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講題：Central dogma and PCR

摘要：1. PCR (polymerase chain reaction)

This process simulates DNA copy mechanism of organism in test tubes (in vitro.) It can create copies of specific fragments of DNA or mRNA, and it is useful in "amplifying" DNA molecules.

Firstly, in 90°C environment, DNA separates into two strands and then hybridize with primers in 50°C. With providing nucleotide bases, DNA synthesizes in 75°C (the Taq polymerase works best — since it was discovered at hot spring in Yellowstone Park.) Eventually a complementary template is formed and thus DNA is doubled. The whole process takes about 5 minutes. Repeating 30 times or more will generate a large numbers of copies.

PCR can only duplicate unique region indicated by oligo-nucleotides (start and stop points).

2. Central dogma

(i) Transcription 轉錄 (from DNA to RNA)

- DNA: A=T, C≡G; double-stranded
- RNA: A=U, C≡G; single-stranded (folded)

- RNA transcription sequence ~ 10^3 b.p.
- DNA replication sequence ~ 10^5 ~ 10^6 b.p.

Tolerance in error: RNA higher (0.01%)

procedure:
1. sigma factor (RNA) find DNA promoter.
2. unwind DNA strands
3. RNA polymerase latch on DNA
4. Synthesize complementary RNA ($5' \rightarrow 3'$)
5. ~10 b.p. sigma factor leave
6. detect "end-of-transcription" codon. (terminator).
7. RNA polymerase leave and release RNA segment.

(ii) splicing (for eucaryotes.)

Remove introns (unused regions) on mRNA.
snRNPs enzyme fold the RNA and cut the introns, connect the exons in the end.

(iii) Translation 轉譯 (from RNA to protein)

Three cosecutive nucleotides ($4 \times 4 \times 4 = 64$ types) as codon to form 20 types of amino acid.

AUG — Met (use as start of translation)
UAA, UAG, UGA — stop (use as stop indication)

Redundancy: codon: 61 types; tRNA: 31 types; a.a. 20 types.

tRNA forming complementary nucleotides to mRNA on the anticodon loop.

Using tRNA as interface between mRNA and amino acid. (helped by aminoacyl-tRNA synthetases).

Place for synthesize protein: ribosomes
(for procaryotics, the RNA could move to ribosomes easier since there is no nucleus.)

Procedure
1. small ribosomal subunit (rRNA) combine with mRNA
2. large ribosomal attached (there are 3 binding sites for tRNA: E, P, A)
3. tRNA + a.a arrive site A (satisfying the complementary codon on mRNA)
4. break tRNA and a.a.; form peptide bond in the large subunit (move to E/P site)
5. used tRNA leave site E, leaving the growing polypeptide at site P.
6. keep on synthesizing next codon until the terminator codons (UAA, UGA, UAG).
7. release factor protein, add H_2O (OH)
8. free carboxyl end the polypeptide
9. folding

※ "Pipelining" may be used for higher efficiency. (polyribosomes)