Independent modulation of the kinase and polo-box activities of Cdc5 protein unravels unique roles in the maintenance of genome stability

Hery Ratsima, Anne-Marie Ladouceur, Mirela Pascariu, Véronique Sauvé, Zeina Salloum, Paul S. Maddox, and Damien D’Amours

Institute for Research in Immunology and Cancer and Département de Pathologie et Biologie Cellulaire, Université de Montréal, Montréal, QC, Canada H3C 3J7

AUTHOR SUMMARY

Complex cellular processes are often regulated by a large number of distinct biochemical activities. The functional units responsible for performing these specific biochemical activities are short sections of proteins known as functional domains. Some proteins, such as polo-like kinases (PLKs), can perform many different cellular functions because they possess multiple functional domains. However, discovering the specific function of each domain in multidomain proteins, such as the PLKs, is a formidable challenge because inactivation of any single activity can lead to cell death. To address this problem, we used structure-based approaches to identify nonlethal mutations that would exclusively affect the functions of either the kinase domain or the polo-box domain (PBD) of the budding yeast PLK, Cdc5. Using this approach, we discovered that the biochemical activity of each domain of Cdc5 contributes to cell division (mitosis) in distinct ways. Specifically, the activity of the kinase domain of Cdc5 promoted several stages of mitosis independently of PBD activity. On the other hand, the activity of the PBD of Cdc5 was crucial for the regulation of spindle pole bodies (SPBs), the structures corresponding to centrosomes in yeast. As a consequence, loss of SPB regulation in mutants of Cdc5, these mutants do not provide information on the specific roles of each functional domain in Cdc5 because most of the mutant alleles affect overall protein stability. This particular limitation prompted us to create cdc5 alleles carrying mutations that can effectively separate the biochemical activities of the kinase domain from those of the PBD of Cdc5.

We first performed a targeted genetic screen to isolate mutants that affect Cdc5 kinase activity. Mutagenesis of a particular region of the kinase domain generated multiple temperature-sensitive alleles. This particular limitation prompted us to create cdc5 alleles carrying mutations that can effectively separate the biochemical activities of the kinase domain from those of the PBD of Cdc5.

Confl ict of interest statement: The authors plan to file a patent on the discovery that mutations in the catalytic domain of protein kinases can regulate their enzymatic activity. This Direct Submission article had a prearranged editor.

1To whom correspondence should be addressed. E-mail: damien.damours@umontreal.ca.

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To address this question, we used the budding yeast Saccharomyces cerevisiae as model organism because it allows for powerful genetic analyses that are not available with other species. Moreover, the budding yeast genome encodes a single PLK family member, CDC5, whereas other organisms, such as fruit flies or mammals, contain several members of this family of kinases [as many as 5 different PLKs (1)]. This corresponds to a singular advantage in our analysis of CDC5 functions because the effects of mutations in a gene of interest can often be hidden by the presence of other family members in a cell. An interesting peculiarity of previous studies on Cdc5 in budding yeast is that such analyses relied largely on the use of mutant versions, or alleles, of CDC5 that are sensitive to changes in temperature. Although very useful in characterizing the global functions of Cdc5, these mutants do not provide information on the specific roles of each functional domain in Cdc5 because most of the mutant alleles affect overall protein stability. This particular limitation prompted us to create cdc5 alleles carrying mutations that can effectively separate the biochemical activities of the kinase domain from those of the PBD of Cdc5.

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ature-sensitive mutants of *cdc5*. The use of these kinase-specific mutants allowed us to show that Cdc5 catalytic activity was absolutely required to promote exit from mitosis after chromosome separation (Fig. P1). In particular, we showed that Cdc5 kinase activity acted in this process by stimulating the activation of a key effector of mitotic exit, the protein phosphatase Cdc14. Interestingly, earlier mitotic events appeared to be less dependent on Cdc5 kinase activity. The apparent increase in the dependency of late mitotic processes on Cdc5 kinase activity is reminiscent of the quantitative model proposed by Stern and Nurse (2) to explain the mode of action of another cell cycle kinase, Cdk1.

