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Abstract

The exit from mitosis is the last critical decision a cell has to make during a division cycle. A complex regulatory system has evolved to evaluate the success of mitotic events and control this decision. Whereas outstanding genetic work in yeast has led to rapid discovery of a large number of interacting genes involved in the control of mitotic exit, it has also become increasingly difficult to comprehend the logic and mechanistic features embedded in the complex molecular network. Our view is that this difficulty stems in part from the attempt to explain mitotic exit control using concepts from traditional top-down engineering design, and that exciting new results from evolutionary engineering design applied to networks and electronic circuits may lend better insights. We focus on four particularly intriguing features of the mitotic exit control system: the two-stepped release of Cdc14; the self-activating nature of Tem1 GTPase; the spatial sensor associated with the spindle pole body; and the extensive redundancy in the mitotic exit network. We attempt to examine these design features from the perspective of evolutionary design and complex system engineering.

Top-down design versus evolutionary engineering

Fundamentally, the mitotic exit system, which includes the Cdc14 early anaphase release (FEAR) and mitotic exit network (MEN) pathways, has relatively simple functionality. Yet an intricate complex control system has evolved to make the basic functions robust and precise under a variety of circumstances. From an engineering perspective, the mitotic exit system, like many other biological pathways, appears to exhibit characteristics of a complex adaptive system.

Simple systems, even complicated ones, can be decomposed into modules or pieces at all scales. An automobile or modern jet aircraft, as a complicated but non-complex systems, can be understood as the sum of their subsystems: the computers, the engine, braking systems, the flight stabilizers, and other major subsystems all have a clear function in the whole. Each of these can also be broken down and understood in terms of yet smaller components, down to the most basic mechanical and electronic parts. Viewed from the design perspective, this complicated system can be put together by many engineers, each working independently on separate components according to a master, top-down design plan. Traditional engineering design depends on this top-down, modular approach and the decomposability of the system. The system must perform precisely as the sum of all the components: it is designed under this assumption. Complex systems generally cannot be decomposed and built this way. Attempts to do so have met with spectacular failure. For example, the US government

spent billions of dollars designing a new air traffic control system that was ultimately scrapped. The system required was far too complex for the traditional design methods being used (Bar-Yam, 2003). On the other hand, the global internet, arguably the most complex human-engineered project to date, had no master blueprint, but was evolved (Berners-Lee, 2000). In fact, it was realized early that the number of different kinds of computers, different communication standards, and the desire of programmers to do things their own way, required an evolutionary design strategy.

An important result of evolutionary design is that the dynamics of a complex system cannot be understood from its components and their interactions alone. The whole is *more than* the sum of the parts, which also imposes a natural scale on the system, below which system functions are lost. Some system functions cannot be found in any single component, but exist only when components are combined in a certain configuration. However, some components may play critical roles in the system and their function is quite clear. In general, evolutionary design proceeds by allowing natural selection to manipulate components to construct a (complex) system that achieves the desired global behavior. The resulting designs often look very different from those that an engineer following traditional design principles would concoct (Antonsson and Cagan, 2001). An interesting observation is that biological systems tend to defy modular design. Although attempts have been made to view biochemical networks in neat modular packages {Hartwell, 1999 #87}, many interconnections

between modules prohibit the black-box modularity that is a hallmark of top-down engineering design {Antonsson, 2001 #64}. Often, proteins that are key components in one biological pathway can be found performing other functions in another pathway. The term "pathway" is used to group proteins conceptually, but in fact it is recognized that the black box is in fact rather transparent as proteins are routinely harnessed in multiple pathways.

It is important, however, to emphasize that there are significant differences between evolutionary algorithms applied to engineering design and the evolutionary processes that occur in biological systems. Nevertheless, both natural and artificial evolved systems exhibit properties unlike traditionally engineered systems, which proceed from a predetermined overall plan. Evolution only tinkers (Alon, 2003) with existing parts until a working solution is found; it does not optimize or coordinate functions in advance. This perspective may help to understand large regulatory networks such as the mitotic exit control system. The purpose of this article is not to provide a comprehensive review of mitotic exit regulators and pathways (for that, several excellent recent reviews are available (Morgan, 1999; Murray, 2004; Seshan and Amon, 2004; Simanis, 2003). Instead, we focus on several important yet puzzling features of the mitotic exit system and attempt to examine the underlying design principles from the perspective of complex systems constructed through evolutionary processes.

The beginning of the end: basic features of the mitotic exit system

Mitotic exit is the last major step in the cell cycle that controls the decision to physically divide a cell into two, an event known as cytokinesis. The decision to undergo cytokinesis is an important one since cytokinesis leads to a point of no return: errors that arise during previous steps in the cell cycle, in particular, chromosome segregation, can no longer be repaired. This critical cell cycle transition is termed "mitotic exit", because cytokinesis occurs with an interphase state of Cdk (cyclin dependent kinase) activity. On paper, the decision to undergo mitotic exit is made based on a simple criterion: the genetic materials (chromosomes) must be segregated fully along an axis that is perpendicular to, and divided by the plane of cleavage. The axis of cell division is often pre-determined by a cell's environment, contacts and developmental program. The spatial organization of cell division in budding yeast, like asymmetric cell divisions in many metazoan organisms, is ultimately determined by the axis of cell polarity (Figure 1) (Pruyne et al., 2004; Roegiers and Jan, 2004). Cell polarity directs asymmetric segregation and inheritance of proteins and organelles between the two progeny cells (called the mother and the bud). The actin cytoskeleton, established in a polarized manner early in the cell cycle, and a number of proteins localized in the bud ensure that the mitotic spindle is aligned and positioned such that elongation of the spindle in anaphase results in distribution of sister chromosomes to the two sides of the bud neck where the cytokinetic machine is assembled. The mitotic exit control system in yeast ensures the temporal order between chromosome segregation and cytokinesis and also entails a spatial sensor to monitor the position of the elongated anaphase

