MODULARITY AND RELIABILITY IN THE ORGANIZATION OF ORGANISMS

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An organism persists only if it satisfies internal and external constraints. Within the organism networks of processes meet the constraints. In such networks a principle of matching often obtains: The pattern of coupling among processes matches the correlation among constraints. That is, a module -- a cluster of coupled processes -- meets a constraint. Dissociable modules meet dissociable constraints. A hierarchy of modules meets a hierarchy of constraints. We have inquired whether such matching is predicted by an optimality criterion in a simple example. We find that in an ensemble of networks with unreliable processes, the networks that meet the constraints with highest reliability obey the principle of matching. The difference in reliability between modular and nonmodular networks that meet the same constraints is a function of the probability of success per process. Our results suggest that this difference is maximal at a probability of success that increases monotonically with the number of processes in the network.

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; NADH, reduced nicotinamide adenine dinucleotide; NADPH, phosphorylated NADH.

1. Introduction.

Organisms characteristically show structure-function matching. Usually such matching refers to the competence of a physical structure such as the gut or the heart to perform actions that meet constraints. However, a kind of structure-function matching also characterizes networks of processes. Several observers (*e. g.* Schmalhausen, 1949; Olson and Miller, 1958; Simon, 1962; Riedl, 1978, p. xix; Bonner, 1988, p. 175; Gelernter, 1989, p. 69) have noted an empirical principle of matching: The structure of a network -- the pattern of coupling among its processes -- tends to match the correlation among constraints that the processes meet. Evidence gathered elsewhere (Mittenthal, in preparation) suggests that this kind of network matching operates at levels of organization from molecular circuits to organ systems and characterizes much of the organization in development, physiology, and evolution.

We have investigated the hypothesis that obedience to the principle of matching gives a high probability that a network of coupled processes meets constraints reliably. (Evolution favors a high probability of meeting constraints but does not require optimality.) We shall show that in a simple model problem the hypothesis is valid: The networks that meet the constraints with greatest reliability obey the principle of matching. The mathematical methods used in this inquiry extend methods used in the engineering analysis of reliability (Barlow and Proschan, 1981); our methods can be used to evaluate the reliability of networks of coupled processes more generally.

1.1. Examples of network matching.

The following example illustrates network matching and suggests a rationale for its occurrence. In vertebrates the pattern of arterial supply often matches the pattern of functional demand in distributed systems, in which several centers cooperate to perform a function. For example, the muscles of the body wall (and of its outgrowths, the limbs) cooperate to move the body relative to the environment. Also, several gastrointestinal organs cooperate in digestion. Over a brief period, on a time scale of minutes, these constraints are dissociable; the intensity of digestion is only weakly correlated with the intensity of external movement. (On a time scale of days the correlation is strong; we must digest food to continue moving.) Corresponding to the short-term dissociability of constraints, the supplies of blood to the body wall and the gut are partially dissociable; separate sets of arteries supply these structures. The body wall receives blood through arteries that extend into the arms and legs and through the dorsal segmental arteries, whereas the gut is supplied by a separate set of visceral arteries (Netter, 1989). The principle of matching posits this matching between the correlation among constraints (digestion, external movement) and the coupling among processes that meet the constraints (activity of the gut and the body wall, including their arterial supply). Several other cases are known in which a set of arteries supply

functionally cooperating centers but do not supply adjacent noncooperating centers (review: Oldendorf, 1990).

Further consideration shows that activity of the gut and the body wall are part of a hierarchy of modules that meet a hierarchy of constraints (Fig. 1). A module is a cluster of coupled processes; it is activated by few inputs, and it meets a constraint that is largely dissociable from other constraints. (Constraints A and B are dissociable if either can be satisfied independently of the other.) At the highest level in the hierarchy there is one module, the entire organism. It meets the constraint that organisms must survive and reproduce if their lineage is to persist. Satisfaction of this constraint implies other constraints that are met by modules at lower levels. These modules include a gut module that meets the constraint of processing food and a wall module that moves the organism in its environment.

The gut module and the wall module present constraints to other modules, the outputs of which they require as inputs. Some of these inputs are particular to each module; for example, the gut module uses food which an ingestion module provides. The gut and wall modules present constraints to the neural and circulatory systems; both modules require neural control and blood. However, the activities of the gut and body wall modules are dissociable on a short time scale. Hence by the principle of matching, the supplies of neural control and blood to the gut and wall modules are probably dissociable. Evidently these modules cluster into higher-level modules: A somatic module includes activity of the body wall, somatic neural control, and somatic blood supply. A visceral module includes activity of the gut, visceral neural control, and visceral blood supply. Thus the matching of processes to constraints occurs throughout a hierarchy of modules.

It is important to note that here a module is a cluster of processes, not a physical structure. A module may represent the operation of a localized structure such as the heart, of a distributed but connected structure such as an arterial tree, or of a set of disjoint structures such as co-acting muscles.

Matching extends from the upper levels of the hierarchy of processes to the molecular level. The supply of reducing agents to catabolic and biosynthetic processes within an individual cell is analogous to the supply of blood to the gut and the body wall. Catabolic and biosynthetic pathways meet dissociable constraints, and they can be regulated dissociably because in general they use different reducing agents: Typically catabolic pathways use NADH, whereas biosynthetic pathways use NADPH (Alberts et al., 1989, p. 77). Other modules at the molecular level mediate replication, transcription, translation, and developmental and physiological cascades.

Thus the coupling among processes matches the correlation among constraints at diverse levels of organization. For constraints that are in effect synchronously, dissociable clusters of processes meet dissociable constraints, whereas coupled clusters meet correlated constraints.

1.2. A rationale for network matching. Why should network matching be common? Consider alternative ways to supply the body wall and the gut with blood. Both regions might receive blood from the same arteries. Such a shared supply might be expected if all cells were supplied by arteries traveling roughly the shortest distance from the heart, as leaves of a tree are supplied by branches. In a tree-like supply arteries supplying the gut would extend into the abdominal body wall. This does not occur. Rather, the supply in the trunk is modular: The gut and the body wall receive blood from different sets of arteries.

