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MACMILLAN WORK OUT SERIES

Molecular Genetics

Peter Smith-Keary

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Introduction

This book is intended as a revision manual for students taking first-year university courses in Molecular Genetics or Genetics and is particularly directed at those intending to pursue further courses in Molecular Genetics and/or Molecular Biology. It is not intended that it should replace a standard introductory text but rather that one should complement the other, and for this reason many features of a textbook, such as historical perspective, detailed descriptions of experimental techniques, multiple examples and lists of further reading, are not included. However, all the basic information required for most introductory courses in Molecular Genetics is included.

The first 11 chapters contain material that is common to nearly all introductory courses in Genetics and Molecular Genetics. Chapter 12 introduces some highly selected aspects of the molecular genetics of eucaryotes, and Chapter 13 outlines some fundamental aspects of genetic engineering; the material in these chapters will be relevant to some courses but not to others.

The Lay-out of the Book

Each chapter is in three parts:

- (1) A concise description of basic concepts.
- (2) A wide range of specimen answers to examination-style questions. Considerable emphasis is placed on these answers and, when using this book for revision, it is important to appreciate that most of the detailed information together with any more advanced material is *only* given in these answers; thus, these model answers are a fundamental part of the text.
- (3) A short selection of further questions, brief answers to which are given at the end of the book.

The Questions and Answers

The questions require answers of varied length, ranging from a few lines to a short essay (30–40 minutes in an examination), but, to increase their usefulness, some of the longer questions are sectionalised and so can be regarded as several shorter questions and answers. Most of the text itself is also written in the style of answers to questions suggested by the chapter sub-titles.

Since the answers are a fundamental part of the text, it has frequently been necessary to provide some information and explanation additional to that contained in the examination-style answers; this further material, which would

not be relevant to an examination answer, either is enclosed in parentheses or appears as notes following the answer.

For convenience in cross-referencing and to increase their use for revision, the figures in the specimen answers have both serial numbers and descriptive legends; these would not normally be required in an examination answer.

The student is strongly recommended to read each question and to plan carefully his or her answer before proceeding to read the model answers.

Answering Examination Questions

Three particular points should always be adhered to:

(1) *Read the question carefully* It is surprising how frequently students misread a question and so fail to answer the question as set.

(2) *Keep to the point* Do not waste time by including irrelevant material. If, for example, you are asked to describe how DNA replicates, it is quite pointless to include a description of DNA structure; you will ONLY be marked on what is relevant to the question as set.

(3) *Apportion your time* This is necessary so that you have time to attempt all the questions that you are required to answer. If you answer less than the required number of questions, you will get one or more zero marks.

Good luck to all who use this book!

Leominster, 1990

P.S.-K.

Glossary

- Alleles** Alternative forms of the same gene (for example, vg and vg^+). (47)
- Anticodon** The sequence of three nucleotides in a molecule of tRNA that recognises a complementary sequence of nucleotides (the codon) on a molecule of messenger RNA. (132)
- Antisense strand** The strand of the DNA duplex that acts as the template for messenger RNA synthesis. (130)
- Autoradiograph** The photographic image produced by labelling a molecule, such as a phage or bacterial chromosome, with a radioactive label and overlaying it with a photographic emulsion. The emissions from the radioactive label expose the emulsion and, after development, reveal the size and shape of the underlying molecule. (36)
- Autosome** Any chromosome that is not directly involved in the determination of sex. (7)
- Auxotroph** A mutant micro-organism that can only grow when a particular amino acid, nucleotide or vitamin is provided in the medium. (98, 113)
- Back-cross** A cross between an organism and one of its parents. (49)
- Back-mutation** A mutation which results in a mutant gene regaining its normal activity by restoring the exact nucleotide sequence present in the wild-type gene. (94, 192, 249)
- Bacteriophage** A virus that infects bacteria. (9, 88)
- Base substitution** The replacement of one base (or base pair) in a nucleic acid by a different base (or base pair). (162, 183)
- Bivalent** The pair of synapsed homologous chromosomes present during the prophase of meiosis. (5)
- Carcinogen** A physical or chemical agent that causes cancer. (190)
- Centromere** A specialised region of a chromosome to which the spindle fibres attach at cell division. (3, 35)
- Chain-termination (nonsense) triplet (CTT)** A codon signalling the termination of polypeptide synthesis. These codons are UAG (amber), UAA (ochre) and UGA (opal). (135, 185)
- Chiasma** The crossed-over strands of two non-sister chromatids seen at the first meiotic division in eucaryotes; the position at which two homologous chromosomes appear to exchange genetic material during meiosis. (5, 11)
- Chromatid** In eucaryotes one of the two identical strands of a newly replicated chromosome. (3)
- Chromatin** The nucleoprotein complexes that form the chromosomes. (33)
- Chromomere** A laterally differentiated region of a chromosome. In meiotic chromosomes the chromomeres appear like beads on a string but in the giant salivary gland chromosomes each chromomere is seen as a transverse band. (39)

