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(54) Title: YEAST STRAINS TO IDENTIFY SPECIFIC INHIBITORS OF POLO KINASES

(57) Abstract: The disclosure provides, in certain embodiments, yeast strains that over-express a polo-box domain from Cdc5 or Plk and thereby exhibit a cytokinesis-defective, chaining growth pattern. These strains, and equivalent cell lines from other species, can be used to identify compounds that modulate polo-like kinase activity. Also described are methods of using polo-box related peptides, and polo-like kinase activity modulatory compounds, to inhibit or enhance cellular proliferation. These peptides and compounds can, for instance, be used to treat a hyper-proliferative disease, disorder or condition, such as neoplasm, through inhibition of polo kinase(s).

YEAST STRAINS TO IDENTIFY SPECIFIC INHIBITORS OF POLO KINASES

FIELD

This disclosure relates to methods of identifying and using compounds and peptides that interact with a kinase or kinase target protein, particularly a polo kinase, as well as such compounds and peptides. Such compounds can be, for instance, inhibitors of a kinase.

BACKGROUND

Cancer is a genetic disease caused by alterations in the genes controlling the cell cycle. Efforts have been made to develop useful tools to regulate uncontrolled proliferation properties of cancer cells, but so far these efforts have met with little success. Members of the polo subfamily of protein kinases have been identified in various eukaryotic organisms, and they appear to play pivotal roles in cell proliferation and cell division. A mammalian polo family kinase Plk is expressed at high levels in tumors of various origins, and uncontrolled Plk expression has been implicated in the development of cancers in humans.

The polo kinase subfamily members are characterized by the presence of a distinct region of homology in the C-terminal non-catalytic domain, termed the polo-box (Clay *et al.*, *Proc. Natl. Acad. Sci. USA*. 90:4882-4886, 1993). Studies in various organisms have shown that polo kinases regulate diverse cellular and biochemical events at multiple stages of M phase (mitosis). In addition to their function in mitosis, some evidence supports a theory that polo kinases may play an important but undefined role in cytokinesis (see reviews, Fishkind and Wang, *Curr. Opin. Cell Biol.* 7:23-31, 1995; and Field *et al.*, *Curr. Opin. Cell Biol.* 11:68-80, 1999).

Cytokinesis is preceded by inactivation of cyclin-dependent kinase Cdc2 (not a polo-like kinase). In budding yeast, degradation of mitotic cyclin by the anaphase-promoting complex (APC) is a prerequisite for cytokinesis. The Cdc5 gene has been identified in the budding yeast *S. cerevisiae* as encoding a polo-like kinase. A *cdc5-1* temperature sensitive mutant arrests at late mitosis at the restrictive temperature, and exhibits reduced APC activity. Overexpression of the Cdc5 protein results in an increased APC activity. Although the role of Cdc5 in the mitotic exit pathway is well established, it is not known if Cdc5 activity is required for cytokinesis. There are no identified *cdc5* mutants that exhibit a cytokinetic defect without inhibiting mitotic exit.

SUMMARY

The disclosed methods take advantage of the finding that ectopic expression of a polo-box in a eukaryotic cell causes a severe cytokinetic defect in the cell. Methods are described herein that use this effect to isolate compounds that modify polo-like kinase function, for instance by inhibiting or enhancing the binding of a polo-box region of a polo-like kinase to a target molecule (*e.g.*, receptor or other specific binding partner). Compounds that modify polo-like kinase expression and/or function can be used in the treatment of different diseases or conditions, including hyper-proliferative disorders such as a neoplasm.

Certain disclosed embodiments are methods of identifying a compound with polo-like kinase modulating activity. These methods involve contacting a eukaryotic cell that exhibits a dominant negative cytokinesis-defective growth pattern with a test compound and assessing an effect of the test compound on the cell. In certain disclosed embodiments, the eukaryotic cells express at least one of the following: (a) a polo-box or polo-box related peptide; (b) a binding peptide

comprising at least 25 residues from a polo-like kinase C-terminal region; or (c) a functional fragment, variant, mimetic, or analog of the peptide of (a) or (b). Compounds with polo-like kinase modulating activity are identified based on whether they change the dominant-negative cytokinesis-defective growth pattern in budding yeast and a mitotic defect in other organisms.

In certain embodiments, an effect of the test compound may include an observed alteration in the growth pattern of the eukaryotic cell, or a subcellular localization of a polo-like kinase (*e.g.*, its association with neck filament proteins, such as septins, or with spindle pole components, including Bbp1 or Mps2), or a measurable phosphorylation activity of a polo-like kinase. These are specific examples of assessment techniques, however, and others could be used.

Another embodiment includes further assessing a compound identified by one of the disclosed methods for anti-neoplastic activity (*e.g.*, the ability of the compound to reduce growth of a cancer cell).

Other embodiments are the compounds identified by any of these methods.

Also encompassed herein are methods for modifying a polo-like kinase function (*e.g.*, a mitotic and/or cytokinetic function), wherein a eukaryotic cell is contacted with a substance that alters (either enhancing or inhibiting) a specific interaction between a polo-box and a polo-box specific binding partner. Such binding partners include elements of neck-filaments and elements of spindle pole bodies. Substances used in these methods are further embodiments.

In further embodiments, these methods of modifying a polo kinase function are methods for treating a hyper-proliferative disorder (*e.g.*, a neoplasm, such as a cancer) in a subject in need of such treatment. When the method is a method of treatment, the substance can be applied to the eukaryotic cell in a pharmaceutically acceptable carrier.

Also encompassed by this disclosure are methods for producing eukaryotic cells that exhibit mitosis- and/or cytokinesis-defective growth, such methods including expressing a heterologous nucleic acid in the eukaryotic cell. The expressed heterologous nucleic acid can encode, for instance, a polo-box, or a functional fragment, variant, mimetic, or analog thereof. In certain embodiments, however, the heterologous nucleic acid does not encode a kinase with substantial kinase activity.

The eukaryotic cells produced by these methods are further embodiments provided herein.

This disclosure also encompasses kits for the treatment of a hyper-proliferative disorder. Such kits include a therapeutic substance that inhibits binding of a polo-box to a polo-box binding partner. Some such kits will also include instructions (*e.g.*, directions for administering at least one dose of the therapeutic substance to a subject in need of such treatment).

The therapeutic substance in the disclosed kits may be provided in the form of a pharmaceutical composition. Specific examples of substances that can be included in these kits are the disclosed compounds with polo-like kinase modulatory activity or with polo-box modulatory activity and those that alter specific interactions between a polo-box and a polo-box specific binding partner.

Eukaryotic cells include fungal, animal, and plant cells. Specific examples include human cells or yeast cells. Specific yeast cells include cells of the yeast *S. cerevisiae*, such as those carrying a mutation in either a *cyk2* or a *myo1* encoding sequence. In specific embodiments wherein the eukaryotic cell is a yeast cell, the yeast cells are of strain KLY1083 or KLY1212.

Polo-boxes include those that contain the sequence of SEQ ID NO: 1, and functional fragments or variant thereof. In certain embodiments, the polo-box corresponds to that of a polo-like

kinase (*e.g.*, Cdc5, *Polo*, $Plk1_{mammalian}$, Plo1p, Snk, FNK/Prk, Plx1, Tbplk, $Plk1_{C. elegans}$). Particular specific examples include polo-boxes that correspond to those found in Cdc5 or mammalian Plk.

A specific embodiment disclosed herein is a method of specifically inhibiting certain mitotic functions of polo kinases (such as their role in cytokinesis, *e.g.*, in budding yeast) without substantially inhibiting mitosis. This method involves exposing a cell to a substance that inhibits a specific interaction between a polo-box and its binding partner(s) (such as septins Cdc11 or 12, Bbp1 and/or Mps2), wherein the substance does not have assayable protein phosphorylation activity.

Another specific embodiment is a method of specifically inhibiting cytokinesis without substantially inhibiting mitosis. This method involves exposing a cell to a substance that inhibits a specific interaction between a polo-box and a septin, wherein the substance does not have assayable protein phosphorylation activity.

The foregoing and other features and advantages will become more apparent from the following detailed description of several embodiments, which proceeds with reference to the accompanying sequence listing and figures.

SEQUENCE LISTING

The nucleic acid and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and the three-letter code for amino acids. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood to be included by any reference to the displayed strand.

SEQ ID NO: 1 shows the consensus amino acid sequence of a polo-box.

BRIEF DESCRIPTION OF THE FIGURES

FIG 1A and 1B show a time course of the expression level of HA-tagged fusion polo-family protein in two yeast strains, and the proportion of cells arrested at different at various points of the cell cycle. Depletion of Plk or cdc5-1 protein revealed a large fraction of large-budded cells with disassembled spindles. Strains KLY1046 (expressing *GAL1-HA-EGFP-PLK*) and KLY1047 (expressing *GAL1-HA-EGFP-cdc5-1*) growing exponentially in YEP-galactose medium were transferred into YEP-glucose to deplete Plk and cdc5-1 proteins. Upon transfer, samples were taken to analyze the levels of HA-EGFP-Plk and HA-EGFP-cdc5-1 proteins using an anti-HA antibody; representative westerns are shown.

Chromosomal DNA was stained with DAPI. Due to an apparent cell lysis phenotype after a prolonged incubation, cells were not taken beyond the last indicated time point. Strain KLY1047, but not KLY1046, accumulated a significant number of cells with elongated spindles.

FIG 2 shows a western blot of cell extracts from a control and cells expressing a functional (*cdc5ΔN*) or localization-defective (*cdc5ΔN* /FAA) C-terminal region of Cdc5, showing that the *FAA* mutations in the polo-box do not influence the level of *cdc5ΔN* expression. An equal amount (30 μg) of cell lysate prepared from various strains shown in panel A was loaded onto each lane. Control, strain KLY1080; cdc5ΔN, strain KLY1082; cdc5ΔN/FAA, strain KLY1081; EGFP-cdc5ΔN, EGFP-fused cdc5ΔN proteins expressed; EGFP, control EGFP lacking Cdc5. Cdc28 protein serves as a loading control for each lane.

FIG 3A shows two graphs illustrating that strain KLY1083 (expressing *EGFP-cdc5ΔN*) grows without increasing cell numbers under the induction conditions. Cells expressing control *EGFP* (KLY1080), *EGFP-cdc5ΔN* (KLY1083), or *EGFP-cdc5ΔN/FAA* (KLY1229) were taken at the indicated time points upon transferring cultures into YEP-galactose. Cell number was determined by plating serial dilutions on YEP-glucose and counting the colony numbers. The number of cells at time 0 was 1.2 x 10^6 cells per ml with an OD_{600} of 0.05. The resulting cell number and OD_{600} at each time point were divided by those at time 0 to give relative cell number and OD_{600}.

FIG 3B shows the relative quantity (by Western blot) of EGFP-cdc5ΔN protein in strains expressing the indicated fusions; the *FAA* mutations do not influence the stability of EGFP-cdc5ΔN. EGFP-cdc5ΔN was detected by an anti-GFP antibody, whereas Cdc28 (loading control) was recognized by an anti-Cdc28 antibody. 1X, one copy of *cdc5ΔN*; 3X, three copies of *cdc5ΔN*.

FIG 4 is a table of the interactions between the indicated constructs in a two hybrid system, and the structures of various Cdc5 constructs used in these analyses. To enhance the protein stability, a destruction-box deficient form of Cdc5 (Song *et al.*, *Mol. Cell. Biol.* 20:286-298, 2000) was used in place of the wild-type Cdc5. Grey boxes indicate the kinase domain in the N-terminus of Cdc5. Hatched boxes in the C-terminal domain indicate the polo-box, whereas the same box with vertical lines indicate the polo-box with *FAA* mutations. The numbers in the table indicate the Miller units of β-galactosidase activity averaged from two independent experiments. Cl, Cla I; RV, Eco RV; Sa, Sna BI; Cdc5, Cdc5 lacking the N-terminal residues 6 to 71 (Song *et al.*, *Mol. Cell. Biol.* 20:286-298, 2000); cdc5ΔC, C-terminal domain deletion; cdc5ΔN, N-terminal domain deletion; cdc5ΔN/FAA, N-terminal domain deletion with FAA mutations in the polo-box; a and b, control plasmids: pEG202-NLS (DBD fusion vector) and pJG4-5 (AD fusion vector).

FIG 5 shows a comparison of the amino acid sequences of the mammalian polo-like kinase Plk (query) and the yeast Cdc5 (subject). The polo-box consensus is at amino acid residues 410 to 430 in Plk.

FIG 6 is a schematic drawing of a budding yeast cell, showing the interactions or lack of interactions of the indicated polo-like kinase molecules and derived proteins with specific binding proteins (represented by a gray circle marked with an "X"). Over-expression of the C-terminal domain of Cdc5, but not the corresponding FAA mutant, results in competitive inhibition of the cytokinetic function of endogenous Cdc5 by binding to essential Cdc5 targets at spindle poles and cytokinetic neck-filaments.

Endogenous Cdc5 has both an N-terminal kinase domain (K.D.) and a C-terminal polo box (P.B.); interactions of this molecule with at least some of its specific binding partners are mediated by the polo domain. The polo box, expressed on its own (marked as WT), can also interact with these specific binding partners such as septins at the neck filaments and Bbp1 at the spindle poles. In contrast, a binding-incompetent version of a polo box (marked FAA, indicative of point-mutated amino acids within the polo box consensus sequence) cannot specifically interact with polo-box binding proteins.

FIG 7 is a series of schematic drawings of polo-box action during cytokinesis, and models for compound interactions that disrupt this action.

Wild type yeast express Cdc5 (a polo-like kinase, PLK) and at least one cytokinesis-linked specific binding partner, referred to here as a "target" (**FIG 7A**). At some point prior to cytokinesis, at least a portion of the Cdc5 molecules within the cell relocates to the bud neck; this localization is mediated by the interaction between the polo-box and at least one specific binding partner such as

septins (Cdc11 and Cdc12) (**FIG 7B**). Relocalization of the Cdc5 to the bud neck (at the neck filament complex) permits initiation of cytokinesis that leads to the separation of the mother and daughter cells (**FIG 7C**).

FIG 7D illustrates the dominant negative effect of over-expressing a polo-box peptide. In cells over-expressing a peptide or protein containing a polo-box (or a polo box-related protein that is receptor-binding competent), ectopically expressed polo box competes for native polo-like kinase for binding sites at the bud neck. Insufficient native kinase (*e.g.*, Cdc5) re-locates to the bud neck, and cytokinesis is blocked. Likewise, this effect would also be seen if polo box was supplied to the cell by a method other than by expressing it (*e.g.*, by contacting the cell with a composition that included a peptide or protein that contains a polo box).

FIG 7E illustrates more generally that non-polo-box substances can be used to compete for receptor binding sites at the bud neck. As in **FIG 7D**, when a substance (either peptide or non-peptide) that can bind at a polo-box specific binding partner (receptor) is added to a wild type cell, it competes with the native polo-like kinase for binding sites. A sufficient amount of the binding-inhibiting substance will prevent enough of the kinase from re-localizing to the bud neck that cytokinesis is blocked.

FIG 7F illustrates the action of substance that interacts with the polo-box itself, thereby inhibiting the cytokinesis-related functions of the polo box. When a compound (an "inhibitor" or "binding inhibitor") that interacts with the polo-box of native polo-kinase (*e.g.*, Cdc5) is added to wild type cells, the native polo kinase cannot interact with its neck filament receptor(s), and therefore does not re-locate to the bud neck. In the absence of such re-localization, these cells cannot complete cytokinesis.

FIG 8 is a series of schematic drawings, illustrating the disclosed method of isolating compounds by complementation of the polo-box over-expression dominant negative phenotype.

FIG 8A shows a eukaryotic cell (*e.g.*, a yeast cell) expressing a polo-box peptide. Because the ectopically expressed polo-box competes for binding at the mother-bud neck, this cell is arrested and cytokinesis (**FIG 8B**) cannot complete.

FIG 8C shows that the addition of a compound that binds to a polo-box can overcome this dominant negative cytokinetic arrest. This compound is referred to generally as an "inhibitor" or "binding inhibitor." Depending on the number of polo kinase molecules and ectopically expressed polo boxes there are in the cell, an amount of the binding inhibitor molecule will "scavenge" sufficient of the over-expressed polo-boxes to allow the polo kinase to re-locate to the mother-bud neck specific binding partner(s), thereby rescuing the arrest phenotype. The amount of the inhibitor compound necessary to overcome arrest will be influenced also by its relative affinity for the polo box of the native kinase and for the ectopically expressed polo-box.

As illustrated in **FIG 7F**, when a compound with these characteristics is applied to a wild-type cell (not over-expressing a polo-box), the compound inhibits re-location of the native kinase to the mother-bud neck, and thereby inhibits cytokinesis.

FIG 9 shows a schematic representation of four deletion variants of Plk (**FIG 9A**), and a Western blot showing expression of these constructs in HEK293 cells (**FIG 9B**). Expression of recombinant HA-GFP-PLK in HEK293 cells. The diagram shows four constructs expressing different regions of the Plk coding sequence. Construct #1 and #3 are two different versions of the C-terminal domain of Plk, whereas construct #2 and #4 lack polo-box 2 and polo-box 3 of #1 and #3, respectively. Recombinant HA-GFP-Plk was generated by N-terminally tagging Plk with HA

(hemagglutinin epitope) and GFP (green fluorescence protein). Adenoviral expression constructs bearing various forms of HA-GFP-PLK cDNA were expressed in HEK293 cells using the pAdEasy-1 expression system (He, Proc. Natl. Acad. Sci., USA, 1998. 95:2509-2514). Expression of these proteins was detected with anti-HA antibody using enhanced chemiluminiscence Western analysis.

DETAILED DESCRIPTION

I. Abbreviations

APC: anaphase promoting complex
cdc: mutant in the cell division cycle
cdk: cyclin dependent kinase
DIC: differential interference contrast
FAA: shorthand designation for the triple mutation W517F/V518A/L530A in a polo-box
EGFP: enhanced green fluorescent protein
plk: polo-like kinase

II. Explanations of terms

Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art. Definitions of common terms in molecular biology may also be found in Rieger *et al.*, *Glossary of Genetics: Classical and Molecular,* 5th edition, Springer-Verlag: New York, 1991; and Lewin, *Genes VI*, Oxford University Press: New York, 1997. The nomenclature for DNA bases as set forth at 37 C.F.R. § 1.822 is used. The standard one- and three-letter nomenclature for amino acid residues is used. The terms "a" and "the" are understood to include the plural of the object referred to, as well as the singular, unless the context indicates otherwise.

In order to facilitate review of the various embodiments, the following explanations of specific terms are provided:

Analog, derivative or mimetic: An analog is a molecule that differs in chemical structure from a parent compound, for example a homolog (differing by an increment in the chemical structure, such as a difference in the length of an alkyl chain), a molecular fragment, a structure that differs by one or more functional groups, a change in ionization. Structural analogs are often found using quantitative structure activity relationships (QSAR), with techniques such as those disclosed in Remington (The Science and Practice of Pharmacology, 19th Edition (1995), chapter 28). A derivative is a biologically active molecule derived from the base structure. A mimetic is a biomolecule that mimics the activity of another biologically active molecule. Biologically active molecules can include both chemical structures and peptides of protein entities that mimic the biological activities of the compounds or peptides.

Animal: Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects.

Anti-proliferative activity: An activity of a molecule, *e.g.*, a compound or peptide as described herein, which reduces proliferation of at least one cell type, but which may reduce the proliferation (either in absolute terms or in rate terms) of multiple different cell types (*e.g.*, different

cell lines, different species, etc.). In specific embodiments, the anti-proliferative activity will be apparent against cells (either *in vitro* or *in vivo*) that exhibit a hyper-proliferative condition, such as is characteristic of certain disorders or diseases.

In certain embodiments, an anti-proliferative activity can be an anti-tumor or anti-neoplastic activity of a compound or peptide. Such molecules will be useful to inhibit or prevent cellular proliferation or growth, *e.g.* in a tumor, such as a malignant neoplasm.

Antisense, Sense, and Antigene: Double-stranded DNA (dsDNA) has two strands, a 5'→ 3' strand, referred to as the plus strand, and a 3' → 5' strand (the reverse compliment), referred to as the minus strand. Because RNA polymerase adds nucleic acids in a 5' → 3' direction, the minus strand of the DNA serves as the template for the RNA during transcription. Thus, the RNA formed will have a sequence complementary to the minus strand and identical to the plus strand (except that U is substituted for T).

Antisense molecules are molecules that are specifically hybridizable or specifically complementary to either RNA or the plus strand of DNA. Sense molecules are molecules that are specifically hybridizable or specifically complementary to the minus strand of DNA. Antigene molecules are either antisense or sense molecules directed to a dsDNA target.

