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A FUNCTION-BASED FRAMEWORK FOR UNDERSTANDING BIOLOGICAL SYSTEMS

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■ **Abstract** Systems biology research is currently dominated by integrative, multidisciplinary approaches. Although important, these strategies lack an overarching systems perspective such as those used in engineering. We describe here the Axiomatic Design approach to system analysis and illustrate its utility in the study of biological systems. Axiomatic Design relates functions at all levels to the behavior of biological molecules and uses a Design Matrix to understand these relationships. Such an analysis reveals that robustness in many biological systems is achieved through the maintenance of functional independence of numerous subsystems. When the interlinking (coupling) of systems is required, biological systems impose a functional period in order to maximize successful operation of the system. Ultimately, the application of Axiomatic Design methods to the study of biological systems will aid in handling cross-scale models, identifying control points, and predicting system-wide effects of pharmacological agents.

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INTRODUCTION

After decades of reductionistic studies focused on molecular-level details, there has been a recent emphasis among biologists to develop integrated models of biological systems. Armed with the near-comprehensive "parts list" that has resulted from the genome sequencing project, efforts are increasing to build models that illustrate how these parts function in the context of the overall system. The following describes a framework for developing models of biological systems in a way that concisely and explicitly relates the functions of biological systems to increasingly well-understood molecular-level behaviors. This framework, the Design Matrix of Axiomatic Design (11), organizes complicated, multiscale systems such that functional modules are disambiguated. A clear picture of functional modules and their control points can emerge from this approach.

In the long-term the application of Axiomatic Design (AD) will help researchers develop an integrated picture of how biological systems achieve their various functions. Moreover, the AD approach will help simplify biological models by providing formalisms with which functional redundancy, multifunctionality, and nonspecific interactions can be reduced to a small subset of key structure-function relationships. AD methods provide a unique framework for building the multiscale models necessary to relate molecular-level functions to cellular, organismal, and population-level functions.

Commonalities Between Engineered and Biological Systems

AD principles have been developed over the past two decades to help designers of human-engineered systems create robust systems (12). The thrust of the AD approach is to maximize the certainty that a system will perform required functions. Compared with most human-engineered systems, biological systems are extremely robust, operating with a high degree of certainty in achieving the functions required for life. AD theory predicts that robust systems must conform to key axioms (discussed below). Evaluation of biological systems from the perspective of AD reveals that biological systems do indeed conform to these axioms.

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Axiomatic Design and System Robustness

Axioms are truths that cannot be derived but for which there are no counter-examples or exceptions. Sir Isaac Newton's three laws of mechanics are examples of such axioms. Darwin's theory of natural selection is another. The following two axioms form the foundation of AD (11):

- The Independence Axiom: maintain the independence of the functional requirements.
- The Information Axiom: minimize the information content of the design.

Adherence to these axioms confers robustness to engineered systems. System robustness is characterized by (*a*) adaptability and (*b*) insensitivity to random environmental variation. Both biological and robust engineered systems are capable of adapting to long-term changes in the environment. The Independence Axiom states how adaptability is achieved: by keeping the system's various functions independent from one another. The prevalence of functional modules in biological systems (3, 7) supports the assertion that biological systems do conform to the Independence Axiom. Robust systems are also resistant to random environmental variations (noise). The Information Axiom states that robust designs should minimize the amount of information input required in order to be robust. Clearly, biological systems function over a broad range of environmental variations, including broad fluctuations in climate, diet, toxin exposure, and the prevalence of predators, parasites, and infectious agents. As discussed below, the abundance of periodic behavior and reinitialization functions is further evidence that biological systems also adhere to the Information Axiom.

Goals for Interpreting Biological Systems Using Axiomatic Design Principles

AD principles are helpful in understanding biological systems for the following reasons:

1. Specifying functions is the starting point for AD methods. How well a species or individual can achieve specific functions determines its fitness; thus evolution operates at this same functional level.
2. AD provides a cross-scale, hierarchical framework called the Design Matrix, which integrates organismal, cellular, and molecular levels of structure and function and thereby bridges knowledge previously codified into subdisciplines such as physiology, cellular biology, and genomics.
3. When applied to a biological system, the Design Matrix can help reveal:
   - Functional modules
   - Critical interdependencies
   - Sources of robustness
   - Points for pharmaceutical intervention

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4. Analysis of a system using AD methods can help identify sources of functional periodicity.

AXIOMATIC DESIGN PRINCIPLES IN ENGINEERING AND BIOLOGY

Design Domains

According to AD theory, the design world comprises four domains (11) (Figure 1*A*) that formalize the design process by relating "what is to be achieved" to "how will it be achieved." The customer domain is determined by the customer's needs from the system, product, or process. In biological systems the equivalent of the customer domain is Darwinian fitness: the ability to compete for limited resources and reproduce. The functional domain is where customer needs are specified in terms of functional requirements (FRs). FRs common in biological systems include obtain fuel, reproduce, locomote, or maintain genetic variation. Whereas the functional domain is composed of actions, the physical domain is composed of physical entities called design parameters (DPs). The physical domain is the

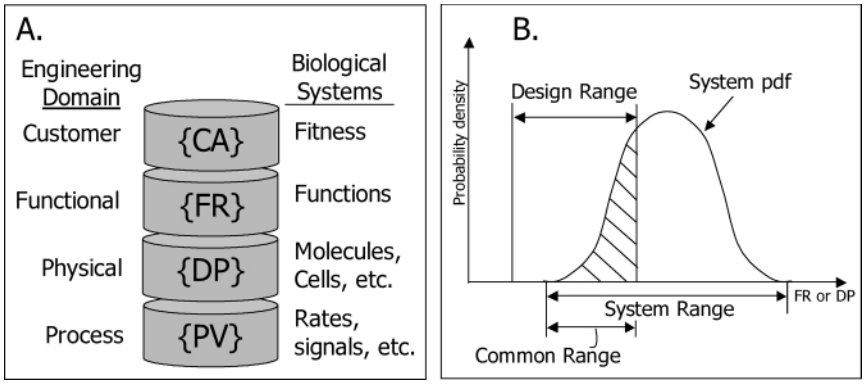

Figure 1 Design domains and ranges. Figure 1*A* shows the hierarchy of design domains. The highest level is the customer domain, or in biological systems, fitness, and is described in terms of goals (CAs). Functions required to achieve stated goals are specified in the functional domain. How those functions are achieved is specified in the physical and process domains. Figure 1*B* illustrates the probability functions that ultimately determine system robustness. The system range is the set of conditions in which a system may need to operate and is a probability density function. The design range is the set of conditions that a system is designed to operate within. The overlap between the system range and design range is the common range. Thus the probability that a system will perform successfully is determined by the area under the system probability density function bounded by the common range (*shaded*).

