STRATEGIES FOR RISK ASSESSMENT AND INSTITUTIONAL OVERSIGHT OF EMERGING RNAi TECHNOLOGIES

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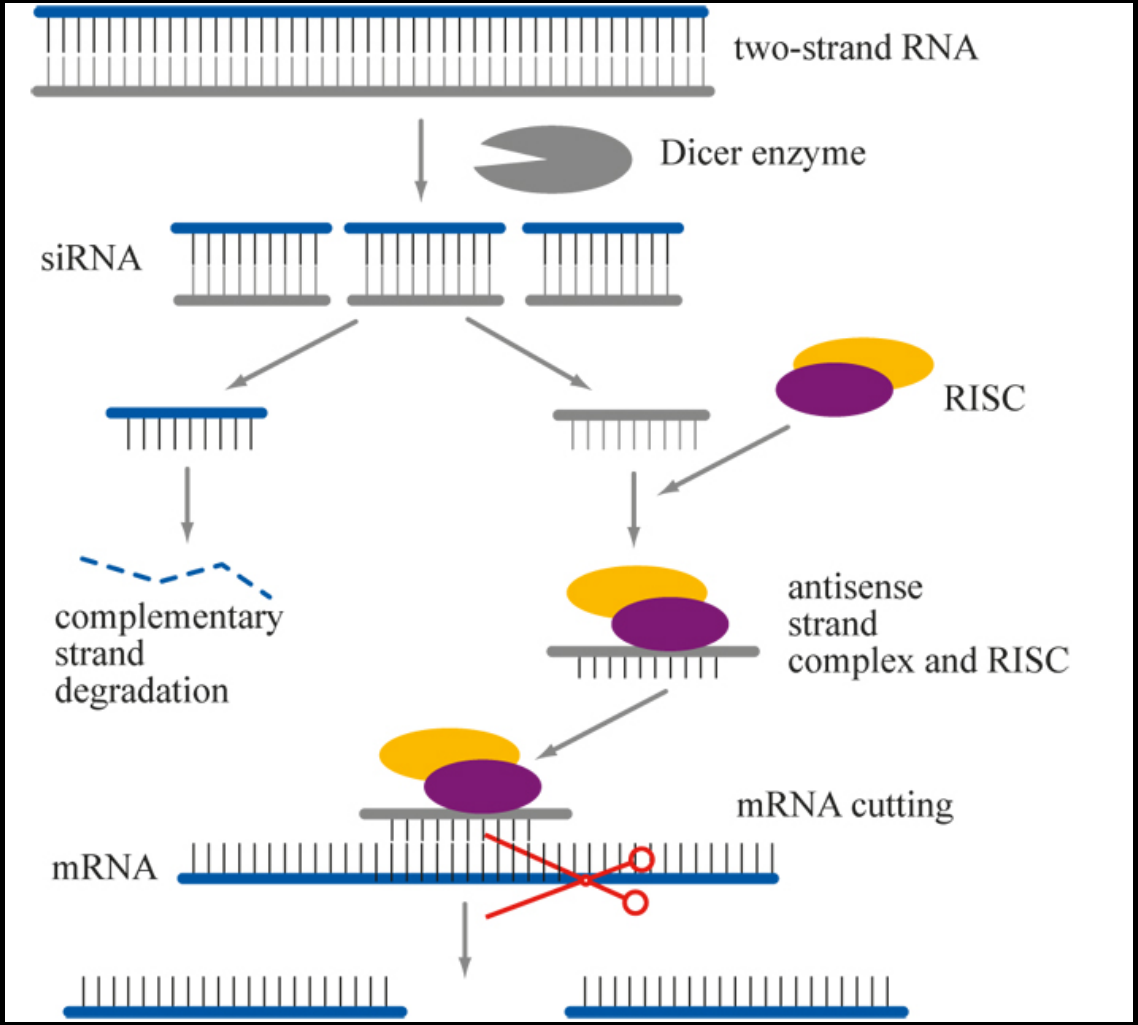
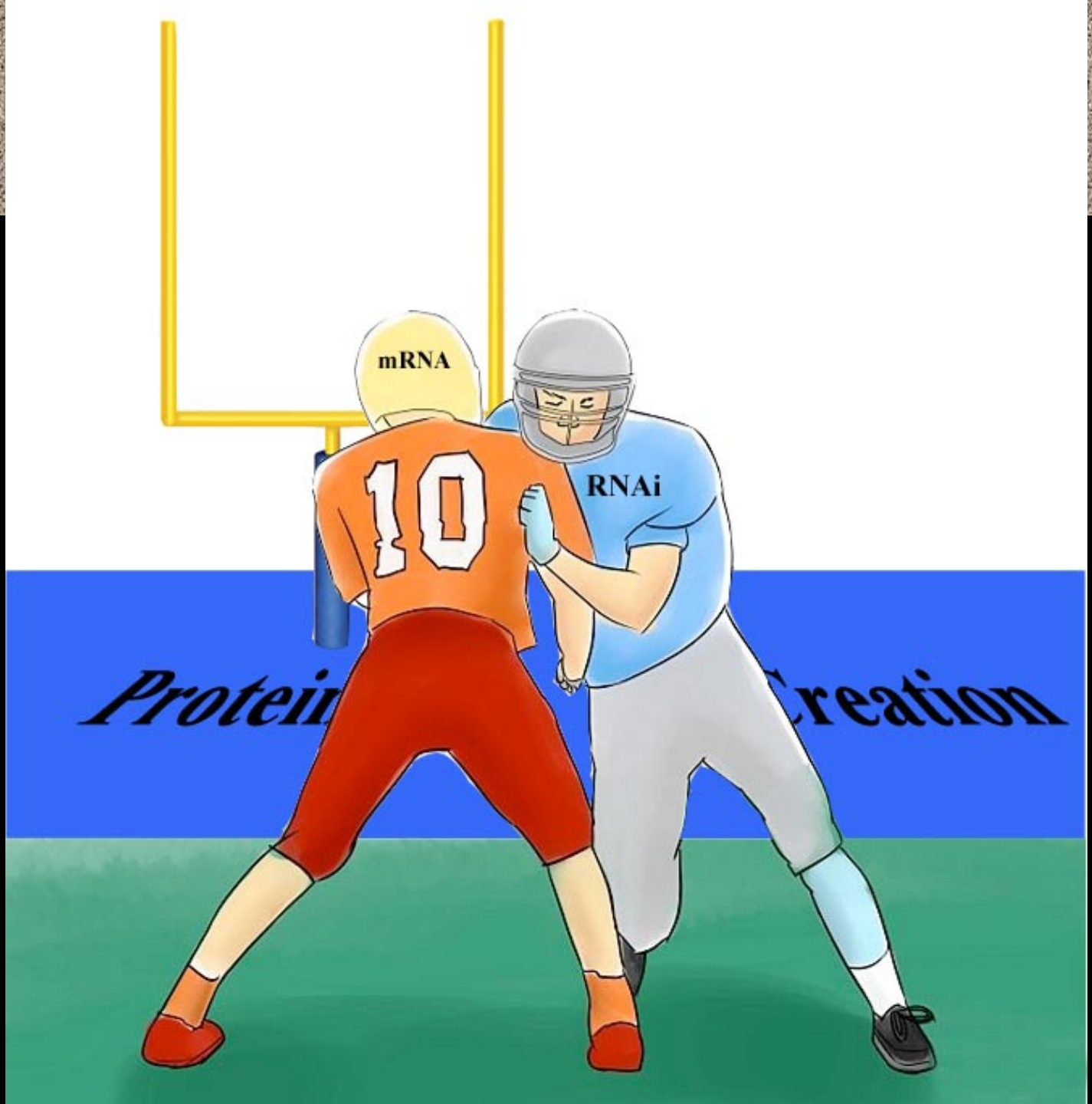
RNAi Background- Brief History

