Lee RC, Feinbaum RL, Ambros V. *Cell. 1993 75:843-54.*

The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14.

lin-4 is essential for the normal temporal control of diverse postembryonic developmental events in C. elegans. lin-4 acts by negatively regulating the level of LIN-14 protein, creating a temporal decrease in LIN-14 protein starting in the first larval stage (L1). We have cloned the C. elegans lin-4 locus by chromosomal walking and transformation rescue. We used the C. elegans clone to isolate the gene from three other Caenorhabditis species; all four Caenorhabditis clones functionally rescue the lin-4 null allele of C. elegans. Comparison of the lin-4 genomic sequence from these four species and site-directed mutagenesis of potential open reading frames indicated that lin-4 does not encode a protein. Two small lin-4 transcripts of approximately 22 and 61 nt were identified in C. elegans and found to contain sequences complementary to a repeated sequence element in the 3' untranslated region (UTR) of lin-14 mRNA, suggesting that lin-4 regulates lin-14 translation via an antisense RNA-RNA interaction.

Wightman B, Ha I, Ruvkun G. *Cell. 1993 75:855-62.*

Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans.

During C. elegans development, the temporal pattern of many cell lineages is specified by graded activity of the heterochronic gene Lin-14. Here we demonstrate that a temporal gradient in Lin-14 protein is generated posttranscriptionally by multiple elements in the lin-14 3'UTR that are regulated by the heterochronic gene Lin-4. The lin-14 3'UTR is both necessary and sufficient to confer lin-4-mediated posttranscriptional temporal regulation. The function of the lin-14 3'UTR is conserved between C. elegans and C. briggsae. Among the conserved sequences are seven elements that are each complementary to the lin-4 RNAs. A reporter gene bearing three of these elements shows partial temporal gradient activity. These data suggest a molecular mechanism for Lin-14p temporal gradient formation: the lin-4 RNAs base pair to sites in the lin-14 3'UTR to form multiple RNA duplexes that down-regulate lin-14 translation.

lin-4 loss-of-function mutations display reiterations of early fates at inappropriately late developmental stages; cell lineage patterns normally specific for the L1 are reiterated at later stages. The consequences of these heterochronic developmental patterns include the absence of adult structures (such as adult cuticle and the vulve) and the prevention of egg laying.

lin-14 null mutations cause a phenotype opposite to that of *lin-4* and are completely epistatic to lin-4, which is consistent with lin-4 acting as a negative regulator of lin-14.
lin-14 mutants skip the expression of L1-specific events and precociously execute programs normally specific for L2, L3, L4 and adult stages.

Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G.
Nature. 2000 403:901-6.

The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans.

The C. elegans heterochronic gene pathway consists of a cascade of regulatory genes that are temporally controlled to specify the timing of developmental events. Mutations in heterochronic genes cause temporal transformations in cell fates in which stage-specific events are omitted or reiterated. Here we show that let-7 is a heterochronic switch gene. Loss of let-7 gene activity causes reiteration of larval cell fates during the adult stage, whereas increased let-7 gene dosage causes precocious expression of adult fates during larval stages. let-7 encodes a temporally regulated 21-nucleotide RNA that is complementary to elements in the 3' untranslated regions of the heterochronic genes lin-14, lin-28, lin-41, lin-42 and daf-12, indicating that expression of these genes may be directly controlled by let-7. A reporter gene bearing the lin-41 3' untranslated region is temporally regulated in a let-7-dependent manner. A second regulatory RNA, lin-4, negatively regulates lin-14 and lin-28 through RNA-RNA interactions with their 3' untranslated regions. We propose that the sequential stage-specific expression of the lin-4 and let-7 regulatory RNAs triggers transitions in the complement of heterochronic regulatory proteins to coordinate developmental timing.

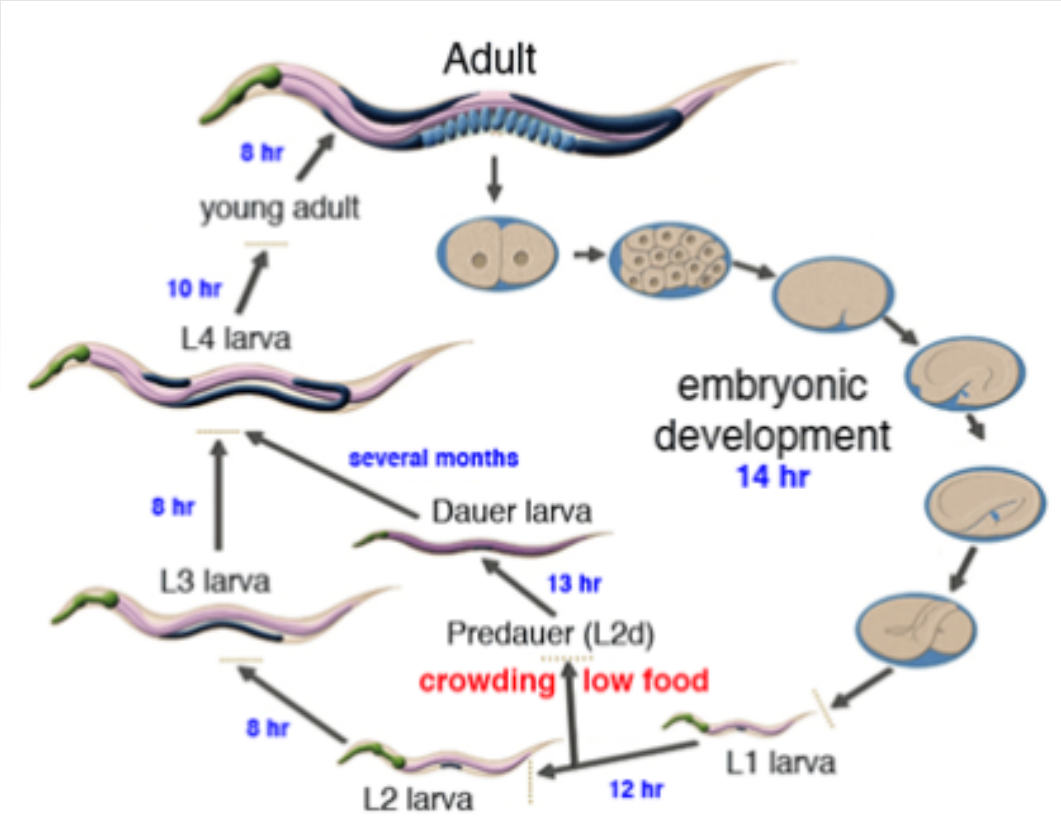
The C. elegans Heterochronic Gene *lin*-4 Encodes Small RNAs with Antisense Complementary to *lin*-14

di Ambros V.

