Short history of post-transcriptional gene silencing

Definition: the ability of exogenous double-stranded RNA (dsRNA) to suppress the expression of the gene which corresponds to the dsRNA sequence.

- **1990 Jorgensen :**

  Introduction of transgenes homologous to endogenous genes often resulted in plants with both genes suppressed!

  - Called Co-suppression
  - Resulted in degradation of the endogenous and the transgene mRNA

1995 Guo and Kemphues:

injection of either antisense or sense RNAs in the germline of *C. elegans* was equally effective at silencing homologous target genes

1998 Mello and Fire:

-extension of above experiments, combination of sense and antisense RNA (= dsRNA) was 10 times more effective than single strand RNA

What is RNA interference /PTGS?

- dsRNA needs to be directed against an exon, not an intron in order to be effective
- homology of the dsRNA and the target gene/mRNA is required
- targeted mRNA is lost (degraded) after RNAi
- the effect is non-stoichiometric; small amounts of dsRNA can wipe out an excess of mRNA (pointing to an enzymatic mechanism)
- ssRNA does not work as well as dsRNA

double-stranded RNAs are produced by:

– transcription of inverted repeats

– viral replication

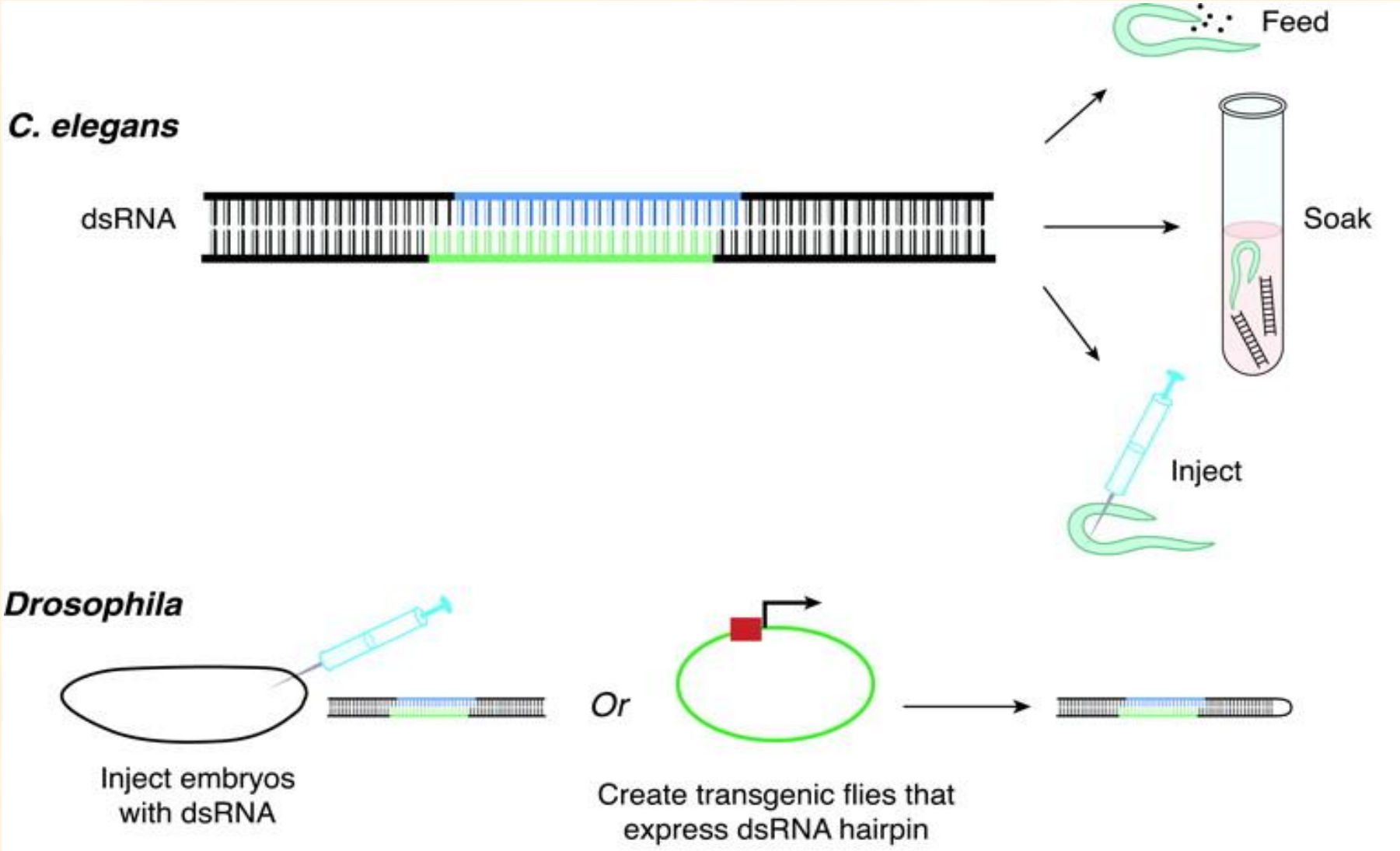
– transcription of RNA by RNA-dependent RNA-polymerases (RdRP)

double-stranded RNA triggers cleavage of homologous mRNA

PTGS-defective plants are more sensitive to infection by RNA viruses

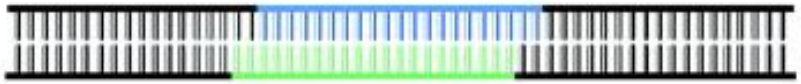
in RNAi defective nematodes, transposons are much more active

RNAi can be induced by:



Cultured Mammalian cells

Embryonic cultured cells or oocytes or early embryos

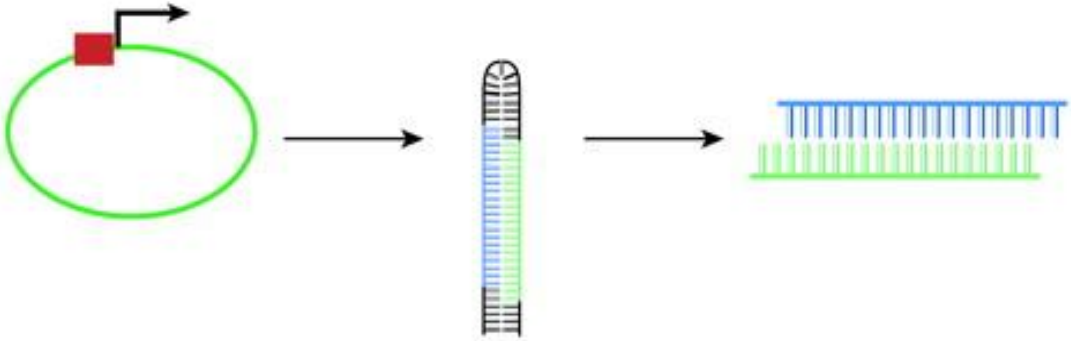


Transfect or inject dsRNA

Differentiated cultured cells or whole organisms (in vivo)

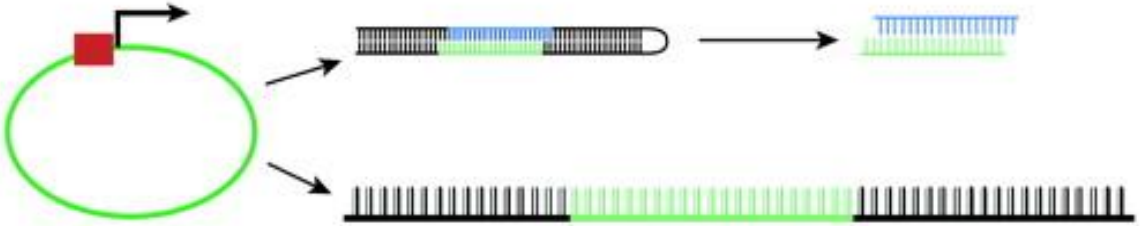
Transfect synthetic siRNA

Or



Transfect plasmids expressing miRNA precursor-like stem-loop to generate siRNA in cells

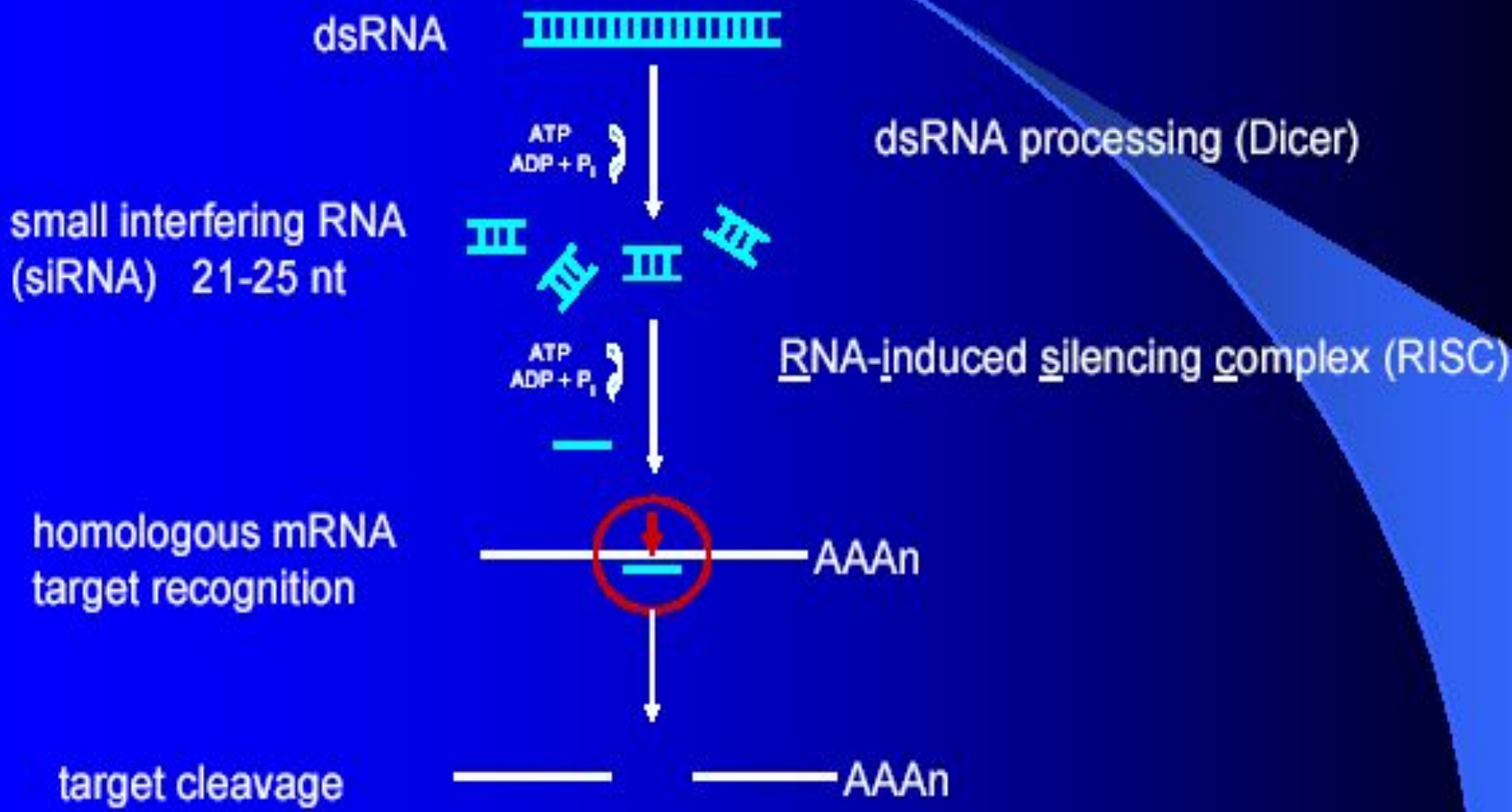
Plants

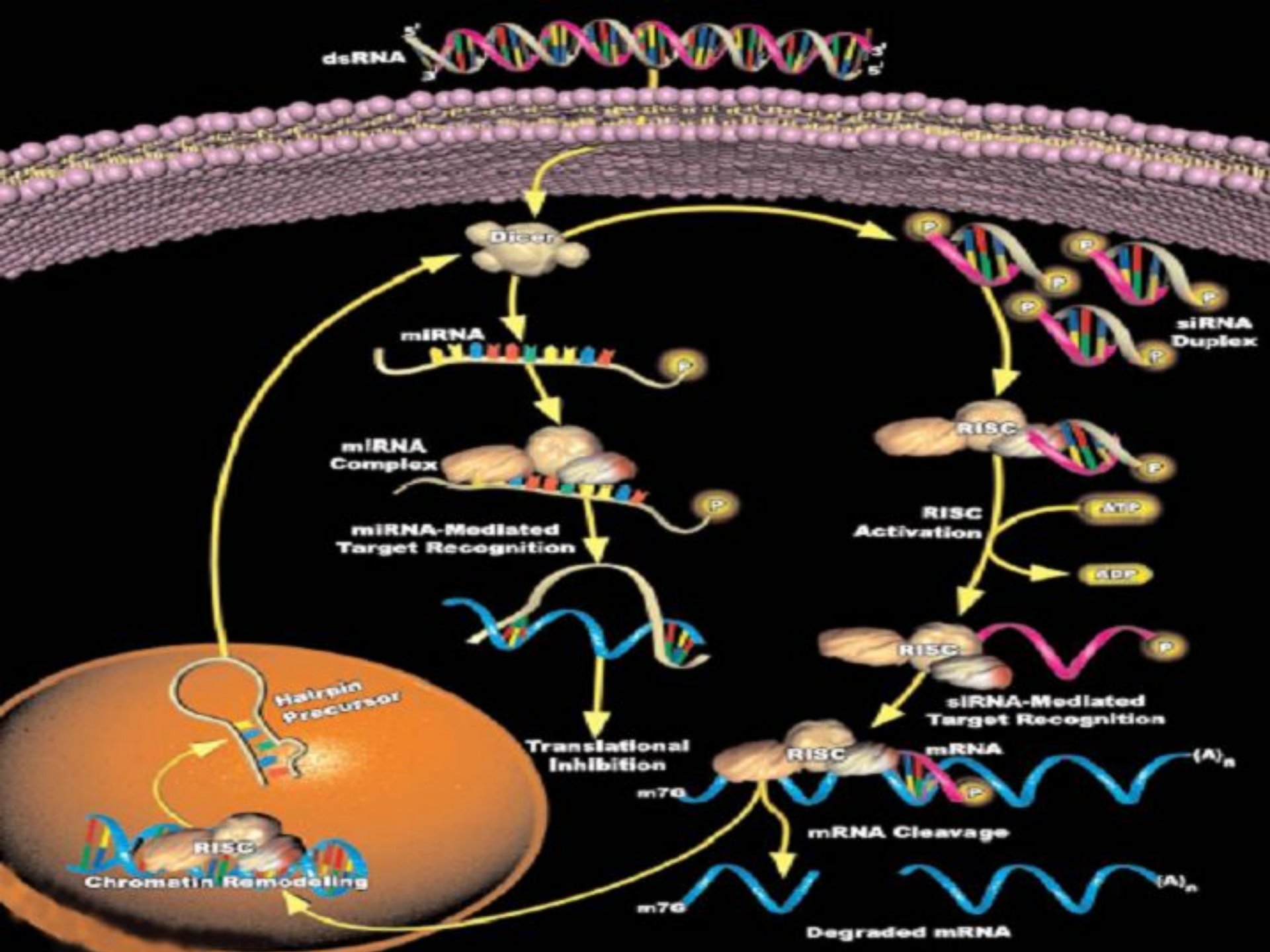


Overexpress dsRNA or a dsRNA hairpin from a transgene

Overexpress single-stranded RNA from a transgene or virus to trigger cosuppression

Mechanism of RNAi




dsRNA
5'
3'
3'
5'
Dicer
miRNA
P
miRNA
Complex
P
miRNA-Mediated
Target Recognition
Translational
Inhibition
Hairpin
Precursor
RISC
Chromatin Remodeling
P
P
P
P
P
P
siRNA
Duplex
RISC
P
ATP
RISC
Activation
ADP
RISC
P
siRNA-Mediated
Target Recognition
RISC
mRNA
P
(A)n
m7G
mRNA Cleavage
m7G
(A)n
Degraded mRNA

Dicer

Double-stranded RNA triggers processed into siRNAs by enzyme RNAseIII family, specifically the Dicer family Processive enzyme - no larger intermediates.

Dicer family proteins are ATP-dependent nucleases.

These proteins contain an amino-terminal helicase domain, dual RNAseIII domains in the carboxy- terminal segment, and dsRNA-binding motifs.

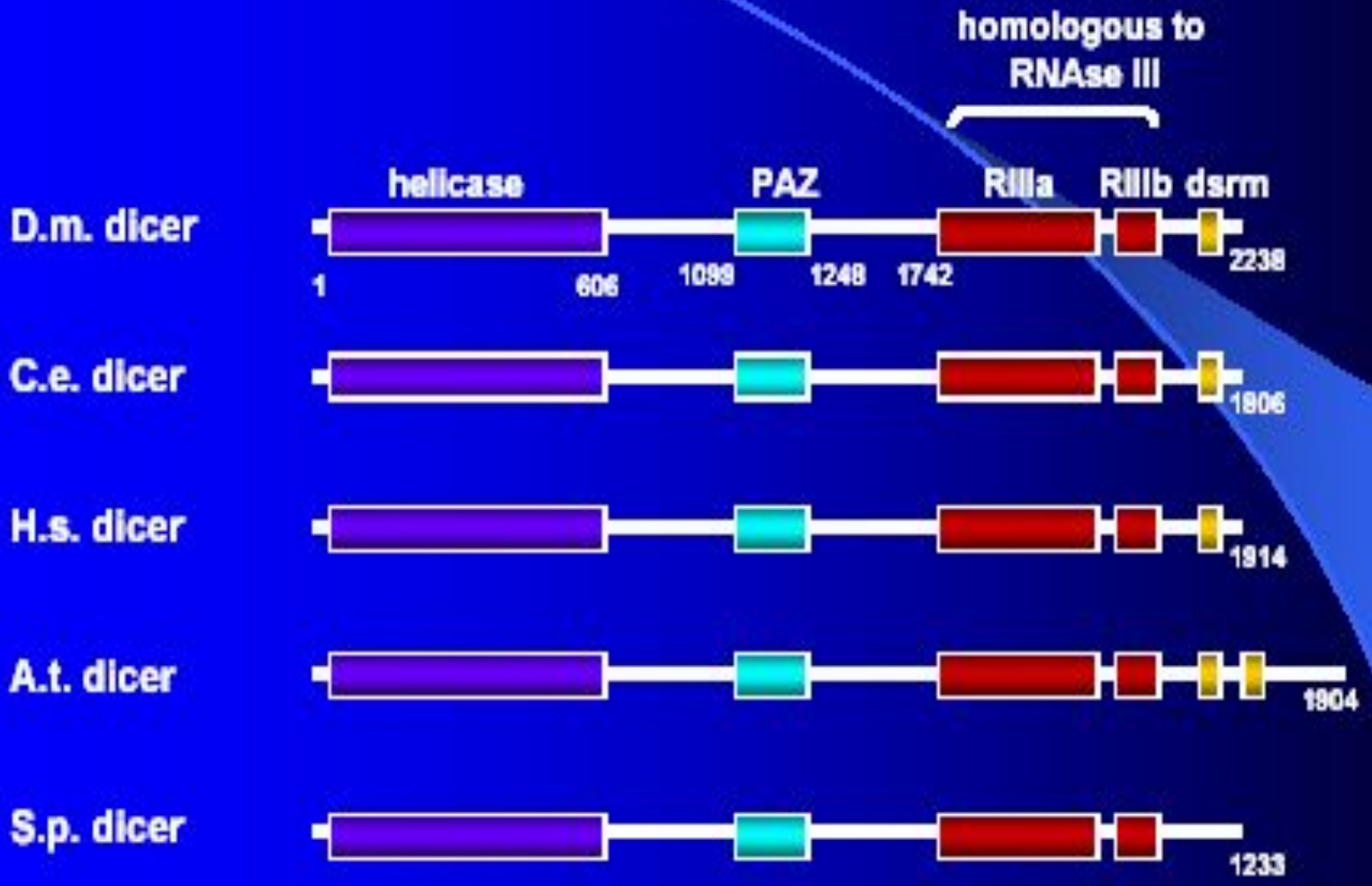
They can also contain a PAZ domain, which is thought to be important for protein-protein interaction.