spindle relative to the polarity axis and the plane of cytokinesis. The output of the sensor must be able to influence the basic modules that control the timing of cell cycle transitions, such as Cdk/cyclin complexes and the ubiquitin-mediated proteolysis system (Ingolia and Murray, 2004; Morgan, 1999; Murray, 2004).

Figure 2 shows an overview of the mitotic exit control system. The onset of anaphase is marked by the sudden separation of sister chromatids attached to opposite poles of the mitotic spindle. Sister chromatid separation is initiated by the APC/Cdc20 complex, which also triggers degradation of mitotic cyclins, and the Cdc **F**ourteen **E**arly **A**naphase **R**elease (FEAR) pathway (Stegmeier et al., 2002). Since Cdk1/cyclin is needed to sustain APC/Cdc20 activity, only partial cyclin degradation is achieved by APC/Cdc20 (Geymonat et al., 2002a). The FEAR network has a dual role: it is required for completion of chromosome separation (D'Amours et al., 2004) and also causes transient release of Cdc14 from its "prison" - the nucleolus (Stegmeier, 2002; Azzam, 2004;D'Amours and Amon, 2004). Cdc14 is a protein phosphatase that triggers mitotic exit by dephosphorylating multiple targets (see below). Therefore, the FEAR pathway performs a control and timing function that connects the initiation of chromosome separation to mitotic exit and also accomplishes the first step in cyclin degradation that reduces the threshold for mitotic exit.

If APC/Cdc20 and the FEAR pathway set the stage for finishing mitosis, the **m**itotic **e**xit **n**etwork (MEN) provides the eventual trigger (McCollum and Gould, 2001). In a general sense, the MEN is a signal transduction system that monitors the position of the anaphase spindle relative to the polarity axis and the bud neck and then turns on

a second wave of cyclin degradation and the cytokinetic machine (Figure 3). The MEN also provides a control device through which mitotic exit can be delayed, by returning Cdc14 to the nucleolus, if the spindle is improperly positioned (Geymonat et al., 2002a). The design of the spatial sensor in the MEN is clever: the orientation of the anaphase spindle is monitored by measuring the proximity of one of the spindle pole bodies (SPB) to the polar cortex in the bud. This proximity is only achieved if the anaphase spindle is properly aligned and one of the spindle poles successfully penetrates the bud neck. The sensor is composed of two general parts: components that mark the polar cortex in the bud, and components that mark the SPB destined for the bud (Seshan and Amon, 2004). A central component capable of switch-like function is a small GTPase called Tem1, which localizes preferentially to the bud SPB. The full activity of Cdc14 leads to mitotic exit at least in part through dephosphorylation of Cdh1, another APC cofactor involved in cyclin degradation, and Sic1, an inhibitor of Cdk1 (Prinz and Amon, 1999). APC/Cdh1 and Sic 1 together eliminate mitotic Cdk1 activity, leading to mitotic exit, though additional targets of Cdc14 may yet be identified (D'Amours and Amon, 2004).

FEAR and MEN: Double-clutch control of Cdc14 release

Cdc14 holds the key for mitotic exit. Prior to anaphase onset, Cdc14 is imprisoned in the nucleolus by binding to a nucleolar protein called Net1/Cfi1 (Torres-Rosell et al., 2005). Its release and hence activation, strangely, is controlled sequentially

by the FEAR network and the MEN. Cdc14 is bound in the nucleolus until early anaphase, when activation of the FEAR network initiates its release, a process that is thought to require phosphorylation of both Cdc14 and Net1/Cfi1 (Visintin et al., 2003) (Azzam et al., 2004). After the initial release, Cdc14 would return to its imprisonment in the nucleolus unless the MEN is activated to sustain its release. What mechanisms could allow Cdc14 to be released in two pulses? In the first step, Cdc14's short-lived freedom could result from two negative feedback loops: 1) the released Cdc14 catalyzes dephophorylation of itself and Net1/Cfi1, which enables their interaction, leading to re-sequestration of Cdc14 into the nucleolus (Jaspersen and Morgan, 2000); and 2) the activities that promote Cdc14 release, such as Cdk1 (Azzam et al., 2004) decline due to APC-mediated proteolysis that occurs downstream from Cdc14.