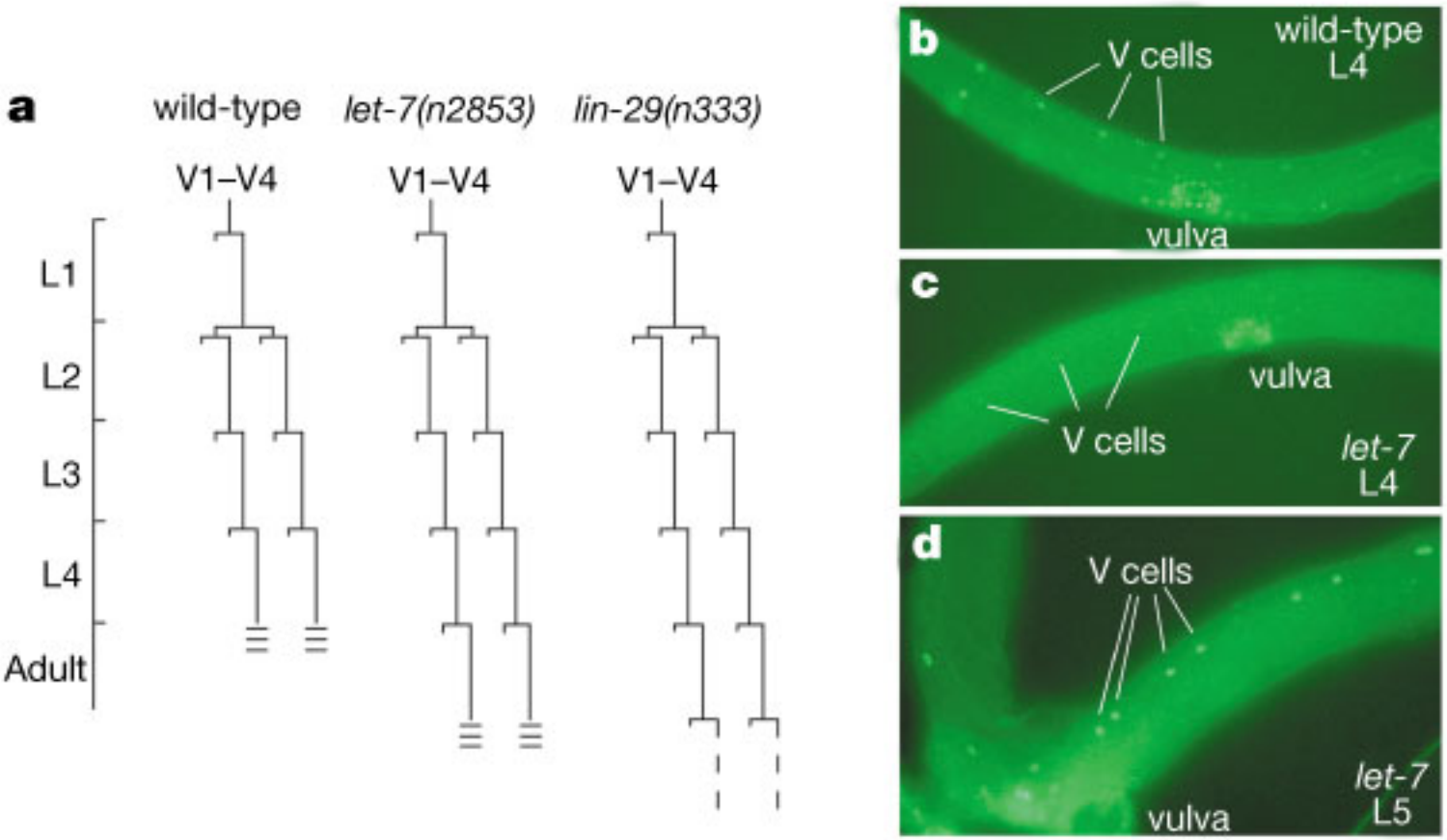
Caenorhabditis elegans

Caenorhabditis elegans è un verme nematode lungo circa 1 mm. Il C. elegans è trasparente. Pur essendo un organismo relativamente semplice, possiede molti dei sistemi e degli apparati presenti negli altri animali.

Ciclo vitale C. elegans



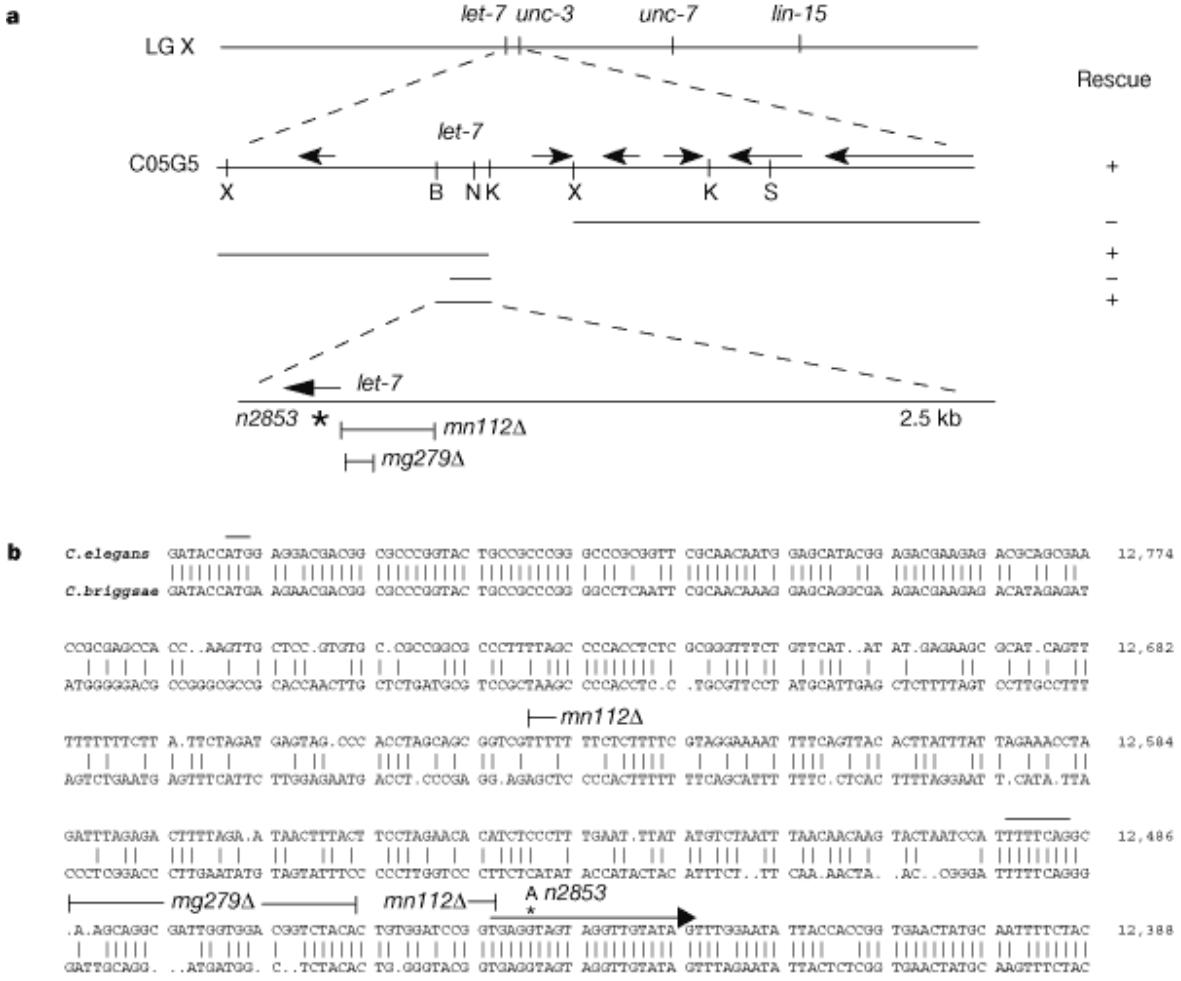
Il ciclo vitale di C. elegans presenta stadi ben precisi con una durata pressoché fissa. Essendo il destino delle cellule ben studiato, la ricerca si concentra sui meccanismi del differenziamento.