Chromosome The molecule of nucleic acid (in procaryotes and viruses) or the complex of DNA, RNA and protein (in eucaryotes) carrying the genetic information. (2, 31, 33)

Cis-arrangement Describes the situation where two mutant sites or genes are on the same chromosome or molecule of DNA; in eucaryotes a double heterozygote in the coupling phase (*AB/ab*). (115)

Cis-trans test A genetic test to determine whether two mutations are in the same or in different genes. (115)

Cloning The production of very many genetically identical molecules of DNA, cells or organisms. (223)

Codon The three consecutive nucleotides in RNA or DNA encoding a particular amino acid or signalling the termination of polypeptide synthesis. (151)

Cohesive (sticky) ends Single-stranded and base-complementary sequences of nucleotides at opposite ends of a molecule of DNA. (42)

Complementation The ability of two mutant genes to make good each other's defects when present in the same cell but on different molecules of DNA. (114)

Concatamer An end-to-end (tandem) array of identical DNA molecules. (29)

Conditional lethal mutant A mutant able to grow under one set (permissive) of environmental conditions but lethal under different (restrictive) conditions. (183)

Consensus sequence An idealised nucleotide sequence where the base at each particular position is the one most frequently observed when many different actual sequences are compared. (138)

Constitutive gene expression A gene or operon which is expressed at all times and under any environmental conditions. (169, 171)

Coupling Two pairs of linked genes are in the coupling phase when both dominant alleles were contributed by one parent and both recessive alleles by the other parent (for example, *AB/ab*). (75)

Crossing-over The process of genetic recombination that gives rise to new combinations of linked genes. (10, 65)

Deletion The loss of one or more base pairs from a molecule of DNA. (152, 185)

Denaturation The treatment of a molecule of double-stranded DNA so that it separates into its two component strands. (27, 141)

Dihybrid cross A cross between two dihybrids (for example *A/a B/b* × *A/a B/b*). (51)

Diploid A eucaryotic cell or organism in which the chromosomes exist in pairs. (3)

Direct repeats Two identical (or nearly identical) nucleotide sequences sometimes separated by a sequence of non-repeated DNA, for example
5' ATTCGAATTCGA 3'
3' TAAGCTTAAGCT 5' (199)

DNA ligase The enzyme that joins together the 5' and 3' ends of polynucleotide chains by forming a phosphodiester bond between them. (22)

DNA polymerases The enzymes that polymerise deoxyribonucleotides on to existing polynucleotide chains using the complementary DNA strands as templates. (21)

DNA primase The enzyme that normally synthesises the RNA primers required for initiating DNA synthesis. (22)