Dicer homologs exist in many organisms including *C. elegans*, *Drosphila*, yeast and humans

Loss of dicer: loss of silencing, processing *in vitro*

Developmental consequence in *Drosophila* and *C. elegan*

Dicer is a conserved protein



RISC complex

RISC is a large (~500-kDa) RNA-multiprotein complex, which triggers mRNA degradation in response to siRNA

some components have been defined by genetics, but function is unknown, e.g.

– unwinding of double-stranded siRNA (Helicase !?)

– ribonuclease component cleaves mRNA (Nuclease !?)

– amplification of silencing signal (RNA-dependent RNA polymerase !?)

cleaved mRNA is degraded by cellular exonucleases

Different classes of small RNA molecules

During dsRNA cleavage, different RNA classes are produced:

- siRNA
- miRNA
- stRNA

siRNAs

Small interfering RNAs that have an integral role in the phenomenon of RNA interference(RNAi), a form of post-transcriptional gene silencing

RNAi: 21-25 nt fragments, which bind to the complementary portion of the target mRNA and tag it for degradation

A single base pair difference between the siRNA template and the target mRNA is enough to block the process.

miRNAs/stRNAs

micro/small temporal RNAs derive from ~70 nt ssRNA (single-stranded RNA),

which forms a stemloop; processed to 22nt RNAs found in:

– *Drosophila*, *C. elegans*, HeLa cells genes

– *Lin-4, Let-7*

stRNAs do not trigger mRNA degradation role: the temporal regulation of *C. elegans* development, preventing translation of their target mRNAs by binding to the target's complementary 3'

untranslated regions(UTRs)

conservation: 15% of these miRNAs were conserved with 1-2 mismatches across worm, fly, and mammalian genomes

expression pattern: varies; some are expressed in all cells and at all developmental stages and others have a more restricted spatial and temporal expression pattern

Protocol for „gene silencing" (for a 6-well plate):

tube1: 12 µl of 20uM siRNA
200 µl MEM

tube2: 10 µl Oligofectamine
50 µl MEM
5-10 min at RT

mix tube1 with tube2, incubate 15-20 min at RT

add complex solution onto cells and incubate for 4h at 37 °C

add medium and incubate cells for 2 days at 37 °C

assay for gene silencing (Western/Northern blot)

Why is PTGS important?

Most widely held view is that RNAi evolved to protect the genome from viruses (or other invading DNAs or RNAs)

Recently, very small (micro) RNAs have been discovered in several eukaryotes that regulate developmentally other large RNAs

May be a new use for the RNAi mechanism besides defense

Recent applications of RNAi

Modulation of HIV-1 replication by RNA interference.

Hannon(2002).

Potent and specific inhibition of human immunodeficiency virus type 1 replication by RNA interference.

An *et al.*(1999)

Selective silencing of viral gene expression in HPV-positive human cervical carcinoma cells treated with siRNA, a primer of RNA interference.

Jung *et al.* 2002.

RNA interference in adult mice.

Mccaffrey *et al.2002*

Successful inactivation of endogenous Oct-3/4 and c-mos genes in mouse pre implantation embryos and oocytes using short interfering RNAs.

Le Bon *et al*.2002

Possible future improvements of RNAi applications

Already developed:

in vitro synthesis of siRNAs using T7 RNA Polymerase

U6 RNA promoter based plasmids

Digestion of longer dsRNA by *E. coli* Rnase III

Potentially useful:

creation of siRNA vectors with resistances cassettes

establishment of an inducible siRNA system

establishment of retroviral siRNA vectors (higher efficiencies, infection of suspension cell lines)

Conclusions

begun in worms, flies, and plants - as an accidental observation.

general applications in mammalian cells.

probably much more common than appreciated before:

- it was recently discovered that small RNAs correspond to centromer heterochromatin repeats
- RNAi regulates heterochromatic silencing

Faster identification of gene function

Powerful for analyzing unknown genes in sequence genomes.

efforts are being undertaken to target every human gene via miRNAs

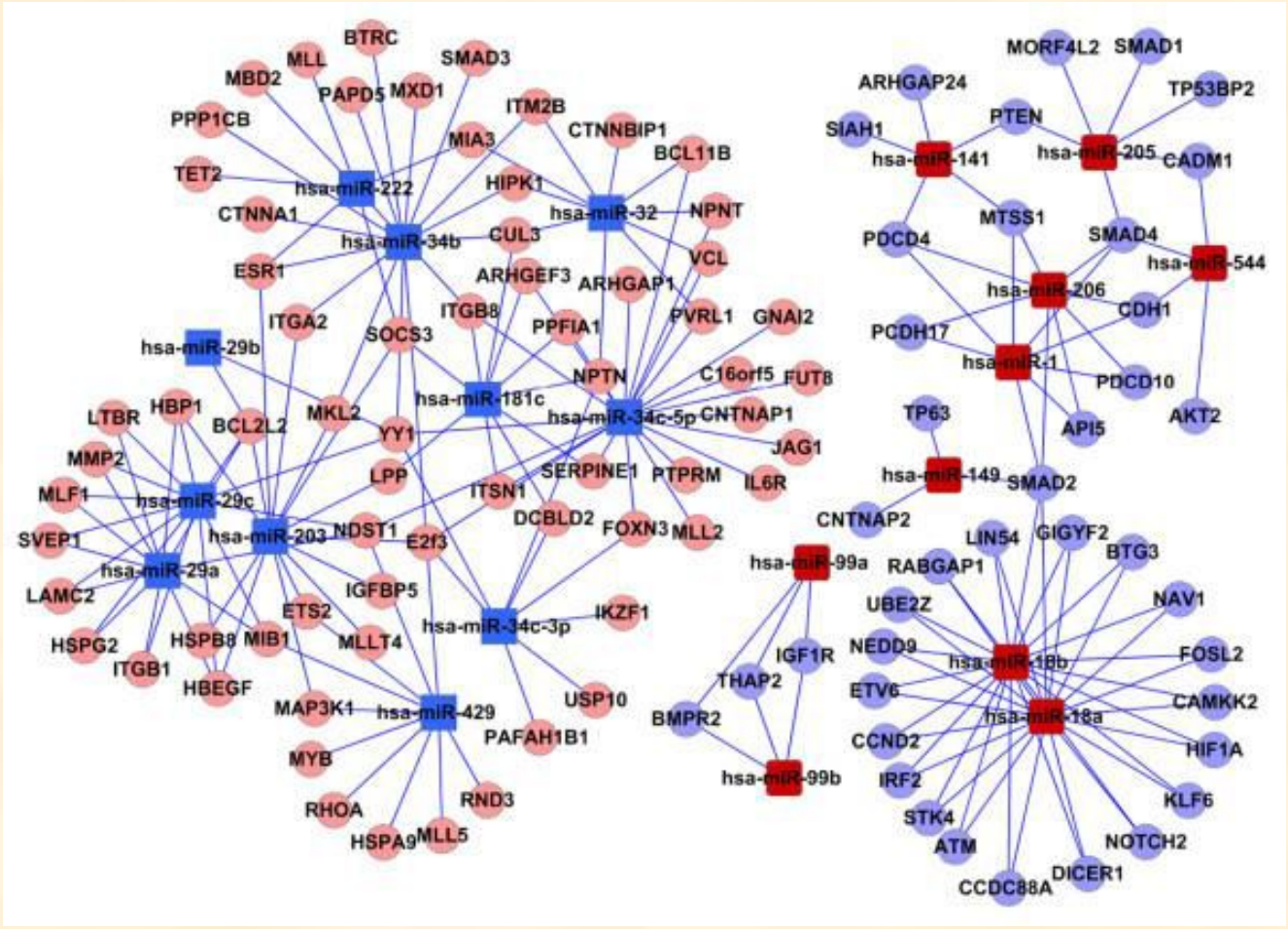
Gene therapy: down-regulation of certain genes/mutated alleles

Cancer treatments

- knock-out of genes required for cell proliferation
- knock-out of genes encoding key structural proteins

Agriculture

Регуляция экспрессии генов с помощью miRNA



DNA-интерференция DNA-guided DNA interference by a prokaryotic Argonaute. Swarts DC, Jore MM, Westra ER, Zhu Y, Janssen JH, Snijders AP, Wang Y, Patel DJ, Berenguer J, Brouns SJ, van der Oost J. **Nature**. 2014 Mar 13;507(7491):258-61.

- Механизм РНК-интерференции осуществляется за счет очень консервативного семейства белков Argonaute (Ago)
- Белки семейства Argonaute есть даже у прокариот, но механизма RNA-интерференции нет.
- Оказалось, что у одной эубуктерии Thermus thermophilus белок TtAgo реализует механизм DNA-интерференции, аналогичным образом.
- Затравкой для него являются 5’-фосфорилированные ДНК олигонуклеотиды длинной 13-25 нуклеотидов.
- Считается, что бактерия тем самым защищается от чужеродной ДНК.

Защита от ДНК → Защита от РНК → Регуляция экспрессии

Функции siРНК

1. Сайленсинг мобильных генетических элементов;
2. Сайленсинг гетерохроматиновых повторов;
3. Сайленсинг генетического материала вирусного происхождения;
4. Ограничение степени экспрессии гена в определенных тканях.

При выделение фракций коротких РНК (19-25 нуклеотидов) из различных организмов обнаружен еще один класс малых РНК – **микроРНК.**

МикроРНК (miRNAs - micro RNAs) – класс 19-25 нуклеотидных одноцепочечных РНК, ***закодированных в уникальных генах геномов*** многоклеточных организмов.

Функция miРНК

Обеспечивают сайленсинг различных генов, обычно, за счет частично комплементарного связывания с мРНК, в результате которого блокируется ее трансляция.

- один тип miРНК может регулировать трансляцию мРНК более 100 различных генов;
- степень ингибирования зависит от количества связывающихся miРНК (в 3’UTR мРНК содержится несколько сайтов связывания).

Отличия miРНК и siРНК

miРНК

- Продукт dsРНК, закодированных в уникальных генах геномов многоклеточных организмов (>1% от всех генов у человека);
- мРНК может не разрушаться;
- Один тип miРНК регулирует разные гены.

siРНК

- Продукт dsРНК, образующихся в результате транскрипции транспозонов, гетерохроматиновых повторов или генетического материала вирусного происхождения ;
- мРНК разрушается;
- Один тип siРНК обычно регулирует только один тип мРНК.

- созданы библиотеки коротких РНК и ДНК-векторов, кодирующих короткие РНК, мишенями которых является около 8000 генов генома человека;

- внедряется в практику терапевтическое применение синтетических коротких РНК для целенаправленного подавления генетической экспрессии при некоторых заболеваниях.

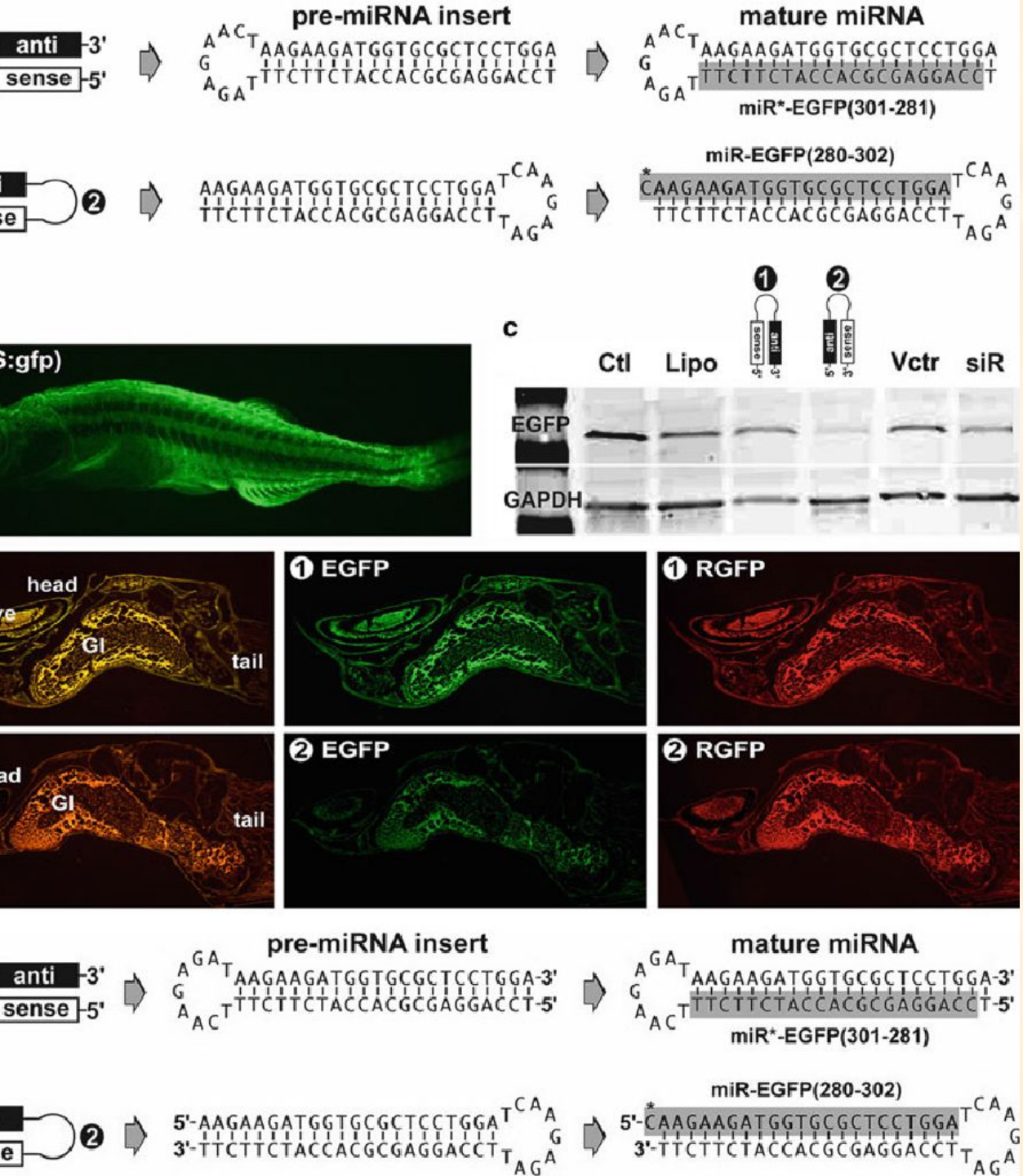
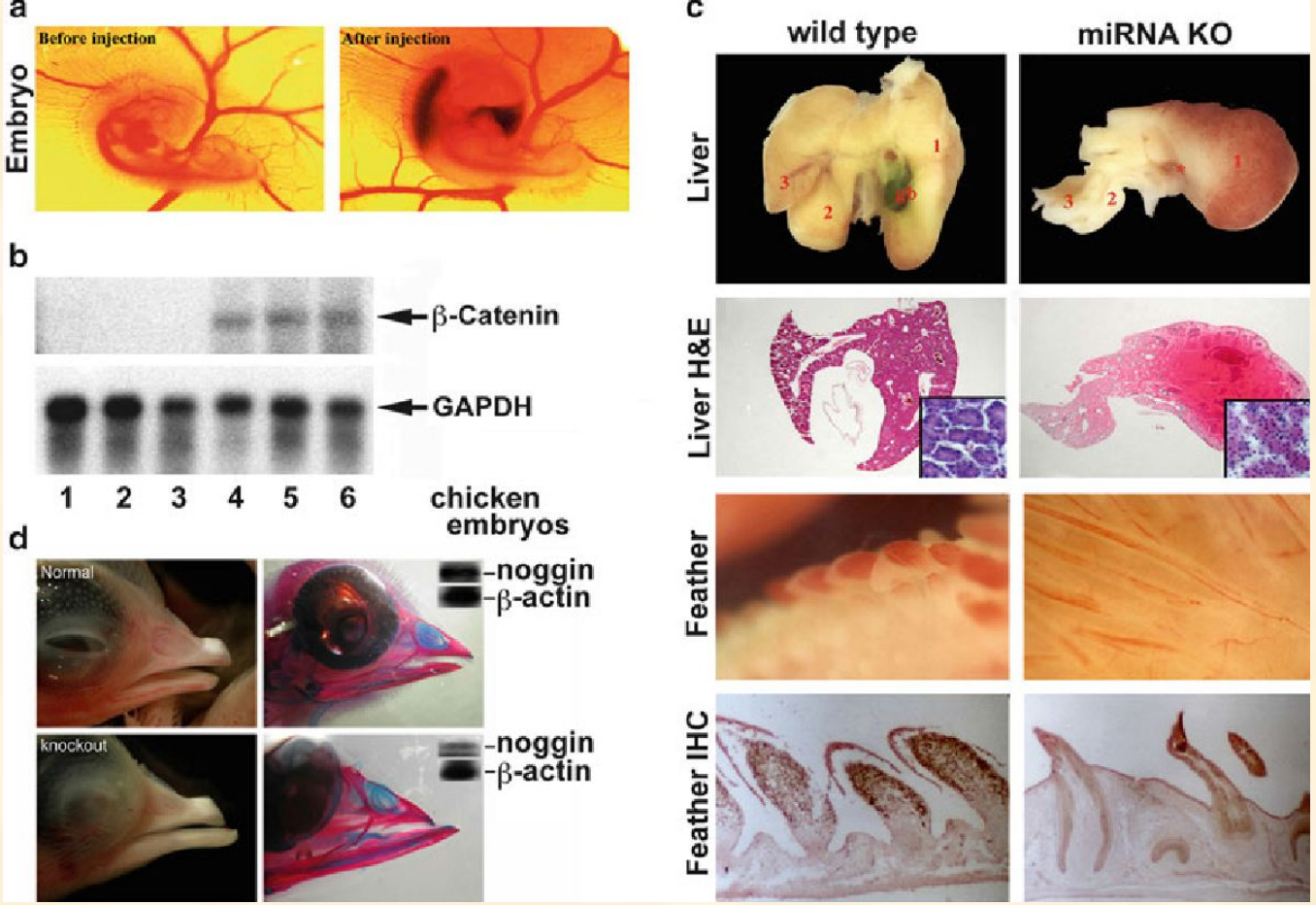
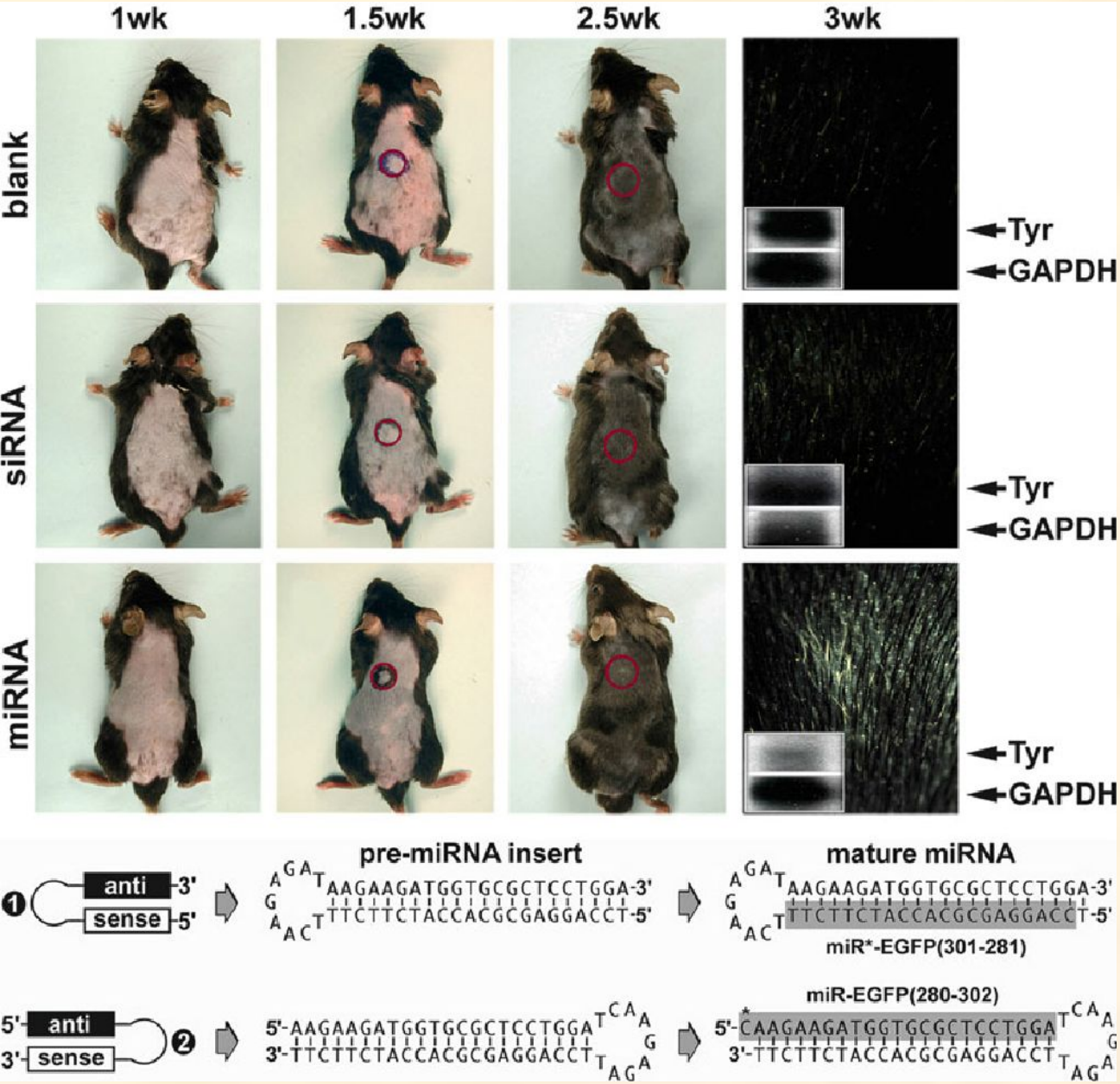


