
# Molecular Biology (7)

## Measurement of transcriptional activity

the amount of RNA give you an indecation about amount of protein

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# Resources

- This lecture
- Cooper, Chapter 8

# How can we measure RNA levels and site of expression?

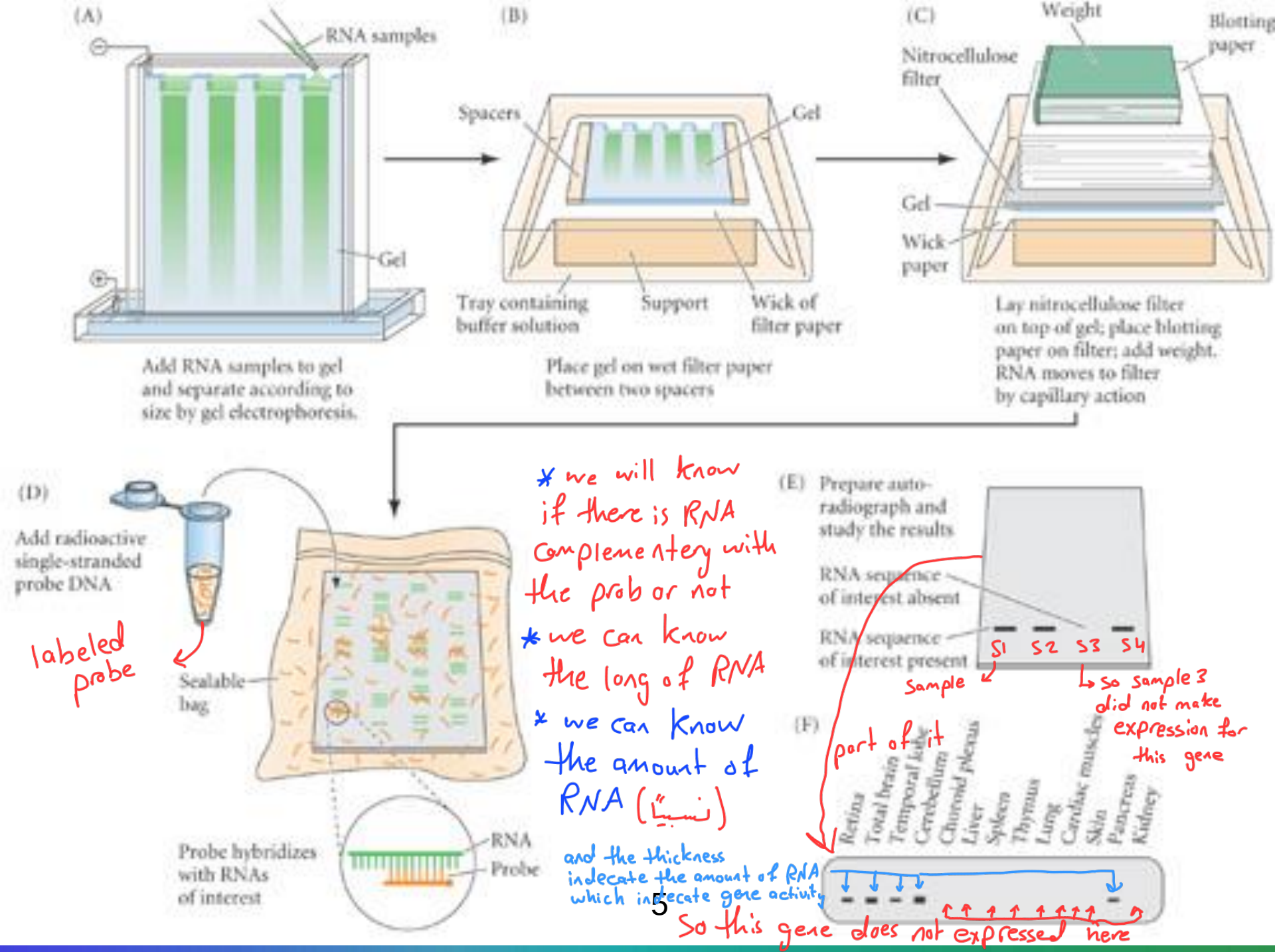
*Basic methods: Northern blotting, in situ hybridization*