- Dominant gene** The gene (or, more correctly, the character) that is expressed in a heterozygous or partially heterozygous cell (for example, vg^+ in vg^+/vg). (48)
- Endonuclease** An enzyme that makes breaks in a molecule of DNA by hydrolysing internal phosphodiester bonds. (237)
- Eucaryote** An organism with nuclear membranes and certain organelles, such as mitochondria, and mitotic spindles. (1)
- Euchromatin** The parts of a eucaryotic chromosome that show the normal cycle of condensation at cell division. (33)
- Exonuclease** An enzyme that digests a molecule of nucleic acid by removing successive nucleotides from the 5' or 3' end. (21, 237)
- F⁺ cell** A bacterial cell harbouring an F plasmid. (91)
- F plasmid or F factor** The fertility factor of *Escherichia coli*; it enables cells to conjugate. (91)
- F-prime plasmid (F')** An F plasmid carrying a segment of the bacterial chromosome. (95)
- Frameshift** A mutation which adds or deletes one or two base pairs from a coding sequence in a molecule of DNA, so that the genetic code is read out-of-phase. (152, 185)
- Gene** The genetic unit of function; it may (i) encode a polypeptide, (ii) encode a molecule of ribosomal or transfer RNA or (iii) be a regulatory sequence involved in the control of gene expression. (47)
- Genome** The complete gene content of a cell or organism.
- Genotype** A specific description of the genetic constitution of an organism. (48)
- Haploid** A cell or organism with only one set of chromosomes. (3)
- Hemizygous** Describes the situation in a diploid organism whereby a certain gene or chromosome is only present in a single copy, for example, the X-linked genes and the X chromosome in an XY male. (54)
- Heterochromatin** Chromatin that is relatively over- or under-condensed at cell division. (34)
- Heteroduplex** A molecule of double-stranded nucleic acid where the two strands are of different origin and so may not have exactly complementary base sequences. (208)
- Heterozygote** A cell or organism where different alleles are carried by the two members of a pair of homologous chromosomes. (3, 47)
- Histones** Basic proteins found complexed with DNA in eucaryotic chromosomes. (33)
- Homozygote** A cell or organism where the same allele is carried by each member of a pair of homologous chromosomes. (3, 47)
- Inducible system** A regulatory system where the genes are only turned on and enzymes synthesised when the appropriate substrate is present. (165)
- Insertion sequence (IS)** A transposable nucleotide sequence that only encodes functions related to its own transposition. (190, 198)
- Inverted repeats (IR)** Where the sequence of nucleotides along one strand of DNA is repeated in the opposite physical direction along the other strand; inverted repeats are often separated by a tract of non-repeated DNA. For example
- $$\begin{array}{ccccccc}
 5' & & \text{GATG} & \text{.....} & \text{CATC} & & 3' \\
 3' & & \text{CTAC} & \text{.....} & \text{GTAG} & & 5'
 \end{array}$$
- (23)
- Karyotype** A description of the chromosome content of a cell or organism. (7)

Kilobase (kb) A unit of length of 1000 nucleotides or 1000 nucleotide pairs.

Lagging strand The strand of newly replicated DNA that is synthesised discontinuously and away from the replication fork. (20)

Leading strand The strand of newly replicated DNA that is synthesised continuously and towards the replication fork. (20)

Ligation The formation of a phosphodiester bond between two adjacent bases separated by a single-strand break. (42)

Linkage The tendency of genes close together on the same molecule of DNA (chromosome) to be inherited together. (65)

Linkage map A map, assembled from recombination data, showing the order of mutant sites and genes along a chromosome. (66)

Locus The site on a chromosome where a particular gene is located. (3)

Lysogen A bacterial cell carrying a phage genome as a repressed prophage. (88)

Lytic response When an infecting phage genome replicates, matures and eventually lyses the bacterial cell, releasing free phage. (88)

Meiosis The special type of cell division, which halves the number of chromosomes in a diploid organism so as to form haploid gametes or spores. (4)

Messenger RNA (mRNA) The transcript of a segment of chromosomal DNA which is a template for polypeptide synthesis. (130)

Micrometre (μm) 1×10^{-6} metres, 1000 nm.