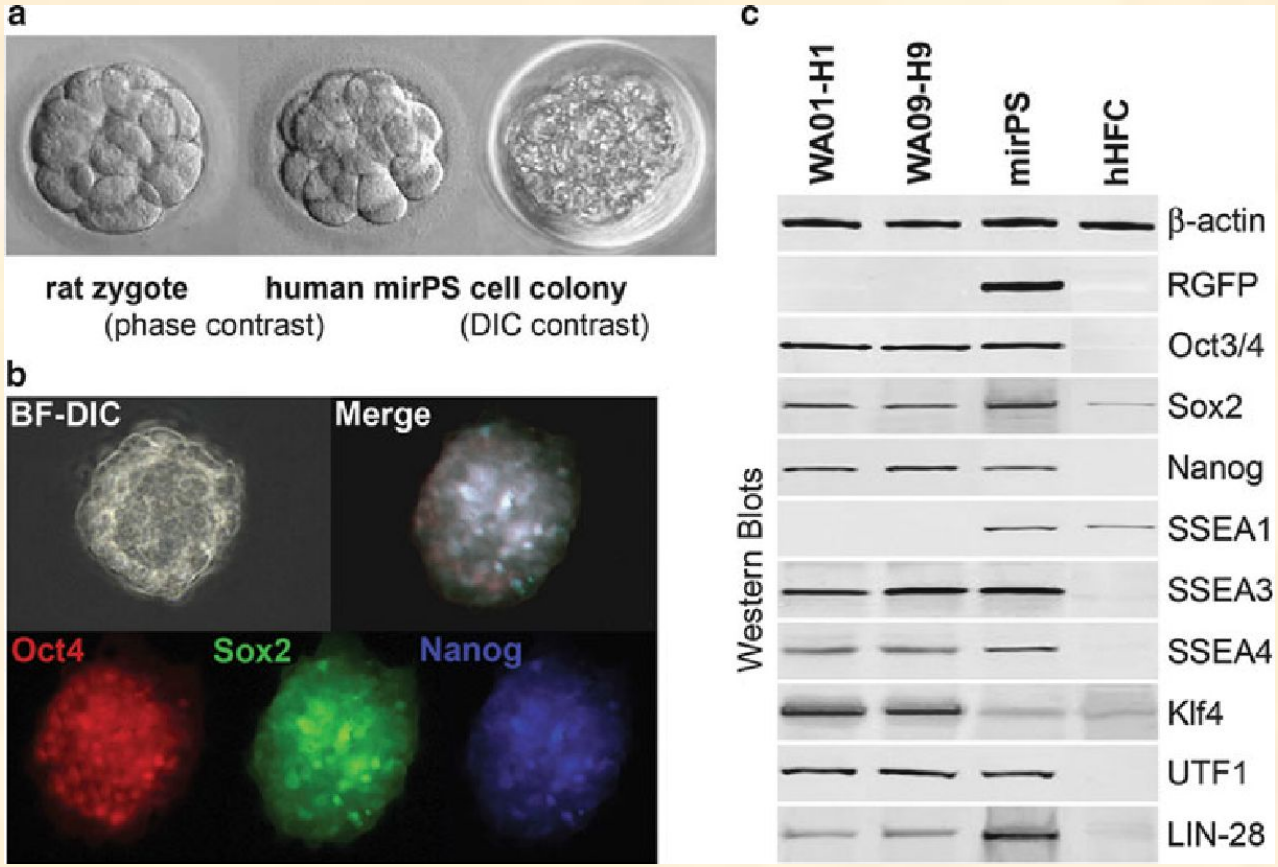
Fig. 3. Structural preference of miRNA–miRNA* asymmetry in miRNA-induced gene silencing complex (RISC) in vivo.
Different preferences of RISC assembly were observed by transfection of 5 ŷ -miRNA*-stem-loop-miRNA-3 ŷ (❶) and
5 ŷ -miRNA-stem-loop-miRNA*-3 ŷ (❷) pri-miRNA constructs in zebra fi sh, respectively. (**a**) Based on the RISC assembly ruleof siRNA, the processing of both ❶ and ❷ should result in the same siRNA duplex for RISC assembly; however, the experiments demonstrate that only the ❷ construct was used in RISC assembly for silencing target EGFR. Due to the fact that
miRNA is predicted to be complementary to its target messenger RNA, the “antisense” (*black bar*) refers to the miRNA and
the “sense” (*white bar*) refers to its complementarity, miRNA*. One mature miRNA, namely miR-eGFP-(280/302), was
detected in the ❷-transfected zebra fi shes, whereas the ❶ transfection produced different miRNA: miR*-EGFR(301–281),
which was partially complementary to the miR-eGFP(280/320). (**b**) In vivo gene silencing ef fi cacy was only observed in the
transfection of the ❷ pri-miRNA construct, but not the ❶ construct. Because the color combination of EGFP and RGFP
displayed more red than green (as shown in *deep orange*), the expression level of target EGFP (*green*) was signi fi cantly
reduced in ❷, while miRNA indicator RGFP (*red*) was evenly present in all vector transfections. (**c**) Western blot analysis of
the EGFP protein levels con fi rmed the speci fi c silencing result of (**b**). No detectable gene silencing was observed in fi shes
without (Ctl) and with liposome only (Lipo) treatments. The transfection of either a U6-driven siRNA vector (siR) or an empty
vector (Vctr) without the designed pri-miRNA insert resulted in no gene silencing signi fi cance.



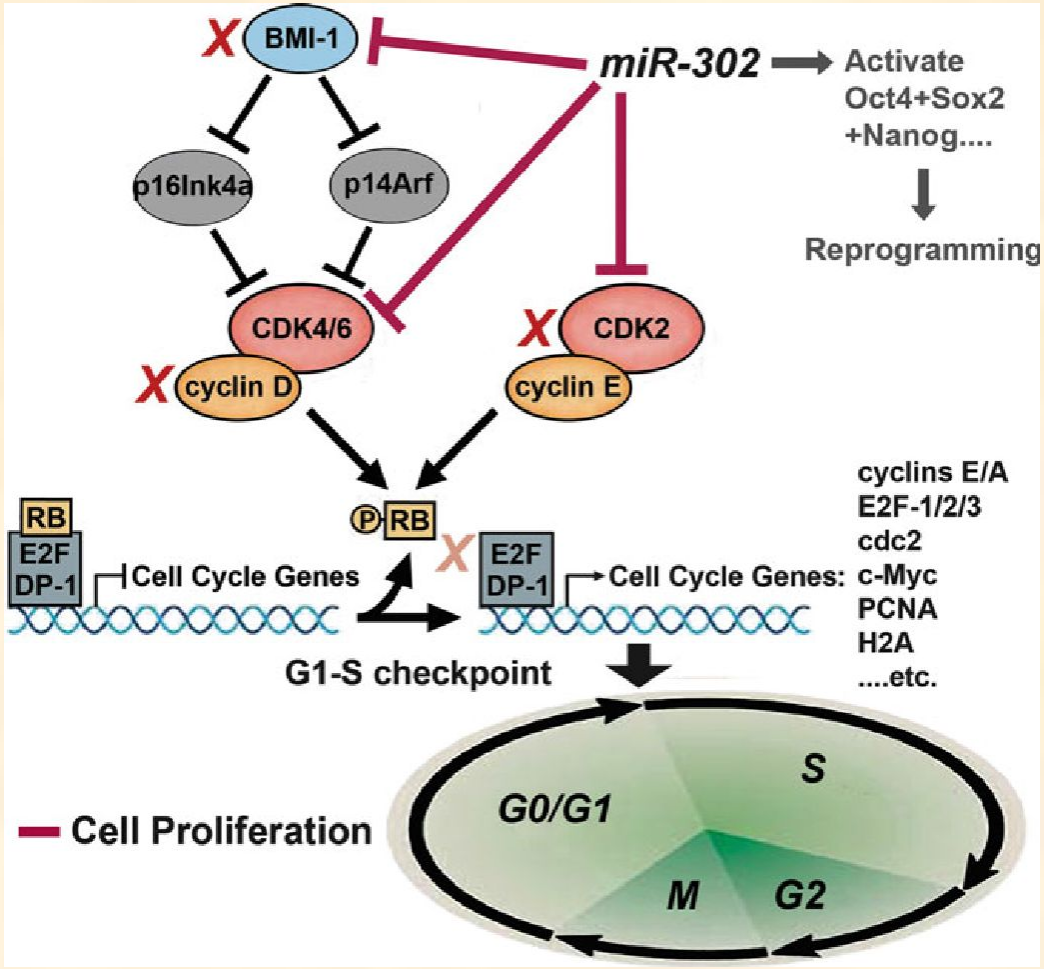
In vivo gene-silencing effects of anti- b -catenin miRNA and anti-noggin miRNA (d) on special organ development in embryonic chicken. (a) The pre-miRNA-expressing construct and fast green dye mixtures were injected into the chickenembryos near the liver primordia below the heart. (b) Northern blots of extracted RNAs from chicken embryonic livers with(*lanes 1–3*) and without (*lanes 4–6*) anti- b -catenin miRNA treatments were shown. All three knockouts (KO) showed a greater than 98% silencing effect on b -catenin mRNA expression but housekeeping genes, such as *glyceraldehyde phosphate dehydrogenase* , was not affected. (c) Liver formation of the b -catenin KOs was signi fi cantly hindered (*upper right two panels*). Microscopic examination revealed a loose structure of hepatocytes, indicating the loss of cell–cell adhesion caused by breaks in adherins junctions formed between b -catenin and cell membrane E-cadherin in early liver development. In severely affected regions, feather growth in the skin close to the injection area was also inhibited (*lower right two panels*). Immunohistochemistry for b -catenin protein expression (*brown*) showed a signi fi cant decrease in the feather follicle sheaths. *H&E* Hematoxyline and eosin staining. (d) The lower beak development was increased by the mandible injection of the anti-noggin pre-miRNA construct (*down panel*) in comparison with the wild type (*upper panel*). *Right panels* showed bone (alizarin red) and cartilage (alcian blue) staining to demonstrate the outgrowth of bone tissues in the lowerbeak of the *noggin* KO. Northern blot analysis (inserts) con fi rmed a 60–65% decrease of *noggin* mRNA expression in thelower beak area.



In vivo effects of anti-tyrosinase (*Tyr*) miRNA on the mouse pigment production of local skins. Transfection of the miRNA-induced strong gene silencing of tyrosinase (*Tyr*) messenger RNA (mRNA) expression but not housekeeping glyceraldehyde phosphate dehydrogenase (*GAPDH*) expression, whereas expression of U6-directed small interfering RNA (siRNA) triggered mild nonspeci fi c RNA degradation of both *Tyr* and *GAPDH* gene transcripts. Because *Tyr* is an essential enzyme for black pigment melanin production, the success of gene silencing can be observed by a signi fi cant loss of the black color in mouse hairs. The *red circles* indicate the location of intracutaneous injections. Northern blot analysis of *Tyr* mRNA expression in local hair follicles con fi rmed the effectiveness and speci fi city of the miRNA-mediated gene-silencing effect (inserts).



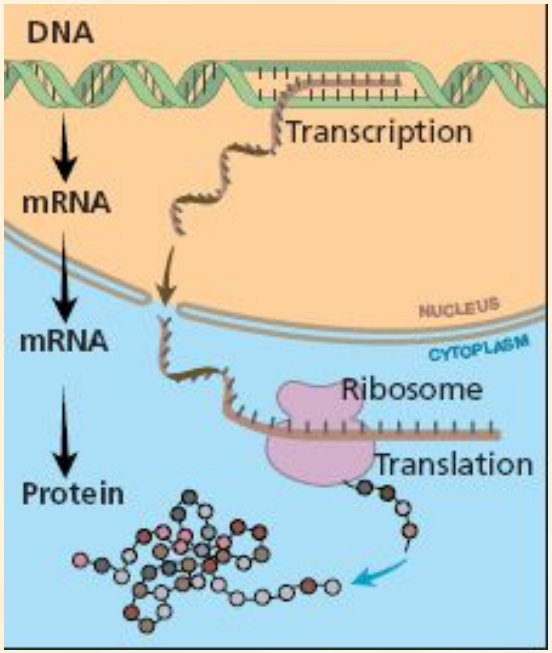
Morphological and genetic properties of mirPSCs. (a) A morphological comparison between a morula-staged rat embryo and an mirPSC colony at 16–32-cell stage. *BF-DIC* bright field with differential interference contrast. (b) Fluorescent microscope examination showing the homogeneous expression of the core reprogramming factors Oct3/4, Sox2 and Nanog in an mirPSC-derived embryoid body. (c) Western blots con fi rming the expression patterns of major human embryonic stem cell (hESC)-speci fi c markers in mirPSCs compared to those found in hESCs H1 and H9 ($n = 4$, $p < 0.01$).

