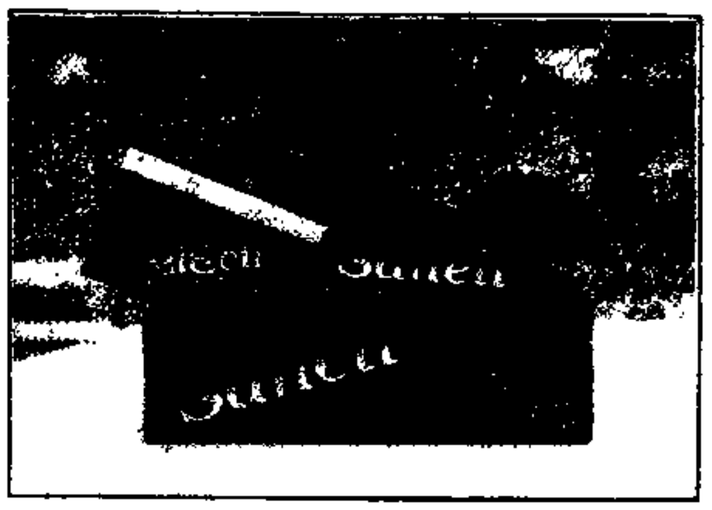
India launches new weekly contraceptive pill

The Central Drug Research Institute (CDRI) in Lucknow, a research organization of the Council of Scientific and Industrial Research (CSIR), has released a new oral contraceptive for women. The new once-a-week contraceptive, Centchroman, is the first nonsteroidal, nonhormonal birth-control pill.

The new pill is an original product of CDRI and represents a major breakthrough in the international effort to develop better contraceptives. Releasing the drug, A. S. Paintal, director-general of the Indian Council of Medical Research (ICMR), said the drug has been under development for over 20 years and its release to the market was delayed because of extensive efficacy and safety studies. B. N. Dhawan, director of CDRI, added that Centchroman has been approved for inclusion in the National Family Welfare Programme and has been cleared for marketing under the Social Marketing Programme by the Drugs Controller of the Government of India. CDRI has licensed it for marketing to the public-sector Hindustan Latex Ltd under the trade names 'Choice-7' and 'Saheli'. It is expected to cost one rupee a tablet.

Unlike the existing steroidal pills, which prevent ovulation, Centchroman prevents attachment of the fertilized egg in the uterus. The contraceptive action is due to a unique combination of a weak oestrogenic and a potent antioestrogenic property which inhibits the uterus' preparation for nidation of the fertilized egg. The contraceptive effect is readily reversible.

The compound has been subjected to extensive safety studies in several species of laboratory animals. It does not produce teratogenic, mutagenic or carcinogenic effects and has an excellent therapeutic index. In the contraceptive-efficacy multicentric trials, with Centchroman at 30 mg once a week, about 1600 women of reproductive age have been covered, giving a total of over 20,000 months of use, with excellent pregnancy protection (Pearl index 3.05; Pearl index is an indirect measure of contraceptive failure and a value of up to 6.0 is considered acceptable). The protection improves (Pearl index 2.53) if, during the first 12 weeks, a 30 mg dose is given twice a week. Users experience no side-effects except that about 8% of the menstrual cycles are of a longer-than-normal duration. Babies born owing to user failures present normal milestones. Intensive monitoring



by clinical examination, haematological and biochemical tests, as well as ultrasonographic examination of the ovaries and uterus have shown that the drug is quite safe. The drug has also been found useful for treatment of selected cases of breast cancer.

Applied molecular evolution: The shape of things to come

Vidhya Gopalakrishnan

The days of recombinant-DNA technology or genetic engineering on the pedestal of the biotechnology industry may soon come to an end. The early and middle nineties brought forth, in the form of five independent research papers[1–5], an entirely new area of biotechnology known as applied molecular evolution. This new approach may be able to generate an infinite range of new and novel biomolecules, from vaccines to industrial catalysts, all based on random variation followed by natural selection of the best shape.

The whole basis of this new theme is shape. It is well known that shape or a lock-and-key type of fit governs the interactions of most of the large biomolecules—antibodies recognizing antigens, enzymes binding reactants together as in a reaction complex or transition state, hormones slotting perfectly into receptors All these recognition and interaction phenomena are based on the above concept. So, in principle, anything that mimics the shape of a biologically important molecule may be able to replace that molecule in its function. Applied molecular evolution, therefore, deals with the ability to produce the shape mimic or replica of interest. If a hormone is shape-mimicked, the result is a potential drug; if the target is a virus coat protein, the shape mimics would be potential vaccines; when a reaction complex or transition state is mimicked, we would have candidate enzymes. These shape mimics constitute the components of applied molecular evolution.

How are these shape mimics obtained? The answer lies in the screening of libraries of peptides and oligonucleotides generated by random variation of sequence, followed by selection of the right shape. The selection is provided by the lock-and-key fit of the target or its surrogate to different substrates.

The recent crop of five reports that heralded the approach of this new area were all based on the above principle. Two of them were on RNA while the other three reported peptide mimics. Of the two papers on RNA, one, by Ellington and Szostak[1], reported sub-populations of RNA molecules, 100 nucleotides long, that bound specifically to a variety of organic dyes (ligands), like Cibacron Blue. These were screened from a population of RNA molecules of random sequence. The idea was that the shape of the dye molecule—the ligand—is like the transition state of a reaction complex. Any RNA molecule whose shape would complement that of the dye would therefore be a potential RNA enzyme. The hit was roughly one in 10^8, or, in other words, roughly one in 10^8 random-sequence RNA mole-

cules folds in such a way as to create a specific binding site for the dye ligands.

