Bacteriocin

- Antagonistic proteinaceous compounds by MO
- Small heat-stable peptides
- Large heat-labile proteins
- Modified peptides I.e. lantibiotics
- Examples: nisin, diplococcin, pediocin, helvetican J

Nisin

- Antimicrobial peptide
- Nisin A (NisA, *nisA*) and nisin Z
- Ribosomally synthesized precursor peptide undergoes extensive post-translational modification with 5-ring structures—lantibiotics
- Wide antimicrobial spectrum
  - G+
  - G-

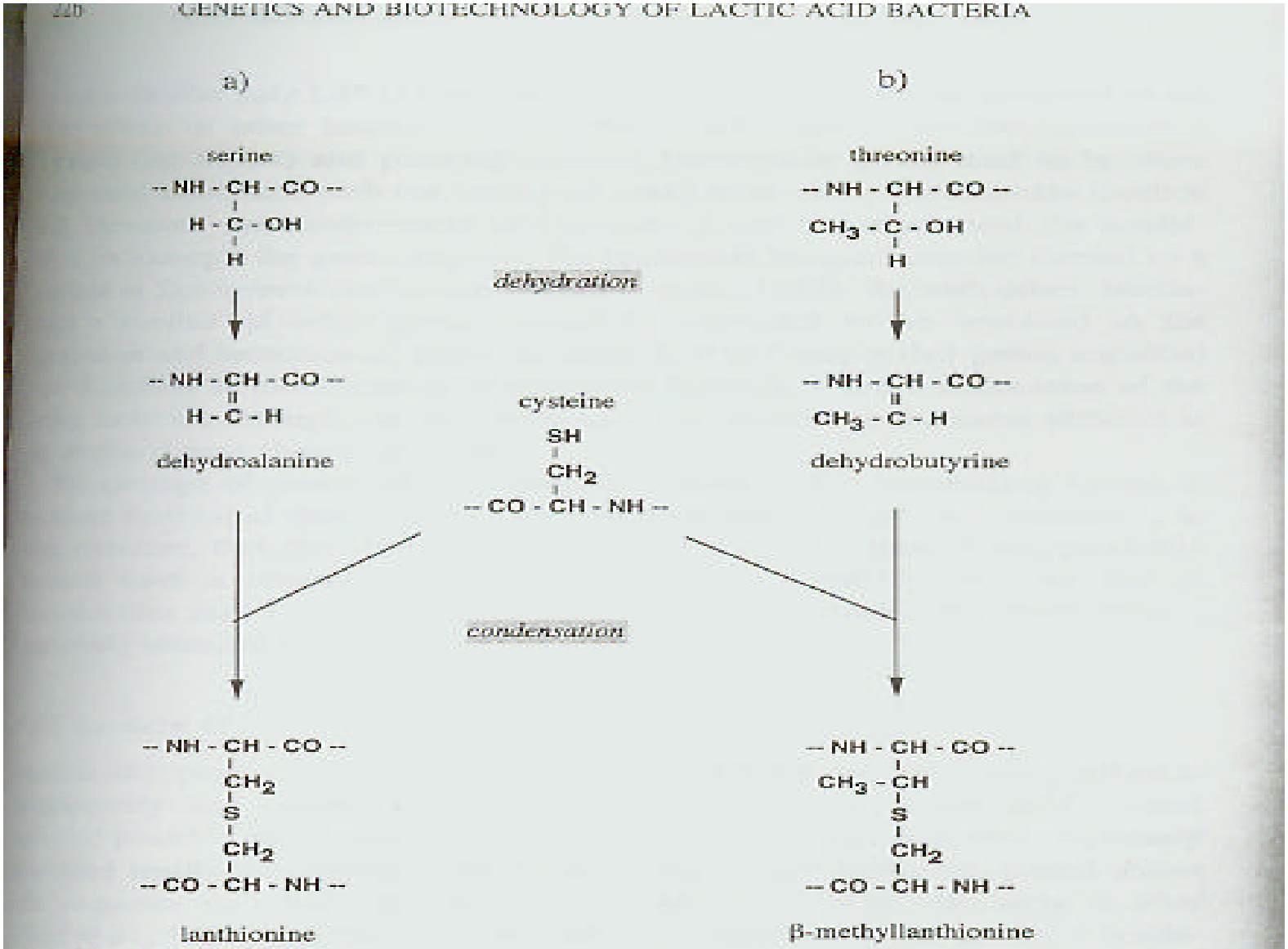


Figure 5.3 Mechanism of biosynthesis of non-coded residues present in mature nisin. (a) dehydroalanine and lanthionine and (b) dehydrobutyrine and β-methyllanthionine synthesis.