- Discovered in 1998 by Andrew Fire and Craig Mello.
- Demonstrated in 2001 to be an effective methods for silencing specific genes in mammalian cells.
- Has since been used to knock down specific genes in cell lines and animal models.
- Growing interest in using RNAi processes in human therapies by blocking the creation of harmful proteins.

RNAi Background- Basic Function




mRNA
10
RNAi

RNAi Methods

There are different methods of RNAi that can be used, depending on the experiment.

- Mammalian culture and animal models: short interfering RNA (siRNA), short hairpin RNA (shRNA)
- Other organism models (nematodes, insects, plants): long double stranded RNA (dsRNA) may also be used.

siRNA

- siRNA is an intermediate in the (RNAi) pathway. It occurs naturally in the cytoplasm or may be synthesized externally and introduced to the cell.
- A RNA oligonucleotide of this sequence and its complement are synthesized, mixed and allowed to anneal to form an RNA duplex.
- This duplex can then be transfected into cells or introduced into animals.
- The resulting suppression of the target gene's mRNA is transient as the amount of siRNA within the cells is reduced by degradation and cell division.
- Limited to cells that are amenable to synthetic oligos

shRNA

- shRNA is produced inside the target cell from a DNA construct that has been delivered to the nucleus.
- The construct contains a nucleotide target sequence, a loop domain, and then a nucleotide sequence complementary to the target sequence.
- shRNA DNA cassettes placed into plasmid vectors and either directly administered to cells or animals or used to make viral vectors which then introduce the construct by viral infection.
- When transcribed, the RNA twists into the short hairpin structure which brings the complementary target RNA sequences together to form an RNA duplex.
- Gene suppression can be transient or persistent.

dsRNA

- Many organisms, most notably *C. elegans* and *Drosophila*, have the capacity to take up dsRNA segments of 200-400 nucleotides in length and then process them into nucleotide pieces which are taken up by the RNAi as described.
- Whole *C. elegans* and *Drosophila* cell culture can passively absorb dsRNA. Targeted microinjection of dsRNA is also used.
- Gene suppression can be persistent. Some RNAi mediated suppression in *C. elegans* persists into first generation offspring.

Biosafety Risk Assessment Considerations

- Inherent laboratory risk of organism or cell type in which knockdown will occur
- Gene function/biological activity
- Method of introduction
  - ✓ Via vector- vector amount, nature of vector
  - ✓ Via polymers, synthetics, or lipids to cytoplasm

IBC Oversight

- Overall Risk assessment
- Agent
- Gene Inserts
- Risk Group
- Biosafety Level
- Special Hazards
- NIH Guidelines
- BMBL Guidelines

COMS Review- Case Study #1

Project Summary: The laboratory is studying lentivirus vector packaging and expression in cells infected with herpes simplex virus 1 (HSV1) or cytomegalovirus (CMV). The lentivirus vector will deliver interfering ribonucleic acids (RNAi) into human cell lines. Cells expressing shRNA will be infected with either HSV-1 or HCMV. Growth of HSV-1 and HCMV will be tested to determine if the proteins depleted with RNAi are essential for virus replication. The lentivirus is a VSV-G pseudotyped, self-inactivating, 2" generation system.

Agents	BL (species, if applicable)	NIH RG/ CDC BL	Special Hazards
Lentiviral vector, Human cell lines infected with HSV1 or CMV TRIPZ inducible shRNA	BL2	RG2	n/a
Stipulations: Aerosol-proof rotors or centrifuge buckets with safety caps. Elimination of sharps. Annually certified BSC for all procedures having potential to create infectious aerosols, splashes, high concentrations, or large volumes. Agent-specific training.			

COMS Review- Case Study #2

Project Summary: The laboratory works with the mechanisms of bone homeostasis and metabolism in relation to diseases such as osteoporosis. They utilize over expression and knockout of bone metabolism genes in cell culture. They also perform the same process in mice utilizing lentiviral and adenoviral vectors to introduce the bone metabolism genes in mice.

Agents	BL	NIH RG/ CDC BL	Special Hazards
Primary mouse osteoclast cells, pLenti, lentivirus-commercial. Gene Inserts: Dnm2, actin, Bin1 shRNA, clathrin shRNA, Dnm1 shRNA, Dnm2 shRNA, GFP-beta actin	BL2	RG2	n/a
Stipulations: Aerosol-proof rotors or centrifuge buckets with safety caps. Elimination of sharps. Annually certified BSC for all procedures having potential to create infectious aerosols, splashes, high concentrations, or large volumes. Agent-specific training.			

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