

The Cold Shock Domain  
Protein LIN-28 Controls  
Developmental Timing  
in *C. elegans*

And Regulated by the *lin-4* RNA

# Introduction

- Animal development consists of a complex schedule of **stage specific developmental events**
- The **heterochronic genes** regulatory hierarchy that developmental timing in
- A notable feature of the as developmental regula **specificity** of their action





o the heterochronic gene *lin-28*

o another heterochronic gene *lin-14*

o heterochronic gene *lin-4* :

*lin-4* encodes a 22 nt RNA through complementary elements in the 3'UTR of the *lin-14* mRNA

& negatively regulate *lin-14* protein accumulation

o the relationship between *lin-28* and *lin-14*

# Lin-28

- o *lin-28* Encodes a Protein with a Cold Shock Domain and Retroviral-Type Zinc Finger Motifs
- o *lin-28:GFP* Is Expressed in the Cytoplasm of Diverse Cell Types
- o expression pattern of *lin-28*

<i>lin-28</i>	5'	-UUG.CAC.....UCUCAGGGA-
<i>lin-14</i> -1		-UCA-UGCU.....CUCAG.GAA-
<i>lin-14</i> -2		-UCA.....-.CUCAG.GAA-
<i>lin-14</i> -3		-UCG-CAUUU...-.CUCAGGGAA-
<i>lin-14</i> -4		-UCA.U.....-.CUCAG.GAA-
<i>lin-14</i> -5		-UC..UAC.....CUCAGGGAA-
<i>lin-14</i> -6		-UU-.UGU......UCAG.GAA-
<i>lin-14</i> -7		-UCA.....-.CUCAGGG.A-
<i>lin-4S</i>	3'	AGU.GUGAACUCCAGAGUCCCUU



- *lin-28:GFP* Expression Decreases from Early to Late Postembryonic Development
- A posttranscriptional developmental regulation of *lin-28*
- Decreasing Expression of *lin-28:GFP* after the L1 Stage

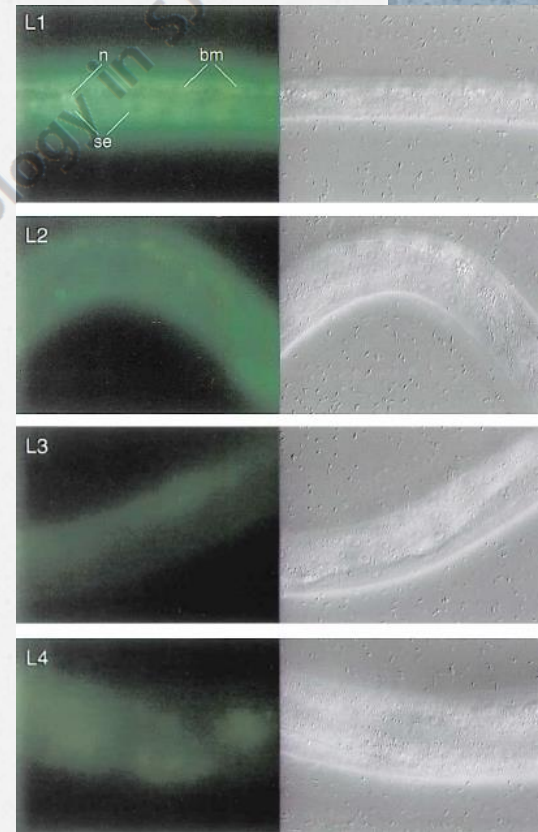


Table 1. Regulation of *lin-28* Expression by *lin-4* and *lin-14*

Strain	Genotype <sup>a</sup>	Percentage of Fluorescent L4 Animals <sup>b</sup>	Percentage of Adult Animals with Alae <sup>c</sup>
VT800	<i>lin-28:GFP</i>	0 (n = 105)	100 <sup>d</sup> (n = 16)
VT802	<i>lin-4(-); lin-28:GFP</i>	100 (n = 45)	ND <sup>e</sup>
VT803	<i>lin-28(gf):GFP<sup>f</sup></i>	98 (n = 44)	0 (n = 21)
VT805	<i>lin-4(-); lin-14(ts); lin-28:GFP</i>	4 <sup>g</sup> (n = 52)	30 <sup>g</sup> (n = 10)