These two negative feedbacks ensure that Cdc14 release would not be sustained without an additional activation step where the MEN comes into play. The MEN possibly sustains Cdc14 release by using a kinase Dbf2, activated downstream of Tem1 GTPase, to keep Cdc14 and Net1 in the phosphorylated state (Visintin et al., 2003). Interestingly, the MEN-induced Cdc14 release might also be self-terminating due to a negative feedback loop: the released Cdc14 localizes to the SPB where it dephosphorylates Bfa1, a subunit of the GTPase-activating protein (GAP) for Tem1, resulting in GAP activation and Tem1 assuming the inactive GDP-bound state (Pereira et al., 2002). To make the matter even more complicated, the two steps of Cdc14 release are connected with a positive feedback loop: Cdc14 released by FEAR stimulates MEN activity by dephosphorylation of Cdc15 (Stegmeier et al., 2002). Figure 4 illustrates the

intricate interconnectedness of these feedback loops. The complicated interconnections that have so far been identified suggest that it may not be possible to decompose the mitotic exit process into distinct modules, as might be required for analyzing a top-down engineering design.

Why does yeast employ this two-clutch, self-limiting system to control Cdc14 release? Negative feedback loops in electronic circuits are commonly used to remove distortion from amplified signals, a way of adding robustness to the system by damping out noise. Noise is a common problem in biological control systems because biochemical interactions are often reversible and incomplete. Fluctuation in the level of the reaction components, variation in reaction rates as a function of environmental parameters all contribute to the noise. For example, as discussed below, the Tem1 GTPase, a key MEN component, can self-activation and its rate of nucleotide exchange is sensitive to temperature. Fluctuation in Tem activity can seriously affect timing of cell cycle events by influencing Cdc14 release. The negative feedback loops could damp out the spontaneous fluctuation of free Cdc14 level due to noisy components of the mitotic exit control system. Negative feedback loops in biological networks indeed have also been found as parts of an elegant structure for creating natural oscillatory or timing functions that are robust to noisy input signals (Becskei and Serrano, 2000). The sequence of events from chromosome separation, to segregation and cytokinesis requires strict ordering. This need may have caused negative feedback structures to evolve in the mitotic exit control apparatus.

Another possible explanation for the two-clutch release of Cdc14 is that the MEN plays a surveillance role. If the FEAR network ignites the fuse that leads to mitotic exit, the MEN seems to be a separate control device inserted halfway in the fuse and allows another input into the decision to undergo mitotic exit. In this capacity, the MEN acts as a spatial sensor monitoring the orientation and position of the anaphase spindle. An interesting question is how this additional control has evolved. One possibility is that in a primitive cell, where the orientation of mitosis might be inconsequential, the FEAR network could represent the sole mechanism for Cdc14 release, and the MEN emerged later coevolving with oriented cell division. FEAR and MEN are structured quite differently, and yet the two modules are redundant for mitotic exit: whereas FEAR is not required for mitotic exit with normally functioning MEN, the requirement for the MEN in mitotic exit can be diminished by slight over-expression of Spo12, a FEAR network component (Toyn and Johnston, 1993). Thus, it is possible that the original FEAR control of mitotic exit became less effective during the evolution process to allow additional control by the MEN.

Tem1: an unusual self-activating GTPase

GTPases are often used as biological switches because these proteins adopt different conformations when bound to GTP or GDP and can convert between the two

nucleotide bound states through GTP hydrolysis and nucleotide exchange reactions (Bourne, 1995). The GTP-bound state is usually the "on" state where the GTPase interacts with downstream effectors and elicits specific signaling effects. For most Ras super family GTPases, the exchange of GDP to GTP is limited by the rate of GDP dissociation, a property that positions the guanine nucleotide exchange factor (GEF) as a critical regulator of the "on" switch. The reverse switch, from the GTP- to GDP-bound state, is catalyzed by the GTPase itself and is accelerated by the GTPase activating proteins (GAP). Tem1, however, has an unusually high intrinsic nucleotide exchange ability under physiological conditions and thus exhibits little dependence on the GEF for activation. The intrinsic nucleotide exchange reaction appears to be temperature dependent, and only at 13 ^{o}C the GDP dissociation rate was slow enough to be measured in a previous study (Geymonat et al., 2002b).

The self-activating property of Tem1 forms the basis for several important properties of the mitotic exit network. First, the fast intrinsic GDP-to-GTP exchange introduces high flexibility to the Tem1 GTPase switch, allowing fine tuning of the relative levels of $Tem1^{GTP}$ and $Tem1^{GDP}$ by both the GEF (Lte1) and the GAP (Bub2/Bfa1 complex). For example, a high level of $Tem1^{GTP}$ can be achieved by either promoting GEF action or by inhibiting GAP activity. Conversely, $Tem1^{GTP}$ can be reduced either by restricting the interaction with the GEF, or by activating the GAP. Indeed, the activity and localization of Lte1 and Bub2/Bfa1 complex are regulated in many ways (see below), allowing Tem1 to function as a dynamic switch that integrates spatial and temporal inputs through multiple pathways.