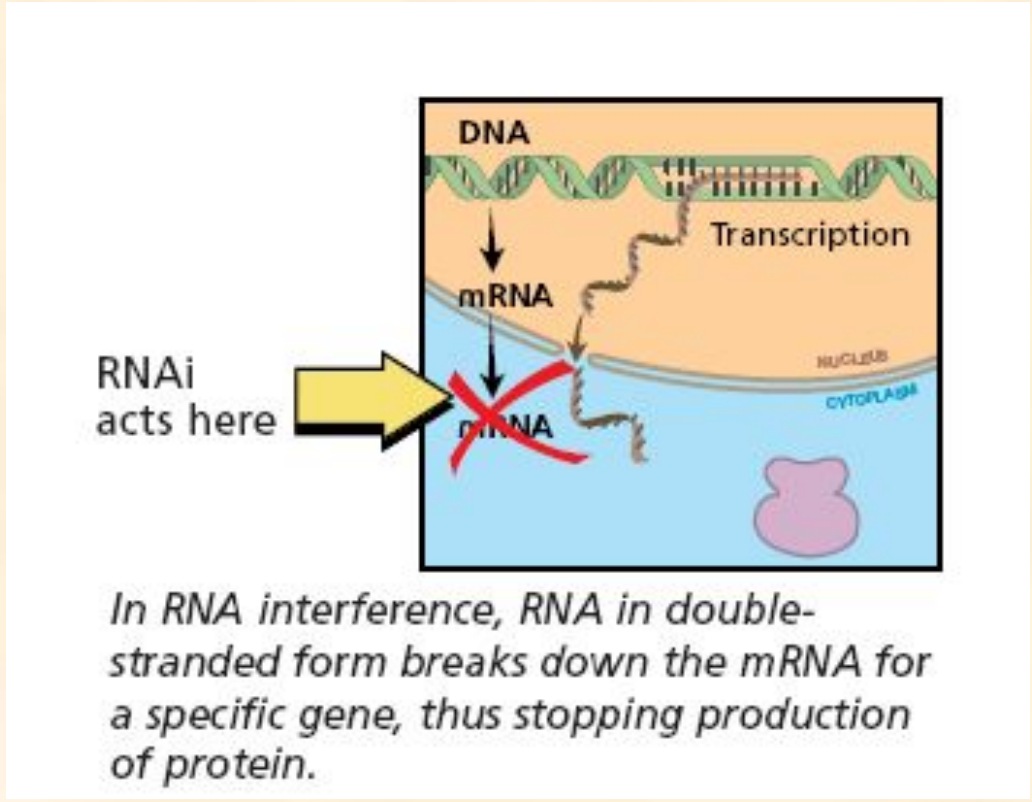


Mechanism of miR-302–mediated tumor suppression in human iPSCs. miR-302 not only concurrently suppresses G1-phase checkpoint regulators cyclin-dependent kinase 2 (CDK2), cyclin D and BMI-1 but also indirectly activates p16Ink4a and p14/p19Arf to quench most (>70%) of the cell cycle activities during somatic cell reprogramming (SCR). E2F is also a predicted target of miR-302. Relative quiescence at the G0/G1 state may prevent possible random growth and/or tumor-like transformation of the reprogrammed iPSCs, leading to a more accurate and safer reprogramming process, by which premature cell differentiation and tumorigenicity are both inhibited

What is RNA interference (RNAi)?

- "*The Process by which dsRNA silences gene expression...*"
- Degradation of mRNA or translation inhibition



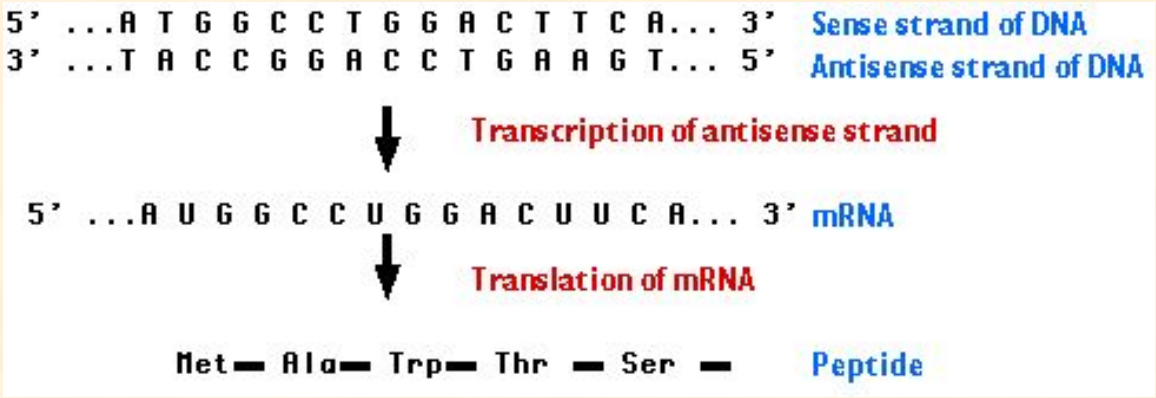


In RNA interference, RNA in double-stranded form breaks down the mRNA for a specific gene, thus stopping production of protein.

www.nobelprize.org

What are sense and antisense RNA?

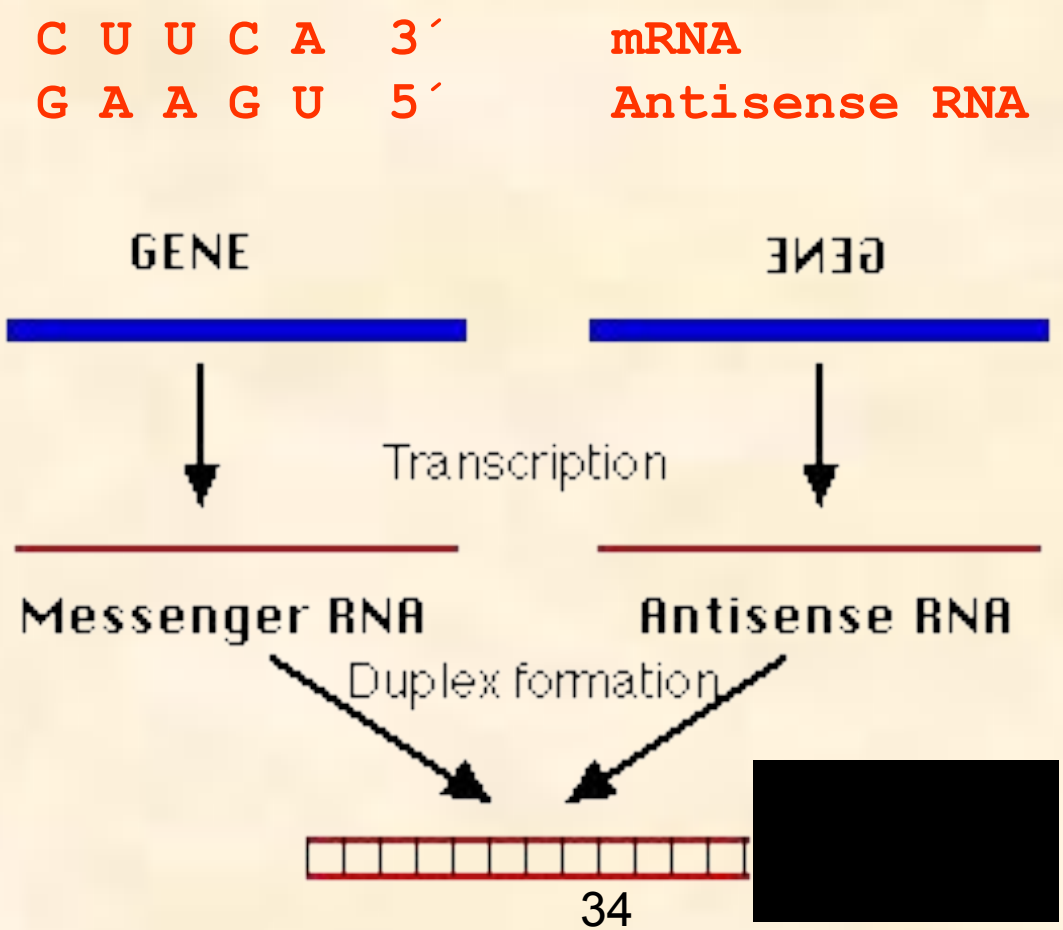
- Messenger RNA (mRNA) is single-stranded, called "sense" because it results in a gene product (protein).



5´ C U U C A 3´ mRNA
3´ G A A G U 5´ Antisense RNA

What are sense and antisense RNA?

- Antisense molecules interact with complementary strands of nucleic acids, modifying expression of genes.



RNAi terms

- dsRNA: double stranded RNA, longer than 30 nt
- miRNA: microRNA, 21-25 nt.
  - Encoded by endogenous genes
- siRNA: small-interfering RNA, 21-25 nt.
  - Mostly exogenous origin

RNAi like phenomena

- Plants
  - Petunias
- Fungi
  - *Neurospora*
- Animals
  - *Caenorhabditis elegans*

Alternate terms to RNAi

- PTGS (Posttranscriptional Gene Silencing)
- Cosuppression
- Quelling
- Virus-induced gene silencing

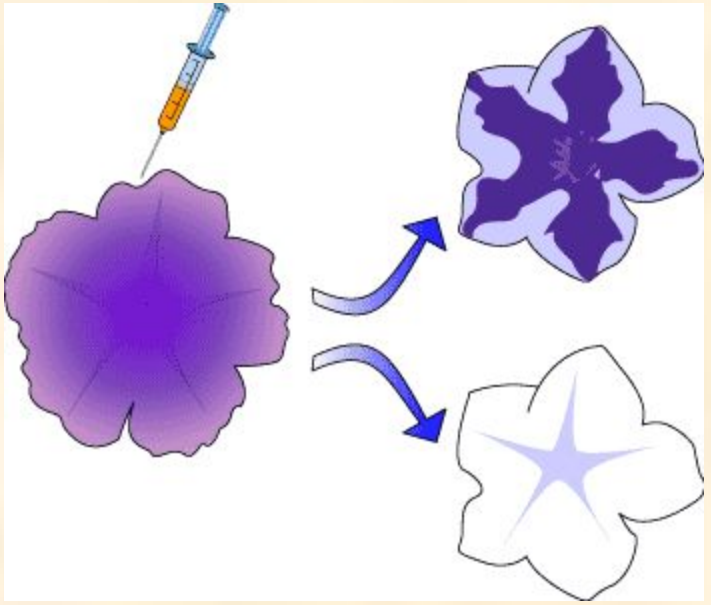
1990-Petunias

- Napoli et al. defined an RNAi-like phenomenon and called it "*cosupression*."

- chalcone synthase (CHS), a key enzyme in flavonoid biosynthesis, the rate-limiting enzyme in anthocyanin biosynthesis, responsible for the purple coloration.

Overexpression of chalcone synthase in petunias unexpectedly resulted in white petunias

- The levels of endogenous as well as introduced CHS were 50-fold lower than in wild-type petunias, which led the authors to hypothesize that the introduced transgene was "**<u>cosuppressing</u>**" the endogenous CHS gene.



http://www.scg.ubc.ca/?p=265

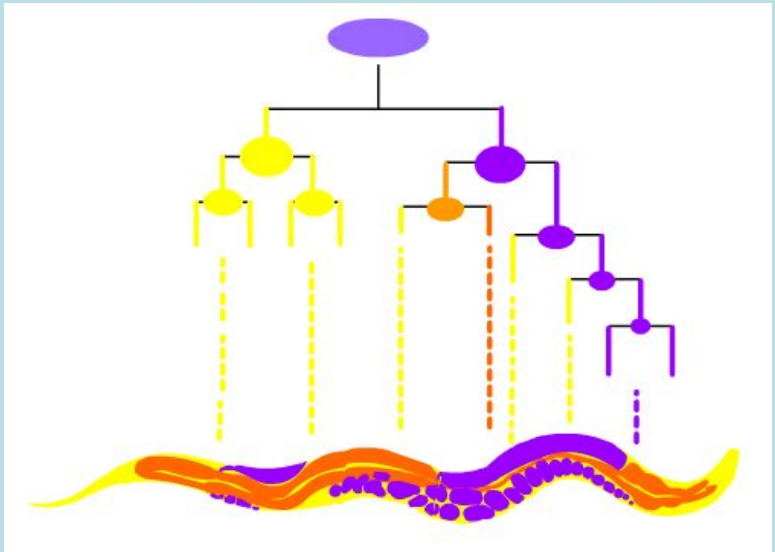
1992-The mold

A rosette of the asci

- Carlo Cogoni and Guiseppe Macino of the Università di Roma La Sapienza in Italy introduced a gene needed for carotenoid synthesis in the mold *Neurospora crassa:*
  - The introduced gene led to inactivation of the mold's own gene in about 30% of the transformed cells. They called this gene inactivation "**quelling**."

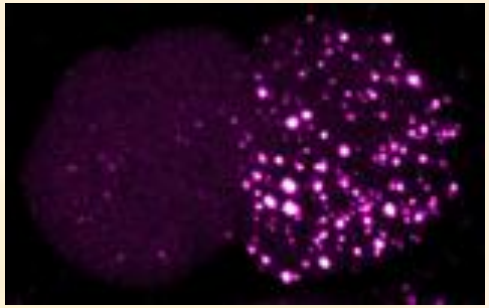
1995-The worm

- Guo and Kemphues studied **par-1** gene during embryogenesis
- The worm, *C. elegans*
  - has a fixed lineage: hypodermis, intestine, gonads
  - asymmetric divisions



1995- The worm

- Guo and Kemphues first studied Par-1 gene mutants
  - Division: Asymmetric symmetric
  - P-granule distribution



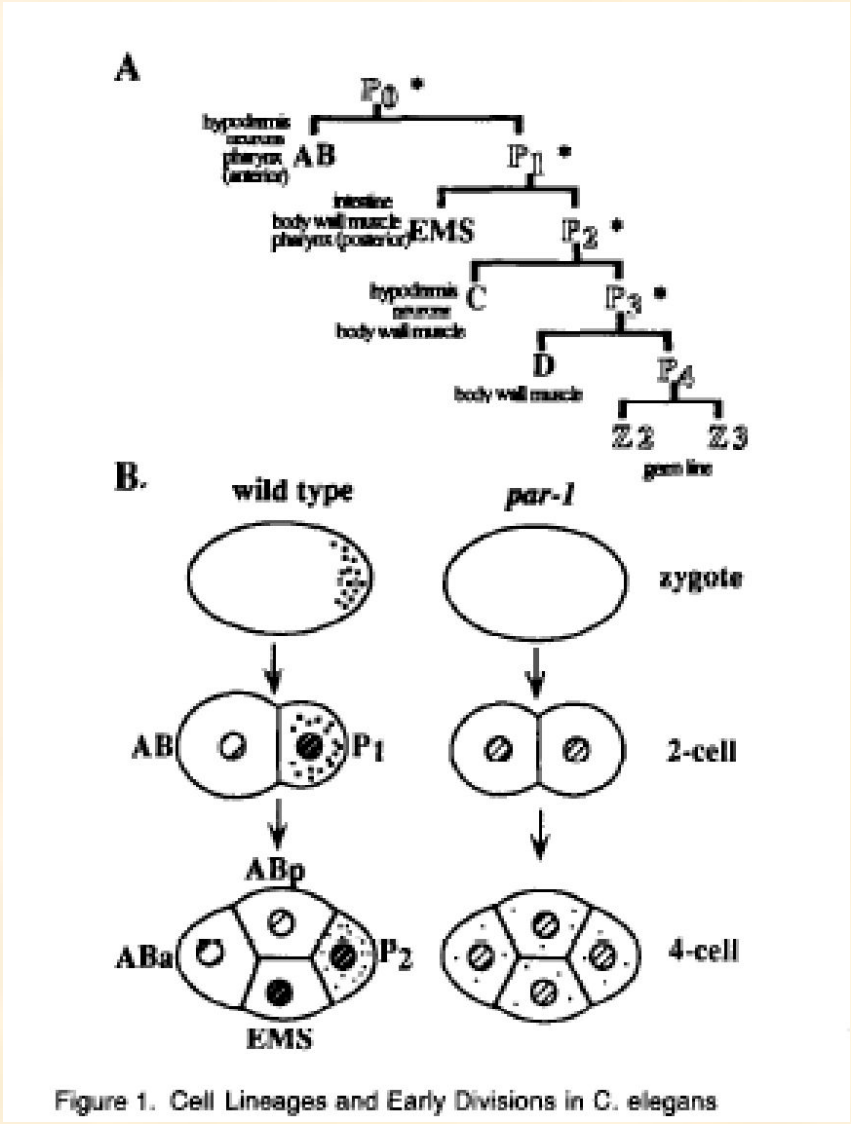
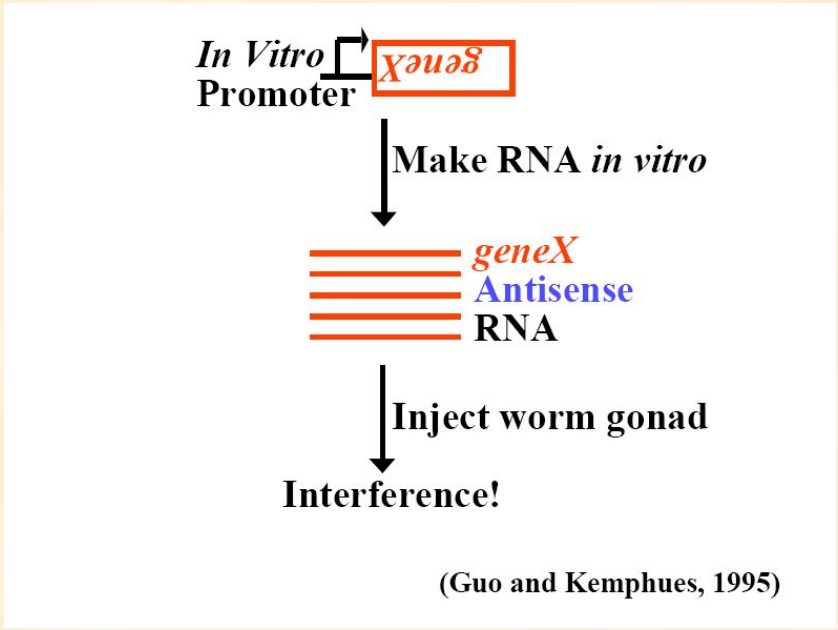
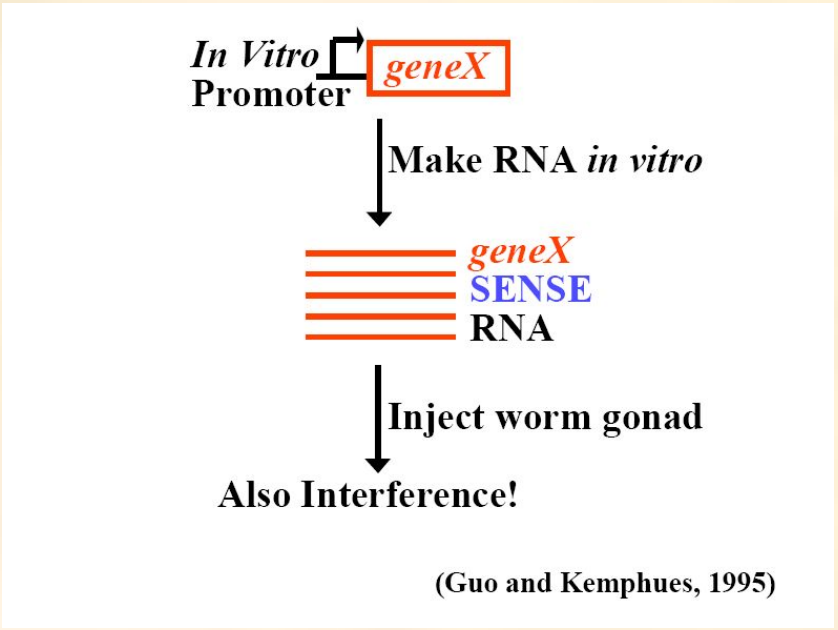


Figure 1. Cell Lineages and Early Divisions in C. elegans

Guo and Kemphues, 1995

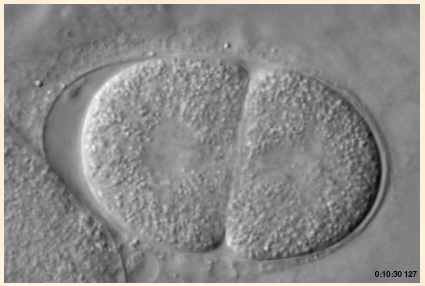




Both the antisense and sense strands effectively silenced

the antisense and sense effects appear to be separable and probably involve different mechanisms. The basis for the sense effect is under investigation and will not be discussed further. Overall, the specificity of the antisense and sense phenocopies provides strong evidence that the ZC22 cDNA represents the *par-1* transcript. Additional evi-

wildtype



Par-1 RNAi

‘Antisense’ Technology?