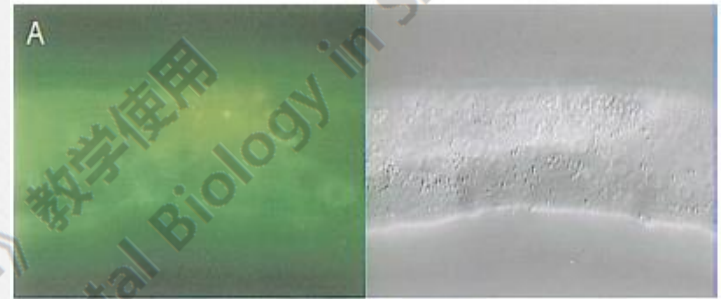
# Lin-4

o *lin-28:GFP* Is Regulated by *lin-4*

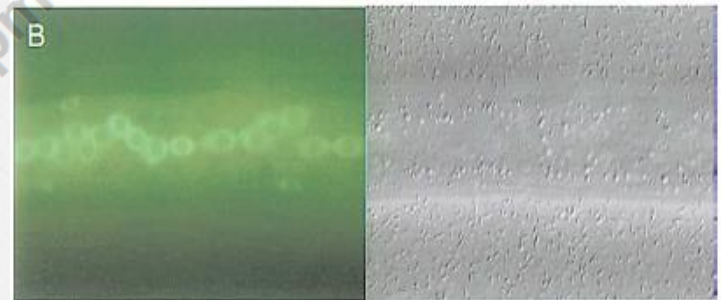
o The presence of an LCE suggested that *lin-28* expression is regulated by *lin-4*.

o The LCE Is Required for Regulation of *lin-28:GFP* Expression??

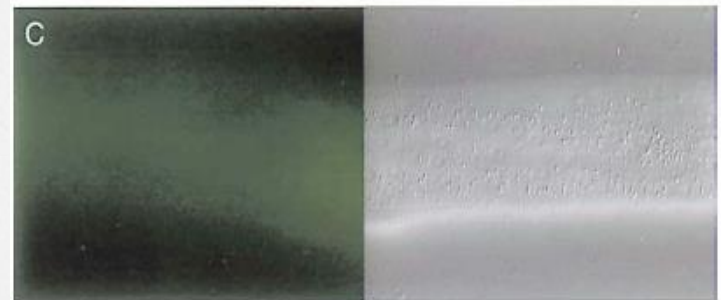
o *lin-4* Regulates *lin-28* Activity Independently of *lin-14* ??