Nisin gene cluster

- 11-gene cluster
- *nisA*: encode nisin A 57 a.a. precursor peptide
- *nisB*, *nisC*: post-translational modification
- *nisT*: transport protein (ABC translocator)
- *nisP*: subtilisin-like proteinase
- *nisI*: lipoprotein (immunity)
- *nisFEG*: putative transporter proteins
- *nisR* and *nisK*-two component regulatory system
  - *nisR*: response regulator
  - *nisK*: sensor histidine kinase

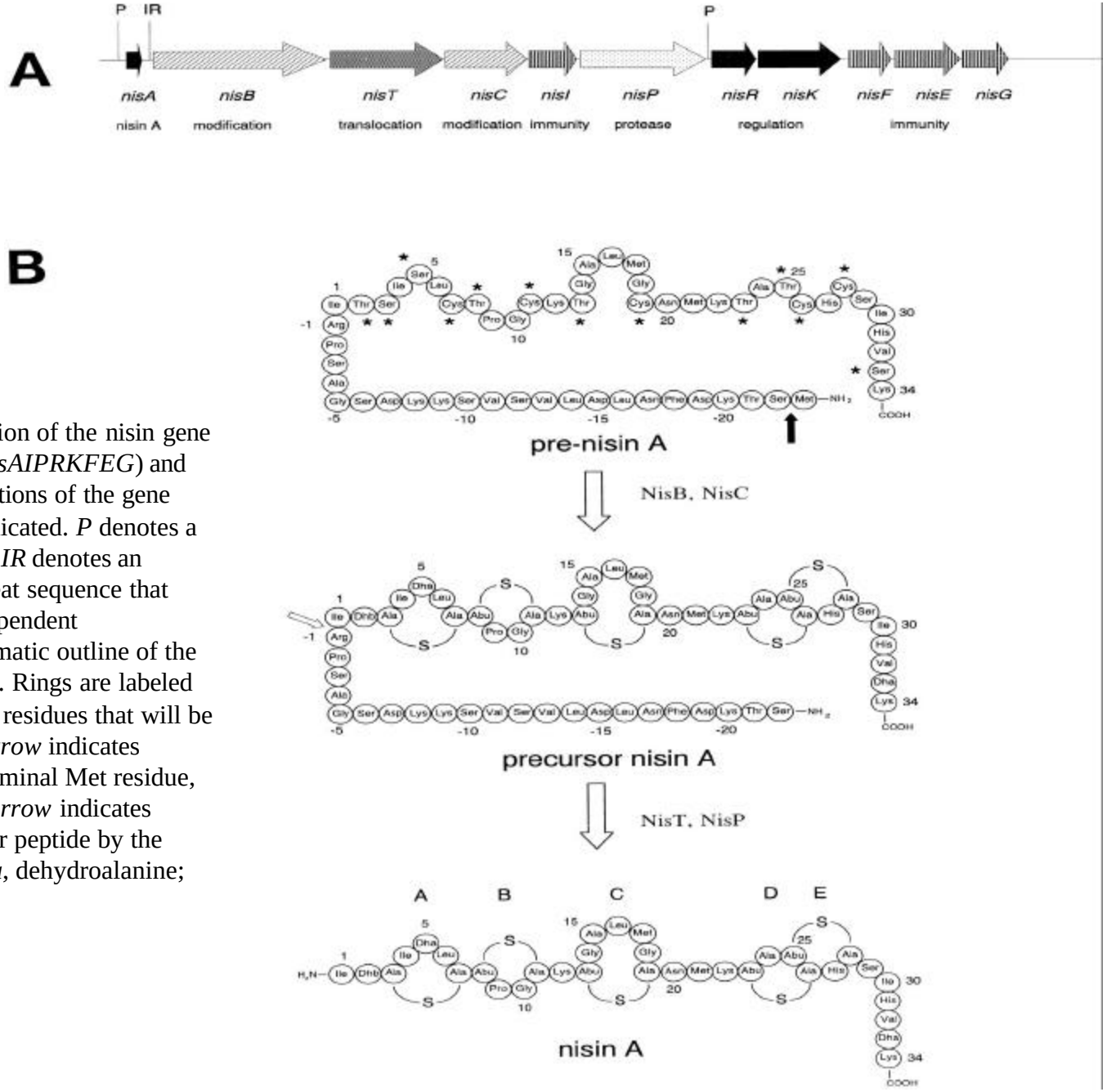
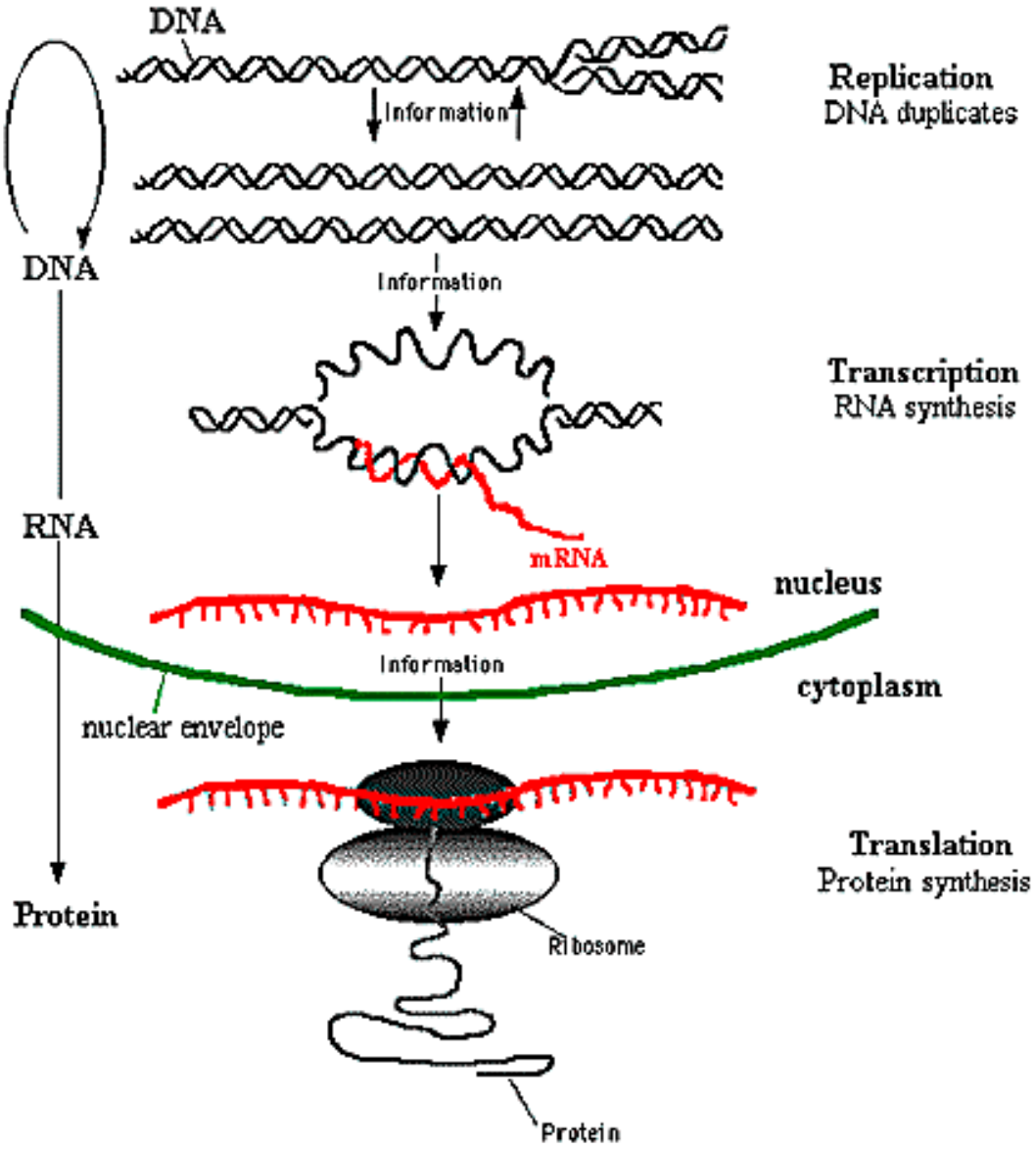
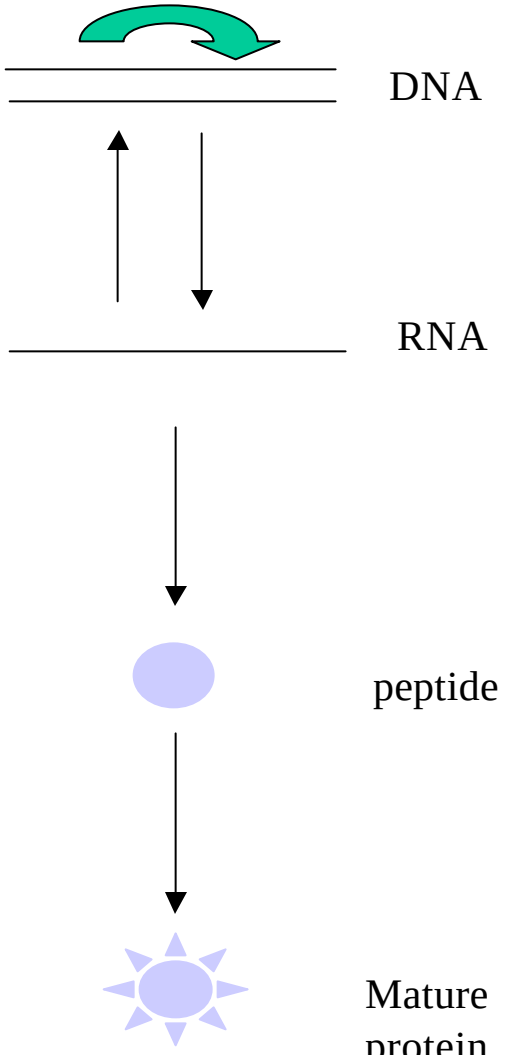