We next wanted to isolate a mutant completely defective in Cdc5 PBD activity. We deleted the entire PBD of Cdc5, but this deletion had drastic effects on protein stability and strains carrying this deletion were not viable. This led us to envision a more subtle approach to inactivate the PBD. Indeed, informed by the structure of the PBD of human polo-like kinase 1 (Plk1) (3), we introduced three point mutations that are known to completely abrogate the binding of the PBD to phosphorylated proteins. The resulting PBD-deficient mutant was viable, allowing us to investigate the contribution of the PBD domain of Cdc5 to cell cycle progression. Our first surprise was to discover that the PBD mutant of Cdc5 did not cause any major defect in mitosis beyond a mild delay in the progression of cells through anaphase (i.e., the stage when cells separate their chromosomes).

Interestingly, in a routine assay used to monitor progression in the cell cycle, we noticed that the number of chromosomes in the genome of PBD-deficient mutants of Cdc5 was different from that in normal cells. The nature of the chromosomal change observed in mutant cells suggested that Cdc5 did not regulate chromosomes individually but as an ensemble (i.e., the total complement of chromosomes within a cell, or ploidy). Further analyses verified this prediction by showing that PBD-deficient mutants accumulated twice the normal number of chromosomes in one discrete step (Fig. P1). Doubling of genome size is a relatively uncommon phenomenon that is typically observed in mutants with defective SPB functions. This prompted us to investigate whether PBD mutants showed defects in SPB formation and/or function. To achieve this, we tagged a key component of SPBs with GFP. GFP is a protein that can generate a green fluorescent light signal, and therefore acts as a microscopic marker for the localization of a protein to particular structures within the cell. We then monitored the number of GFP foci formed in cells carrying this marker, designated Spc42-GFP. In normal mitotic cells, Spc42-GFP forms two foci that correspond to the two SPBs that are needed for the assembly of a functional mitotic spindle. However, in the *cdc5* mutants lacking PBD activity, we observed unusual numbers of Spc42-GFP foci, with some cells showing up to four or five distinct Spc42-labeled structures. Subsequent analyses of other SPB components showed that some but not all SPB components were included in the extra SPB-like structures in *cdc5* mutants. This indicated that the extra Spc42-GFP foci created in the absence of PBD activity did not correspond to fully functional SPBs.

The association of only a subset of SPB components within aberrant Spc42-containing structures also suggested that the balance and/or levels of SPB components in the real SPBs could be altered in *cdc5* mutants. This, in turn, may affect SPB function and the ability of PBD-deficient cells to segregate chromosomes effectively. We tested this hypothesis by monitoring the behavior and composition of active SPBs in *cdc5* mutants using live cell microscopy. As predicted, loss of PBD activity led to aberrant levels of Spc42 within active SPBs and, as a consequence, to defective SPB activity. Collectively, these results show that the PBD of Cdc5 regulates cellular ploidy by promoting the formation of fully functional SPBs.

The role of PLK family members in the control of cellular ploidy is likely conserved from yeast to humans. Indeed, a specific type of bone marrow cell in mammals, the megakaryocyte, becomes naturally polyploid (i.e., it contains more than 2 sets of chromosomes) during its development. This process is associated with the down-regulation of Plk1 (4). Reintroduction of Plk1 in developing megakaryocytes prevents their polyploidization (4), suggesting that mammalian Plk1, like its yeast counterpart, also regulates cellular ploidy. Importantly, our work suggests that this process in megakaryocytes occurs via regulation of centrosome function. Changes in the ploidy of mammalian genomes are also likely to be relevant for tumorigenesis. The putative role that PLKs play in this process may explain, at least in part, the benefit of targeting this family of kinases for cancer treatment (5).