PROTEINS TARGETING ORTHOLOGS

FIELD OF THE INVENTION

[0001] In a particular aspect, the present invention is relevant to proteins and to their generation by protein evolution.

BACKGROUND

[0002] Protein engineering via site-directed mutagenesis and, more recently, molecular evolution has been successfully employed to improve enzymatic properties in industrial applications and therapeutic properties in antibodies. Characteristics such as thermostability, pH optimum, enantioselectivity, specificity and binding affinity have all been altered to better adapt proteins and antibodies for specific purposes.

[0003] Since its inception, many different methods for molecular evolution have been described and applied to improve characteristics of the target protein. For example, sets of single point mutants can be generated and screened for upmutants. Beneficial single amino acid substitutions can then be recombined and screened to further optimize the desired characteristics in the target molecule.

[0004] However, the successful evolution of a target molecule starting with single point mutations requires that the (sometimes) subtle changes in performance can be accurately measured to identify the upmutants. In cases where a sensitive assay does not exist, single point mutations cannot be successfully screened. Simultaneous mutations of several sites can be done, however the number of combinations created, increases very quickly and reaches the limits of cloning efficiency and screening capability.

SUMMARY

[0005] The present disclosure relates to methods of identifying cross-species active mutant polypeptides formed from, or based upon, a template polypeptide. The template polypeptide shows an activity on a target from a first species. The method comprises the steps of generating a library of mutant polypeptides from the template polypeptide; selecting mutant polypeptides that show the activity on an ortholog of the target from a second, different species; and screening the selected mutant polypeptides for a cross-species mutant polypeptide that also shows the activity on the target from the first species.

[0005a] In one aspect, there is provided a method of identifying a cross-species mutant polypeptide from a template polypeptide of a species selected from human and non-human animals, wherein the template polypeptide shows an activity on a target from the species and has a total of n amino acid residues, the method comprising the steps of:

i. generating a library of n-1 separate sets of mutant polypeptides from the template polypeptide, each set comprising mutant polypeptides having X different predetermined amino acid residues at a single predetermined position of the template polypeptide, each set of mutant polypeptides differs in the single predetermined position, and a number of different mutant polypeptides generated is equivalent to [n-1] x X;

ii. selecting mutant polypeptides that show the activity on an ortholog of the target in another species; and

iii. screening the selected mutant polypeptides for a cross-species mutant polypeptide that shows the activity on the target.

[0005b] In another aspect, there is provided a method of identifying a cross-species mutant peptide from a template peptide from a species selected from human and non-human animals, wherein the template peptide has a total of n amino acid residues and shows an activity in a system from the species, the method comprising the steps of: generating a library of n-1 separate sets of mutant peptides from the template peptide, each set comprising mutant peptides having X different predetermined amino acid residues at a single predetermined position of the template peptide, each set of mutant peptides differs in the single predetermined position, and a number of different mutant peptides generated is equivalent to [n-1] x X; selecting mutant peptides that show the activity in an orthologous system from another species which is orthologous to the system from the species; and screening the selected mutant peptides for one or more cross-species mutant peptides that shows the activity in the system from the species.

[0006] The present disclosure relates to comprehensive methods of identifying and mapping mutant polypeptides formed from, or based upon, a template polypeptide. Typically, the polypeptide will comprise n amino acid residues, wherein the method comprises (a) generating n

Claims

What is claimed is:

1. A method of identifying a cross-species mutant polypeptide from a template polypeptide of a species selected from human and non-human animals, wherein the template polypeptide shows an activity on a target from the species and has a total of n amino acid residues, the method comprising the steps of:

    i. generating a library of n-1 separate sets of mutant polypeptides from the template polypeptide, each set comprising mutant polypeptides having X different predetermined amino acid residues at a single predetermined position of the template polypeptide, each set of mutant polypeptides differs in the single predetermined position, and a number of different mutant polypeptides generated is equivalent to [n-1] x X;

    ii. selecting mutant polypeptides that show the activity on an ortholog of the target in another species; and

    iii. screening the selected mutant polypeptides for a cross-species mutant polypeptide that shows the activity on the target.

2. The method of claim 1, wherein the template polypeptide is an antibody and the target is an antigen that the antibody specifically binds with.

3. The method of claim 1, wherein the template polypeptide is an enzyme and the target is a substrate of the enzyme.

4. The method of claim 1, wherein the template polypeptide is a hormone and the target is a receptor of the hormone.

5. The method of claim 1, wherein the generating step comprises using comprehensive positional evolution.

6. The method of claim 1, wherein X is 19.

7. The method of claim 1, wherein each set of the mutant polypeptides is generated by incorporating N,N,N at a predetermined codon position in a polynucleotide that encodes the template polypeptide.

8. The method of claim 1, wherein the species is human.

9. The method of claim 8, wherein the another species is selected from rat, mouse, rabbit, hamster, guinea pig, monkey, dog, cat and non-human primates.

10. The method of claim 1, wherein the species is a non-human species selected from rat, mouse, rabbit, hamster, guinea pig, monkey, dog, cat and non-human primates.

11. The method of claim 1, wherein the another species is a non-human species selected from rat, mouse, rabbit, hamster, guinea pig, monkey, dog, cat and non-human primates.

12. The method of claim 1, wherein the species and the another species are different and independently selected from rat, mouse, rabbit, hamster, guinea pig, monkey, dog, cat and non-human primates.

13. A method of identifying a cross-species mutant peptide from a template peptide from a species selected from human and non-human animals, wherein the template peptide has a total of n amino acid residues and shows an activity in a system from the species, the method comprising the steps of:

generating a library of n-1 separate sets of mutant peptides from the template peptide, each set comprising mutant peptides having X different predetermined amino acid residues at a single predetermined position of the template peptide, each set of mutant peptides differs in the single predetermined position, and a number of different mutant peptides generated is equivalent to [n-1] x X;

selecting mutant peptides that show the activity in an orthologous system from another species which is orthologous to the system from the species; and

screening the selected mutant peptides for one or more cross-species mutant peptides that shows the activity in the system from the species.

14. The method of claim 13, wherein the species is human.

15. The method of claim 14, wherein the another species is selected from rat, mouse, rabbit, hamster, guinea pig, monkey, dog, cat and non-human primates.