Binding or stable binding: An oligonucleotide binds or stably binds to a target nucleic acid if a sufficient amount of the oligonucleotide forms base pairs or is hybridized to its target nucleic acid, to permit detection of that binding. Binding can be detected by either physical or functional properties of the target:oligonucleotide complex. Binding between a target and an oligonucleotide can be detected by any procedure known to one skilled in the art, including both functional and physical binding assays. Binding may be detected functionally by determining whether binding has an observable effect upon a biosynthetic process such as expression of a gene, DNA replication, transcription, translation and the like.

Physical methods of detecting the binding of complementary strands of DNA or RNA are well known in the art, and include such methods as DNAse I or chemical footprinting, gel shift and affinity cleavage assays, Northern blotting, dot blotting and light absorption detection procedures. For example, one method that is widely used, because it is so simple and reliable, involves observing a change in light absorption of a solution containing an oligonucleotide (or an analog) and a target nucleic acid at 220 to 300 nm as the temperature is slowly increased. If the oligonucleotide or analog has bound to its target, there is a sudden increase in absorption at a characteristic temperature as the oligonucleotide (or analog) and target disassociate from each other, or melt.

The binding between an oligomer and its target nucleic acid is frequently characterized by the temperature (T_m) at which 50% of the oligomer is melted from its target. A higher (T_m) means a stronger or more stable complex relative to a complex with a lower (T_m).

Binding inhibitory amount: An amount of a substance sufficient to inhibit, to some measurable extent, the binding of one substance to another. In some embodiments, at least one of the substances in a complex for which binding inhibition is being studied will be a protein, a peptide, or a fragment, mimetic, analog or derivative thereof. By way of example, such a complex may include a polo-like kinase and one (or more) of its cognate "receptor" molecules, for instance a protein that interacts with the kinase by way of a polo-box region within the kinase. In this example, a binding inhibitory amount of a substance (for instance, a peptide or other compound) would be an amount of that substance which is sufficient to disrupt at least a portion of the specific association between the kinase and its "receptor" molecule. In certain embodiments, the "receptor" molecule for a polo-like

kinase will be a protein involved in cytokinesis, for instance a neck filament protein (such as a septin, as described herein).

A binding inhibitory amount of a substance is merely an amount sufficient to disrupt some of the binding between two molecules, or two populations of molecules, and is not intended to be an absolute term. However, in certain embodiments a binding inhibitory amount of a substance will be an amount necessary to disrupt at least about 30% of the specific association between two molecules. In other embodiments, it will be an amount sufficient to disrupt at least about 40%, about 50%, about 60%, about 70%, or about 80% of such specific binding. Under some circumstances, binding inhibitory amounts will be sufficient to inhibit an even greater proportion of the binding between two molecules, and may be as high as at least about 90%, 95%, or even 98%. Such particularly high levels of binding inhibition are not required in all circumstances, however.

It is often advantageous to measure the amount of binding inhibition that is caused by the addition of a compound or peptide to a binding complex, or by different concentrations of that compound being added to the binding complex. One method for measuring an amount of binding inhibition is to compare the proportion of chained cells (and/or the length of such cell chains) in a culture of cells in the presence and absence of the test compound (and/or at different compound concentrations). Other methods for measuring inhibition are described herein.

cDNA (complementary DNA): A piece of DNA lacking internal, non-coding segments (introns) and transcriptional regulatory sequences. cDNA may also contain untranslated regions (UTRs) that are responsible for translational control in the corresponding RNA molecule. cDNA is usually synthesized in the laboratory by reverse transcription from messenger RNA extracted from cells.

Chaining (connected) growth pattern: A growth pattern (phenotype) in a eukaryote, particularly a yeast (*e.g.*, *S. cerevisiae*) wherein cytokinesis is inhibited to an extent sufficient to prevent a proportion of mature daughter cells from cleaving completely from the mother cell. The higher the percentage of "chained" cells (or the greater the average number of cells in a chain) in a culture, the "worse" or more cytokinesis-defective the phenotype is in that culture.

The chaining (connected) growth pattern is typified by yeast strains that over-express a C-terminal peptide that contains a polo-box from a polo-like kinase. Several such strains are described herein in detail.

Complementarity and percentage complementarity: Molecules with complementary nucleic acids form a stable duplex or triplex when the strands bind, (hybridize), to each other by forming Watson-Crick, Hoogsteen or reverse Hoogsteen base pairs. Stable binding occurs when an oligonucleotide remains detectably bound to a target nucleic acid sequence under the required conditions.

Complementarity is the degree to which bases in one nucleic acid strand base pair with the bases in a second nucleic acid strand. Complementarity is conveniently described by percentage, *i.e.* the proportion of nucleotides that form base pairs between two strands or within a specific region or domain of two strands. For example, if 10 nucleotides of a 15-nucleotide oligonucleotide form base pairs with a targeted region of a DNA molecule, that oligonucleotide is said to have 66.67% complementarity to the region of DNA targeted.

"Sufficient complementarity" means that a sufficient number of base pairs exist between the oligonucleotide and the target sequence to achieve detectable binding, and in the case of the binding of an antigen, disrupt expression of gene products (such as a polo-like kinase). When expressed or

measured by percentage of base pairs formed, the percentage complementarity that fulfills this goal can range from as little as about 50% complementarity to full, (100%) complementary. In general, sufficient complementarity is at least about 50%, about 75% complementarity, about 90% or 95% complementarity, and or about 98% or even 100% complementarity.

A thorough treatment of the qualitative and quantitative considerations involved in establishing binding conditions that allow one skilled in the art to design appropriate oligonucleotides for use under the desired conditions is provided by Beltz *et al. Methods Enzymol* 100:266-285, 1983, and by Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

Complex (complexed): Two proteins, or fragments or derivatives thereof, or between one protein (or fragment or derivative) and a non-protein compound, are said to form a complex when they measurably associate with each other in a specific manner. Such association can be measured in any of various ways, both direct and indirect. Direct methods may include co-migration in non-denaturing fractionation conditions, for instance. Indirect measurements of association will depend on secondary effects caused by the association of the two proteins or protein domains. For instance, the formation of a complex between a kinase and a receptor or localization anchor may be demonstrated by an alteration in the subcellular localization of the kinase, or a consequent growth defect (*e.g.*, a cytokinesis-defective growth pattern).

By way of example, the formation of a complex between a polo-like kinase (or a polo-box peptide) and a compound with polo-like kinase modulating activity can be measured by determining the degree to which the compound alters a cytokinesis-defective growth pattern in a cell expressing a polo-box domain of a polo-like kinase. In the case of a polo-box **inhibitory** compound, the inhibitory activity of the compound will be proportional to the extent to which the compound inhibits cytokinesis. Alternatively, in the test system described herein, the inhibitory activity of such a compound can be measured as the extent of recovery from a cytokinesis-defective phenotype of a cell over-expressing a polo-box domain (*e.g.*, a reduction in the proportion of chained cells in a yeast system). In the case of a polo-box-**enhancing** compound, activity of the compound can be measured by an intensification of a cytokinesis-defect observed in a cell over-expressing a polo-box domain (*e.g.*, an increase in the proportion of chained cells in a yeast system).

Compound with polo-like kinase modulatory activity: A compound that, when applied to a cell or cell free system, has a measurable effect on a polo-like kinase. In particular, such effects include any or all of the following: a modification in the subcellular localization of the polo-like kinase; a modification in binding affinity of polo-domain or the polo-box (or related peptide) for one or more specific binding partners; a change in the phosphorylating activity of the kinase; or an alteration (either stimulation or inhibition) in the stability of the polo-like kinase.

As used herein, the term "modulatory activity" generally includes (unless context clearly implies that one or the other is not appropriate) both inhibitory and enhancing (activating) activities. It is rare but not impossible that a single compound exhibits both kinase inhibitory and enhancing properties.

Cytokinesis-defective growth pattern: A growth pattern or phenotype that is measurably abnormal as a result of a defect in a cell's ability to progress through cytokinesis (cell division of, for instance, a daughter cell from its mother). The prototypical cytokinesis-defective growth pattern (phenotype) is exhibited by yeast cells that over-express a functional copy of a polo-box (*e.g.*, one sharing substantial sequence homology with the Cdc5 polo-box), thereby having an inhibition in

polo-like kinase mediated cytokinesis events. As described herein, such yeast cells form chains of connected cells, wherein cytokinesis has begun but is unable to finish. Such cultures produce branched, filamentous cell chains, for instance as illustrated in Song and Lee, "A Novel Function of Saccharomyces cerevisiae CDC 5 in Cytokinesis," *J. Cell Biol.* 152:451-470, February 5, 2001, incorporated herein by reference in its entirety.

Mammalian cells exhibit a different growth pattern in response to introduction of an over expressed polo-box. In particular, rather than leading to a visible chaining phenotype, mammalian cells exhibit a mitotic arrest with a rounded phenotype. Since over-expression of polo box induces multiple spindle poles by interaction with Bbp1 in budding yeast, the rounded phenotype see in mammalian cell culture suggests that in mammalian cells polo box function is also required for an essential event prior to cytokinesis (such as centromere maturation). Though this phenotype is visually different from that exhibited by a yeast cell, a change in the mammalian cell phenotype in response to application of a compound is still indicative of a compound that exhibits polo-like kinase/polo-box modulatory activity.

DNA (deoxyribonucleic acid): DNA is a long chain polymer which comprises the genetic material of most living organisms (some viruses have genes comprising ribonucleic acid (RNA)). The repeating units in DNA polymers are four different nucleotides, each of which comprises one of the four bases, adenine, guanine, cytosine and thymine bound to a deoxyribose sugar to which a phosphate group is attached. Triplets of nucleotides (referred to as codons) code for each amino acid in a polypeptide, or for a stop signal. The term codon is also used for the corresponding (and complementary) sequences of three nucleotides in the mRNA into which the DNA sequence is transcribed.

Unless otherwise specified, any reference to a DNA molecule is intended to include the reverse complement of that DNA molecule. Except where single-strandedness is required by the text herein, DNA molecules, though written to depict only a single strand, encompass both strands of a double-stranded DNA molecule. Thus, a reference to the nucleic acid molecule that encodes a polo-like kinase, or a fragment thereof (*e.g.*, a polo-box), encompasses both the sense strand and its reverse complement. Thus, for instance, it is appropriate to generate probes or primers from the reverse complement sequence of the disclosed nucleic acid molecules.

Deletion: The removal of a sequence of DNA, the regions on either side of the removed sequence being joined together. Correspondingly, a deletion in a protein is the removal of a region of amino acid sequence of the protein or peptide.

Dominant negative phenotype: A phenotype resulting form inhibiting endogenous protein function by expression of a protein, or a competing peptide. The term implies competition between the native protein and the expressed protein/peptide for a binding site.

The term as used herein more specifically refers to the effect of expressing or over-expressing a polo-box (or related peptide) in a eukaryotic cell and thereby blocking association of native polo-like kinase with one or more specific binding partners essential for their mitotic and/or cytokinetic functions, through competition for one or more polo-box specific binding site(s). This phenotype is illustrated, for instance, in FIG 7B. The phenotype is "dominant" in that it is apparent in cells that are expressing a native polo-like kinase, and "negative" in that it causes a recognizable growth defect (definable cell cycle arrest).

Genomic target sequence: A sequence of nucleotides located in a particular region in the human genome that corresponds to one or more specific genetic abnormalities, such as a nucleotide

polymorphism, a deletion, or an amplification. The target can be for instance a coding sequence; it can also be the non-coding strand that corresponds to a coding sequence.

Hybridization: Oligonucleotides and their analogs hybridize by hydrogen bonding, which includes Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary bases. Generally, nucleic acid consists of nitrogenous bases that are either pyrimidines (cytosine (C), uracil (U), and thymine (T)) or purines (adenine (A) and guanine (G)). These nitrogenous bases form hydrogen bonds between a pyrimidine and a purine, and the bonding of the pyrimidine to the purine is referred to as "base pairing." More specifically, A will hydrogen bond to T or U, and G will bond to C. "Complementary" refers to the base pairing that occurs between to distinct nucleic acid sequences or two distinct regions of the same nucleic acid sequence.

"Specifically hybridizable" and "specifically complementary" are terms that indicate a sufficient degree of complementarity such that stable and specific binding occurs between the oligonucleotide (or its analog) and the DNA or RNA target. The oligonucleotide or oligonucleotide analog need not be 100% complementary to its target sequence to be specifically hybridizable. An oligonucleotide or analog is specifically hybridizable when binding of the oligonucleotide or analog to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide or analog to non-target sequences under conditions where specific binding is desired, for example under physiological conditions in the case of *in vivo* assays or systems. Such binding is referred to as specific hybridization.

Hybridization conditions resulting in particular degrees of stringency will vary depending on the nature of the hybridization method of choice and the composition and length of the hybridizing nucleic acid sequences. Generally, the temperature of hybridization and the ionic strength (especially the Na^+ concentration) of the hybridization buffer will determine the stringency of hybridization, though waste times also influence stringency. Calculations regarding hybridization conditions required for attaining particular degrees of stringency are discussed by Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, chapters 9 and 11, herein incorporated by reference.

For purposes of the present disclosure, "stringent conditions" encompass conditions under which hybridization will only occur if there is less than 25% mismatch between the hybridization molecule and the target sequence. "Stringent conditions" may be broken down into particular levels of stringency for more precise definition. Thus, as used herein, "moderate stringency" conditions are those under which molecules with more than 25% sequence mismatch will not hybridize; conditions of "medium stringency" are those under which molecules with more than 15% mismatch will not hybridize, and conditions of "high stringency" are those under which sequences with more than 10% mismatch will not hybridize. Conditions of "very high stringency" are those under which sequences with more than 6% mismatch will not hybridize.

Hyper-proliferative disorder: A disorder characterized by abnormal proliferation of cells, and generically includes skin disorders such as psoriasis as well as benign and malignant tumors of all organ systems. This latter class of hyper proliferative disorder includes, for instance, breast carcinomas (including lobular and duct carcinomas) and other solid tumors, carcinomas, sarcomas, and cancers including carcinomas of the lung like small cell carcinoma, large cell carcinoma, squamous carcinoma, and adenocarcinoma, mesothelioma of the lung, colorectal adenocarcinoma, stomach carcinoma, prostatic adenocarcinoma, ovarian carcinoma such as serous

cystadenocarcinoma and mucinous cystadenocarcinoma, ovarian germ cell tumors, testicular carcinomas, and germ cell tumors, pancreatic adenocarcinoma, biliary adenocarcinoma, heptacellular carcinoma, bladder carcinoma including transitional cell carcinoma, adenocarcinoma, and squamous carcinoma, renal cell adenocarcinoma, endometrial carcinomas including adenocarcinomas and mixed Mullerian tumors (carcinosarcomas), carcinomas of the endocervix, ectocervix, and vagina such as adenocarcinoma and squamous carcinoma, tumors of the skin like squamous cell carcinoma, basal cell carcinoma, melanoma, and skin appendage tumors, esophageal carcinoma, carcinomas of the nasopharynx and oropharynx including squamous carcinoma and adenocarcinomas, salivary gland carcinomas, brain and central nervous system tumors including tumors of glial, neuronal, and meningeal origin, tumors of peripheral nerve, soft tissue sarcomas and sarcomas of bone and cartilage.

Injectable composition: A pharmaceutically acceptable fluid composition comprising at least one active ingredient, *e.g.*, a compound with polo-like kinase modulatory activity, a polo-box related peptide, or a polo-box binding peptide. The active ingredient is usually dissolved or suspended in a physiologically acceptable carrier, and the composition can additionally comprise minor amounts of one or more non-toxic auxiliary substances, such as emulsifying agents, preservatives, and pH buffering agents and the like. Such injectable compositions that are useful for use with the compounds and peptides of this disclosure are conventional; formulations are well known in the art.

Isolated: An "isolated" biological component (such as a nucleic acid molecule, protein or organelle) has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, *i.e.*, other chromosomal and extra-chromosomal DNA and RNA, proteins and organelles. Nucleic acids and proteins that have been "isolated" include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

Kinase: An enzyme that transfers a phosphate group, usually from ATP.

Kinase (phosphorylating) activity: Measurable phosphorylating activity of a protein (a kinase). Kinase activity can be quantified using well known assays (see, *e.g.*, Jaspersen *et al.*, Mo. Biol. Cell, 9:2803-2817, 1998), which measure, for instance, incorporation of a radioactive isotope of phosphorous into a test compound (*e.g.*, incorporation of [γ-^{32}P] from [γ-^{32}P]ATP into a protein with a known phosphorylation site). Alternatively, kinase activity can be measured by the degree of autophosphorylation or on the basis of a phosphorylation-dependent protein mobility shift.

Mimetic: A molecule (such as an organic chemical compound) that mimics the activity of a protein or peptide, such as the specific binding activity of a polo-box peptide. Peptidomimetic and organomimetic embodiments are within the scope of this term, whereby the three-dimensional arrangement of the chemical constituents of such peptido- and organomimetics mimic the three-dimensional arrangement of the peptide backbone and component amino acid sidechains in the peptide, resulting in such peptido- and organomimetics of the peptides having substantial specific inhibitory activity. For computer modeling applications, a pharmacophore is an idealized, three-dimensional definition of the structural requirements for biological activity. Peptido- and organomimetics can be designed to fit each pharmacophore with current computer modeling software (using computer assisted drug design or CADD). See Walters, ("Computer-Assisted Modeling of Drugs" in Klegerman & Groves, eds., 1993, Pharmaceutical Biotechnology, Interpharm Press:

Buffalo Grove, IL, pp. 165-174 and Principles of Pharmacology (ed. Munson, 1995), chapter 102) for a description of techniques used in computer assisted drug design.

Neck filament components: Molecular components of the structures involved in cytokinesis, *e.g.*, in a yeast cell. Neck filament components include, for instance, septins (such as Cdc3, Cdc10, Cdc11, and Cdc12). Data reported herein indicate that the polo-box of polo-like kinases is required for localization of this protein to the bud neck during cytokinesis. This cytokinesis-associated localization of at least a sub-population of polo-like kinase molecules in a cell to the neck filaments may indicate that a direct interaction between polo-box peptide and a septin occurs, and is essential for completion of cytokinesis.

Nucleotide: "Nucleotide" includes, but is not limited to, a monomer that includes a base linked to a sugar, such as a pyrimidine, purine or synthetic analogs thereof, or a base linked to an amino acid, as in a peptide nucleic acid (PNA). A nucleotide is one monomer in a polynucleotide. A nucleotide sequence refers to the sequence of bases in a polynucleotide.

Oligonucleotide: An oligonucleotide is a plurality of joined nucleotides joined by native phosphodiester bonds, between about 6 and about 300 nucleotides in length. An oligonucleotide analog refers to moieties that function similarly to oligonucleotides but have non-naturally occurring portions. For example, oligonucleotide analogs can contain non-naturally occurring portions, such as altered sugar moieties or inter-sugar linkages, such as a phosphorothioate oligodeoxynucleotide. Functional analogs of naturally occurring polynucleotides can bind to RNA or DNA, and include peptide nucleic acid (PNA) molecules.

Particular oligonucleotides and oligonucleotide analogs can include linear sequences up to about 200 nucleotides in length, for example a sequence (such as DNA or RNA) that is at least 6 bases, for example at least 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100 or even 200 bases long, or from about 6 to about 50 bases, for example about 10-25 bases, such as 12, 15 or 20 bases.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

Open reading frame: A series of nucleotide triplets (codons) coding for amino acids without any internal termination codons. These sequences are usually translatable into a peptide.

Ortholog: Two nucleic acid or amino acid sequences are orthologs of each other if they share a common ancestral sequence and diverged when a species carrying that ancestral sequence split into two species. Orthologous sequences are also homologous sequences.

Parenteral: Administered outside of the intestine, *e.g.*, not via the alimentary tract. Generally, parenteral formulations are those that will be administered through any possible mode except ingestion. This term especially refers to injections, whether administered intravenously, intrathecally, intramuscularly, intraperitoneally, or subcutaneously, and various surface applications including intranasal, intradermal, and topical application, for instance.

Peptide Nucleic Acid (PNA): An oligonucleotide analog with a backbone comprised of monomers coupled by amide (peptide) bonds, such as amino acid monomers joined by peptide bonds.

Pharmaceutical agent or drug: A chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers useful in this disclosure are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of the proteins and other compositions herein disclosed.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (*e.g.*, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Polo-like kinase: A member of a family of kinase proteins that are characterized by the presence of a distinct region of homology in the C-terminal non-catalytic domain of the kinase. This domain is termed the polo-box, and it appears (as reported herein) to be important for subcellular localization of polo-like kinase proteins. The name of this family of kinases is derived form the *polo* gene, which encodes the first polo-like kinase identified; *polo* is a *Drosophila* gene.

Members of the polo subfamily of protein kinases (*e.g.*, Cdc5, *Polo*, $Plk1_{mammalian}$, Plo1p, Snk, FNK/Prk, Plx1, Tbplk, and $Plk1_{C.\ elegans}$) have been identified in various eukaryotic organisms. These kinases are known to play pivotal roles in cell division and proliferation. Studies in various organisms have shown that polo kinases regulate diverse cellular and biochemical events at multiple stages of M phase. These include centrosome maturation, bipolar spindle formation, and activation of anaphase promoting complex (APC).