Figure 1: A, organization of the nisin gene cluster. Established (*nisAIPRKFEG*) and putative (*nisBCT*) functions of the gene products have been indicated. *P* denotes a mapped promoter, and *IR* denotes an extensive inverted repeat sequence that could act as a rho-independent terminator(7) . *B*, schematic outline of the biosynthesis of nisin A. Rings are labeled *A-E*. *Asterisks* indicate residues that will be modified. The *black arrow* indicates processing of the N-terminal Met residue, while the *small white arrow* indicates processing of the leader peptide by the action of NisP(8) . *Dha*, dehydroalanine; *Dhb*, dehydrobutyrine.



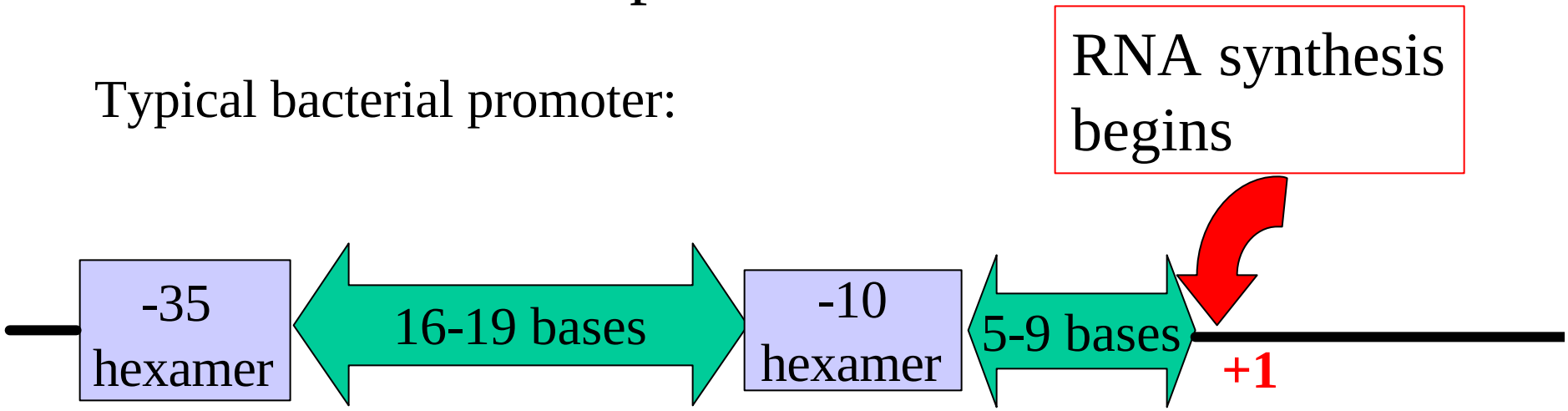
The Central Dogma of Molecular Biology



Bacterial transcription

Promoter = a DNA sequence involved in binding RNA polymerase and required for the initiation of transcription

Typical bacterial promoter:



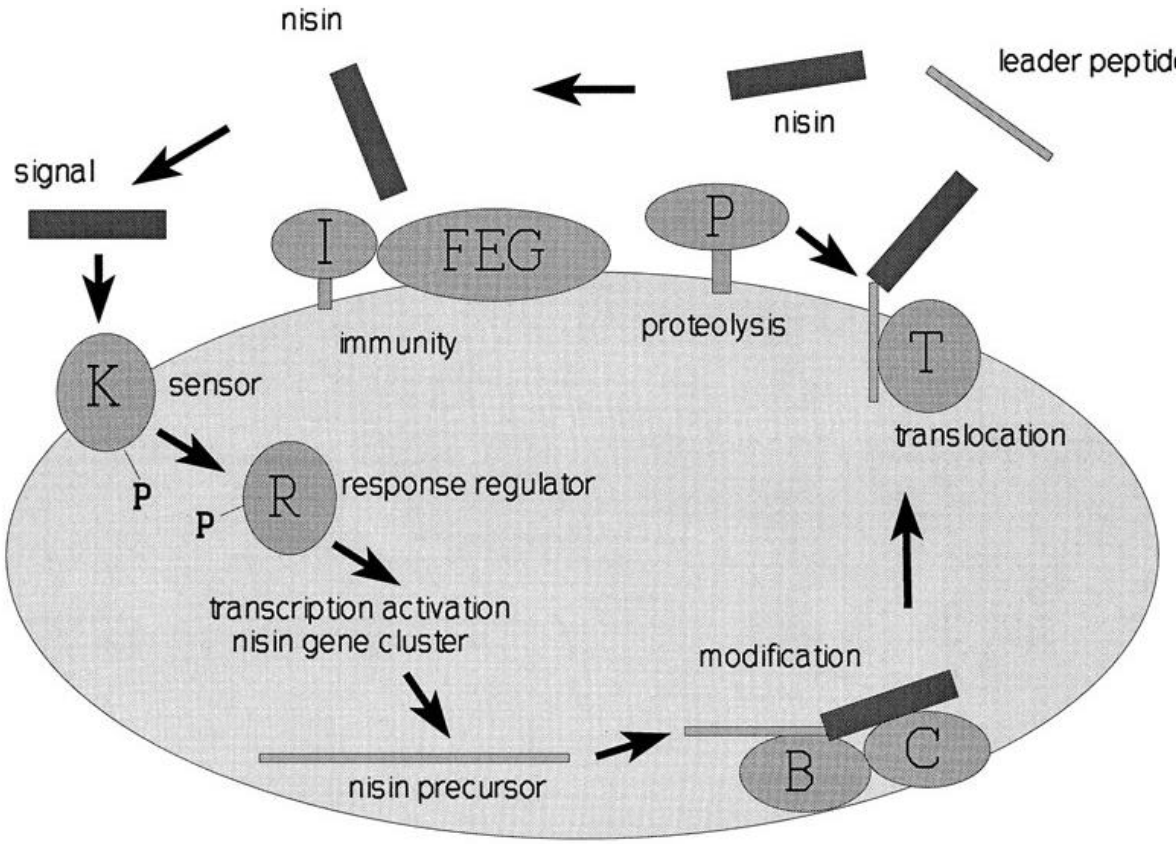


Figure 4: Model for nisin biosynthesis and regulation. In Step 1, NisK senses the presence of nisin in the medium and autophosphorylates. In Step 2, the phosphate group is transferred to NisR, which acts as a transcriptional activator, followed by mRNA synthesis and ribosomal synthesis of unmodified precursor nisin and of biosynthetic proteins. In Step 3, the precursor is modified by the putative enzymes NisB and NisC(7, 9) . In Step 4, the fully modified precursor peptide is translocated across the membrane by the putative ABC transporter NisT(7, 9) . In Step 5, fully modified precursor nisin is extracellularly