Missense mutation A mutation that changes a codon for an amino acid into a codon for a different amino acid. (184)

Mitosis The normal process of cell division. (3)

Mutagen An agent that increases the frequency of mutation. (186)

Mutant A cell or organism with a defective gene and displaying a specific altered phenotype. (47, 182)

Mutation The process that produces a sudden heritable change in the nucleotide sequence of an organism; any such change in the nucleotide sequence. (182)

Nanometer (nm) 1×10^{-9} metres.

Negative control A regulatory system where gene activity only occurs in the *absence* of a specific regulatory protein. (169)

Nonsense mutation A mutation which alters a codon for an amino acid to a chain-termination triplet. (155, 185)

Nucleolus A granular structure found in eucaryotic cells attached to a specific chromosomal site, the **nucleolar organiser**. It is the site of ribosomal RNA synthesis. (2)

Nucleoside A purine or pyrimidine base linked to a molecule of ribose or deoxyribose. (15)

Nucleotide A nucleoside with an attached phosphate group. (16)

Okazaki fragments The short discontinuously synthesised fragments that are ligated together to form the lagging strand during DNA replication. (20)

Operator The DNA sequence to which a repressor protein reversibly binds so as to regulate the activity of one or more structural genes. (167)

Operon A group of co-ordinately controlled structural genes, together with the operator and promoter sequences that control them. (166)

Palindrome A pair of adjacent inverted repeat sequences. (224)

Phage (bacteriophage) A virus that infects a bacterial cell. (9, 88)

- Phenotype** The appearance or other observable characteristics of an organism. (48)
- Phosphodiester bond** The covalent bond joining the 3'-hydroxyl of the sugar moiety of one (deoxy) ribonucleotide to the 5' hydroxyl of the adjacent sugar. (17)
- Plasmid** An extra-chromosomal, covalently closed circular molecule of DNA carrying non-essential genetic information and replicating independently of the chromosomal DNA. (91)
- Point mutation** A mutation involving the addition, deletion or substitution of a single base pair (or, occasionally, several adjacent base pairs). (183)
- Polypeptide** A compound made up of two or more amino acids joined together by peptide bonds. (109)
- Positive control** A regulatory system where gene activity only occurs in the *presence* of a gene-encoded regulatory protein. (170)
- Primer** The short strand of RNA which provides the free 3'—OH end on to which DNA polymerase can then add deoxyribonucleotides. (20)
- Prokaryotes** Organisms lacking nuclear membranes and certain organelles, such as mitochondria, and mitotic spindles. (8)
- Promoter** The DNA sequence to which RNA polymerase binds in order to initiate transcription. (130, 138)
- Prophage** A phage genome whose lytic functions are repressed and which replicates in harmony with the bacterial chromosome; it is, as with lambda, frequently integrated into the chromosome of the host bacterium. (88)
- Prototroph** A micro-organism able to grow on minimal medium containing only certain inorganic salts and a carbon source. (113)
- Reading frame** One of the three ways by which a coding sequence of nucleotides can be read in consecutive groups of three. (152)
- Recessive** The gene (or, more correctly, the character) that is not expressed in a heterozygote (for example, vg in vg^+/vg). (48)
- Recombinant DNA** A DNA molecule made by joining together two different DNA molecules. This term is usually applied to molecules made by *in vitro* ligation. (225)
- Recombination** The process which produces new combinations of the genetic material following the occurrence of crossing-over between two homologous molecules of DNA. (65)
- Redundant** Applies to a gene or nucleotide sequence that is present in more than one (usually many) copies. (48, 204)
- Replication fork** The Y-shaped region of a DNA molecule where the two strands have separated and replication is taking place. (19, 21)
- Replicon** A molecule of DNA able to initiate its own replication.
- Repressor** The protein product of a regulator gene; when it binds to its own operator it prevents transcription of the adjacent structural genes. (168)
- Repulsion** Two pairs of linked genes are in the repulsion phase when the paternal and maternal chromosomes each carry one dominant and one recessive gene (for example, Ab/aB). (75)
- Restriction endonuclease** A large group of endonucleases each of which recognises and attacks a specific DNA sequence; they are extensively used in recombinant DNA technology. (224)
- Reversion** Any mutation that restores (or partially restores) the wild phenotype of an organism. (158, 191)

