MOLECULAR CELL BIOLOGY II.

Institute of Biochemistry and Molecular Biology
Department of Molecular Biology
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Credit: 5
Number of lessons per week: lectures: 3; practicals: 2
Subject code: **AOKMBT795_2A**

The subject provides the foundations of modern molecular medicine, emphasizing points of interest for diagnostics, intervention and therapeutic applications. It serves as a base for several fields in medicine, such as molecular pathology, molecular diagnostics, pharmacology, gene therapy and medical biotechnology.

Prerequisites for subject registration:
Successful exams in Molecular Cell Biology I and Medical Biochemistry II

Lectures:

1. Control of the cell cycle 1
2. Control of the cell cycle 2
3. Control of the cell cycle 3
4. Control of the cell cycle 4
5. Programmed cell death – apoptosis 1
6. Programmed cell death – apoptosis 2
7. Molecular basis of tumorigenesis
8. Types of non-apoptotic programmed cell death
9. Methods in molecular biology 1: genetic polymorphisms, PCR
10. Methods in molecular biology 2: investigation of genetic variations
11. Methods in molecular biology 3: gene expression analysis
12. Methods in molecular biology 4: DNA cloning
13. Methods in molecular biology 5: knock-out techniques
14. Methods in molecular biology 6: gene therapy
15. COVID-19 – molecular biological aspects
16. Signal transduction 1
17. Signal transduction 2
18. Signal transduction 3
19. Signal transduction 4
20. Intracellular signals: mTOR, AMP kinase, hypoxia
21. Mechanisms of ageing
22. Components of the cytoskeleton. Molecular motor proteins
23. Vesicular transport. Mechanisms of exo- and endocytosis
24. Formation of the metabolome of cellular compartments
25. Signaling in organelle stress
26. Extracellular matrix, tissue architecture, adhesion molecules
27. Methods in cell biology (cell cultures, cell fractionation, *in vivo* microscopy)

Practicals: *(4x45 min every other week):*

1. Genotyping of a taste receptor SNP by PCR-RFLP 1: *in silico* design
2. Genotyping of a taste receptor SNP by PCR-RFLP 2: DNA isolation and PCR
3. Genotyping of a taste receptor SNP by PCR-RFLP 3: restriction digestion and electrophoresis
4. Molecular cloning and *in vitro* protein synthesis 1: recombinant DNA construction, bacterial transformation
5. Molecular cloning and *in vitro* protein synthesis 2: analysis of the DNA construct, *in vitro* mRNA synthesis
6. Molecular cloning and *in vitro* protein synthesis 3: protein expression by *in vitro* translation
7. Lab midterm exam, consultation

Acknowledgment of the semester:
Attendance of at least 75% of the practical lessons is prerequisite of acknowledging the semester. Students can collect “practical points” during the labs. An optional, written practical midterm test can be taken during the last practical lesson (topics: practicals of the whole subject, i.e. both semesters). Performance at the laboratory activity during the whole semester is also taken into account at the evaluation. Students, who achieve a

minimal score of 25 are exempt from taking a laboratory question in the final exam. This bonus is valid throughout the entire exam period of the semester.

Examination and grading system:
The oral exam is based on the topic list announced in the beginning of the semester, and it takes place before a committee of two (examiner and co-examiner). Students pick five random questions from each group of the following topic list. The exam can be passed if all five topics are sufficiently answered.

Topic list

I. DNA and RNA

1. Chemical structure of nucleotides; primary and secondary structure of nucleic acids (DNA and different types of RNA)
2. Condensation of the genetic material in pro- and eukaryotic cells; the role of topoisomerases and chromatin proteins
3. Structure of the human genome: coding and regulatory sequences; noncoding genomic sequences: introns, pseudogenes, repetitive sequences
4. The role of genetic variations in the pathogenesis of diseases; methods to study genetic factors
5. Principles of the semiconservative DNA replication; replication fork, leading and lagging strand synthesis
6. Comparison of DNA-replication in pro- and eukaryotes
7. Telomeric repeat sequences: replication of the telomeric regions of eukaryotic chromosomes; functions and importance of the telomerase
8. The most important types of DNA damage; repair of base deamination
9. Formation and repair of thymine dimers; mismatch repair
10. Types of point mutations; mechanisms of their development; possible effects of DNA sequence variations on the corresponding protein
11. Structure and function of the RNA-polymerase of *E. coli*; initiation and termination of transcription in prokaryotes
12. Characterization, role and synthesis of different types of RNA
13. Regulation of transcription in prokaryotes, definition of operon; positive and negative regulation
14. Structure of the eukaryotic genes, initiation and termination of transcription in eukaryotes
15. Regulation of the transcription in eukaryotes
16. Processing of the eukaryotic mRNA
17. Function and application of PCR and real-time PCR
18. Methods for the investigation of genetic mutations and polymorphisms (RFLP, allele-specific PCR, DNA-sequencing and primer extension)
19. Generation and application of recombinant DNA, reporter- and expression vectors