↳ but now they used new techniques

# Northern blotting

Same concept of Southern Blotting but the biggining will be from RNA

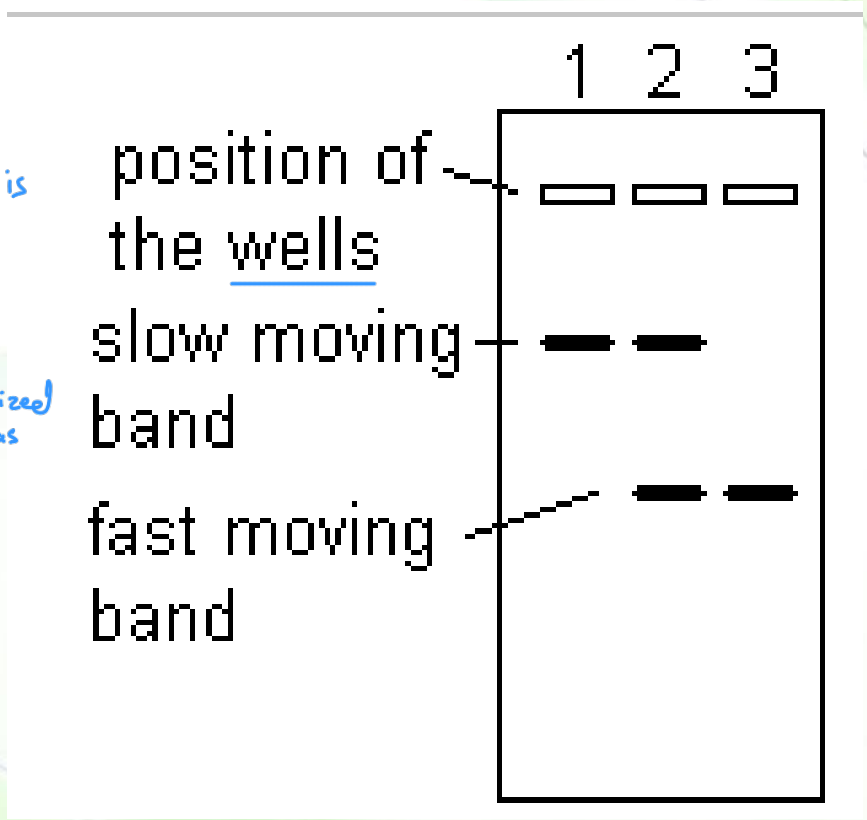
- This is done exactly like Southern blotting except that:
  - RNA from cells is isolated instead of DNA.
  - RNA molecules are fractionated based on size by gel electrophoresis. (but we have large amount of DNA from (24 bp → thousands bp) So in electrophoresis they will not be shown as bands but as سطح ممسوح)
  - The fractionated RNA molecules are transferred onto a membrane.
  - RNA molecules are targeted by a labeled DNA probe with sequence that is complementary to a specific RNA molecule. So we can have A-U pairing.
- What information can you deduce from it?


(A)
RNA samples
Gel
Add RNA samples to gel and separate according to size by gel electrophoresis.
(B)
Spacers
Gel
Tray containing buffer solution
Support
Wick of filter paper
Place gel on wet filter paper between two spacers
(C)
Weight
Blotting paper
Nitrocellulose filter
Gel
Wick paper
Lay nitrocellulose filter on top of gel; place blotting paper on filter; add weight. RNA moves to filter by capillary action
(D)
Add radioactive single-stranded probe DNA
labeled probe
Sealable bag
Probe hybridizes with RNAs of interest
RNA
Probe
(E) Prepare auto-radiograph and study the results
RNA sequence of interest absent
RNA sequence of interest present
S1 S2 S3 S4
Sample
So sample 3 did not make expression for this gene
(F)
part of it
Retina
Total brain
Temporal lobe
Cerebellum
Choroid plexus
Liver
Spleen
Thymus
Lung
Cardiac muscle
Skin
Pancreas
Kidney
So this gene does not expressed here
* we will know if there is RNA complementary with the prob or not
* we can know the long of RNA
* we can know the amount of RNA (نسبياً)
and the thickness indecate the amount of RNA which indecate gene activity

