Poster H-35

Identification and biological interpretation of correlated amino acid changes in human Influenza A virus (H3N2)

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Short Abstract: We have identified and categorized the correlated amino acid substitutions responsible for the evolution of yearly predominant H3N2 isolates. Through analysis of correlated amino acid mutations, we propose the mechanism that drives the emergence of recent H3N2 since 2004 is mainly due to human memory CTL response.

Long Abstract:
The burden of influenza has been a global concern since it was introduced into human. It is estimated currently that the average global burden of inter-pandemic influenza may be on the order of ~1 billion cases of flu, ~3–5 million cases of severe illness and 300 000–500 000 deaths annually. Epidemics and outbreaks of influenza occur in different seasonal patterns depending on the region in the world. The currently circulating influenza viruses that cause human disease are divided into two groups: A and B. Influenza A has 2 subtypes which are important for humans: A(H3N2) and A(H1N1), of which the former is currently associated with most deaths. Currently, large amount of complete genomic sequences are available for human H3N2. To understand the proteomic base underlying H3N2 virus evolution, we developed a novel computational approach and have identified correlated amino acid mutations based on over 600 recently sequenced H3N2 isolates. Many of these correlated amino acid substitutions are interwoven interactions between multiple viral proteins, which indicate that the H3N2 evolution has been achieved through a highly coordinated/collective evolutionary dynamics. We further analyzed the correlated amino acid substitutions according to an extensive literature study. Based on our analysis, we proposed that the emergence of the recent H3N2 during 2004-2005 were mainly driven by human memory CTL response rather antigenic shift induced mainly by HA.

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Identifying functional domains in a family of restriction endonucleases where DNA recognition, methyltransferase and endonuclease functions reside in a single polypeptide.

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Short Abstract: Restriction-Modification systems of the MmeI family consist of a single polypeptide performing DNA recognition, DNA methylation and DNA cleavage. We speculate

these enzymes use a single DNA recognition domain to mediate both methylation and endonuclease functions. Replacing the recognition domain may allow engineering endonucleases with new DNA specificity.

Long Abstract:

Restriction-Modification systems of the MmeI family consist of a single polypeptide performing three functions; sequence specific DNA recognition, DNA methylation and site specific DNA endonuclease. MmeI has the distinction of the greatest reach between the recognition sequence and DNA cleavage position of type II endonucleases: 5'-TCCRACN20/N18-3', and recently this characteristic has made MmeI useful for the Long SAGE technique.1, 2 MmeI modifies only one DNA strand, 5'-TCCRmAC-3', and DNA modified by MmeI in the absence of Mg++ is not subsequently cut by MmeI when Mg++ is added. This reliance on only one strand modification for protection is unique among type II systems. BLAST searches revealed a number of sequences highly similar to MmeI, suggesting that MmeI represents a family of single polypeptide restriction-modification enzymes. We have cloned a number of these genes in E. coli and have identified several that functions like MmeI as an endonuclease. One such enzyme is CstMI, from a Corynebacterium striatum M82B plasmid, which recognizes a novel 6 base sequence, AAGGAG, and cuts downstream from this sequence at the same distance as MmeI, AAGGAGN20/N18.

We speculate that enzymes of the MmeI family might use a single DNA recognition domain to mediate both methylation and endonuclease functions. If so, these enzymes may prove more tractable to engineering endonucleases with new DNA specificity, since changing this single DNA recognition domain may allow both host protection and new endonuclease specificity, whereas altering typical type II endonucleases requires simultaneously altering the separate DNA methyltransferase to the same new DNA specificity. We use multiple sequence alignments to predict the functional domains of members of this family. We are investigating the predictions for the DNA sequence recognition domain through shuffling portions of several members of the enzyme family. We hope to learn to predict the DNA sequence recognized from the amino acid sequence of new members of the family.

1. Boyd, A.C., Charles, I.G., Keyte, J.W., Brammar, W.J.; Nucleic Acids Res. 14: 5255-5274 (1986).
2. Velculescu, V.E., Zhang, L, Vogelstein, B., and Kinzler, K.W.; Science 270: 484-487 (1995).