Second, the flexibility in the way by which Tem1 can be activated explains the high degree of functional redundancy in mitotic exit regulation observed in genetic experiments. Although Tem1 is required for mitotic exit, Lte1 is not required at temperatures above 30 ^{o}C (Yoshida et al., 2003). In the absence of Lte1, Tem1 can be activated through its intrinsic nucleotide exchange reaction coupled with inhibition of its GAP, Bfa1/Bub2, through phosphorylation by Cdc5, a FEAR network component (Geymonat et al., 2003; Hu et al., 2001). Lte1 and the temperature sensitivity of Tem1 self-activation through its intrinsic nucleotide exchange may have co-evolved to deal with low temperature situations, when spindle orientation and movement are slowed down due to impaired microtubule assembly (Huffaker et al., 1988; Richards et al., 2000). Under this situation, failure in spindle positioning could be frequently, thus necessitating a spatial sensor that facilitates the coupling between spindle orientation and Tem1 activation. Experiments with evolutionary algorithms demonstrate how natural selection can exploit specific or unusual properties of network components in the design process. (Thompson, 1997) used computational algorithms to directly manipulate a semiconductor medium, called a Field Programmable Gate Array (FPGA), to automatically construct an electronic circuit. Note that the computer algorithm manipulated the network connections between real transistors. The system evolved by this process is not an idealized mathematical system, but a physical system, where the laws of physics and the subtle variability of real materials determine system behavior. The final evolved circuit operated perfectly over the 10 ^{o}C temperature range that the population experienced during evolution. Some circuit elements appeared to be

disconnected from the main circuit, but interacted through subtle electrical coupling properties, showing that physical characteristics not included in the design algorithm were nevertheless exploited by the evolutionary process. The circuit was also much smaller – by one or two orders of magnitude – than would be expected from conventional design experiments, demonstrating a very efficient use of resources. Whether this is a general tendency of evolutionary design that would be operative in biological systems also is not known and should be explored further.

Additional experiments showed that greater robustness was built into the system when it was exposed to a wider range of conditions during the evolutionary process (Thompson, 1998a; Thompson and Wasshuber, 2000). The surprising result of this experiment was that natural selection resulted in an efficient, robust system that incorporated unique characteristics of the components in ways that were bizarre and unlike anything an engineer would do following traditional design practices. The unusual biochemical property of Tem1 may have been incorporated into, or coevolved with the MEN in a similar manner in response to a need for integrating multiple input signals and robustness to a range of external or internal variations.

SPB and bud cortex: sensing without touching

The Tem1 GTPase and its regulators form the core of the spatial sensor that monitors the correct orientation of the anaphase spindle. The simple model for the function of this spatial sensor has been that the correct spindle orientation is sensed

through an interaction between the bud-bound SPB, which carries Tem1 and the GAP complex, and the bud cortex, to which the GEF and inhibitors of the GAP localize. This interaction causes an increase in the steady state concentration of the GTP-bound Tem1 on the SPB, resulting in recruitment and activation of downstream MEN components such as Cdc15 and Dbf2/Mob1 kinase complex. However, later experiments found that the bud-bound SPB does not necessarily contact the bud cortex, and that both Lte1 (GEF) and Gic1,2 (inhibitors of the GAP) are released from the bud cortex in late anaphase (Hofken and Schiebel, 2004; Seshan et al., 2002). These findings raise the following questions: 1) how is the spatial relationship sensed precisely without a physical contact? 2) What controls the timing for the release of the bud cortex components?

An important insight came from analysis of Tem1 dynamics by fluorescence recovery after photobleaching, which showed that Tem1 is not stuck on the SPB, but exchanges with a cytosolic pool at an appreciable rate (half recovery time = ~30s) (Molk et al., 2004). Additionally the amount of Tem1 on the bud-bound SPB increases as the distance between the SPB and the bud neck increases. These observations suggest that the spatial sensor could operate based on dynamic localization of Tem1 on the SPB. We propose that distance sensing occurs continuously through GTPase dependent cycling of Tem1 on and off the SPB (Figure 5). In this model, we assume 1) $Tem1^{GTP}$ has a higher affinity with the SPB than $Tem1^{GDP}$, 2) most Tem1 on the SPB initially (when the SPB is far away from bud cortex) is GDP-bound due to activity of the SPB-associated GAP complex; 3) $Tem1^{GDP}$ dissociated from the SPB is converted to $Tem1^{GTP}$ (which

subsequently binds tightly to the SPB) at the bud cortex. As the SPB approaches the bud cortex, the rate of nucleotide exchange increases due to increased concentration of $Tem1^{GDP}$, resulting in an increased contraction of $Tem1^{GTP}$ on the SPB. This increase in $Tem1^{GTP}$ brings mitotic exit to the brink but may not be sufficient to trigger it. The final trigger is accomplished through a positive feedback loop: a threshold level of Cdc14 following FEAR and MEN activation triggers the release of the bud cortex associated Lte1 by dephosphorylating Lte1 at sites phosphorylated by Cdk1 early in the cell cycle (Bardin et al., 2000; Jensen et al., 2002).

Validation of above model requires further experimentation and quantitative simulations. Regardless of the detailed mechanism, it appears that the MEN-based spatial sensor is composed of parts that lack absolute precision: a self-flipping GTPase and diffusible components. Stochastic variations are expected of this system, and yet the final outcome of this system is mind-bogglingly precise: anucleate cells are never observed in a wild-type population; and even in spindle orientation-defective mutants, the Tem1-based sensor limits anucleate and poly-nucleate cells to less than 10% (Bardin et al., 2000; Bloecher et al., 2000; Pereira et al., 2002). The emergence of these abnormal cells may not even be due to sensor failure. Achieving precision using imprecise components is another common feature of evolved complex systems (Hartmann, 2002; Hartmann, 2004; Schmid, 2004). Electronic circuits designed by evolutionary processes are able to attain robustness with noisy components (Hartmann, 2004; Schmid, 2004; Thompson, 1998a; Thompson, 1998b; Thompson and Wasshuber, 2000). An interesting observation was that evolution appears to prefer small circuits in noisy environments

and that noisy or imprecise components actually seem to speed the evolutionary search process (Hartmann, 2004).

