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The *NK* Model of Rugged Fitness Landscapes And Its Application to Maturation of the Immune Response

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Adaptive evolution is, to a large extent, a complex combinatorial optimization process. Such processes can be characterized as "uphill walks on rugged fitness landscapes". Concrete examples of fitness landscapes include the distribution of any specific functional property such as the capacity to catalyze a specific reaction, or bind a specific ligand, in "protein space". In particular, the property might be the affinity of all possible antibody molecules for a specific antigenic determinant. That affinity landscape presumably plays a critical role in maturation of the immune response. In this process, hypermutation and clonal selection act to select antibody V region mutant variants with successively higher affinity for the immunizing antigen. The actual statistical structure of affinity landscapes, although knowable, is currently unknown. Here, we analyze a class of mathematical models we call *NK* models. We show that these models capture significant features of the maturation of the immune response, which is currently thought to share features with general protein evolution. The *NK* models have the important property that, as the parameter K increases, the "ruggedness" of the *NK* landscape varies from a single peaked "Fujiyama" landscape to a multi-peaked "badlands" landscape. Walks to local optima on such landscapes become shorter as K increases. This fact allows us to choose a value of K that corresponds to the experimentally observed number of mutational "steps", 6–8, taken as an antibody sequence matures. If the mature antibody is taken to correspond to a local optimum in the model, tuning the model requires that K be about 40, implying that the functional contribution of each amino acid in the V region is affected by about 40 others. Given this value of K, the model then *predicts* several features of "antibody space" that are in qualitative agreement with experiment: (1) The fraction of fitter variants of an initial "roughed in" germ line antibody amplified by clonal selection is about 1–2%. (2) Mutations at some sites of the mature antibody hardly affect antibody function at all, but mutations at other sites dramatically decrease function. (3) The same "roughed in" antibody sequence can "walk" to many mature antibody sequences. (4) Many adaptive walks can end on the same local optimum. (5) Comparison of different mature sequences derived from the same initial V region shows evolutionary hot spots and parallel mutations. All these predictions are open to detailed testing by obtaining monoclonal antibodies early in the immune response and carrying out *in vitro* mutagenesis and adaptive hill climbing with respect to affinity for the immunizing antigen.

Introduction

The evocative imagery created by Wright's notion of an adaptive landscape (Wright, 1932) is one of the most powerful concepts in evolutionary theory. The simplest version of his idea pictures a space of genotypes, each "next to" those other genotypes which differ by a single mutation, and each assigned a fitness. The distribution of

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the fitness values over the space of genotypes constitutes the fitness landscape. Maynard Smith (1970) borrowed Wright's image in defining "protein space" and adaptive walks in that space. The space consists of all 20^N proteins, length N, arranged such that each protein is a vertex next to all $19N$ single mutant variants obtained by replacing one amino acid at one position by one of the 19 remaining possible coded amino acids. Each protein in the space is assigned some "fitness" with respect to a specific property, such as binding a specific ligand, where "fitness" can be defined as the affinity of binding. An adaptive walk can be conceived as a process which begins at a single protein in the space and passes via ever fitter 1-mutant variants. Ultimately, such an adaptive walk on a fixed landscape must climb to a locally optimal protein, better than all $19N$ 1-mutant variants. These ideas can be generalized to walks proceeding via 2-mutant or more distant neighbors, or those allowed to pass via less fit neighbors as well. More recently, other authors have taken up the idea of protein space, or more generally of sequence space, (Ninio, 1979; Eigen, 1985; Schuster, 1986, 1987; Fontana & Schuster, 1987; Kauffman & Levin, 1987; Kauffman *et al.*, 1988; Kauffman, 1989 *a,b*).

It is clear that the character of such adaptive walks depends upon the actual structure of the fitness landscape, whether it is smooth with few adaptive peaks, or highly mountainous and multipeaked. In addition the adaptive process depends upon the actual mechanisms of adaptive "flow" by the population: for an asexual population these include the mutation rate, and population size. Gillespie (1983, 1984) has shown that in the limit where the mutation rate is low and the relative fitness differences between less fit and more fit mutant neighbors sufficiently great, the general flow of a population over a landscape can be simplified to a process where the population as a whole remains fixed at a single "genotype", protein, or point in the space for long times then moves as a unit to a fitter 1-mutant variant. We use this limiting case of an adaptive walk in this article, because it allows us to focus on the statistical structure of rugged adaptive landscapes. Nevertheless, the actual flow of a population of maturing B cells on the real affinity landscape is very likely to be a more complex process which "spreads out" along fitness ridges in the affinity landscape in ways depending upon the details of mutation rate, cell population sizes, and fitness differences. Once the statistical structure of affinity landscapes are understood, these further issues must also be addressed.

The actual structure of fitness landscapes in protein space for specific catalytic or ligand binding functions is unknown, but increasingly open to direct investigation by current genetic engineering and site directed mutagenesis studies. Our aim in this article is to discuss further a spin-glass-like model of random epistatic interactions, called the NK model, introduced and considered elsewhere, (Kauffman *et al.*, 1988; Kauffman, 1989*a,b*). N is the number of "sites" in the model genotype or protein, while K is the number of sites whose alternative states, "alleles" or amino acids, bear on the fitness contribution of each site. Thus K measures the richness of epistatic interactions among sites.

This model generates a family of increasingly rugged multipeaked landscapes as its main parameters are tuned, (Kauffman *et al.*, 1988; Kauffman, 1989*a,b*). Thus as K increases relative to N, landscapes pass from smooth and single peaked to jagged and multipeaked. Of course, since protein space is a discrete "sequence

space", the fitness values are only defined on this discrete space. The general interest in this family of landscapes lies in understanding the implications of the richness of epistasis on the expected structure of fitness landscapes. Thus the model can be interpreted as a haploid genetic model and used to study the effects of epistasis in population genetics. Our particular purpose in the present article is to show that the *NK* model predicts a number of features of a well known example of rapid adaptive protein evolution: maturation of the immune response.

We wish to contrast our approach, which considers only the statistical structure of the landscape, with more familiar theoretical approaches that involve detailed simulation of actual protein molecules. We believe we are proposing a kind of "statistical mechanics" of the immune response in particular, and protein evolution in general. This analogy with physics seems apt, because the theory is motivated by a desire to understand the *ensemble* properties of evolution among proteins even at the risk of ignoring important details regarding individual proteins. There are two reasons for taking this position, one practical and the other theoretical. First, the practical reason: there is an extensive literature which discusses detailed mathematical models of proteins that include the main chain, electrostatic, van der Waals and hydrogen bonding forces, and, perhaps, forces due to interactions with a solvent (Karplus & Kusick, 1983; Karplus *et al.*, 1987; Shenkin *et al.*, 1987). Unfortunately, detailed analysis of the kind of adaptive walks in the space of antibodies which we propose is computationally intractable on current computers. From a theoretical perspective, we need to understand the actual statistical structure of fitness landscapes underlying protein evolution. If simple statistical models such as the *NK* model we discuss predict actual adaptive landscapes in protein evolution, then we may hope that the *NK* model or improved variants point to the underlying basis for the structure of protein adaptive landscapes. Such models may help teach us how proteins work and evolve.

The rest of this paper is laid out as follows: In the first section, we present a more detailed discussion of the idea of peptide spaces, which we choose to rename "affinity landscapes", to reflect our interest in the binding of antigens by antibodies. This leads us to note a number of natural features of mountainous fitness landscapes which are open to experimental and theoretical investigation. In the second section we discuss the *NK* class of mathematical models, and discuss enough of its properties to motivate the modeling steps employed subsequently. The third section sketches the biological facts regarding the maturation of the immune response. In this section, we suggest that this well studied system, during which hypermutation and clonal selection amplify V region mutant antibodies with successively higher affinity for the immunizing antigen, is a natural testbed for the application of the *NK* model to protein evolution. The fourth section summarizes the predictions of the model and its qualitative agreement with relevant experiments. We conclude with a discussion of the significance and limitations of our findings and some suggestions for avenues of future investigation.

The Structure of Affinity Landscapes

In this section, we set forth the concept of an affinity landscape in more detail. The

set of all 20^N proteins of length N can be represented as points in an abstract N dimensional space in which proteins that differ at exactly one residue are "neighbors." Although it is difficult to draw a picture of such high dimensional spaces, a sense of their structure can be captured by considering proteins with only two amino acids, e.g. alanine and glycine. In Fig. 1(a), all $2^4 = 16$ possible peptide sequences of length 4 for these two amino acids are shown, using the representation "1" for alanine and "0" for glycine. Each vertex in this figure corresponds to a specific sequence, e.g. '1101" or "0101". There are also four lines emanating from each vertex. These lines connect the vertex with the four other "neighbor" verticies that differ from the first by a single amino acid substitution. It is thus clear from the figure that the neighbors of e.g. "0110" are "1110", "0010", "0100", and "0111". Peptide sequences involving all 20 amino acids would be structurally similar, but more complicated to draw. Each dimension would have 20 verticies, and each vertex would be connected to the 19 others in the same hyperplane. Because there are N hyperplanes, there are $19N$ total connections for each vertex. The "protein space" construction allows us to specify exactly what we mean by neighboring sequences, the minimum number of changes to pass from one sequence to another, etc. We also remark in passing that the concept is very general, and can be used to represent entire organisms or other ensembles of related objects that are "one mutant neighbors" of each other.

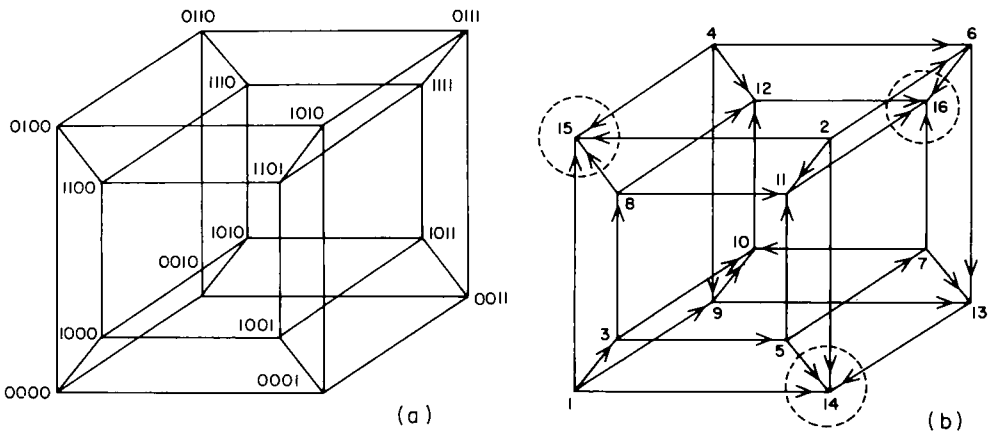

FIG. 1(a). A four-dimensional "Boolean hypercube" showing all 16 possible peptides length four comprised of only two amino acids, alanine = 1, glycine = 0. Each peptide is "next" to those which are accessible by mutating a single amino acid. (b) Each peptide has been assigned, at random, a rank order fitness, one low, 16 high. Arrows connect adjacent peptides, and point to peptide of higher fitness. Circles surround locai optima in this small peptide space.