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primary focus of much current biological research owing to recent advances in the characterization of biological structure (e.g., DNA sequencing, protein structure determination, and high-throughput methods for determining molecular interactions such as the yeast two-hybrid system or chromosome immunoprecipitation). The fourth and final domain is the process domain, where the process necessary to manufacture the product DPs is specified by process variables (PVs). Examples of PVs include temperature, precision, and specific assembly methods. Examples of PVs in biological systems include transcription and translation rates, mRNA decay rates, and error rates in DNA replication.

Design Range Versus System Range

The key determinant of robustness is the probability that the system range of a system falls within the bounds of a design range. The design range of a function is the range in which the design must operate within, whereas the system range is determined by the system performance. The system range is a probability density function (Figure 1*B*). Typically, the design range consists of an upper and lower bound. Robustness is measured by the common range: the area in common between the system range and design range. Perfect designs share 100% of the common range. System failure occurs when the system performance is outside the design range, i.e., where the design range and system range do not overlap (Figure 1*B*). For a biological system such as a species, a common consequence of such a mismatch is extinction, where typically the rate of change of the design range (owing to introduction of a predator or habitat destruction) is greater than the rate at which the species can adapt its system range by mutation and natural selection.

A FRAMEWORK FOR MODELING BIOLOGICAL SYSTEMS

Many current models of biological systems use symbols to connote biological entities (objects such as proteins and genes) and arrows (vectors) to connote interactions between them (Figure 2*A*, see color insert). Although these traditional object/vector representations can be deeply meaningful to biologists focused on a specific aspect of a system, they are limited in several ways that make them undesirable for representing biological systems. Specifically, object/vector models do not explicitly relate system components to functions, they are typically nondynamic and hence difficult to update, and they handle cross-scale dimensionality informally (often using cartoon depictions).

In the subsequent paragraphs an alternative method for representing biological systems is presented. It is called the Design Matrix. The Design Matrix offers many features that are useful in modeling biological systems, including (*a*) explicit relationships between components and functions, (*b*) formal representation of cross-scale and hierarchical relationships, and (*c*) dynamic models that can be readily modified and greatly expanded using commercially available software (Acclaro Designer available from www.axiomaticdesign.com). Most importantly,

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the Design Matrix can reveal much about how biological systems operate, including which functions are coupled to other functions, sources of robustness, the location of control points, and the impact of pharmaceutical modulation.

V-Model for Construction of the Biological Functions from Molecular Behavior

To understand the biological functions on the basis of an understanding of molecular interactions, a V-model shown in Figure 3 is proposed as a conceptual framework. The left arm of the V-model represents the "top-down" decomposition of a system from the highest-level FRs of the system to the leaf-level DPs. The right arm of the V-model represents the "bottom-up" integration process.

A model of a biological system is developed first by defining FRs and DPs relative to system needs (high-level functions). Once the molecular-level moieties are determined by the decomposition process (coming down the left arm of the V-Model), these molecules and other moieties are assembled to form a biological

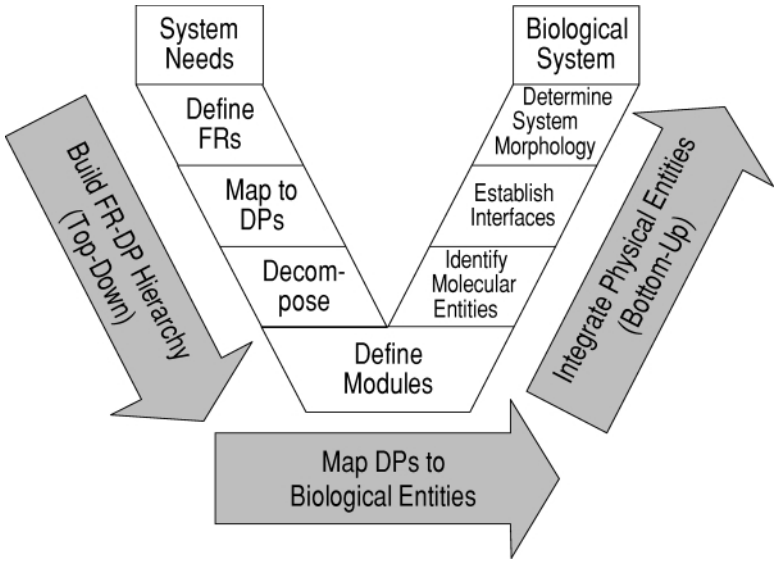

Figure 3 V-Model overview of system analysis using the Design Matrix. The V-Model describes how the Design Matrix of AD is used to study biological systems. First, one describes the functions a system must achieve (system needs and then FRs). FRs are then mapped to processes, tissues, cells, or other components (DPs) that provide the given function. This is done iteratively, zigzagging between FRs and DPs, in order to decompose the FR-DP hierarchy to the necessary completeness. Once the FR-DP relationships are specified, one can then integrate physical entities into a model that is based on functions.

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system by going up the right leg of the V-Model. In this process certain DPs are placed in geometric proximity because they interact or satisfy the geometric conditions in an optimum way. In other words, the physical pieces are put together to form a biologically functioning system, using the most likely physical moieties and the most probable configurations that can be made to work as an integrated system.

Decomposition of the Lung

The left arm of the V-model and the use of the Design Matrix are illustrated using the example of the human lung. In this example, "lung" is the highest-level DP. It satisfies the highest-level FR, which may be stated as:

$$DP_0 = \text{Lung}$$

$$FR_0 = \text{Supply } O_2 \text{ to blood and remove } CO_2 \text{ from blood}$$

In engineered designs, FRs are stated first and then solved in terms of DPs. However, because knowledge of most biological systems is dominated by DPs, this example maps the DP (lung) in the physical domain to the FR (exchange O_2 with CO_2) in the functional domain.