*lin-4(0); lin-28:GFP*



*lin-28(gf):GFP*



*lin-4(0); lin-14(ts); lin-28:GFP*



## o *lin-14* Regulates *lin-28:GFP* Expression

Table 2. Regulation of Developmental Timing by *lin-4* Independent of *lin-14*

Strain	Temperature	Genotype <sup>a</sup>	Percentage of Adult Alae <sup>b</sup>	
			L3 Molt	L4 Molt
MT1388	20°C	<i>lin-28(+)</i> <i>lin-4(+)</i> <i>lin-14(gf,ts)</i>	0 (n = 93)	100 (n = 62)
VT785	20°C	<i>lin-28(+)</i> <i>lin-4(-)</i> <i>lin-14(gf,ts)</i>	0 (n = 270)	10 (n = 279)
VT799	20°C	<i>lin-28(-)</i> <i>lin-4(+)</i> <i>lin-14(gf,ts)</i>	100 (n = 81)	NA <sup>c</sup>
VT798	20°C	<i>lin-28(-)</i> <i>lin-4(-)</i> <i>lin-14(gf,ts)</i>	100 (n = 97)	NA <sup>c</sup>
MT1388	25°C	<i>lin-28(+)</i> <i>lin-4(+)</i> <i>lin-14(gf,ts)</i>	100 (n = 134)	
VT785	25°C	<i>lin-28(+)</i> <i>lin-4(-)</i> <i>lin-14(gf,ts)</i>	6 (n = 200)	
			L2/L3 Molts <sup>d</sup>	
VT799	25°C	<i>lin-28(-)</i> <i>lin-4(+)</i> <i>lin-14(gf,ts)</i>	97 (n = 67)	
VT798	25°C	<i>lin-28(-)</i> <i>lin-4(-)</i> <i>lin-14(gf,ts)</i>	100 (n = 140)	

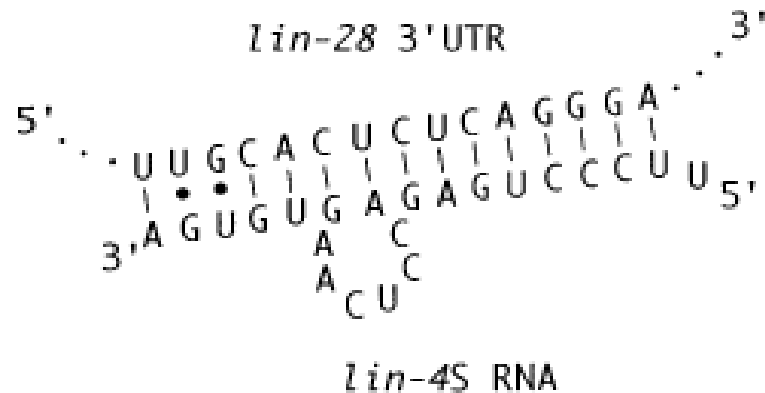
# Structure and Function of LIN-28 Protein

- o Because LIN-28 is primarily localized to the cytoplasm and consists of two domains that contain putative RNA-binding motifs, it is likely to function in posttranscriptional regulation
- o CSD and RRM proteins may interact with nucleic acids in similar ways
- o LIN-28 resembles other eukaryotic CSD proteins in that its N-terminal CSD is coupled to a C-terminal domain that is also implicated in RNA binding
- o The C-terminal region of LIN-28 has two zinc finger motifs



# Regulation of *lin-28* Expression by *lin-4* RNA

- *lin-4* RNA directly regulate the *lin-28*
- Other than the LCEs, no significant similarities between the 3'UTRs of *lin-28* and *lin-14*



# The *lin-28(gf)* Retarded Phenotype

- By deleting the LCE from the 3'UTR of *lin-28*, a *gain-of-function* allele of *lin-28* can cause a retarded phenotype.

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Only for Teaching of Cell and Developmental Biology in SJTU

