Gene	Protein	dictyBase gene ID	Binding partner
<i>cycA</i>	Cyclin A	DDB_G0279085	Cdk1 (predicted)
<i>cycB</i>	Cyclin B	DDB_G0275493	Cdk1 [36, 40]
<i>cycC</i>	Cyclin C	DDB_G0274139	Cdk8 [87] ^a
<i>cycD</i>	Cyclin D	DDB_G0277439	Cdk1 (predicted)
<i>cycH</i>	Cyclin H	DDB_G0268668	Cdk7 (predicted)
<i>cycK</i>	Cyclin K	DDB_G0286617	Cdk9 (predicted)
<i>cycL</i>	Cyclin L	DDB_G0285553	Cdk11 (predicted)
<i>psaA</i>	Puromycin-sensitive aminopeptidase	DDB_G0270994	Cdk5 [43]
<i>calA</i>	Calmodulin	DDB_G0279407	Cdk5 [60]
<i>cksl</i>	Cyclin-dependent kinases regulatory subunit	DDB_G0271642	Unknown

^a Although a direct interaction between Cdk8 and CycC has not yet been shown, the two proteins are detected in high molecular weight fractions isolated from *Dictyostelium* nuclei

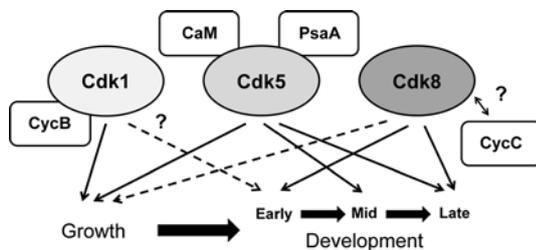


Fig. 1 The functions of Cdk1, Cdk5, and Cdk8 during *Dictyostelium* growth and development. Cdk1 interacts with CycB and is required for growth. Its function during early development remains unclear (i.e., indicated by a *dashed arrow* and *question mark*). Cdk1 activity is also dependent on CycB expression levels. Cdk5 is a CaMBP that also binds to PsA. Cdk5 is required for efficient growth and for mid- and late developmental processes. Cdk8 is required for early and late developmental processes. Cdk8 also functions during growth, however its activity is not essential for this cellular process (i.e., indicated by a *dashed arrow*). Cdk8 and CycC are detected in high molecular weight complexes isolated from *Dictyostelium* nuclei, however a direct interaction between Cdk8 and CycC has not yet been shown

Cdk1 is involved in regulating developmental processes, including the mitotic events that occur during later developmental stages [22, 36, 37, 39]. Although Luo et al. [36] reported an increase in Cdk1 histone H1 kinase activity during early development, a follow-up study by Sharma et al. [39] concluded that this increase in kinase activity is likely not important for *Dictyostelium* development. This conclusion was based upon observations of cells that expressed a dominant-negative mutant *cdk1* gene under the control of the discoidin promoter, which drives gene expression only during growth and early development [38]. Thus the mutant Cdk1 protein was only expressed during these stages of the life cycle. Although the mutant cells showed slow rates of growth, thus supporting a function for Cdk1 during cell proliferation, they were observed to develop and differentiate normally [39]. In other words,

expression of the mutated Cdk1 protein during early development did not negatively affect later developmental processes. It is important to note that the *cdk1* mutant cells still expressed endogenous Cdk1. Therefore it is likely that the effect of the mutant Cdk1 protein during growth and early development was somewhat mitigated by the presence of the endogenous protein. Furthermore, the absence of an increase in Cdk1 histone H1 kinase activity during later development coupled with the relatively low expression of *cdk1* mRNA and protein during this stage of the life cycle, also supports the contention that Cdk1 is not involved in regulating later developmental processes, including the mitotic events that occur during this time [36].

Cyclin B interacts with Cdk1 and regulates its activity during *Dictyostelium* growth

Like its mammalian counterpart, *Dictyostelium* Cdk1 is activated through its association with cyclin B (CycB) and its activity during growth and development is dependent on CycB expression levels (Fig. 1) [36]. During growth, *cycB* mRNA and protein expression increases upon entry into mitosis and the Cdk1/CycB complex has been suggested to regulate the transition from G2 to M phase since overexpression of truncated CycB results in mitotic arrest [36, 40]. In addition, CycB degradation is required for vegetative cells to leave mitosis, which has also been reported to occur during the early development of *Xenopus* [40, 41]. During *Dictyostelium* development, *cycB* mRNA and protein expression reaches a maximum between the tipped aggregate and multicellular slug stage (i.e., 12–16 h), which closely adheres to the observed expression profiles of *cdk1* mRNA and protein [36, 40] (Fig. 2). Together these findings indicate that Cdk1 and CycB are required for growth, however the function of these proteins during early development (i.e., aggregation) is still unclear.