Specific examples of sequences of polo-lik kinases include those disclosed in the following GenBank Accession Nos.: P32562 (*S. cerevisiae* Cdc5); P53351 (*M. musculus* Snk); P50528 (*S. pombe* Plo1); P52304 (*D. melanogaster* Polo); P34331 (*C. elegans* Ykz4); P53350 *(H. sapiens* Plk1); Q07832 (*M. musculus* Plk1); and CAA02714 (*H. sapiens* serine-threonine kinase). These sequences are incorporated herein by reference.

Polo-box: A distinct region of homology in the C-terminal non-catalytic domain of a polo-like kinase. This domain appears (as reported herein) to be important for subcellular localization of these kinases. The core sequence of a polo-box (corresponding to residues 513 through 542 of Cdc5 and residues 410 through 439 of mammalian Plk) is as follows:

KWVDYSxKxGxxYQLxxxxxxVxFN (SEQ ID NO: 1).

The specified residues in this sequence are highly conserved across members of the polo-like kinase family of proteins. In particular, as discussed herein and by Song *et al.* (*Mol. Cell. Biol.* 20:286-298, 2000; incorporated herein by reference, in its entirety), mutation of the second residue (a tryptophan) to a phenylalanine essentially completely disrupts subcellular localization of polo-like kinase to cytokinesis-related structures, and can cause a defect in mitotic functions of the polo-like kinase.

In general, as discussed herein, a polo-box (related) peptide is a linear sequence of amino acids, usually longer than the consensus sequence depicted in SEQ ID NO: 1, that is capable of competing with a native polo-box for binding to or specific association with at least one specific binding partner (target) of the native polo-box. At a minimum, such a peptide contains at least 25 residues from a polo-like kinase C-terminal region, or a functional (*e.g.*, binding functional) variant, analog, or mimetic thereof. A polo-box related peptide can include further linear amino acid sequences upstream and/or downstream of the core consensus box. For instance, there may be about an additional about 10 amino acids on either or both the upstream and downstream sides of the consensus box. In certain embodiments, there will be at least about 20, at least about 30, at least about 40, at least about 50, at least about 75, or more additional amino acids on the upstream and/or downstream sides of the consensus box to form the polo-box-related peptide. The length of any additional amino acid sequence does not need to be the same on the upstream and downstream sides of the consensus box.

Additional amino acid sequences can be from a native polo-like kinase (as in certain of the specific examples disclosed herein), or they can be sequences taken from other proteins or purely or partially random or other non-naturally occurring amino acid sequences. One possible purpose for the addition of additional up- and/or downstream sequences in a polo-box peptide is to provide stability of the peptide in a biological system (*e.g.*, a cell or the body of a subject). In certain embodiments, these additional sequences of amino acids can serve to tag the peptide in order to enable tracking and localization (*e.g.*, with a green fluorescent protein, as described herein, or with an epitope or other peptide tag), to target the peptide to specific cells or tissues (*e.g.*, through interaction of a targeting or binding domain to a receptor, especially when the peptide is applied to a subject as a pharmaceutical preparation rather than through gene therapy), or for other purposes.

Functional fragments and variants of a polo-box are those peptide sequences share sequence homology with a native polo-box domain, but have one or more sequence change (for instance, a conservative residue change, deletion, addition and so forth), and still maintain a polo-box functional activity (*e.g.*, a binding activity or other activity as described herein).

Primers: Primers are short nucleic acid molecules, for instance DNA oligonucleotides 10 nucleotides or more in length. Longer DNA oligonucleotides may be about 15, 20, 25, 30 or 50 nucleotides or more in length. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then the primer extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, *e.g.*, by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art.

Methods for preparing and using nucleic acid primers are described, for example, in Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual*, CSHL, New York, 1989), Ausubel *et al.* (ed.) (In *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, 1998), and Innis *et al.* (*PCR Protocols, A Guide to Methods and Applications*, Academic Press, Inc., San Diego, CA, 1990). Amplification primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, © 1991, Whitehead Institute for Biomedical Research, Cambridge, MA). One of ordinary skill in the art will appreciate that the specificity of a particular probe or primer increases with its length.

Probes: A probe comprises an isolated nucleic acid attached to a detectable label or other reporter molecule. Typical labels include radioactive isotopes, enzyme substrates, co-factors, ligands, chemiluminescent or fluorescent agents, haptens, and enzymes. Methods for labeling and guidance in the choice of labels appropriate for various purposes are discussed, *e.g.*, Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual*, CSHL, New York, 1989) and Ausubel *et al.* (In *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, 1998).

Promoter: A promoter is an array of nucleic acid control sequences that direct transcription of a nucleic acid. A promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements that can be located as much as several thousand base pairs from the start site of transcription.

Protein: A biological molecule expressed by a gene and comprised of amino acids.

Purified: The term "purified" does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified protein preparation is one in which the protein referred to is more pure than the protein in its natural environment within a cell or within a production reaction chamber (as appropriate).

Recombinant: A recombinant nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, *e.g.*, by genetic engineering techniques.

Sequence identity: The similarity between two nucleic acid sequences, or two amino acid sequences, is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith & Waterman *Adv. Appl. Math.* 2: 482, 1981; Needleman & Wunsch *J. Mol. Biol.* 48: 443, 1970; Pearson & Lipman *Proc. Natl. Acad. Sci. USA* 85: 2444, 1988; Higgins & Sharp *Gene,* 73: 237-244, 1988; Higgins & Sharp *CABIOS* 5: 151-153, 1989; Corpet *et al. Nuc. Acids Res.* 16, 10881-90, 1988; Huang *et al. Computer Appls. in the Biosciences* 8, 155-65, 1992; and Pearson *et al. Meth. Mol. Bio.* 24, 307-31, 1994. Altschul *et al.* (*J. Mol. Biol.* 215:403-410, 1990), presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al. J. Mol. Biol.* 215:403-410, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx.

An alternative indication that two nucleic acid molecules are closely related is that the two molecules hybridize to each other under stringent conditions. Stringent conditions are sequence-dependent and are different under different environmental parameters. Generally, stringent conditions are selected to be about 5° C to 20° C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence remains hybridized to a perfectly matched probe or complementary strand. Conditions for nucleic acid hybridization and calculation of stringencies can be

found in Sambrook *et al.* ((1989) In *Molecular Cloning: A Laboratory Manual*, CSHL, New York) and Tijssen ((1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes* Part I, Chapter 2, Elsevier, New York). Nucleic acid molecules that hybridize under stringent conditions to a human polo-like kinase encoding sequence (*e.g.*, a plk-1 encoding sequence) will typically hybridize to a probe based on either an entire human plk-1 encoding sequence or selected portions of the encoding sequence under wash conditions of 2 x SSC at 50° C.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences, due to the degeneracy of the genetic code. It is understood that changes in nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid molecules that all encode substantially the same protein.

Specific binding agent: An agent that binds substantially only to a defined target. Thus a polo kinase (*e.g.*, Plk) protein-specific binding agent binds substantially only the polo kinase protein. As used herein, the term "polo kinase specific binding agent" includes anti-polo kinase antibodies (and functional fragments thereof) and other agents (such as soluble receptors) that bind substantially only to a polo kinase protein. For instance, a Plk -specific binding agent would bind substantially only to Plk.

Anti-polo kinase antibodies may be produced using standard procedures described in a number of texts, including Harlow and Lane (*Antibodies, A Laboratory Manual*, CSHL, New York, 1988). The determination that a particular agent binds substantially only to the selected polo kinase may readily be made by using or adapting routine procedures. One suitable *in vitro* assay makes use of the Western blotting procedure (described in many standard texts, including Harlow and Lane (*Antibodies, A Laboratory Manual*, CSHL, New York, 1988)). Western blotting may be used to determine that a given polo kinase binding agent, such as an anti- Plk protein monoclonal antibody, binds substantially only to the chosen polo kinase (*e.g.*, Plk). By way of example, a specific method for the production of polyclonal anti- Plk antibodies can be found in Lee *et al.* (*Mol. Cell Biol.* 15:71433-7151, 1995), incorporated herein by reference.

Shorter fragments of antibodies can also serve as specific binding agents. For instance, FAbs, Fvs, and single-chain Fvs (SCFvs) that bind to Plk would be Plk -specific binding agents. These antibody fragments are defined as follows: (1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain; (2) Fab', the fragment of an antibody molecule obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule; (3) (Fab')2, the fragment of the antibody obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; (4) F(ab')2, a dimer of two Fab' fragments held together by two disulfide bonds; (5) Fv, a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and (6) single chain antibody ("SCA"), a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. Methods of making these fragments are routine.

Subject: Living multi-cellular vertebrate organisms, a category that includes both human and non-human mammals.

Target sequence: "Target sequence" is a portion of ssDNA, dsDNA or RNA that, upon hybridization to a therapeutically effective oligonucleotide or oligonucleotide analog, results in the inhibition of expression of a polo kinase, *e.g.*, Plk or an ortholog thereof. Either an antisense or a

sense molecule can be used to target a portion of dsDNA, since both will interfere with the expression of that portion of the dsDNA. The antisense molecule can bind to the plus strand, and the sense molecule can bind to the minus strand. Thus, target sequences can be ssDNA, dsDNA, and RNA.

Therapeutically effective amount of (a substance): A quantity of a compound with polo-like kinase modulating activity, a polo-box or related peptide, or a polo-box binding peptide sufficient to achieve a desired effect in a subject being treated. For instance, this can be the amount necessary to inhibit cellular proliferation or to measurably inhibit (or enhance) a polo-like kinase phosphorylating activity. For those embodiments in which the compound or peptide is used to inhibit cell proliferation, in general, a therapeutically effective amount will be sufficient to measurably inhibit proliferation of a target cell. Techniques for measuring such activity are discussed herein.

An effective amount of kinase modulating compound, polo box (or related peptide), or binding peptide may be administered in a single dose, or in several doses, for example daily, during a course of treatment. However, the effective amount of a composition will be dependent on the compound or peptide applied, the subject being treated, the severity and type of the affliction, and the manner of administration of the composition. For example, a therapeutically effective amount of composition can vary from about 0.01 mg/kg body weight to about 1 g/kg body weight.

The therapeutic substances (compounds and peptides) disclosed have equal application in medical and veterinary settings. Therefore, the general term "subject being treated" is understood to include all animals (*e.g.* humans, apes, dogs, cats, horses, and cows) that are or may suffer from a cellular proliferation-related disease, disorder or condition that is susceptible to polo-like kinase pathway-mediated modulation.

Transformed: A transformed cell is a cell into which has been introduced a nucleic acid molecule by molecular biology techniques. As used herein, the term transformation encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration.

Tumor: A neoplasm that may be either malignant or non-malignant. "Tumors of the same tissue type" refers to primary tumors originating in a particular organ (such as breast, prostate, bladder or lung). Tumors of the same tissue type may be divided into tumor of different sub-types (a classic example being bronchogenic carcinomas (lung tumors) which can be an adenocarcinoma, small cell, squamous cell, or large cell tumor).

Variant polo-box related peptides: Polo-box related peptides having one or more amino acid substitutions, one or more amino acid deletions, and/or one or more amino acid insertions, so long as the peptide retains the property of binding to a polo-box and thereby modulating a polo-box kinase function. Conservative amino acid substitutions may be made in at least 1 position, for example 2, 3, 4, 5 or even 10 positions, as long as the peptide retains modulatory activity, as readily measured by the cytokinesis defect, yeast-based assay disclosed in the present specification.

Conserved residues in the same or similar proteins from different species (*e.g.*, from different members of the polo-like kinase family) can also provide guidance about possible locations for making substitutions in the sequence. A residue that is highly conserved across several species (see SEQ ID NO: 1) is more likely to be important to the function of the protein than a residue that is less conserved across several species.

Vector: A nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art.

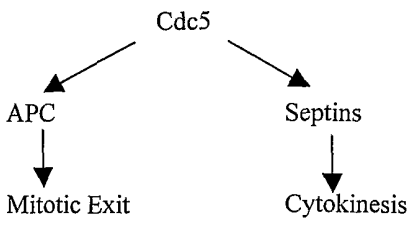
Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

II. Yeast Strains Overexpressing a Polo-box

Cells depleted of Cdc5 function arrest at multiple points of M phase, including an arrest in cytokinesis. Consistent with this observation, inhibition of Cdc5 function by overexpressing the C-terminal domain of Cdc5 (cdc5ΔN), but not the corresponding polo-box mutant, leads to a dominant-negative cytokinetic defect. This defect is likely due to an inhibition of a direct interaction between endogeneous Cdc5 and septins, Cdc11 and Cdc12, by the kinase activity-deficient cdc5ΔN. Cells expressing cdc5ΔN appear to proceed through the cell cycle normally as evidenced by cycling Cdc28/Clb2 activity, which indicates that an intact polo-box is critical for selective inhibition of cytokinesis without disrupting mitotic exit. Polo-box-dependent localization of Cdc5 at cytokinetic neck-filaments likely provides the temporal and spatial regulation of Cdc5, which may be important in coordinating the completion of mitosis with the initiation of cytokinesis.

Overexpression of the C-terminal domain of Cdc5 (*cdc5ΔN*), but not the corresponding polo-box mutant, resulted in connected cells without interfering with nuclear division cycle. These cells shared cytoplasms with incomplete septa, and possessed aberrant septin ring structures. Provision of additional copies of endogeneous *CDC5* remedied this phenotype; this indicates that over-expression of the polo box results in a dominant-negative inhibition of cytokinesis. The polo-box-dependent interactions between Cdc5 and septins (Cdc11 and Cdc12) and genetic interactions between the dominant-negative cdc5ΔN and Cyk2/Hof1 or Myo1 indiate that direct interactions between cdc5ΔN and septins resulted in inhibition of Cyk2/Hof1- and Myo1-mediated cytokinetic pathways. Cdc5 is therefore believed to coordinate mitotic exit with cytokinesis by participating in both APC activation and a polo-box-dependent cytokinetic pathway, as illustrated in the following diagram:

CDC5 pathway diagram:



At late anaphase/telophase, but prior to full spindle elongation and assembly of contractile ring, Cdc5 carries out two important functions. First, in a polo-box-independent manner, Cdc5 activates APC, which leads to inactivation of Cdc28/Clb2, thereby permitting mitotic exit (left-hand pathway). Second, in a polo-box-dependent manner, Cdc5 localizes at cytokinetic neck-filaments and promotes the cytokinetic functions of septins (right-hand pathway).

MATERIALS AND METHODS

Plasmid Construction

All plasmids (Table 1, part 2) and yeast strains (Table 1, part 1) used in this study are listed below. In order to generate a *GAL1-cdc5-1* construct, a PCR-amplified *cdc5-1* allele from strain KKY921-2BY was digested with *Ppu*MI and *Bst*EII to generate pSK877 (Song *et al.*, *Mol. Cell. Biol.* 20:286-298, 2000). A Plk-expressing integration construct was generated by inserting a *Spe*I-*Sph*I fragment of *GAL1-HA-EGFP-Plk* from YCplac111-*GAL1-HA-EGFP-Plk* (Lee *et al.*, *Proc. Natl. Acad. Sci. U S A.* 95:9301-9306, 1998) into a pUC19-*TRP1* vector to yield pSK1267.

To visualize the subcellular structures of spindle, septin, or contractile rings, *GFP*-tagged *TUB1*, *CYK2*, *MYO1*, or *CDC3* constructs were integrated into the genome. *Kpn*I-*Not*I fragments containing either *TUB1-GFP* (from pAFS125; provided by Aaron F. Straight, Harvard Medical School, Boston, MA) or *CYK2-GFP* (from pLP2; provided by Rong Li, Harvard Medical School, Boston, MA) were inserted into YCplac111 vector digested with *Bgl*II and *Kpn*I to generate pUC19-*TUB1-GFP::LEU2* (pSK1050) and pUC19-*CYK2-GFP::LEU2* (pSK1051). The resulting constructs lack both *ARS* and *CEN* loci, but bear the pUC19 backbone and *LEU2* gene. pUC19-*MYO1-GFP::LEU2* (pSK1052) was generated by inserting a *Pst*I-*Not*I fragment of *MYO1-GFP* (from pLP8; a gift of Rong Li) into the *Pst*I-*Bgl*II fragment of YCplac111 lacking both *ARS* and *CEN* loci. pUC19-*YFP-CDC3::LEU2* (pSK1059) was created by inserting the *Sst*I-*Sph*I fragment of *CDC3* tagged N-terminally with *YFP* (a yellow-green variant of GFP; Clontech, Palo Alto, CA) into a pUC19-*LEU2* construct.

Gene disruption constructs for *CYK2* (pSK1330), *CDC10* (pSK1332), and *MYO1* (pSK1348) were generated by inserting a 1.5 kb *Bgl*II-*Pme*I fragment of *KanMX6* from pFA6a-13Myc-*KanMX6* (Longtine *et al.*, *Yeast.* 14:953-961, 1998) into the open reading frame of the corresponding genes in pLP1 (*CYK2*-Myc, a gift of Rong Li), pSK1060 (YFP-*CDC10*), or pSK1052 (*MYO1*-GFP), respectively.

Several dominant-negative *cdc5ΔN* constructs were used to inhibit the function of endogenous Cdc5. A fragment of GAL1-2X*(EGFP)-cdc5ΔN* or its *FAA* mutant (Song *et al.*, *Mol. Cell. Biol.* 20:286-298, 2000) was inserted into a pUC19-*URA3* plasmid to generate pSK983 or pSK984, respectively. To generate a *GST*-tagged *cdc5ΔN*, a 0.75 kb *Sal*I-*Xho*I fragment containing *GST* open reading frame was PCR-amplified using pGEX-KG (Pharmacia, Peapack, NJ) as a template. This fragment was inserted into pSK983 digested with *Xho*I digestion to replace *GFP* coding sequence.

To construct plasmids for two-hybrid analyses, *CDC3*, *CDC10*, *CDC11*, and *CDC12*, were PCR-amplified using genomic clones (provided by John Chant, Harvard University, Cambridge, MA) as templates. The *Eco*RI-*Xho*I (artificially introduced restriction enzyme sites at 5' end and 3' end of each open reading frame) fragments were ligated into pJG4-5 plasmid digested with corresponding enzymes. This results in in-frame fusion of full-length septins to the activation

domain (AD). To generate a LexA DNA binding domain (DBD)-fused *CDC5* construct, a 2-kb *Xba*I fragment from pSK1006 was inserted into *Eco*RI-digested, end-filled pEG202-NLS (Origene Technologies Inc., Rockville, MD). Various forms of *CDC5* mutants were constructed by PCR amplification or enzymatic deletion (see Table 1 and FIG 4).

Strain Construction

All strains constructed in this study were confirmed by PCR or Southern hybridization. A *cdc5Δ::KanMX6* mutation was introduced into strain 1783 harboring either pSK877(*GAL1-cdc5-1*) or pSK1267(*GAL1-Plk*) by a one step gene disruption method described previously (Longtine *et al.*, *Yeast.* 14:953-961, 1998). Overexpression of *cdc5ΔN* under *GAL1* promoter control was achieved by the integrating pSK983, pSK984, or pSK1041 into strain 1783 at the *URA3* locus.

Strain KLY1229 was generated by integrating two additional copies of *GAL1-HA-2X(EGFP)-cdc5ΔN/FAA::TRP1* (pSK929) into strain KLY1081. To generate a *swe1Δ* strain (KLY1439), a 2.3 kb *Xba*I fragment from p*SWE1*-10g (Booher *et al.*, *EMBO J.* 12:3417-3426, 1993) was transformed into strain KLY1083. To disrupt *CYK2*, a 2 kb *Bam*HI fragment from pSK1330 that contains the *KanMX6* and flanking *CYK2* sequences was transformed into strain KLY1083. To generate *cdc10Δ* and *myo1Δ* strains, 1.8 kb and 2 kb *Bam*HI-*Sph*I fragments from pSK1332 and pSK1348, respectively, were introduced into KLY1083 to yield KLY1589 and KLY1593, respectively.

Growth Conditions, Media, and Zymolyase Treatment

Yeast cell cultures and transformations were carried out by standard methods (Sherman, Fink, and Hicks, *Methods in yeast genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986). For cell cycle synchronization, *MAT***a** cells were arrested with 5 μg/ml α-factor mating pheromone (Sigma, St. Louis, MO). Treatment of the connected cells with zymolyase was performed as described previously (Frazier *et al.*, *J. Cell Biol.* 143:737-749, 1998; Lippincott and Li, *J. Cell Biol.* 140:355-366, 1998). Loss of the refractile appearance (indicating that cell wall removal was efficient under these conditions) was evident under the microscope.