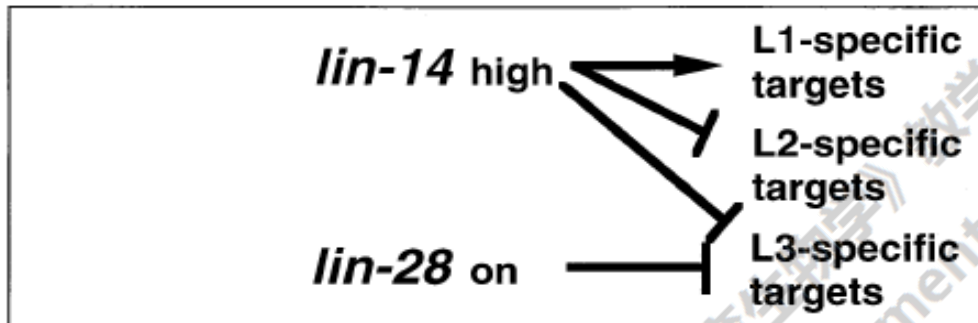


# The Heterochronic Gene Hierarchy

- *lin-28* functions as a developmental timing switch affecting the L2/L3 cell fate decision
- *lin-4* down-regulates both *lin-14* and *lin-28* and thereby controls two developmental timing switches in parallel
- *lin-14* and *lin-28* become mutually dependent for their expression at some time during or after the L1
- this mutual positive regulation between *lin-14* and *lin-28* may serve to coordinate the decrease of both of these critical gene activities and allow the proper succession of L1- to L2- to L3-specific cell fates

## o The Heterochronic Gene Hierarchy

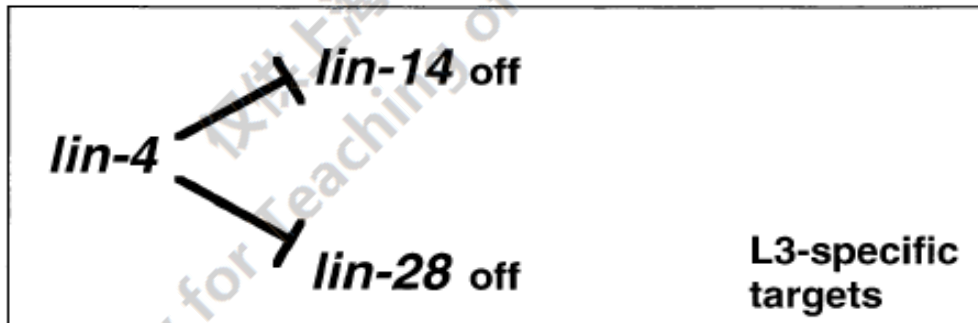
L1



L2



L3





# Acknowledge

Lei Duan

Yue Huang

Ze Liu

Xingyu Wang

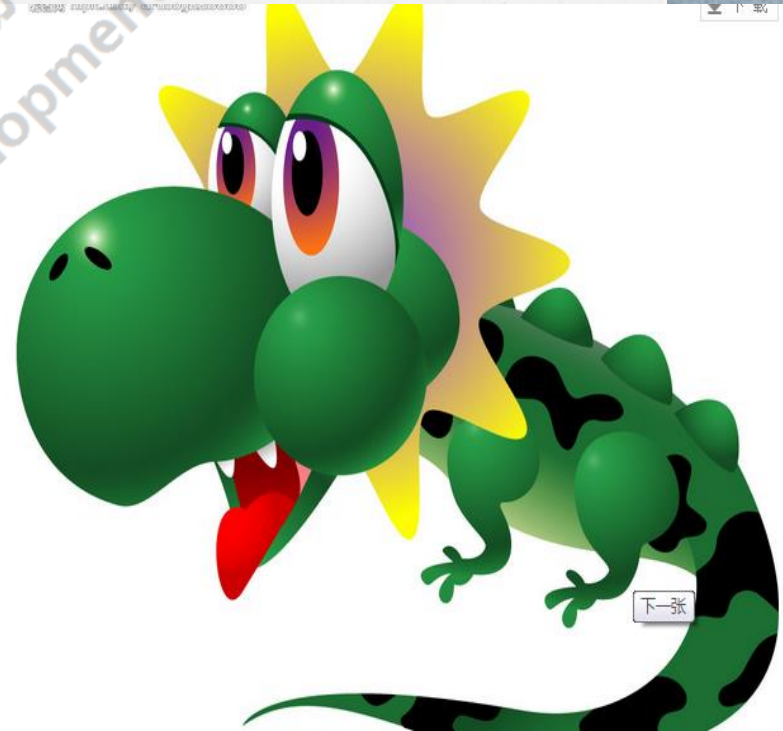
Huihui Chen

Lin Liu

Gong Zhao

Palash Chandra Mondol

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Thanks for attention

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