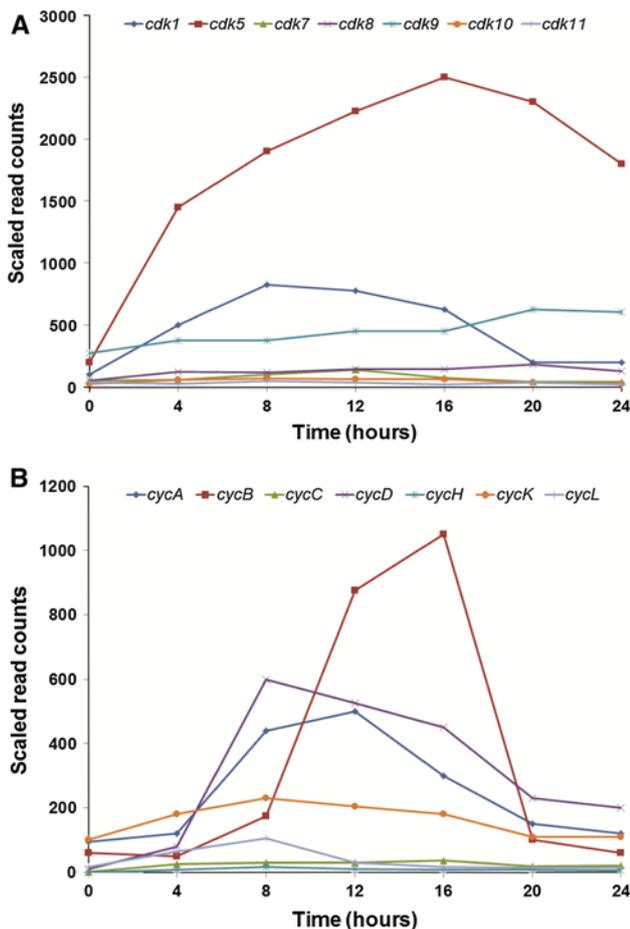


Fig. 2 Gene expression analysis of *Dictyostelium* Cdk5 and cyclins during development. RNA-Seq data was obtained from dictyExpress (www.dictyexpress.biolab.si, [38]) and re-plotted using Microsoft Excel. **a** Expression profiles of *cdk* mRNA during development. **b** Expression profiles of *cyclin* mRNA during development

Cyclin-dependent kinase 5

Cdk5 is required for growth and later development processes in *Dictyostelium*

Cdk5 is required for growth and for processes that occur during the later stages of *Dictyostelium* development (Fig. 1) [42–44]. The kinase activity of the protein has been confirmed and Cdk5 protein levels remain constant during all stages of axenic growth (i.e., exponential and stationary phases) [39, 44, 45]. *cdk5* mRNA and protein expression increases significantly during development reaching peak expression levels after 16 h and remains relatively high during terminal differentiation [38, 39, 45] (Fig. 2). Overexpression of a dominant negative form of Cdk5 that contains an aspartate to asparagine substitution at amino acid position 144 reduces the rate of growth in suspension culture as well as the rates of fluid-phase endocytosis and

phagocytosis [42]. In mammals, this amino acid substitution causes the binding between Cdk5 and its activator p35 to be two to threefold more efficient than the binding of normal Cdk5 to p35 [46]. Thus, when over-expressed, the mutant protein blocks the activation of endogenous Cdk5 by depleting the available pool of p35. During *Dictyostelium* development, overexpression of this mutant Cdk5 protein delays aggregation and fruiting body formation as well as significantly reduces the number of fruiting bodies and spores that form from these cells indicating that Cdk5 is required for processes that occur during later development [42]. These results also suggest that Cdk5 may regulate cell cycle events during mid- to late development. Although the *Dictyostelium* genome does not encode an obvious homologue of mammalian p35, Cdk5-binding partners have been identified in *Dictyostelium* (discussed below).

