Introduction to Gene Silencing

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Introduction

- Gene + Silencing = Prevention of gene's expression or turning "off" the formation of protein.
- Often confused with "gene knockout", gene silencing (GS) is the regulation of gene expression wherein gene expression is reduced.
- Gene knockout is the deletion of the particular gene from the genome.
- GS is similar to "gene knockdown" in terms of reduction in the expression of genes involved (by around 70%) without their complete deletion.
- Stages of GS:
  - Transcription
  - Translation

What is the need of GS?

- Gene silencing - molecular process - downregulate expression of certain specific genes.
- It may have evolved to defend the host organism against the invading viruses or nucleic acids.

What are the types of GS?

- PTGS (Post transcriptional gene silencing) or RNA interference (RNAi)
- micro RNA based gene silencing
- Transcriptional gene silencing
- Antisense RNA based gene silencing
- Ribozyme based gene silencing
- Co-suppression
- Virus induced gene silencing (VIGS)

Mechanisms

- Early insights – attempts for transgenic petunias – amplification of chalcone synthase – increased anthocyanin production
- White/chimeric flowers were obtained in place of deeper purple flowers.
- Transgene expression failed – a homologue endogenous gene was silenced (Co-suppression)
- Unstable transmission of co-suppression within generations.
- Hypothesis: A nucleic acid (probably RNA) mediated co-suppression.

mRNA
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Introduction of extra copies of a gene encoding an enzyme involved in production of pigment

mRNA
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How does introduction of additional copies of gene involved in pigment synthesis result in decreased pigmentation?

How does introduction of additional copies of gene lead to A decrease in mRNA levels?

- Similar phenomena in:
  - *N. crassa:* "quelling"
  - *Caenorhabditis elegans:* "RNA interference" (RNAi)

- dsRNA (absent in normal cells) – sequence homologue gene silencing.

- Quelling, RNAi and co-suppression seemed to be activated by dsRNA.

- These phenomena originated as a shield against viruses and transposable elements.

- RNA activity in cell – majority of GS phenomena.
- GS is described as RNA silencing.
- RNA silencing – regulatory mechanisms for gene expression.
- RNAi, co-suppression, VIGS – mechanistically similar
- Underlying pathways for GS induced by dsRNA are present in many eukaryotes.
- Similar GS phenomena – found in different organisms: proposal of models for different & interacting GS mechanisms.

What are the applications of GS?

- Study of animal essential genes
- Study of disease development
- Medical diagnostics & treatment
- Improving the quality of plant products
- Stock improvement in plants/animals

GS in therapeutics

- Gene therapy: disease is cred by replacement of a defective copy by a functional copy of the gene. e.g. Cystic fibrosis, haemophilia etc.
- Through RNAi, gene therapy can be extended to the diseases caused by an overexpressed protein.
- Viral infection treatment can be achieved by RNAi mediated blockage.
- It can stop the overproduction of the protein causing macular degeneration, and hence prevent blindness.

Haibin Xia et al. Nature Medicine 10, 816 - 820 (2004)

- Spinocerebellar ataxia type 1 (SCA1) and Huntington disease: progressive, untreatable, neurodegenerative disorders

- Expanded paolyglutamine repeats are present.

- RNAi ↓ polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia (SCA1) – mutant allele expression is repressed

- Disease phenotypes show improvements - inducible mouse models of SCA1 and Huntington disease

Vira Bitko et al., Nature Medicine 11, 50 - 55 (2004)

- siRNA is nasally administered - Respiratory syncytial virus (RSV) and parainfluenza virus (PIV) infection is prevented.
- Both viruses can be targeted jointly or separately.
- Even in lower dosages, inhaled siRNAs may act fast and potently to provide an antiviral treatment.

References

- NPTEL
- Amancio José de Souzal; Beatriz Madalena Januzzi Mendes; Francisco de Assis Alves Mourão Filho. Gene silencing: concepts, applications, and perspectives in woody plants. Sci. agric. (Piracicaba, Braz.) vol.64 no.6 Piracicaba Nov./Dec. 2007