As noted above, we can assign a "fitness" to each protein by measuring its capacity to perform a specific function, such as catalyzing a given reaction, binding a given ligand, etc. In Fig. 1(b), we have assigned each of the 16 peptides on the verticies with the hypothetical fitness values shown. This assignment gives a rank order to the peptides from the worst (1) to the best (16). As we will see, the properties of interest in the subsequent discussion depend only on these rank orderings. The

adaptive walks we focus on might begin with any of the 16 peptides and will "step" to a one mutant neighbor peptide only if the second peptide is fitter (has higher rank order) than the first. In Fig. 1(b) this is represented by arrows from each peptide directed to those 1-mutant neighbors with higher rank order. The sequence of arrows connecting a series of adjacent verticies in the Figure represent such a walk. The walk must terminate when it reaches a peptide which is fitter than all of its one mutant neighbors. Such a peptide is a *local optimum* of the space. In Fig. 1(b) three of the 16 peptides are local optima.

To completely specify the character of the walk, it is necessary to choose a step selection mechanism. Two choices are natural. The first is to step to the neighbor with the highest fitness. Such walks are called *greedy walks.* The second involves selecting the fitter neighbor *at random* from among all the fitter 1-mutant neighbors. Either of these two is an idealization of the actual flow of an adapting population on a rugged landscape under the drive of mutation and selection. The former roughly represents a case where more than a single mutant is encountered in a short time period and the fittest variant sweeps the population. The latter roughly represents the case considered by Gillespie, when the rate of finding a fitter variant is low compared to selection differences ensuring the rapid establishment of any fitter variant, once it is found and present in sufficient numbers to obviate loss by chance drift. We will use Gillespie's limiting case and consider fitter 1-mutant variants to be chosen at random.

The simple landscape shown in Fig. 1(b) has rank order fitness values which were assigned at random to the 16 possible peptides in the space. In considering maturation of the immune response the natural measure of fitness is the *affinity* with which each possible antibody binds the immunizing epitope on the specific antigen with respect to which maturation is occurring. Since the diversity of antibodies in the mammalian immune response is thought to be greater than 10^8 (Honjo *et al.*, 1983; Berek *et al.*, 1985), while each antibody is a 1-mutant neighbor of thousands of other antibody molecules, affinity landscapes are far more complex. Yet whatever the other cellular mechanisms may be which underlie hypermutation, clonal selection, and other parts of the maturation process, it seems obvious that the unknown mountainous structure of affinity landscapes in antibody space must be central to the maturation process.

The primary virtue of the landscape construct is that it raises a number of theoretically and experimentally accessible questions about the nature of uphill walks and the optima that they reach. All are evident in Fig. 1:

(1) How many local optima exist in a landscape?
(2) What is the distribution of optima in the landscape? Are they near one another in special subregions of the space, or randomly scattered?
(3) What are the lengths of uphill walks to local optima?
(4) As an optimum is approached, the fraction of fitter neighbors must dwindle to 0. How rapidly does the fraction of fitter neighbors dwindle?
(5) Because the fraction of neighbors which are fitter dwindles to 0, there is some characteristic relation between the number of mutations "tried" and the number "accepted" on an adaptive walk. How are the two related?

(6) How many alternative optima are accessible from a given starting point? Can a "low fit" peptide typically climb to all possible local optima, or only a small fraction of those optima? Among the accessible alternative optima, how often will each be "hit" on independent adaptive walks from the same starting point?
(7) How many of the possible peptides can climb to any specific optimum, including the global optimum? A small fraction? Almost all?
(8) Since most adaptive walks end on local optima, what are the fitnesses of such optima and how do they compare with the global optimum in the space?
(9) The 1-mutant variants of a local optimum must be less fit than the optimum. But do all of the variants lead to nearly the same loss of fitness or is there high variance indicating precipitous cliffs and gentle ridges in different directions in the high dimensional space?

Previous work (Kauffman & Levin, 1987; Weinberger, 1988; Macken & Perelson, 1989) has analyzed many of these questions with respect to the limiting case of *fully random fitness landscapes* in which the fitness of 1-mutant neighbors are assigned at random from some fixed underlying distribution. In such an uncorrelated landscape, the fitness of one protein carries no information about the fitness of its 1-mutant neighbors. Presumably the real fitness landscapes underlying protein evolution are not uncorrelated, although they are correlated in as yet unknown ways. In order to begin to gain insight into the structure of *correlated* fitness landscapes we previously introduced the *NK* family of rugged landscapes, (Kauffman *et al.*, 1988; Kauffman, 1989*a*,*b*). Our hope is that this family of correlated landscapes, characterized by a few major parameters, may make reasonable predictions about the actual structure of antibody affinity landscapes. We present the *NK* family next.

The *NK* Model, a Spin Glass-like Model of an Affinity Landscape

The *NK* model is meant to apply to systems of many, N, parts, where the functional contribution of each part depends upon the "state", among A alternatives, of that part, and is epistatically affected by an average of K other parts. In the case of genotypes, the N parts are interpreted as genes, the A alternative states of a part as the alleles, and the K epistatic interactions as functional effects of the alleles at other loci upon the fitness contribution of an allele at a specific locus to overall fitness. In the case of a protein, the N parts are the amino acids in the primary sequence, the A states are the 20 possible amino acids, and K measures the average number of other sites in the primary chain whose amino acids bear on the functional contribution of the amino acid at a given site to overall function. In reality, K presumably varies from site to site. We treat it as a constant for the moment. In short, K is a parameter which measures how richly interconnected the parts of the system are. As we shall see, increasing K from 0 to $N-1$ increases the number of peaks and valleys, and thus the ruggedness of the corresponding fitness from single peaked and smooth to multipeaked and fully uncorrelated. In turn, the ruggedness of the landscape alters the character of adaptive walks towards optima under biologically reasonable mutation selection models or any of a variety of optimization

procedures. In addition to specifying the values of N, A, and K, it is also necessary to specify for each site the specific K among the N which affect it. For example, one might wish to assume reciprocity. If site I affects J, then J affects I. Alternatively, reciprocity might not be assumed. More generally, if the sites are located in a linear structure such as a chromosome or protein, the K sites bearing on any site might be its neighbors, might be chosen at random, or in some non-random spatial distribution.

The central idea used in the NK model is that the epistatic effects of the A^K different combinations of A alternative states of the K other sites on the functional contribution of Ath state of each part are so complex that their *statistical* features can be captured by assigning fitness consequences *at random* from a specified distribution. It is in this sense that the NK model is a model of random epistatic interactions. Given N, A, K, the distribution of K among the N assigned to each site, and the underlying fitness distribution from which random fitness contributions assignments are made, the NK model is specified and in turn determines an ensemble of fitness landscapes. The model is similar to spin glass models of disordered magnetic materials which have received extensive attention in solid state physics recently (Edwards & Anderson, 1975; Sherrington & Kirkpatrick, 1975; Anderson 1985). In fact, for the case where the K epistatic sites are a site's flanking neighbors, the model can rigorously be shown to be a type of short range spin glass (Binder & Young, 1986). Conversely the case in which $K = N-1$ corresponds to the Derrida random energy spin glass (1981), as becomes clear below.

Consider the simplest version of the NK model which assumes that each site has only $A =$ two states. This corresponds to an N locus two allele haploid genetic model, or, as in the previous section, to a restricted peptide space in which only two of the 20 biologically important amino acids are present (e.g. alanine = 0 and glycine = 1). Each amino acid makes a fitness contribution depending on whether it is 0 or 1, and whether the K other amino acids which impinge upon it are 0 or 1. Thus the fitness contribution of each of the N sites depends upon the state at $K+1$ sites.

The NK model assigns a "fitness contribution", w_i in (0, 1), to each amino acid, i, $1 \leqslant i \leqslant N$, of the N residue chain such that w_i depends on i and $K < N$ other bits. Since each amino acid can be 0 or 1, there are $2^{(K+1)}$ combinations of states of the $K+1$ amino acids which determine the fitness contribution of each amino acid. The fitness contributions associated with each of these combinations is assigned by selecting an independent random variable from the uniform distribution on (0, 1). This constitutes the "fitness table" for the ith amino acid. There is a different, independently generated table for each of the N amino acids. Then, given any "string" of N amino acids, the total fitness of the string, W, is defined as the average of the fitness contributions of each part, (i.e. each of the w_i's) each in the context of the K which impinge upon it:

$$W = \frac{1}{N} \sum_{i=1}^{N} w_i. \tag{1}$$

Figure 2(a) shows a simple example of the NK model for a hypothetical three gene system each with two alleles, where the fitness contribution of each allele at

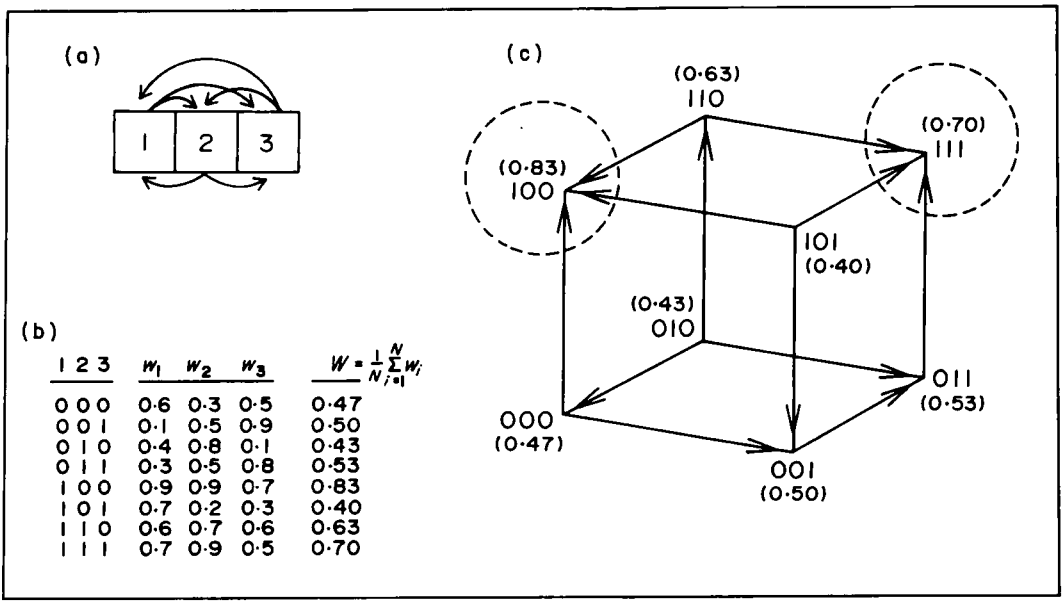

FIG. 2(a). Three adjacent sites in the *NK* model, each receives epistatic inputs from the other two; $N = 3$, $K = 2$. (b) The fitness contribution of each site, w_i, $i = 1, 2, 3$, as a function of the allele, 1 or 0, at that site and at the $K = 2$ other sites which bear upon it. The fitness, W, of each genotype, or tripeptide is the average of the fitness contributions of the three sites. (c) The fitness of each of the $2^3 = 8$ possible genotypes, or tripeptides, from 2(b), on the three dimensional Boolean cube representing this small sequence space. Note that two local optima exist.

each locus depends on the alleles at that locus and the two other loci. Equivalently, this models a tripeptide with three amino acids, each of which makes a contribution to overall function depending upon the amino acid at that site and the remaining two sites. As shown, the resulting fitness tables for the three sites, Fig. 2(b) yield a fitness for each of the $2^3 = 8$ possible tripeptides, and in turn induce a fitness landscape like that in Fig. 1, with adaptive walks to local optima, Fig. 2(c).