The modular supply allows an organism to survive in more diverse circumstances than the shared supply. Suppose one of the two systems is active and elicits an increased blood supply to its centers. The modular supply can provide more blood to the active system while the supply to the inactive system is unchanged or reduced (Burton, 1972; Mchedlishvili, 1986). Thus during digestion the flow of blood is increased to the intestines and reduced to the muscles of the limbs; during exercise this pattern of flow is reversed. With a shared supply either system can receive more blood only if both do. (For simplicity, this argument neglects regulation of blood flow by physiological processes that supplement arterial anatomy.) Thus the modular organization of blood supply allows a limited resource (cardiac output) to be distributed in diverse ways that meet the demands of function. This allows the organism to survive in more diverse environments.

The same argument applies on an evolutionary time scale: Suppose that as a lineage of organisms evolves, one of two distributed systems enlarges more than the other. (The increased development of hearing relative to vision in bats is an example.) A modular supply can accommodate the increased demand by enlarging one of the arterial trees. A shared supply must enlarge both trees, and increase the demand for cardiac output correspondingly.

In summary, modular arterial supply is a special case of modular organization, in which a network of processes contains functional modules. Each module is a cluster of coupled processes that meets a constraint. Within an organism collections of modules form higher-level modules that meet higher-level constraints.

In the following section we present a simple model for coupled processes that meet a hierarchy of constraints. The analysis shows that a network consisting of a hierarchy of functional modules has a high probability of meeting the constraints.

2. Model.

We propose a simple model of network organization and consider some biologically plausible alternative sets of constraints that the networks might meet. Suppose that two centers, A and B, cooperate to perform function AB. Likewise, C and D cooperate to perform CD. The overall constraint may be that AB and CD are to be performed dissociably, in various combinations: At some times only AB is appropriate; at other times only CD without AB meets the constraints; and sometimes AB and CD together are suitable. This constraint approximates the requirement that the network increases the variety of circumstances in which an organism can meet constraints. Alternatively, perhaps AB and CD are to be performed jointly, to perform the larger function ABCD; this is a hierarchy of constraints.

These requirements are an abstraction of the constraints on arterial supply. Rather than dealing with the complexities of this example, we treat a simpler alternative interpretation of the ABCD problem in terms of molecular processes, as discussed preliminarily by Mittenthal (1989).

2.1. Assumptions of the model.

In the molecular interpretation A, B, C, and D can be regarded as proteins (monomers) that can associate to form heterodimers AB and CD, which can aggregate to form a tetramer ABCD. A network of genes synthesizes the monomers. Each gene has an input region containing cis-regulatory elements (promoters, enhancers; here called cis elements), and an output region that codes for a protein. Structural genes synthesize the four monomers. A regulatory gene synthesizes a protein that is a messenger, a transcription factor that can bind to one kind of cis element and activate synthesis of a protein.

Two genes are coupled if one produces a messenger that binds to a cis element of the other. A messenger may activate the synthesis of more than one protein. If two or more genes are coupled, they form a network; the pattern of coupling specifies the topology of the network.

We assume that each network occurs in a spatial unit, and networks in different units do not interact. That is, messengers or monomers produced in one network are not available to other networks. The assumption that individual networks are independent simplifies the analysis. This assumption is plausible because the dispersion of intermediate products in a network is often restricted by membrane boundaries. Or, macromolecules often associate to form a multicomponent complex that mediates an entire network of processes. In the ABCD model the messengers are nuclear transcription factors, produced by single copy genes; these factors are likely to remain in the cell where they are produced. Modifications of the analysis would allow treatment of interacting networks.

In our model time occurs in discrete steps. In each time step each gene either synthesizes one protein molecule or none. Each protein only persists for a specified number of time steps after it is synthesized. The lifetimes of the dimers must also be specified. If the monomers that make a dimer are present simultaneously, they combine instantly. Likewise AB and CD combine instantly to make ABCD. The tetramer is removed as it is produced.

These assumptions represent simplifications that should be explained. Here all processes occur in one cell; there are no intercellular messengers. We ignore cytoplasmic messengers, the machinery of protein synthesis (including messenger RNA), and the distinction between nucleus and cytoplasm. We assume that any input to a gene stimulates synthesis of its protein; the inhibitory interactions that occur among real genes are neglected. We assume no feedback; a protein can not bind to cis elements in any of the genes leading to its production. Here each gene has only one cis element, whereas a real gene may have several cis elements. Because there is no feedback and each gene has only one cis element, each network that synthesizes monomers has a tree-like structure.

Here each gene can only synthesize one type of protein, whereas a real gene may synthesize alternative proteins, depending on the combination of messengers it has bound. In our model production of a protein is all-or-none; a real gene may synthesize protein at various rates, depending on the combination of messengers it has bound. Binding, synthesis, and degradation of proteins are likely to be stochastic processes in continuous time, rather than processes occurring in discrete time steps.

In reality the input-output relation of a gene may change during evolution as the sequence of nucleotides in it changes, altering cis elements and coding regions. Here we are not concerned with evolutionary changes, but with the reliability of processes within the lifetime of an organism.

The preceding assumptions are made for convenience; they represent simplifications of the biological complexity rather than limitations on the mathematical techniques. However, real chemical processes occur in continuous time. Our use of a discrete time approximation is a limitation on the mathematical technique since discrete time stochastic processes do not always converge to continuous time stochastic processes as the time step decreases.

We assume that the control of gene expression is a stochastic process: In a time step a gene does not always synthesize a functional protein (monomer or messenger) in response to a messenger for which it has a cis element. The messenger might fail to bind to its cis element, or a messenger-cis element complex may fail to stimulate synthesis of a protein. Kirkwood *et al.* (1986) discuss some of the aberrations of gene expression and its reliability. In our model, on receipt of its input each gene succeeds or fails in synthesizing a protein independently of

other genes. We assume that every gene has the same probability of success, p. This assumption is convenient though not essential.

Because genes are coupled together the operation of a network as a whole is a stochastic process. We are modeling this process as a Markov chain with discrete states, operating in discrete time; therefore, with exceptions not encountered here, it converges to a unique stationary distribution. That is, asymptotically, the probability that the Markov chain is in a particular state is independent of the initial state. We refer to the stationary distribution as the stochastic steady state. The reliability of a network is defined as the probability that in the stochastic steady state the network meets the constraints imposed on it. For example, the making of AB is a possible constraint; the constraint is met if the probability of making AB is appreciable. Our aim is to find the networks that meet constraints with high reliability.

To this end we consider an ensemble of networks that can synthesize the four monomers but that differ in topology. We proceed in two stages. We first display the complete ensemble of minimal networks. Each branch of a minimal network depends on only one gene. As we shall see, most minimal networks can not synthesize all four monomers in synchrony, because paths from an initial messenger to different monomers involve different numbers of genes. To allow synchronous synthesis of monomers in every network we analyze phased networks, in which more than one gene is allowed per branch.