FR_0 and DP_0 can be decomposed further into the children FRs, FR1–4, which describe the FRs of DP (lung). These children-level FRx, which as an aggregate perform the function FR_0, may be stated as:

$$FR1 = \text{Supply } O_2 \text{ to blood as required}$$

$$FR2 = \text{Remove } CO_2 \text{ from blood}$$

$$FR3 = \text{Filter particulates in air}$$

$$FR4 = \text{Remove particulates and infectious agents from lung}$$

The DPx that can satisfy the FRx are as follows:

$$DP1 = O_2 \text{ supply mechanism}$$

$$DP2 = CO_2 \text{ removal mechanism}$$

$$DP3 = \text{Mucous in large airways}$$

$$DP4 = \text{Cough reflex, ciliary action, macrophage activity}$$

To understand the system behavior, the relationship between these FRs and DPs must be understood. This is achieved by constructing the Design Matrix, as illustrated in Table 1.

Symbol X indicates a strong relationship between the FR and the DP and symbol O indicates no relationship. For example, X indicates that the answer is "yes" to the question: "Does the DP_i affect function FR_j?" In the above matrix, DP1 is assumed to affect FR2 because if fresh air does not come into the lung, there will

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TABLE 1 Design Matrix for the lung[a]

	DP1	DP2	DP3	DP4
	O_2 supply mechanism	**CO_2 removal mechanism**	**Mucous in large airways**	**Cough reflex, ciliary action, macrophage activity**
FR1 Supply O_2 to blood	X	O	O	O
FR2 Remove CO_2 from blood	X	X	O	O
FR3 Filter particulates	O	O	X	O
FR4 Remove particulates and infectious agents	O	O	O	X

[a]This matrix depicts the relationship between the highest level FRs and DPs. The secondary relationship between DP1 and FR2 shows that FR2 is effected by FR1.

not be any need to expel CO_2. Ultimately Xs are replaced by transfer functions, phenomenological equations, or sensitivity parameters that provide a quantitative representation of the relationship between the DP and the FR. In biological systems many of these relationships are unknown and this limits one's ability to make quantitative predictions for the system. Even without this information, the Design Matrix helps specify the elements in the Design Matrix that should be measured and can thereby help reduce unnecessary experimentation. For the purpose of demonstrating the Design Matrix, this step is omitted.

FR1 and DP1 may be further decomposed as:

$$\text{FR11} = \text{Bring in fresh air to the lung cavity}$$

$$\text{FR12} = \text{Allow diffusion across the alveolar membrane}$$

$$\text{DP11} = \text{Expansion of the lung cavity}$$

$$\text{DP12} = O_2 \text{ gradient across alveolar membrane}$$

These too can be represented in the Design Matrix, as shown in Table 2.

Because each DP relates to a single FR, the Design Matrix for (FR11, FR12)/(DP11, DP12) is a diagonal matrix. The significance of this is discussed below.

FR2 and DP2 may be decomposed, as illustrated in Table 3.

The Design Matrix for the above set of FR2x and DP2x is also a diagonal matrix. The full Design Matrix for the lung, decomposed to the levels described above, is shown in Figure 4.

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TABLE 2 Decomposition of FR1[a]

	DP1.1	**DP1.2**
	Expansion of lung cavity	**O_2 gradient across alveolar membrane**
FR1.1 Bring fresh air into lung cavity to create O_2 gradient	X	O
FR1.2 Allow diffusion across alveolar membrane	O	X

[a]This matrix shows the "child" FRs and DPs for FR1 (supply O_2 to blood). The one-to-one relationship between these FRs and DPs indicates an uncoupled design at this level.

Significance of a Full Design Matrix

To achieve the goal of relating the FRs of the biological system to molecular-level interactions, the decomposition of the lung demonstrated above should be continued further. Once the mapping is complete to a reasonable level of detail (for example, to the molecular but not atomic level), the relationship between the FRs and DPs is specified by a full Design Matrix. The full Design Matrix relates the highest-level FRs to the molecular-level DPs, a goal of systems biology. In the example of the lung, the full matrix becomes quite complex and its utility in keeping track of FR-DP relationships becomes readily apparent.

Uncoupled and Decoupled Designs

The Independence Axiom mandates that to achieve a robust design, one must maintain the independence of FRs. The Design Matrix helps engineers ensure the independence of FRs through careful consideration of the relationship between FRs and DPs. The Design Matrix reveals whether designs are coupled, decoupled, or uncoupled. In uncoupled designs each DP supports a different FR and hence

TABLE 3 Decomposition of FR2 (remove CO_2 from blood)

	DP2.1	**DP2.2**
	Contraction of lung cavity	**CO_2 gradient across alveolar membrane**
FR2.1 Expel CO_2 from lung cavity	X	O
FR2.2 Allow diffusion across alveolar membrane	O	X

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			[DP0] Lung					
			[DP1] O2 supply mechanism		[DP2] CO2 removal mechanism			
			[DP1.1] Expansion of lung cavity	[DP1.2] Oxygen gradient across alveolar membrane	[DP2.1] Contraction of lung cavity	[DP2.2] CO2 gradient across alveolar membrane	[DP3] Mucous in large airways	[DP4] Cough reflex, ciliary action, macrophage activity
[FR0] Supply oxygen and remove CO2	[FR1] Supply O2 to blood	[FR1.1] Bring fresh air into lung cavity to create oxygen gradient	X	O	O	O	O	O
		[FR1.2] Allow diffusion across alveolar membrane	O	X	O	O	O	O
	[FR2] Remove CO2 from blood	[FR2.1] Expel CO2 from lung cavity	O	O	X	O	O	O
		[FR2.2] Allow diffisuion across alveolar membrane	O	X	O	X	O	O
	[FR3] FIlter particulates in air		O	O	O	O	X	O
	[FR4] Remove particulates and infectious agents from lung		O	O	O	O	O	X

Figure 4 This full Design Matrix, generated by Acclaro Designer, shows that the lung normally functions as a decoupled system, in which the various functions it performs are satisfied by independent system components. The Design Matrix reveals that the link between FR2 and DP1 occurs because both the O_2 and CO_2 gradients must occur simultaneously at the alveolar membrane. (Maintenance of the O_2 gradient is dominant, but because it is controlled in the CNS, it is not specified in the decomposition of the lung.)

these matrices are characterized by a diagonal in the Design Matrix (Table 4*A*). Truly uncoupled designs are the most robust because they are highly adaptable and controllable. It is therefore likely that many biological systems employ uncoupled designs.