Cdk5 localizes to the nucleus and cytoplasm in *Dictyostelium* and translocates from the nucleus to the cytoplasm during mitosis

In *Dictyostelium*, Cdk5 localizes to both the nucleus and cytoplasm of vegetative cells suggesting a functional role in both cellular locales [43, 44]. Interestingly, this finding mirrors observations in mammalian cells. Unlike other members of the Cdk protein family that function primarily in the nucleus, Cdk5 localizes and functions in both the nucleus and cytoplasm of mammalian cells. Early studies on Cdk5 function in mammals focused on the cytoplasmic functions of the protein, however the nuclear functions of Cdk5 are slowly being revealed [47]. In neurons, Cdk5 has a death-promoting activity when localized to the nucleus and a pro-survival activity when localized to the cytoplasm [48]. In cycling NIH 3T3 cells, the localization of Cdk5 shifts from the nucleus to the cytoplasm suggesting that Cdk5 acts as a cell cycle suppressor when localized to the nucleus [49]. The change in localization occurs before or shortly after the initiation of the cell cycle and blocking the migration of Cdk5 out of the nucleus suppresses the cell cycle. During the early stages of *Dictyostelium* mitosis, Cdk5 gradually moves from a punctate nucleoplasmic distribution to localize adjacent to the inner nuclear envelope, eventually condensing as an intranuclear ring [43]. During anaphase and telophase, Cdk5 is absent from the nucleoplasm and localizes to the cytoplasm. The protein then returns to the nucleus during cytokinesis. Together these results indicate that the nucleocytoplasmic translocation of Cdk5 is an evolutionarily conserved process. In addition to its localization to the nucleus and cytoplasm, a small amount of Cdk5 has also been detected in the cytoskeleton of *Dictyostelium* cells [44]. This observation fits with the established role of Cdk5 in regulating fluid-phase endocytosis, phagocytosis, and cytoskeletal dynamics in *Dictyostelium* and adheres to

studies in mammalian cells, which have shown that mammalian Cdk5 and its activators p35 and p39 localize to the actin cytoskeleton possibly playing a role in the regulation of actin-cytoskeletal dynamics [42, 50–52].

Cdk5 binds to a puromycin-sensitive aminopeptidase in *Dictyostelium*

As discussed above, Cdk5 is one member of the mammalian Cdk protein family that is not activated by cyclins. Instead, Cdk5 is activated through its association with the neuron-specific activator molecules p35, p39, and p67 [10]. The *Dictyostelium* genome does not encode obvious homologues of mammalian p35, p39, or p67, however immunoprecipitations coupled with immunolocalizations have identified puromycin-sensitive aminopeptidase A (PsaA) as a Cdk5-binding partner in *Dictyostelium* (Fig. 1) [43]. PSAs are highly conserved metalloproteases that hydrolyze N-terminal amino acids from oligopeptides and are linked to a number of cellular processes [53]. In *Dictyostelium*, PsaA localizes to the nucleoplasm [54]. Bestatin, which inhibits the final step of intracellular protein degradation (i.e., degradation of small peptides into free amino acids) and represses epidermal growth factor-induced DNA synthesis and cell division in rat hepatocytes, binds to PsaA and inhibits cell division [54–56]. These results thus support a functional role for PsaA in regulating cell proliferation and mitosis in *Dictyostelium*. The interaction between Cdk5 and PsaA is interesting given that proteolytic activity has been shown to be a critical regulator of the cell cycle [57]. Inhibitors of aminopeptidase activity suppress cell proliferation by arresting the cell cycle and causing an accumulation of cells in the G2/M phase [55, 58, 59]. These findings indicate that PSA activities are required to complete mitosis and are essential for cell division and viability. In *Dictyostelium*, the association of Cdk5 with PsaA suggests that PsaA proteolytic activity may modulate the cell cycle by regulating the function and/or localization of Cdk5 during mitosis. Several potential phosphorylation sites have been identified in mammalian PSA, however *Dictyostelium* PsaA does not appear to be phosphorylated in vivo indicating the protein is likely not a target of Cdk5 kinase activity [43, 59].