2.2. Minimal networks. Fig. 2 shows the ensemble of 52 minimal networks. Four independent inputs could elicit synthesis of the four monomers directly in only one way (network #1, Fig. 2). Three inputs could elicit the synthesis directly if one input stimulated synthesis of two monomers. This could occur in six ways (#2 - #7), since any two of the four syntheses could be coupled. Two inputs could stimulate the four syntheses directly in seven ways (#8 - #14). However, an input might stimulate syntheses by activating two or more processes in sequence. By such indirect paths two inputs could produce monomers in twelve ways (#15 - #26). One input could stimulate the syntheses in 26 ways (#27 - #52), directly or through one or two intermediate processes.

Only certain networks allow the synthesis of A and B to be dissociated from the synthesis of C and D. In these five networks (#1, 2, 4, 12, 32) the presence of particular messengers will elicit synthesis of AB but not CD, CD but not AB, or AB and CD. The most reliable of these networks are #12 and #32; they only require the arrival of two initial messengers, one to activate a module that synthesizes A and B and another to activate a module that synthesizes C and D. In each of these modules two structural genes synthesize two monomers in response to one messenger. The monomers then associate to form a dimer. The least reliable of these networks is #1; it requires four initial messengers that evoke independent production of all four

monomers. One may expect that under natural selection a network that works with high reliability will tend to evolve; in the problem with synthesis of AB and CD dissociable, networks #12 and #32 with modular organization meet this constraint most reliably.

The network may be constrained to make ABCD in response to a single input. AB and CD then can not be produced dissociably. If messengers and monomers only last one time step then most minimal networks can not make ABCD in response to a single stimulus, because the four monomers are not made synchronously; the monomers made earlier will be gone by the time the remaining monomers are synthesized. Allowing more than one gene per branch in a network solves this problem, as we now show.

2.3. Phased networks. We define phased networks as follows. Suppose all messengers and monomers have a lifetime of one time step. In a phased network a single messenger called "start" activates synchronous synthesis of the four monomers through a branching network of regulatory genes. As Fig. 2 shows, the minimal networks have at most three genes in series. In a phased network every path from start to a monomer contains three genes in series and so requires three time steps to traverse. A generic path from start to a monomer can be represented as in Fig. 3a. In a phased network each gene is at one of three levels; at each level there may be as many as four genes.

Each of the 52 minimal networks can be converted to one or more phased networks. Only networks 41 - 52 convert to unique phased networks. The other minimal networks may be converted to two or more phased networks; Figs. 3b-e show some phased analogs of networks 27, 32, 29, and 33. If there are several phased analogs of a minimal network the analog having the fewest processes will have the highest reliability. We call this analog the natural analog, and we use it without further comment.

As just assumed, a monomer has a lifetime of one time step. If the lifetime of a dimer is also one time step the reliability of a network with N genes is p^N, independent of the coupling among genes: In response to an initial messenger all four monomers are synthesized if and only if each synthesis of a messenger or monomer succeeds. However, if the lifetime of a dimer is at least two time steps then the reliability of a network depends on the coupling among genes as well as on the number of genes. This is so because, dimers made at different times can combine to form a tetramer. Therefore, as the dimer lifetime increases the reliability of a network increases.

Given the lifetimes of the molecular species, the reliability of the phased networks decreases monotonically with the number of genes in the network. Therefore we shall only consider the relative merits of the networks with fewest processes, which are the most reliable

networks. The minimum number of genes, six, occurs only in the analog of network 27 (Fig. 3b, top). In network 27 a single messenger elicits all four syntheses of monomers directly. This will be called a shotgun pattern of activation, since it resembles the cluster of shot emerging from a shotgun. The analogs of several networks (#28-31, 33, 34) have seven genes. Figs. 3c, d, e (top) show the analogs for networks 32, 29, and 33. All other networks have more genes, and we shall not consider them further.

The Appendix shows how Fig. 4 was generated. For a dimer lifetime of two time steps Fig. 4a shows the reliability of the natural analogs of networks 27, 29, 32, and 33 as a function of p, the probability of success. In this case the shotgun network (#27 analog) is most reliable. The modular network (the analog of #32) is more reliable than the other networks that use seven processes. The latter networks are not modular: All messengers that stimulate syntheses of monomers must be produced in the same time step to produce either dimer. However, in the modular network AB can be produced in the absence of CD but can survive long enough to combine with a CD produced in a later time step. The pseudomodular networks #28-31 perform better than the nonmodular networks (#33, 34), but not as well as the modular ones.

We have not calculated the reliability of networks in which the monomer lifetime is greater than one. However, qualitatively it is clear that longer-lived monomers made at different times could interact to produce dimers. Such interaction would increase the reliability of the pseudomodular and nonmodular networks toward the reliability of the shotgun network.

Fig. 4b shows the difference in reliability between the modular network #32 and the nonmodular network #33. This difference is the improvement in reliability achieved by a network with modules that meet constraints over the most reliable network that lacks such modules.

A minimal network uses fewer genes than its phased natural analog. Results for minimal networks analogous to those in Fig. 4 (not shown) indicate that the maximum difference between the modular and nonmodular networks occurs at a lower value of p for the minimal networks than for the phased networks. These results suggest a conjecture: If molecular complexes formed in different time steps can interact, the difference in reliability between modular and nonmodular networks that meet the same constraints is maximal at a probability of success that increases monotonically with the number of processes in the network. If this conjecture is valid, then in real biological networks having many processes, the increase in reliability associated with modular organization may be maximal at the high probabilities of success that are characteristic of molecular processes. The accuracy of molecular processes can be remarkable; for example, in replication of DNA roughly one incorrect base pairing occurs per billion base pairs replicated (see Kirkwood *etal.*, 1986).

4. Discussion

Within organisms the coupling among processes that meet constraints often matches the correlation among the constraints. Such matching may increase the reliability with which networks of processes meet constraints; we have investigated this possibility with a simple model. A network of genes synthesizes the monomer proteins A, B, C, and D. In each gene a messenger protein binds to a cis-regulatory element and, with imperfect reliability, activates the synthesis of a messenger or monomer protein. The monomers associate to form the dimers AB and CD, which form the tetramer ABCD. Diverse networks of coupling among genes are compatible with these constraints. To evaluate the reliability of a network from the reliability of gene expression we modeled the operation of a network as a Markov chain.