The *let-7* heterochronic phenotype. a, Lineage of the lateral hypodermal cells V1, V2, V3 and V4 in wild-type, *let-7* and *lin-29* animals. L3 and L4 stage V cell lineages are equivalent in hermaphrodites and cannot be distinguished. b, Wild-type L4 stage animal with LIN-29 expression in the lateral hypodermis and vulva. c, *let-7(n2853)* L4 stage animal with LIN-29 expression reduced in V cells but at normal levels in vulval cells. d, An L5 stage *let-7(n2853)* animal showing accumulation of LIN-29 at high levels one stage later than wild-type animals.

Introduzione

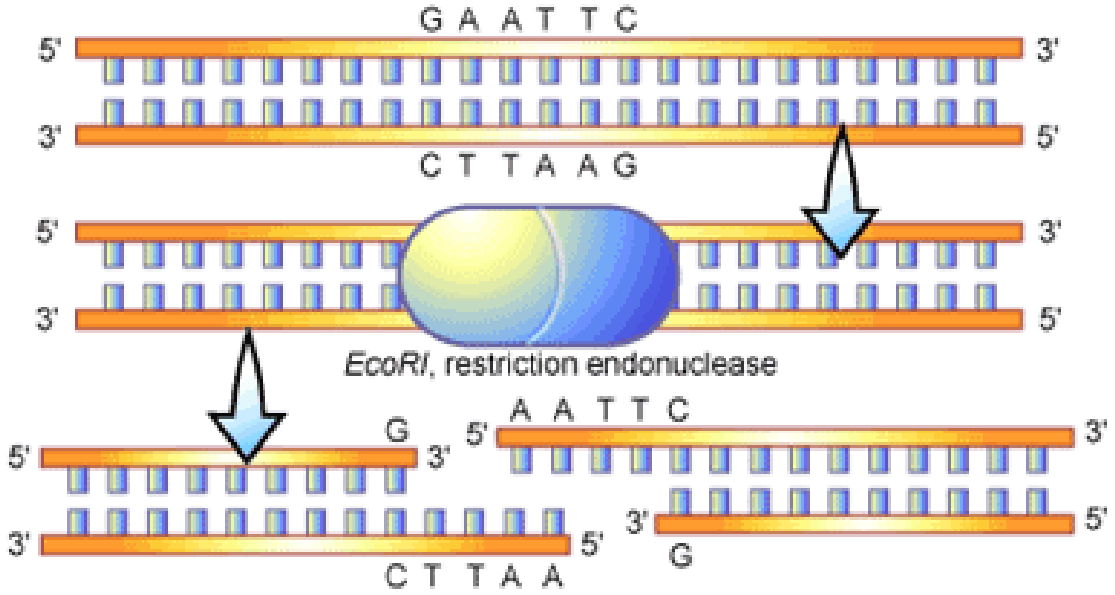
- Il gene lin-14 codifica per una proteina che svolge un ruolo specifico nella fase L1
- Il gene lin-4,attivandosi in tarda fase L1 reprime l'espressione di lin-14 e permette il corretto proseguimento dello sviluppo negli stadi successivi
- Gli studi sono condotti a partire da un mutante che presenta una delezione dell'intero gene lin-4



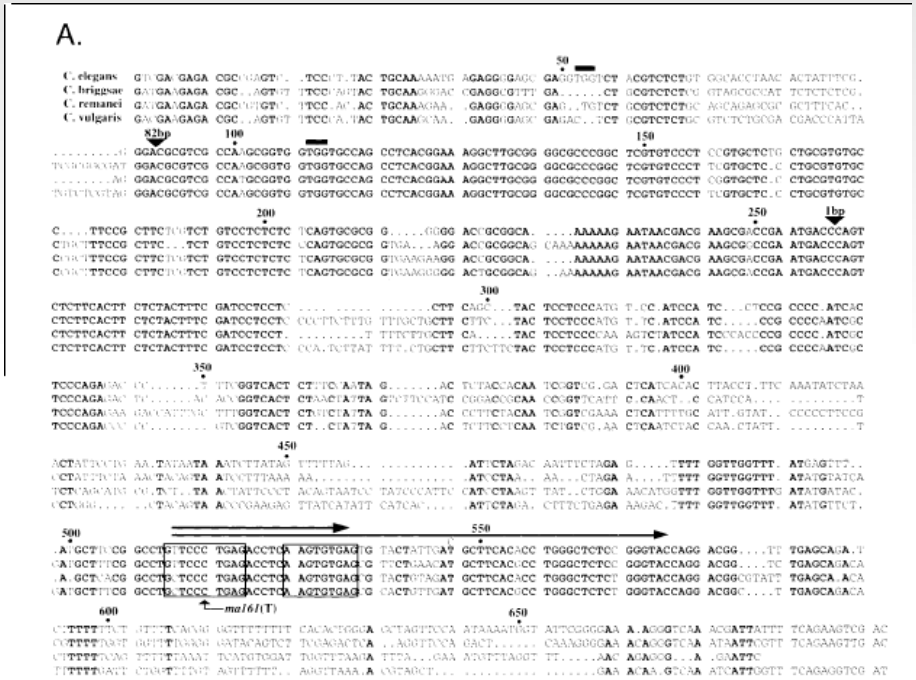
The *let-7* gene sequence. a, Transgenic rescue. +, more than 90% rescue in multiple lines; -, no rescue. Arrows above C05G5 indicate predicted genes. b, Sequence comparison of *let-7* from *C. elegans* and *C. briggsae*. The 2.5-kb genomic fragment that fully rescues *let-7(mn112)* corresponds to nucleotide positions 14,208–11,749 of cosmid C05G5. The 21-nucleotide *let-7* transcript is indicated by an arrow. A truncation of the 2.5-kb fragment (to 12,446) deleting this transcript no longer rescues *let-7(mn112)* . The ATG at 12,857 and a 3' splice consensus TTTTCAG of a non-conserved open reading frame at 12,494 are indicated by bars.