Immunoprecipitation, Kinase assays, and Western analyses

Cell lysates were prepared in TED buffer (40 mM Tris-Cl (pH 7.5), 0.25 mM EDTA, 1 mM DTT, 1 mM AEBSF (Pefabloc; Boehringer Mannheim, IN), 10 μM/ml pepstatin A, 10 μM/ml leupeptin, and 10 μM/ml aprotinin) with an equal volume of glass beads (Sigma, St. Louis, MO). To measure Clb2-associated Cdc28 kinase activity, lysates were spun at 15, 000 x g for 10 minutes, and the resulting supernatants subjected to immune complex kinase assays using an anti-Clb2 antibody (provided by David Morgan, University of California, San Francisco, CA) as described previously (Jaspersen *et al.*, *Mol. Biol. Cell.* 9:2803-2817, 1998). Western analyses were carried out with an anti-GFP antibody (Clontech, CA), anti-Cdc28 antibody (provided by Raymond Deshaies, California Institute of Technology, Pasadena, CA), anti-Clb2 antibody, and anti-HA antibody as described previously (Tinker-Kulberg and Morgan, *Genes Dev.* 13:1936-1949, 1999; Song *et al.*, *Mol. Cell. Biol.* 20:286-298, 2000). Proteins that interact with antibodies were detected using the enhanced chemiluminescence (ECL) Western detection system (Amersham, Princeton, NJ).

Table 1. Yeast Strains and plasmids used in this study.

Strain	Relevant genotype	Source
1783	*MATa leu2-3, 112 ura3-52 trp1-1 his4 can1^r* (isogenic of EG123)	I. Herskowitz
KKY921-2B	*MATa cdc5-1 leu2 trp1 ura1*	A. Sugino
KKY902	*MATa/α leu2-3, 112 trp1-289 ura3-52 HIS7/his7-1 CAN1/can1 CDC5/cdc5Δ::LEU2*	A. Sugino
KLY969	*MATa leu2 trp1 ura3 (a segregant of KKY902)*	This work
KLY1046	as KKY921-2B except *cdc5Δ::KanMX6 TUB1-GFP::LEU2 GAL1-HA-EGFP-PLK::TRP1*	This work
KLY1047	as KKY969 except *cdc5Δ::KanMX6 TUB1-GFP::LEU2 (YCplac22-GAL1-HA-2X(EGFP)-cdc5-1)*	This work
KLY1053	as 1783 except *GAL1-HA-GST-cdc5ΔN::URA3*	
KLY1069	as 1783 except *GAL1-HA-GST-cdc5ΔN::URA3 TUB1-GFP::LEU2*	This work
KLY1071	as 1783 except *GAL1-HA-GST-cdc5ΔN::URA3 CYK2-GFP::LEU2*	This work
KLY1073	as 1783 except *GAL1-HA-GST-cdc5ΔN::URA3 MYO1-GFP::LEU2*	This work
KLY1075	as 1783 except *GAL1-HA-GST-cdc5ΔN::URA3 YFP-CDC3::LEU2*	This work
KLY1080	as 1783 except *GAL1-HA-2X(EGFP)::URA3*	This work
KLY1081	as 1783 except *GAL1-HA-2X(EGFP)-cdc5ΔN::URA3*	This work
KLY1082	as 1783 except *GAL1-HA-2X(EGFP)-cdc5ΔN/FAA::URA3*	This work
KLY1083	as 1783 except *3X[GAL1-HA-2X(EGFP)-cdc5ΔN::URA3]*	This work
KLY1229	as 1783 except *GAL1-HA-2X(EGFP)-cdc5ΔN/FAA::URA3 2X[GAL1-HA-2X(EGFP)-cdc5ΔN/FAA::TRP1]*	This work
KLY1253	as KKY921-2B except *TUB1-GFP::LEU2*	This work
KLY1256	as KKY921-2B except *CYK2-GFP::LEU2*	This work
KLY1258	as KKY921-2B except *MYO1-GFP::LEU2*	This work
KLY1260	as KKY921-2B except *YFP-CDC3::LEU2*	This work
KLY1439	as KLY1083 except *swe1Δ::LEU2*	This work
KLY1589	as KLY1083 except *cdc10Δ::KanMX6*	This work
KLY1590	as KLY1083 except *cdc10Δ::KanMX6*	This work
KLY1591	as KLY1083 except *cyk2Δ::KanMX6*	This work
KLY1593	as KLY1083 except *myo1Δ::KanMX6*	This work

Table 1. continued

Name	Description	Source and reference
pSWE1-10g	pUC118-*swe1Δ::LEU2*	Booher et al., 1993
pSK615	YCplac111-*CDC5*	Song et al., 2000
pSK772	YCplac111-*cdc5(N209A)*	Song et al., 2000
pSK785	YCplac111-*cdc5(W517F/V518A/L530A)*	Song et al., 2000
pSK856	pUC19-*GAL1-HA-2X(EGFP)-cdc5ΔN/FAA::TRP1*	This work
pSK877	YCplac22-*GAL1-HA-2X(EGFP)-cdc5-1(P511L)*	This work
pSK983	pUC19-*GAL1-HA-2X(EGFP)-cdc5ΔN::URA3*	This work
pSK984	pUC19-*GAL1-HA-2X(EGFP)-cdc5ΔN/FAA::URA3*	This work
pSK986	pUC19-*GAL1-HA-2X(EGFP)::URA3*	This work
pSK1041	pUC19-*GAL1-HA-GST-cdc5ΔN:URA3*	This work
pSK1050	pUC19-*TUB1-GFP::LEU2*	Derived from pAFS125 (Straight et al., 1997)
pSK1051	pUC19-*CYK2-GFP::LEU2*	Derived from pLP2 (Lippincott and Li, 1998b)
pSK1052	pUC19-*MYO1-GFP::LEU2*	Derived from pLP8 (Lippincott and Li, 1998a)
pSK1059	pUC19-*YFP-CDC3::LEU2*	This work
pSK1267	pUC19-*GAL-HA-EGFP-PLK::TRP1*	This work
pSK1330	pUC19-*cyk2Δ::KanMX6*	This work
pSK1332	pUC19-*cdc10Δ::KanMX6*	This work
pSK1348	pUC19-*myo1Δ::KanMX6*	This work
pSK1366	pJG4-5-*CDC3*	This work
pSK1367	pJG4-5-*CDC10*	This work
pSK1368	pJG4-5-*CDC11*	This work
pSK1369	pJG4-5-*CDC12*	This work
pSK1390	pEG202-NLS-*CDC5ΔN*	This work
pSK1405	pEG202-NLS-*CDC5*	This work
pSK1407	pEG202-NLS-*CDC5ΔN/FAA*	This work
pSK1408	pEG202-NLS-*CDC5ΔC*	This work

Cell Staining and Immunofluorescence Microscopy

To visualize plasma membranes, cells were stained with DiI (Molecular Probes, Eugene, OR) as described previously (Lippincott and Li, *J. Cell Biol.* 143:1947-1960, 1998). To determine whether septums are formed between the cell bodies, calcofluor staining was carried out as previously described (Pringle, Staining of bud scars and other cell wall chitin with calcofluor. *In Guide to Yeast Genetics and Molecular Biology,* Vol. 194, (Guthrie and Fink, editors) Academic Press, San Diego, CA. 732-735, 1991; Lippincott and Li, *J. Cell Biol.* 143:1947-1960, 1998) with a

fluorescent brightener 28 (Sigma, St. Louis, MO), then serial sections were obtained using a confocal microscope with 0.1 nm interval.

Indirect immunofluorescence was performed as described previously (Lee *et al.*, *Proc. Natl. Acad. Sci. USA.* 95:9301-9306, 1998). Briefly, cells cultured under induction conditions for the indicated time were fixed with 3.7% formaldehyde. Actin was localized using rhodamine-conjugated phalloidin (Molecular Probe, Eugene, OR). DNA was visualized with a 4',6-diamidino-2-phenylindole (DAPI). Confocal fluorescent images were collected with a Bio-Rad MRC 1024 confocal scan head mounted on a Nikon Optiphot microscope with a 60X planapochromat lens or with a Leica TCS spectrophotometer confocal microscope.

Two-hybrid Analyses

Two-hybrid analyses were performed using a system described by Gyuris *et al.* (*Cell.* 75:791-803, 1993). Full-length *CDC3, CDC10, CDC11,* and *CDC12* were fused to transcriptional activation domain in pJG-5, whereas various forms of *CDC5* were fused to DNA binding domain in pEG202-NLS (Origene Technologies, Inc., Rockville, MD). These constructs were co-transformed with a reporter plasmid pSH18-34 into yeast strains EGY48 and EGY194, respectively. After combinatorial matings, duplicate samples of each combination were subjected to quantitative β-galactosidase assays.

RESULTS

Loss of *CDC5* Function Results in Arrests in Multiple Points of M Phase

Although polo kinases are believed to play important roles in multiple stages of M phase in various eukaryotic organisms, it has not been clear whether budding yeast polo kinase Cdc5 has a role other than in the mitotic exit pathway. The results reported herein demonstrate that Cdc5 does play additional roles, as illustrated by the terminal morphology of a *cdc5Δ* mutant. Since overexpression of Cdc5 inhibits cell growth (Kitada *et al.*, *Mol. Cell. Biol.* 13:4445-4457, 1993) and induces abnormal bud elongation (Song *et al.*, *Mol. Cell. Biol.* 20:286-298, 2000), *cdc5Δ* strains were generated which were kept viable by expressing either its murine functional homolog Plk (KLY1046) or the less-stable temperature sensitive mutant *cdc5-1*-encoded protein (Cheng *et al.*, *Mol. Cell. Biol.* 18:7360-7370, 1998) (KLY1047), under control of the *GAL1* promoter. These strains possess an integrated copy of a green fluorescent protein (*GFP*)-fused *TUB1* (*TUB1-GFP*) under control of its own promoter, to facilitate examination of dynamic spindle structures in mitosis.

Provision of a single copy of endogeneous *CDC5* into these strains complemented the growth defect associated with the *cdc5Δ* mutation. Both strains (*GAL1-PLK*, strain KLY1046; *GAL1-cdc5-1*, strain KLY1047), incubated for three days at 30° C, grow well on YEP-galactose medium, but are unable to grow on YEP-glucose. When exponentially growing cells were transferred to YEP-glucose medium, Plk was undetectable after 10 hours, whereas the cdc5-1 protein disappeared after three to four hours (FIG 1). The fast removal of cdc5-1 protein, in comparison to Plk, may be due to the presence of two putative destruction boxes in the N-terminal sequence of Cdc5 (Shirayama *et al.*, *EMBO J.* 17:1336-1349, 1998). Consistent with the protein removal kinetics, large-budded cells become abundant approximately six hours after depletion of Plk or three hours after depletion of cdc5-1. Cells with disassembled spindles or with separate chromatids increased and reached plateau in eight hours for KLY1046 or four hours for KLY1047 (FIG 1).

To determine terminal arresting phenotypes associated with depletion of Plk or cdc5-1 protein, strain KLY1046 was depleted of Plk for ten hours, whereas KLY1047 cells were depleted of cdc5-1 protein for four hours. Cells arrested at different phases of the cell cycle were scored based on the spindle morphologies. Numbers shown are the average of two independent experiments. About 65% of strain KLY1046 (*GAL1-PLK*) arrested at early anaphase with a short spindle elongated to, or extended just beyond, the mother-bud neck, whereas 25% of the population were arrested at cytokinesis with disassembled spindles (Table 2). In the case of depleted strain KLY1047 (*GAL1-cdc5-1*), 53% of the cells arrested in cytokinesis, whereas 3% and 44% of the cell population arrested at early anaphase and late anaphase/telophase, respectively (Table 2). The complete absence of unbudded (G1) cells indicates that cells were arrested in terminal phenotypes under these conditions. In contrast, consistent with a late mitosis arrest as reported previously (Kitada *et al., Mol. Cell. Biol.* 13:4445-4457, 1993; Toczyski *et al., Cell.* 90:1097-1106, 1997), the *cdc5-1* mutant arrested homogeneously with elongated spindles (93% of the population) when cultured at 35° C (a non-permissive temperature) for 3.5 hours (Table 2). Since the contraction of cytokinetic rings occurs at the time of spindle breakdown (Lippincott and Li, *J. Cell Biol.* 143:1947-1960, 1998), a large population of *cdc5Δ* cells with disassembled spindles in both strain KLY1046 and strain KLY1047 indicates that Cdc5 activity is required at a step in cytokinesis. In addition, these data demonstrate that Cdc5 function is required at multiple points of M phase, including cytokinesis.

Table 2

% cells arrested at various points of the cell cycle						
phenotype:	***A***	***B***	***C***	***D***	***E***	
cdc5Δ IGAL1-PLK	0	2	65	8	25	(n=420)
cdc5Δ IGAL1-cdc5-1	0	0	3	44	53	(n=345)
cdc5-1	0	1	2	93	4	(n=427)

A: Prophase
B: Metaphase
C: early anaphase
D: late anaphase/telophase
E: Cytokinesis

The differences observed between cdc5-1 and Plk depletion may reflect the different capacity of these two proteins to replace the function of Cdc5. In addition, the relatively high percentage of cells arrested with elongated spindles in strain KLY1047 (44%), in comparison to that seen with KLY1046 (8%) (Table 2), may be attributable to the specific defect of the *cdc5-1* allele at this stage of the cell cycle. The population exhibiting a cytokinetic arrest is likely underrepresented, since cells escaping earlier arrests can only achieve arrests at later points.

TUB1-GFP, *CDC3-YFP*, *CYK2-GFP*, and *MYO1-GFP* constructs were integrated into the *cdc5-1* mutant strain to compare the *cdc5Δ* phenotype with that of the *cdc5-1* mutation. This produced strains KLY1253 (*TUB1-GFP*), KLY1260 (*YFP-CDC3*), KLY1256 (*CYK2-GFP*), or KLY1258 (*MYO1-GFP*). When these cells were cultured at 23° C, then shifted to 35° C for 3.5 hours, they exhibited homogeneous arrest at late anaphase/telophase, as indicated by elongated spindles. In addition, Cdc3 was present as double rings at the mother-bud neck, prior to relocalization to the future budding sites. The Cyk2 ring was never seen as a single ring in these cells and the Myo1 ring appears to maintain normal size prior to contraction. Since the Cyk2 double ring merges to a single ring after the completion of spindle elongation and the contraction of the Cyk2 ring follows during spindle breakdown (Lippincott and Li, *J. Cell Biol.* 143:1947-1960, 1998), these

cells arrested at a point prior to contraction. This is approximately 10-15 minutes into the cell cycle, between double Cyk2 ring stage with incompletely elongated spindle and initiation of contraction (see Lippincott and Li, *J. Cell Biol.* 143:1947-1960, 1998). Consistent with these observations, close examination of the cells revealed that spindles were not yet fully elongated. In contrast to the large fraction of *cdc5Δ* cells arrested in cytokinesis, the *cdc5-1* mutant arrests in late mitosis at a step before the initiation of cytokinesis.

Induction of Connected (Chained) Cells by Overexpression of Localization-Competent C-terminal Domain of Cdc5

The non-catalytic C-terminal domain of Cdc5, which contains the polo-box, is sufficient to localize a protein at both the mother-bud neck and spindle poles, whereas introduction of a triple W517F/V518A/L530A mutation (referred to hereafter as FAA) in the polo-box abolishes localization (Song *et al.*, *Mol. Cell. Biol.* 20:286-298, 2000). In addition, over-expression of Cdc5, but not the FAA polo-box mutant, can induce ectopic septin ring structures in abnormally elongated buds (Song *et al.*, *Mol. Cell. Biol.* 20:286-298, 2000). Therefore, the polo-box is critical in the localization and cytokinetic function of Cdc5.

Over-expression of the C-terminal domain of Cdc5 (*cdc5ΔN*) inhibits polo-box function. A single copy of *GAL1-EGFP* (KLY1080), *GAL1-EGFP-cdc5ΔN* (KLY1082), or *GAL1-EGFP-cdc5ΔN/FAA* (KLY1081) was integrated separately into strain KLY1783 (*MATa* EG123). Each of these cells grew normally on YEP-glucose, without any detectable morphological defect. However, when grown in YEP-galactose (induction conditions) for 12 hours, expression of *EGFP-cdc5ΔN* (KLY1082) resulted in connected (chained) cells. A similar chained cell phenotype was also observed in a W303-1A and S288C genetic background. In contrast, cells expressing either control *EGFP* (KLY1080) or *EGFP-cdc5ΔN/FAA* (KLY1081) did not yield any detectable morphological changes.

Microscopic examinations revealed that cells expressing *cdc5ΔN* yield two fluorescent bands at the mother-bud neck and fluorescent dots in the cytoplasm, whereas the *EGFP* control and the corresponding *FAA* mutant show only diffuse signals. The apparent multiple dot signals in strain KLY1082 may be attributable to the ability of cdc5ΔN to induce multiple subcellular structures containing Spc42, a component of spindle pole bodies (Song *et al.*, *Mol. Cell. Biol.* 20:286-298, 2000). However, strains KLY1082 and KLY1083 (expressing three copies of *EGFP-cdc5ΔN*; described below) do not possess any detectable growth defect in the presence of the anti-microtubule drug benomyl; these strains grow without a significant defect in the spindle checkpoint pathway. Both *EGFP-cdc5ΔN* and *EGFP-cdc5ΔN/FAA* were expressed at similar levels, indicating that the observed phenotype associated with *EGFP-cdc5ΔN* expression was not due to a difference in protein abundance (FIG 2).

Connected (Chained) Cell Phenotype is the Result of a Dominant-negative Activity of cdc5ΔN

The observed chained cell phenotype (growth pattern) indicates that overexpression of the cdc5 kinase-activity-deficient C-terminal domain (*cdc5ΔN*) may have resulted in a dominant-negative inhibition of a cytokinetic event normally regulated by endogeneous Cdc5. To test this theory, endogeneous *CDC5* was expressed in a low copy centromeric plasmid to determine if this could alleviate the chained cell growth pattern. Cells expressing *EGFP-cdc5ΔN* under the control of *GAL1* promoter (KLY1082) were transformed with wild type and various mutant forms of Cdc5.

When cultured under induction conditions for 12 hours, KLY1082 cells transformed with control vector showed typical connected cell morphology in 48% of the total population. In contrast, provision of wild-type *CDC5* into strain KLY1082 efficiently restored this phenotype to a wild-type morphology. In contrast, neither the polo-box mutated *cdc5/FAA* nor the kinase-inactive *cdc5/NA* (the N209A mutation that inactivates Cdc5 kinase activity; see Hardy and Pautz, *Mol. Cell Biol.*, 16:6775-6782, 1996) were capable of alleviating this defect (Table 3). The data shown in this table represent the quantitation of reversion of the connected cell phenotype by various *CDC5* constructs. Both the kinase activity and an intact polo-box appear to be required for reversing the chained cell morphology.

Table 3

	Number of cells per chain					
	1 or 2	**3**	**4**	**5**	**6 or more**	**% cells in chains**
vector	52	20	10	8	10	48 % (222/464)
CDC5	97	2	1	0	0	3 % (14/467)
cdc5/FAA	54	22	10	8	6	46 % (201/437)
cdc5/NA	61	19	10	4	6	39 % (164/420)

These data indicate that the observed cytokinesis-defective phenotype associated with *EGFP-cdc5ΔN* over-expression is due specifically to the inhibition of endogeneous Cdc5 function, and both the kinase activity and the intact polo-box are required to remedy this phenotype. Thus, it appears that the observed chained growth pattern is the result of dominant-negative inhibition of endogeneous Cdc5 function important for normal cell division. In addition, cells possessing three copies of *EGFP-cdc5ΔN* (KLY1083) exhibited a uniformly connected cell morphology in almost all cells, whereas cells expressing the same three copies of *EGFP-cdc5ΔN/FAA* (KLY1229) did not (see below). Provision of multiple copies of wild type *CDC5* into strain KLY1083 alleviated the chained cell morphology.

Inhibition of Cytokinesis by a Dominant-negative C-terminal Domain of Cdc5

Strain KLY1083 was cultured under induction conditions in YEP-galactose; control strains expressing either *EGFP* alone (KLY1080) or an equal dosage of *EGFP-cdc5ΔN/FAA* (KLY1229) were grown under the same conditions.

When induced, KLY1083 cells yielded a connected cell phenotype in a time-dependent manner, whereas cells expressing the corresponding FAA mutant (KLY1229) appear to divide normally with wild-type morphology. Upon inducing for nine hours, 98% of the population exhibited this phenotype, and 30% of them possess more than ten connected cell bodies. In contrast, cells expressing the FAA mutant did not show this morphology (Table 4). This table shows the time-dependent accumulation of chained cells, and length of cell chains. Percentage of cells in chains was determined by dividing the sum of cells in chains by the total number of cells.