Redundancy in MEN: complexity for a reason?

A glaring feature of the MEN, as revealed by genetic analysis, is a high degree of redundancy in functional parts, generating complexity seemingly without necessity. For example, as mentioned above, the FEAR network and the MEN are partially redundant for achieving Cdc14 release. Within the MEN, deletion of the all important *LTE1* gene does not appear to have any detrimental effects on mitotic exit at temperatures above 30 ^{o}C (Hofken and Schiebel, 2004). Ste20, an effector kinase of the polarity mediator, Cdc42 GTPase, appears to be part of this alternative pathway (Figure 3), since *Δlte1* and *Δste20* deletions are lethal only in combination (Hofken and Schiebel, 2002; Seshan et al., 2002). At low temperatures, *Δlte1* alone is lethal, suggesting that the alternative Ste20 pathway is too inefficient to completely substitute for Lte1 at this temperature (Hofken and Schiebel, 2004). The pathway by which Ste20 functions presumably leads to an enhanced level of $Tem1^{GTP}$ by preventing GTP hydrolysis instead of through another GEF-like protein, since Lte1 homologs have not been found. The difference in the biochemical activities of Lte1 and Ste20 also indicates that these redundant pathways have different origins.

A later suppressor screen suggested that there are yet other alternative strategies for dealing with the lack of both Lte1 and Ste20 (Hoefken and Schiebel, 2004). The

lethality of *Δlte1 Δste20* can be rescued by over-expression of proteins involved in a wide spectrum of functions, including Bem1 (polarity), Gic1 (polarity), Pup3 (proteosome), Spo12 (FEAR network), Sic1, Tem1 and Cdc42. The mechanism of suppression by Gic1 is particularly interesting: Gic1 binds to and inhibits the activity of one of the GAP components – Bub2. Over-expression of Gic1 reduces the GAP activity and tips the balance in favor of $Tem1^{GTP}$. Another mechanism for inhibiting the GAP is through phosphorylation of the other GAP component, Bfa1, by the Polo-like kinase Cdc5. Redundancy between the Gic1-based inhibition and the Cdc5-based inhibition is demonstrated by the fact that in the absence of the GEF Lte1, *cdc5-1 Δgic Δgic2* (Gic2 is a redundant homolog of Gic1) triple mutant is inviable under conditions permissive for *cdc5-1* or *Δgic1 Δgic2* double mutant. Taken together, there are at least four mechanisms, redundant to varying degrees, to generate high [$Tem1^{GTP}$] to trigger mitotic exit: two involving inhibition of the GAP; and two acting directly on the production and/or stabilization $Tem1^{GTP}$. The ability of yeast cells to survive various mutations in genes involved in mitotic exit suggests that the system is robust, and this robustness is accomplished through system design and redundant functions rather than by using duplicated, identical redundant components.

Robust engineering systems (also called fault tolerant systems) are traditionally designed using redundant components arranged in one or more parallel configurations (Mitra et al., 2002). Electronic circuits, large database servers on the internet, and spacecraft have all used this approach. For example, the space shuttle, a complicated

but non-complex system, carries three identical onboard computers, each capable of handling all flight operations in case the others fail. With this kind of simple redundancy, the spare parts generally perform no useful function unless needed due to failure. But robustness can also arise without duplication of components but is hidden in the system design itself (Tyrrell, 2001). Complex systems built through evolutionary processes tend to achieve robustness this way and can meet stringent fault tolerance requirements (Antonsson, 2003; Bar-Yam, 2003; Bentley, 1999; Holland, 1995; Thompson and Layzell, 1999b).

Perspective

Analyzing the novel designs produced by evolutionary strategies is a new challenge for electronics (Thompson and Layzell, 1999b), but the methods they develop may also prove useful for studying biological networks. Engineers, inspired to develop design approaches based on natural selection in biological systems, may point the way to analysis methods for systems biology. Analysis is important for understanding the range of complex system dynamics and possible failure conditions. Thompson and Layzell (1999a) suggest several steps for analyzing evolved circuits, even when the circuit complexity is radically different from conventional designs. Some, as listed below, may be appropriate for analyzing biological pathways and are already used.

- *Probing abnormal conditions by manipulating environmental conditions such as temperature, varying external signals, or inhibiting components.*

- *Mathematical techniques: If a whole unconventional circuit is mathematically intractable, there may still be limited parts of the circuit which are amenable to mathematical analysis.*
- *Computer simulation of a circuit allows rapid and interactive exploration. Modeling techniques for biological pathways will be needed to fully exploit this approach.*
- *Evolutionary history: It may be possible to identify the innovation giving rise to the behavior's origin in an ancestor, and to relate this to the operation of the final circuit.*
- *Population diversity: Sometimes there can be several slightly different forms of high-fitness circuit in an evolutionary population, which can help reveal the basic mechanisms used.*