RNA polymerase The enzyme that normally synthesises RNA against a DNA template. (23)

RNase An enzyme that hydrolyses RNA molecules. (237)

Rolling-circle (sigma) replication The type of replication where a replication fork moves round and round a circular molecule of DNA producing a single-stranded concatamer which may then become double stranded by the synthesis of a complementary strand. (29)

Same-sense mutation A mutation which changes the nucleotide sequence of a codon so that it still encodes the same amino acid. (184)

Screening A technique to increase the proportion of a rare phenotype in a population so making its recovery easier. (191)

Selection A technique, using a specific set of environmental conditions, which allows the survival of mutant or recombinant cells having a particular phenotype and excludes any other more common phenotypes. (90, 191)

Semi-conservative replication The method whereby DNA normally replicates so as to produce two daughter molecules each consisting of one parental and one newly synthesised strand. (19)

Sense strand The strand of the DNA duplex that is *not* transcribed and which has the same nucleotide sequence as the messenger RNA. (131)

Silent mutation A mutation which changes the nucleotide sequence but which has no detectable effect on the phenotype. (182)

Sister chromatids In eucaryotes, two identical chromatids held together by a common centromere. (3)

Site The position of a mutation within a gene; a specific base pair. (95, 196)

Suppressor mutation A mutation that restores, or partially restores, the loss of function caused by a different mutation. Many suppressor mutations are in genes encoding a species of tRNA; the altered tRNA can recognise the original mutant codon and, during translation, insert an acceptable substitute amino acid into the polypeptide. (193)

Tandem duplication A directly repeated DNA sequence. (211)

Temperate phage A phage that can either establish itself as prophage or can enter the lytic response when it infects a sensitive cell. (88, 99)

Terminator The DNA sequence that signals the end of transcription. (131, 139)

Tetrad The four products of a single meiosis. (6, 73)

Theta-type (Cairns-type) replication Replication of a circular molecule of double-stranded DNA by initiation at a unique origin and proceeding in one or both directions around the circular molecule. (32)

Trans-arrangement Describes the situation where two mutant sites or genes are on different chromosomes or molecules of DNA; in eucaryotes a double heterozygote in the repulsion phase (*Ab/aB*). (114)

Transcription The process by which RNA polymerase (or DNA primase) produces a molecule of RNA using one strand of a DNA duplex as a template. (130)

Transduction The transfer of genes from one bacterium to another by a phage vector. (93)

Transfer RNA (tRNA) Adaptor molecules which, on the one hand, bind to a particular amino acid and, on the other hand, recognise the corresponding amino acid codon on the messenger RNA, thus correctly aligning the amino acids for assembly into a polypeptide. (132)

Transformation The transfer of genes from one bacterium to another by

'infecting' a recipient strain with purified DNA extracted from a genetically different donor. (26, 95)

Translation The assembly of amino acids into polypeptides using the genetic information encoded in the molecules of messenger RNA. (131)

Transposon A transposable genetic element which, in addition to encoding the proteins required for its own transposition, confers one or more new recognisable phenotypes (usually resistance to one or more drugs) on the host cell. (190)

Transposition The transfer of a discrete segment of DNA from one location in the genome to another. (190)

Vector An independent replicon, usually a small plasmid or viral genome, used to introduce a 'foreign' gene into a host cell. (223)

Virulent phage A phage that can only enter the lytic cycle when it infects a sensitive host cell. (88, 99)

Zygote The diploid formed by the fusion of the male and female gametes. (4)

*The numbers refer to the pages where the definitions are more fully explained. The explanations of technical terms not listed in this glossary can be found in the text by reference to the index.