4.1. The reliability and structure of networks. The most reliable network activates the synthesis of all four monomers directly with a single messenger, in a shotgun pattern of activation. The next most reliable network consists of a hierarchy of functional modules; a module is a cluster of coupled processes that meet a constraint. A module activates synthesis of each dimer: One messenger activates the synthesis of two monomers (A and B, or C and D) that then associate to form a dimer. In a higher level module a messenger activates these two modules, stimulating the formation of a tetramer. This modular network has higher reliability than the two nonmodular networks with the same number of genes, if dimers formed in two different time steps last long enough to form a tetramer. Pseudomodular networks have reliability intermediate between the modular and nonmodular networks. A pseudomodular network has a partially modular structure that couples processes forming one dimer but not the other. All other networks that can synthesize a tetramer use more processes and are less reliable than the preceding networks.

Our results raise several issues that bear discussion. First, as Fig. 4 shows, the difference in reliability among the most reliable networks (shotgun, modular, pseudomodular, and nonmodular networks) is small. However, such small differences are likely to have a large cumulative effect on the reliability of higher-level processes. The network of processes in an organism is a hierarchy in which molecular processes such as the synthesis of the ABCD tetramer are at a low level. In a high-level process such as morphogenesis or a neuroendocrine response, many low-level processes occur in series and in parallel. A slight change in the reliability of low-level processes can be amplified in the reliability of the high-level process; for example, this occurs for low-level processes in series. Such amplification can increase with the number of low-level processes (Barlow and Proschan, 1981).

Second, note that the shotgun and modular networks both show a matching of coupling to constraints, and they are the two networks with highest reliability. In fact, the shotgun network

is a degenerate case of the modular network: In the modular network one messenger elicits synthesis of A and B, and a different messenger elicits synthesis of C and D. The shotgun network has the same topology, but these two messengers happen to be identical. Because it is a degenerate modular network, a shotgun pattern of activation will always use fewer processes than an explicitly modular pattern that meets the same constraints; hence the shotgun pattern will be more reliable. Thus in the ABCD model modular organization and a matching of coupling to constraints are associated with high reliability.

4.2. Relevance of the ABCD problem to modules and matching more generally. The ABCD problem offers a useful framework for generating hypotheses and developing methods to investigate the evolution and reliability of networks in other cases. The model shows that a hierarchy of modules can meet a hierarchy of constraints with high reliability. The mathematical methods used in the analysis can be used to calculate the asymptotic reliability of any network of unreliable processes operating in discrete time with a finite number of states. By dealing with repeated responses of a network in which the outputs of different responses can interact, the analysis goes beyond studies of the reliability of individual responses (*cf.* Barlow and Proschan, 1981). However, the task of characterizing properties of classes of stochastic networks, rather than analyzing individual networks, largely remains to be accomplished.

Our analysis suggests some cautionary notes for those who analyze other molecular networks. In the modular network for the ABCD problem the pattern of processes leading to synthesis of the monomers is mirror-symmetric to the pattern of associations among monomers that leads to ABCD; the line of mirror symmetry passes through the monomers. This mirror symmetry occurs because all the constraints are imposed after the monomers are synthesized, whereas the alternative networks of processes operate before the syntheses. In more typical situations where constrained structures and alternative networks are intermingled, as in Fig. 1, simple patterns of symmetry should not be expected in modular networks.

One should also note that in contrast to the networks that make ABCD, real molecular networks meet many constraints. One might think that avaoidance of waste is a significant constraint. The importance of this constraint is unclear; many biological processes seem to utilize resources inefficiently (*e.g.* Cavener, 1989). Requirements for the performance of specific actions (such as synthesizing proteins) may be self-evident, but other constraints may not. Such background constraints include limitations on the resources allocated to each process, and maintenance of plasticity that allows the lineage to respond to variations in the environment and in the organism. As Cavener (1989, p. 463) has emphasized, "... the potential harm that [a gene product] may be causing and the cost to make it when it is not needed must be weighed against the evolutionary opportunity of adding more- complex controls and the day-to-day cost

of providing that control." Analyses of the structure and reliability of molecular networks should take the diverse possible constraints into account to generate biologically interesting results.

APPENDIX

Here we give the mathematical details behind the results stated in the paper. We compare the probability of successfully making a tetramer in the shotgun, modular, pseudomodular and nonmodular phased networks. Recall the assumptions: A network of unreliable processes makes monomers A, B, C, D; these associate to form dimers AB and CD, which associate to form the tetramer ABCD. These associations occur instantaneously when the components are present. Time passes in discrete steps. At each time step a messenger arrives with certainty at the first process so as to trigger the network and thereby initiate the synthesis of the monomers. For simplicity we take the conditional probability that a process is successful, given that its predecessor was successful, to be the same for all processes and denote it by p. When $p=1$ the network works with certainty; when $p=0$ it is certain that no part of the network makes anything. Between these two extremes the operation of the network is probabilistic.

We assume that the monomers last one time step; the dimers last two time steps. It is easy to see that if the dimers last only one time step then the reliability is a function of the number of processes coupled together. In this case calculations similar to those presented below show that although the modular (#32) and nonmodular (#33) networks produce ABCD with the same probability, the modular network produces each dimer with higher probability than the nonmodular network does. As we shall see, with dimer lifetimes of two time steps the modular network also produces the tetramer with higher reliability.

The operation of the network is described by a first order Markov chain. (For treatment of Markov chains see Doob, 1953; Hoel et al., 1972.) We compare networks by calculating the probability of making the tetramer under the stationary distribution. It is well known that positive recurrent, aperiodic, irreducible discrete time Markov chains converge to their

stationary distributions; see Theorem 7, page 73, Hoel *et al.* (1972). The positive recurrence is always true in finite state Markov chains. In addition, in all the Markov chains used here, the irreducibility is satisfied because there is a sequence of transitions from any one state to any other state which occurs with strictly positive probability. The aperiodicity is satisfied in all four classes of networks because for each chain one can identify a state which leads to itself in one transition with positive probability. The uniqueness of the stationary distribution holds under the same hypotheses; see Theorem 5, page 64, Hoel *et al.* (1972). As a result, the technique we use here can be applied to any network, no matter how complex, that can be represented as a Markov chain with stationary transition probabilities that satisfies these conditions.