processed by NisP(8) , resulting in the release of active nisin. NisI(9) , together with NisF, NisE, and NisG(11) , protects the cell from the bacteriocidal action of nisin by a thus far unknown mechanism.

Nisin: mode of action

- Form voltage-dependent pores in the bacterial cytoplasmic membrane
- Spcific binding of nisin to the cell wall precursor lipid II coincides with pore formation

Nisin immunity

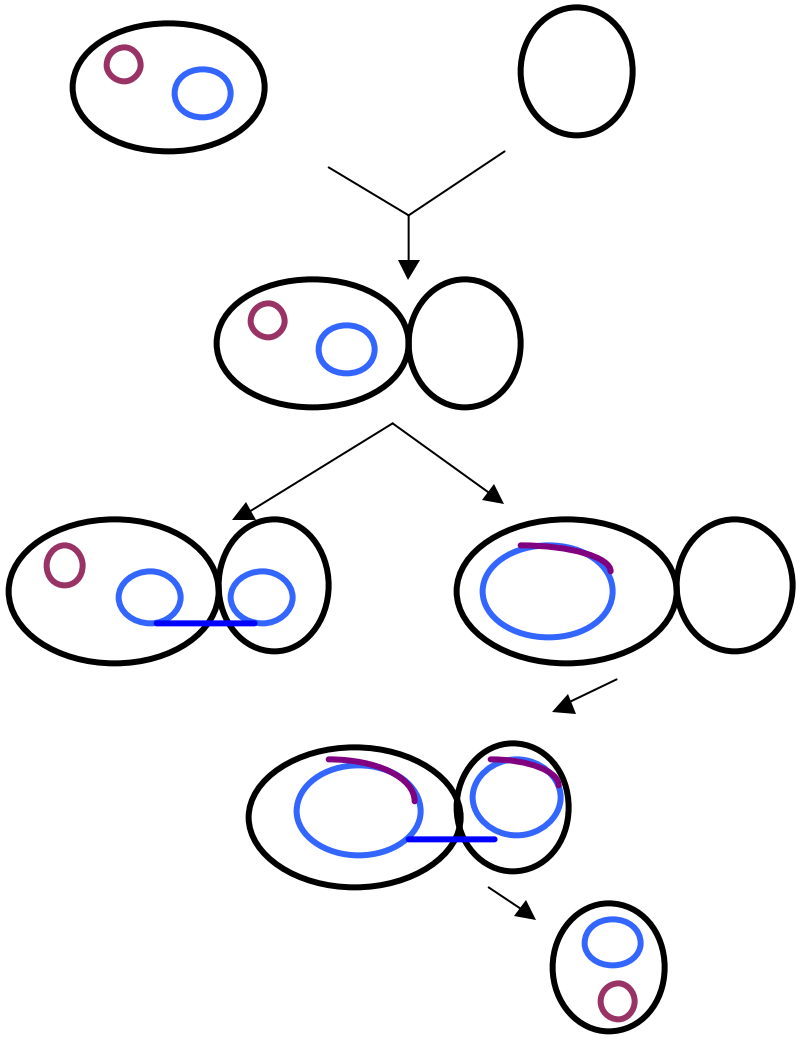
- Mechanisms for nisin producing strain to survive the bacteriocin
- *nisI*-encoding a lipoprotein
- *nisFEG*, encoding a putative ATP binding cassette exporter-nisin extrusion
- Both required for full immunity