Studi genomici su lin-4

Attraverso RFLP e southern blot si è individuata la posizione del gen lin-4 e si è visto che esso giace all'interno di un introne di un gene la cui funzione non è rilevante per lo sviluppo di C. elegans.



Comparazione filogenetica



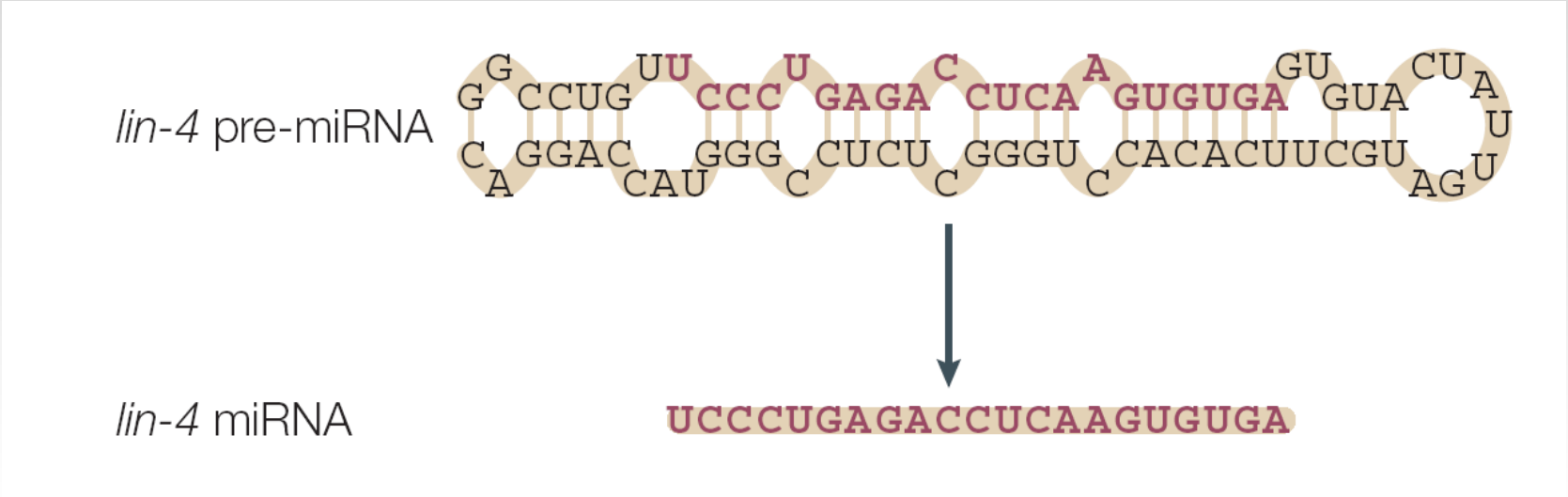
Utilizzando DNA plasmidico proveniente dalle altre specie di Caenorhabditis, si recupera il fenotipo wt in un individuo mutante.
Il sequenziamento mostra la presenza di una sequenza conservata.

Proteina LIN-4?

Per individuare la proteina LIN-4 si è studiata la sequenza del gene lin-4:

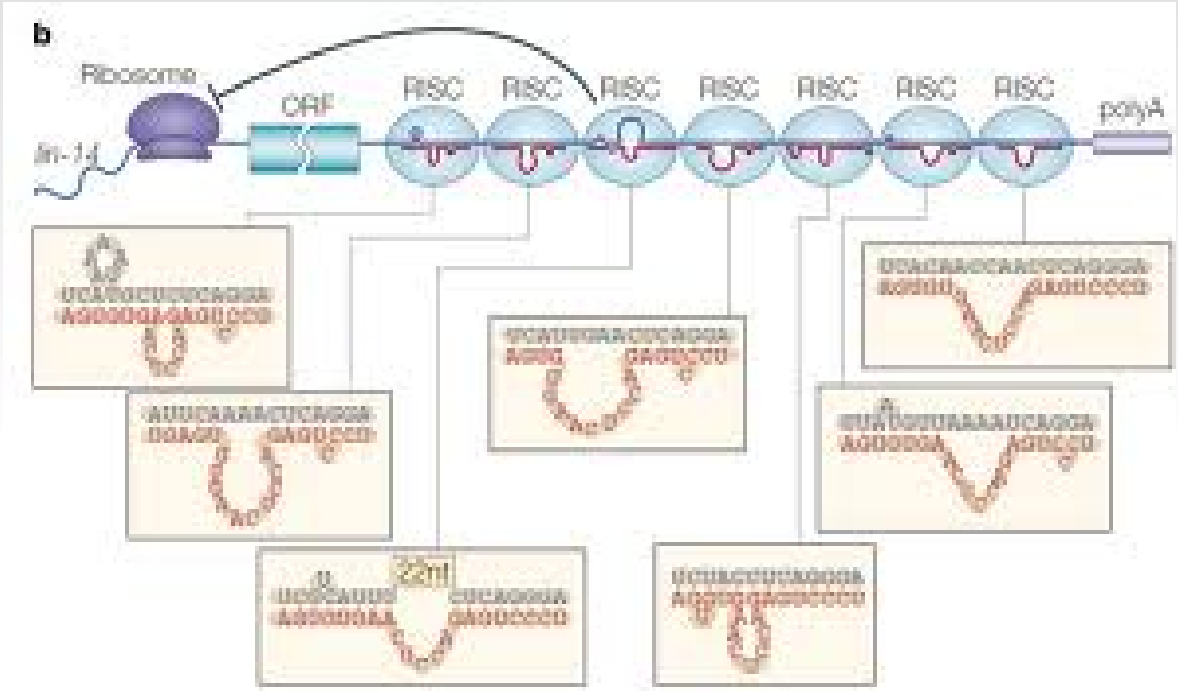
- il confronto con i cDNA posseduti ha avuto esito negativo;
- il confronto con le sequenze delle altre specie non mostra alcuna open reading frame conservata;
- l'inserzione di mutazione puntiformi permette la conservazione del fenotipo wild type.