To calculate the stationary probability of making ABCD we find the transition matrices. This requires that we have a list of the possible states of the Markov chain, with the allowed transitions and their conditional probabilities. The possible states of the Markov chain are the possible states of the network. These states depend on processes in the present time step and in the preceding three time steps. To specify the state of the network we must know in each time step whether each gene has been activated, whether each dimer is present, and if so how old it is. As Table 1 shows, the shotgun and nonmodular networks can be regarded as Markov chains each having 48 states, which we number 1 through 48. The modular and pseudomodular networks can be regarded as Markov chains having 96 states which we number 1 through 96. The transitions and conditional probabilities associated with each network can be derived by inspection from the states. The major difficulty is bookkeeping since there are so many states and possible transitions. This results from the fact that the possible states must represent all possible outcomes of all processes within four time steps.

We denote the transition matrices for the shotgun, modular and pseudomodular and nonmodular networks by T_{shot}, T_{mod}, T_{pseud}, and T_{non} respectively. The dimension of each matrix is the number of states in the Markov chain for the network. The matrices can be calculated explicitly. To see the calculation of sample elements, consider first the phased analog of the modular network, #32 (Fig. 3c, top). We examine the possible transitions from an initial state in which only the level 1 process has been successful. In the next time step the level 1 and level 2 genes all have bound messengers. The probability of transition to the state in which all three genes have produced their products is p^3; similarly, the probability that all three genes fail is $(1-p)^3$. The probability that any one of them works - and there are three such possibilities - is $p(1-p)^2$; the probability that any two work is $p^2(1-p)$ and again there are three possibilities.

Because the outcomes of level 3 processes are combined according to their success or failure in producing dimers, these processes only contribute to the transition probabilities

through the conditional probabilities of making dimers. For the nonmodular network these conditional probabilities are

$$P \text{ (no dimers)} = (1-p)^4 + 4p(1-p)^3 + 4p^2(1-p)^2,$$

$$P \text{ (AB only)} = P \text{ (CD only)} = 2p^3(1-p) + p^2(1-p)^2,$$

$$P \text{ (ABCD)} = p^4.$$

For the shotgun network note that we have essentially the same states as with the nonmodular network: The success at level 2 that occurs with probability p^2 in the nonmodular network is replaced by success with probability p for the shotgun network, because only one process need be successful for ABCD to be made. Thus, we derive T_{shot} from T_{non} by changing all p^2's to p's when they arise from a transition from level 1 to level 2. The pseudomodular network has the same number of states as the modular network but different transition probabilities. The transition probabilities for making the dimers are as in the nonmodular case; the rest are much like the modular case.

There are now four stationary distributions, one for each transition matrix. We denote them

$$\Lambda_{shot} = (\lambda_{shot,1}(p), ..., \lambda_{shot,48}(p)),$$

$$\Lambda_{mod} = (\lambda_{mod,1}(p), ..., \lambda_{mod,96}(p)),$$

$$\Lambda_{pseud} = (\lambda_{pseud,1}(p), ..., \lambda_{pseud,96}(p)),$$

$$\Lambda_{non} = (\lambda_{non,1}(p), ..., \lambda_{non,48}(p)).$$

The components of each stationary distribution are the entries of the eigenvector corresponding to eigenvalue 1 for the corresponding transition matrix. These components can be found by solving the equations

$$T_{shot}(p)\Lambda_{shot}(p) = \Lambda_{shot}(p),$$

$$T_{mod}(p)\Lambda_{mod}(p) = \Lambda_{mod}(p),$$

$$T_{pseud}(p)\Lambda_{pseud}(p) = \Lambda_{pseud}(p),$$

$$T_{non}(p)\Lambda_{non}(p) = \Lambda_{non}(p),$$

using the fact that their entries are positive and sum to unity. It is not the stationary distributions that interest us, but rather the reliability of each network -- its asymptotic probability of making ABCD. We denote these reliabilities by R_{shot}, R_{mod}, R_{pseud} and R_{non}. They can be calculated from the entries of the corresponding stationary distributions by summing the probabilities of the states of the network that result in making ABCD.

The stationary probabilities in all four cases were found numerically by using the software package Mathematica (Wolfram, 1988) so as to generate the graphs in Fig. 4.

We conclude that among all phased networks arising from the minimal networks the shotgun network has the highest reliability, followed by the modular network, the pseudomodular network, and the nonmodular network.

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FIGURE AND TABLE CAPTIONS

Figure 1. A greatly simplified hierarchy of modules involved in digestion and movement. A box encloses each module, words within a box indicate the process it performs, an arrow on the right points to the constraint it meets, and stalks on the left show inputs to it.

Figure 2. The 52 minimal networks. Each network is a directed graph in which a branch represents a process (gene expression) which proceeds from left to right. Only #27 shows the constraints explicitly: Once monomers have been made they must combine to form dimers AB and CD before forming the tetramer ABCD. The minimal networks that allow the synthesis of A and B to be dissociated from the synthesis of C and D are numbered 1, 2, 4, 12, 32. The minimal networks that can make ABCD in response to one input are numbered 27 (shotgun, s); 32 (modular, m); 33 and 34 (nonmodular, n). The pseudomodular (p) minimal networks (#28-31), and the remaining minimal networks, can not make ABCD because they do not produce all four monomers synchronously in response to one input.

Figure 3. Phased networks. (a) A generic path from start to a monomer. Each horizontal line represents a gene; the line segment to the left of the vertical bar represents the cis element, while the right segment represents the output region. (b-e) Phased analogs of minimal networks 27, 32, 29 and 33. In each case the natural analog is on top. (b) #27, the shotgun networks with the fewest processes (6) and with the most processes (12); other analogs have intermediate numbers of processes. (c) #32, modular networks. (d) #29, the pseudomodular networks in which A and B (and C) are coupled, but C and D are not directly coupled. (e) #33, nonmodular networks. (For #34 the corresponding networks are mirror images obtained by reflection across the dashed lines.)

Figure 4. The reliability R of networks for synthesizing ABCD, as functions of the probability p of success. Monomer lifetimes = 1; dimer lifetimes = 2. (a) R vs. p for shotgun, modular, pseudomodular, and nonmodular phased networks, for the natural analogs in Figs. 3b-e. (b) The curve shows the difference in reliability for the modular and nonmodular networks, from (a). The maximum difference occurs at $p = .85$ approximately.

Table 1: Alternative states of activity and products for the shotgun, modular, pseudomodular and nonmodular networks. Rather than listing all 16 possible outcomes of structural gene

activation at level 3 (success or failure of each of the 4 structural genes), we have combined these outcomes according to their success or failure in producing dimers. An analogous condensation has been done at level 2 for the nonmodular network.