Decoupled designs (Table 4*B*) are characterized by a triangular matrix in which some DPs support multiple FRs. If DPs are modified in the correct sequence, then a system can adapt in one step without iterations. In a decoupled design the sequence

TABLE 4 Uncoupled, decoupled, and coupled designs

	DP1	DP2	DP3	DP4
(*A*) Uncoupled design				
FR1	X	O	O	O
FR2	O	X	O	O
FR3	O	O	X	O
FR4	O	O	O	X
(*B*) Decoupled design				
FR1	X	O	O	O
FR2	X	X	O	O
FR3	O	X	X	O
FR4	O	X	O	X
(*C*) Coupled design				
FR1	X	X	O	O
FR2	X	X	O	X
FR3	X	X	X	X
FR4	O	O	O	X

In an (*A*) uncoupled design, each DP satisfies a single FR. If the FR needs to be modified in order to accommodate changes in the system range, the relevant DP can be modified without affecting other FRs. Complexity is also minimized by a (*B*) decoupled design. Although some DPs satisfy multiple FRs, there is a specific sequence with which DPs can be modified in order to adapt. (*C*) Coupled designs are difficult to adapt because changes in a single DP can affect multiple FRs and necessitate changes in other DPs.

of modifications is specified by the Design Matrix. Signal transduction cascades are examples of such decoupled designs. In some cases there is a secondary relationship between a DP and an FR. These secondary FR-DP relationships are referred to as off-diagonal elements. The many genes that have no abnormal phenotype when knocked out may be examples of such off-diagonal elements.

The exact relationship between DPs and FRs distinguishes decoupled designs from coupled designs. If there does not exist a hierarchy of FR-DP relationships such that a matrix can be drawn in which the upper-right triangle is blank (i.e., has zero values for FR-DP relationships), then the design is coupled (Table 4*C*). The Design Matrix reveals that coupled systems are difficult to adapt to changes in the system range. Modifying a single DP can affect a large number of FRs, which in turn necessitates the modification of additional DPs, and so on.

Although there is rarely sufficient knowledge of FRs and DPs to determine the absolute structure of a Design Matrix for a given biological system, AD theory offers important clues for understanding how biological systems operate. For example, biological systems are likely to obey the Independence Axiom. Consistent with this view, many have noted the abundance of functional modularity in biological systems (3, 7).

The Independence Axiom may explain the frequent occurrence of gene families in metazoans. The need to maintain the independence of FRs may be the driving

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force for maintaining redundancy of gene function, as is often seen in gene families. Redundant genes may be equivalent to "spare parts." As the design range for a given species changes (owing to migration to a new habitat or the introduction of a predator), mutation and natural selection can draw upon the available spare parts to achieve the new FRs required for the new design range without introducing coupling.

Nonetheless some biological systems are coupled. As is apparent from the existence of multifunctional proteins, adaptive evolutionary processes make use of pre-existing components (DPs) and thereby create coupling, at least transiently. Coupling is evident in the decomposition of the G1/S transition in the mammalian cell cycle (Figure 2).

In the above decomposition of the lung the highest-level decomposition reveals a decoupled design, since DP1 affects FR2 as well as FR1, but DP2 does not affect FR1. Evidence for this comes from the phenomenon of altitude sickness, or respiratory alkalosis (1a). In response to decreased O_2 levels at higher altitudes, the rate of respiration is increased. Because the function of delivering O_2 is coupled to the function of removing CO_2, the rate of CO_2 removal is also increased, resulting in reduced blood CO_2 levels. Because dissolved CO_2 is acidic, the blood pH increases and causes the symptoms of altitude sickness. Mountain climbers avoid altitude sickness by spending a few days at elevation before climbing to the summit. Their bodies adapt to the increased breathing rate (changes in DP1) by modulating the level of bicarbonate buffer in the blood (modified design range). Because the design is decoupled, the modification in blood buffering (changes in the setting of DP2) does not affect O_2 delivery (FR1).

Coupling does interfere with robustness in biological systems. For example, certain cytochrome P450 proteins (a DP) metabolize multiple drugs. In certain circumstances, this multifunctionality (multiple FRs) can cause what is observed clinically as a drug-drug interaction. Individuals taking drug A for some period of time will have increased levels of the metabolizing P450 protein in order to efficiently eliminate drug A. If these patients are given a second drug, drug B, metabolized by the same protein, drug B may be metabolized so rapidly that it does not reach a therapeutic level (14). The coupled metabolism of different drugs can affect the robustness (efficacy) of drug therapy. To avoid this coupling, pharmaceutical companies routinely determine whether a drug candidate is metabolized by a single type of P450 protein early in the clinical trial process.

The Relevance of the Design Matrix to the Study of Biological Systems

The decomposition for G1/S transition (Figure 2*B*,*C*) demonstrates some interesting aspects of the G1/S transition. First, many FRs in the G1/S transition behave independently. Although this matrix represents the combined activities of several dozen proteins and numerous subsystems, the overall system behavior as

