Keys to quiz 2

Part I: Describe the following 10 terms (3 points each).

(1) EF-G:

a. its structure likes tRNA
b. it is a translational elongation factor
c. it binds to ribosome when associated with GTP
d. GTP hydrolysis allows it to reach into the small subunit
e. the previous step triggers translocation of the A-site tRNA

(A full point is given if you wrote three out of five points listed above)

(2) SsrA:

a. a tmRNA that is partly tRNA and partly mRNA
b. it rescues ribosomes stalled by prematurely terminated mRNA
c. the rescue concerns EF-Tu-GTP
d. SsrA binding adds a ten-amino-acid tag that is recognized by cellular proteases

(A full point is given if you wrote three out of four points listed above)

(3) frameshift mutations:

a. caused by insertions or deletions of one or a few base pairs (1')
b. the above change alters the reading frame of a protein (2')

(4) gRNA:

a. it stands for guide RNA
b. it directs Uridine insertions or deletions
c. involved in the process of RNA editing
d. deved into 3 regions: 5' anchor, editing region, and poly U

(A full point is given if you wrote three out of four points listed above)

(5) ADAR:

a. it stands for Adenosine Deaminase Acting on RNA (1')
b. it converts Adenosine into Inosine through a process of site-specific deamination (2')

(6) **RRF** refers to ribosome recycling factor, a factor serves in the termination of translation in prokaryotic cells (1'). RRF cooperates with EF-G and IF3 to recycle ribosomes after polypeptide release (1'). It binds to the empty A site of the ribosome, where it mimics a tRNA (1').

(7) **U2 snRNP** is one of the five small nuclear ribonuclear proteins, a component of the spliceosome (1'). After the formation of Early complex, U2 snRNP binds to the branch site, aided by U2AF and displacing BBP (1'). This arrangement results in the extruded branch site A residue, which is unpaired and available to react with the 5'

splice site (1').

8. **Poly-A polymerase** mediates polyadenylation in the termination of transcription in eukaryotes (1'). It adds about 200 As to the 3' end of the newly synthesized mRNA without a template (2').

9. **Gre factors** stimulate hydrolytic editing, a proofreading mechanism in the prokaryotic transcription (2'). They also serve as elongation stimulating factors (1').

10. **Cre-*lox*** is a simple example of recombination by the tyrosine recombinase family: Cre is an enzyme encoded by phage P1, and *lox* sites are its recombination sites on the DNA (2'). Only Cre protein and the lox sites are needed for completing recombination (1'). Cre-*lox* is widely used as a tool in genetic engineering.

Part II

1. What is the relationship between DNA damage/lesion and mutation? Which repair mechanisms are error-free, and which are error-prone? Is the error-prone repair mechanism useful? (10 points)

1) The relationship between DNA damage/lesion and mutation:
If DNA damage/lesion is not repaired before the next round of replication, it may result in mutation because the incorrect nucleotides could be incorporated into the newly synthesized DNA because of the damaged DNA structure. In another word, mutation could be resulted from DNA damage/lesion. (2')

2) Error-free repair mechanisms include Mismatch repair (1'), Direct reversal of DNA damage (e.g. photoreactivation, methyltransferase, etc.) (1'), Base excision repair (1'), Nucleotide excision repair (1') and Recombination repair (1')
Error-prone repair mechanism refers to the translesion DNA synthesis (1')

3) The error-prone repair mechanism (translesion DNA synthesis) is useful. It is a fail-safe mechanism that allows the replication machinery to bypass the damaged sites. Although this repair mechanism tends to introduce mutations, it saves the cells from the worse fate of an incompletely replicated chromosome (chromosome broken). (2')

2. What are the possible causes of double stranded breaks in E. coli cells? How does E. coli repair this kind of damage? (10 points)

1) The possible causes of double stranded breaks in *E. coli*:
Ionizing radiation and other damaging agents (reactive oxygen species) directly break both strands of DNA backbone. 2'
Interfering with the progress of a replication fork (an unrepaired nick or lesion in template strand) indirectly causes double stranded breaks. 2'

2) The ways that *E. coli* repairs double stranded breaks:
Homologous recombination via the DSB-repair pathway (major mechanism): the broken DNA retrieves sequence information from the sister chromosome. 4'
Nonhomologous end joining (NHEJ): the two ends of the broken DNA are directly joined to each other by misalignment between single strands protruding from the

broken ends. 2’

(The above was the criteria that TA used to grade. Prof. Zhang suggested that 3 points are assigned to answer the causes of DSB, and 7 points are assigned to answer the RecBCD repair pathway in *E. coli*. Answer of nonhomologous end joining will be awarded to 1 point at most.这题主要是 test 大家是不是熟练掌握了细菌里的 RecBCD 修复机制)

3. What are the three principle classes of transposable elements? Who discovered the transposition? What do you learned from the research career of the person who discovered transposition? (10 points)

1) The three principal classes of transposable elements:

DNA transposons; 2’

Viral-like retrotransposons (LTR retrotransposons); 2’

Poly-A retrotransposons (nonviral retrotransposons) 2’

2) Barbara McClintock discovered transposable elements in maize in the late 1940s. 2’
3) This is an open question, so just write down your own opinions (e.g. about perseverance, strong interest, hard-working, etc.). 2’

4. Please describe how the transcription in bacteria is initiated, elongated and terminated? (10 points)

(1) Initiation (4’): The initiation factor σ recognizes and binds to promoter (1’). In the case of *E. coli*, the most common σ factor is σ^{70} that contains two conserved sequences: -35 and -10 regions. Binding of the σ factor facilitates RNA polymerase to bind the promoter DNA, which forms a close complex, then the promoter is melted to form the open complex, and synthesis of RNA is then started. RNA polymerase usually has to synthesize several short RNAs before escaping from the promoter region to enter the elongation phase. Promoter escape requires the dissociation of the σ factor from the core polymerase.

