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Synthesis And Selection Of Antibacterials Using Codon-Shuffling As Method For De Novo Protein Design.

Authors:
Alka Rao (*ICGEB, New Delhi*)
Sidharth Chopra (*ICGEB, New Delhi*)
Geeta Ram (*ICGEB, New Delhi*)
Anand Ranganathan (*ICGEB, New Delhi*)

Short Abstract: The concept of de novo design and laboratory evolution has gained great importance especially as an invaluable tool in designing novel bioactive molecules. Using codon shuffling as a method of de novo design, five different proteins were generated successfully that proved bacteriostatic on their expression in E. coli.

Long Abstract:
The approach of recasting or constructing proteins from scratch in order to achieve altogether new entities that are then able to carry out a predetermined catalytic or structural function is currently an area of intense scientific research. In this report, we describe synthesis and selection of antibacterials using a recently developed directed-evolution technique, called “codon-shuffling”. We also describe how, through codon-shuffling, libraries are generated that: (a) can be both non-rational as well as part-rational, (b) have members large enough to be classified as proteins and not just peptides, (c) are not restricted by limits of length of their corresponding genes. As an application of codon-shuffling method an inducible protein library was constructed by shuffling of judiciously designed hexamer DNA duplexes, called dicodons. Upon induction with IPTG, some of these de novo synthesized library members were found to arrest the growth of the E. coli cultures. The bactereostatic/lytic nature of the dicodon proteins was monitored by growth curves, by zone clearance studies as well as Transmission Electron Microscopy of the affected cells. Good quantities of the soluble proteins of isolated antibacterials could be obtained only as fusion partners. One such purified protein was found to strongly bind heparin, an indication that the interaction of the de novo proteins may be with the nucleic acids of the host cell, much like many of the naturally occurring antibacterial peptides, e.g. Buforin. We have also explored an exclusive property of the “codon-shuffling” method, which is the allowance for skewing library properties by inclusion, exclusion or predominance of some dicodons, thereby narrowing down the search for proteins for specific needs. Furthermore, the recent sequencing of organisms such as M. tuberculosis and P. facliparum has unearthed many virulence-determining proteins that are exclusively composed of short sequence repeats. Proteins rich in dicodon -repeats, constructed using codon-shuffling method may therefore help in generating a multitude of finely tuned anti-bacterial proteins that can potentially be regarded as lead compounds.