The other paper on RNA dealt with the search for novel RNA molecules that bound, in a specific way, to the DNA polymerase of bacteriophage T4. This enzyme is essential for replication of T4. Normally a short RNA sequence of eight nucleotides binds to the polymerase and controls its activity. Tuerk *et al.*[2] generated a pool of RNA molecules ($\simeq 4^8$ molecules) eight nucleotides long and of random sequence, and exposed them to T4 DNA polymerase immobilized on nitrocellulose filters. The unbound RNA was washed out, leaving behind only those whose shape fitted the shape of the control area of the enzyme. This study revealed that an RNA, other than the natural one, did bind to the enzyme. The hit was approximately one in 70,000. Thus this new RNA sequence could do the job of the natural one in inhibiting the activity of the T4 enzyme. The novel sequence therefore has the potential to prevent T4 infection and, as a molecule that controls an essential viral function, could thus be a potential vaccine.

The three reports on peptide mimics were also based on the same principle. All three papers dealt with the construction and characterization of epitope libraries. Cwirla *et al.*[3] reported the screening of a large library of peptides with N-terminal hexapeptide sequences. The vast library was screened with a monoclonal antibody (mAb) specific for the Tyr–Gly–Gly–Phe sequence present in the natural opiate β-endorphin. Out of a pool of 3×10^8 peptides, 51 bound to the target, giving a one-in-10^7 hit rate.

In another study, Devlin and his colleagues[4] screened a large library of phage expressing random 15-residue peptide sequences to check whether proteins other than antibodies to epitopes of linear peptides are able to bind to some random peptide. The library was screened on the basis of binding to the protein streptavidin, which is not known to have any affinity for peptides. This gave a one-in-2×10^6 hit rate.

Lastly, Scott and Smith[5] screened tens of millions of short peptides (hexapeptides) in an epitope library for tight binding to an antibody. The library was tested using two monoclonal antibodies (A2 and M33) that are specific to an epitope (hexapeptide) of the protein myohaemerythrin. Their results also showed a one-in-two-million hit rate.

As can be seen from these examples, applied molecular evolution deals with gigantic numbers of peptides and oligonucleotides. On the face of it this may impose some restriction on the chance of obtaining at least one with the right shape from a pool of random molecules. In other words, one may have to deal with extremely large pools in order to obtain at least one right hit. For example, if the protein under consideration is about 100 amino acids long, the number of amino-acid sequences would be 20^{100}, or 10^{130}—a number that far exceeds even the number of particles in the Universe. If every single sequence represented a single shape then applied molecular evolution would have been in the realm of the impossible. But according to Kauffman (see ref. 6) this is not so. He believes that there are a lot fewer shapes than there are polymer sequences.

On the basis of the report by Oster and Perelson (see ref. 6) on (i) the idea of a 'shape space' as representative of all the three-dimensional possibilities of polymers and (ii) their result that about 10^8 antibodies, from the entire pool of antibodies, effectively cover all of shape space, Kauffman extrapolated the same idea to catalysis. He concluded that the number of enzymes required to cover all of catalytic task space (or all the existing reaction-complex transition states) is again about 10^8. Thus the whole world of biopolymers is effectively represented by a mere 100 million shapes, give or take an order of magnitude. Amazingly, this is also comparable to the kinds of numbers dealt with in the above five reports.

The inhibition of T4 DNA polymerase by an octaribonucleotide provides a potential route for production of highly specific antiviral drugs that attack the basic molecular machinery of viruses. The other example, of RNA binding to specific ligands, makes it possible to isolate novel ribozymes from pools of random-sequence RNAs. This has the potential to throw light on the prebiotic world, which was possibly based on RNA enzymes.

Libraries of peptides in phage will have immediate application in epitope mapping. A much larger number of potential epitopes can be screened than is feasible with methods based on chemical synthesis of candidate peptides. These libraries should also be useful in discovering new ligands for other important binding proteins, such as hormone receptors and enzymes. This approach does not require a priori information on ligand structure, and the work of Devlin *et al.* fosters the prospect that an epitope library can also be used to find peptide mimics of nonprotein ligands, thereby broadening the applicability of the epitope library. Future development and screening of peptide libraries should lead to the discovery of novel ligands for many purposes, including the identification of new drug candidates.

Last but not the least, working with peptides is also more technically demanding than working with DNA or RNA. The random-peptide pool has to be generated from a random DNA library cloned and translated to give the random-sequence peptides. Amplification of peptides is also much more cumbersome than just doing a polymerase chain reaction (PCR) for DNA. This provides much scope for improving the technology.

The idea of applied molecular evolution is therefore the search for the right molecular shape. Even if the screening is limited to only a 100 million shapes, the shape hit on is not always the best fit. Site-directed mutation provides scope for improvement of the fit. Thus applied molecular evolution can be redefined as the technique of selection, mutation and further selection—the rules that govern organismic survival and replication on the one hand and the modern computational approach to complex mathematical calculations based on iterative numerical analysis on the other.

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