FIGURE 1

FIGURE 2

1   2   3   4   5   6

7   8   9   10   11   12

13   14   15   16   17   18

19   20   21   22   23   24

25   26   27, s

28, p   29, p   30, p   31, p

32, m   33, n   34, n
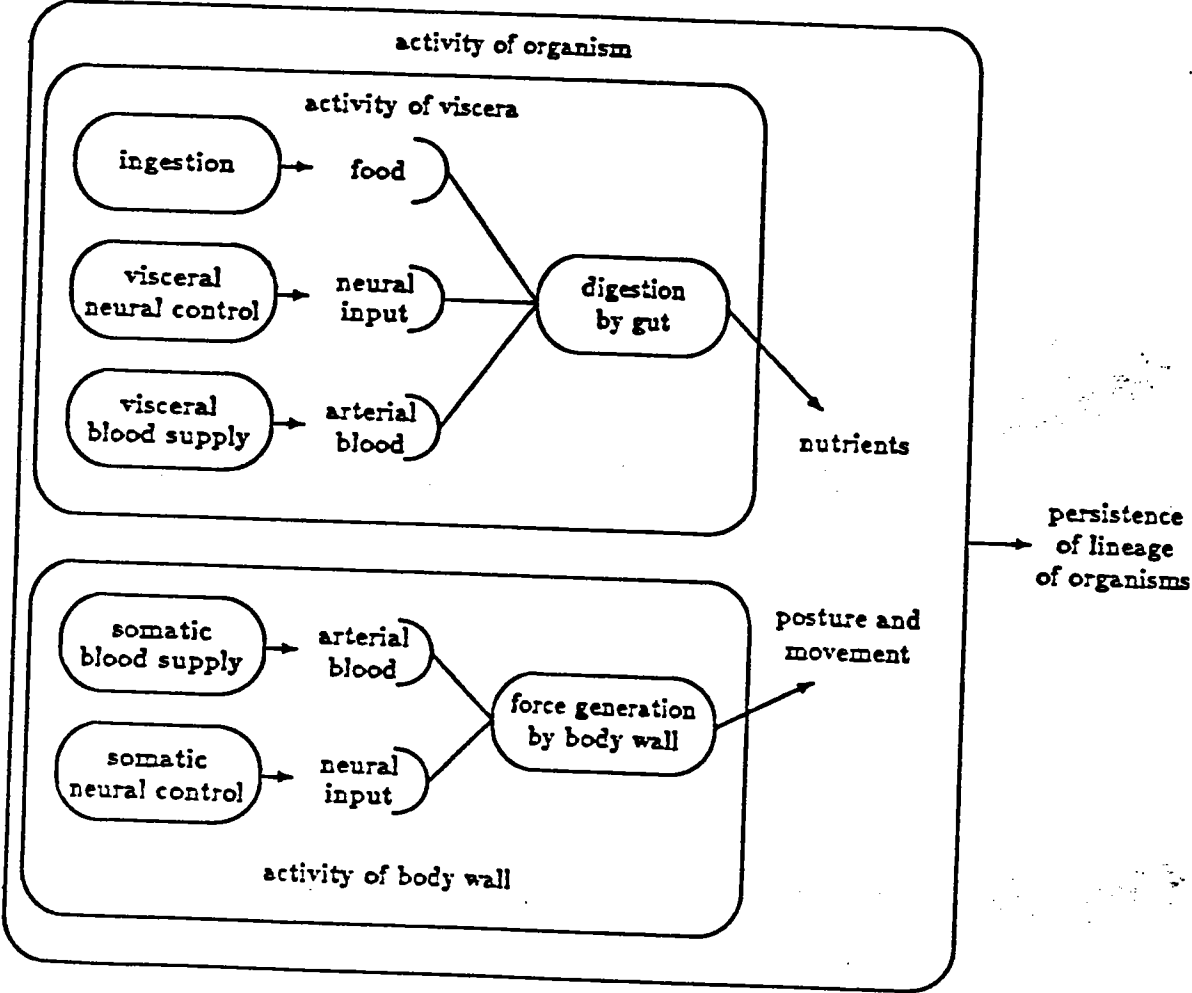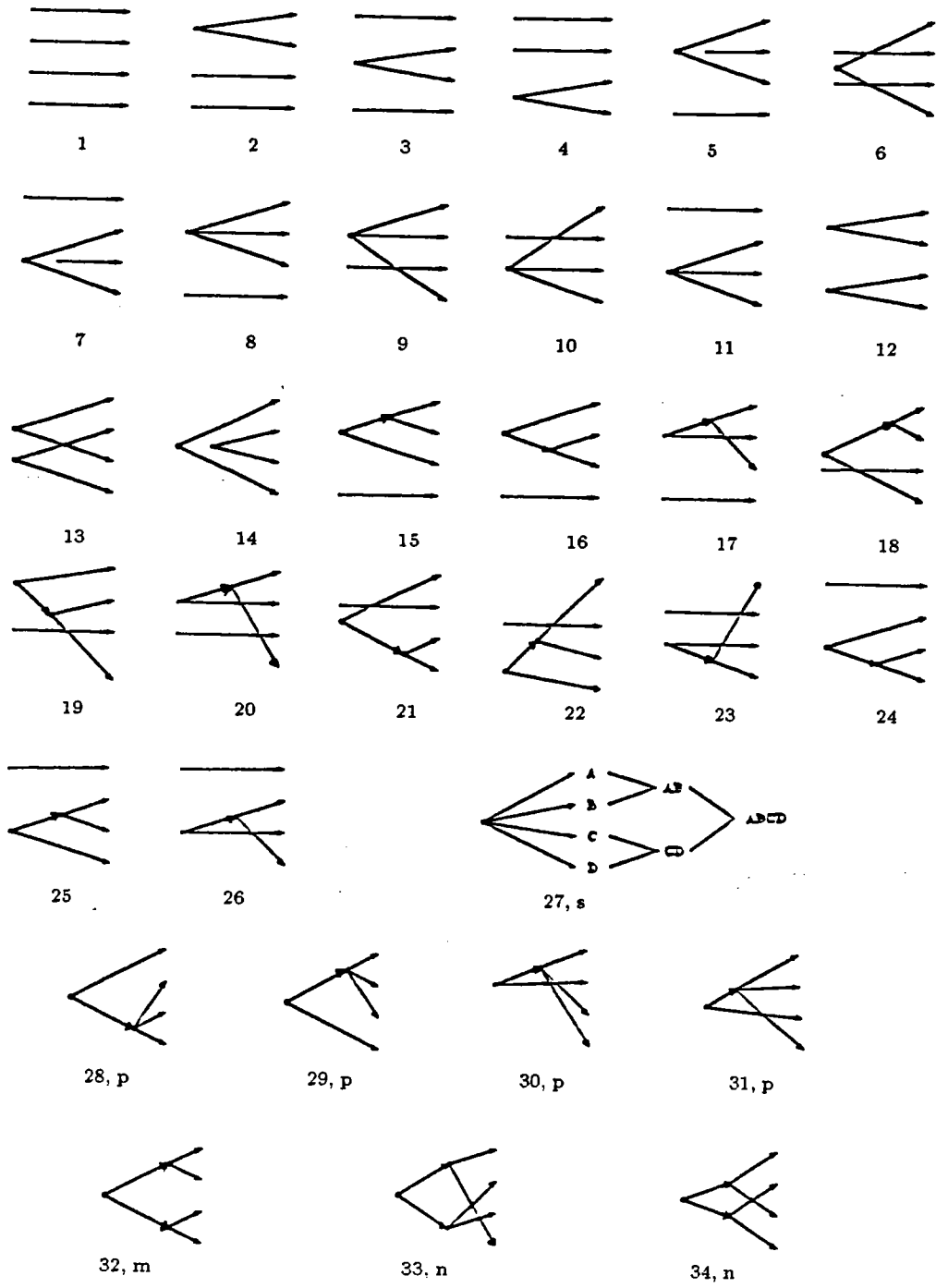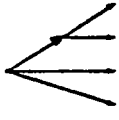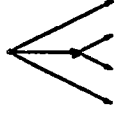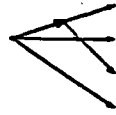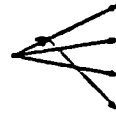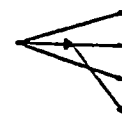
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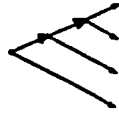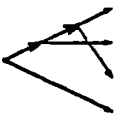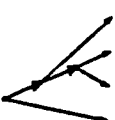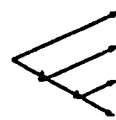
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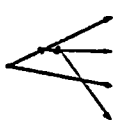
47 48 49 50 51 52