These principles for analyzing evolved complex circuits can be applied to analysis of complex biological pathways such as the mitotic exit system. The first method is precisely what classical genetics is all about. Mathematical analysis and computer simulation of biological pathways are areas of active research, but are not nearly as well developed as mathematical circuit theory. Computer simulation of biological pathways and their evolution will be a useful tool when there is enough data to make this feasible. The last two approaches are of particular interest. If robustness of the MEN is primarily due to the complex organization of the network, it may be helpful to consider the functional requirements that might act on simpler precursor systems to favor evolution of new complexity. The remarkable functional redundancy in the MEN might have evolved under the selective pressure to accomplish a high degree of accuracy in cell division as well as robustness in the ability to continue proliferation under adverse conditions.In conclusion, traditional top-down engineering requires that

all system behavior can be determined by specific components and that the behavior of the whole is precisely the sum of the parts. A defining characteristic of complex systems, also that of many biological systems, is that the whole is more than the sum of the parts. The clear implication is that at some point, system function cannot be anticipated by consideration of each of the components. The system design itself, the arrangement of the particular components, results in emergent functionality to meet complicated, unanticipated requirements. There are profound ramifications of this idea both in terms of building complex systems out of parts and for trying to understand a complex system, such as a cellular pathway. A comprehension of the system dynamics and functionality that result from natural selection may prove necessary for understanding the origins of molecular networks and our ultimate ability to manipulate biological pathways to achieve therapeutic goals.

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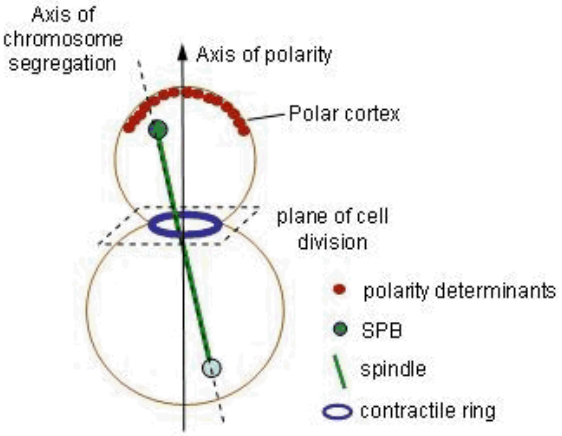
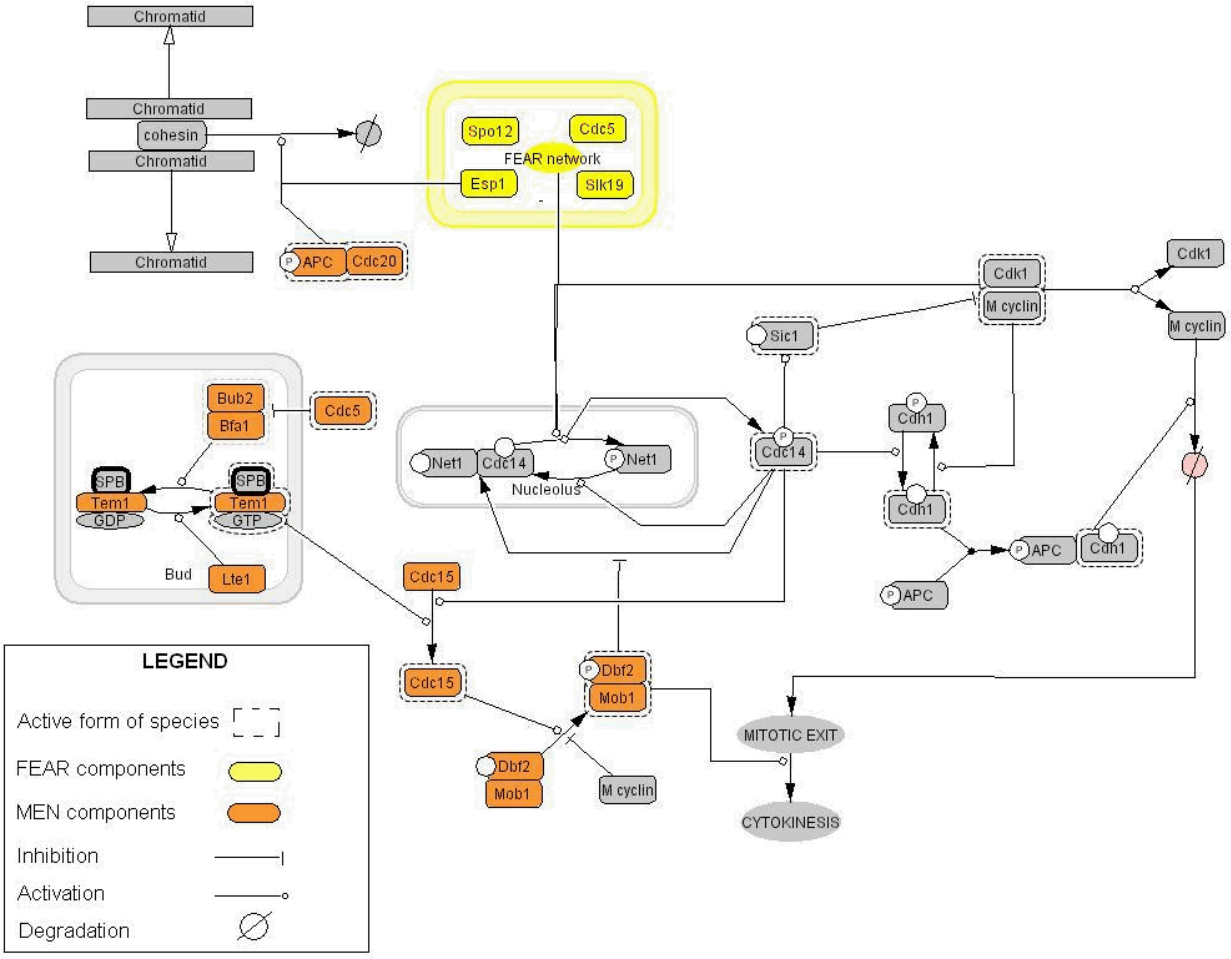
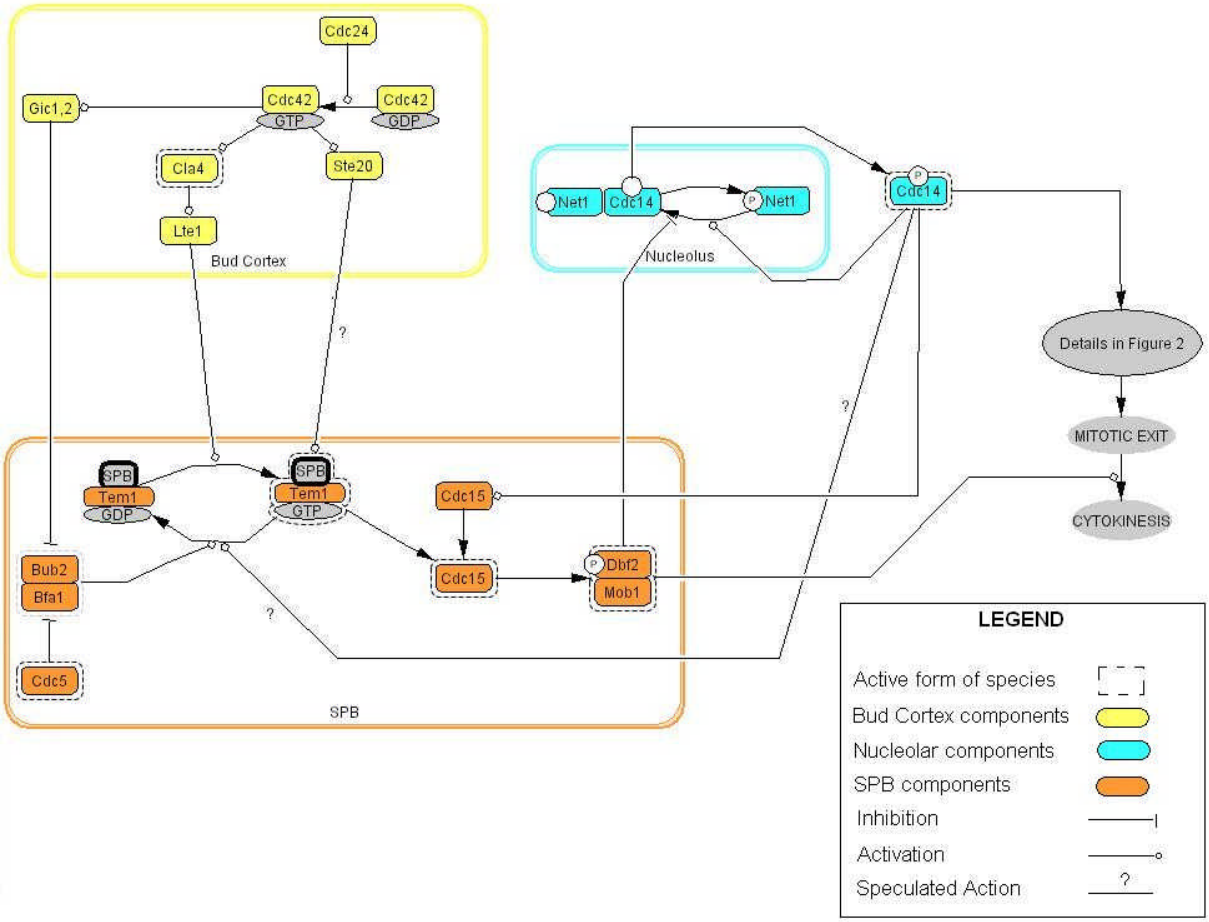
Figure 1