Table 4

		Number of cells per chain						
	hrs	1 or 2	3	4	5	6 to 10	10 or more	% cells in chains
cdc5ΔN	0	100	0	0	0	0	0	0% (0/529)
	3	84	9	6	1	0	0	16% (82/512)
	6	12	30	31	16	11	0	88% (224/254)
	9	2	3	12	14	39	30	98% (258/264)
	12	1	3	9	12	35	40	99% (268/271)
Cdc5ΔN /FAA	0	100	0	0	0	0	0	0% (0/450)
	12	100	0	0	0	0	0	0% (0/450)

In addition, the cell number of strain KLY1083 (when counting the connected cells as one cell) did not increase after shifting to the induction conditions, indicating that cells remained unseparated. However, these cells continued to grow, although slowly, as indicated by an increase in optical density of the cell culture. In contract, cells expressing either control *EGFP* (KLY1080) or *EGFP-cdc5ΔN/FAA* (KLY1229) exhibited a normal increase in both cell number and optical density (FIG 3A). Western analyses revealed that EGFP-cdc5ΔN and EGFP-cdc5ΔN/FAA were expressed at similar levels, and that they were slightly more abundant than that of a single, integrated copy of *EGFP-cdc5ΔN* (FIG 3B).

To determine whether the connected cell morphology could be caused by various defects, such as a defect in cytokinesis, septum formation, or cell separation, the extent to which the chained cells maintained cytoplasmic connections was examined. KLY1083 cells cultured under induction conditions for 12 hours were treated with zymolyase to remove the cell wall, and the cell (spheroplast) number was counted. Even after extensive zymolyase treatment, cells largely remained as clumps of unseparated cells without a significant increase in the number of individual cells. The connected cell phenotype, therefore, is the result of a cytokinetic defect as defined by the criteria described previously (Hartwell, *Exp. Cell Res.* 69:265-276, 1971).

To confirm a cytokinetic defect in strain KLY1083, cells induced for nine hours were stained with calcofluor to examine chitin deposition in cell walls and septum. Strong calcofluor bands were observed between connected cell bodies. Calcofluor signals were discontinuous (with gaps within the apparent necks) in large fractions (approximately 70%) of mother-bud necks of the connected cell bodies. This is indicative of a failure of septum formation.

Overexpression of *cdc5ΔN* results in an inhibition of septum formation between mother-bud necks. Strain KLY1083 was cultured under the induction conditions for nine hours, fixed with formaldehyde, and stained with calcofluor or DiI as described in Materials and Methods.

Serial optical sectioning using a confocal microscope was performed to examine whether septum formation was completed in these cells. In most of the mother-bud necks examined, calcofluor signals were discontinuous in focal planes bisecting the cell bodies longitudinally, indicating that septa were not completely formed between the connected cell bodies. Among the connected cells (n=56) that were examined, only one cell body, either in the center or at one edge of chains, possessed bud scars (revealed as fluorescent chitin rings), indicating that other cell bodies did not generate daughter cells. Since these cells do not increase in number under the induction conditions (see FIG 3A), the bud scars on the presumed mother cells are likely to be the result of budding events that occurred prior to transferring into induction conditions.

The same cells used for calcofluor staining were stained with DiI in order to examine the structure of cytoplasmic membrane in the connected cells. Membrane closure was observed to be impaired in approximately 50% of the necks examined. Heavy patches of DiI stain were present in other bud necks, indicating that membrane closure was impaired but not completely inhibited by overexpression of *cdc5ΔN*. Most of connected cell bodies share cytoplasm, however, a fraction of mother-bud necks have closed membranes. Hence, some mother-bud necks overcame the cytokinetic defect imposed by overexpression of *EGFP-cdc5ΔN*, but failed to complete cell division.

Cell Division Cycle is Unaffected in an Early Stage of Connected Cells

To demonstrate that the cell cycle is altered in connected cells, flow cytometry analyses were carried out for the cells expressing control *EGFP* (KLY1080), *EGFP-cdc5ΔN* (KLY1083), or *EGFP-cdc5ΔN/FAA* (KLY1229). Overexpression of *EGFP-cdc5ΔN*, but not the corresponding *FAA* mutant, causes accumulation of cells with greater than a 2C (G2/M) DNA content.

Cells expressing control *EGFP* (KLY1080), *EGFP-cdc5ΔN* (KLY1083), or *EGFP-cdc5ΔN/FAA* (KLY1229) were arrested in YEP-raffinose for 3 hours, washed and transferred into YEP-galactose medium to an OD_{600} of 0.05 to induce protein expression upon release from a G1 arrest. Strains KLY1080 and KLY1229 appeared to progress through the cell cycle normally, regenerating the 1C DNA-containing (G1) population in approximately 120 minutes after release. In contrast, strain KLY1083 expressing *EGFP-cdc5ΔN* failed to regenerate a distinct 1C (G1) peak, and continued to accumulate DNA to greater than 2C (G2/M) peak. Accumulation of cells with higher DNA contents appears to be the result of induction of connected cells.

To examine the cell cycle progression, cellular lysates from cells used in the flow cytometry experiment were subjected to Western blot analyses to examine changes in Clb2 and EGFP-cdc5ΔN levels upon release from a α-factor arrest. The level of Cdc28 was determined as a loading control for each lane. The same lysates were also used to carry out anti-Clb2 immune complex kinase assays to measure the Clb2-associated histone H1 kinase activities.

Control strain KLY1080 achieved the maximum Cdc28/Clb2 activity (onset of M phase) at 60 minutes and 150 minutes after release from cell cycle arrest. (Samples beyond 240 minutes after release were not taken because of lack of cell cycle synchrony.

Strain KLY1229 (cdc5ΔN/FAA) exhibited a slight delay in the cell cycle in comparison to the *EGFP* control strain, whereas strain KLY1083 (cdc5ΔN) had a 20 minute delay. In all cases, however, Clb2 protein levels and Clb2-associated kinase activities appeared to fluctuate normally and with similar kinetics, hence overexpression of *cdc5ΔN* did not efficiently prevent endogeneous Cdc5 from activating APC. Upon induction of construct expression after release from cell cycle arrest, approximately equal amounts of EGFP control, EGFP-cdc5ΔN, and EGFP-cdc5ΔN/FAA proteins were expressed in these cells.

Cdc28/Clb2 activity appeared to fluctuate normally in strain KLY1083, which overexpresses *EGFP-cdc5ΔN*. Thus, overexpression of EGFP-cdc5ΔN appears to selectively inhibit cytokinesis without significantly perturbing the nuclear division cycle during the early stage of connected cells.

Disturbed Septin Structures Leads to the Cytokinetic Defect

The chained (connected) cell phenotype could be achieved by continuous division of peripheral cells in the absence of cytokinesis. To examine the cell cycle stage of individual cell

bodies in the connected cells, a new strain (KLY1069) was created. To make this strain, a copy of a *TUB1-GFP* fusion was integrated into the genome of KLY1053 (expressing a GST-fused cdc5ΔN (*GST-cdc5ΔN*) under control of the *GAL1*). Strain KLY1069 grew normally and exhibited normal spindle structures in YEP-glucose. Cultured under induction conditions for 12 hours, cells of this strain showed either a dot or short line of fluorescent signal in most of internal cell bodies, indicating that these cell bodies were arrested at a point after spindle disassembly. In contrast, mitotic spindle structures were often observed in cell bodies present at the periphery of chained cells, suggesting that edges cells are active in cell division. A similar observation was made when connected strain KL1083 cells were immunostained with an anti-tubulin antibody. These observations indicate that a prolonged inhibition of cytokinesis in these cell bodies may have resulted in an inhibition of the next round of the cell cycle.

Inhibition of cytokinesis in connected cells could be the result of a failure either in recruitment of cytokinetic materials or in the function of cytokinetic structures. To investigate these possibilities, strain KLY1053 was additionally integrated with *CDC3-YFP* (KLY1075), *CYK2-GFP* (KLY1071), or *MYO1-GFP* (KLY1073) under control of their own promoters. These strains grew normally with apparently normal ring structures when cultured in YEP-glucose. Upon induction of GST-cdc5ΔN, cells were examined to determine whether these components formed normal ring structures.

At an early stage of induction, distinct septin ring structures were observed at all mother-bud necks of the connected cells, indicating that continuous budding events have occurred in the absence of complete cytokinesis at the previous mother-bud necks. Upon 12 hours of induction, double YFP-Cdc3 rings were distantly placed at the mother-bud neck, or remnants of YFP-Cdc3 were placed at one side of the mother-bud neck, indicative of a disturbance in maintaining properly organized septin ring structures or a defect in septin ring disassembly and relocalization. Unusually wide or tiny rings of Cdc3 were occasionally observed at the axial position of incipient budding site without formation of a noticeable bud. This may indicate that a fraction of cell bodies were capable of relocalizing septins to the future budding sites but failed to assemble or maintain proper septin ring structures. In the presence of dominant-negative cdc5ΔN, septin rings appear to be assembled normally at the growing edge of the connected cells, thereby permitting normal budding events. These ring structures are cytokinesis-incompetent, resulting in the generation of the connected cell morphology. As with septin ring structures persisting at most of the mother-bud necks of connected cells, both Cyk2-GFP and Myo1-GFP ring structures were also remained at these sites. This observation further supports the notion that cytokinetic processes have failed in these cells.

In contrast to double Cyk2-GFP rings observed with the *cdc5-1* mutant at the restrictive temperature, connected cells possess single Cyk2-GFP rings between cell bodies. Since double Cyk2 rings become a single ring structure at the time of cytokinesis (Lippincott and Li, *J. Cell Biol.* 143:1947-1960, 1998), *cdc5-1* mutant at the restrictive temperature is defective in cytokinesis. Consistent with the occasional membrane closure observed with DiI staining, contraction-size Myo1 and Cyk2 rings were occasionally evident.

Cyk1 and actin have been shown to form a ring after activation of the Cdc15 pathway (Lippincott and Li, *J. Cell Biol.* 140:355-366, 1998). Consistent with this, actin was rarely relocalized to mother-bud necks in the *cdc5-1* mutant at the restrictive temperature, although it was polarized normally to the tip of peripheral cells. Strain KLY1083 cultured under the induction conditions for 12 hours were fixed and stained with rhodamine-phalloidin and DAPI to visualize

actin and nuclei, respectively. In KLY1083, actin polarizes normally, but fails to relocalize to bud-necks. Therefore some contractile components were recruited to these sites in the presence of aberrant septin ring structures, and these components were able to overcome the cytokinetic defect imposed by the overexpression of the C-terminal domain of Cdc5, albeit poorly. Completion of cell division does not appear to occur significantly within 12 hours of induction period, since cell number does not increase and nearly all the cells remain as connected cells.

Polo-box-Dependent Interactions Between Cdc5 and Septins

A yeast two-hybrid system was used to investigate whether Cdc5 directly interacts with septins. A destruction-box deficient form of Cdc5 (Song *et al.*, *Mol. Cell. Biol.* 20:286-298, 2000) was used in place of the wild-type protein to increase protein stability for these experiments.

The Cdc5 interacted with Cdc11 and Cdc12 strongly, whereas the corresponding polo-box mutant, cdc5/FAA, did not. Consistent with this observation, cdc5ΔN, but not the cdc5ΔN/FAA, interacted strongly with both Cdc11 and Cdc12 (FIG 4). No interaction was detected between the N-terminal domain of Cdc5 (cdc5ΔC) and septins. Western blot analyses revealed that septins and various forms of Cdc5 were expressed at similar levels. The polo-box domain is critical for the interactions between Cdc5 and septins, Cdc11 and Cdc12. The connected morphology could be the result of a disturbance in septin ring structure through direct interactions between cdc5ΔN and septins.

Enhancement of Cytokinetic Defects by Overexpression of *cdc5ΔN* in *cyk2*Δ or *myo1*Δ Mutants.

Although septin ring structures are largely disturbed in the connected (chained) cells, the presence of contraction-size Cyk2 or Myo1 rings suggests that overexpression of *cdc5ΔN* did not completely eliminate the function of septin rings in these cells. The two cytokinetically important components, Myo1 and Cyk2, function in parallel pathways and localize at neck-filaments in a septin-dependent manner. Since overexpression of *cdc5ΔN* partially inhibits septin function, introduction of either a *cyk2*Δ or a *myo1*Δ mutation into the connected cells may enhance the severity of the chained cell phenotype.

Strain KLY1083 was modified with the additional of a *cyk2*Δ (KLY1591), *myo1*Δ (KLY1593), or *cdc10*Δ (KLY1589) mutation, and examined under induction conditions (streaked onto YEP-galactose and, for comparison, YEP-glucose). Introduction of additional *cyk2*Δ or *myo1*Δ into KLY1083 strain resulted in a synthetic growth defect. Introduction of *cdc10*Δ, in contrast, did not reveal this phenomenon, likely because septin ring structures are already impaired by overexpression of *cdc5ΔN*. Both *cdc10*Δ and *cyk2*Δ mutants possess an apparent temperature-sensitivity for growth; plates for these experiments were therefore incubated at 23° C for five days prior to examination. Two independently generated *cdc10*Δ mutants (KLY1589 and KLY1590) were used to confirm the absence of a synthetic defect with overexpression of *EGFP-cdc5ΔN*.

During the initial stage of induction (up to six hours), both KLY1591 and KLY1593 cells displayed a significantly enhanced connected cell morphology, in comparison to those of the corresponding *cyk2*Δ or *myo1*Δ single mutants or cells overexpressing *cdc5ΔN* alone. The observed synthetic growth defect is the result of the synthetic cytokinetic defect and indicates that the cytokinetic pathways regulated by Cyk2 and Myo1 are mediated by septins.

Inactivation of Cdc28/Clb2 results in a "filamentous" phenotype that appears grossly similar to the dominant-negative phenotype induced by *cdc5ΔN* overexpression, due to the inability of buds

to switch to isotropic growth (Barral *et al.*, *Genes·Dev.* 13:176-187, 1999; Edgington *et al.*, *Mol. Cell. Biol.* 19:1369-1380, 1999). Hsl1 acts as a negative regulator of Swe1 (Ma *et al.*, *Genes Dev.* 10:1327-1340, 1996), which inhibits Cdc28 by phosphorylation of conserved tyrosine 19 (Booher *et al.*, *EMBO J.* 12:3417-3426, 1993). In addition, the morphological defects resulting from the triple *hsl1Δ, kcc4Δ, gin4Δ* mutant require the Swe1 kinase (Barral *et al.*, *Genes Dev.* 13:176-187, 1999).

Swe1, however, is not necessary for the dominant-negative phenotype induced by *cdc5ΔN* overexpression. Introduction of *swe1Δ* into KLY1083 strain did not influence the cell growth and chained cell phenotype associated with overexpression of *cdc5ΔN*. In addition, overexpression of *cdc5ΔN* was still capable of inducing a connected cell morphology in a strain that possesses an activated *CDC28* allele, *CDC28/Y19F*, as a sole *CDC28* locus. These observations indicate that the cytokinetic defect induced by overexpression of *cdc5ΔN* differs from the phenotype associated with Cdc28 inactivation, and that the connected cells observed with overexpression of *cdc5ΔN* is not the result of inhibition of Cdc28/Clb2 activity by a Swe1-dependent mechanism.

DISCUSSION

The Role of Cdc5 in Cytokinesis

Over-expression of *cdc5ΔN*, but not the corresponding polo-box mutant, results in dominant-negative inhibition of cytokinesis without interfering with mitotic exit. This over-expression disturbs septin ring structures, likely through interaction of Cdc5 with two septins, Cdc11 and Cdc12. These interactions occur in a polo-box-dependent manner in a yeast two-hybrid system. Thus, overexpression of *cdc5ΔN* may prevent endogeneous Cdc5 from interacting with septins and carrying out its cytokinetic function.

The septins are thought to be the major structural components of the neck filaments (Frazier *et al., J. Cell Biol.* 143:737-749, 1998). Interactions between Cdc5 and septins suggest that localization of EGFP-cdc5ΔN at the mother-bud neck may require septins and higher-order cytokinetic neck-filaments. Since a *cdc10Δ* mutant grows normally at 23° C, but does not possess detectable neck-filaments (Frazier *et al., J. Cell Biol.* 143:737-749, 1998), a *cdc10Δ* mutation was introduced into KLY1083 strain (KLY1589). When strain KLY1589 was cultured under the induction conditions, localization of EGFP-cdc5ΔN was greatly diminished, but was not completely eliminated. Intact neck-filaments are likely to be important for the localization of cdc5ΔN. Instability of the EGFP-cdc5ΔN protein at elevated temperatures has made confirmation that cdc5ΔN localization is septin-dependent difficult. Thus, although it appears that septins are likely the targets of cdc5ΔN binding, it is possible that cdc5ΔN also interacts with additional component(s) other than septins at the mother-bud necks.

Cdc5 activity may not be required for initiation of septin ring formation, since Cdc5 is expressed in a late phase of the cell cycle and its kinase activity peaks in mitosis. In addition, septin ring structures are weakened but not completely disassembled in a *cdc5-1* mutant at the restrictive temperature (Kim *et al.*, *J. Cell Biol.* 112:535-544, 1991). Thus, Cdc5 activity may not be important for the assembly of septin rings into a higher-order structure, but rather it may be important for maintaining or reinforcing a cytokinetically competent structure. Data reported here indicate that septin rings at the edge cells of cell chains appear to be relatively normal, as evidenced by continuous budding events. Only the cells at the periphery possess polarized actin patches, suggesting that the septins at the peripheral cells are capable of providing spatial cues for actin polarization, thereby

supporting continuous budding events in the absence of cytokinesis. In contrast, the internal septin rings are largely disturbed.

In summary, in the presence of dominant-negative cdc5ΔN, septins form a ring at the neck of growing buds, but fail to maintain a cytokinesis-competent structure. Since remnants of septin materials are found between mother-bud necks without proper relocalization to the future budding sites, overexpression of *cdc5ΔN* may also disturb one or more steps that are important for disassembly of septin ring structures.

Overexpression of the dominant-negative cdc5ΔN protein appears to induce a partial inhibition of cytokinesis as demonstrated by the presence of Cyk2 and Myo1 rings the size of contractile ring, and occasional membrane closures between budding cells. Since both Myo1 and Cyk2 localization is depend on septin ring structures, it is likely that septins play a central role in regulating both cytokinetic pathways. Introduction of either *myo1Δ* or *cyk2Δ* into the strain overexpressing *cdc5ΔN* enhances the cytokinetic defects.

Dominant-negative cdc5ΔN mutant vs. septin mutants

Loss of septin function results in a cytokinetic defect with a chained-cell morphology (Cooper and Kiehart, *J. Cell Biol.* 134:1345-1348, 1996; Longtine *et al.*, *Curr. Opin. Cell Biol.* 8:106-119, 1996; Frazier *et al.*, *J. Cell Biol.* 143:737-749, 1998), visually similar to that observed with overexpression of *cdc5ΔN*. These phenotypes differ from each other in several important aspects. At the restrictive temperature, septin mutants accumulate multinucleated cells with hyperpolarized buds (Hampsey, *Yeast.* 13:1099-1133, 1997), due to inability of buds to switch to isotropic growth in the absence of the septin-ring-dependent Cdc28 activation pathway (Barral *et al.*, *Genes Dev.* 13:176-187, 1999; Edgington *et al.*, *Mol. Cell. Biol.* 19:1369-1380, 1999). In addition, septin-defective cells lose viability, likely because septins play additional roles in other stages of the cell cycle. In contrast, cells overexpressing *cdc5ΔN* exhibit round cell bodies that are connected (chained), and which form colonies on YEP-glucose medium. These cells go through the cell cycle essentially normally, with cycling Cdc28/Clb2 activity. In the absence of complete cell division, these cultures continue to increase cell mass (though not individualized cell number) by continuous budding events at the periphery of the chained cells.

Mutations in septins have been shown to cause defects in bud site selection. Similarly, the chained cells observed with strain KLY1083 (an **a**-type haploid) exhibited a largely unipolar budding pattern, whereas these cells develop a new bud at the proximal pole of previous cytokinetic site when grown in YEP-glucose medium. These observations indicate that, in the absence of cytokinesis, the distal pole of the daughter cell is favored for subsequent budding event. Thus, changes in budding pattern may be attributable to the absence of proper septin relocalization to the future budding sites in the internal cell bodies, while maintaining polar budding at the peripheral cells. The disturbance in septin ring structures in the connected cells results in a cytokinetic defect that is accompanied by a budding defect.

Most internal cells in the disclosed cell chains possess short spindles, indicating that these cells are arrested at a point after spindle disassembly. In addition, cell bodies within the chains fail to generate buds, suggesting an arrest in G1. Inactivation of Cdc28, and thereby mitotic exit, occurs normally in these cells in an early stage of the cell cycle. Failure in cytokinesis in these cells therefore may be detected by a previously unknown mechanism that leads to the cell cycle arrest in G1.