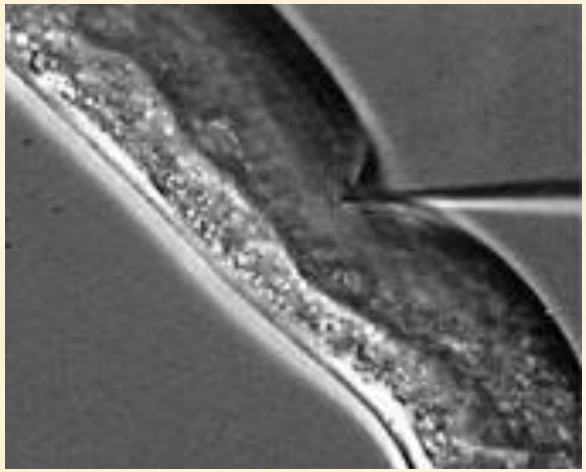
- Sense RNA silences yet no hybridization of sense RNA with sense mRNA is expected!
- Intronic and promoter sequences do not silence.
- ssDNA or dsDNA does not work!

- Craig Mello at the Worm Meeting in Madison, Wisconsin coined the term ‘RNAi’ and said that:
  - “ We can’t call it ‘antisense’ when ‘sense’ works as well”*

*Montgomery (2006) RNA interference: unraveling a mystery

Craig Mello

- In 1996, C. Mello and his student S. Driver also reported that sense RNAs mimic antisense phenotype.
  - Injection is made into a single site yet acts more systemically.



Andrew Fire

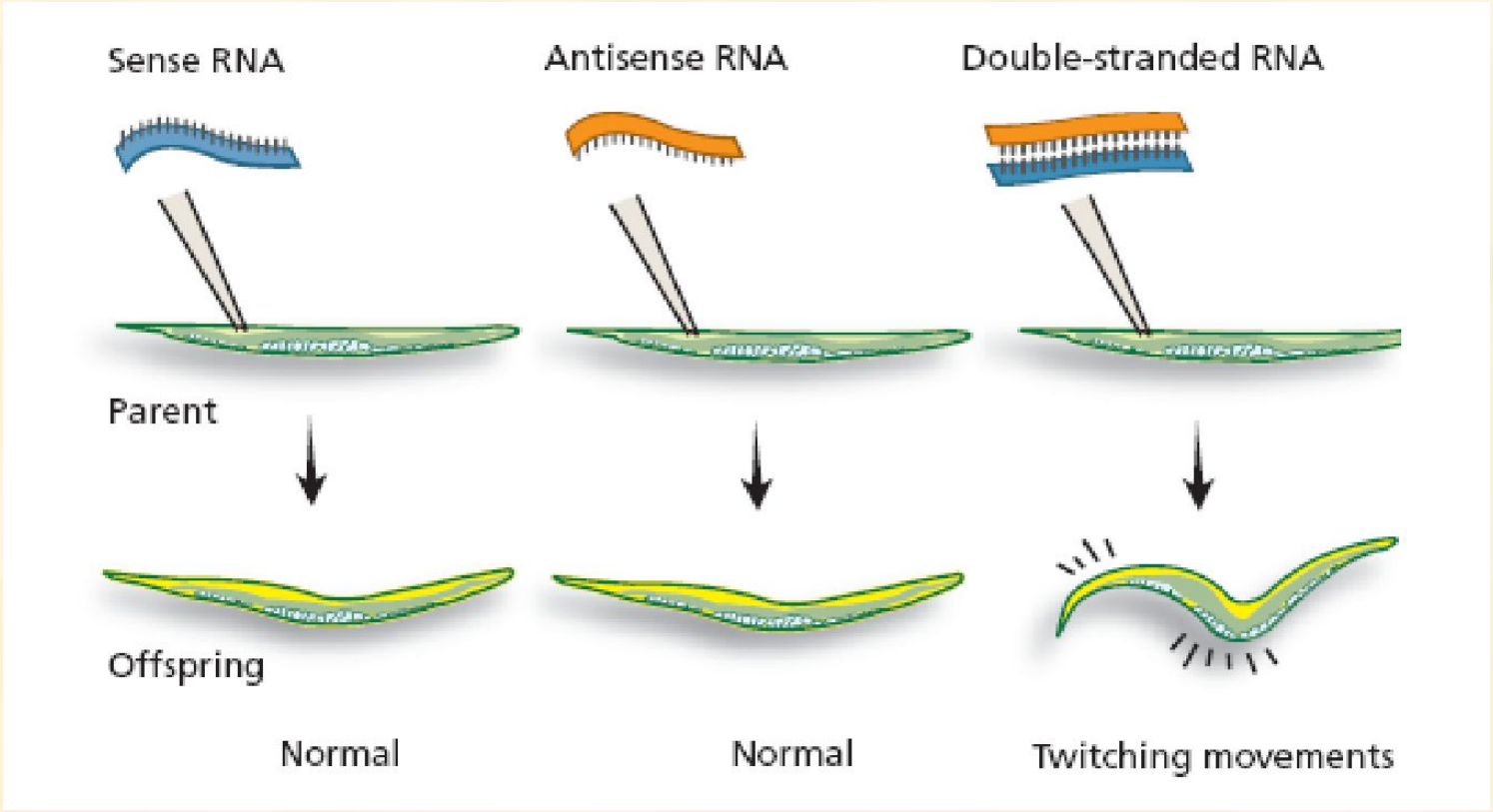
- In 1991, A. Fire successfully targeted genes by antisense constructs from transgenes.
- Sense constructs also exhibited silencing activity.

1998-Fire et al and Mello

- Gel-purified ssRNA
- Used purified ssRNA (antisense and sense) separately and also together.
- Tested ssRNA against different genes for specificity
- Tested whether a general post-transcriptional silencing is in place.

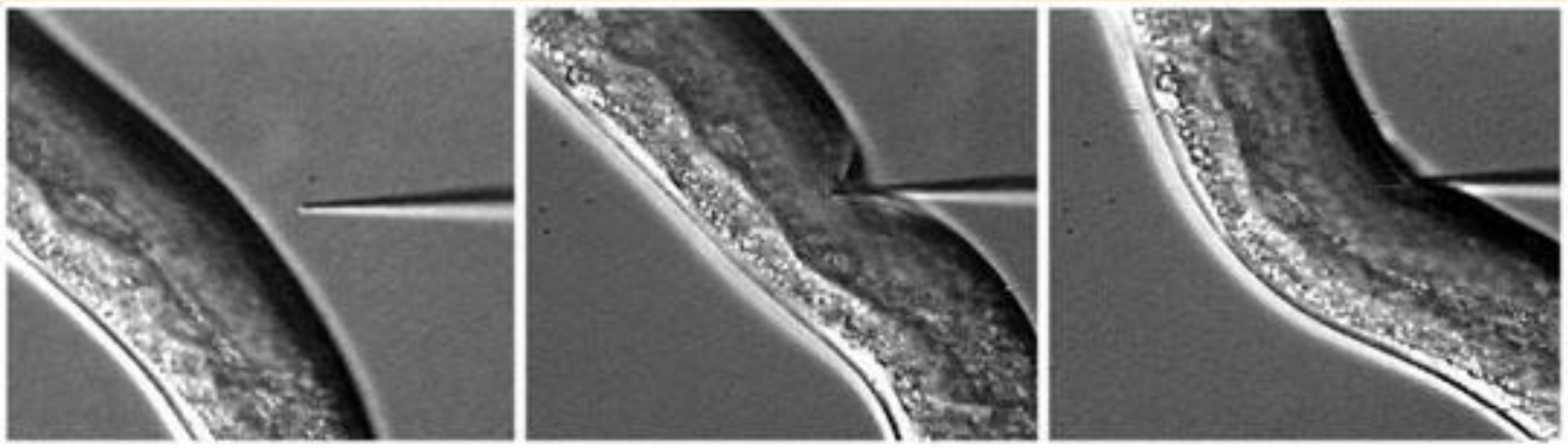
Unc-22 (Uncoordinated 22)

- Codes for a non essential myofilament
- It is present several thousand copies/cell



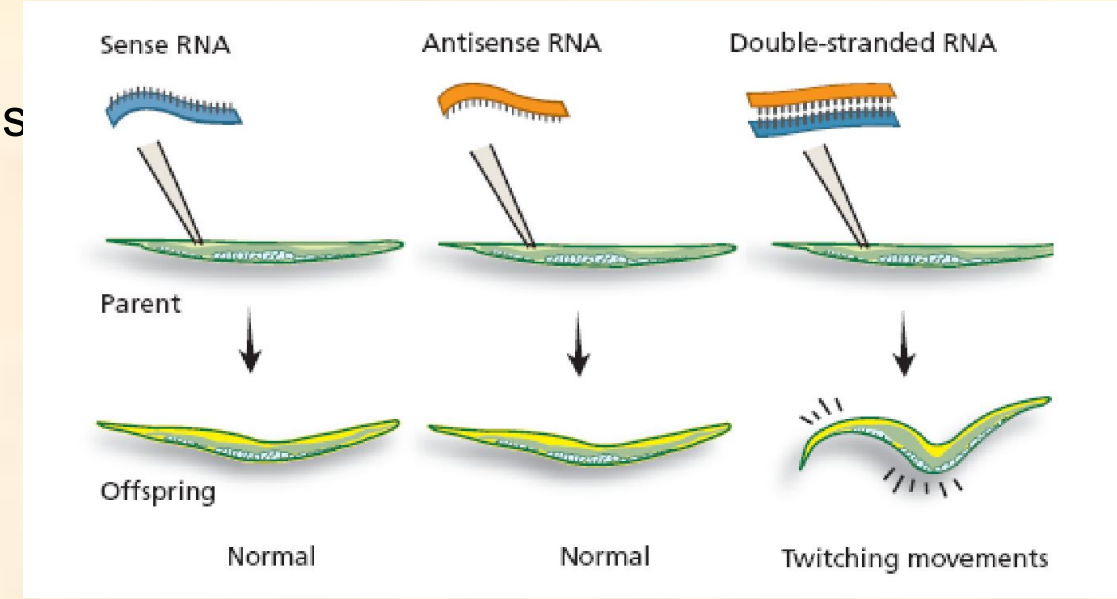
Injection for RNAi

- 6-10 adult hermaphrodites were injected with $0.5x10^6$-$1x10^6$ molecules into each gonadal arm.



Unc-22 phenotype

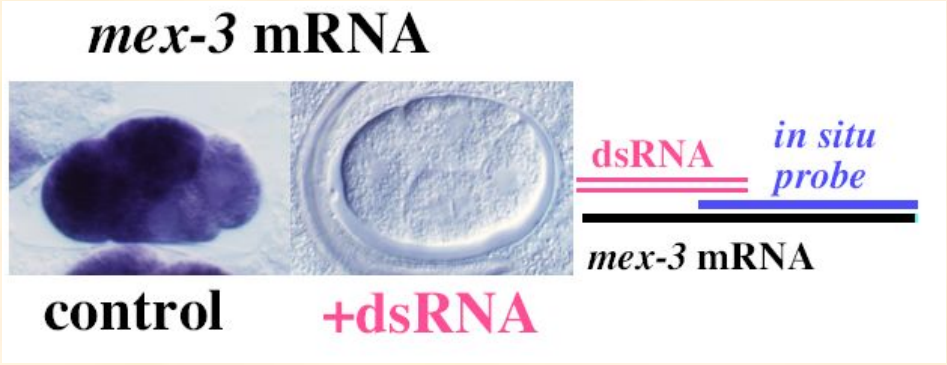
- 4-6 hours after injection, eggs collected.
- Screened for phenotypic changes
  - twiching

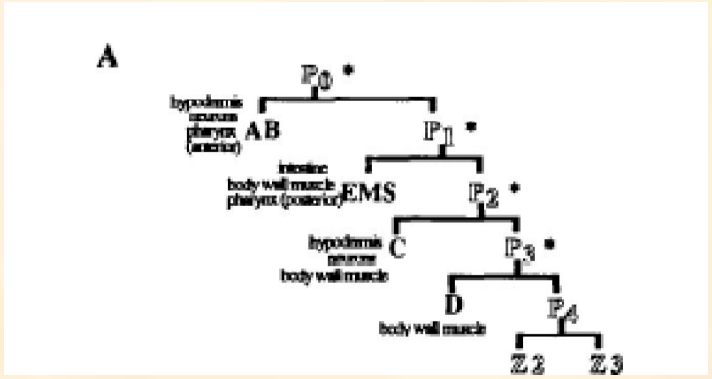


Exon	Size	RNA	Phenotype
Exon 21-22	742	Sense Antisense Sense+antisense	Wildtype Wildtype Twicher (100%)
Exon 27	1033	Sense Antisense Sense+antisense	Wildtype Wildtype Twicher (100%)

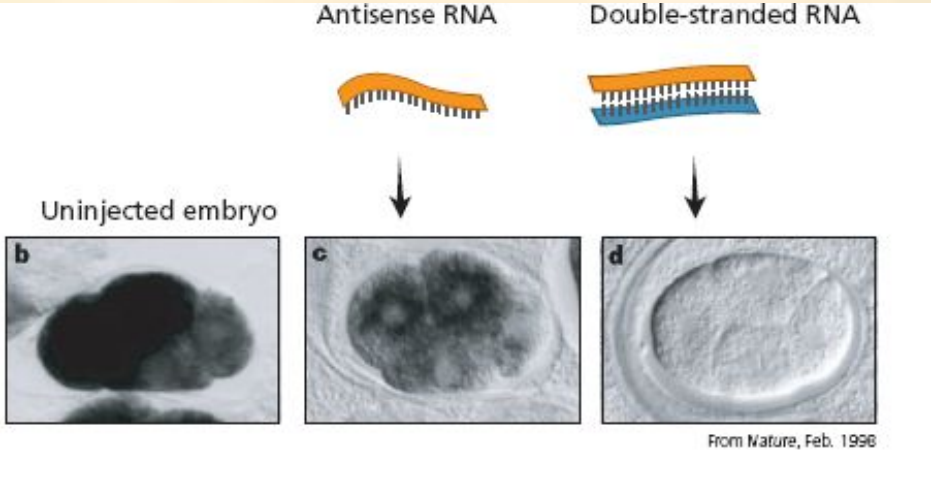
Mex-3

- mex-3 encodes two RNA binding proteins; in the early embryo, maternally provided
- Mex-3 is required for specifying the identities of the anterior AB blastomere and its descendants, as well as for the identity of the P3 blastomere and proper segregation of the germline P granules





Mex-3 RNAi



b, Embryo from uninjected parent (showing normal pattern of endogenous *mex-3* RNA20).

c, Embryo from a parent injected with purified *mex-3B* antisense RNA. Retain the *mex-3* mRNA, although levels may be somewhat less than wild type.

d, Embryo from a parent injected with dsRNA corresponding to *mex-3B*; no *mex-3* RNA is detected.

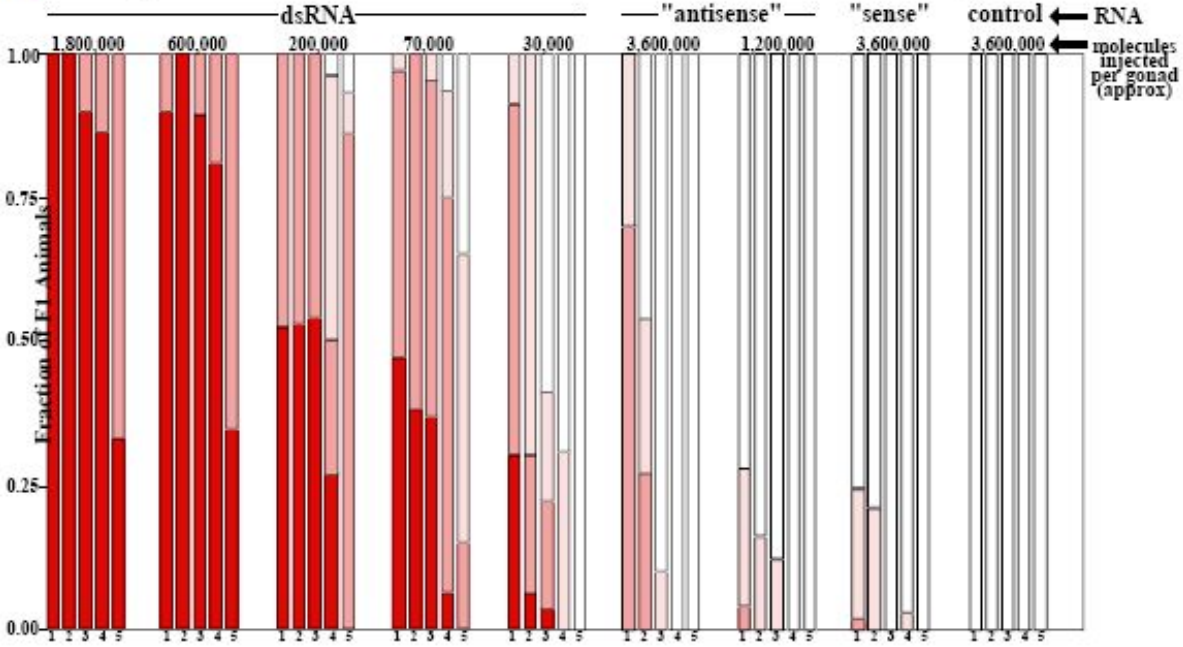
RNAi concentration and dose response

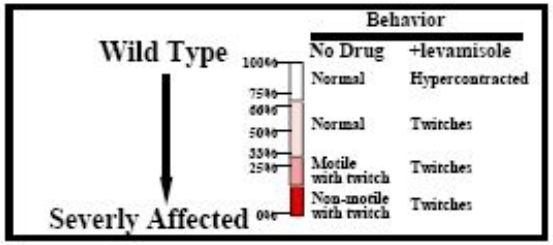
- $3.6x10^6$ molecules/gonad
  - Sense phenocopied 1% of progeny
  - Antisense phenocopied 11% of progeny
  - dsRNA phenocopies 100% progeny and at even $3x10^8$ molecules/gonad.