Axis of chromosome segregation
Axis of polarity
Polar cortex
plane of cell division
polarity determinants
SPB
spindle
contractile ring


Chromatid
Chromatid
cohesin
Chromatid
Chromatid
Spo12
Cdc5
FEAR network
Esp1
Slk19
APC
Cdc20
Cdk1
M cyclin
Cdk1
M cyclin
Sic1
Bub2
Bfa1
Cdc5
SPB
Tem1
GDP
SPB
Tem1
GTP
Bud
Lte1
Net1
Cdc14
Net1
Nucleolus
Cdc14
Cdh1
Cdh1
APC
Cdh1
APC
Cdc15
Cdc15
Dbf2
Mob1
Dbf2
Mob1
M cyclin
MITOTIC EXIT
CYTOKINESIS
LEGEND
Active form of species
FEAR components
MEN components
Inhibition
Activation
Degradation


Cdc24
Gic1,2
Cdc42
GTP
Cdc42
GDP
Cla4
Ste20
Lte1
Bud Cortex
Net1
Cdc14
Net1
Nucleolus
Cdc14
Details in Figure 2
MITOTIC EXIT
CYTOKINESIS
SPB
Tem1
GDP
SPB
Tem1
GTP
Cdc15
Bub2
Bfa1
Cdc15
Dbf2
Mob1
Cdc5
SPB
LEGEND
Active form of species
Bud Cortex components
Nucleolar components
SPB components
Inhibition
Activation
Speculated Action