# What are your interpretations?

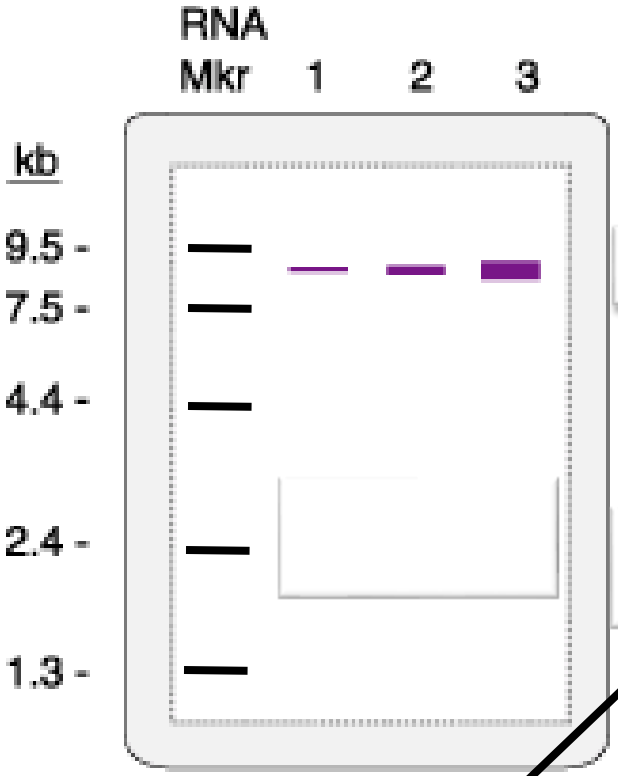
why the tall of genes is different ?

① alternative splicing
② prob is not specific so the prob is also hybridized with another gene which has hemologus sequence part.




* the thickness is same so gene expression is the same (same amount of DNA each band)

# What are your interpretations?




Gene of interest

* is there any expression of gene in sample 1, 2, 3 ?
yes

* How long the mRNA ?
9 kb = 4.5 k bp

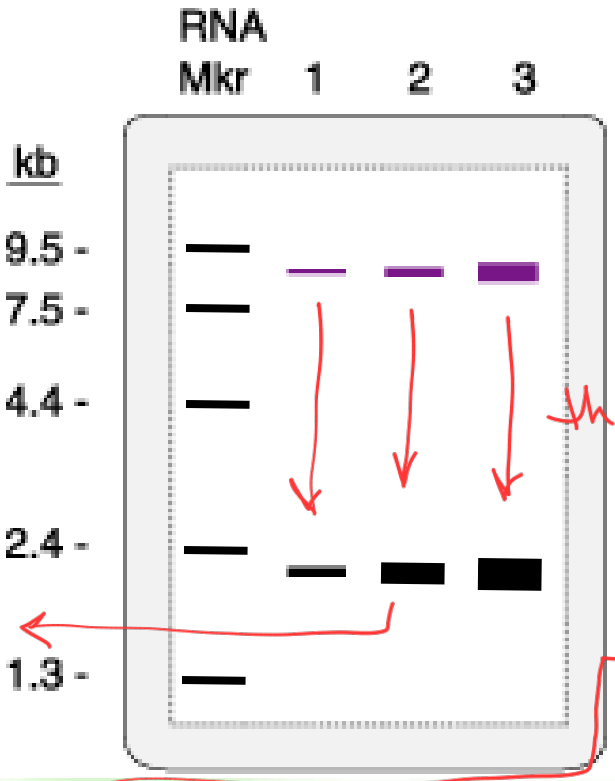
* what is the most active sample ? (more mRNA)
not Sample 3 (explination in the next slide)

A gene with constant expression
(examples: actin, tubulin)

# What are your interpretations?

* we use prob for housekeeping mRNA to ensure that the amount of mRNA in the wells is same (to know a real result about thickness)