Immunity

- NisI (lipoprotein) acts as nisin-sequestering protein
- NisFEG as nisin exporter expels nisin molecules from the cytoplasmic membrane into the environment

Gene transfer systems in LAB

- Conjugation
  - Donor, recipient
  - Phenotypic traits
  - Natural gene transfer mechanism
  - Plasmid, transposons
  - Self-transmissible; mobilization
  - Cointegrate
  - Plasmid incompatibility



Gene transfer systems in LAB

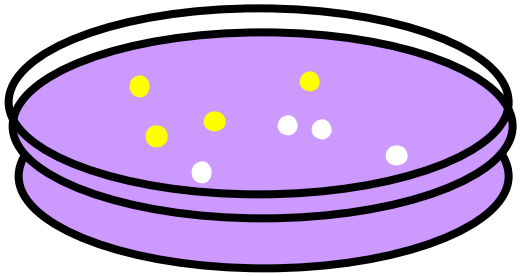
- Transduction
  - Bacterial genetic information erroneously packaged into bacteriophage headss and passively transferred to a phage sensitive host
- Protoplast fusion
  - Protoplast formation; fusion

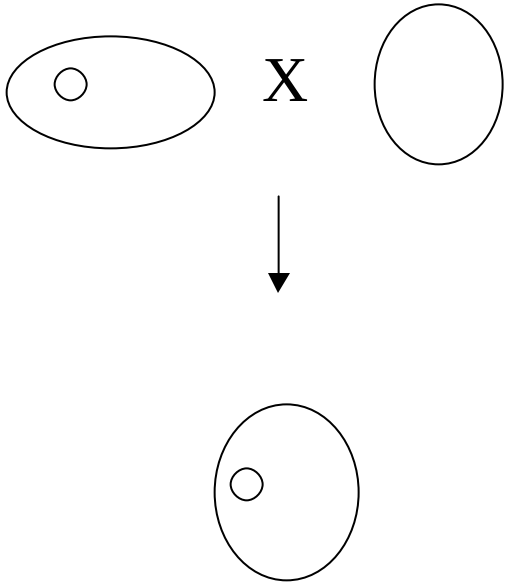
Gene transfer systems in LAB

- Transformation
  - Introduce free DNA in its native form or following in vitro manipulation into a recipient cell
  - Natural competence
  - artificial competence
  - Electroporation
  - Protoplast transformation
    - Enzyme treatment; osmotic stabilizer; PEG
  - Transfection
    - Bacteriophage DNA introduced into recipient cells
    - Plaque instead of colony

Selectable traits

- Antibiotic resistant (recipient)
- Sugar fermentation, i.e., lactose fermentation (donor)
- Selective media for transconjugants





Summary

- Bacteriocin production
- Immunity mechanisms
- Signaling-sense the environment
- Gene transfer mechanisms

Genetic Analysis

- Promoter activity analysis
  - Develop promoter fusion vectors
- The requirement of the *nisR* and *nisK* in the regulation of he promoters of the nisin gene cluster
  - Inactivate chromosomal gene
  - Introduce another intact gene on a plasmid
  - Study the expression of reporter system

Reporter systems

- The Gus system
  - ß-Glucuronidase hydrolyzes conjugated glucuronides

    HOCO-CH2CH2CH(NH2)CO2H-→HOCO-CH2CH2CH2NH2+CO2
  - Enzyme assay: X-Gluc as substrate (Fishman *et* al., 1948)

Reporter Systems

- The Lac system
  - ß-galactosidase
  - Hydrolyze lactose →galactose + glucose
  - Enzyme assay: use X-gal as substrate
- Lac operon