Quantitative Assays

Quantitative assays for silencing: *unc-22*

- **dsRNA is >100-fold more effective than sense or antisense**
- **dsRNA can produce interference at a few molecules per cell**





Progeny cohort group

1: 0-6 hr
2: 6-15 hr
3: 15-27 hr
4: 27-41 hr
5: 41-56 hr

Other possibilities

- Sense+antisense in low salt
- Rapid sequential injection of sense & antisense
  - Both cause interference
  - 1 hour apart injection of sense and antisense leads to reduction in interference.

Conclusions

- Gene silencing was highly effective when double-stranded RNA was injected but weak or non-existent after injection of single-stranded RNA (sense RNA or antisense RNA for the gene).
- The mRNA affected by the double-stranded RNA disappeared – it was apparently broken down and eliminated.
- The double-stranded RNA injected must match the mature, "trimmed" mRNA sequence for the gene. Interference could not be elicited by intron sequences (that is, segments of molecules that do not contain coding information). This implies that the interference takes place after transcription, probably in the cytoplasm rather than in the cell nucleus.

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Conclusions

- Only the mRNA corresponding to the sense RNA strand of the double-stranded RNA was silenced – no other mRNA in the cells was affected. RNA interference was specific for the gene with a code corresponding to that of the mRNA molecule.
- Just a few double-stranded RNA molecules were needed to silence a gene completely. The effect was so strong that Fire and Mello suggested that enzymes were involved in the process.
- The effect of double-stranded RNA could spread from cell to cell and from tissue to tissue and could even be passed on to offspring.

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Ways to induce silent phenotypes

- Timmons and Fire showed that feeding dsRNA works!
- Reversible and gene-specific effects…

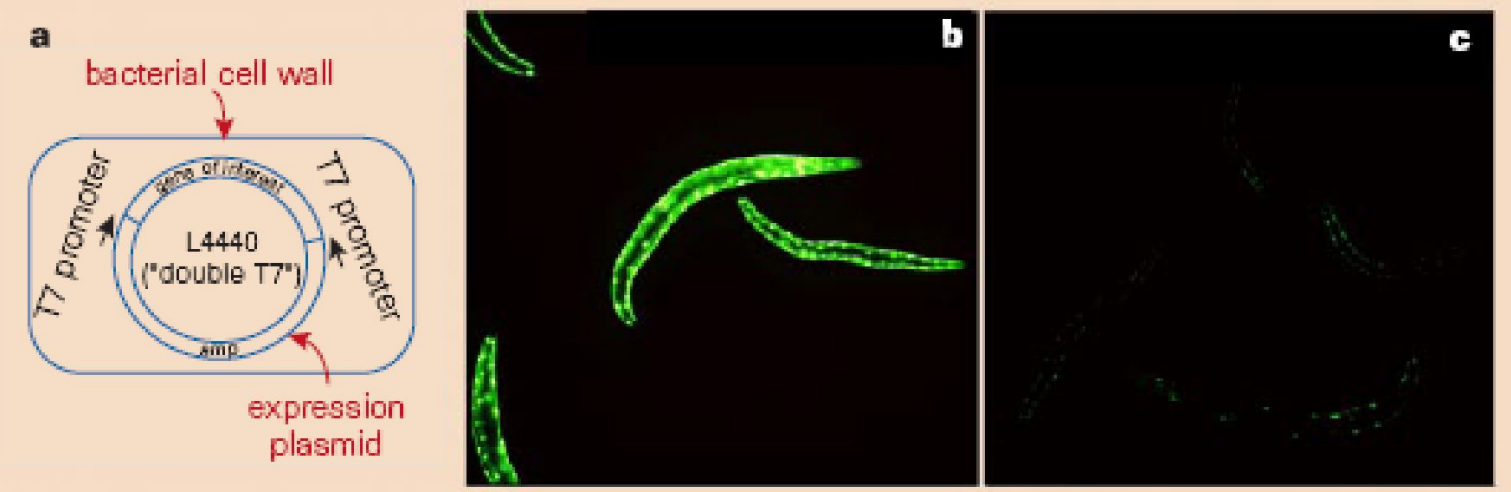

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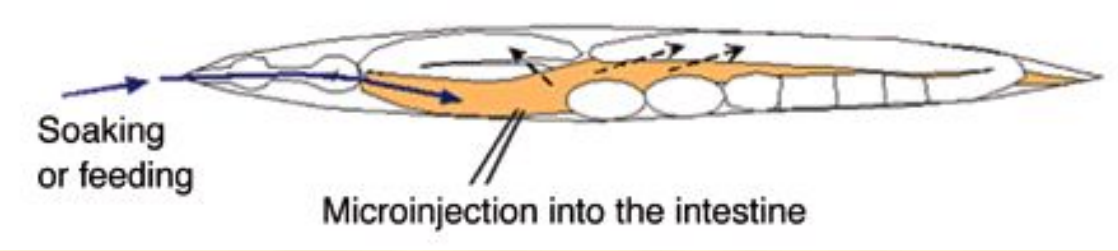
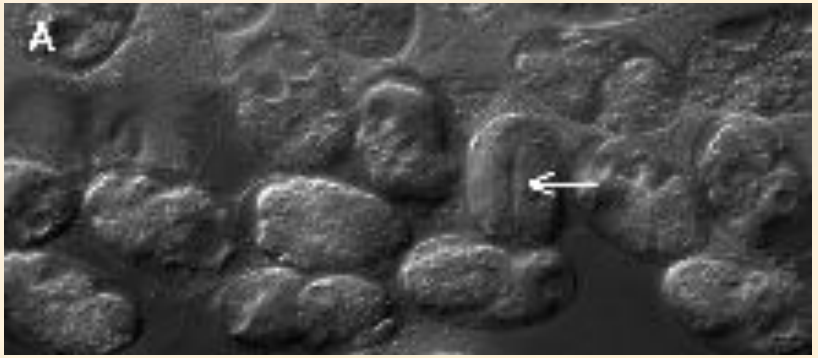
Figure 1 Genetic interference following ingestion of dsRNA-expressing bacteria by *Caenorhabditis elegans*. **a,** General scheme for dsRNA production. Segments were cloned between flanking copies of the bacteriophage T7 promoter into a bacterial plasmid vector (pPD129.36; J. Fleenor and A. F., unpublished). A bacterial strain (BL21/DE3; ref. 7) expressing the T7 polymerase gene from an inducible (Lac) promoter was used as a host. As an alternative strategy, we used a single copy of the T7 promoter to drive expression of an inverted duplication for a segment of the target gene (*unc-22* or *gfp*). A nuclease-resistant dsRNA was detected in lysates of these bacteria. The two bacterial expression systems gave similar interference results. **b,** A GFP-expressing *C. elegans* strain (PD4251)[1] fed on a naive bacterial host. Animals show high GFP fluorescence in body muscles. **c,** PD4251 animals reared on bacteria expressing dsRNA corresponding to the *gfp* coding region. Under the conditions of this experiment, 12% of these animals show a dramatic decrease in GFP.

Ways to induce silent phenotypes

- Tabarra, Grishok, and Mello in 1998 demonstrated that soaking in dsRNA also works!



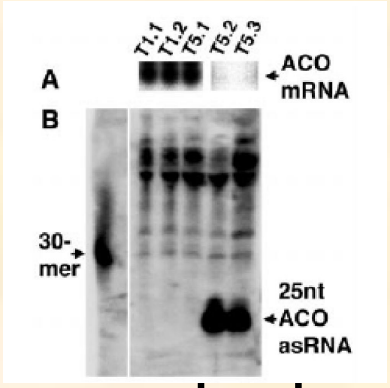
Nomarski image showing embryos produced by a wild-type mother treated with pos-1 RNAi by soaking. All except one embryo (arrow) show the distinctive pos-1 embryonic arrest with no gut, no body morphogenesis, and extra hypodermal cells



pos-1 encodes a CCCH-type zinc-finger protein; maternally provided POS-1 is essential for proper fate specification;

Mechanisms revealed

- 25bp species of dsRNA found in plants with co-suppression [Hamilton and Baulcombe, 1999]
  - Sequence similar to gene being suppressed

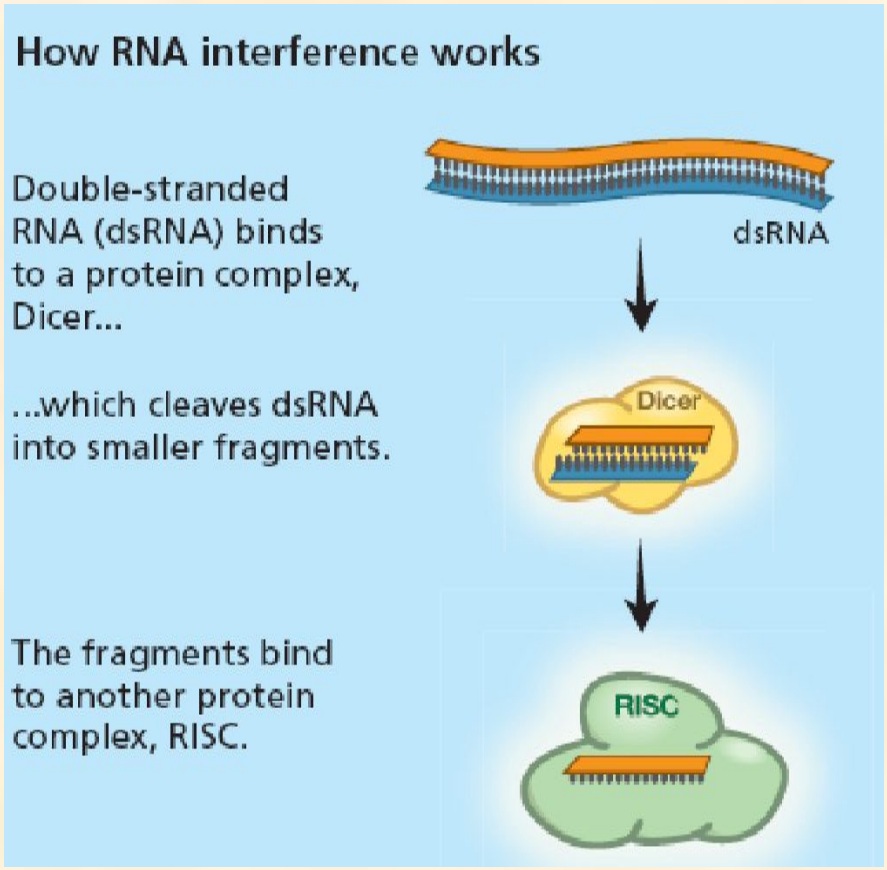


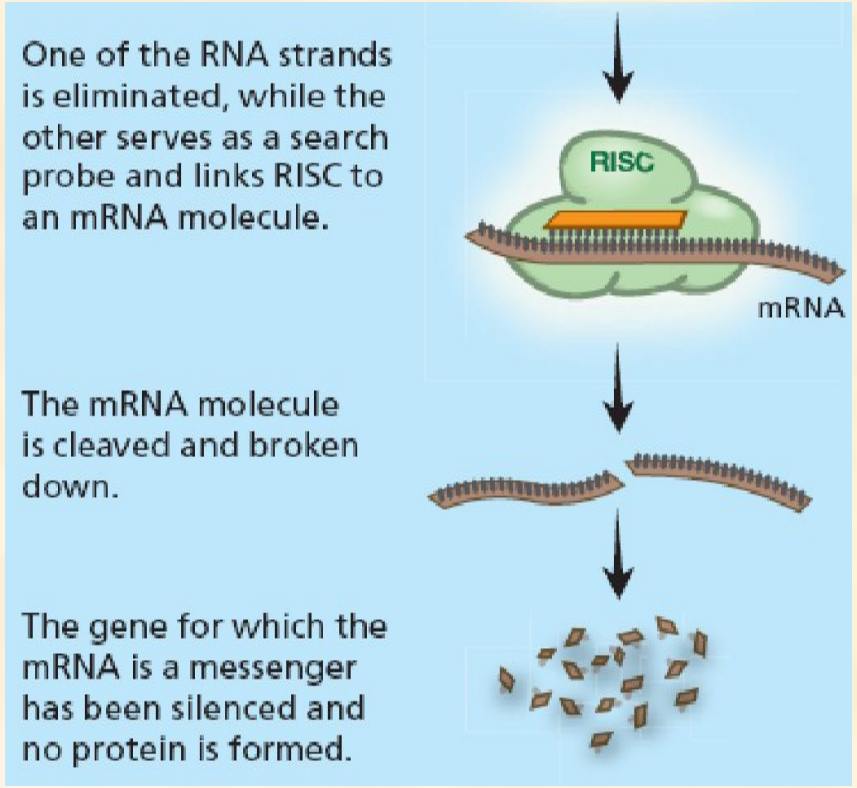
- Drosophila: long dsRNA "triggers" processed into 21-25bp fragments [Elbashir et al., 2001]
  - Fragments = short interfering RNA (siRNA)
  - siRNA necessary for degradation of target

RNAi: two phases

- Initiation
  - Generation of mature siRNA or miRNA
- Execution
  - Silencing of target gene
  - Degradation or inhibition of translation

How does RNAi work?





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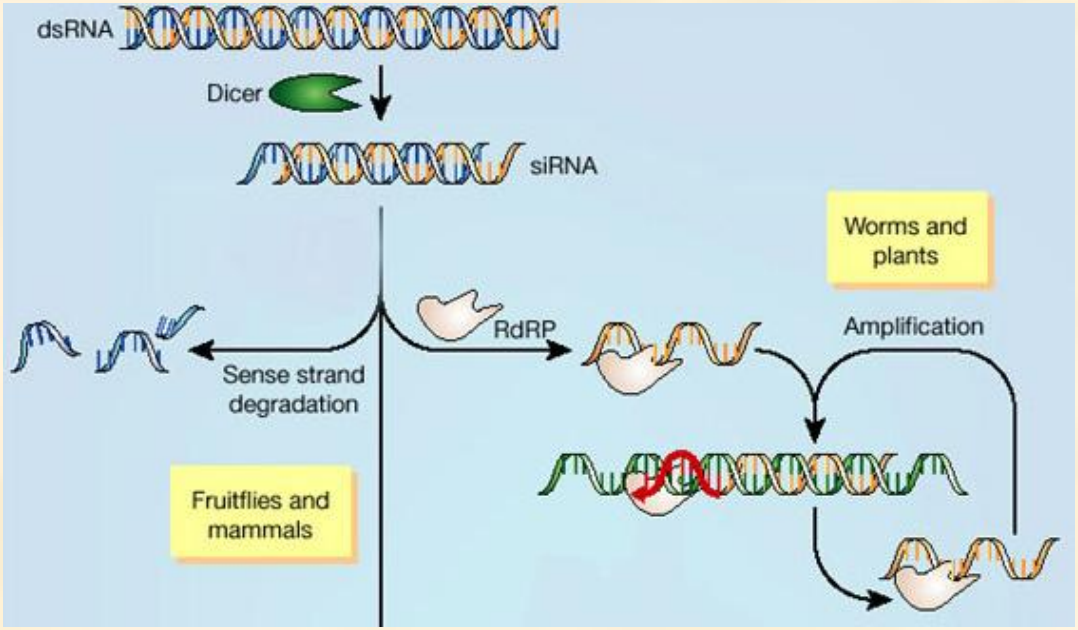
siRNA biogenesis

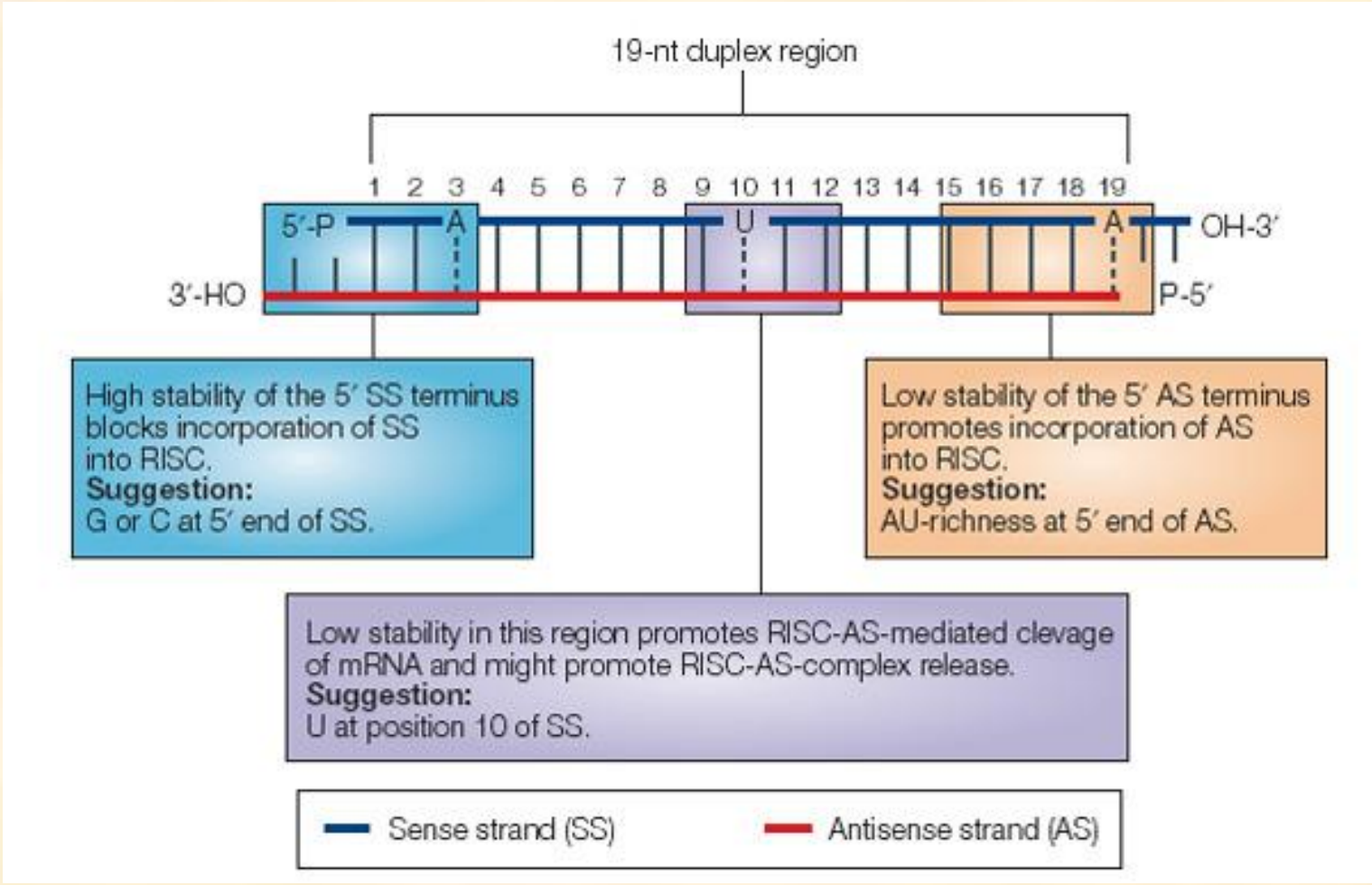
- Dicer (type III RNAse III) cleaves long dsRNA into siRNA 21-25nt dsRNA from exogenous sources
  - Symmetric 2nt 3' overhangs, 5' phosphate groups
  - Evidence for amplification in C. elegans and plants