* So we have a technical error




Gene of interest

that make sense

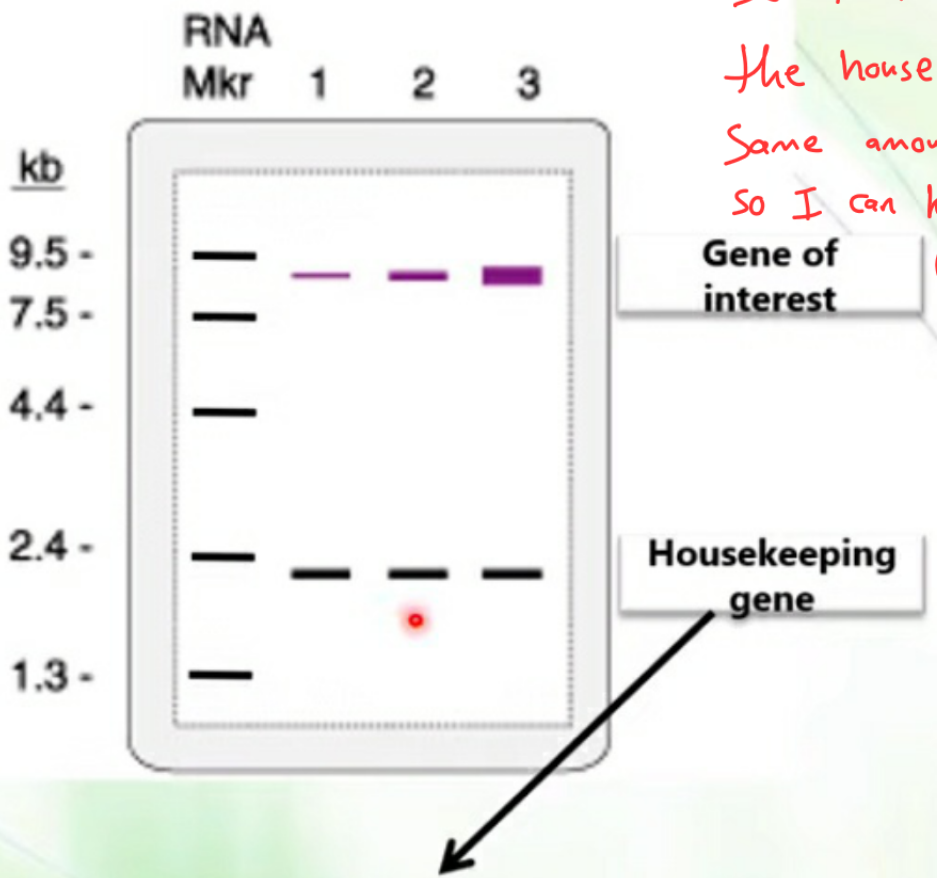
Housekeeping gene

↳ gene is needed in all cell (to to be expressioned) and the expression is not affected by cell factors (so it always be expressioned)

oops the housekeeping mRNA is not same so I know that the amount of RNA is not the same so the thickness of the Bands is no longer indicate the amount.

A gene with constant expression (examples: actin, tubulin) and Histons and GAP

# What are your interpretations?




So this is another sample the housekeeping RNA is the same amount in all samples so I can know the amount of RNA upon the thickness

A gene with constant expression
(examples: actin, tubulin)