FIGURE 3

a)
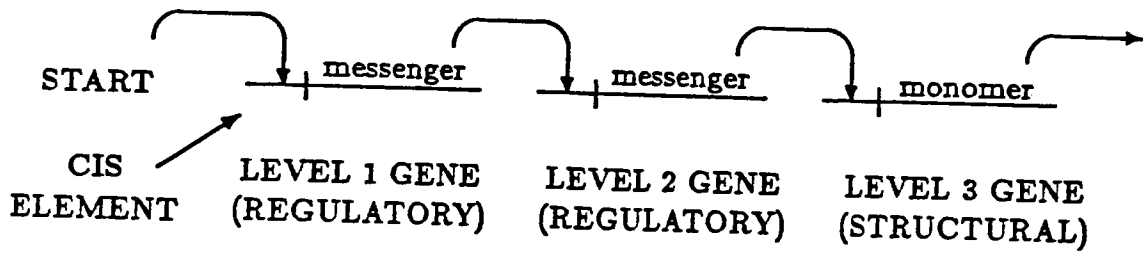
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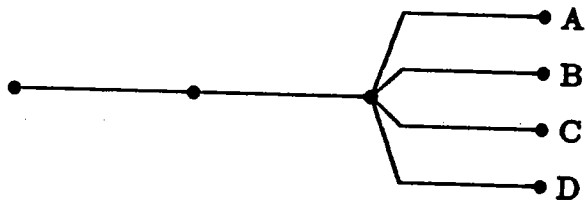
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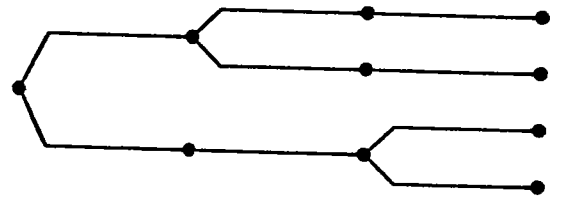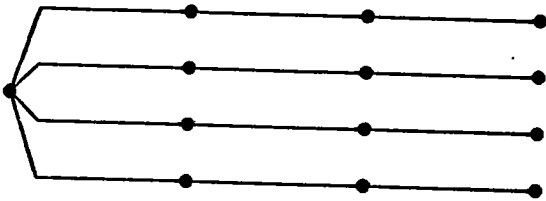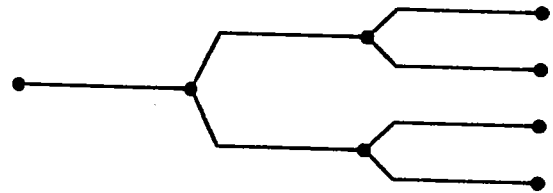
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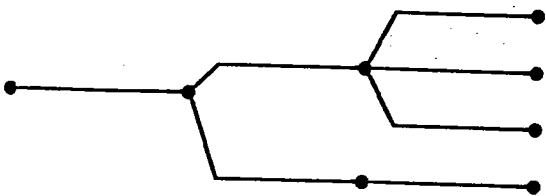
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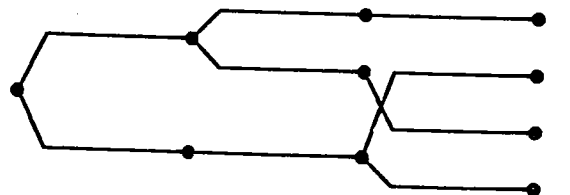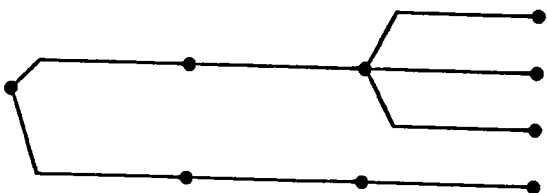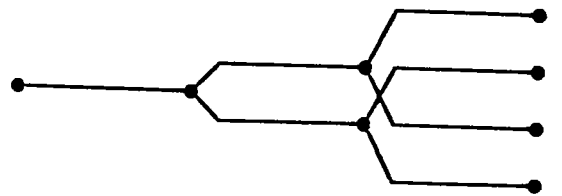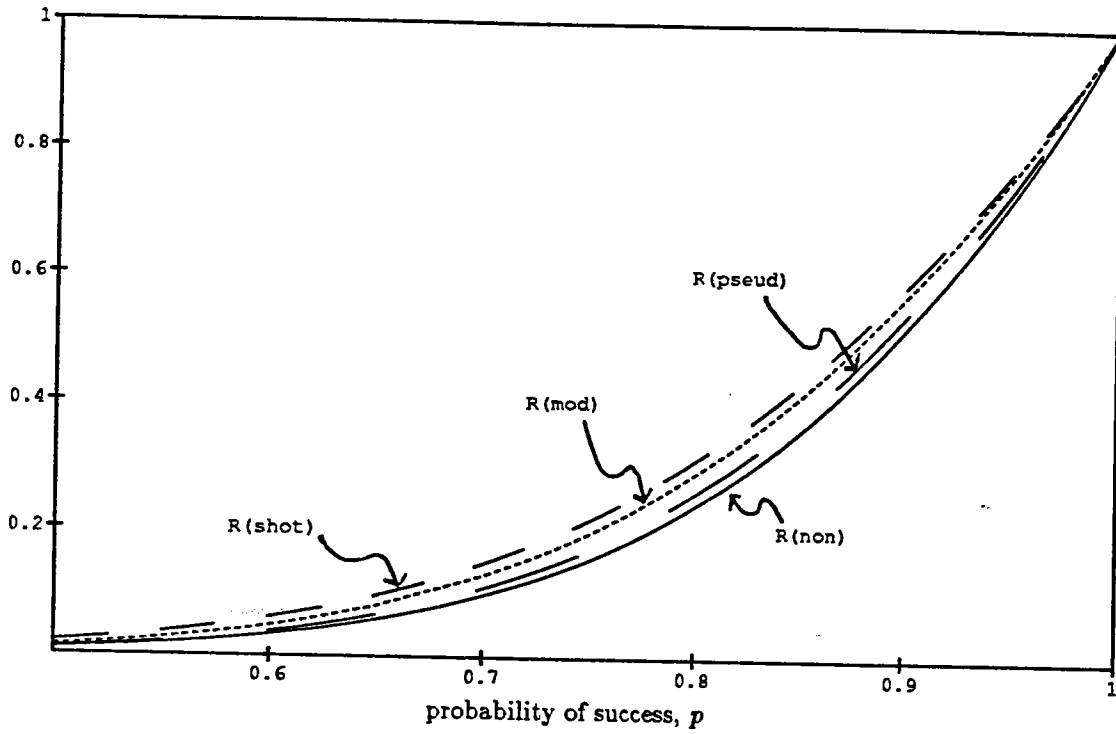
FIGURE 4