The *NK* model affords a "tuneably rugged" fitness landscape, since tuning K alters how rugged the landscape is. This can be seen from the following: For $K = 0$, each site is independent of all other sites. Except for rare "ties" which we ignore, either the bit value 0 or the bit value 1 is "fitter" than the other; hence, a single specific sequence comprised of the fitter bit value in each position is the single, global optimum in the fitness landscape. This simple case corresponds to the familiar haploid multilocus, two allele additive genetic model found in population genetics (see, e.g. Ewens, 1979), The "correlation" of a fitness landscape measures how similar the fitness values of "1-mutant" variants in the space of systems are. Specifically, each N bit sequence has N 1-mutant neighbors, obtained by mutating any bit to the opposite state. Since such a mutation can only alter fitness by $1/N$ or less in $K = 0$ landscapes, such landscapes are highly correlated. Furthermore, in such landscapes, the statistics of local hill climbing walks are simply obtained. There is a single sequence which is the only and global optimum. Any other sequence is

suboptimal, and lies on a connected walk via 1-mutant fitter variants to the global optimum by mutating bits from less fit to more fit values. The length of the walk is just the number of bits by which the initial sequence differs from the global optimum. For a randomly chosen initial string, half the bits will be in their less fit state, hence the expected walk length is just $N/2$. Thus the lengths of adaptive walks to the global optimum scale linearly with N. Further, at each adaptive step the number of fitter 1-mutant neighbors decreases by one, hence the directions uphill dwindle slowly as the global optimum is approached. These properties are in sharp contrast to the limit when $K = N-1$.

The fully connected NK model yields a completely random fitness landscape. For $K = N-1$, the fitness contribution of each site depends on all of the other sites in the sequence and therefore altering any site from one to the other value, 0–1, alters the fitness contribution of each site to a new random value. Thus the fitness of any 1-mutant neighboring sequence is completely random with respect to the initial sequence. The landscape is fully random. As was shown in Kauffman & Levin (1987), Weinberger (1988), and Macken & Perelson (1989), such random landscapes have very many local optima, on average, $2^N/(N+1)$. Walks to optima are short, scaling as ln N. The expected fraction of fitter neighbors falls by half at each adaptive step. The "time" or number of mutants "tried" to reach a local optimum scales as N, the number of 1-mutant neighbors of a sequence. Thus the ratio of accepted to tried mutations itself scales as (ln N/N), (Kauffman, 1989*a*,*b*; Macken & Perelson, 1989). Only a small fraction of local optima are accessible from any initial string, and only a small fraction of peptides can climb to any optimum, including the global optimum, (Kauffman & Levin, 1987; Kauffman, 1989*a*). Finally, as N increases, and K remains equal to $N-1$, the *fitness of local optima fall* toward the mean of the space, 0·5, in a kind of *complexity catastrophe.* On the other hand, simulation results and some analytic work suggests that the fitness of the global optimum in the space appears not to fall as N increases, (Kauffman, 1989*a*). In short, adaptive walks vary dramatically as the ruggedness of the landscape varies.

To gain insight into the behavior of the model for $0 < K < N-1$ with two amino acids, we simulated 100 different instances of uphill walks for each of the N and K values given in Tables 1(a), (b) and 2(a), (b). Walks started from random initial states. These tables show the means and, in parentheses, the standard deviations of the maximal fitnesses attained, Tables 1(a), 2(a), and mean walk lengths, Tables 1(b), 2(b), for the cases in which the K sites are nearest neighbors, Table 1(a),(b), or randomly chosen, Table 2(a), (b). The largest possible value of K is $N-1$. For simplicity, in these tables we use $K = N$. Figure 3 shows that as K increases relative to N, the fraction of fitter neighbors at each adaptive step dwindles more rapidly. The reciprocal of the fraction of fitter neighbors is the expected waiting time to find a fitter variant, or equivalently the expected number of mutants "tried" to take the next adaptive step.

Note that as N increases for $K = N-1$, the fitness of optima fall. The complexity "catastrophe" inexorably sets in. Indeed Tables 1(a) and 2(a), suggest that K need only increase linearly with N for this ultimate decline in the fitness of accessible optima to occur. Conversely Tables 1(a) and 2(a) indicate that if K remains small

TABLE 1(a)

Mean fitness of local optima attained on walks on 100 *different landscapes for each different value of* N *and* K. *Standard deviations shown in brackets.* K *sites bearing on each site were its* K/2 *flanking neighbors on each side. Circular sequences were assumed to avoid boundary effects. Two alternative "alleles" or amino acids are possible at each site*

	N				
K	8	16	24	48	96
0	0·65 (0·08)	0·65 (0·06)	0·66 (0·04)	0·66 (0·03)	0·66 (0·02)
2	0·70 (0·07)	0·70 (0·04)	0·70 (0·08)	0·70 (0·02)	0·71 (0·02)
4	0·70 (0·06)	0·71 (0·04)	0·70 (0·04)	0·70 (0·03)	0·70 (0·02)
8	0·66 (0·06)	0·68 (0·04)	0·68 (0·03)	0·69 (0·02)	0·68 (0·02)
16		0·65 (0·04)	0·66 (0·03)	0·66 (0·02)	0·66 (0·02)
24			0·63 (0·03)	0·64 (0·02)	0·64 (0·01)
48				0·60 (0·02)	0·61 (0·01)
96					0·58 (0·01)

TABLE 1(b)

Mean walks lengths to local optima attained on walks on 100 *different landscapes for each different value of* N *and* K. K *adjacent*

	N				
K	8	16	24	48	96
0	1·5 (1·2)	8·6 (1·9)	12·6 (2·2)	24·3 (3·4)	48·8 (4·6)
2	4·1 (1·9)	8·1 (3·2)	11·2 (3·1)	22·5 (4·6)	45·2 (6·6)
4	3·2 (1·8)	6·6 (2·5)	9·4 (2·9)	19·3 (3·9)	37·3 (6·1)
8	2·7 (1·5)	4·7 (2·3)	7·7 (3·0)	15·3 (4·3)	27·7 (5·3)
16		3·3 (1·7)	4·8 (2·1)	9·6 (3·0)	19·3 (4·2)
24			3·5 (1·4)	7·4 (3·0)	5·0 (3·9)
48				3·9 (1·9)	8·9 (3·0)
96					5·1 (2·4)

while N increases, local optima do not fall in fitness. This hints at a construction requirement: as the number of interacting parts in a complex system increase, the adaptive landscape will tend to retain highly fit and accessible local optima if the epistatic interactions remain low. Note also that as K increases relative to N mean walk lengths to optima decrease. We use this fact below in apply the NK model to maturation of the immune response. Table 3 shows the number of local optima found for different values of N and K. An important general features of these results is that the basic structure of the landscape is quite insensitive to whether the K sites affecting each site are its neighbors or assigned at random. This will recur in our application of the NK model to maturation of the immune response. We comment that for the limiting case of $K = N-1$, $K = 15$, $N = 16$, the number of local optima

TABLE 2(a)

As in Table 1(*a*), *except the* K *sites bearing on each site were randomly chosen. Sequences were not assumed to be circular*

	N				
K	8	16	24	48	96
2	0·70 (0·06)	0·71 (0·04)	0·71 (0·03)	0·71 (0·03)	0·71 (0·02)
4	0·68 (0·05)	0·71 (0·04)	0·71 (0·04)	0·72 (0·03)	0·72 (0·02)
8	0·66 (0·06)	0·69 (0·04)	0·69 (0·04)	0·70 (0·02)	0·71 (0·02)
16		0·65 (0·04)	0·65 (0·03)	0·67 (0·03)	0·68 (0·02)
24			0·63 (0·03)	0·65 (0·02)	0·66 (0·02)
48				0·60 (0·02)	0·62 (0·02)
96					0·58 (0·01)

TABLE 2(b)

As in Table 1(*b*), *except the* K *sites bearing on each site were randomly chosen*

	N				
K	8	16	24	48	96
2	4·4 (1·8)	8·1 (2·8)	12·5 (3·8)	26·5 (5·1)	46·9 (6·1)
4	3·6 (1·8)	7·3 (2·9)	10·9 (3·3)	22·9 (5·6)	44·5 (7·9)
8	2·7 (1·5)	5·3 (2·5)	8·0 (3·2)	17·0 (4·3)	34·7 (6·5)
16		3·3 (1·7)	4·8 (2·1)	10·1 (3·4)	21·6 (4·8)
24			3·5 (1·4)	7·4 (2·6)	16·0 (4·3)
48				3·9 (1·9)	9·3 (2·6)
96					5·1 (2·4)

encountered appears higher than the predicted $2^N/(N+1)$. The reasons for this disparity are not clear.

NK landscapes have other quite striking properties, most but not all of which are quite insensitive to the detailed assumptions of the model, as discussed elsewhere (Kauffman, 1989). For example, for $A=2$ and $K=2$, whether the epistatic sites are adjacent or random, the general configuration of the landscape is very non-random. The highest optima are both nearest one another and also have the largest drainage basins. The landscape has a *Massif Central.* As A and K increase these ordered features decay, rapidly with respect to the nearness of high local optima to one another, more slowly for the tendency of the highest optima to drain the largest region of the space. Since these features and their implications are described elsewhere, (Kauffman, 1989*a*), we do not comment upon them further here.

Conclusions for the *N* Locus Two Allele or Two Amino Acid Case

The *NK* model is a very general approach to understanding complex epistatic interactions and their effects upon the structure of correlated but mountainous

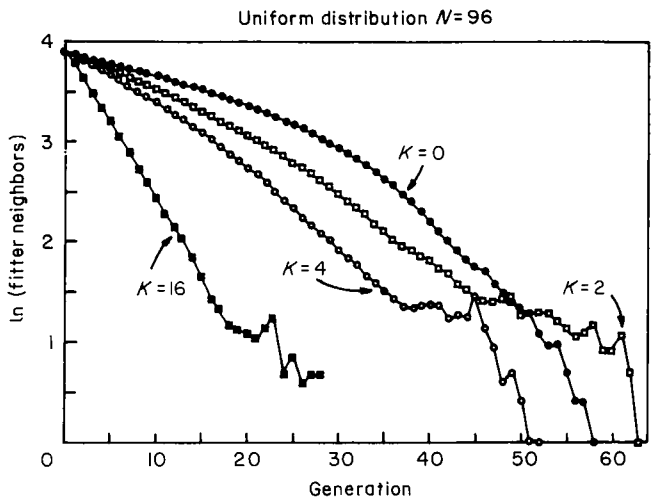

FIG. 3. Logarithm of the mean number of fitter 1-mutant neighbors at each adaptive step, plotted against the adaptive step, or generation, for the NK model with $N = 96$, and fitness values chosen uniformly between 0 and 1. As K increases the rate of fall of in the fraction of fitter 1-mutant neighbors increases as well.