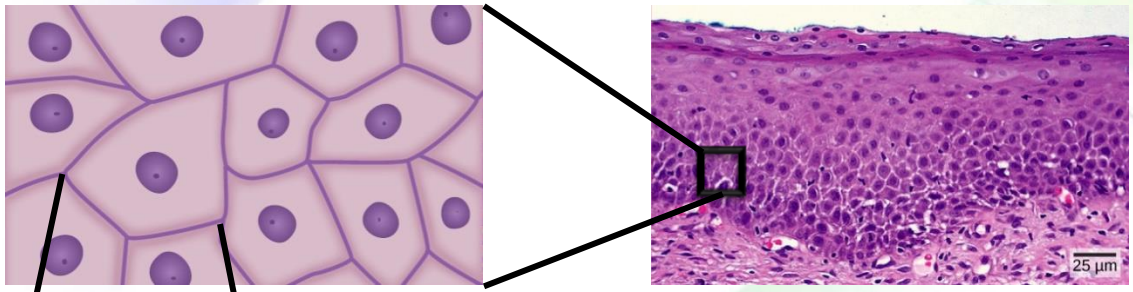
# In situ hybridization

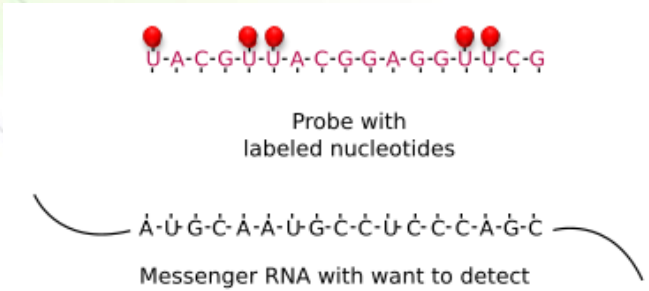
in place. So I see the hybridization of two molecule in place which third molecule exist which I want

- In situ hybridization methods reveals the distribution of specific RNA molecules in cells in tissues.
- RNA molecules can hybridize when the tissue is incubated with a complementary DNA or RNA probe.
  and the sample is tissue section
- In this way the patterns of differential gene expression can be observed in tissues, and the location of specific RNAs can be determined in cells.

give me three pieces of information ① is there gene expression ② how much expression is done upon the intensity ③ where the expression happened. (which tissue)

# Procedure of in situ hybridization

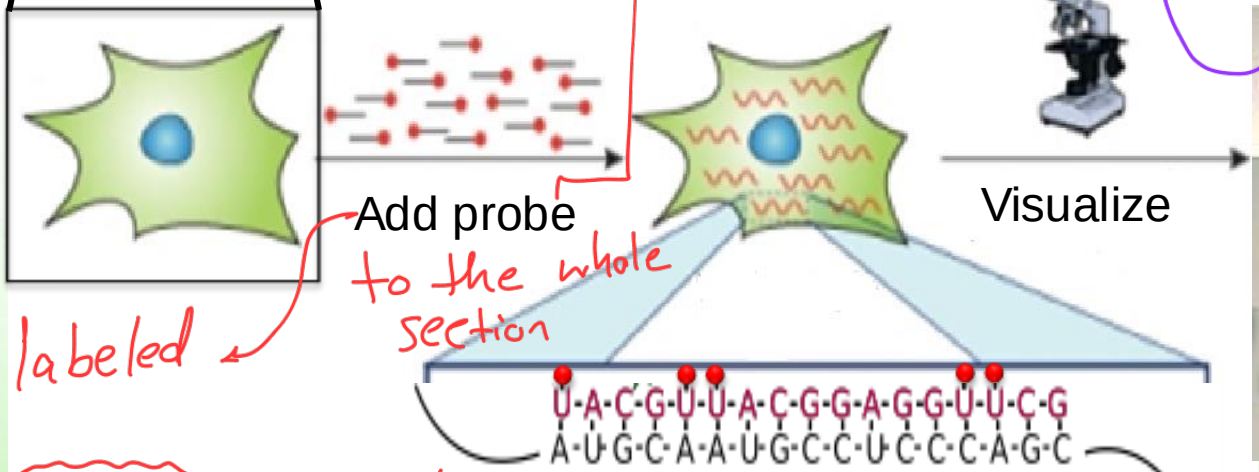



which will bind to the RNA in cells (but why not DNA ? because we degredaded it)

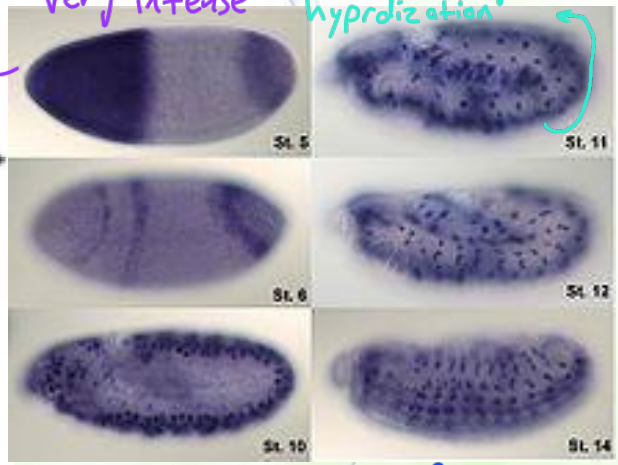
the probe hybridize here and very intense