Temporal and spatial regulation of mitotic exit and cytokinesis

Cdc5 plays dual roles in a late stage of M phase. In addition to activation of APC, Cdc5 activity is required for proper function of septin ring structures required for cytokinesis. During or shortly after activation of APC, a fraction of Cdc5 relocalizes to the bud-neck to carry out its cytokinetic function(s), a relocalization event that requires an intact polo-box. A dominant-negative cdc5ΔN, which localizes at the neck-filament, does not interfere with APC activation or the mitotic exit pathway. In addition, expression of this protein it does not disturb cell cycle progression, since the septin-dependent Hsl1/Gin4/Kcc4 pathway is already turned on to activate Cdc28 at the onset of M phase. The dominant-negative cdc5ΔN, however, inhibits Cdc5 localization to the neck-filaments, thereby impairing septin function(s) critical for cytokinesis. This model helps explain the phenotypes associated with overexpression of intact Cdc5 (Song *et al.*, *Mol. Cell. Biol.* 20:286-298, 2000) or expression of an activated form of Plk (Lee *et al.*, *Proc. Natl. Acad. Sci. U S A.* 96:14360-14365, 1999). In both cases, unregulated Cdc5/Plk activity may have induced abnormally elongated buds by activation of APC, whereas ectopic septin rings may have arisen by reinforcing septin ring organization. Activation of the APC apparently does not require polo-box-dependent localization of Cdc5 at the septin rings, whereas overexpression of the polo-box domain is sufficient to inhibit cytokinesis. Thus, temporal and spatial regulation of Cdc5 provides an important mechanism to coordinate mitotic exit pathway with initiation of cytokinesis.

III. Mammalian Cultured Cells Overexpressing a Polo-box

Deletion constructs, shown in **FIG 9A**, were constructed using standard molecular biology techniques to examine importance of the polo boxes of Plk in mammalian cells. Constructs #1 and #3 are different deletions of Plk, missing the sequence encoding the kinase domain (contained on the XhoI to BamHI fragment or the NaeI to SmaI fragment, respectively). Constructs #2 and #4 are polo-box deletion variants of #1 and #3, respectively, wherein polo-boxes 2 and 3 have been removed by deletion of the SstI to SstI fragment.

Recombinant HA-GFP-Plk was generated by N-terminally tagging Plk with HA (hemagglutinin epitope) and GFP (green fluorescence protein). Adenoviral expression constructs bearing various forms of HA-GFP-PLK cDNA were expressed in HEK293 cells using the pAdEasy-1 expression system (He, *Proc. Natl. Acad. Sci., USA*, 1998. 95:2509-2514). Expression of these proteins was detected with anti-HA antibody using enhanced chemoluminiscence Western analysis (**FIG 9B**).

Each of constructs #1 - #4 induce a prometaphase arrest in HeLa cells, as judged by immunostaining with an anti-tubulin antibody and an anti-centrosome antibody. In addition, flow cytometry analyses with the cells infected with these viruses show a mitotic arrest, which is accompanied by cell death.

Polo-box 2 and 3 of Plk are required to localize at midbody in HeLa cells.

Endogenous Plk localizes at both centrosomes and midbody (Lee, *Mol. Cell. Biol.* 15:7143-51, 1995). To examine which domain of Plk is critical for its subcellular localization, adenoviral constructs expressing various forms of recombinant HA-GFP-Plk were infected into HeLa cells. Both construct #1 and #3 localized at midbody, whereas construct #2 and #4 lacking both the polo-

box 2 and 3 did not. These observations indicate that polo-box 2 and 3 are required for targeting the kinase domain of Plk to midbody.

Polo-box 1 of Plk is sufficient to localize at centrosomes in HeLa cells.

To examine which domain of Plk is important for centrosome localization, adenoviral constructs expressing various deletion variants of recombinant HA-GFP-Plk (as described herein and illustrated in **FIG 9A**) were infected into HeLa cells. Centrosomes were visualized by staining the cells with an anti-alpha tubulin antibody, whereas Plk localization was detected as GFP signals. All four deletion constructs were able to localize at centrosomes. These observations indicate that, unlike midbody localization, the polo-box 2 and 3 are not required for targeting the kinase domain of Plk to centrosome.

EXAMPLES

Example 1. Expression and purification of polo-box-related peptides

A. Expression of polo-box-related peptides.

One skilled in the art will understand that there are myriad ways to express a recombinant protein (*e.g.*, a polo-box-related peptide as described herein) such that it can subsequently be purified. In general, an expression vector carrying the nucleic acid sequence that encodes the desired protein will be transformed into a microorganism for expression. Such microorganisms can be prokaryotic (bacteria) or eukaryotic (*e.g.*, yeast). One example species of bacteria that can be used is *Escherichia coli* (*E. coli*), which has been used extensively as a laboratory experimental expression system. An eukaryotic expression system can be used where the protein of interest requires eukaryote-specific post-translational modifications such as glycosylation. Also, protein can be expressed using a viral (*e.g.*, vaccinia) based expression system.

Protein can also be expressed in animal cell tissue culture, and such a system can be used where animal-specific protein modifications are desirable or required in the recombinant protein.

Vectors suitable for stable transformation of culturable cells are also well known. Typically, such vectors include a multiple-cloning site suitable for inserting a cloned nucleic acid molecule, such that it will be under the transcriptional control of 5' and 3' regulatory sequences. In addition, transformation vectors include one or more selectable markers; for bacterial transformation this is often an antibiotic resistance gene. Such transformation vectors typically also contain a promoter regulatory region (*e.g.*, a regulatory region controlling inducible or constitutive expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, and a transcription termination site, each functionally arranged in relation to the multiple-cloning site. For production of large amounts of recombinant proteins, an inducible promoter can be used. This permits selective production of the recombinant protein, and allows both higher levels of production than constitutive promoters, and enables the production of recombinant proteins that may be toxic to the expressing cell if expressed constitutively.

In addition to these general guidelines, protein expression/purification kits are produced commercially. See, for instance, the QIAexpress™ expression system from QIAGEN (Chatsworth, CA) and various expression systems provided by INVITROGEN (Carlsbad, CA). Depending on the

details provided by the manufactures, such kits can be used for production and purification of the disclosed polo-box related peptides.

B. Purification.

One skilled in the art will understand that there are myriad ways to purify recombinant polypeptides, and such typical methods of protein purification may be used to purify the disclosed therapeutic polo-box peptides. Such methods include, for instance, protein chromatographic methods including ion exchange, gel filtration, HPLC, monoclonal antibody affinity chromatography and isolation of insoluble protein inclusion bodies after over production. In addition, purification affinity-tags, for instance a six-histidine sequence, may be recombinantly fused to the protein and used to facilitate polypeptide purification. A specific proteolytic site, for instance a thrombin-specific digestion site, can be engineered into the protein between the tag and the fusion itself to facilitate removal of the tag after purification.

Commercially produced protein expression/purification kits provide tailored protocols for the purification of proteins made using each system. See, for instance, the QIAexpress™ expression system from QIAGEN (Chatsworth, CA) and various expression systems provided by INVITROGEN (Carlsbad, CA). Where a commercial kit is employed to produce a protein, the manufacturer's purification protocol is a particularly disclosed protocol for purification of that protein. For instance, proteins expressed with an amino-terminal hexa-his tag can be purified by binding to nickel-nitrilotriacetic acid (Ni-NTA) metal affinity chromatography matrix (*The QIAexpressionist*, QIAGEN, 1997).

If the polo-box related peptide is produced in a secreted form, *e.g.* secreted into the milk of a transgenic animal, purification will be from the secreted fluid. Alternately, purification may be unnecessary if the peptide can be applied directly to the subject in the secreted fluid (*e.g.* milk).

Example 2. Variation of a Polo-box Related Peptide

A. Sequence Variants

The binding characteristics, and therefore polo-like kinase function modulatory activity, of the polo-box peptides disclosed herein lie not in the precise amino acid sequence, but rather in the three-dimensional structure inherent in the amino acid sequences encoded by the DNA sequences. It is possible to recreate the binding characteristics of any of these peptides, proteins or protein domains of this disclosure by recreating the three-dimensional structure, without necessarily recreating the exact amino acid sequence. This can be achieved by designing a nucleic acid sequence that encodes for the three-dimensional structure, but which differs, for instance by reason of the redundancy of the genetic code. Similarly, the DNA sequence may also be varied, while still producing a functional binding peptide.

Variant polo-box related peptides include those that differ in amino acid sequence from the disclosed sequence (SEQ ID NO: 1), but that share structurally significant sequence homology with any of the provided proteins.

Table 5

Original Residue	Conservative Substitutions
Ala	ser
Arg	lys
Asn	gln; his
Asp	glu
Cys	ser
Gln	asn
Glu	asp
Gly	pro
His	asn; gln
Ile	leu; val
Leu	ile; val
Lys	arg; gln; glu
Met	leu; ile
Phe	met; leu; tyr
Ser	thr
Thr	ser
Trp	tyr
Tyr	trp; phe
Val	ile; leu

More substantial changes in protein structure may be obtained by selecting amino acid substitutions that are less conservative than those listed in Table 5. Such changes include changing residues that differ more significantly in their effect on maintaining polypeptide backbone structure (*e.g.*, sheet or helical conformation) near the substitution, charge or hydrophobicity of the molecule at the target site, or bulk of a specific side chain. The following substitutions are generally expected to produce the greatest changes in protein properties: (a) a hydrophilic residue (*e.g.*, seryl or threonyl) is substituted for (or by) a hydrophobic residue (*e.g.*, leucyl, isoleucyl, phenylalanyl, valyl or alanyl); (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain (*e.g.*, lysyl, arginyl, or histadyl) is substituted for (or by) an electronegative residue (*e.g.*, glutamyl or aspartyl); or (d) a residue having a bulky side chain (*e.g.*, phenylalanine) is substituted for (or by) one lacking a side chain (*e.g.*, glycine).

Variant binding peptides or peptide-encoding sequences may be produced by standard DNA mutagenesis techniques, for example, M13 primer mutagenesis. Details of these techniques are provided in Sambrook (In *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989), Ch. 15. By the use of such techniques, variants may be created which differ in minor ways from the polo-box peptide-encoding sequences disclosed. Peptide sequences which are derivatives of those specifically disclosed herein and that differ from those disclosed by the deletion, addition, or substitution of amino acids while still encoding a peptide or protein that competes with a native polo-box for binding to a specific site, thereby modulating a polo-like kinase function in a cell or cell-free system, are comprehended by this disclosure.

Alternatively, the coding region may be altered by taking advantage of the degeneracy of the genetic code to alter the coding sequence such that, while the nucleotide sequence is substantially altered, it nevertheless encodes a protein having an amino acid sequence substantially similar to the disclosed fusion sequences. Based upon the degeneracy of the genetic code, variant DNA molecules may be derived from encoding sequences disclosed herein using standard DNA mutagenesis techniques as described above, or by synthesis of DNA sequences. Thus, this disclosure also encompasses nucleic acid sequences which encode a polo-box related peptide, but which vary from the disclosed sequences by virtue of the degeneracy of the genetic code.

B. Peptide Modifications

The present disclosure includes biologically active molecules that mimic the action of the polo-box peptides and polo-box related peptides of the present disclosure, and specifically compete with a polo-like kinase for binding at a specific cellular site. The proteins and peptides of the disclosure include synthetic embodiments of naturally-occurring proteins described herein, as well as analogues (non-peptide organic molecules), derivatives (chemically functionalized protein molecules obtained starting with the disclosed peptide sequences) and variants (homologs) of these proteins that specifically compete with native polo-like kinase for binding, *e.g.* binding to an element of the neck filaments or other cytokinetic structure. Each protein or peptide is comprised of a sequence of amino acids, which may be either L- and/or D- amino acids, naturally occurring and otherwise.

Proteins and peptides may be modified by a variety of chemical techniques to produce derivatives having essentially the same activity as the unmodified proteins, and optionally having other desirable properties. For example, carboxylic acid groups of the protein, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a C_1-C_{16} ester, or converted to an amide of formula NR_1R_2 wherein R_1 and R_2 are each independently H or C_1-C_{16} alkyl, or combined to form a heterocyclic ring, such as a 5- or 6-membered ring. Amino groups of the protein, whether amino-terminal or side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric and other organic salts, or may be modified to C_1-C_{16} alkyl or dialkyl amino or further converted to an amide.

Hydroxyl groups of the protein side chains may be converted to C_1-C_{16} alkoxy or to a C_1-C_{16} ester using well-recognized techniques. Phenyl and phenolic rings of the protein side chains may be substituted with one or more halogen atoms, such as fluorine, chlorine, bromine or iodine, or with C_1-C_{16} alkyl, C_1-C_{16} alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids. Methylene groups of the protein side chains can be extended to homologous C_2-C_4 alkylenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Those skilled in the art will also recognize methods for introducing cyclic structures into the proteins to select and provide conformational constraints to the structure that result in enhanced stability.

It also may be advantageous to introduce one or more disulfide bonds to connect the frameworks of a recombinant protein that contains a polo-box-related peptide sequence. This modification often enhances the stability and affinity of some proteins (*e.g.*, SCFvs; see Reiter *et al.*, *Protein Engineering* 7:697-704, 1994).

Peptidomimetic and organomimetic embodiments are also within the scope of the present disclosure, whereby the three-dimensional arrangement of the chemical constituents of such peptido- and organomimetics mimic the three-dimensional arrangement of the protein backbone and component amino acid side chains in the protein, resulting in such peptido- and organomimetics of the proteins of this disclosure having measurable or enhanced inhibiting or enhancing ability. For computer modeling applications, a pharmacophore is an idealized, three-dimensional definition of the structural requirements for biological activity. Peptido- and organomimetics can be designed to fit each pharmacophore with current computer modeling software (using computer assisted drug design or CADD). See Walters, "Computer-Assisted Modeling of Drugs", *in* Klegerman & Groves, eds., 1993, *Pharmaceutical Biotechnology*, Interpharm Press: Buffalo Grove, IL, pp. 165-174 and *Principles of Pharmacology* Munson (ed.) 1995, Ch. 102, for descriptions of techniques used in

CADD. Also included within the scope of the disclosure are mimetics prepared using such techniques that produce proteins.

C. Domain length variation.

It will be appreciated that different length portions of a polo-like kinase C-terminal region, which includes a polo-box, or a derivative or variant of a polo-box, may be either shorter or longer than the specific sequences disclosed herein. Thus, though the reported constructs and strains include a 465 amino acid fragment of a polo-like kinase (corresponding to residues 240-705 of Cdc5), it is believed possible to use a shorter or longer fragment of the protein domain. The core polo-box consensus sequence is found at residues 513 through 542 of Cdc5 or residues 410 through 439 of mammalian Plk. By way of example only, other polo-box related peptides can include about residues 493 through 562 of Cdc5 (having about 20 additional residues on either side of the consensus box), or about residues 473 through 582 of Cdc5 (with about 40 additional residues on each side), or about residues 413 through 652 (with about 100 additional residues on each side).

Example 3. Activity of Polo-box-Related Peptides

The chemical, physical and biological activity of the disclosed polo-box related peptides can be readily assessed. Among other uses, functional assays of peptide function permit optimization of the length of the peptide either chosen from the C-terminal domain of a polo-like kinase, or the design (including length) of a heterologous peptides containing a polo-box like sequence.

Polo-box related peptide activity is the ability of the peptide to bind to a polo-box specific interacting molecule, for instance a polo-box receptor. More specifically, interaction of the polo-box related peptide with a neck filament component (such as septins Cdc11 and Cdc12) or a spindle pole component (such as Bbp1) is measured to determine the activity of the peptide. Methods for examining such interactions include those disclosed herein, such as direct measurement of protein-peptide interactions, interaction in a yeast two hybrid system, or alteration/modification of a cytokinesis-related eukaryotic growth phenotype. Control molecules may be included in each assay. Usually at least one control peptide will include one or more point mutations within its conserved polo-box domain (see SEQ ID NO: 1). Specific examples of such mutations are described herein.

Example 4. Use of Eukaryotic Cells Overexpressing a Polo-box or Related Peptide to Isolate Compounds with Polo-like Kinase Modulating Activity

The eukaryotic cells disclosed herein, which express or over-express a polo-box or related peptide, can be used to identify compounds that modify a polo-box-dependent function of a polo-like kinase. In certain embodiments, these identified compounds will inhibit polo-like kinase function; in other embodiments, compounds are isolated that enhance (activate) polo-like kinase function. Inhibitory compounds identified in this manner can be, for instance, used to inhibit or reduce cellular proliferation, such as proliferation caused by a hyper-proliferative disorder, disease or condition.

The cytokinesis-defective phenotype that is exploited for identification of kinase function modulatory compounds, as described herein, is recognizable but may be slightly different in different experimental organisms (*e.g.*, yeast cultures versus animal cell lines). Stably expressing the C-terminal domain of Plk (*plkΔN*) or Cdc5 (*cdc5ΔN*) induces chains of connected yeast cells. Specific examples of yeast strains that exhibit this cytokinetic-defective phenotype include the *S. cerevisiae* strains KLY1212 (which expresses *EGFP-plkΔN*) and KLY1083 (which expresses *EGFP-cdc5ΔN*). Though these specific strains express constructs containing the EGFP to aid in easy subcellular

localization studies of the truncated polo-like kinase protein, this fluorescent label may not be necessary for the function of the polo-box related peptide.

Cells from non-yeast organisms can be similarly used to identify compounds that have polo-like kinase modulating activity. As described herein for yeast cells, a C-terminal domain of a polo-like kinase (or a derivative or fragment thereof that maintains polo-box activity) is heterologously expressed or over-expressed in cells of a eukaryotic organism. Prior to contacting the cells with a test compound, the phenotype resulting from this heterologous expression is examined. In the case of yeast (particularly *S. cerevisiae*, as disclosed herein), such over expression causes a chaining/connected phenotype that is directly linked to polo-box dependent defects in cytokinesis. In addition, multiple spindle poles were also induced, likely through interaction with spindle pole component Bbp1. In the case of mammalian cells (in particular, the mouse-derived cell line S1), expression of two different *plkΔN* constructs results in a mitotic arrest phenotype, in which the cultured cells round up on growth dishes. Similar observations can readily be made with cells from other organisms, including other mammalian cell lines (*e.g.*, human-derived cell lines), cells from *C. elegans*, fungi (including *S. pombe*), etc.

In any experimental system, it is not necessary to use a polo-box peptide derived from the text organism in the methods described herein. Thus, the yeast polo-like kinase Cdc5 can serve as a source of the polo-box used to generate a peptide that is over expressed in mammalian cells. Likewise, a mammalian polo-like kinase (such as Plk1) can be expressed in yeast cells.

Eukaryotic cells expressing or over expressing a polo-box as described herein can be used for screening compounds for therapeutic activity (*e.g.*, to identify compounds that modify cellular proliferation and/or cytokinesis), including compounds developed by combinatorial library technology. Combinatorial library technology provides an efficient way of testing a vast number of different potentially therapeutic substances for an activity, *e.g.*, the ability to inhibit binding of a polo-box (or related peptide) to a specific receptor/binding site. Construction of such combinatorial libraries and their use are known in the art (see, *e.g.*, U.S. Patent Nos. 5,045,755, 5,565,324, 5,663,046, 5,723,598, and 6,061,636).

Briefly, a method of screening for a compound that modulates a polo-like kinase function, using the described yeast strains, may include contacting one or more test compounds (*e.g.*, from a combinatorial library) with cells of the yeast strain in a suitable reaction chamber (such as a microtiter plate well). The cells are then incubated for a time sufficient to permit an effect of the test compound to be felt by the cells, and the cells are then assessed for whether or not the compound affected any change to the cells. Such assessment can include, for instance, visual (*e.g.*, microscopic) examination of the cells to determine if the proportion of cells exhibiting a cytokinesis-defective phenotype has changed. Alternatively, such assessment can include determination of the subcellular localization of one or more proteins or peptides within the yeast cells. In certain embodiments, for instance, the subcellular localization of a polo-like kinase or another cytokinesis-functional protein (such as a septin) can be examined. A difference in a polo-like kinase in the cell after exposure to a compound is indicative of the compound having polo-like kinase modulatory activity.

The nature of the difference in a polo-like kinase in the cell after exposure to a compound is indicative of the nature of the compound. For instance, a compound with polo-box inhibitory activity may be identified as reducing an apparent cytokinesis-/mitosis-defective phenotype in a eukaryotic cell expressing a heterologous polo-box related peptide; this same compound will exhibit

cytokinesis-/mitosis -inhibitory (and/or other polo-like kinase function inhibitory) activity when applied to a cell that is **not** expressing the heterologous peptide.

On the other hand, a compound identified with the same identification assay as enhancing the cytokinesis-/mitosis -defective phenotype (*e.g.*, by increasing the proportion of chained cells, or the length of the cell chains or the number of rounded up cells in mammalian culture) is a polo-box activating compound. This class of compounds, when applied to a cell that is not expressing a heterologous polo-box peptide, may cause an increase in apparent polo-box activity within the cell. Such enhancing/activating compounds can, for instance, include compounds that stabilize the association between a polo-box (either in a peptide or heterologous protein, or in a native polo-like kinase) and a specific polo-box binding target (such as a component of the neck filaments or spindle poles). Since activation of polo kinases results in an increase in cytokinesis in budding yeast, it is likely that a specific polo-box enhancing compound can potentiate the ability of polo kinases to induce bud elongation and ectopic cytokinetic structures.