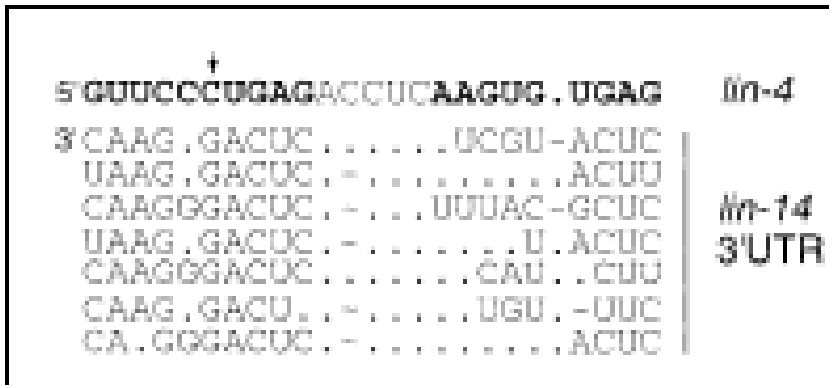
Prodotti genici di lin-4



Attraverso northern blot si è rilevata la presenza di due piccoli trascritti di 61 e 22 nt.
L'RNA lin-4L (Larger) si è scoperto essere il pre-miRNA.
L'RNA lin-4S (Smaller) si è scoperto essere il miRNA maturo

Azione di lin-4





Il piccolo RNA di lin-4 presenta complementarietà per sette siti presenti nella sequenza 3'UTR dell'mRNA lin-14

Variabilità allelica di lin-4 e lin-14

Le mutazioni dei geni lin-4 e lin-14 permettono di capire il meccanismo attraverso il quale lin-4 e lin-14 regolano lo sviluppo di C. elegans in L1:

- lin-4 (lf) è una delezione che causa la reiterazione del fenotipo di L1 negli stadi larvali successivi
- lin-14 (0) causa un fenotipo opposto a lin-4 (lf)
- lin-14 (gf) causa un fenotipo simile a lin-4 (lf).

Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G.
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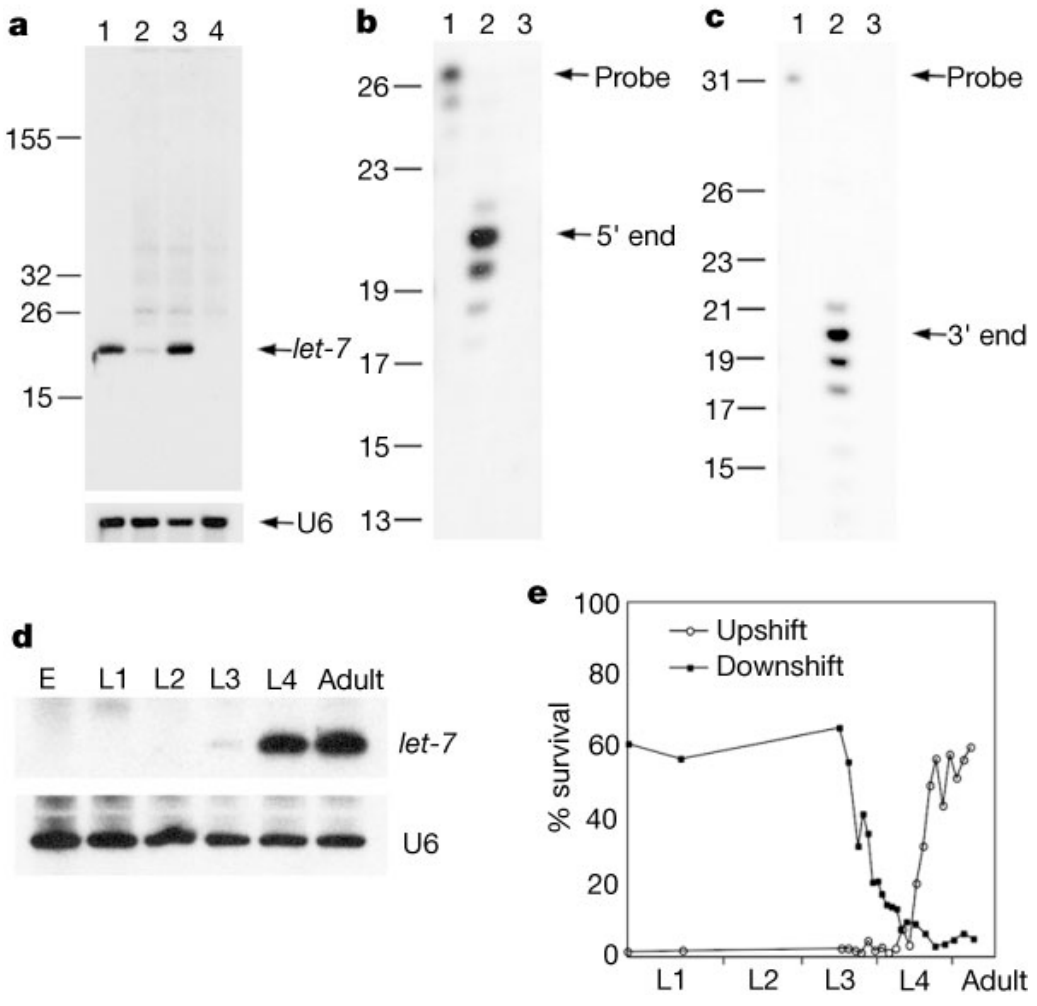
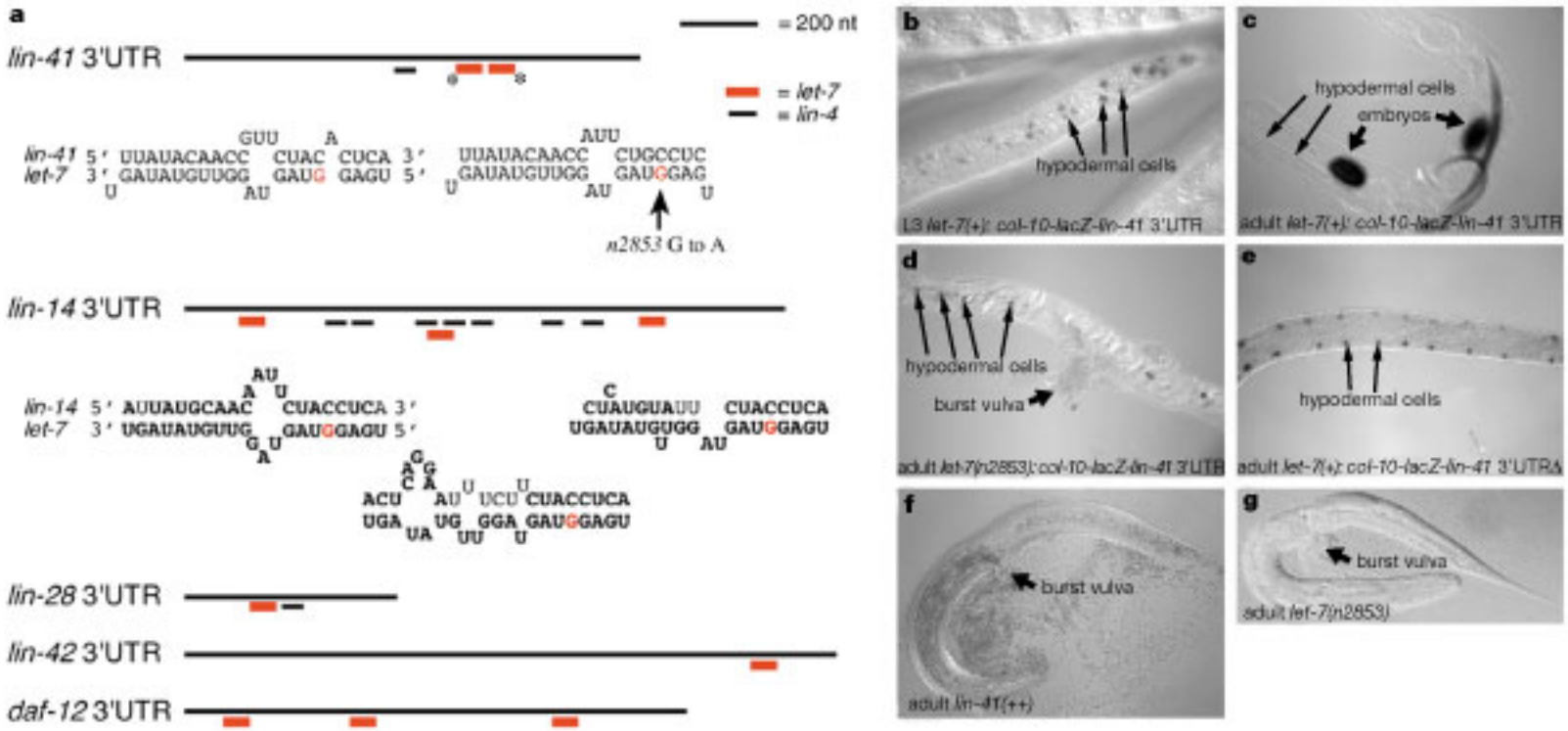
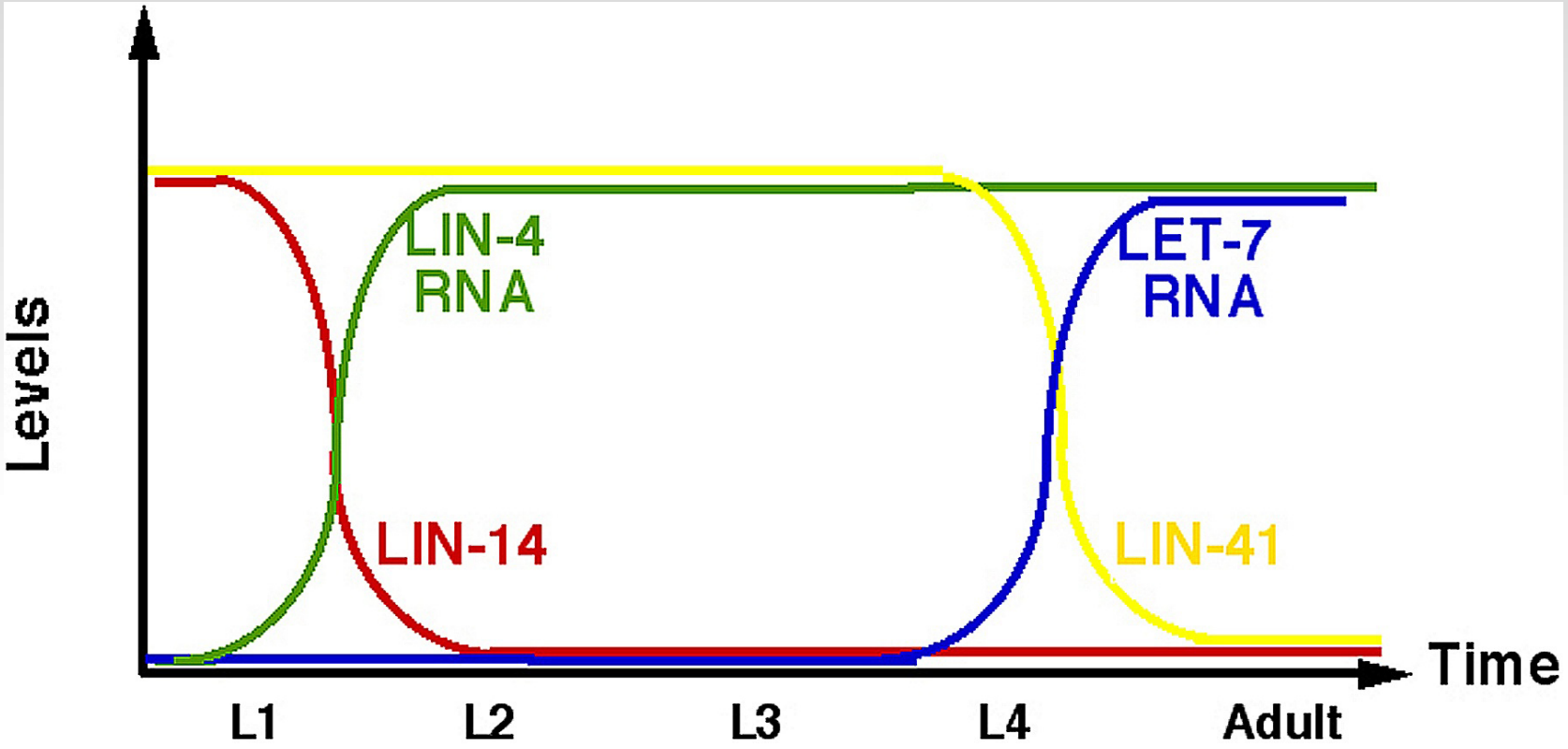


The 21-nucleotide *let-7* RNA. a, Northern blot of total RNA from mixed stage wild-type (lane 1), *let-7(n2853)* (lane 2), *lin-28(n719)* (lane 3) and *lin-28(n719); let-7(mn112) unc-3(e151)* animals (lane 4) probed with p249N. b, c, S1 nuclease transcript mapping. b, 5' probe p263 undigested (lane 1), and digested after hybridization to wild-type RNA (lane 2) or tRNA (lane 3). c, 3' probe p267 undigested (lane 1), and digested after hybridization to wild-type RNA (lane 2) or tRNA (lane 3). Sizing 1 nucleotide. d, Northern blot of wild-type RNA from the first 3 hours of each developmental stage. e, Temperature-sensitive period of *let-7(n2853)* viability.