(2) Elongation (3’): RNA polymerase synthesis RNA, unwinds DNA in front, re-anneals it behind, dissociate the growing RNA chain. In addition, RNA polymerase carries out two proofreading functions as well. RNA polymerase catalyzes the removal of one incorrectly inserted ribonucleotide by reincorporation of PPi, called pyrophosphorolytic editing. RNA polymerase can also backtracks by one or more nucleotides and cleaves the RNA product stimulated by Gre factors, called hydrolytic editing.

(3) Termination (3’): Transcription in bacteria can be terminated by Rho-independent or Rho-dependent mechanism. Rho-independent termination requires the terminators contain a short inverted repeat rich in G and C followed by a stretch of about eight A:T base pairs. After the terminator transcription, the mRNA can form a strong hairpin structure followed by a stretch of weak A:U base pairs with its DNA template, and mRNA easily dissociates from the DNA template. Rho-dependent termination requires no intrinsic terminator RNA structure but requires Rho factor that uses the energy derived from ATP hydrolysis to stop transcription.

[Notes: (1) Some students still confused the process of transcription and translation, as well as the bacterial transcription and eukaryotic transcription. For example, CTD tails was mentioned when explaining the initiation of bacterial transcription. (2) Students usually do not know how to express elongation. And most of students forgot the proofreading process in elongation.]

5. Please describe the feature and biological roles of the CTD tail of RNA Polymerase Ⅱ. (10 points)

The feature of the CTD tail (2'): The largest subunit in RNA polymerase II has a carboxy-terminal domain (CTD), which consists of multiple repeats of a consensus sequence of 7 amino acids. The CTD can be highly phosphorylated on serine or threonine residues.

Roles (8):

(1) During promoter escape, phosphorylation of the CTD by the kinase activity of TFIIH may help the polymerase leave behind the promoter and GTFs and enter elongation phase. (3')

(2) During transcription elongation and processing, many elongation factors and RNA professing proteins are loaded to polymerase, nascent mRNA and/or processing machinery through the CTD tails. Therefore, through the CTD tail, synthesis and processing of the new mRNAs are highly coupled and coordinated to ensure the production of high quality mRNA for translation. (5')

(Note: 区分真核和原核。题目中指明为 RNAP ii,说明为真核生物。另外原核生物 pol 的 CTD 称为α-CTD. 很多学生回答 CTD tail 可以和 up-element 作用，这是错误的)

6. What are the chemistry and mechanism of the spliceosome-mediated splicing, group Ⅱ intron splicing and group Ⅰ intron splicing? (10 points)

Spliceosome-mediated splicing of mRNA (6')

Chemistry (2'): splicing occurs as two sequential ester-transfer reactions. Firstly, the 2'OH of the branch point A attacks the phosphoryl group of a conserved 5' G at he 5' splice site, resulting in the free of 5' exon from the intron. Then, the 3' OH of the free 3' end of 5' exon attacks a phosphoryl group at the 3' splice site, resulting in the ligation of the 5' and 3' exons and release of a lariat form of intron.

Mechanisms (4'):

Step 1, formation of the E (early) complex by recognition of the 5' splice site, 3' splice site and A branch point by U1 snRNP, U2AF and BBP respectively.

Step 2, U2 snRNP bind to the branch site to replace BBP forms A complex. The base-pairing between U2 snRNA with the branch site make the conserved A residue in the branch site extruded from the paired region, and thus this A is ready to carry the nucleophile attack. The tri-snRNP U4/U6/U5 joins and A complex is arranged to B complex in which the three splice sites are brought together. In this complex U4/U6 snRNPs are held together tightly by extensive base-pairing between U4 and U6 snRNAs.

Step 3, U1 leaves the complex, and U6 occupies the 5' splice site by base-pairing.

U4 leaves the complex, allowing the RNA components of U2 and U6 to base pair to produce the active site. The branch site A attacks the 5' splice site, forming the 3-way junction and C complex. The 5' splice site then attacks the 3' splice site, freeing the intron lariat and forming the mRNA product.

Group Ⅱintron splicing (2'): the chemistry is the same as that of the spliceosome-mediated mRNA splicing, but the splicing is catalyzed by the intron RNA itself, which is also named as self-splicing.

Group Ⅰintron splicing.

