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Searching sequence space for ATP-binding proteins

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Abstract

A library of random protein sequences has yielded novel, ATP-binding proteins not found in any organism.

Significance and context

How difficult is it to create a functional protein? Evolutionary biologists ask this question when studying the origin of life; protein designers ask it in reference to theories of protein folding. Keefe and Szostak address the question using a brute-force protein-sequence sampling technique. They synthesized 10^{12} random proteins of 80 amino acids (80-mers), each covalently fused to its mRNA (to allow simpler sequencing), and then selected for fusions that bound an ATP affinity column. Keefe and Szostak identified at least one protein that bound free ATP tightly and specifically. Their results enable an upper limit to be estimated for the rate at which evolution would uncover a functional protein in a random sequence search.

Key results

Keefe and Szostak began with a library of 10^{12} 80-mers. Following eight rounds of selection and amplification, 6.2% of the library bound the ATP column; none of the binders at this stage shared sequence similarity with other known proteins. After mutagenesis and further selection, 34% of the library bound the ATP column. From the final generation, eight clones were purified in milligram quantities and characterized as free proteins. All shared about 80% sequence identity among themselves but are not related to any other known sequence. The isolate with the highest affinity for ATP, called clone 18-19, bound the nucleotide with a K_d of 100 nM at 4°C. This clone bound other nucleotides at least three orders of magnitude more weakly than ATP; affinity dropped by a factor of between two and 600 when moieties were removed from the ATP structure. EDTA chelation and metal replacement experiments suggested that Zn^{2+} may be coordinated by a CXXC motif in clone 18-19.

Conclusions

From their first phase of selection - 6.2% functional proteins in a library of 10^{12} sequences - the authors estimate that about one in 10^{11} random 80-mers has any given biological activity. They also conclude that bound metals may be a useful structural linchpin for proteins with low melting temperatures. In general, Keefe and Szostak argue that new functional proteins may be accessible through simple selection schemes.

Reporter's comments

As a protein design study, this paper hits the mark expertly. As a study of sequence evolution rates, there are some gray areas. For example, proteins from Keefe and Szostak's first eight rounds of selection appear to use their tethered mRNA in binding ATP; thus, strictly speaking, 10^{11} 80-mers alone may not yield one functional protein. The next step for Keefe and Szostak will be to estimate whether nature has exhaustively searched functional protein space. To do this, they will need to characterize their isolates structurally and compare them with known ATP-binding proteins.

Table of links

Nature

References

1. Keefe AD, Szostak JW: Functional proteins from a random-sequence library. Nature. 2001, 410: 717-718. 0028-0836