TABLE 3

Number of optima in landscapes for different values of N *and* K, K < N. *Data are means of ten landscapes, each explored from* 10,000 *different initial points*

	N	
K	8	16
2	5	26
4	15	184
7	34	—
8	—	1109
15	—	4370

landscapes. The main conclusions to bring away are that increasing K relative to N increases the number of local optima, shortens the lengths of walks to optima, increases the rate at which fitter neighbors dwindle to 0 along adaptive walks, increases the ruggedness of the landscape, reduces the fraction of optima accessible from a given point, reduces the number of points which can climb to a given optimum and leads to a complexity catastrophe in which accessible optima fall toward the mean of the space. All these features presumably reflect the fact that, as K increases, more conflicting constraints, or what in spin glass models is called *frustration*, (Anderson, 1985), sets in.

While the *NK* family of landscapes is of interest and appears to be one useful model of correlated fitness landscapes, there may be very many such families of landscapes. Ultimately we must be interested in the actual structure of the fitness landscapes underlying adaptive protein evolution. We turn in the next section to describe maturation of the immune response, then ask whether the *NK* landscapes have approximately the right statistical features to fit the known features of that adaptive landscape.

Maturation of the Immune Response

The purpose of this section is to suggest that maturation of the immune response is the kind of adaptive process that we have described previously, so that the *NK* model has a natural interpretation in this context. We also show that our ideas about "adaptive walks on rugged landscapes" give rise to interesting and experimentally accessible immunological questions.

To do this, we will first discuss the remarkable and rapid adaptive evolution which occurs during the immune response. This last term refers to the process by which the immune system, in response to a specific antigen, "tunes" the antibody molecules that it secretes. These antibody molecules accumulate successive mutations which progressively increase their affinity for the incoming antigen.

THE NATURE OF THE IMMUNE RESPONSE

When an organism such as a human is exposed to an antigen and mounts an immune response, the complex sequence of events which ensues includes binding of the antigen to immature antibody bearing B cells. Those B cells whose antigen receptors, each with the same specificity antibody molecule it will later secrete, best match the incoming antigen, proliferate most rapidly. This process is called *clonal selection*, (Burnet, 1959) and leads to an abundance of antibodies in the blood serum which match the antigen.

The antigen specificity of an antibody immunoglobulin is determined by the amino acid sequence of its heavy (H) and light (L) chain variable regions. The variable region's diversity is generated by the combinatorial assembly of five different variable (V) gene segments by genomic rearrangement events, during the formation of the particular complete V genes, which are between 330 and 360 base pairs long. A complete heavy chain V domain results from the joining of V_H, diversity (D), and H chain joining (J_H) gene segments in the genomic rearrangements in each particular stem cell. Similarly, the L chain V domain is created by joining of V_L and J_L gene segments. Each of these gene segments is chosen from a repertoire of several to several hundred alternatives, to build up combinatorially a very large number of alternative heavy variable and light variable regions (reviewed in Honjo, 1983; Yancopoulos & Alt, 1986). Honjo (1983) estimates the minimal diversity in the mouse generated by these mechanisms to be $5{\cdot}1 \cdot 10^7$, whereas Berek *et al.* (1985) estimate the diversity at 10^9.

In addition to this combinatorial diversity, two other sources of diversity are generated by variability in the exact locations of joining at the junctions of V gene segments during assembly with insertion of random nucleotides (Tonegawa, 1983). In addition another process results in nucleotide replacement, and is termed *somatic mutation.* In principle, such somatic mutation allows almost limitless V region diversity. From analysis of clonally related cells, it now appears that there exists a special hypermutation system which specifically alters bases in the V region at a rate of 10^{-3} per base pair per generation, a rate approximately six orders of magnitude higher than the spontaneous mutation rate (McKean *et al.*, 1983; Clark *et al.*, 1985; Manser *et al.*, 1985; Wysocki *et al.*, 1986; Sablitzky *et al.*, 1985).

We also note that, within either the heavy or light chain V region are three special sub-regions called *complementarity determining regions* (CDRs). These complementarity determining regions are thought to be the parts of the V region that actually bind the antigen. Presumably, the remaining amino acids, the *framework*, provide a superstructure for the CDR amino acids.

ANTIGEN SELECTION THEORIES

The cellular and molecular mechanisms by which the maturation of the immune response occurs are still being uncovered. Classical theories suggest that competition for limited amounts of antigen may drive a selection process (cf. Siskind & Benacerraf, 1969). The argument goes as follows: the amount of antigen bound to cell surface immunoglobulin depends upon the product of the antigen concentration and the affinity of the receptor for antigen. During the course of an immune response the antigen concentration should decrease. If there is a critical amount of bound antigen required to stimulate a B cell into antibody production, then as antigen concentration falls only those B cells with increasing affinities for the antigen will remain stimulated and continue to secrete antibody. Because the antibody secreted by a cell has the affinity for the antigen as its receptor, one should see the average affinity of serum antibody increase during the course of an immune response. According to this theory, based on clonal selection, smaller and smaller subsets of preexisting B cells are selected by antigen during the immune response. Mathematical models based on this theory were developed by Bell (1970, 1971).

SOMATIC MUTATION THEORIES

Doria (1982) pointed out that certain patterns of affinity changes actually observed are not consistent with the classical theory. For example, antigen selection theories would predict that low doses of antigen would lead to antibodies of higher affinity than would be found after giving higher doses. Instead, lower doses lead to antibodies with lower affinity (Siskind *et al.*, 1968).

Recent evidence has been obtained by studying the messenger RNA (mRNA) sequences of monoclonal antibodies. Sequencing of mRNAs from different stages of the immune response to a single antigen indicate a more complex process. In particular, it now appears that somatic mutation plays a major role in maturation

of the immune response such that the affinities of the antibodies secreted increase over time.

In response to a specific antigen, clonal proliferation of those germ line genes whose variable regions most precisely match the antigen leads to amplification in the serum of an initial set of "roughed in" antibodies from a restricted number of V region containing cells. The initial fraction of B cells which responds to an antigen is on the order of 10^{-5} (Press & Klinman, 1974). These germ line genes have little or no somatic mutation evident, (Kaartinen *et al.*, 1983; Tonegawa, 1983; Manser *et al.*, 1985; Wysocki *et al.*, 1986). Later in the primary or secondary response the majority of antibodies no longer directly correspond to germline varieties, but show extensive somatic point mutations. The accumulation of these point mutations is correlated with an increase in the affinity of the antibody for the antigen. According to present somatic mutation theories, the increased affinity is itself a direct consequence of further clonal selection. Those somatic mutations which result in an alteration of the protein sequence of the V region may alter the binding affinity of the antibody molecule for the antigenic determinant. Then those mutated B cells whose antibodies bind the antigen with higher affinity proliferate more rapidly, and come to dominate the immune response by clonal selection.

Over a succession of somatic mutations to the V region of the initial germ line B cells, the mean affinity of the antibodies increases sharply. Typical changes in affinity over the course of maturation are increases from 5×10^{-4} M to 5×10^{-7} M, (Fish & Manser, 1987; Kaartinen *et al.*, 1983).

Maturation of the immune response is therefore an adaptive walk in antibody space from the initial roughed in crudely matching germ line recombinant V region amplified by clonal selection through a succession of higher affinity variants, to or towards some local optimum antibody which is of higher affinity than its mutant neighbors. All the questions we have posed previously regarding the character of adaptive walks come to the fore, and point to a central experimental question: how correlated is the landscape?

We define the "landscape" in question precisely. Consider the incoming antigen and a single epitope, or molecular feature on that antigen. Then consider measuring the affinity of all possible antibody molecules for that single epitope. The distribution of the affinity values across antibody sequence space constitutes a well defined affinity landscape with respect to that specific epitope. Presumably it is the statistical character of that landscape which largely determines the character of adaptive walks in antibody space. Therefore, we would hope that studies of a model like the *NK* model might provide this kind of statistical information. We discuss a method of applying the *NK* model to explore these issues next.

APPLICATION OF THE *NK* MODEL TO THE MATURATION OF THE IMMUNE RESPONSE

Our fundamental assumptions in applying the *NK* model to the maturation of the immune response are that a representative member of the population of maturing antibodies can be identified at any time, and that these antibodies steadily increase

in fitness due to fortuitous point mutations until a locally optimal antibody is obtained. We define a locally optimal antibody to have higher affinity for the antigen than any of its 1-mutant neighbors. The experimental results in the preceding section confirm that this is, in general, a plausible scenario. However, it is not known whether mature antibodies are, in fact, local optima. It is known that the V regions continue to mutate without substantial changes in affinity even after they have attained maximum affinity for the antigen. This may reflect mutational dispersal among near neutral mutants in the immediate vicinity of the local optimum. We list our more detailed assumptions below:

(1) *Choice of the parameter N.* We identify the parameter N with the number of amino acid sites in the V region. As indicated in the previous discussion, there are between 110 and 120 amino acids in a typical V region. We use $N = 112$ because it was slightly easier to simulate a chain whose length is a multiple of eight.

(2) *Choice of starting place.* We assume that the fact that one in one hundred thousand B cells responds to a given antigen implies that those that do respond secrete antibodies that are in the 99·999th percentile in ability to bind to the antigen. Walks start well up on adaptive hillsides. From the point of view of our simulations, the fitness contribution of each amino acid in the model antibody is a random number. Therefore, finding antibodies in the appropriate percentile was reduced to the problem of finding random number seeds that give a sequence of $N = 112$ random numbers whose average is in the same percentile. Although our use of this procedure implies that there will be some fluctuation in the starting fitness of the model antibody, departure from the bottom boundary of this top percentile was insignificant.

(3) *Choice of neighborhoods.* Preliminary simulations reported above suggest that the lengths of walks to optima and the fitnesses of the optima achieved do not depend strongly on the details of which sites interact with each other. However, those preliminary simulations assumed that there could only be two amino acids per site and that the walks started from randomly selected initial peptides rather than peptides that are already in the 99·999th percentile in fitness. In modeling V regions we again consider both extremes: each amino acid only interacts directly with its K neighbors; to avoid "boundary" effects we therefore idealized the V region as a circular protein. Alternatively, each amino acid interacts with K amino acids drawn randomly from the chain. In this case we do not assume the peptide chain is a circle.

(4) *Choice of $19N$ neighbors or neighbors via the genetic code.* A V region length N can be thought of as having $19N$ 1-mutant neighbors. However, at the DNA level, many single amino acid substitutions require two or three base pair changes. Restriction to single base changes at the DNA level implies a reduction on the number of 1-mutant neighbors at the protein level. Both cases were studied. We incorporated "coding" into the model by explicitly including translation. In particular, we modeled this by assuming that the evolving entity was a pair of polymers, a "protein molecule", consisting of the 112 "amino acids", as before, and a (single stranded) "DNA molecule"

consisting of $112 \times 3 = 336$ sites, each with one of the four "bases". The initial DNA molecule was "back-translated" from the starting model V region to a DNA sequence coding for that model V region. The codon assigned to each position in the back-translation was chosen randomly from the synonymous codons for that amino acid. A "step" consisted in a point mutation of one of the DNA sites, translating the new DNA sequence into the corresponding protein using the genetic code, and then computing the fitness of the protein. Since adaptation passes only to *fitter* neighbors, in this procedure the adaptive walk does not pass to a 1-mutant neighbor which is a silent mutation to a synonomous codon. A DNA mutation which resulted in an internal stop codon in the model V region was scored as a lethal mutation with fitness 0.