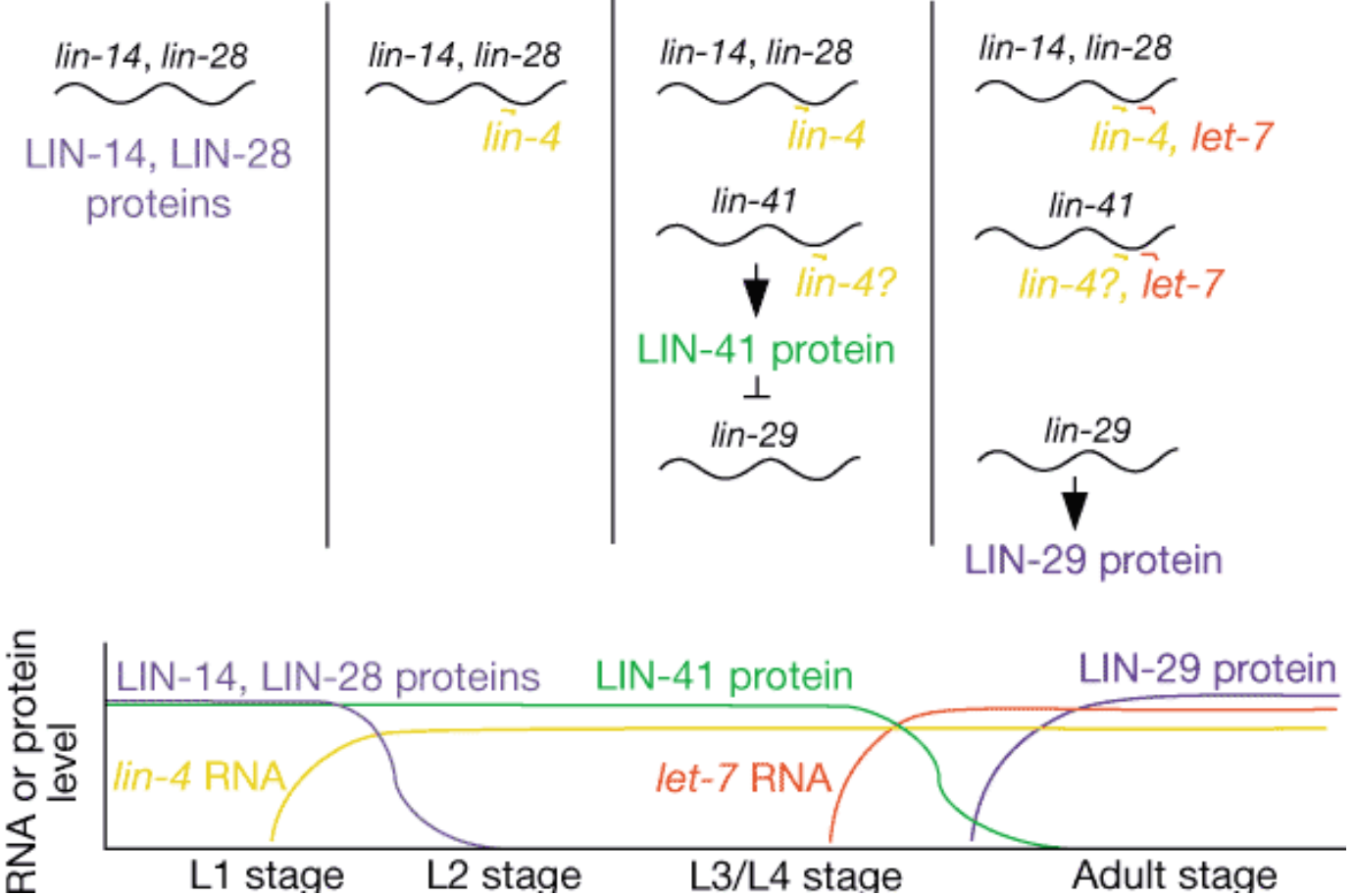


let-7 regulation of heterochronic genes. a, *let-7* complementary sites in heterochronic genes. Bases shown in bold are conserved in genes with known *C. briggsae* homologues. b–e, *let-7* regulation of *lin-41*. A *col-10/lacZ/lin-41* 3' UTR reporter gene was expressed in hypodermal cells of *let-7(+)* L3 larvae (b), downregulated in *let-7(+)* adults (c), but expressed at high levels in *let-7(n2853)* adults (d); deletion of the *let-7* complementary sites in this reporter gene 3' UTR (between asterisks in a allows expression in *let-7*(+) adults (e). f, g, *lin-41* is a major *let-7* target. High dosage of *lin-41*(+) in wild-type (f) caused a bursting

Regolazione temporale

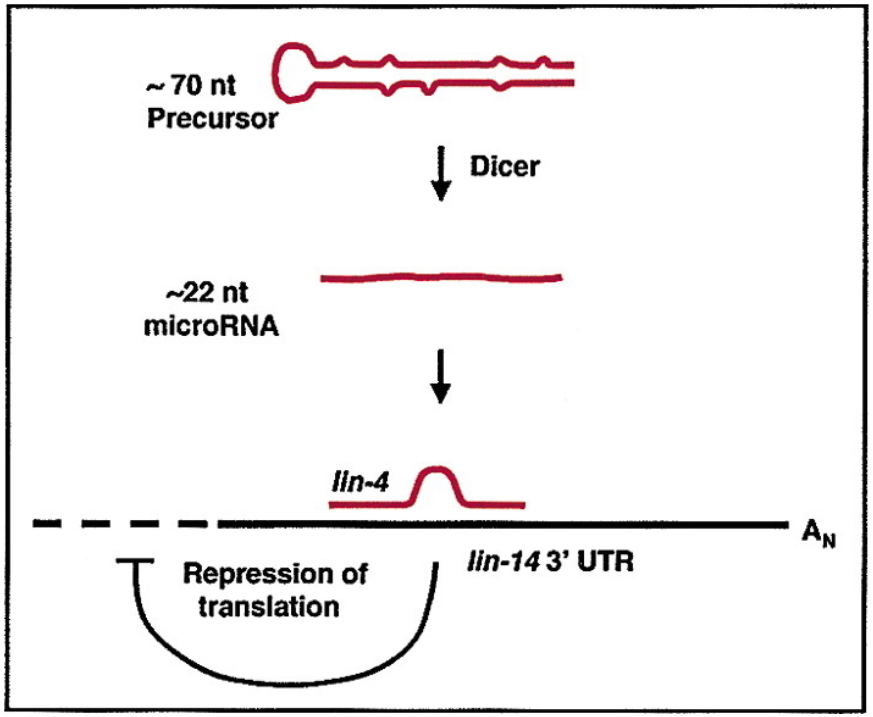


Alcuni geni downregolano l'espressione di geni target, importanti per lo sviluppo dell'individuo con meccanismi non ancora noti.