RNA Induced Silencing Complex (RISC)

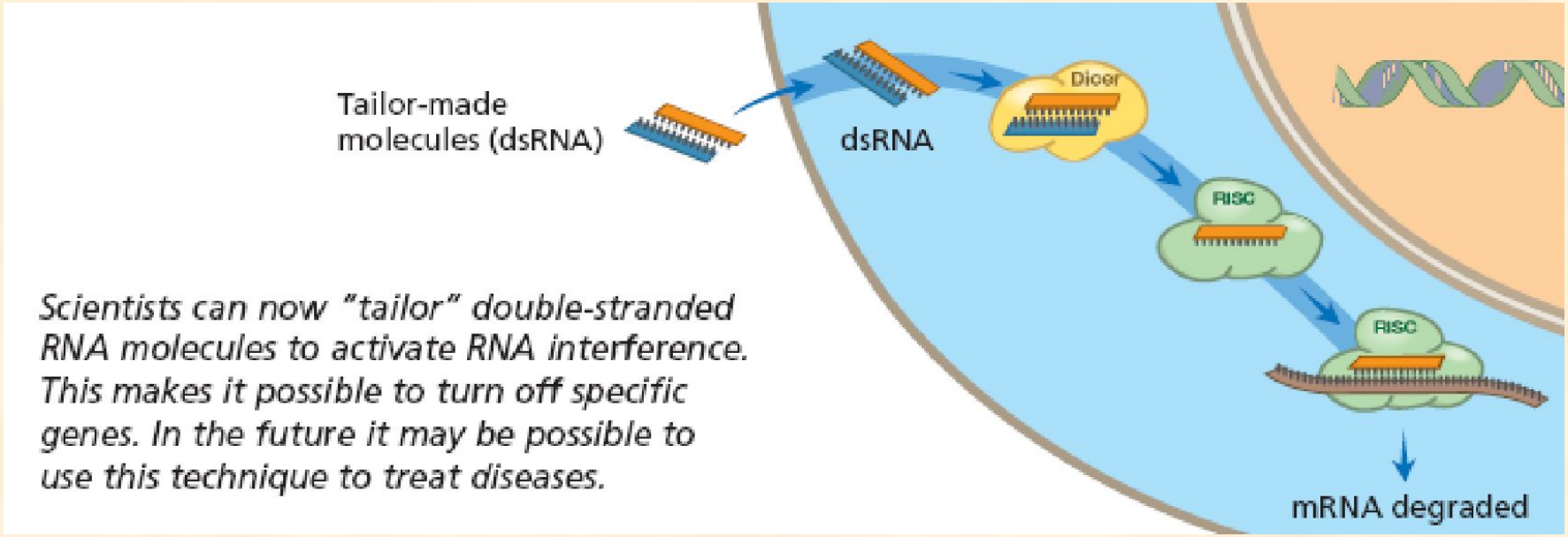
- RNAi effector complex
- Preferentially incorporates one strand of unwound RNA [Khvorova et al., 2003]
  - Antisense
- How does it know which is which?
  - The strand with less 5' stability usually incorporated into RISC [Schwarz et al., 2003]

siRNA design



Mittal, 2004

Custom-made siRNAs

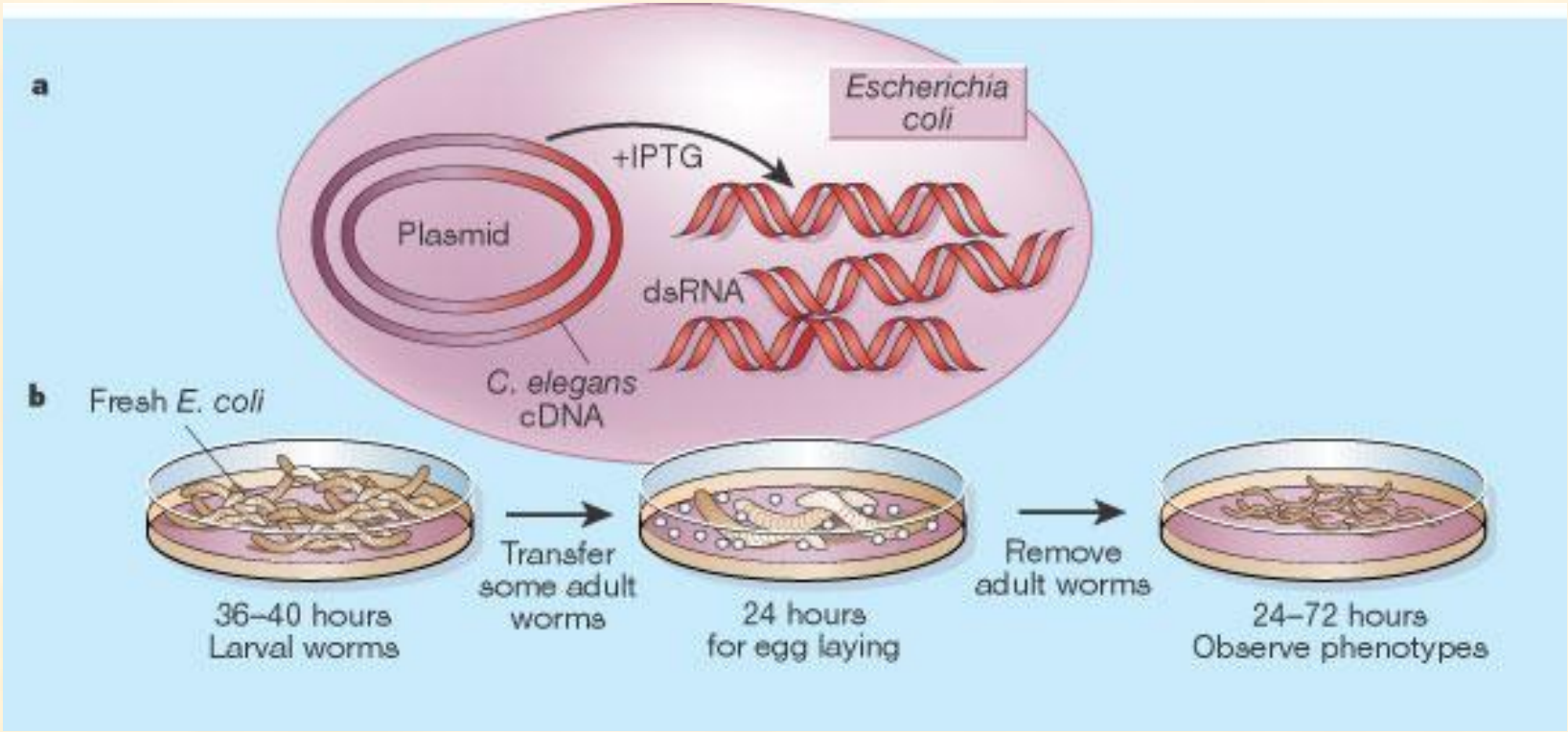


Scientists can now "tailor" double-stranded RNA molecules to activate RNA interference. This makes it possible to turn off specific genes. In the future it may be possible to use this technique to treat diseases.

siRNA libraries

siRNA libraries
- Result: 16 757 bacterial strains
- 86.3% of predicted genes with RNAi phenotypes assigned

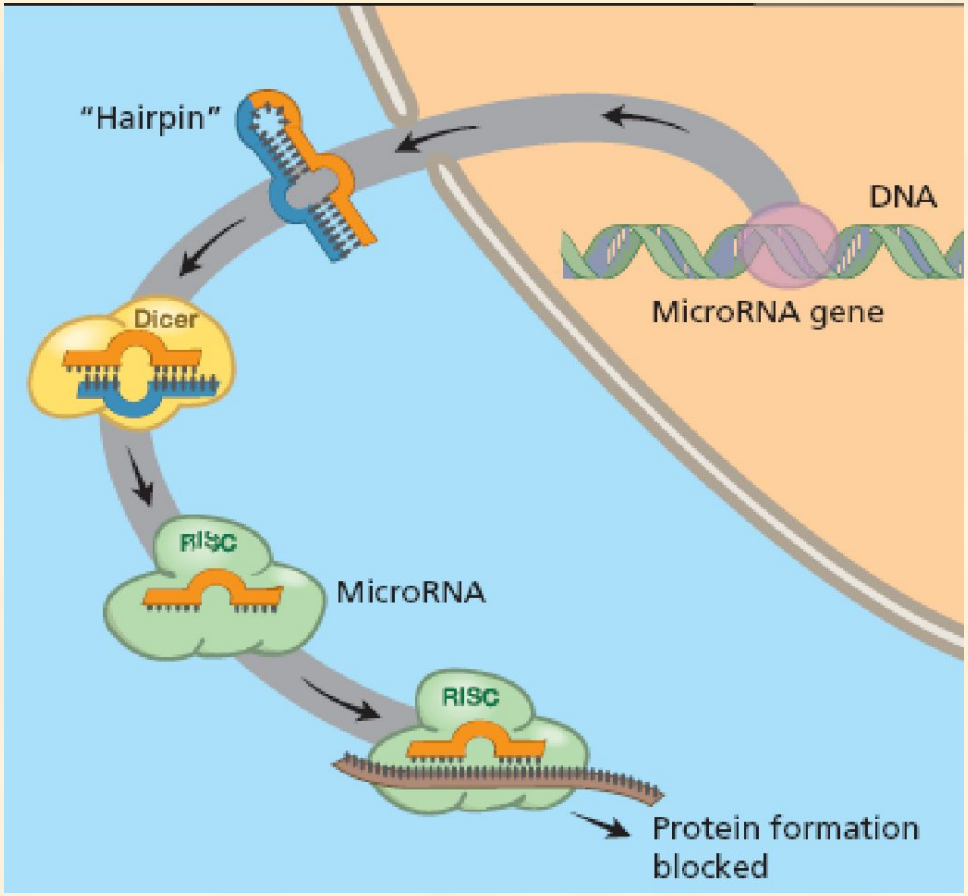
- Generation of a feeding clone



Tuschl, 2003

Endogenous RNAi-miRNA

- We have hundreds of different genes that encode small RNA (collectively, microRNA) whose precursors can form double-stranded RNA. These can activate the RNA interference process and thus switch off the activity of various genes with matching segments.
- First miRNA is lin-4



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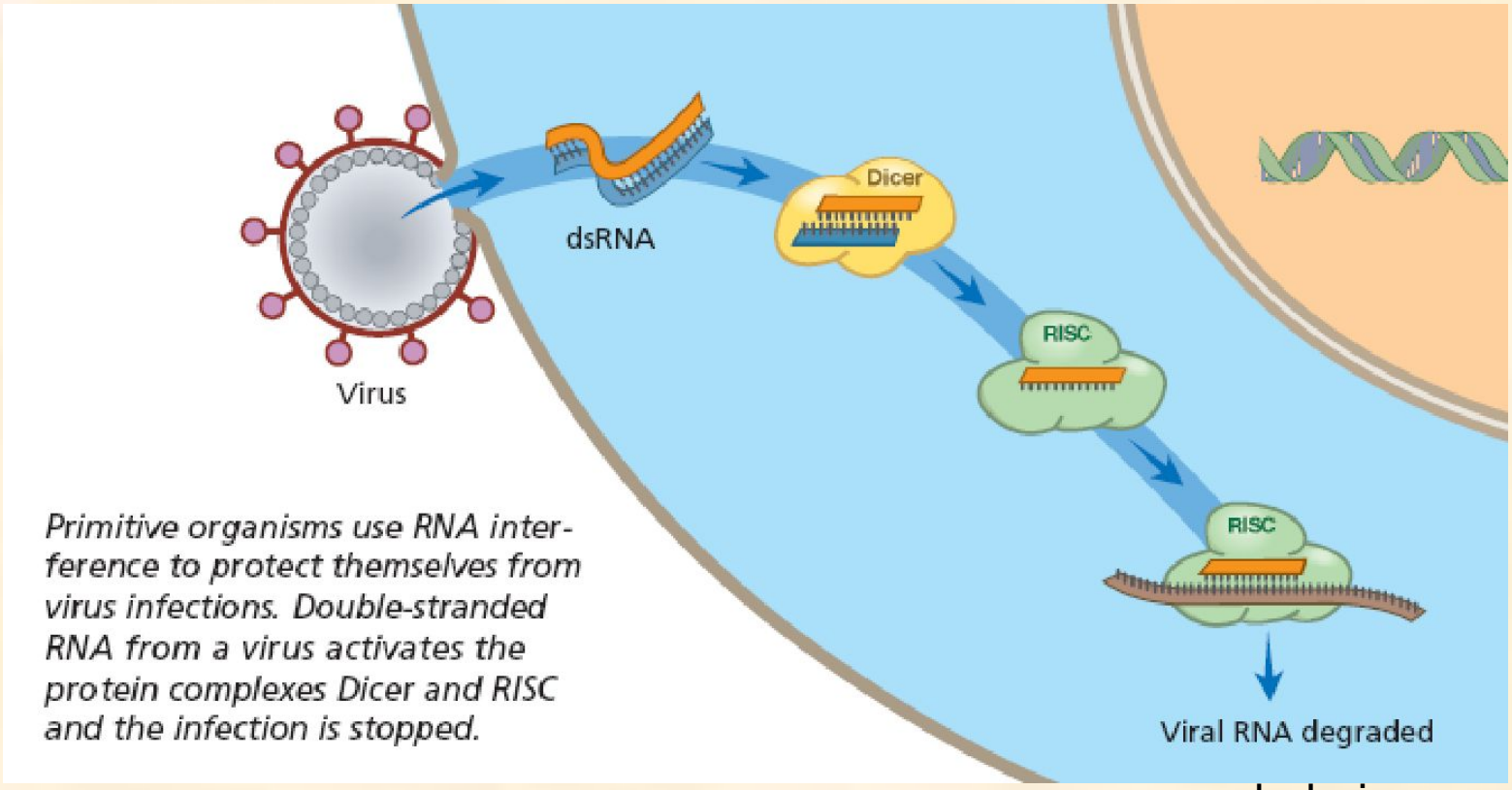
Defense Against Viruses



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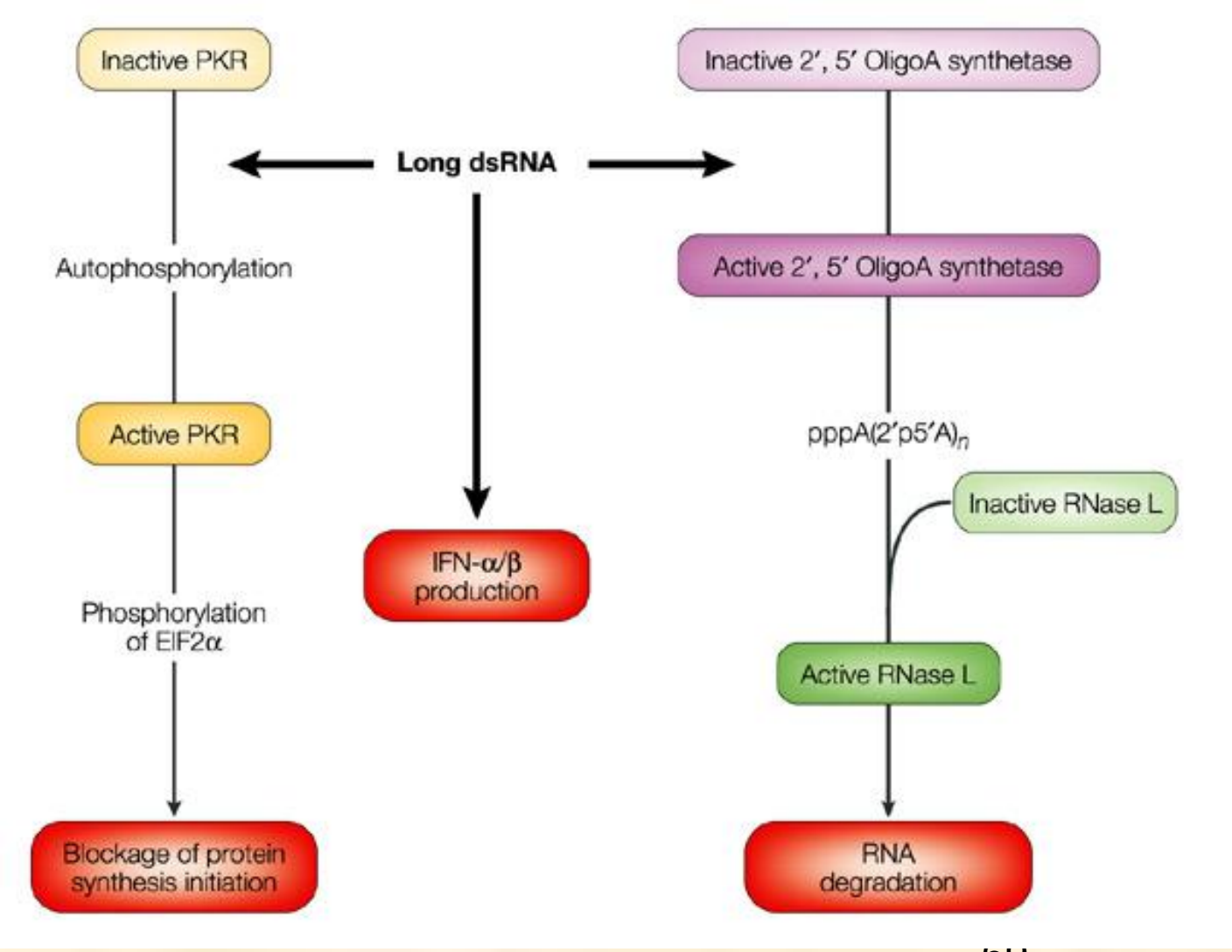
- Indeed, Baulcombe, Vance, and others have shown that, in the continuing evolutionary war to survive and reproduce, plant viruses have evolved genes that enable them to suppress silencing.