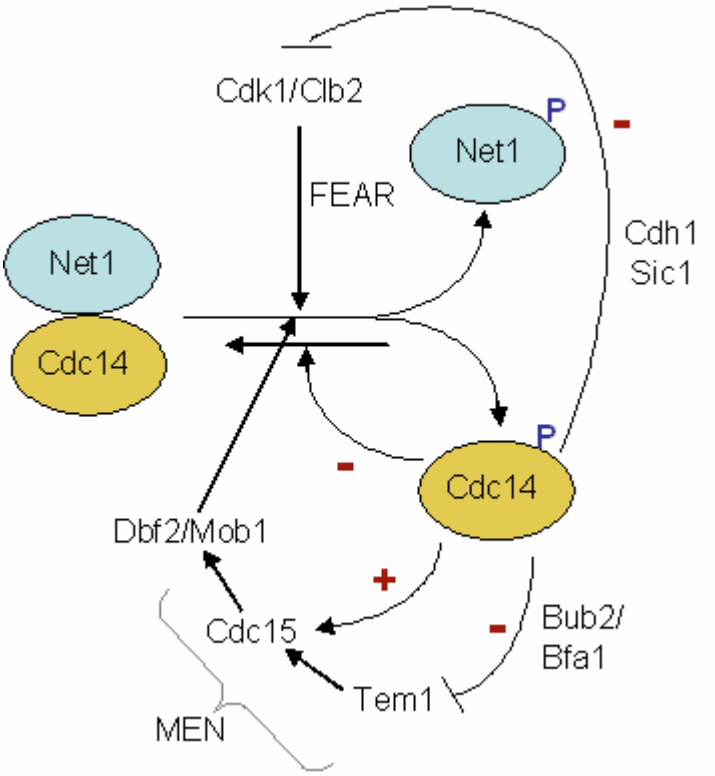

Cdk1/Clb2
P
Net1
FEAR
Cdh1
Sic1
Net1
Cdc14
P
Cdc14
Dbf2/Mob1
Cdc15
Bub2/
Bfa1
Tem1
MEN

Figure 4.

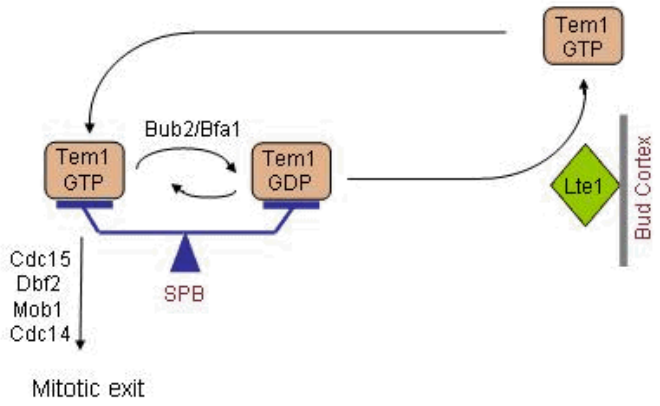

Tem1
GTP
Bub2/Bfa1
Tem1
GTP
Tem1
GDP
Lte1
Bud Cortex
Cdc15
Dbf2
Mob1
Cdc14
SPB
Mitotic exit

Figure 5

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Figure Legends

Figure 1. Asymmetric cell division of budding yeast. The diagram illustrates the organization of the axes of polarity and chromosome segregation and the plane of cell division.

Figure 2. A simple overview of the mitotic exit system proceeds sequentially from top to bottom in this diagram. Sister chromatid separation is initiated by activation of the APC/Cdc20 complex, which also triggers the FEAR pathway. FEAR has a dual role: it enables completion of chromatid separation and also causes transient early release of Cdc14 from its prison in the nucleolus. The MEN sustains Cdc14 release, detects proper spindle pole migration into the bud, begins the breakdown of mitotic cyclins and initiates cytokinesis, the final step in cell division.

Figure 3. A detailed look at the MEN pathway. Proper movement of the spindle pole body into the bud activates Tem1, which triggers the MEN pathway. The MEN sustains release of Cdc14 from the nucleolus, continues the breakdown of mitotic cyclins, and promotes cytokinesis. The MEN acts as a control switch on the "fuse" lit by the FEAR network when Cdc14 is initially released and connects chromosome separation to the breakdown of mitotic cyclins and cytokinesis.

Figure 4. The positive (+) and negative (-) feedback loops involved in the network that controls Cdc14 release, which requires dissociation of the Cdc14-Net1 complex through phophorylation by Cdk1, FEAR and MEN components.

Figure 5. Spatial sensing through the dynamic GTPase cycle of Tem1. When the SPB is distant from the bud cortex, the balance tips toward Tem1GDP at the SPB. As the SPB approaches the bud cortex, $Tem1^{GDP}$ dissociated from the SPB is quickly converted to $Tem1^{GTP}$ by the cortex-bound Lte1. Association of $Tem1^{GTP}$ with SPB results in progressive tipping of the balance toward $Tem1^{GTP}$, eventually triggering mitotic exit