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represented by the Design Matrix is not complex. Second, the matrix describing the G1/S transition shows two apparent instances of coupling. In the first instance of coupling, signals from extracellular mitogens (DP2) are coupled to signals from growth factors (DP3), because of a bifunctional signal transduction pathway (Ras pathway) (Figure 2*C*). Hence cells appear to integrate these two classes of signals and thereby couple cell growth (FR2.2) to cell division (FR3.2). The second instance of coupling is between "Inactivate cdk inhibiting mechanism" (FR5.6) and "Inactivate Rb" (FR5.2), which are both affected by S-Cdk. Both examples of coupling in Figure 2*B*,*C* reflect current knowledge of the cell cycle. However, it is possible this coupling, created in both cases by bifunctional proteins, may not reflect the real situation. Instead, it may be that the two functions occur at a separate time or place. It may also be that one of the two functions is really a minor function achieved by that protein. If these alternatives are correct, then the system is in fact a decoupled system. As described above, decoupled systems are more robust than coupled systems because FRs behave independently if modified in a specific sequence. If the system is in fact coupled, then cells are less likely to successfully adapt to changes in FR2.2, FR3.2, FR5.2, or FR5.6. It is interesting to consider that adipocytes must have somehow uncoupled or decoupled FR2.2 and 3.2, because in adults they can grow greatly in size but do not (normally) divide. In applying AD theory in nonbiological disciplines, a common mistake is to assume that the system is coupled when a single component (DP) of a system participates in multiple functions. However, when the system is considered only in regard to components, the true structure of the Design Matrix cannot be known.The Design Matrix is helpful in describing and understanding any biological system. By identifying and abstracting functions into a hierarchy, one can obscure molecular details for simplicity and clarity. Additionally, it reveals and highlights hidden FRs, DPs, and their interactions. During the process of decomposition, it is required that all FRs be identified and corresponding DPs be specified along with interrelationships. Thus, ambiguity related to any of those will stand out and require attention. Last, by examining the Design Matrix, it is possible to pose some hypotheses. Testing such hypotheses is an interesting problem from both cell biology and AD perspectives.

Many biologists, when introduced to the Design Matrix, express concern that many FRs and DPs are unknown in biological systems. In fact the decomposition process is tolerant of missing knowledge and the Design Matrix need not be fully determined or completely accurate in order to reveal important features of a biological system. The lack of information raises interesting research questions.

The challenge of understanding biological systems is similar to trying to understand a complicated electric circuit that is designed by someone else and consists of many circuit elements such as resistors, capacitors, and inductors. By observing what the system does as a whole, one can understand its high-level functions. At a lower, more-detailed level one can recognize parts (for example, resistors) by their appearance and then measure their activity by measuring the voltage drop across the resistor. Similarly, biologists measure subcellular location, relative abundance,

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protein sequence, and other parameters to characterize system components. Owing to historical factors, there is a predominance of knowledge of high- or low-level FRs in biology and a relative dearth of mid-level FRs. High-level FRs such as "reproduce" or "obtain nutrition" are satisfied by many supporting FRs and DPs and are thus described by a Design Matrix containing many levels of decomposition. Low-level FRs in biology include activities such as "transcribe mRNA" or "phosphorylate substrate protein on tyrosine" and comprise functional annotation typically found in highly used databases such as Locuslink (9). Although these low-level FRs accurately reflect the molecular function of a given gene product, they do not provide insight into that gene product's role in specific cellular functions or relate it to high-level FRs. Mid-level FRs such as "open potassium channel" or "ubiquitinate cyclin-dependent kinase to permit cell cycle progression" link molecules to functions and bridge low- and high-level FRs in the Design Matrix. The Design Matrix reveals missing FRs and DPs that are important areas for research. Because the Design Matrix considers high-level, mid-level, and low-level FRs simultaneously, it represents an important tool for understanding how biological systems operate.

COMPLEXITY

AD theory and the Design Matrix make important inferences about system complexity (13). Most definitions of "complexity" treat it as a synonym of "complicated." From the AD perspective, complexity and complicatedness are different from one another. In fact, a complicated system may have little complexity. Biological systems are undoubtedly complicated. However, the robustness of biological systems suggests that they operate with little true complexity.

According to AD theory, complexity is defined as the uncertainty of satisfying the FRs. This occurs when the system ranges of FRs are not inside the design ranges and is defined as real complexity. On the other hand, imaginary (artificial) complexity is a result of not knowing the exact relationship between FRs and DPs. Imaginary complexity, like complicatedness, is relevant only to the human perception of a system and thus has no true relevance to the system per se.

When the system range changes as a function of time, there is time-dependent combinatorial complexity. As discussed below, nonmodular systems fail when faced with time-dependent combinatorial complexity. Biological systems cope with time-dependent combinatorial complexity by (*a*) using modular designs and (*b*) operating with functional periodicity.

Complexity in Biological Systems

There are two classes of complexity in biological systems. Imaginary complexity is complexity associated with one's ability to predict. When one cannot predict the consequence of certain input to the biological system, the system appears to be

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complex. The complexity associated with predicting system-level behaviors from molecular-level knowledge measures the uncertainty in predicting the biological behavior on the basis of the existing knowledge. AD theory predicts then that biological systems are far less complex than they appear. Subsequent sections only consider the real complexity associated with the behavior of biological systems.

Real complexity is the complexity associated with the behavior of biological systems. When the complexity associated with how well a biological system performs its functions is measured, we have to establish the FRs of the system that must be satisfied and then measure how well these FRs are satisfied by the system. If the FRs of a human are satisfied 100% of the time within the design range, the person should never experience illness and have an immortal life.

An example of real complexity may be illustrated as follows: Humans must have the ability to convert glucose into energy. How well this function is performed by a person can be measured in terms of complexity. The complexity associated with the FR of a person is zero if the person converts the glucose within the physiologically acceptable design range at all times. Whereas for a person who is unable to convert the glucose within the design range (e.g., a Type I diabetic), there is nonzero time-independent real complexity. Some of the FRs of biological systems undergo changes as a function of time, for example, energy requirements may diminish with age. An increasing number of humans develop Type II diabetes with aging. This may be a result of the system becoming one of time-dependent combinatorial complexity. If the time-dependent combinatorial complexity cannot be converted into time-dependent periodic complexity through periodic reinitialization (for example, with medication), the system may not survive.

Functional Periodicity

AD theory states that time-dependent combinatorial complexity can be mitigated by functional periodicity. Functional periodicity is imparted by periodic reinitialization and takes many forms. Examples of functional periodicity in everyday life include rebooting a computer, renewal periods for driver's licenses, and term-limits for elected officials. Functional periodicity does not necessarily have a regular temporal period. Functional periodicity may be created if there is a set of FRs that repeat on a regular basis.

An example that illustrates the importance of functional periodicity is in obtaining the optimal output from a series of robotic production systems that have similar but different throughput rates (Figure 5*A*, see color insert). System 1 produces semifinished part A at rate λ_1. System 2 takes part A to produce part B at rate λ_2. If λ_1 is 50 parts per hour and λ_2 is 60 parts per hour, then the supersystem of A and B ought to have an output that is the lesser of λ_1 and λ_2, or 50 parts per hour. However, in real situations the supersystem has a different output rate, λ_3, which is even less than λ_1 or λ_2.