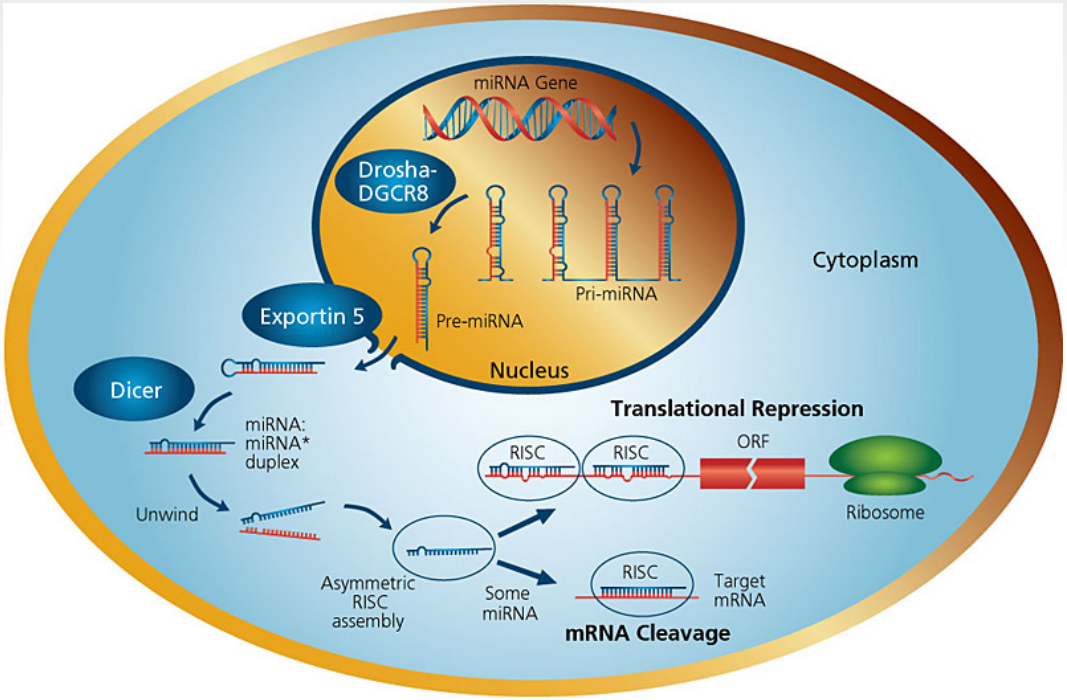


A model for the successive regulation of heterochronic gene activities by the *lin-4* and *let-7* RNAs. LIN-14 and LIN-28 expression levels are decreased by *lin-4* RNA expression at the end of the first larval stage to allow progression to late larval stages. In late larval stages, the expression of LIN-41 and other genes may be similarly downregulated by the *let-7* RNA, relieving their repression of LIN-29 protein expression and allowing progression to the adult stage. Because the *lin-29* mRNA does not contain sites complementary to the *let-7* RNA, *lin-29* is not likely to be a direct target of *let-7*.

stRNAs

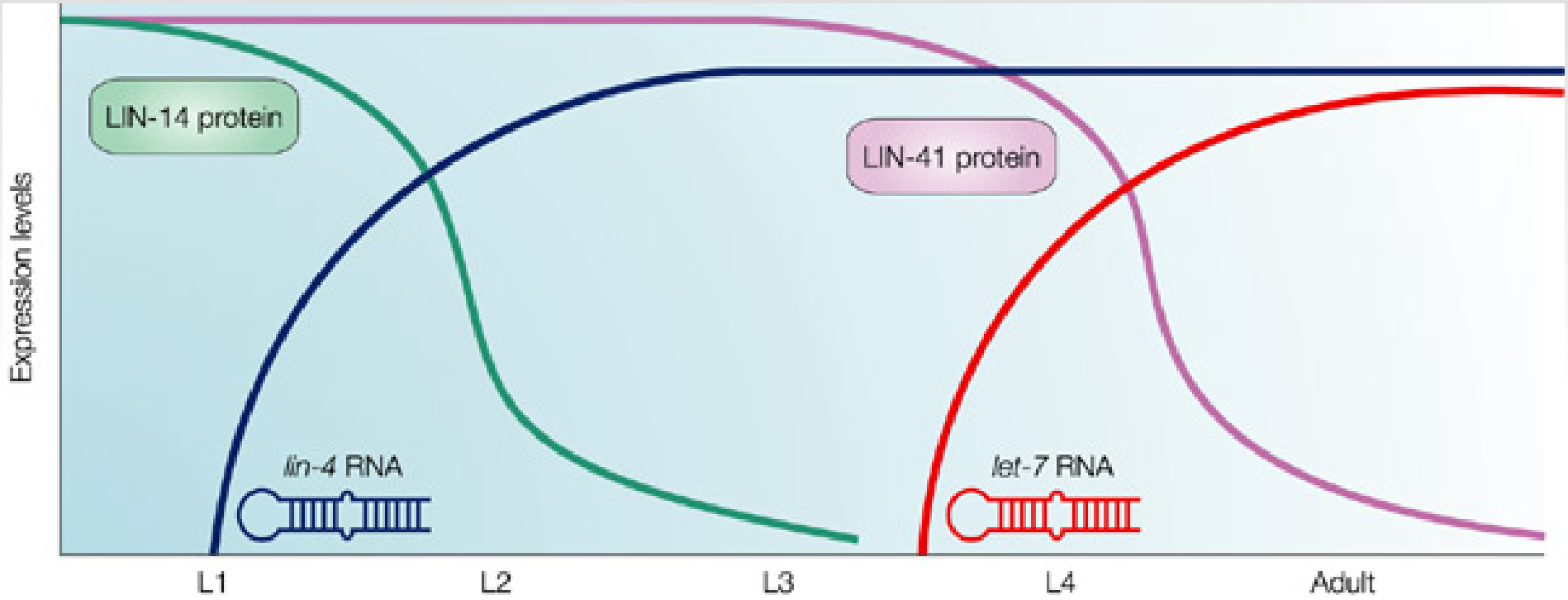

~70 nt
Precursor
Dicer
~22 nt
microRNA
lin-4
A_N
lin-14 3' UTR
Repression of
translation

Biogenesi dei miRna



- Trascrizione
- Modificazioni posttrascrizionali
- Processamento ad opera di Drosha
- Trasporto nel citoplasma ad opera dell'Esportina 5
- Taglio ad opera di Dicer

Regolazione temporale



Il miRNA lin-4 downregola il gene lin-14 a livello post-trascrizionale in fase L1.
Altri miRNA sono stati scoperti ricoprire la stessa funzioni in fasi successive dello sviluppo.