Use of the "code" sharply reduces the number of 1-mutant neighbors. Each DNA sequence has only 1008 1-mutant neighbors, which are obtained by substituting any of the three other possible bases in each of 336 sites. In addition, due to synonymous codons, only about 75% of these result in substitution of a new amino acid. Thus in the versions of the model based on coding, each model V region has about 756 1-mutant neighbors rather than $19 \times 112 = 2128$ 1-mutant neighbors.

(5) *Complementarity determining regions (CDRs) or not.* The *NK* model, in its general form, is isotropic. It assumes that all sites make direct contribution to fitness of the overall string, whether that string is interpreted as a genotype, or protein. Proteins, however, may be more hierarchically constructed, with some sites, e.g. amino acids in the actual active site of an enzyme, or binding site of an antibody molecule, having direct bearing on function, while others play a more modest support role.

As remarked above, in the V region of antibody molecules, special hypervariable regions called complementarity determining regions, are known to play a critical role in antibody diversity and in actual binding of the antigen. The surrounding parts of the V region are thought to be a supporting "framework" for the basic structure of the binding site. A simple way to begin to model the distinction between CDRs and framework is to assume that only the amino acids in the CDRs make direct impacts on the fitness of the V region, while those in the framework act via their influence on the CDR amino acids. Thus as a first effort we have modeled the existence of three CDRs by assigning three contiguous regions of amino acid positions in our model V regions, matching those in observed V regions and measured the fitness contributions only of the amino acids in the model CDRs. Figure 9(a) shows CDRs in a particular V region. We utilized three regions of these sizes and spacing, with a total of 37 amino acids, in our modeled V regions. Because the amino acids in the framework interact with the amino acids in the CDRs, they still have an indirect bearing on fitness.

(7) *Choice of K.* At this point, the remaining free parameter in the model is K. The experimental data we describe in more detail immediately below show that walk lengths in actual affinity landscapes average between six and eight steps, but with considerable variance. Walks start well up on adaptive hill sides where the starting germ line V region initially amplified by clonal selection is in the highest 9999th percentile. Thus we seek a value of K such

that walks to local optima from that starting percentile average six to eight steps. This is the central parameter matching step in applying the *NK* model. We use two features of the immunological data: the fraction of B cells which respond to an antigen sets the starting percentile in affinity space; the number of mutations substituting amino acids in the V region during maturation sets the mean walk length to optima. Given these we can *find* the value of K which yields walks with the appropriate length by carrying out numerical simulations at various trial values of K.

Affinity landscapes are correlated

An immediately interesting point arising from framing these questions is that the appropriate value of K must be less than the maximum, $K = N-1$, and therefore that antibody affinity landscapes must be *correlated.* This follows from examining walk lengths at the upper extreme value for K, $K = N-1$, corresponding to a fully random landscape. Here the probability that a model V region with rank order fitness x is fitter than its $19N$ fitter neighbors is x^{19N}. Thus, any starting peptide that is in the top 99·999th percentile in fitness has roughly a 98% chance of already being fitter than its 2128 1-mutant neighbors. That is, *if affinity landscapes were entirely uncorrelated, initially selected germline variants would already be local optima.* Since maturation of such antibody molecules does occur we can conclude both that affinity landscapes are correlated, and that, within the *NK* model, K must be less than $N-1$.

EXPERIMENTAL FEATURES OF AFFINITY LANDSCAPES RAISED BY THIS ANALYSIS

Maturation of the immune response occurs on a rugged affinity landscape whose structure is only partially known. Whether the *NK* model itself proves to be a good model for the structure of that landscape, a major purpose in this article is to focus attention on the structure of such landscapes. In general, all the questions raised previously regarding abstract landscapes are *a fortiori* of interest with regard to the immune system:

(1) How many improvement steps must be taken from any initial antibody molecule to a local optimum, i.e. how many somatic mutations accumulate in the V region of an initial roughed in germ line variant antibody molecule during maturation? The answer, as mentioned above, appears to be a range, with a mean of six to eight (Bothwell *et al.*, 1982; Tonegawa, 1983; Heinrich *et al.*, 1984; Berek *et al.*, 1985). For example, Crews *et al.* (1981) studying the V_H gene responding to phosphoryl choline find between one and eight residues changed; Bothwell *et al.* (1982) find three mutations in a lambda (λ) light chain and six in a λ_2 light chain V region; McKean *et al.* (1984) studying the V_k region of antibodies against a determinant on influenza found seven or eight replacements; Clark *et al.* (1985) studying the secondary response to influenza found 20, 12, and 19 V_h coding mutations, and 9, 8, and 15 V_k coding mutations.

(2) What fraction of 1-mutant variants of the initial roughed in germ line antibody have higher affinity for the immunizing antigen? How does that fraction

change, presumably dwindling to 0, as successively higher affinity antibody molecules are selected during maturation of the immune response? Here it is known that a large fraction of the 1-mutant variants have lower affinity, but the exact fraction with higher affinity at any step of the maturation processes is unknown.

(3) How rugged is the affinity landscape in the 1-mutant vicinity of local optima? The question of whether affinity falls off dramatically in some directions and slowly in others translates directly to whether mutations at specific positions in the V region cause dramatic loss of affinity while those at other positions cause little loss of affinity. Restated, the distribution of the number of amino acids which can be substituted at a site with retention of function is a direct picture of the local ruggedness of the affinity landscape.

(4) How many alternative local optima can be reached from any initial roughed in germ line antibody amplified by initial clonal selection? Further, what is the probability of climbing to each of those alternative optima, hence the density of their occupancy? Here recent work with inbred mice (Slaughter & Capra, 1984; Perlmutter, 1984) has demonstrated that multiple local optima are accessible. In many cases initial clonal selection opts for the same initial V region, which then climbs to different mature forms by accumulating different somatic mutations. It appears from these and similar experiments that the number of alternative optima accessible from the initial antibody may be at least modestly large. Typically, comparison of five to ten monoclonal antibodies deriving from the same initial V gene shows that all differ from one another. Because only small numbers of sequences have been compared in this way, it is unknown whether a much larger number of local optima are accessible.

These experiments are not entirely unambiguous. As remarked above, we have assumed that mature antibodies are actually local optima, and one of the predictions of the *NK* model will be that many local optima should be accessible. However, the fact that different mature antibodies emerge from the same V gene is insufficient to confirm our reasoning. From the work of Eigen & Schuster (1979), Eigen (1985), Schuster (1987), and Kauffman (1989), as well as classical population genetic analyses (Ewens, 1979), we know that a mutant spectrum around an optimum can be expected, and we know that the rate of hypermutation is high. Thus the diversity seen in mature antibodies derived from one initial V gene may reflect the incapacity of clonal selection to eliminate near neutral variants.

(5) How similar are the local optima? Maturation climbs to alternative local optima from an initial roughed in V region. The typical observations, described more fully below, when several different monoclonal antibodies derived by maturation are compared is that many amino acids are "conserved" while a smaller fraction are repeatedly mutated with respect to the initial V gene. Furthermore, some sites repeatedly have mutated "in parallel" to the same alternative amino acid (Slaughter & Capra, 1984).

As stressed, we use observed walk lengths and starting percentile to "tune" *K*. Hence fitting walk lengths with the *NK* model is just curve fitting. However, the

value of K which we derive, and the remaining features (2)–(5), are aspects of the immune response about which the NK model makes clear and testable predictions. We return to this below after describing our simulation results.

Predictions of the Model and Comparison with Experimental Data

THE APPROPRIATE VALUE OF K IS NEAR 40

Numerical simulations were carried out for all versions of V region models. Of these, presumably the most realistic combination includes both the CDRs and the genetic code. But as we will see, all of the possibilities predict the same features of the landscape and qualitatively agree with the available experimental data. The results are remarkably robust. As Table 4 shows, whether CDRs were included or not, whether all $19N$ protein neighbors of the V gene were used or translation via the genetic code, was used, and whether the K sites were constrained to be flanking adjacent sites or chosen at random, a value of $K = 40$ gives rise to walks of between 6 and 12 steps. $K = 30$ typically yields walks which are too long. $K = 50$ typically yields walks which are too short. Since walk lengths are largely insensitive to the remaining parameters, to a very good first approximation, the dominant parameters are N and K.

There is considerable dispersion about this mean value. As shown in Figures 4(a) and (b), for a given value of N and K and under defined conditions for the rest of the model conditions, walk lengths might range from 2 or 3 to 15 to 20. This dispersion reflects again the ruggedness of the landscape and is encouraging, given the fact that there is a similar dispersion in the experimental data.

Ultimately, the NK model predicts some specific *distribution* of walk lengths to optima, not just a mean and standard deviation. Thus accumulation of adequate experimental data can ultimately establish the actual observed distribution of walk lengths for comparison to the NK model or improved models.

Finding a specific value for K is, in itself, interesting. If the model is taken literally, K stands for the number of amino acids which bear on the fitness contribution of each amino acid. Then, if K is about 40, alteration in a single amino acid could affect the behavior and function of roughly 40 amino acids in the V region. Is this plausible and is there any evidence bearing on the issue?

In a well folded protein, an amino acid is not only open to influence by those which are its neighbors in the primary sequence, but also those which are near it in the folded form due to juxtaposition with amino acids distant in the primary sequence. For example, each of the CDR loops is typically hydrogen bonded to one or more of the other CDRs as well as points in the framework (Shenkin, 1987). In turn, any of those amino acids is itself coupled to its neighbors in the primary sequence.