a) Reliability, R, of four networks.

b) The difference $R_{mod} - R_{non}$.
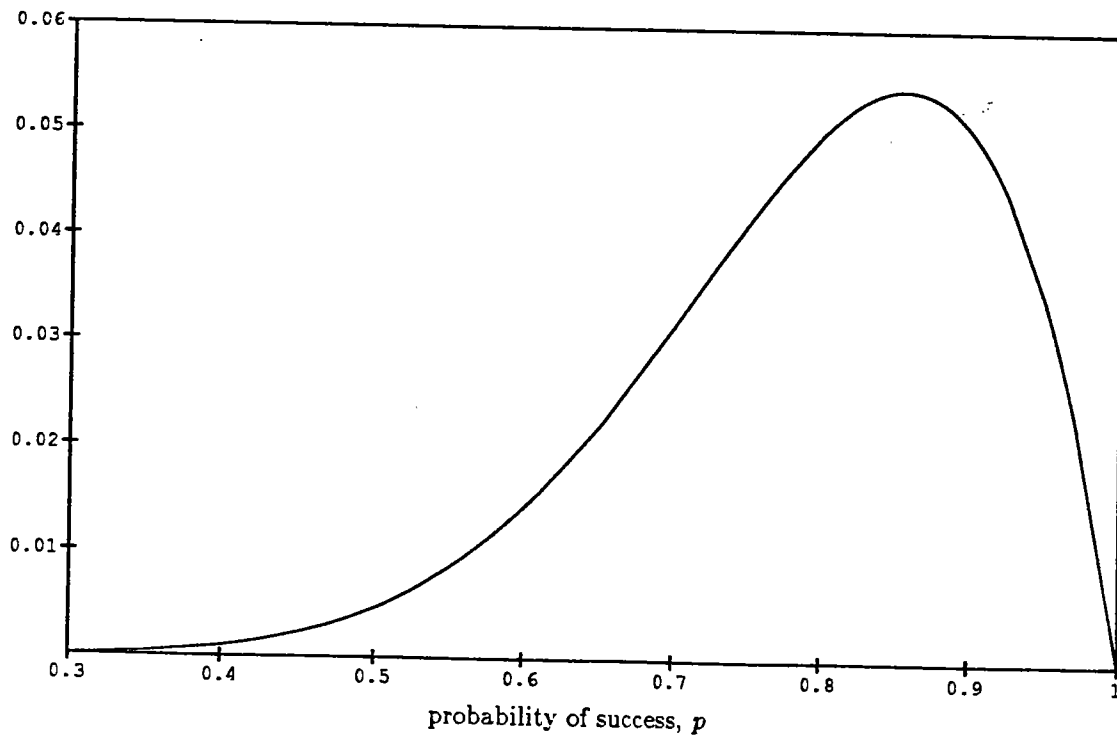
TABLE 1

level 1 processes	level 2 processes	dimers resulting from monomer synthesis		total number of states
		in this time step	in previous time step	
success or failure of one level 1 regulatory gene (2 alternatives)	shotgun: success or failure of one level 2 regulatory gene (2 alternatives)	none	none	shotgun: 48 states
	modular: success or failure of two level 2 regulatory genes (4 alternatives)	AB only		modular: 96 states
	pseudomodular: success or failure of both level 2 regulatory genes (4 alternatives)	CD only	AB only	pseudomodular: 96 states
	nonmodular: success of both level 2 regulatory genes, or failure of at least one. (2 alternatives)	AB and CD (= ABCD)	CD only	nonmodular: 48 states
		(4 alternatives)	(3 alternatives)	