Mammalian RNAi



McManus and Sharp, 2002

Getting Around the Problem

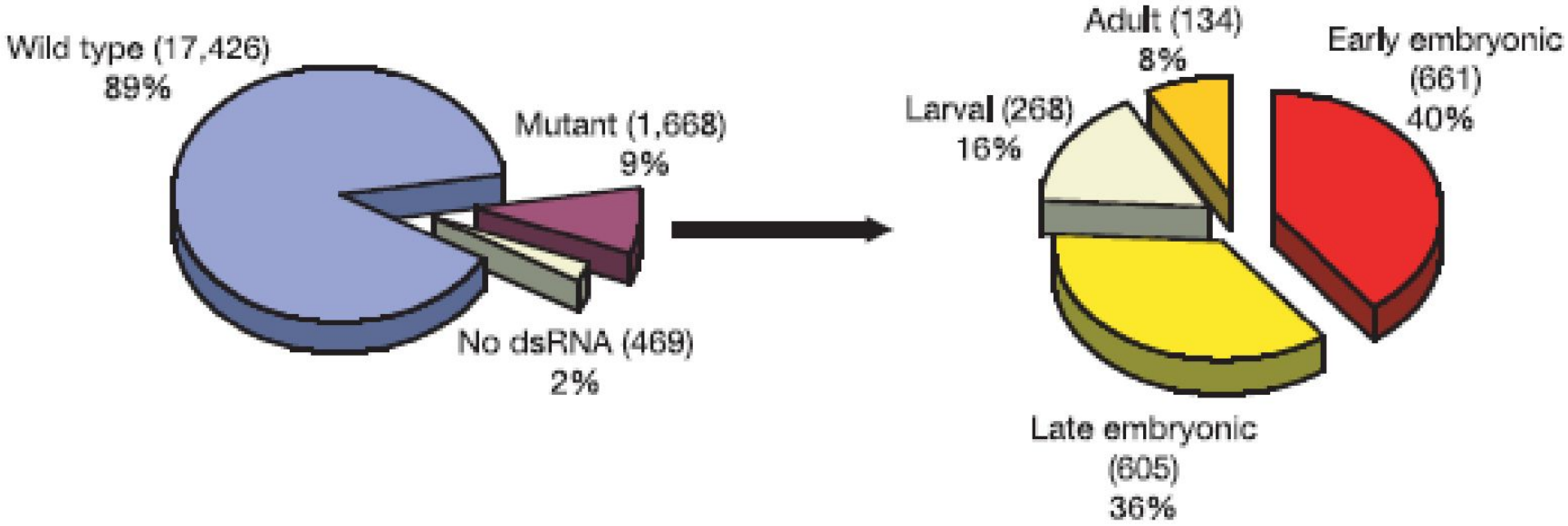
- siRNA (21-22nt) mediate mammalian RNAi
  - Introducing siRNA instead of dsRNA prevents non-specific effects

Some applications of RNAi

- Therapy
  - Candidate genes, drug discovery, and therapy
- Genome-wide RNAi screens
  - Gene function
  - Candidate genes and drug discovery
- Systems biology
  - Models of molecular machines

Genome-wide RNAi

- Only 11% genes showed detectable RNAi phenotype
- Between 600-800 genes are required for early embryogenesis.



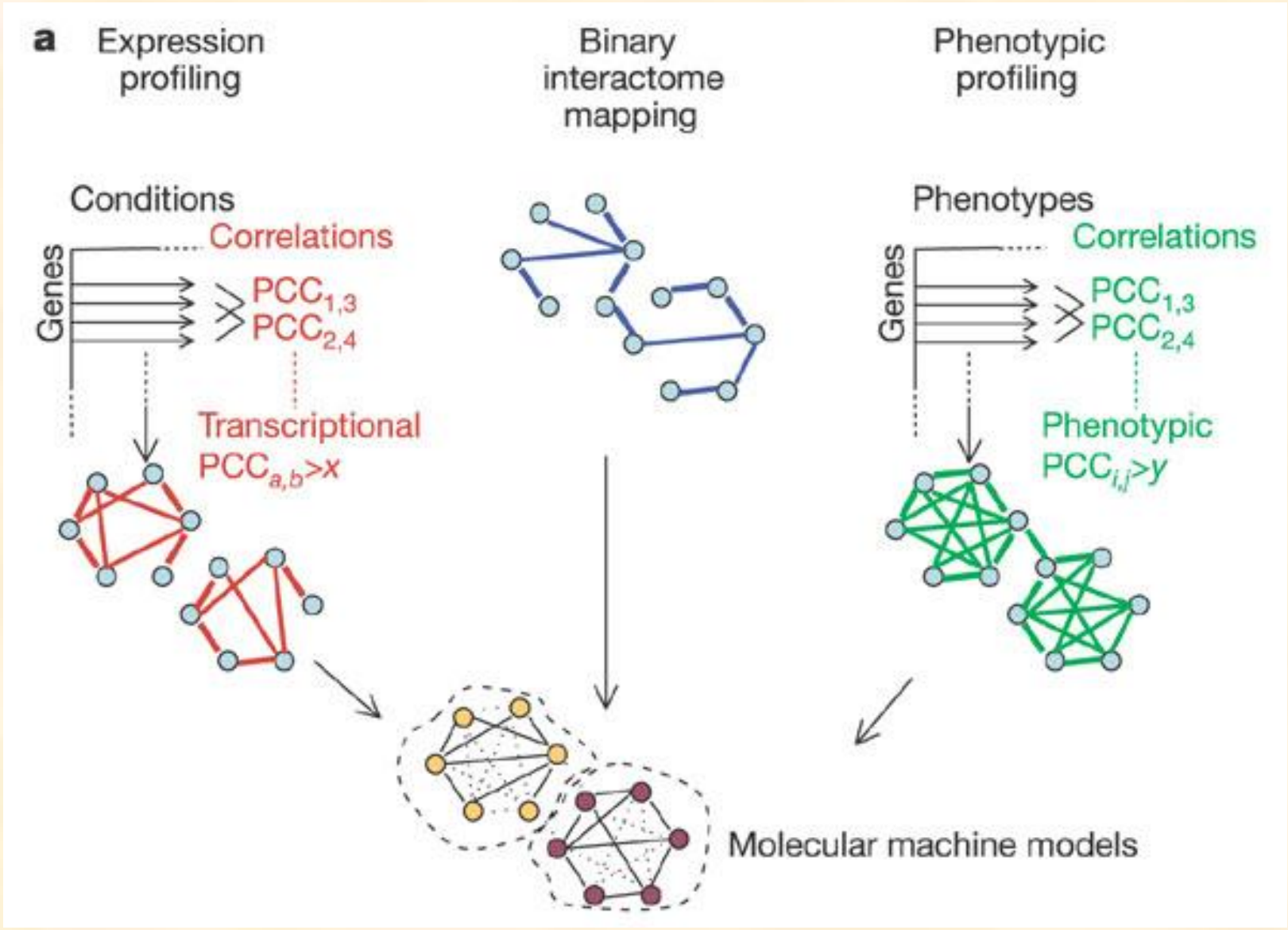
Systems Biology and RNAi

- Cellular systems act as networks of interacting components (genes, RNA, protein, metabolites,…).
- Genome-wide RNAi screens offers the potential for revealing functions of each protein.
- Combining RNAi screen data with other highthroughput data (e.g., protein-protein interaction, mRNA expression profiling) leads to understanding of the organization of the cell system.

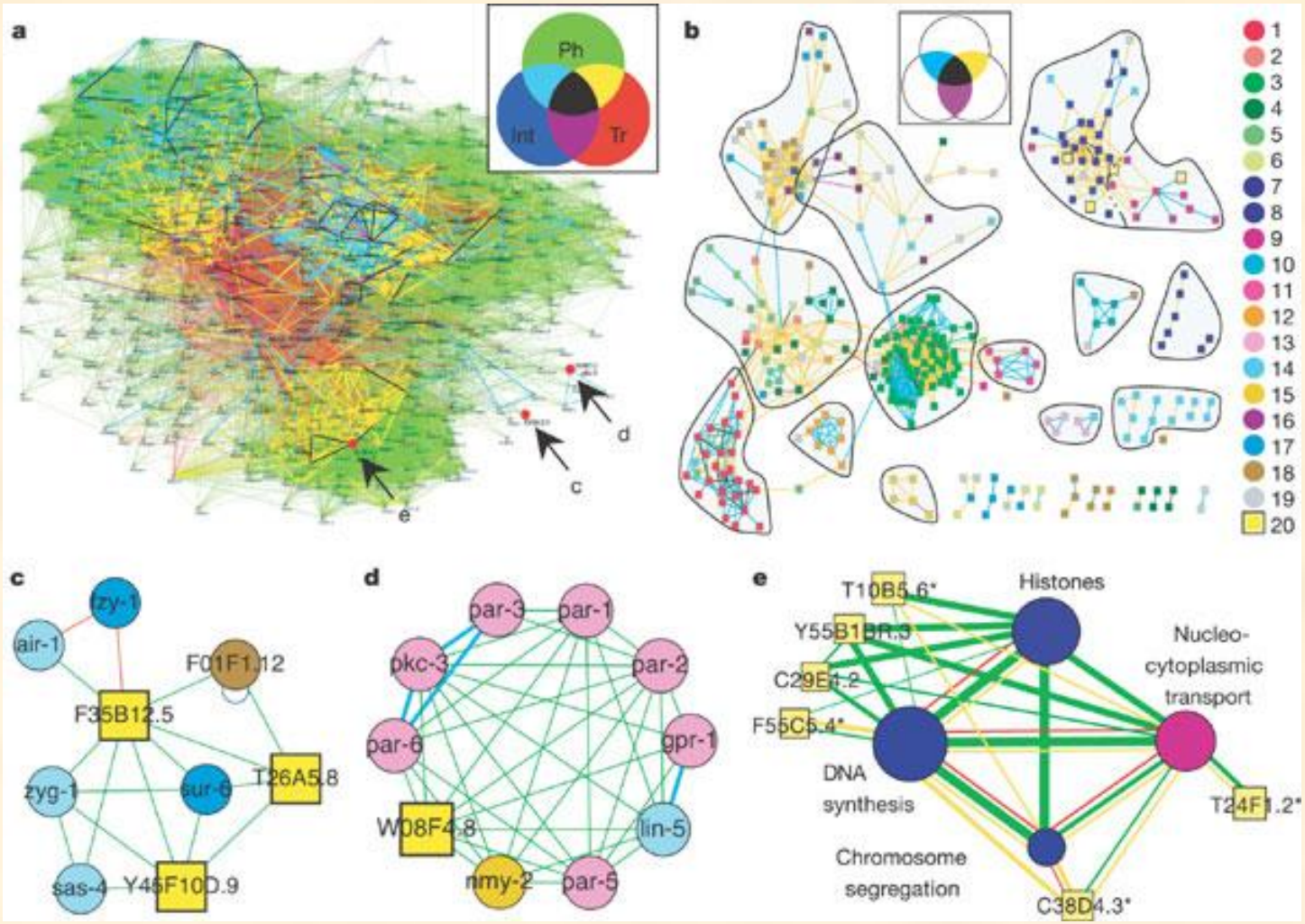
Networks of Early Embryogenesis

- **Protein-protein interaction dataset:** binary physical interactions between 3,848 *C. elegans* proteins
- **Transcriptome dataset:** expression profiling similarity above a given threshold among genes in the network
- **Phenotypic dataset:** phenotypic similarity above another threshold of 661 early embryogenesis genes. RNA interference (RNAi) phenotypic signature consisting of a vector describing specific cellular defects in early embryogenesis.

Systems Biology Approach: Three networks in one

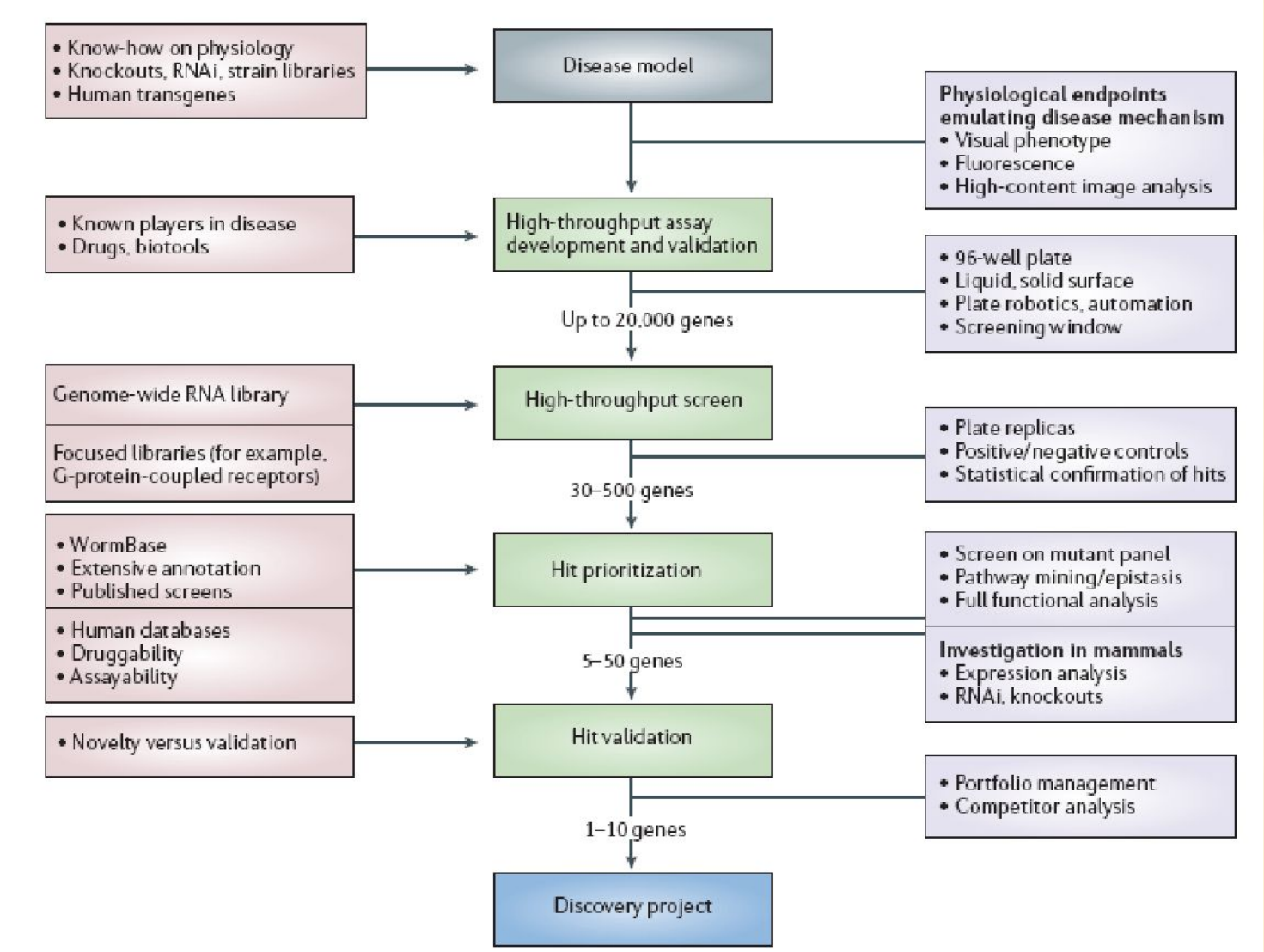


The embryogenesis network



encircled (Supplementary Table S6 provides details). Nodes are colour-coded by function: 1, proteasome; 2, protein degradation; 3, ribosome/protein synthesis; 4, translational control; 5, protein/vesicular trafficking; 6, RNA synthesis/processing/binding; 7, histone; 8, DNA synthesis/replication and chromosome segregation; 9, nucleocytoplasmic transport; 10, APC; 11, mitochondrial F_1F_0 ATPase; 12, vacuolar H^+ ATPase; 13, cell polarity; 14, microtubule cytoskeleton; 15, actin cytoskeleton; 16, cell cycle; 17, signal transduction; 18, metabolism; 19, other/unknown; 20, analysed by protein localization. **c**–**e**, Subnetworks with proteins of unknown function (yellow nodes) analysed by localization (Fig. 4). **c**, Centrosome model. **d**, PAR cell

Discovery Project



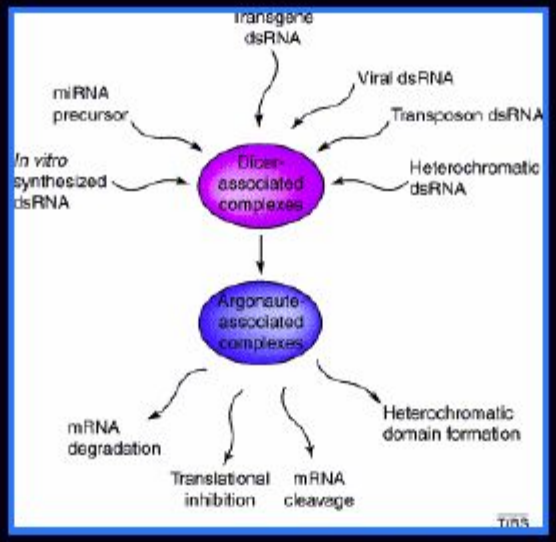
Defense against transposons

- RNAi may also help keep the transposable elements that litter genomes from jumping around and causing harmful mutations. Plasterk's team and Mello, Fire, and their colleagues found that mutations that knocked out RNAi in C. elegans led to abnormal transposon movements.

Why use RNAi?

1. The most powerful way to inhibit gene expression and acquire info about the gene's function fast
2. Works in any cell/organism
3. Uses conserved **endogenous** machinery
4. Potent at low concentrations
5. Highly specific.

Why do organisms have this machinery?



RNAi serves as a cellular defense against foreign DNA/RNA