A compound identified by the herein-described methods as a modulator of polo-like kinase function (*e.g.*, localization or activity) may be of a peptide or non-peptide nature. Non-peptide "small molecules" are preferred for some embodiments, such as certain *in vivo* pharmaceutical uses.

Example 5. Incorporation of Kinase-Activity Modifying Compounds and/or Polo-box Related Peptides into Pharmaceutical Compositions

Pharmaceutical compositions that include, as an active ingredient, at least one kinase-activity modifying compound, polo-box binding peptide, or polo-box related peptide (as described herein) will normally be formulated with a solid or liquid carrier, depending upon the particular mode of administration chosen. The pharmaceutically acceptable carriers and excipients useful in this disclosure are conventional. For instance, parenteral formulations usually comprise injectable fluids that are pharmaceutically and physiologically acceptable fluid vehicles such as water, physiological saline, other balanced salt solutions, aqueous dextrose, glycerol or the like. Excipients that can be included are, for instance, other proteins, such as human serum albumin or plasma preparations. If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Other medicinal and pharmaceutical agents, for instance ifosamide, cisplatin, methotrexate, procarizine, etoposide, BCNU, vincristine, vinblastine, cyclophosphamide, gencitabine, 5-flurouracie, paclitaxel, or doxorubicin, also may be included. It may also be advantageous to include other known anti-cancer drugs.

The dosage form of the pharmaceutical composition will be determined by the mode of administration chosen. For instance, in addition to injectable fluids, topical and oral formulations can be employed. Topical preparations can include eye drops, ointments, sprays and the like. Oral formulations may be liquid (*e.g.*, syrups, solutions or suspensions), or solid (*e.g.*, powders, pills, tablets, or capsules). For solid compositions, conventional non-toxic solid carriers can include pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art.

The pharmaceutical compositions that comprise a kinase-activity-modifying compound and/or a polo-box peptide and/or a polo-box binding peptide may be formulated in unit dosage form, suitable for individual administration of precise dosages. One possible unit dosage contains

approximately 100 μg of the compound or peptide. The amount of active molecule (either a kinase-activity modifying compound, polo-box peptide, or polo-box binding peptide) administered will be dependent on the subject being treated, the severity of the affliction, and the manner of administration, and is best left to the judgment of the prescribing clinician. Within these bounds, the formulation to be administered will contain a quantity of the active component(s) in an amount effective to achieve the desired effect in the subject being treated.

Example 6. Clinical Use of Polo-box Related Peptides or Compounds with Polo-like Kinase Modulating Activity

The potent cytokinesis-modulatory (*e.g.*, inhibitory) activity exhibited by the disclosed compounds and peptides makes them useful for treating proliferative disorders, diseases and conditions in human and other animal subjects. Possibly susceptible diseases, disorders and conditions include neoplasms, and more specifically tumors of various origins (lung, colon, stomach, smooth muscle, esophagus, non-Hodgkin's lymphoma, non-small cell lung cancer, etc.). Kinase-modulatory compounds and peptides disclosed herein can be used, for instance, to prevent or inhibit cellular proliferation or growth, for instance in a tumor, such as a malignant neoplasm.

The compounds and peptides of this disclosure may be administered to humans, or other animals on whose cells they are effective, in various manners such as topically, orally, intravenously, intramuscularly, intraperitoneally, intranasally, intradermally, intrathecally, and subcutaneously. The particular mode of administration and the dosage regimen will be selected by the attending clinician, taking into account the particulars of the case (*e.g.*, the subject, the disease, the disease state involved, and whether the treatment is prophylactic). Treatment may involve daily or multi-daily doses of compound(s) and/or peptide(s) over a period of a few days to months, or even years.

If treatment is through the direct administration to the subject of cells expressing a polo-box (or related peptide) or polo-box binding peptide, such cells (*e.g.* transgenic pluripotent or hematopoietic stem cells or B cells) may be administered at a dose of between about 10^6 and 10^{10} cells, on one or several occasions. The number of cells will depend on the patient, as well as the binding peptide and cells chosen to express the protein.

A general strategy for transferring genes into donor cells is disclosed in U.S. Patent No. 5,529,774, which is incorporated by reference. Generally, a gene encoding a protein or peptide having therapeutically desired effects is cloned into a viral expression vector, and that vector is then introduced into the target organism. The virus infects the cells, and produces the protein sequence in vivo, where it has its desired therapeutic effect. See, for example, Zabner *et al.*, *Cell* 75:207-216, 1993. As an alternative to adding the sequences encoding a polo-box (or related peptide), binding peptide or a homologous protein to the DNA of a virus, it is also possible to introduce such a gene into the somatic DNA of infected or uninfected cells, by methods that are well known (Sambrook *et al.*, In *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989). These methods can be used to introduce nucleic acids encoding the herein-disclosed peptides into human cells to provide long-term inhibition of cellular proliferation. For example, gene therapy can be used to secrete the peptides in cells localized at or near a neoplasm.

The present disclosure also includes combinations of kinase-activity modifying compounds and/or polo-box related peptides with one or more other agents useful in the treatment of a disorder, condition, or disease, *e.g.* a hyper-proliferative disorder such as neoplasm. For example, the compounds and/or peptides of this disclosure may be administered in combination with effective

doses of other anti-proliferatives, anti-cancer agents, immunomodulators, anti-inflammatories, anti-infectives, and/or vaccines. The term "administration in combination" refers to both concurrent and sequential administration of the active agents.

Examples of anti-proliferatives that can be used in combination with the compounds and/or peptides of the disclosure include the following: ifosamide, cisplatin, methotrexate, procarizine, etoposide, BCNU, vincristine, vinblastine, cyclophosphamide, gencitabine, 5-flurouracie, paclitaxel, or doxorubicin.

Examples of immuno-modulators that can be used in combination with the compounds and/or peptides of the disclosure are AS-101 (Wyeth-Ayerst Labs.), bropirimine (Upjohn), gamma interferon (Genentech), GM-CSF (Genetics Institute), IL-2 (Cetus or Hoffman-LaRoche), human immune globulin (Cutter Biological), IMREG (from Imreg of New Orleans, La.), SK&F106528, and TNF (Genentech).

The combination therapies are of course not limited to the lists provided in these examples, but includes any composition for the treatment of a hyper-proliferative disorder.

Example 7. Kits

The kinase-activity modifying compounds and peptides disclosed herein can be supplied in the form of kits for use in prevention and/or treatment of a disorder, condition or diseases (*e.g.*, a hyper-proliferative disorder, such as neoplasm). In such a kit, a clinically effective amount of one or more of the compounds or peptides is provided in one or more containers. The compounds or peptides may be provided suspended in an aqueous solution or as a freeze-dried or lyophilized powder, for instance. In certain embodiments, the compounds or peptides will be provided in the form of a pharmaceutical composition.

Kits can also include instructions, usually written instructions, to assist the user in treating a disorder, condition or disease (*e.g.*, a hyper-proliferative disorder, such as neoplasm) with a kinase-activity modifying compound and/or binding peptide. Such instructions can optionally be provided on a computer readable medium.

The container(s) in which the compound(s) and/or peptide(s) are supplied can be any conventional container that is capable of holding the supplied form, for instance, microfuge tubes, ampoules, or bottles. In some applications, the therapeutic compound or peptide may be provided in pre-measured single use amounts in individual, typically disposable, tubes or equivalent containers.

The amount of a compound or peptide supplied in the kit can be any appropriate amount, depending for instance on the market to which the product is directed. For instance, if the kit is adapted for research or clinical use, the amount of each kinase-activity modifying compound or binding peptide provided would likely be an amount sufficient for several treatments.

Certain kits will also include one or more other agents useful in inhibiting cell proliferation, *e.g.* in treating hyper-proliferation. For example, such kits may include one or more effective doses of other anti-proliferative or anti-cancer drugs.

Example 8. Suppression of Polo-like Kinase Expression

A reduction of Polo-like kinase expression in a transgenic cell may be obtained by introducing (either transiently or permanently) into cells an antisense construct based on a Polo-like kinase encoding sequence. For antisense suppression, a complementary nucleotide sequence or the polo-like kinase transcript from the Polo-like kinase, *e.g.* all or a portion of the kinase cDNA or gene,

is expressed from the transformation vector. Other aspects of the vector may be chosen as discussed above (Example 1).

The introduced sequence need not be a full length Polo-like kinase cDNA or gene, and need not be exactly homologous to the equivalent sequence found in the cell type to be transformed. Generally, however, where the introduced sequence is of shorter length, a higher degree of homology to the complementary sequence of the native Polo-like kinase transcript sequence will be needed for effective antisense suppression. The introduced antisense sequence in the vector may be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. The length of the antisense sequence in the vector advantageously may be greater than 100 nucleotides. For suppression of a Polo-like kinase gene itself, transcription of an antisense construct results in the production of RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous Polo-like kinase gene in the cell.

Although the exact mechanism by which antisense RNA molecules interfere with gene expression has not been elucidated, it is believed that antisense RNA molecules bind to the endogenous mRNA molecules and thereby inhibit translation of the endogenous mRNA.

Suppression of endogenous Polo-like kinase expression can also be achieved using ribozymes. Ribozymes are synthetic RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No. 4,987,071 to Cech and U.S. Patent No. 5,543,508 to Haselhoff. The inclusion of ribozyme sequences within antisense RNAs may be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that bind to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

Suppression of Polo-like kinase expression can be, for instance, used to treat cellular proliferative and other disorders, in particular hyper-proliferative disorders such as neoplasm.

Example 9. Polo-like Kinase (Polo-box) Gene Therapy

Gene therapy approaches for combating neoplasia, and particularly breast cancer, in subjects are now made possible by the present disclosure.

Retroviruses have been considered a preferred vector for experiments in gene therapy, with a high efficiency of infection and stable integration and expression (Orkin *et al., Prog. Med. Genet.* 7:130-142, 1988). A full-length Polo-like kinase gene or cDNA can be cloned into a retroviral vector and driven from either its endogenous promoter or from the retroviral LTR (long terminal repeat). Alternatively, in embodiments wherein only a polo-box or related peptide is to be expressed, a shorter portion of a Polo-like kinase encoding sequence (encoding only the polo-box, or this consensus box and some amount of flanking region) can be cloned into the retroviral vector. Other viral transfection systems may also be utilized for this type of approach, including adenovirus, adeno-associated virus (AAV) (McLaughlin *et al., J. Virol.* 62:1963-1973, 1988), *Vaccinia* virus (Moss *et al., Annu. Rev. Immunol.* 5:305-324, 1987), Bovine Papilloma virus (Rasmussen *et al., Methods Enzymol.* 139:642-654, 1987) or members of the herpesvirus group such as Epstein-Barr virus (Margolskee *et al., Mol. Cell. Biol.* 8:2837-2847, 1988).

Recent developments in gene therapy techniques include the use of RNA-DNA hybrid oligonucleotides, as described by Cole-Strauss, *et al.* (*Science* 273:1386-1389, 1996). This technique may allow for site-specific integration of cloned sequences, thereby permitting accurately targeted gene replacement.

In certain embodiments, gene therapies vectors are preferentially targeted to proliferating cells. Methods and viral strains that can be used for such proliferating cell-biased gene therapy are discussed in U.S. Patent No. 6,045,789, which is incorporated herein by reference in its entirety.

In addition to delivery of Polo-like kinase, or a polo-box or polo-box related peptide to cells using viral vectors, it is possible to use non-infectious methods of delivery. For instance, lipidic and liposome-mediated gene delivery has recently been used successfully for transfection with various genes (for reviews, see Templeton and Lasic, *Mol. Biotechnol.* 11:175-180, 1999; Lee and Huang, *Crit. Rev. Ther. Drug Carrier Syst.* 14:173-206; and Cooper, *Semin. Oncol.* 23:172-187, 1996). For instance, cationic liposomes have been analyzed for their ability to transfect monocytic leukemia cells, and shown to be a viable alternative to using viral vectors (de Lima *et al.*, *Mol. Membr. Biol.* 16:103-109, 1999). Such cationic liposomes can also be targeted to specific cells through the inclusion of, for instance, monoclonal antibodies or other appropriate targeting ligands (Kao *et al.*, *Cancer Gene Ther.* 3:250-256, 1996).

To reduce the level of Polo-like kinase expression, gene therapy can be carried out using antisense or other suppressive constructs, the construction of which is discussed above (Example 8).

Having illustrated and described the principles of the use of polo-box peptides, including their use to isolate compounds with polo-like kinase modulating activity, it should be apparent to one skilled in the art that the disclosed molecules and/or methods can be modified in arrangement and detail without departing from such principles. The scope of the disclosure is defined by the following claims, rather than by the specific examples given above. We therefore claim all that comes within the scope and spirit of these claims.

CLAIMS

We claim:

1. A method of identifying a compound with polo-like kinase modulating activity, comprising:

contacting a eukaryotic cell with a sufficient amount of a test compound to induce an effect; wherein the cell expresses:

(a) a polo-box or polo-box related peptide; or

(b) a binding peptide comprising at least 25 contiguous residues from a polo-like kinase C-terminal region; or

(c) a functional fragment, variant, mimetic, or analog of the peptide of (a) or (b);

and thereby exhibits a dominant negative cytokinesis-defective growth pattern; and

assessing an effect of the test compound on the cell, wherein a change in the dominant-negative cytokinesis-defective growth pattern is indicative of the test compound having polo-like kinase modulating activity.

2. The method of claim 1, wherein assessing at least one effect of the compound comprises:

observing an alteration in the growth pattern of the eukaryotic cell; or

examining a subcellular localization of a polo-like kinase; or

observing an alleviation of the dominant-negative cytokinesis-defective growth pattern; or

measuring a kinase activity of a polo kinase from the cell.

3. The method of claim 1, wherein the change in the dominant-negative cytokinesis-defective growth pattern comprises a change in a subcellular localization of the kinase.

4. The method of claim 3, wherein the change in the subcellular localization of the kinase is due to a change an association of the kinase with a neck filament protein or a spindle pole protein.

5. The method of claim 4, wherein the neck filament protein is a septin.

6. The method of claim 1, further comprising assessing the compound for an anti-neoplastic activity.

7. The compound identified by the method of claim 1.

8. A method of modifying a polo-like kinase function, comprising contacting a eukaryotic cell with a substance that alters a specific interaction between a polo-box and a polo-box specific binding partner.

9. The method of claim 8, wherein the polo-box specific binding partner is a component of a neck filament.

10. The method of claim 8, wherein the method is a method of inhibiting the polo-like kinase function.

11. The method of claim 8, wherein modifying the polo-like kinase function comprises modifying a cytokinesis function or a mitotic function.

12. The method of claim 8, wherein contacting the eukaryotic cell with the substance comprises contacting the cell with:

(a) a polo-box related peptide; or

(b) a binding peptide comprising at least 25 contiguous residues from a polo-like kinase C-terminal region; or

(c) a functional fragment, variant, mimetic, or analog of the peptide of (a) or (b);

(d) a compound identified by the method of claim 1; or

(e) a compound identified by its ability to alleviate a defect of a yeast dominant negative cytokinesis mutant resulting from expression of the peptide of (a) or (b) in the yeast; wherein the substance does not have substantial kinase activity.

13. The method of claim 8, wherein the method is a method for treating a hyper-proliferative disorder in a subject in need of such treatment.

14. The method of claim 13, wherein the hyper-proliferative disorder comprises a neoplasm.

15. The method of claim 13, wherein the substance is contacted with the cell in a pharmaceutically acceptable carrier.

16. A method for producing a eukaryotic cell exhibiting cytokinesis-defective growth, comprising expressing a heterologous nucleic acid in the eukaryotic cell, wherein the heterologous nucleic acid encodes a polo-box, or a functional fragment, variant, mimetic, or analog thereof.

17. The method of claim 16, wherein the heterologous nucleic acid does not encode a kinase with substantial kinase activity.

18. The eukaryotic cell produced by the method of claim 16.

19. The substance used in the method of claim 8.

20. A kit for the treatment of a hyper-proliferative disorder, comprising:

a therapeutic substance that inhibits binding of a polo-box to a polo-box receptor.

21. The kit of claim 20, further comprising instructions.

22. The kit of claim 21, wherein the instructions include directions for administering at least one dose of the therapeutic substance to a subject in need of such treatment.

23. The kit of claim 22, wherein the therapeutic substance is provided in the form of a pharmaceutical composition.

24. The kit of claim 23, wherein the therapeutic substance is the substance of claim 18 or the compound of claim 1.

25. The method of any one of claims 1, 8, or 13, wherein the eukaryotic cell is selected from the group consisting of: a fungal cell, an animal cell, or a plant cell.

26. The method of claim 25, wherein the cell is a human cell.

27. The method of claim 26, wherein the cell is a *S. cerevisiae* cell.

28. The method of claim 27, wherein the *S. cerevisiae* cell has a mutation in either a *cyk2* or a *myo1* encoding sequence.

29. The method of claim 27, wherein the cell is a cell of strain KLY1083 or KLY1212.

30. The method of claim 27, wherein the *S. cerevisiae* cell exhibits a chaining growth pattern

31. The method of any one of claims 1, 8, or 13, wherein the polo-box comprises the sequence of SEQ ID NO: 1, or a functional fragment or variant thereof.

32. The method of claim 31, wherein the polo-box corresponds to a polo-box of a polo-like kinase.

33. The method of claim 32, wherein the polo-like kinase is selected from the group consisting of: Cdc5, *Polo*, $Plk1_{mammalian}$, Plo1p, Snk, FNK/Prk, Plx1, Tbplk, and $Plk1_{C.\ elegans}$.

34. The method of claim 32, wherein the polo-box is derived from Cdc5 or Plk.

35. A method of specifically inhibiting cytokinesis without substantially inhibiting mitosis by exposing a cell to a substance that inhibits a specific interaction between a polo-box and a septin, wherein the substance does not have assayable protein phosphorylation activity.

36. A method of specifically inhibiting cytokinesis in a yeast cell without substantially inhibiting mitosis, comprising:

exposing the yeast cell to a sufficient amount of a compound that inhibits a specific interaction between a polo-box and a septin, wherein a change in the growth phenotype to a chaining phenotype is indicative of specifically inhibiting cytokinesis.

37. The method of claim 36, further comprising exposing the yeast cell to a test compound and determining whether the test compound induces a chaining phenotype.