II. Post-transcription

20. Regulation of eukaryotic gene expression at post-transcriptional level (alternative splicing, RNA editing, regulation of RNA stability, RNA quality control)
21. Regulation of eukaryotic gene expression by RNA interference (miRNA, siRNA)
22. Epigenetic regulation of eukaryotic transcription: the role of DNA methylation and histone modifications
23. The genetic code; structure and function of tRNAs; aminoacyl-tRNA synthetases; codon-anticodon connections
24. The structure of prokaryotic and eukaryotic ribosomes; the ribosome cycle; binding of tRNA to ribosomes
25. Initiation of the translation in pro- and eukaryotes; regulation of eukaryotic translation; the role of phosphorylation of eIF2α
26. Elongation and termination of pro- and eukaryotic translation; pharmacological inhibitors of translation
27. Post-translational modifications of proteins
28. Types, structure and synthesis of collagen
29. Investigation of gene expression by real-time PCR and DNA-microarray methodology
30. Definition of proteostasis; types of intracellular protein degradation
31. Structure and function of the proteasome; role of immune-proteasome and TAP
32. Types, mechanism and role of autophagy
33. Lytic and lysogenic replication cycles of bacteriophages
34. Classification of animal viruses; structure and replication of retroviruses
35. Principles of human gene therapy (*in vivo* vs. *ex vivo* methods, gene augmentation, CRISPR/Cas9 system)
36. Biomedical application of genetic engineering processes (transgenic animals; knock-out, knock-in and knock-down techniques; cloning)

III. Signal transduction and cell cycle

37. Nuclear receptors: steroid, thyroid, retinoid and Ah receptors
38. Classification and function of membrane receptors
39. Types and functions of GTP-binding proteins in signaling
40. Activation mechanisms of serine / threonine protein-kinases with examples
41. cAMP signaling pathway; regulation of gene expression by cAMP
42. Phosphatidylinositol signaling pathways
43. NFκB and TGFβ signaling

44. Structure and function of tyrosine kinase receptors, the Erkl / Erk2 MAP kinase cascade
45. Insulin signaling
46. Integrating role of mTOR, and its effects on translation
47. Role of AMPK in the regulation of the metabolism and autophagy
48. Mechanisms of cellular oxygen sensing; adaption to hypoxia
49. Regulation of the cell cycle in the G_1 phase, transition to S phase
50. Regulation of the cell cycle in the G_2 and M phases
51. Molecular sensors detecting DNA damage and completion of DNA replication during the cell cycle
52. Molecular background of tumor generation
53. Structure and function of the apoptosome, DISC and PIDDosome complexes
54. Members of the Bcl-2 superfamily and their roles in various apoptotic pathways, the „survival signal”
55. Function and activation of apoptotic caspases, role of granzyme B
56. Types of non-apoptotic programmed cell death
57. Regulation of p53 levels and activity; role of p53 in determining the cell’s fate
58. Molecular mechanisms involved in aging

IV. Cell Biology

59. Principles of the organization of the eukaryotic cells; compartmentation; main features of subcellular organelles
60. Structure of cytoskeleton; structure and function of motor proteins
61. The protein secretory pathway; role of the rab cycle in the regulation of vesicular transport
62. Endo- and exocytosis
63. Formation of the proteome of subcellular organelles; principles and mechanisms of protein sorting
64. Protein targeting in the secretory pathway
65. Protein targeting into mitochondria and peroxisomes; uptake of substrates into lysosomes
66. Nuclear import and export of macromolecules
67. Endoplasmic reticulum stress, connection between the Unfolded Protein Response (UPR) and apoptosis
68. Protein quality control in the endoplasmic reticulum; the fate of misfolded proteins; ERAD
69. Characteristics of the proteome of subcellular organelles; maintenance of the internal milieu of subcellular organelles
70. Role of the extracellular matrix in signal transduction (e.g., integrin receptors), role of the extracellular matrix in the formation of metastasis
71. Methods in cell biology: cell culturing, cell fractionation, *in vivo* microscopy, flow cytometry, FACS

V. Lab

72. Quantification of proteins by biuret reaction and Ellmann-method
73. Investigation of subcellular cell fractions
74. Investigation of the gene expression in prokaryotes: induction of *β*-galactosidase in *E. coli*
75. Purification of a bacterially expressed protein by affinity chromatography
76. SDS-polyacrylamide gel electrophoresis and Western blot
77. *In silico* tools in molecular biology
78. Genotyping of the TAS2R38 tasting receptor by PCR-RFLP
79. Analysis and generation of a recombinant DNA
80. *In vitro* transcription and translation