Why is this? Because in real situations, systems rarely operate perfectly, hence the values of λ_1 and λ_2 vary owing to random variation (noise) and errors. These

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propagate downstream and result in suboptimal performance of the supersystem (6). From the perspective of AD, the fundamental problem is that the periodicity in this system depends upon the perfect operation of the subsystems. There is no explicit source of periodicity; rather, periodicity is a consequence of ideal operation. One approach to handling such errors is to make the queue time large; however, in this case, λ is even less than λ_3.

Robust systems can tolerate random variation (noise) and adapt to change, such as an error that might occur in a given cycle. Robustness can be conferred to this robotic supersystem by reinitializing the system on the basis of task completion of each subsystem. The task that initiates the next interval is intentionally delayed until the completion of the functional period. This ensures that the system achieves the same initial state at the beginning of each interval; hence the system becomes predictable and achieves its optimal throughput.

Periodicity is abundant in biological systems. Mammalian behavior typically follows a circadian cycle. The survival of many plant and animal species is dependent on an annual cycle. The rates of many biochemical functions, for example, glycolysis in yeast, oscillate with a regular periodicity (15). The work of Arthur Winfree (15) considers numerous examples of rhythmic behavior in biological systems. Recent work (2) has revealed periodic contractions of advancing lamellipodia during cell movement. The periodic contractions are caused by the periodic transport of a contractile signal from the tip to the back of the lamellipodia.

Could periodicity in biological systems function to provide robustness? Clearly, some periodic behavior in biological systems is not important for robustness but is instead a consequence of the system design. For example, the lag inherent in the negative-feedback regulation of bacterial operon confers periodicity to the transcription of the operon owing to the lag time activation of gene transcription and the negative-feedback signal. However, many biological systems rely on functional periodicity, as is evidenced by abnormal or chaotic behavior when functional periodicity is lost. For example, the mammalian cell cycle relies heavily on functional periodicity: If functional periodicity is lost or malfunctions, the cell either dies or becomes abnormal (cancer). Neuron depolarization relies on the reinitialization (polarization); the loss of this periodicity is seen in epilepsy. Sleep deprivation, studied extensively in humans, leads to suboptimal function of numerous systems.

The Role of Functional Periodicity in Reducing Complexity in the Cell Cycle

The mammalian cell cycle comprises a series of coordinated cyclic events. The manner in which these events are coordinated demonstrates a crucial role of periodicity and the chaotic consequence of breakdown of the periodicity.

In the process of cell division, it is critical that each daughter cell inherits exactly one full complement of DNA from the parent cell to survive and properly perform its functions. Critical events during cell reproduction are thus precise replication of

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its chromosomes and exact partitioning of the duplicated chromosomes into the two daughter cells. Partitioning of the duplicated chromosomes is coordinated by the centrosome, which has its own functional cycle for duplication that is independent of the chromosome cycle. Each of the two daughter cells receives one pair of centrosomes. Duplication of the centrosome occurs during S phase. As discussed below, functional periodicity ensures that the centrosome cycle is complete before mitosis begins.

Proper coordination of the centrosome and chromosome cycles is crucial. Errors in centrosome replication are an important cause of aneuploidy. If there are more than two centrosomes, missegregation of chromosomes is likely. A single centrosome induces the formation of monopolar spindles that do not properly direct cell division and can also cause abnormal segregation of chromosomes (5). Aneuploid daughter cells usually die owing to the lack of essential chromosomes. Occasionally, aneuploid cells survive and, rarely, can acquire a growth advantage over normal cells, i.e., become cancer cells (5). Centrosome aberration is common to most cancer types; extra centrosomes have been described in nearly all cancers that have been surveyed (8).

The chromosome and centrosome cycles can be experimentally dissociated during the rapid early nuclear divisions in the embryos (10). However, in normal conditions they are closely synchronized through the activity of cyclin-dependent kinase 2 (Cdk2), which triggers both centrosome duplication and DNA replication at the G1/S transition. This ensures one level of coordination between these two cycles by making the two cycles begin simultaneously. Prior to cell division the completion of the chromosome cycle is confirmed by the DNA replication checkpoint, which generates a molecular signal that DNA replication is complete. There may be a similar checkpoint mechanism to ensure completion of the centrosome cycle, but it has not yet been described. Cells may rely instead on an indirect mechanism to measure centrosome cycle completion, namely, the spindle attachment checkpoint. It prevents further cell cycle progress until all the chromosomes are properly attached to mitotic spindles. After mitosis and cell division, both the chromosome and centrosome cycles must return to their initial states so that newly generated daughter cells may begin their cell cycle with the same state. This is synchronized by three Cdk-inhibitory mechanisms that bring the activity level of Cdk to zero, reinitializing the cell cycle (and the subcycles).

The means by which the chromosome and centrosome cycles are coordinated are remarkably similar to the effect of imposing functional periodicity on the robotic manufacturing system described above (Figure 5*A*).

In the robotic scheduling example, a disturbance from one of the subsystems breaks down the inherent periodicity of the system. Because of the loss of periodicity, the system eventually yields suboptimal performance of the system. In the case of a cell, the loss of functional periodicity leads to cell death or cancer. This example supports the assertion that biological systems use functional periodicity to mitigate combinatorial complexity. The maintenance of periodicity is an important FR in the cell division cycle rather than a consequence of something else.

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MATHEMATICAL MODELING OF BIOLOGICAL SYSTEMS

Ultimately, we need to model biological systems mathematically to study various behaviors and to simulate the effect of various genes, drugs, and other external and internal variations of the system. Conceptually, the generation of a mathematical model is straightforward once the Design Matrix is created. By replacing Xs with mathematical functions that relate the effect of a particular DP to a given FR, the system behavior can be solved mathematically. Such a mathematical model will enable us to determine the system functions in terms of the molecular behavior in a quantitative manner, which will make biological science more definitive and provide missing links in understanding biological systems.