38. The method of claim 8, further comprising inducing ectopic expression of a polo-box or polo-box related peptide.

FIG. 1A

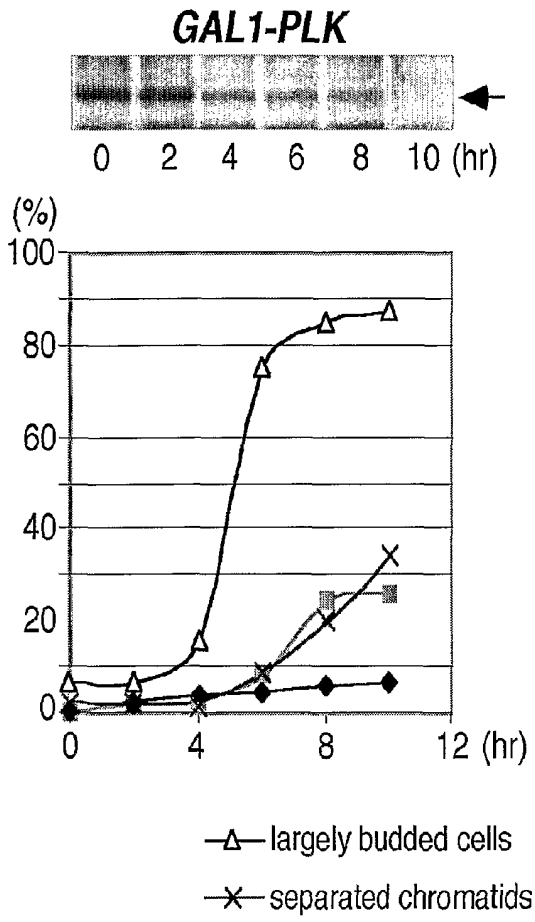


FIG. 1B

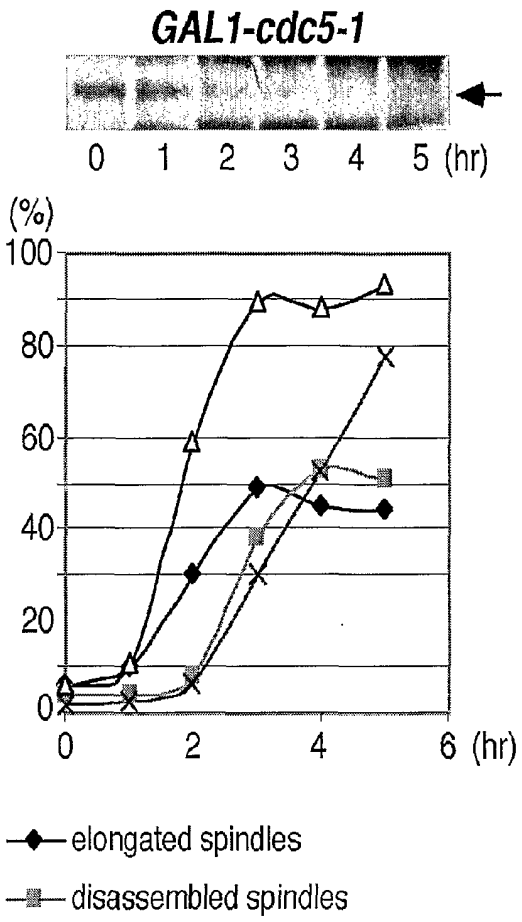
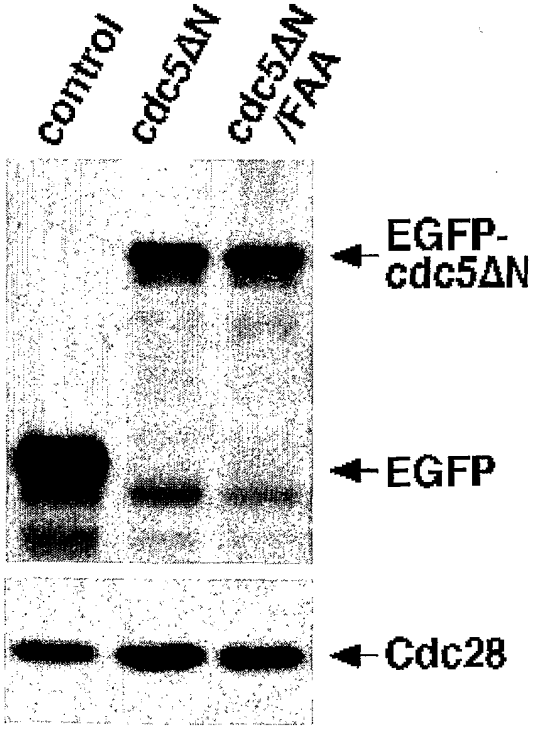


FIG. 2



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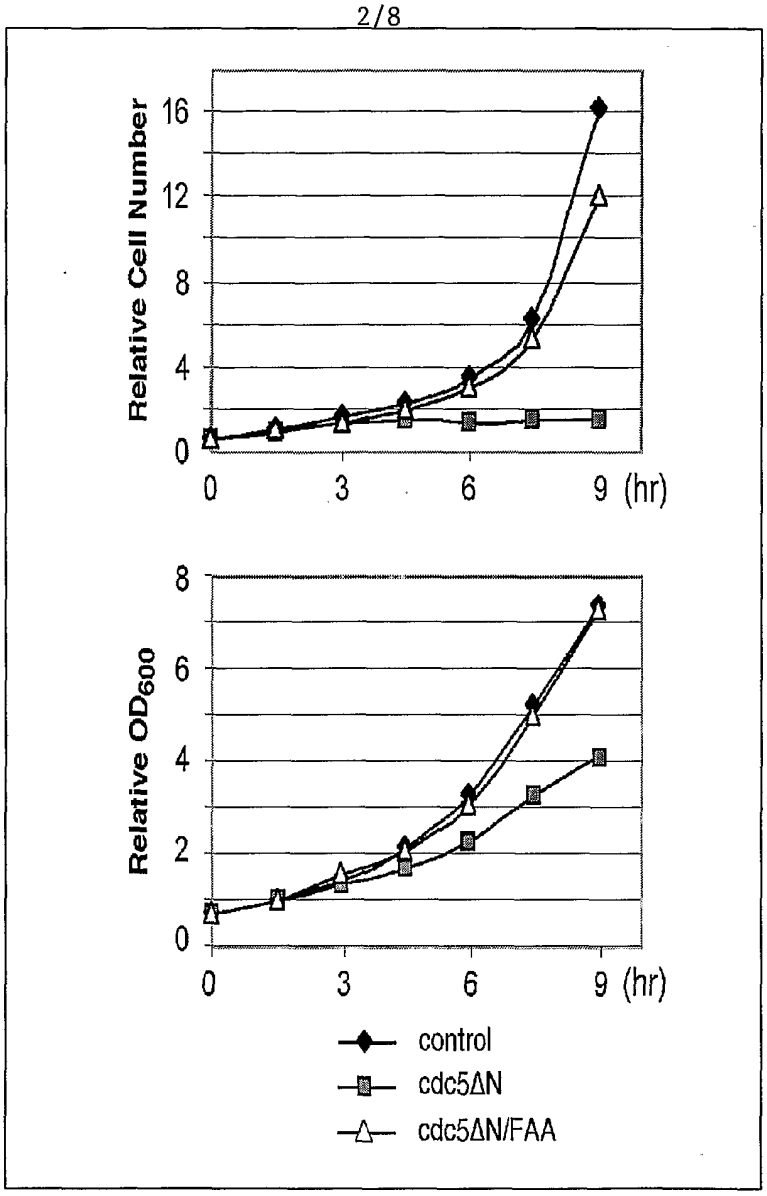
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cdc5ΔN/FAA

FIG. 3B

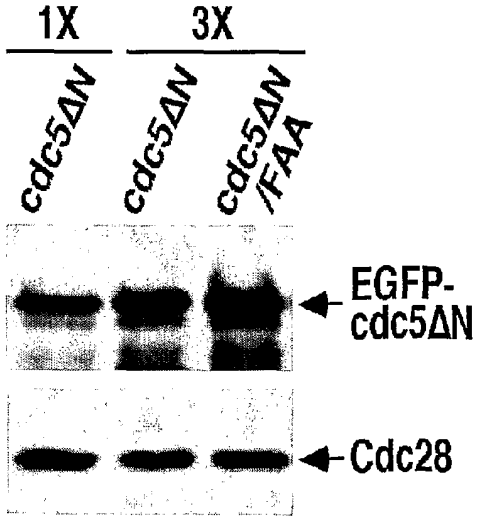
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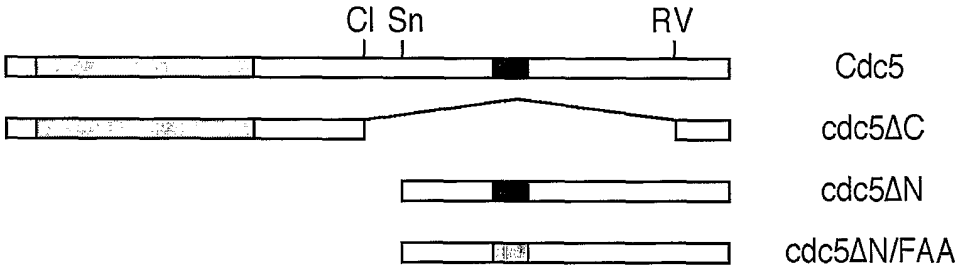
FIG. 4



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cdc5ΔC	4.4	2.6	2.5	4.1	5.1
cdc5ΔN	81.4	82.0	77.1	740.3	428.2
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FIG. 5

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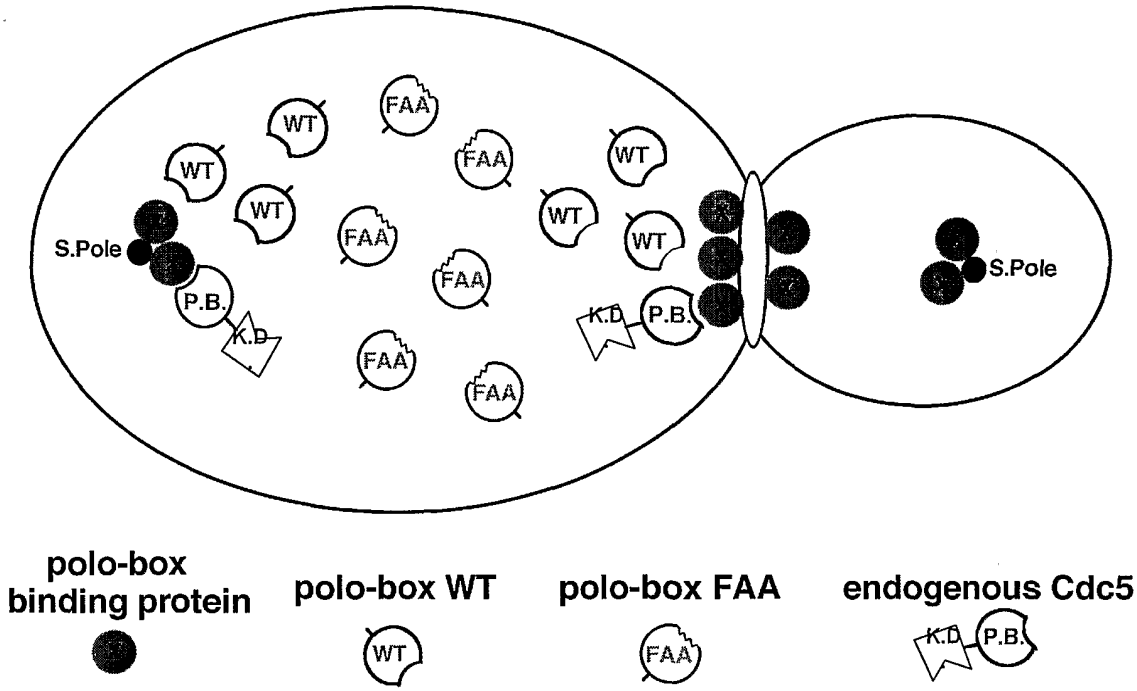
FIG. 6



polo-box binding protein

polo-box WT

WT

polo-box FAA

FAA

endogenous Cdc5

K.D P.B.

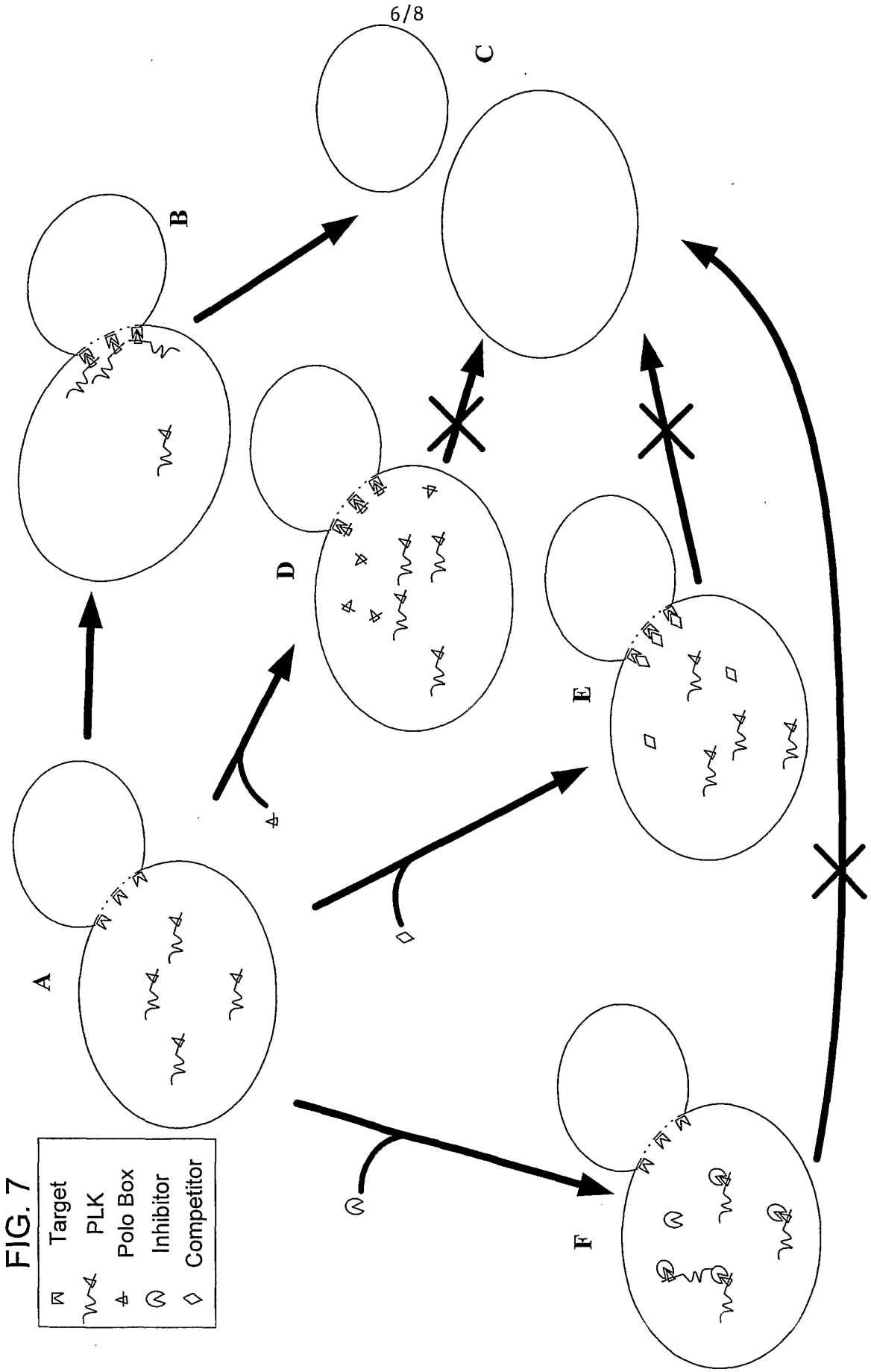


FIG. 7

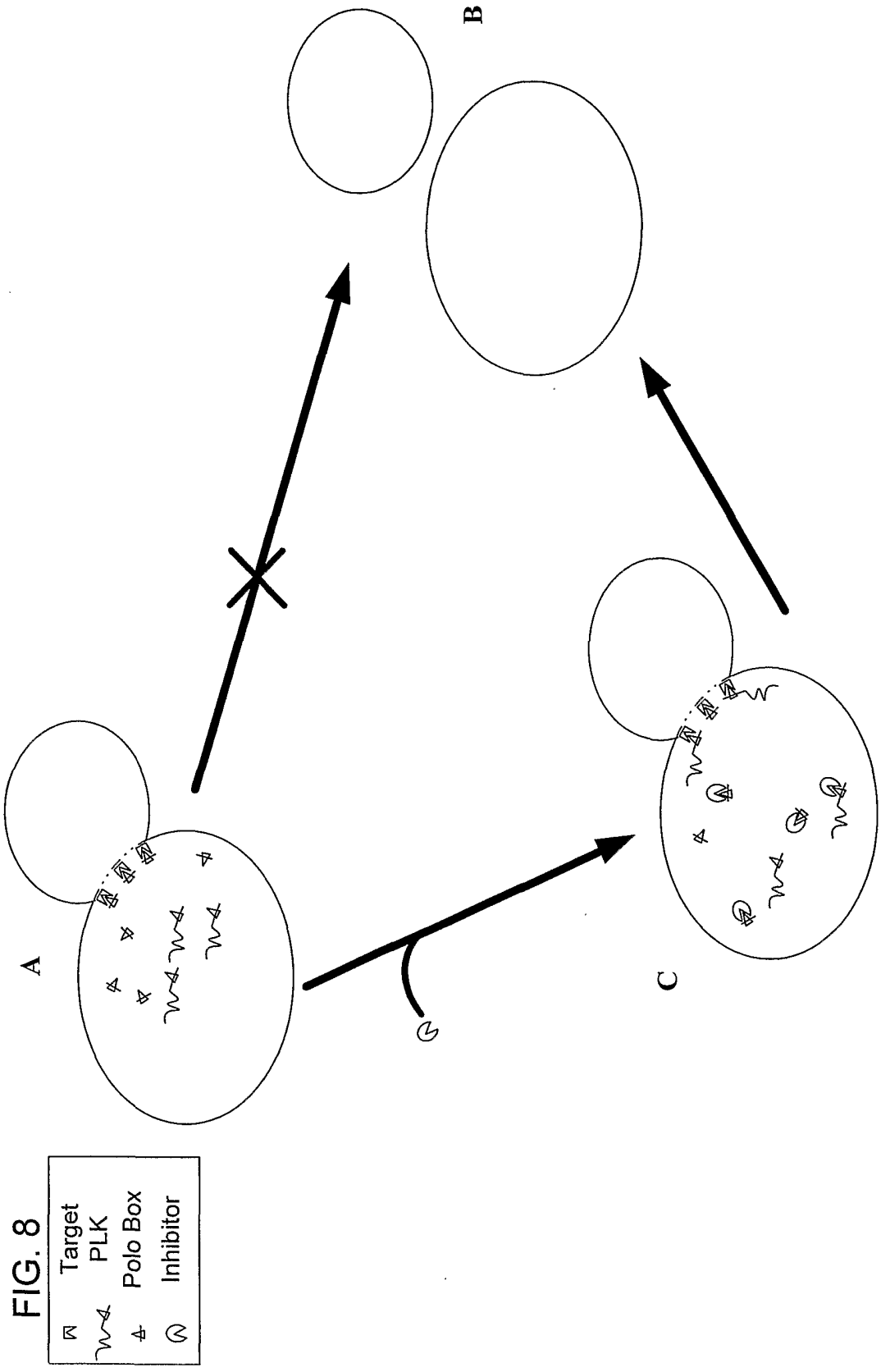


FIG. 8

FIG. 9A

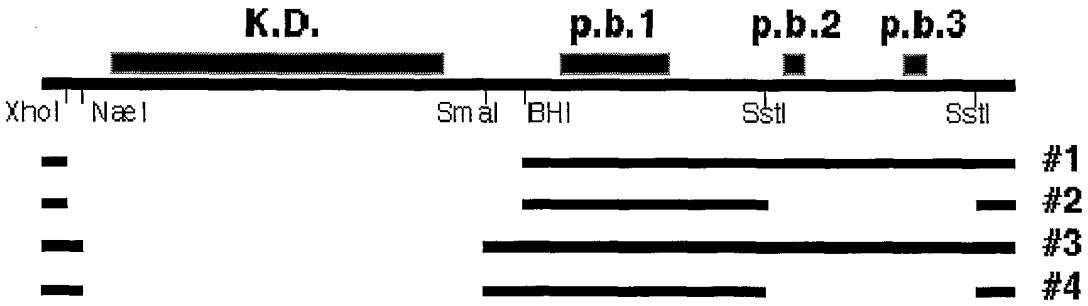

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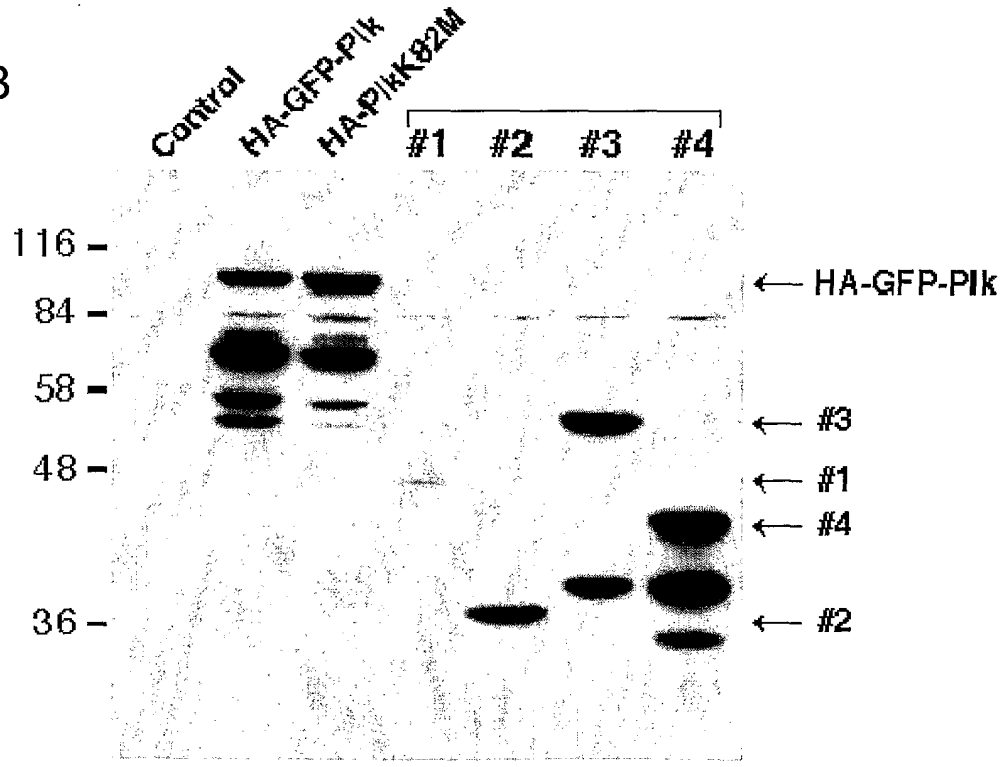
FIG. 9B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