Building proteins with new blocks using the amber suppression system

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If you imagine a cell as a planet (like earth), then proteins are the buildings on it that carry out most of the functions. The process of making a protein within a cell is also similar to constructing a building. Special building blocks called amino acids are linked together to form a long polymer chain during a process called translation. The order (sequence) of the amino acids determines the basic structure and functions of the protein. The blueprint of each protein is encoded in the DNA sequence and a set of rules called the genetic code is required to decode it. The genetic code consists of 64 nucleotide triplets called codons each of which specifies one of the twenty common amino acids. The exceptions are the three stop codons which normally do not code for any amino acid but instead tell cells to terminate the translation process.

Given the limited types of "natural" amino acids, scientists have designed and synthesized numerous "unnatural" amino acids (UAAs) with unique structures and functions. Once incorporated, these new building blocks can add useful features to the original proteins and facilitate the study and manipulation of many biological processes. The site-specific incorporation of UAAs on the target protein can be achieved by adding them to the genetic code. Since all 64 codons are occupied by natural amino acids or termination signals, a chemical biology technique called amber suppression is utilized to expand the limit of the current genetic code. The amber suppression system reprograms one of the three stop codons, the amber codon, into a codon that codes for the desired UAAs. Artificial amber codon sequences can be added to the target protein's blueprints (genes) at desired sites by DNA insertions or mutations. During translation, those amber codons can be recognized by protein-making machinery and lead to efficient incorporation of UAAs at corresponding sites. However, since the amber codon is originally used for translation termination and naturally exists in many endogenous genes, a wide range of non-target proteins may also be incorporated with UAAs during the process and cause unknown side effects to cells.

In this study, the amber suppression system was introduced into two types of mammalian cells for the study of UAAs incorporation under different conditions. We were interested in improving the system's efficiency/specificity in order to reduce the background incorporation level of UAAs. Our results show that by adding certain DNA sequence elements at 5' or 3' of the reporter gene, the amber suppression efficiency could be up- or down-regulated. The results provide interesting insights and suggestions for the design of new amber suppression system in the future.

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