SUMMARY

The Design Matrix of AD provides a cross-scale framework for modeling biological systems. It provides a means of relating the system-level functions to molecular behavior of biological systems. It also clarifies how multiple interactions affect the same functions, some primarily and some secondarily. It also shows the problems created by coupled designs. Analysis of biological systems using the Design Matrix reveals that biological systems exploit modularity (uncoupled and decoupled designs) in order to achieve robustness. Rooted in function, the Design Matrix reveals control points, modules, and sources of robustness. Coupled designs, in which multiple modules must be simultaneously controlled, confer vulnerability (time-dependent combinatorial complexity) to biological systems. Functional periodicity can mitigate time-dependent combinatorial complexity. The ubiquity of functional periodicity in biological systems supports the notion that it is an important source of robustness in biological systems and not just a consequence of other primary principles.

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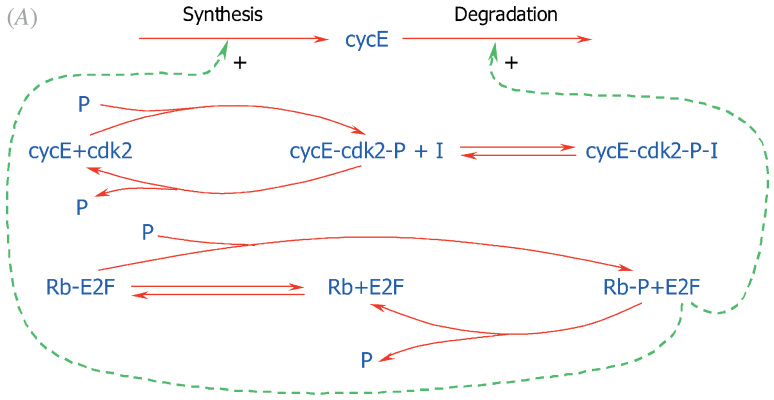
(A)



(B)

			DP0: G1/S Phase activity																	
			DP1		DP2			DP3		DP4				DP5						
										DP4.1										
			DP1.1	DP1.2	DP2.1	DP2.2	DP2.3	DP3.1	DP3.2	DP4.1.1	DP4.1.2	DP4.2	DP4.3	DP5.1	DP5.2	DP5.3	DP5.4	DP5.5	DP5.6
FR0: Prepare cell for duplication	FR1	FR1.1	X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FR1.2	0	X	0	0	0	0	0	0	0	0	0	0	0	0	X	0	X
	FR2	FR2.1	0	0	X	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FR2.2	0	0	X	X	0	X	X	0	0	0	0	0	0	0	0	0	0
		FR2.3	0	0	0	0	X	0	0	0	0	0	0	0	0	0	0	0	0
	FR3	FR3.1	0	0	X	0	0	X	0	0	0	0	0	0	0	0	0	0	0
		FR3.2	0	0	0	X	0	X	X	0	0	0	0	0	0	0	0	0	0
	FR4	FR4.1 FR4.1.1	0	0	0	0	0	0	0	X	0	0	0	0	0	0	0	0	0
		FR4.1 FR4.1.2	0	0	0	0	0	0	0	0	X	0	0	0	0	0	0	0	0
		FR4.2	0	0	0	0	0	0	X	0	0	X	0	0	0	0	0	0	0
		FR4.3	0	0	0	0	0	0	0	0	0	0	X	0	0	0	0	0	0
	FR5	FR5.1	0	0	0	X	X	0	0	0	0	0	0	X	0	0	0	0	0
		FR5.2	0	0	0	0	0	0	0	0	0	0	0	X	X	0	0	0	X
		FR5.3	0	0	0	0	0	0	0	0	0	0	0	0	X	X	0	0	0
		FR5.4	0	0	0	0	0	0	0	0	0	0	0	0	0	X	X	0	0
		FR5.5	0	0	0	0	0	0	0	0	X	X	0	0	0	0	X	X	0
		FR5.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	X	X

Figure 2 Continued on next page

(*C*)

		[DP2] Extracellular signal (Growth factor)			[DP3] Extracellular signal (mitogens)	
		[DP2.1] Growth factor receptor	[DP2.2] Extracellular growth factor pathway	[DP2.3] G1 cyclin inhibitory mechanism	[DP3.1] Mitogen receptor	[DP3.2] MAP kinase – myc (gene regulatory protein)
[FR2] Grow cell to maturity	[FR2.1] Detect growth factor	X	0	0	0	0
	[FR2.2] Stimulate protein synthesis	X	X	0	X	X
	[FR2.3] Suppress the cell cycle progression until it grows enough	0	0	X	0	0
[FR3] Monitor environment	[FR3.1] Detect mitogen	X	0	0	X	0
	[FR3.2] Increase the level of G1-cyclin	0	X	0	X	X

Figure 2 Traditional and AD representations of the G1/S transition in the mammalian cycle. (*A*) Regulation of cyclin E levels. Once cyclin E reaches a critical level, the G1/S transition occurs and the cell cycle is initiated. Red arrows indicate the flow of information, in this case through phosphorylation events that modulate the activity of substrate proteins and then influence cyclin E levels. Objects in blue represent proteins, protein complexes, and phosphorylated forms (Rb, retinoblastoma protein; cycE, cyclin E; I, inositol; P, phosphate; -P, phosphorylated; -P-I, phosphatidyl inositol). Green arrows illustrate how the complex of E2F and phosphorylated Rb impacts cyclin E levels. This diagram is from Reference 4. (*B*) The full Design Matrix, based on Reference 1, reveals a hierarchy of FR-DP relationships that codifies components based upon the function(s) they support. The full matrix includes multiple levels of decomposition and hence colors are used to track coupling; this Design Matrix shows that aspects of the G1/S transition are decoupled (*blue*), and other aspects appear to be coupled (*pink*). (*C*) Expanded view of FR2 and FR3. Because some of the signaling pathways are shared by both the growth factor receptors and the mitogen receptors, these aspects of the G1/S transition are coupled. In the Design Matrix, this is designated by Xs between FR2.2 and DPs 3.1 and 3.2. An expanded view of FRs 2 and 3 is shown in Figure 2*C*. FRs and DPs are as follows:

[FR0] Prepare a cell for duplication
[FR1] Form DNA replication machine
  [FR1.1] Mark the origin of duplication
  [FR1.2] Form Mcm helicase
[FR2] Grow cell to maturity
  [FR2.1] Detect growth factor
  [FR2.2] Stimulate protein synthesis
  [FR2.3] Suppress the cell cycle progression until it grows enough