another gene hyprdization




labeled

to the whole section



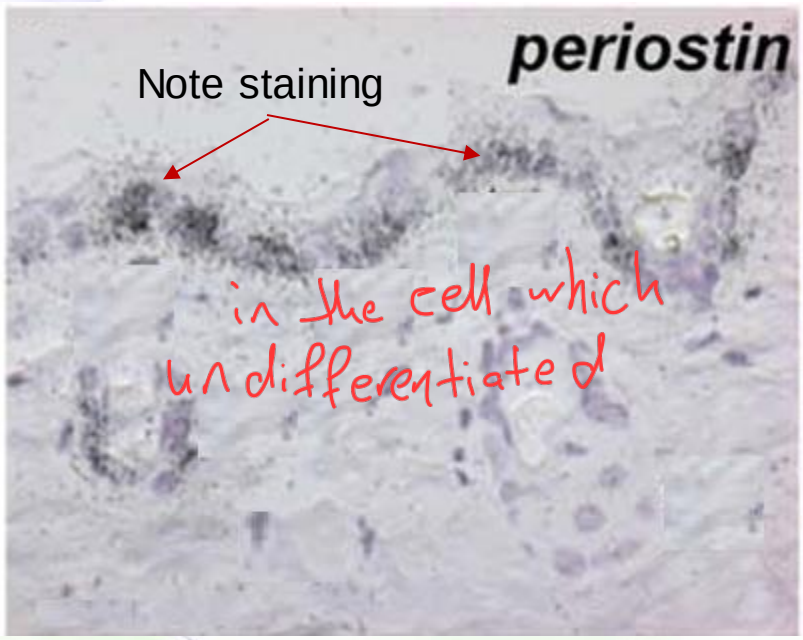

So I know if specific gene was expressioned and where.

important

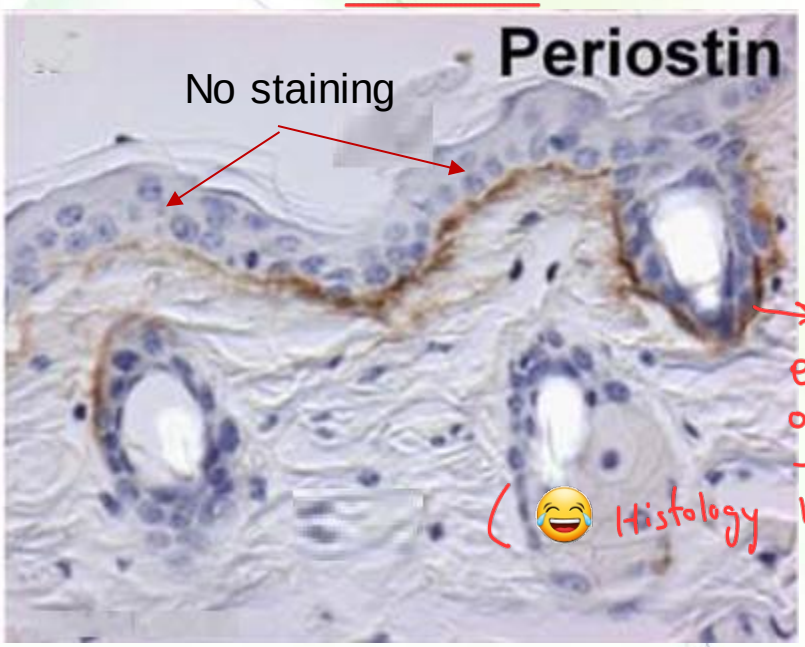
there is difference in Situ hybridization and flurosense in situ hybridization (FISH) → for chromosomes but in situ → RNA

immunehisto chemistry

## ISH (RNA)




## IHC (protein)




**RNA and protein molecules do not coexist and are present in different places.**

***mRNA: inside cells along the basement membrane***

***Protein: outside cells in the basement membrane***