Cdk5 is a calmodulin-binding protein in *Dictyostelium*

A recent study showed that Cdk5 is a calmodulin (CaM)-binding protein (CaMBP) in *Dictyostelium* that binds to apo-CaM (i.e., Ca^{2+} independent CaM-binding) and associates with PsaA and CaM in *Dictyostelium* nuclei (Fig. 1) [60]. This was the first evidence in any system for Cdk5 being a CaMBP. CaM is the primary sensor of Ca^{2+} within the cell and binds to a diversity of CaMBPs that participate

in a variety of cellular processes [61–63]. In HEK293T and PC12 cells, p35 activity is regulated by both Cdk5 phosphorylation and CaM-binding, however a direct binding between Cdk5 and CaM has not been shown [64]. Sequence analysis identified two putative CaM-binding domains (CaMBDs) in *Dictyostelium* Cdk5, however until now, only one of the two putative domains has been verified as a true CaMBD [60]. Deletion of CaMBD2 (¹³²LLIN-RKGELKLADFGLARAFGIP¹⁵⁴) prevented CaM-binding indicating that this region, which contains 1-10, 1-12, 1-14, 1-16, and 1-8-14 hydrophobic motifs, encompasses a functional CaMBD. Interestingly, the two CaMBDs of *Dictyostelium* Cdk5 are strongly conserved in human Cdk5 indicating that this is an evolutionarily conserved interaction that warrants investigation in human cells. Insight into the function of this interaction was provided when Huber et al. [60] showed that deletion of Cdk5 CaMBD2 significantly increased the nuclear distribution of Cdk5 and that this effect was dramatically enhanced by deletion of both CaMBD1 and CaMBD2. In mammalian cells, CaM functions at multiple points in the cell cycle and is required for cell proliferation [65, 66]. CaM function has also been linked to mitosis and cell proliferation in *Dictyostelium* [67, 68]. Together, these results suggest that CaM may regulate the nucleocytoplasmic transport of Cdk5, which could ultimately regulate cell proliferation and mitosis in *Dictyostelium* and other systems. The identification of Cdk5 as a CaMBP opens up new avenues of research to investigate whether Cdk5 binds CaM in other systems and the function of this interaction.

Roscovotine inhibits Cdk5-dependent processes in *Dictyostelium*

Attempts to generate a *cdk5* knockout mutant have been unsuccessful, likely because the protein is essential for cell division and growth [42]. As discussed previously, an early study made use of a mutant strain overexpressing a dominant negative form of Cdk5 [42]. That study showed that Cdk5 is required for optimal growth and differentiation and that the protein is involved in regulating fluid-phase endocytosis, phagocytosis, and cytoskeletal dynamics. The inability to generate a *cdk5* knockout mutant has necessitated the use of other approaches to fully elucidate the function of this biomedically important protein. With this in mind, Huber and O'Day [44] showed that the mammalian Cdk inhibitor roscovotine could be used to study the function of Cdk5 during *Dictyostelium* growth and development.

Roscovotine is a potent, cell-permeable Cdk inhibitor that inhibits kinase activity by binding to the ATP-binding pocket of a number of Cdks (e.g., Cdk1, Cdk2, Cdk5, Cdk7, and Cdk9), however studies have indicated that it preferentially inhibits Cdk5 [69–72]. The ability

of roscovitine to inhibit Cdk10 and Cdk11 has not been reported, however it has no effect on the activity of mammalian Cdk4, Cdk6, and Cdk8 [73, 74]. Its effectiveness at treating nasopharyngeal cancer and non-small cell lung cancer is currently being evaluated in phase 2 and 2B clinical trials, respectively, and it is also being investigated for its potential to treat breast cancer, herpes simplex infection, HIV infection, leukemia, and chronic inflammation disorders such as cystic fibrosis and arthritis [75–79]. Roscovitine has been used to show that Cdk5 is required for long-term potentiation induction and NMDA-induced currents in rat hippocampal neurons, morphine tolerance in rats, cell proliferation and apoptosis of MDA-MB-231 cells, and endothelial cell migration [70, 71, 80, 81]. Roscovitine has also been used to inhibit Cdk5 activity in mouse C2C12 myoblasts [82].