One approach to studying this question is based on hydrogen exchange data. The experimental technique consists in observing a protein in deuterated water. Hydrogen atoms on different amino acids in the protein exchange with the deuterium at different rates which depend upon the product of an intrinsic exchange rate for a

TABLE 4

Mean walk length to local optima, mean number of fitter neighbors from the first model V region on the walk, and mean number of sites of the locally optimal V region attained which could be substituted with maintenance of above "threshold" fitness. Simulations were carried out for different versions of the model, as shown. The number of trials under each condition was ten with conditions using the DNA code and 25 for those without it. Standard deviations shown in brackets

	K	Average walk length	Average no. fitter neighbor on 1st step	Mean no. of allowed subst./site
Protein (no cdr)				
adjacent	30	13·6 (3·2)	65·9 (20·4)	15·4 (3·6)
	40	8·6 (4·5)	24·7 (9·9)	4·1 (3·9)
	50	4·9 (2·3)	10·3 (4·9)	0·8 (1·4)
random	30	17·7 (4·4)	83·7 (13·0)	17·9 (2·4)
	40	11·5 (5·3)	42·1 (6·9)	8·5 (5·8)
	50	6·6 (2·2)	17·5 (3·5)	2·1 (3·0)
Protein (with cdr)				
adjacent	30	26·1 (7·1)	89·7 (27·8)	12·5 (6·6)
	40	9·8 (3·7)	27·7 (11·3)	5·0 (5·5)
	50	7·0 (3·3)	12·7 (6·0)	1·5 (2·9)
random	30	16·1 (5·3)	81·8 (16·2)	15·5 (4·5)
	40	12·0 (3·9)	36·9 (9·3)	7·4 (5·8)
	50	7·3 (3·2)	17·0 (5·8)	1·9 (3·0)
DNA Code (no cdr)				
adjacent	30	8·4 (2·3)	20·1 (12·7)	10·5 (5·1)
	40	5·2 (2·7)	7·3 (4·3)	3·4 (3·8)
	50	2·9 (2·1)	3·9 (2·5)	0·3 (0·7)
random	30	11·7 (4·5)	24·6 (8·2)	14·1 (5·1)
	40	6·9 (3·3)	11·2 (4·9)	5·7 (5·1)
	50	3·1 (1·9)	4·5 (2·8)	1·0 (2·0)
DNA Code (with cdr)				
adjacent	30	15·1 (5·6)	41·8 (17·5)	9·7 (7·5)
	40	6·7 (3·1)	17·1 (12·1)	3·2 (4·6)
	50	3·5 (2·1)	7·0 (7·0)	0·6 (1·7)
random	30	11·2 (4·1)	27·4 (9·3)	11·9 (6·1)
	40	7·6 (2·7)	10·6 (5·5)	3·8 (4·5)
	50	4·1 (2·2)	5·6 (3·5)	0·7 (1·7)

hydrogen exposed to deuterated water and the frequency of exposure. That frequency in turn depends upon subtle "breathing" motions of the protein as it twists and unfolds slightly in different ways. Thus hydrogen exchange is a sensitive measure of protein behavior. Wand *et al.* (1986) and Roder *et al.* (1989) have studied such hydrogen exchange in numbers of proteins. While they have not yet analyzed differences between a protein and a 1-mutant variant, they have looked at the oxidized and reduced form of cytochrome C, a 106 amino acid protein. Oxidization

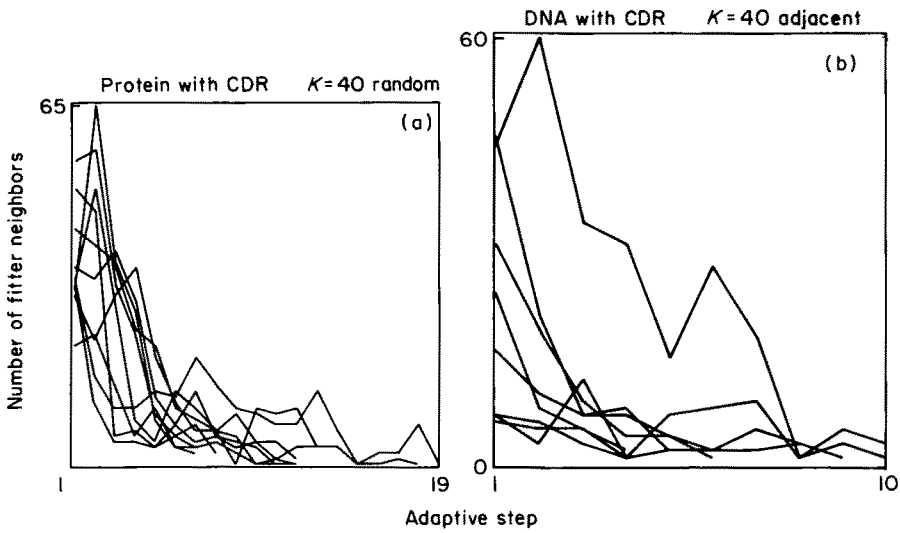

FIG. 4. The number of fitter 1-mutant neighbors at each adaptive step from the initial model V region, the best in 100,000, on several different adaptive walks to local optima for the same values of the *NK* model parameters. (a) Modeling the V region with CDR regions present, and with $19N$ 1-mutant neighboring proteins. (b) As in (a) except that 1-mutant neighbors were determined via the DNA code.

and reduction, due to presence or absence of a charge on the heme group, correspond very roughly to substitution of a charged for an uncharged amino acid in that vicinity. Roder *et al.* (personal communication) have been able to examine 50 hydrogen bonded pairs of amino acids, and find that at least 30 of them alter their exchange behavior in the oxidized and reduced forms. The very crude conclusion to be drawn is that a charge alteration at one point in a protein can affect at least 30 amino acids. Since these authors studied only half the hydrogen bonded atoms, the number of amino acids affected by altering one amino acid may be greater than 30. This point has obvious caveats. The study is not of an amino acid substitution, but of an altered heme group. Further, to have found a statistically significant alteration in hydrogen exchange by an amino acid does not yet say that such alterations are in any way relevant to the function of the protein. Third, the cytochrome C molecule may be well evolved to undergo alterations when the heme group is charged. Many fewer alterations in hydrogen exchange behavior might be found by randomly substituting amino acids in proteins. Nevertheless, the data suggests that any amino acid might be affected by, and affect, as many as 30 amino acids in a protein region of about 106 amino acids. Direct testing in antibody molecules would require study of hydrogen exchange in the V region of a mature antibody and its 1-mutant variants.

THE *NK* MODEL MAKES PLAUSIBLE PREDICTIONS ABOUT THE FRACTION OF FITTER 1-MUTANT VARIANTS

Given a value of $K = 40$, the *NK* model makes clear predictions about the fraction of fitter 1-mutant variants of the first roughed-in V region and about the fraction

of fitter 1-mutant variants of each improved variant on the adaptive walk. The expected number of fitter variants to the first V region is on the order of one or two percent in all of the combinations of conditions mentioned above (Table 4). In those runs that used all $19N$ 1-mutant neighbors, 24–42 among the 2128 1-mutant variants are typically fitter. When translation via the genetic code (and the implicit constraints in the 1-mutant neighbors) were added, typically there are about seven to 17 fitter 1-mutant variants among the 1008 1-mutant nucleotide substitutions and about 756 1-mutant V regions at the protein level (Table 4).

There is moderate variance in the fraction of fitter 1-mutant variants of the initial V region on individual walks. The minimum we have found on the initial step is 1, and the maximum is 70, or over 3%.

The fraction of fitter variants dwindles, but not smoothly on any specific walk, to 0 over the steps to the local optimum, as shown in Fig. 4(a) and (b).

The actual fraction of fitter variants in maturing antibody molecules is not yet known in detail, but the experimental procedure to find this fraction is clear: Monoclonal antibodies at different stages during an adaptive walk must be obtained, the gene cloned, and the 1-mutant spectrum examined for the affinities of the 1-mutant variants. However, studies of the *lac* repressor provide an indirect estimate of the number and the nature of fitter one mutant variants of a roughed in V region.

The *lac* repressor monomeric unit has 360 amino acids. Miller *et al.* (1979), studied a collection of over 300 altered proteins, each by a single substitution, with respect to both ligand binding activities. The mutant forms were generated in a controlled way by use of 90 different nonsense sites in the corresponding *lac* I gene which were suppressed by a set of nonsense suppressors of amber (UAG), ochre (UAA) or UGA mutations. The classes of substitutions allow substitution of similar (e.g. hydrophobic for hydrophobic) and dissimilar amino acids at 25% of the positions in the normal molecule.

Mutant phenotypes due to loss in capacity of the repressor molecule to bind to the operator DNA, or to bind allo-lactose, (or the synthetic inducer IPTG) or due to an increased affinity for the DNA or IPTG, allowed Miller's group to study the consequences of such mutations conveniently. The overall results from 323 single amino acid replacements is that 42% result in a detectable change in either the capacity to bind IPTG, or the operator DNA. The remaining 58% appear to be "silent" and do not result in measurably altered proteins. About 33% of the replacements decrease capacity to bind to the operator DNA, although only 15% of the substitutions destroyed 25% or more of the capacity, and only 8% became fully inactive. About 11% reduced affinity for the inducing metabolite. On the other hand, 1% of all one step mutants actually increased affinity for the operator DNA, in some cases by as much as 100-fold. No one step mutant was found which increased affinity for IPTG.

From this study we can draw at least the following conclusions. First, even well tuned proteins may have rare variants which "improve" a given function. Here, one percent of the 1-mutant neighbors, at a restricted number of sites, showed increased affinity for the operator DNA, and no mutant was found which showed increased affinity for the inducer metabolite. Second, 58% of the single amino acid substitutions

had no obvious effect. Because the assays employed are rough measures, the reported fraction of silent mutants is probably an over estimate. However, since 42% of the mutants clearly do reduce affinity for the operator or inducer or both, it is very unlikely that more than a small fraction of the one step neighbors subtly increase affinity.

Our simulation results suggest that an initial germ line V region with $K = 40$ would be open to improvement by about 1-2% of the 1-mutant variants. This is very close to the observed data for the lac repressor. Thus, tuning K to fit observed walk lengths yields a value which, having tuned the ruggedness of the fitness landscape, predicts a plausible value for the expected fraction of fitter 1-mutant variants of the initial germ line V region amplified by clonal selection.

Note that these predictions of the NK model are fairly sensitive to K. When $K = 30$, roughly 3-5% of the 1-mutant variants of the first clonally selected V region have higher affinity, while walks to optima would average about 13 steps. For $K = 20$, the average walk length is 22 steps and about 7% of the 1-mutant variants of the initial antibody are fitter.

THE *NK* MODEL PREDICTS THE EXISTENCE OF CONSERVED AND VARIABLE SITES IN THE V REGION

In real proteins, antibodies and otherwise, it is widely known that some amino acids cannot be altered without drastic loss of function while amino acids at other positions can be altered with relative impunity. It is therefore of interest to ask whether the NK model, for the parameters given, predicts this phenomenon without further assumptions.

To answer this question, we computed the fitnesses of all of the 1-mutant neighbors of the local maxima obtained in our walk simulations. In order to make valid comparisons between simulations with and without the genetic code, we substituted all 19 other amino acids in each site of the V region, including those amino acids that required several mutations of the corresponding DNA sequence. The results, for different values of K and for different assumptions about whether the entire V region or just the CDRs are used in computing fitness, are as expected. The first main result is that as K increases, the jaggedness of the landscape increases. In other words, landscapes are smoother for low K than for high K. The second major result for $K = 40$ is perhaps more surprising: at some sites, any model amino acid substitution causes a dramatic loss of fitness, while at others, all substitutions cause almost no loss of function. At still other sites, some substitutions cause almost no loss of function while other amino acids in the same site cause drastic loss of function. Thus, without further assumptions, the NK model for these parameters gives a highly rugged landscape in which amino acids at some sites in the locally optimal V region must be entirely conserved to preserve function, and amino acids at other sites can be substituted indiscriminately.

Note that, in constructing the general NK model, no site is *a priori* more important than others. It is instead the fact that K is high, resulting in a rugged landscape which predicts that some sites are conserved and others broadly substitutable.