(*Continued*)

(*Continued*)

- [FR3] Sense environment to determine go/no-go
  - [FR3.1] Detect mitogen
  - [FR3.2] Increase the level of G1-cyclin
- [FR4] Cause the cell cycle arrest if needed
  - [FR4.1] Arrest cell cycle in the event of DNA damage
    - [FR4.1.1] Prevent the cell cycle progression into M phase
    - [FR4.1.2] Prevent the cell cycle pregression into S phase
  - [FR4.2] Arrest cell cycle in the event of abnormal proliferation signals
  - [FR4.3] Stop and terminally arrest when needed
- [FR5] Initiate exit-G1 and entry-S
  - [FR5.1] Accumulate G1-Cdk
  - [FR5.2] Inactivate Rb
  - [FR5.3] Promote E2F protein
  - [FR5.4] Promote G1/S, S-cyclin synthesis
  - [FR5.5] Activate "some" of G1/S, S-Cdk
  - [FR5.6] Inactivate Cdk inhibitory mechanism [DP0] (Events during) G1 phase
- [DP1] Pre-RC forming mechanism
  - [DP1.1] ORC-origin interaction
  - [DP1.2] Cdc6 mechanism
- [DP2] Extracellular signal (growth factor)
  - [DP2.1] Growth factor receptor
  - [DP2.2] Extracellular growth factor pathway (PI 3-kinase)
  - [DP2.3] G1-cyclin inhibitory mechanism
- [DP3] Extracellular signal (mitogens)
  - [DP3.1] Mitogen receptor
  - [DP3.2] MAP kinase—Myc (gene regulatory protein)
- [DP4] Cell-cycle inhibitory mechanism
  - [DP4.1] DNA damage checkpoints (G1 and G2)
    - [DP4.1.1] Signal from damaged DNA for Cdc25 phosphorylation (inactivation)
    - [DP4.1.2] Signal from damaged DNA to initiate p53-dependent mechanism
  - [DP4.2] p19ARF _ p53 mechanism
  - [DP4.3] Intracellular stopping mechanism
- [DP5] G1-Cdk-related mechanism (deactivation of Cdk-suppressing mechanism; S1-Cdk activation pathway)
  - [DP5.1] Available G1-cyclin
  - [DP5.2] G1-Cdk
  - [DP5.3] Inactivation of Rb
  - [DP5.4] E2F
  - [DP5.5] G1/S, S-cyclin
  - [DP5.6] G1/S, S-Cdk.

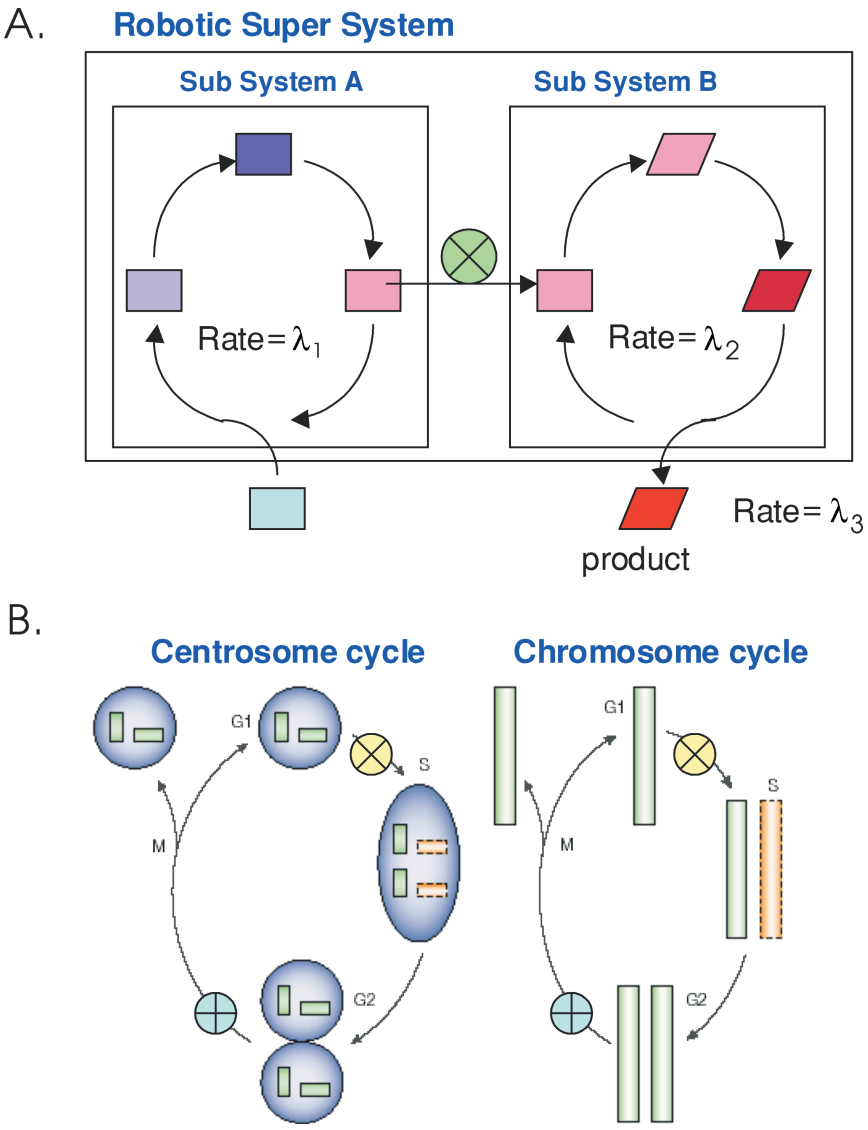


Figure 5 Functional periodicity in manufacturing and the cell cycle. (*A*) Functional periodicity, indicated by the cross in circle, imposes the requirement that subsystem A complete a cycle before subsystem B initiates a cycle, thereby ensuring the shortest interval for product completion (λ_3). (*B*) Functional periodicity imposed on at least two points in the cell cycle ensures the proper synchronization of the chromosome and the centrosome cycles. Functional periodicity is imparted by CDK2 (*cross in yellow circle*) and by the spindle attachment checkpoint (*cross in blue circle*).