In *Dictyostelium*, roscovitine significantly inhibits kinase activity and axenic growth and this inhibition can be partially rescued by the overexpression of Cdk5-GFP [42, 44]. These results thus support the involvement of Cdk5 in the regulation of axenic growth and indicate that Cdk5 is a primary target of the chemical. Roscovitine does not affect the expression of Cdk5 protein during growth, but it does inhibit its translocation to the nucleus; a result that has not been previously reported for any organism [44]. It also increases the amount of cytoskeletal-associated Cdk5. Development up to the mound stage is unaffected by roscovitine, however treatment does inhibit the later stages of *Dictyostelium* development, specifically slug and fruiting body formation. The fruiting bodies that do form are small relative to untreated fruiting bodies and contain relatively few spores. In addition, unlike the elliptical shape of untreated spores, roscovitine causes spores to adopt a round shape. These findings mirror the observations of the mutant Cdk5 dominant negative overexpressing strain, thus providing strong evidence for the ability of roscovitine to inhibit Cdk5 activity in *Dictyostelium* and supporting the involvement of Cdk5 in the regulation of later developmental processes [42]. The relative abundance of *cdk5* mRNA during *Dictyostelium* growth and development compared to other Cdks is also an indication that roscovitine primarily inhibits Cdk5-dependent cellular processes in *Dictyostelium* (Fig. 2). Since it has been reported that at least one round of mitosis occurs during the later stages of development, these findings also indicate that Cdk5 may be required for the mitotic events that occur during this stage of the developmental cycle [22, 83]. The ability of roscovitine to inhibit Cdk5 activity and its nucleocytoplasmic translocation in *Dictyostelium* will allow future studies to clarify the function of Cdk5 during cell proliferation and development by identifying the signaling pathways that regulate its activity and localization. *Dictyostelium* can also be used as a model system to understand the mechanism of action

of roscovitine, which could provide important insight for studies aimed at determining its usefulness as a clinical drug. As discussed previously, it is well established that the cell cycle affects cell type differentiation during *Dictyostelium* development [30, 31]. Therefore, it is also possible that the effect of roscovitine on development is due to the inhibition of the cell cycle during earlier developmental processes rather than roscovitine having a direct effect on later development. In addition, although Huber and O'Day [44] provided strong evidence that roscovitine specifically inhibits Cdk5 activity in *Dictyostelium*, the ability of the chemical to also inhibit other Cdks during *Dictyostelium* growth and development remains unclear. This may require future study since Cdk1, Cdk7, and Cdk9 have also been shown to be sensitive to roscovitine in mammals [69].

Cyclin-dependent kinase 8

Cdk8 is required for early and later developmental processes in *Dictyostelium*

In *Dictyostelium*, Cdk8 is not absolutely required for growth, but is required for early and later developmental processes (Fig. 1) [84–86]. *cdk8* mRNA expression is relatively low during growth (Fig. 2) [84]. Expression increases during early development, reaches peak levels after 12 h, and then quickly decreases between 12 and 15 h of development [84]. Recently published RNA-Seq data supports the increase in *cdk8* mRNA expression during early development, however in that analysis expression peaked at 20 h and remained high during the later stages of development (Fig. 2) [38].

cdk8- cells possess a pleiotropic phenotype and there is no obvious effect of Cdk8 overexpression [84, 85]. Mutant cells grow more slowly than parental cells in axenic medium and form small plaques on bacterial lawns indicating that Cdk8 is required for feeding [84, 86]. Upon starvation, *cdk8*- cells do not aggregate or stream and do not show evidence of pulsatile cell movement [84, 85]. Mutant cells do not express adenylyl cyclase A (*acaA*) transcripts and the expression of 3'-5'-cyclic adenosine monophosphate receptor A (*carA*) mRNA is strongly reduced indicating that the aggregation defect is likely due to abolished cAMP signaling [84]. Lin et al. [85] confirmed this hypothesis by showing that the aggregation defect could be partially overcome by pulsing cells with cAMP thus indicating an impaired signal relay in *cdk8*- cells. Lin et al. [85] also reported a dysregulation of gene expression during early development for the catalytic subunit of protein kinase A (PKAc) and discoidin 1 (Disc1). However, overexpression of PKAc could not rescue the aggregation defect indicating that the low expression levels of PKAc are not solely responsible for the aggregation defect [86].