Chemistry: the 3' OH of an exogenous G nucleotide or nucleoside attacks the 5' splice site to undergo the first ester-transfer reaction. Then the 3'OH of the 5' exon attacks 5' phosphate of at the 3' splice site, resulted in the release of a linear intron and ligated exons. The splicing is catalyzed by the intron RNA itself as the group II intron does.

Conclusion:

7. What is alternative splicing? What is the biological importance of alternative splicing? How alternative splicing is regulated (Note the involvement of the cis-acting elements and trans-acting factors)? (10 points)

Alternative splicing (3'): many protein-encoded genes in higher eukaryotes contain multiple introns and can be spliced in alternative ways to generate two or more different mRNAs.

Biological functions (2')**:** Alternative splicing allows individual genes to produce multiple protein isoforms, thereby playing a central part in expanding protein diversity from a limited number of genes and regulating gene expression in higher eukaryotes (2'). Alternative splicing also has a largely hidden function in quantitative gene control, by targeting RNAs for nonsense-mediated decay. (1')

Regulatory mechanisms (5')**:** alternative splicing is regulated by trans-acting factors (activators or repressors) that recognize an arrangement of positive and/or negative cis-acting sequence elements called exonic (or intronic) splicing enhancers or silencers. An interplay between cis-acting sequences and trans-acting factors modulates the splicing of regulated exons. Activators include members of the SR protein family and can activate splicing by binding to exon splicing enhancers (ESEs) using RNA-recognition domain and recruiting the splicing machinery using RS domain. Repressors are frequently members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family, which bind to exonic/intronic splicing silencers, blocking specific splice site

[Note: 有些同学不懂什么是 trans-acting，什么是 cis-acting；还有的同学回答成 trans-splicing 的机制。*Here is some additional material to explain the cis-acting elements and trans-acting factors.* The basic concept for how transcription is controlled in bacteria was provided by the classic formulation of the model for control of gene expression by Jacob and Monod in 1961. They distinguished between two types of sequences in DNA: sequences that code for trans-acting products; and cis-acting sequences that function exclusively within the DNA (and also RNA element

in our splicing case). Gene activity is regulated by the specific interactions of the trans-acting products (usually proteins) with the cis-acting sequences (usually sites in DNA/RNA). In more formal terms:

A gene is a sequence of DNA that codes for a diffusible product. This product may be protein (as in the case of the majority of genes) or may be RNA (as in the case of genes that code for tRNA and rRNA). The crucial feature is that the product diffuses away from its site of synthesis to act elsewhere. Any gene product that is free to diffuse away from the gene that made it to function elsewhere is described as trans-acting.

The description cis-acting applies to any sequence of DNA/RNA functions exclusively as a DNA/RNA sequence in situ, affecting only the DNA/RNA to which it is physically linked. -----GENE VIII]

8. What are the challenges of translation of the genetic information residing in mRNA into a protein (4 points)? What are the four components of translation machinery (4 points)? Please describe the roles of each component in translation (remember the relationship between the structure and function) (12 points).

Translation faces two main challenges (4 points):

The genetic information in mRNA cannot be recognized by amino acids. Therefore, the genetic code has to be recognized by an adaptor molecule (translator), and this adaptor has to accurately recruit the corresponding amino acid.

Four components of translation machinery and their roles in translation (8 points):

1) Messenger RNA (mRNA) serves as the template in translation (1') The coding region in the mRNA consists of a series of three-nucleotide long units called codons (1'). The prokaryotic mRNA and eukaryotic mRNA employ different strategies to recruit the ribosome--the RBS in prokaryotes and the 5' cap in eukaryotes (1').
2) tRNA transfer RNA is the molecular adaptor between the codon and the corresponding amino-acid (1 point). The tertiary structure of the tRNA resembles an inverted L, in which the acceptor stem and the anticodon loop are positioned at the two extreme ends. The amino acids is attached to the acceptor stem, and the anticodon loop base pair with the three letter code in mRNA (2')
3) Aminoacyl-tRNA synthetase (1'). The enzyme catalyzes the addition of amino acid to its cognate tRNA, which process is termed "charging". The enzyme catalyzes the charging in two separate steps, first attaching AMP to the-COOH group of the amino acid and then the appropriate tRNA displacing the AMP to covalently link with the amino acid (2').
4) Ribosome (1'). A two-subunit complex which comprises of both proteins and rRNAs. The large subunit contains the peptidyl-transferation center, catalyzing the peptide bond formation and the small subunit which contains the decoding center is where the codon-anticodon reaction takes place. (2')

9. Leucine has six codons, UUA, UUG CUU CUC CUA and CUG please predict at

least how many tRNAs shall exist in cells to translate these codons (10 points)

At least three tRNAs are required (5')
According to the Wobble theory, the base at the 5' end of the anticodon is less spatially confined than the other two bases, thus allowing it to base pair with more than one base at the 3'end of a codon. (3 points) UUA and UUG can both be recognized by UAA. In the same way, CUA and CUG can both be recognized by UAG and the remaining CUU and CUC be recognized by GAG. (5')
Or
In another scenario, the codons CUU CUA and CUC can be translated by IAG, with the codon CUG to be translated by GAC. In both cases, at least three tRNAs are required to translate all these codons. (5')