A particularly interesting view of these results is the following. We have no direct scale relating "fitness" in the *NK* model with real affinities of antibody molecules. However, real antibody walks start with those already the best in 100,000, and such antibody molecules typically have affinities of about 10^{-4} M for their antigens. In contrast, matured antibodies have affinities around 10^{-7} M. Then it is sensible to define the fitness of the first member of the model walk as corresponding to a modest affinity of 10^{-4} M, and let this fitness serve as a threshold separating model V regions which do and which do not bind the antigen. Given this threshold, we can test the number of substituted amino acids at each site in the 112 long optimal V region which preserve "at least above-threshold" function. Figures 5(a),(b) show the results for model V regions for different values of K. Similar results are found for the different versions of the model with and without CDR, coding, or choice or adjacent or random epistatic connections (Table 4). Again, it is K that determines the qualitative features of the landscape. For $K = 20$, each of the 112 sites can be substituted by all 19 of the other amino acids and the mutated model V region remains above threshold in affinity. For $K = 30$, most sites are substitutable by 19 amino acids, but some sites can only be substituted by 15, 16, . . . , 18 amino acids. For $K = 50$, almost no sites can be substituted with any amino acids and preserve above threshold affinity. But for K about 40, a very wide distribution is found. Some sites can be substituted by 19 other amino acids, some by 15, some by 10, some by 5 and some by 0. Thus K about 40 yields the broadest distribution. We emphasize that this broad distribution is a *prediction* of the *NK* model.

The experimental data to test this prediction would consist in a high affinity mature monoclonal antibody against a defined epitope, and its entire 1-mutant spectrum with respect to V region mutants. The affinities of that mutant spectrum constitute the data set. It is not available, but the experiment is obviously feasible using cloned antibody molecules. Nevertheless a rough approximation to this experiment is available. Geysen *et al.* (1985, 1986, 1987), Getsoff *et al.* (1987) and Fieser *et al.* (1987) have studied the effects of all possible 1-mutant variations in an *antigen* upon the antigen's affinity for the antibody. More precisely, in these studies, the authors raised polyclonal sera or monoclonal antibodies against a defined six amino acid long epitope on a protein antigen, then made synthetic hexamers identical to that epitope and demonstrated that the hexamer was bound by the sera or monoclonal antibody at high affinity. Then in each case the authors looked at all 20 variants at each of the six positions, one position at a time. The results for nine such peptides are summarized in Fig. 6. In this summary we have utilized the authors data and set an arbitrary threshold of about 10% affinity compared to the "wild type" hexamer as the criterion for "function". The striking feature is that the distribution is again very broad. Some sites are not substitutable at all, others are substitutable by all 19 other amino acids, still other sites accept some fraction of the 19 and retain affinity.

An interesting feature of the observed distribution is that it is not only broad, but bimodal. Sites more likely to be entirely substitutable, or not substitutable at all. A bimodal distribution emerges rather naturally from our model if a *small distribution of* K *values* between 30 and 40 is assumed. Whether the bimodality is to be taken seriously at this stage is uncertain.

(a)

Allowed substitutions per site	$K=20$		$K=30$		
0	0	0	0	0	
1	0	0	0	0	
2	0	1	0	0	
3	0	1	0	0	
4	0	2	0	0	
5	0	2	0	0	
6	0	3	0	0	
7	0	5	0	0	
8	0	4	0	0	
9	0	20	0	1	
10	0	7	0	0	
11	0	18	1	5	
12	0	10	5	6	
13	0	11	6	3	
14	0	8	4	7	
15	0	10	7	12	
16	0	5	12	16	
17	0	4	12	14	
18	0	1	21	25	
19	112	0	44	23	
	19·00	11·13	17·14	16·37	Mean
	0·00	3·27	2·17	2·39	S.D.

(b)

Allowed substitutions per site	$K=40$			$K=50$			
0	7	3	7	105	63	85	
1	4	4	14	6	31	20	
2	11	5	6	1	13	6	
3	10	14	8	0	4	1	
4	15	9	3	0	1	0	
5	15	9	5	0	0	0	
6	12	13	5	0	0	0	
7	11	9	3	0	0	0	
8	7	6	5	0	0	0	
9	6	8	6	0	0	0	
10	3	5	6	0	0	0	
11	5	6	5	0	0	0	
12	2	4	7	0	0	0	
13	2	5	6	0	0	0	
14	1	5	4	0	0	0	
15	0	2	9	0	0	0	
16	1	4	8	0	0	0	
17	0	0	3	0	0	0	
18	0	0	2	0	0	0	
19	0	1	0	0	0	0	
	5·49	7·23	8·17	0·07	0·65	0·31	Mean
	3·36	4·29	5·62	0·29	0·88	0·61	S.D.

FIG. 5(a). The distribution of the number of sites in a locally optimal model V region with 112 sites which are substitutable by 0, 1, ..., 19 other amino acids with maintenance of "affinity" or fitness above a "threshold level" defined by the affinity or fitness at the start of the adaptive walk. $K = 20$ or 30 adjacent. (b) As in (a), except $K = 40$ or 50.

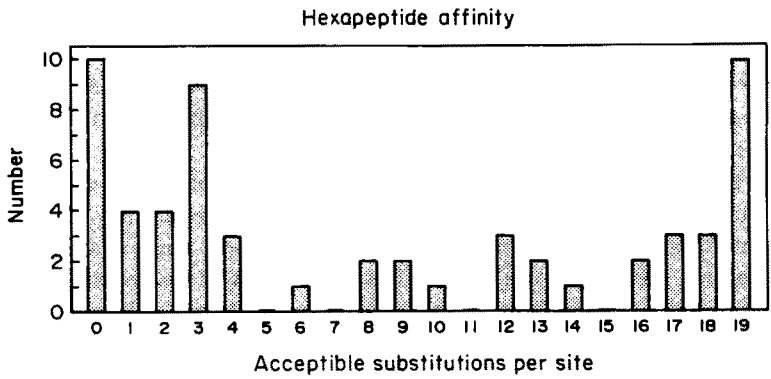

FIG. 6. Experimental distribution of acceptable number of amino acid substitutions per site in hexamers with maintenance of 10% or more of the affinity of the "wild type" hexamer for the polyclonal sera or monoclonal antibody.

Four comments are warranted. First, it is clearly encouraging that the *NK* model predicts a broad distribution for K near 40, and such a broad distribution is found. Second, we have defined a "threshold" affinity as the fitness of the first model V region in the walk, the best in 100,000. We do not know how this threshold bears on the experimental affinities measured. Third, the experimental data need to be used cautiously in this context. They concern free hexamers bound to polyclonal sera, or monoclonal antibodies, not the number of substitutions at each position within the V region of a mature monoclonal antibody molecule. The constraints within a V region may or may not dramatically alter the observed distribution. Fourth, taking data and model for the moment at face value, the same value of K which fits walk lengths to optima also predicts a reasonable fraction of fitter 1-mutant variants, and genuinely predicts that some sites allow no substitutions while others are more permissive. Were K much smaller, say 20, almost all sites would be open to substitution by most model amino acids. The prediction is thus sensitive to K.

THE NUMBER OF ALTERNATIVE LOCAL OPTIMA FOUND FROM AN INITIAL V REGION

The *NK* model for these parameters allows us to examine the number of alternative optima accessible from the initial model V region, and also to test whether alternative accessible optima are typically "hit" equally often on independent walks, or with biased preferences.

The experimental data on repeated walks from the same V region remain scant, as noted, but clearly suggest that multiple optima are accessible from the same initial V region. The true number of such local optima is not known experimentally, but presumably is greater than the five to ten alternatives often observed.

Numerical simulations with $K = 40$ were carried out from initial model V regions, based on use of the DNA code, and were stopped by limitations of computer storage. In two simulations making 797 and 315 such walks from the same initial V region

150 and 235 optima were found. Because many of these optima were found only once, it is difficult to know how many more remain to be accessed from the same initial V region. However, it is a clear prediction of the *NK* model that a given initial germ line V region can give rise to hundreds, perhaps thousands of mature antibodies, each a local optimum in affinity space.

A second feature of these studies is shown in Fig. 7, which is the histogram of the numbers of times each local optimum was "hit" on independent random walks from the same initial model V region. As can be seen, four optima are each encountered about 130 times. Analysis of these four showed that each is a 1-mutant variant of the initial V region from which walks started. Ultimately, the *NK* model predicts a distribution which is open to experimental testing. The density distribution with which nearby local optima are reached is another expression of the ruggedness of the fitness landscape.

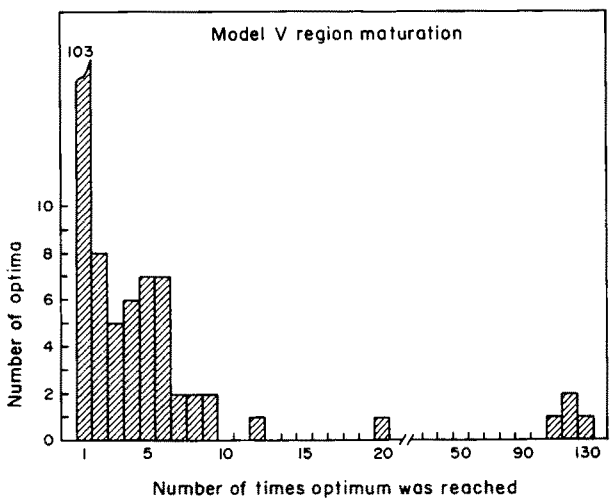

FIG. 7. Multiple adaptive walks from the same initial model V region, $K = 40$, showing how many optima were "hit" once, twice, or many times. Note that some optima were encountered 130 times on independent walks from the same initial V region.

The Similarity of Alternative Local Optima; Conserved Sites and Parallel Mutations

Comparison of alternative mature V regions obtained experimentally reveals that not all sites in the V region accumulate somatic mutations equally. In particular, some sites are rarely mutated, and among the sites which are preferentially mutated, sometimes the *same* amino acid is substituted on two or more independent walks. These are called "parallel" mutations. To see whether these phenomena are observed in the *NK* model landscapes, we compared four to 11 alternative optima accessed from the same initial V region. Similarly, experimental data sets often compare five to seven V regions obtained by independent walks from the same initial V region

	K	Number of observations		Number of experiments	Number of sequences
A	20	79	<	91	9
A	25	68	<	83	9
A	25	55	<	68	8
A	30	48	=	48	7
A	30	52	<	62	10
A	40	41	<	54	5
A	40	36	<	43	9
A	40	35	<	39	4
A	40	27	<	33	10
A	40	41	<	54	7
A	40	34	<	37	7
A	50	20	=	20	4
A	50	18	<	27	11
A	50	15	<	17	4
A	50	21	<	23	6
R	40	47	<	53	5
Antiarsonate vs "germline"		13	<	23	6
Antiarsonate vs prototype antiars		28	<	42	6
Antiphosphocholine vs "germline"		21	<	26	8

FIG. 8. Comparison of numerical results for model V regions with different parameter values, and observed alternative V regions derived from a single initial V region, with respect to the deviation in the number of mutated and non-mutated sites from chance. In all cases, model and real V regions show that fewer sites are mutated than expected by chance, suggesting "hot spots" and conserved regions.