cdk8- cells develop abnormally during the later stages of *Dictyostelium* development indicating that Cdk8 may be involved in regulating the cell cycle during development [84]. Fruiting bodies form only under certain experimental conditions (i.e., grown axenically in nutrient-rich HL5 medium followed by plating on filters) and most of the spores are not capable of germinating [84]. Follow-up studies confirmed the requirement of Cdk8 for spore cell differentiation [85, 86], however a recent study that generated a *cdk8* mutant in a strain lacking detectable gene duplication showed that *cdk8*- cells are able to form phenotypically normal fruiting bodies [86]. In that study, a slight developmental defect was reported with fruiting body formation occurring 3–4 h later than parental cells. Despite the ability to form normal fruiting bodies, spores that formed were less viable than the parental strain, though they looked morphologically normal. Nonetheless, these findings confirmed earlier observations supporting an involvement of Cdk8 in spore cell differentiation [84, 85].

Cyclin C associates with high molecular weight complexes and regulates the activity of Cdk8 during *Dictyostelium* development

Dictyostelium Cdk8 localizes primarily to the nucleus and forms part of a high molecular weight complex that has carboxy terminal domain kinase activity [85, 87]. In mammals, the high molecular weight Mediator complex links regulatory proteins to the basal transcription machinery [7, 9]. *Dictyostelium* cyclin C (CycC) has also been detected in high molecular weight complexes isolated from *Dictyostelium* nuclei however a direct interaction between Cdk8 and CycC has not yet been shown [87]. CycC protein is expressed in growing cells [87]. During development, the protein level begins to decrease after mound formation [87]. *cycC* mRNA follows this expression pattern and the decrease in expression after mound formation, which is triggered by extracellular cAMP, reduces the association of Cdk8 with the nuclear high molecular weight complex [87]. Recently published RNA-Seq data shows that *cycC* mRNA expression increases during early development, but does not decrease after mound formation as reported by Greene et al. [87] (Fig. 2) [38]. Instead, *cycC* mRNA expression continues to increase after mound formation and reaches its peak level once the multicellular slug has formed (i.e., 16 h) (Fig. 2). Expression then decreases during the later stages of development. While it is not immediately apparent why different expression profiles have been reported research to this point still strongly supports a developmental function for Cdk8 and CycC. Finally, increased levels of either Cdk8 or CycC enhance the rate of early development indicating that the two proteins are rate-limiting for multicellular development [87]. Overexpression of both

proteins results in an even greater acceleration of early developmental events. Together, the accumulated evidence indicates that Cdk8 and CycC are not absolutely necessary for growth, but are required during development for aggregation and spore cell differentiation.

Conclusions and future directions

The expression profiles and interacting proteins involved in regulating the activity of Cdk1, Cdk5, and Cdk8 suggest that the proteins likely perform distinct functions during the *Dictyostelium* life cycle (Fig. 1,2). Accumulated evidence indicates that Cdk1 is important for growth, but does not appear to be essential for development including the regulation of developmental mitosis. In contrast, Cdk5, although also required for growth, is important for the later stages of development, specifically slug and fruiting body formation. Cdk5 activity may also be required for the mitotic events that occur during later development indicating that the regulation of developmental mitosis may be different from the mechanisms that regulate mitosis during vegetative growth. Finally, Cdk8 functions during early and late development, specifically aggregation and spore differentiation, indicating that Cdk8 may also be involved in regulating cell cycle events during development. The research community would benefit from future studies that focus on the identification of signaling pathways that mediate the activity of these Cdks as well as studies that characterize the putative Cdks and cyclins that have also been identified in *Dictyostelium* to determine their function during growth and development (e.g., Cdk7, Cdk9, Cdk10, and Cdk11) (Tables 1 and 2; Fig. 2). Future research in this area may also be able to shed light on the precise mechanisms that regulate the cell cycle and cell type differentiation during *Dictyostelium* development. Taken together, these results show that *Dictyostelium* can be used as a model system to study the function of conserved Cdks in an organism that provides an excellent opportunity to examine their role in a number of cellular and developmental processes. Since the Cdk protein family has been identified as a potential target for anti-cancer therapy, research into the function of Cdks in *Dictyostelium* could provide valuable new insight for the development of effective treatments and therapies for various forms of cancer. In addition, studies into Cdk5 function in *Dictyostelium* could provide new insight into the role Cdk5 dysregulation plays in neurodegeneration.

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