(Slaughter & Capra, 1984; Perlmutter, 1984). Figure 8 shows that for experimental and model V regions the numbers of sites which accumulate mutations in sets of the local optima compared to the initial V region is less than expected by chance. This means that some sites are preferentially not mutated, and others are mutated more often than expected in real and model V regions. Figures 9(a),(b) show experimental data sets for two different clusters of V regions, one for the arsonate system (Slaughter & Capra, 1984), the other for phosophocholine (Perlmutter, 1984). In addition Fig. 9(c) shows ten local optima and the initial model V region for an example with $K = 40$. Note that in the real and experimental sets some sites have similar parallel mutations.

Conclusions, Caveates, and Directions for Future Work

How seriously should we take the *NK* model as an account of the structure of affinity landscapes? With considerable, but not unbridled enthusiasm. The *NK* model is the first effort at a statistical model to predict the rugged structure of fitness landscapes in sequence space. A single choice of parameter values, $N = 112$ as set by the known length of the V region, and K about 40 as tuned to fit known walk lengths to mature antibodies predicts a number of features of antibody affinity landscapes well. It is premature to say that the *NK* model predicts these features

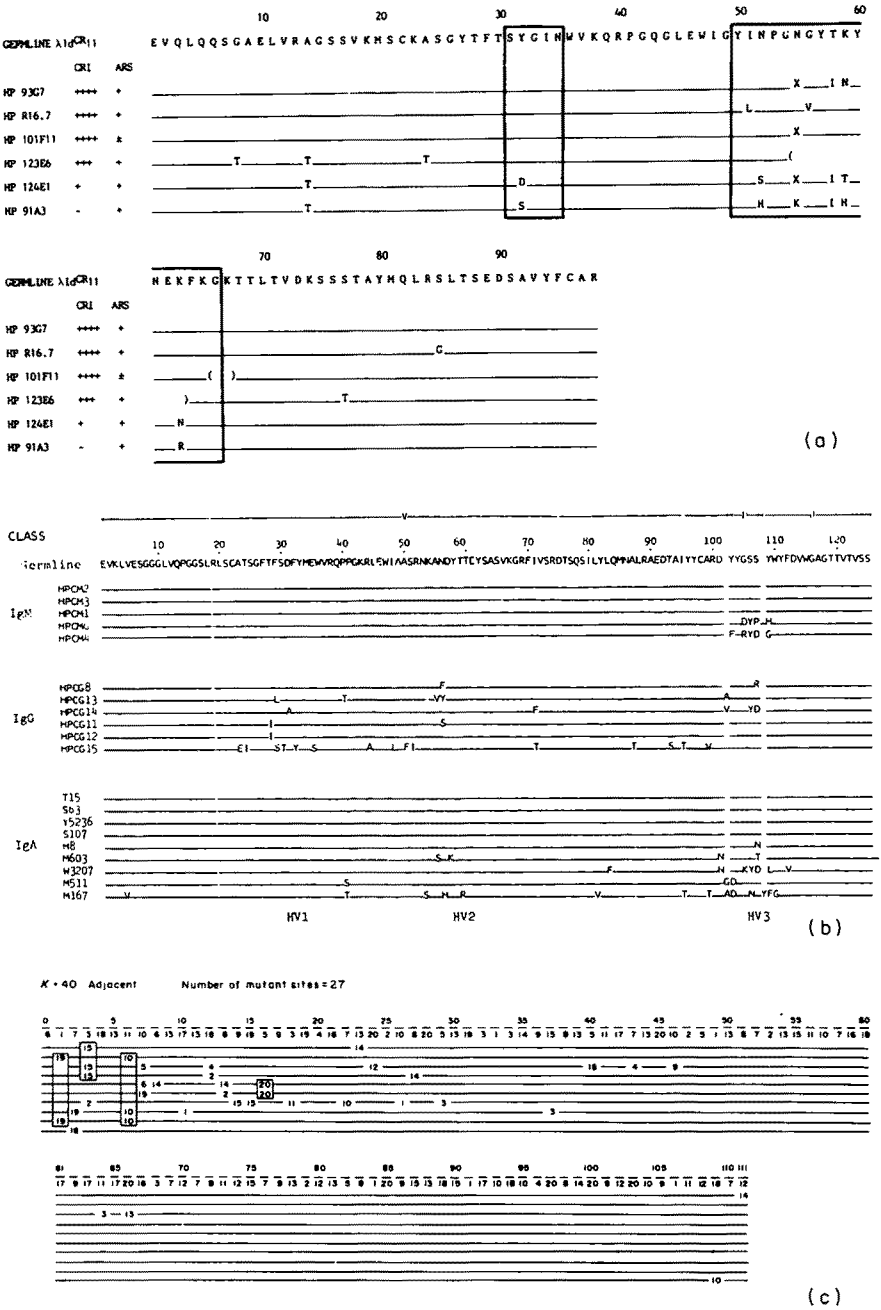

FIG. 9. Observed initial antibody sequence early in the immune response and alternative mature forms which evolve from each. Letters on mature strands show substitutions relative to the initial germ line V region from which maturation occurs, top strand. (a) The arsonate system, Ars-A (Slaughter & Capra, 1984). Boxes correspond to the three CDR regions. (b) The anti-phosphocholine system (Perlmutter, 1984). (c) Comparison of initial model V region and ten different local optima found on adaptive walks from that initial V region. Numbers on different "mature" strands correspond to substitutions with respect to initial model V region. Boxes show parallel substitutions arising independently on independent adaptive walks to different local optima.

accurately. All we can say now is that the predictions are very plausible. Direct experimental investigation with cloned V regions at different stages of maturation are needed to test the predictions. Even more directly, one might imagine carrying out an entire adaptive walk by fitter 1-mutant variants *in vitro* beginning with a cloned V gene from the initial B cells which respond.

Although broadly successful, the *NK* model as tested does exhibit certain failures. In particular, during maturation of the immune response there appears to be a tendency for mutations causing amino acid substitutions to accumulate preferentially in the CDRs. Further, there may be a tendency for less than the expected number of mutations causing substitutions than expected by chance to accumulate in the framework regions outside the CDRs (Shlomchik *et al.*, 1987). If true, these biases are not captured in our current application of the *NK* model to V regions. Such biases might reflect evolutionary specialization of the framework create the fundamental structure of an antibody binding site, while the CDRs specialize for antigen binding. In this view, the framework is highly adapted and easily disrupted, leading to overall loss of binding by the entire V region. Modeling such a *high adapted* character of the framework is ignored in our modeling of CDRs and frameworks. Instead we tested the case in which only CDRs make direct contributions to fitness and the framework acts indirectly via the CDRs. An alternative approach would be to allow the framework amino acids, on average, to affect more than K other sites. Use of a distribution of K values as epistatic "inputs" or "outputs" might provide a better model of the possible hierarchical epistatic relations among amino acids to overall function.

The fact that the *NK* model appears to succeed as well as it does is encouraging in at least three respects. First, it suggests that a statistical model may well capture the actual structure of fitness landscapes. Second, if the *NK* model, or an improved model, can predict the statistical structure of antibody affinity landscapes, it may also be able to predict the structure of fitness landscapes with respect to enzymatic function. Both involve the evolution of a structure with a "business end"—the antigen binding site in the case of the antibody and the active site in the case of the protein. Third, if the *NK* model is close to right, it may be telling us something fundamental about how proteins work. In solid state physics spin glass models (Binder & Young, 1986; Stein *et al.*, 1987) capture the real behavior of physical spin glasses by assuming interactions are so complex that the statistical distribution of their effects can be captured by random assignments of coupling energies. The same may be true for proteins. We comment briefly on these issues.

Certainly the most important implication of the rough success of the *NK* model at this stage is the hint that some statistical theory may some day actually fit well established data on the actual structure of the affinity landscape. Obvious refinements of the model would include more of the details of protein chemistry. Thus, as suggested above, some sites should have more interactions than others, reflecting the fact that some amino acids have more hydrogen bonds, hydrophobic bonding and salt bonds than others. One would therefore like K to be chosen from a distribution of possible values. In this simplest application of the *NK* model the identity of the A amino acids at each site bears no relation to the identity at another

site. Amino acid no. 7 is merely a name specific to each site. In reality, alanine at each site is the same amino acid. Thus, the nature of the interactions should reflect the fact that alanine at each site is the same amino acid and that different amino acids have different chemical properties.

Whether or not the theoretical predictions regarding adaptive walks on correlated landscapes generated by the *NK* model fit the experimental data is far less important than obtaining real insight into the true adaptive landscape of antibody molecules with respect to affinity for a specific epitope. It is worth stressing again the data needed to extend understanding Perhaps most important is analysis of the affinity of *all* 1-mutant variants of a given antibody molecule for the same epitope. Second, as the immune response matures, and higher affinity antibodies are amplified, it is important to examine whether the number of fitter V regions decreases as affinity increases. Third, we need to know the distribution of amino acid substitutions which allow modest affinity for the epitope. Is it broad or not? Fourth, we need good data on sequence similarities during branching walks to alternative optima from the same V region. As noted, perhaps the best way to obtain the requisite data is to carry out *in vitro* adaptive walks from cloned examples of the initial germ line variant amplified by clonal selection. At each step all 1-mutant variants should be generated, and one or more selected to carry on the walk to local optima. The actual structure of affinity landscapes is open to direct investigation and is one piece of the immune system puzzle.

We have focused in this article on the structure of affinity landscapes. But the immune response itself depends upon clonal selection of mutating B cells flowing across this landscape under the drives of antigen stimulation in the context of regulatory effects due to T cells, growth factors, and antiidiotype effects, as well as proliferation of T cells and other cellular components of the immune response. An obvious immediate direction for investigation is exploration of the proliferation and flow of B cell populations across rugged affinity landscapes. As in models of molecular evolution on rugged landscapes (Eigen, 1987; Schuster, 1987; Kauffman 1989*a*,*b*), a variety of behaviors including "freezing" of the adapting population into small regions of the space, and diffusive flow among near neutral mutants along ridges in the affinity landscape, as functions of the mutation rate, population size, and fitness landscape structure, are to be expected.

ARE PROTEIN ADAPTIVE LANDSCAPES AND FOLDING LANDSCAPES RELATED?

We close with a question. Adaptive landscapes appear to be very rugged and may be captured by something like the *NK* model. The clear relation between the *NK* model and spin glasses was noted above. Spin glass models are currently proving useful as models for protein folding itself (Ansari *et al.*, 1985; Stein *et al.*, 1987; Karplus *et al.*, 1987), and protein folding is a complex process of "self binding," rather than binding to another molecule. Spin glass models stress the idea that the potential surface guiding protein folding is likely to be very complex with many local minima. Proteins, once folded, presumably "breathe" by undergoing transitions between these minima. Clothia (1987) and Karplus *et al.* (1987) comment that

families of evolutionary related proteins undergo shape deformations of their crystallized form on the same scale as the breathing deformations of a single protein. This suggests that the range of readily available shape deformations of proteins, guided by intramolecular forces, is closely related to the range of shape and function deformations in protein evolution. In turn, the function of proteins in binding ligands and catalyzing reactions is primarily due to the similar shape and force properties. Might it be the case that the statistical character of the potential surface underlying folding of proteins is intimately related to the statistical character of adaptive landscapes in protein evolution? If so, then spin glass models, or the *NK* model or a similar improved model may capture the right statistical features of both.

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