15

Molecular Biology

Doctor 2019 | Medicine | JU

◉ Sheet ○ Slides

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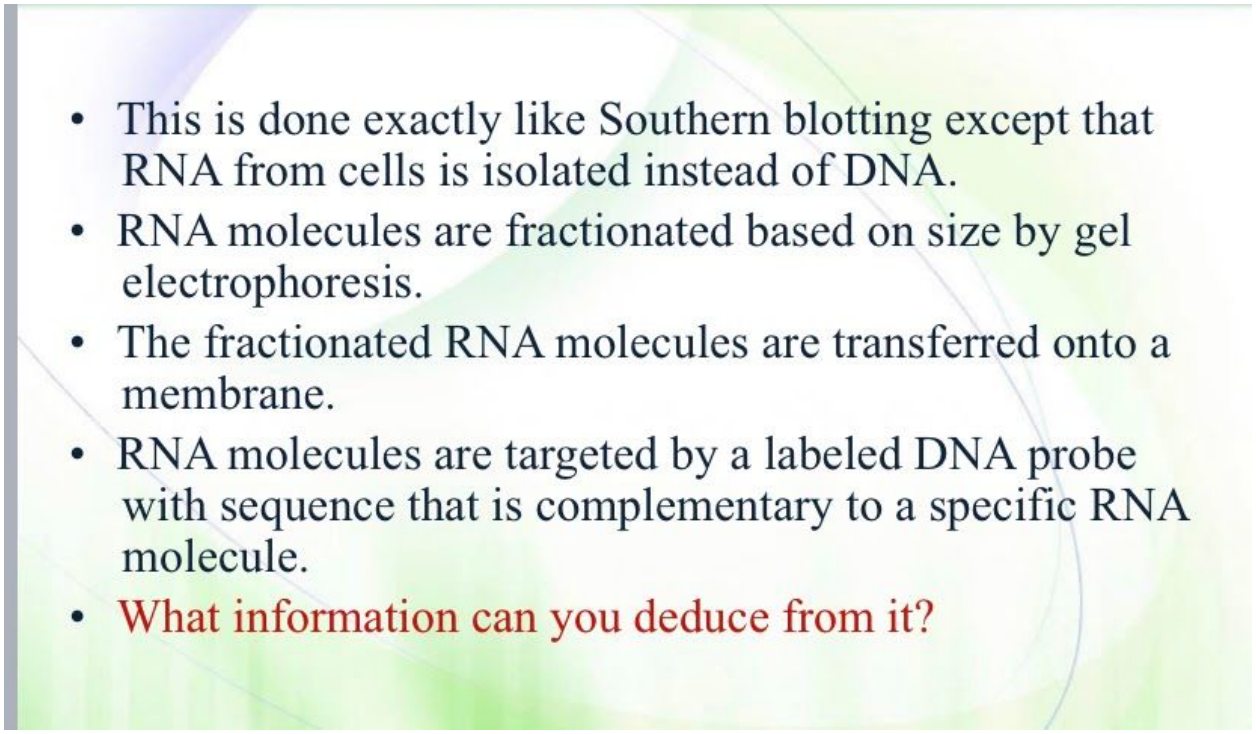
Transcription regulation 3

Why do we want to know the level of RNA ?
Because that tells us something about how active the gene is in terms of expression

For example
Lac operon .. we know that in the presence glucose , there is little expression of the lac operon
However , when glucose is removed , expression of lac operon increases so that the amount of RNA present in bacterial cells is very high

Northern blotting



** the same main idea of southern blotting
** Used to identify - relatively - how much DNA is in biological sample (bacterial cells / human cells)

** Mechanism :
1/ Opening up the sample (cells) releasing all RNA molecules
Note : there are several techniques by which RNA is isolated (not mixed with other biological macromolecules like proteins / lipids /...)

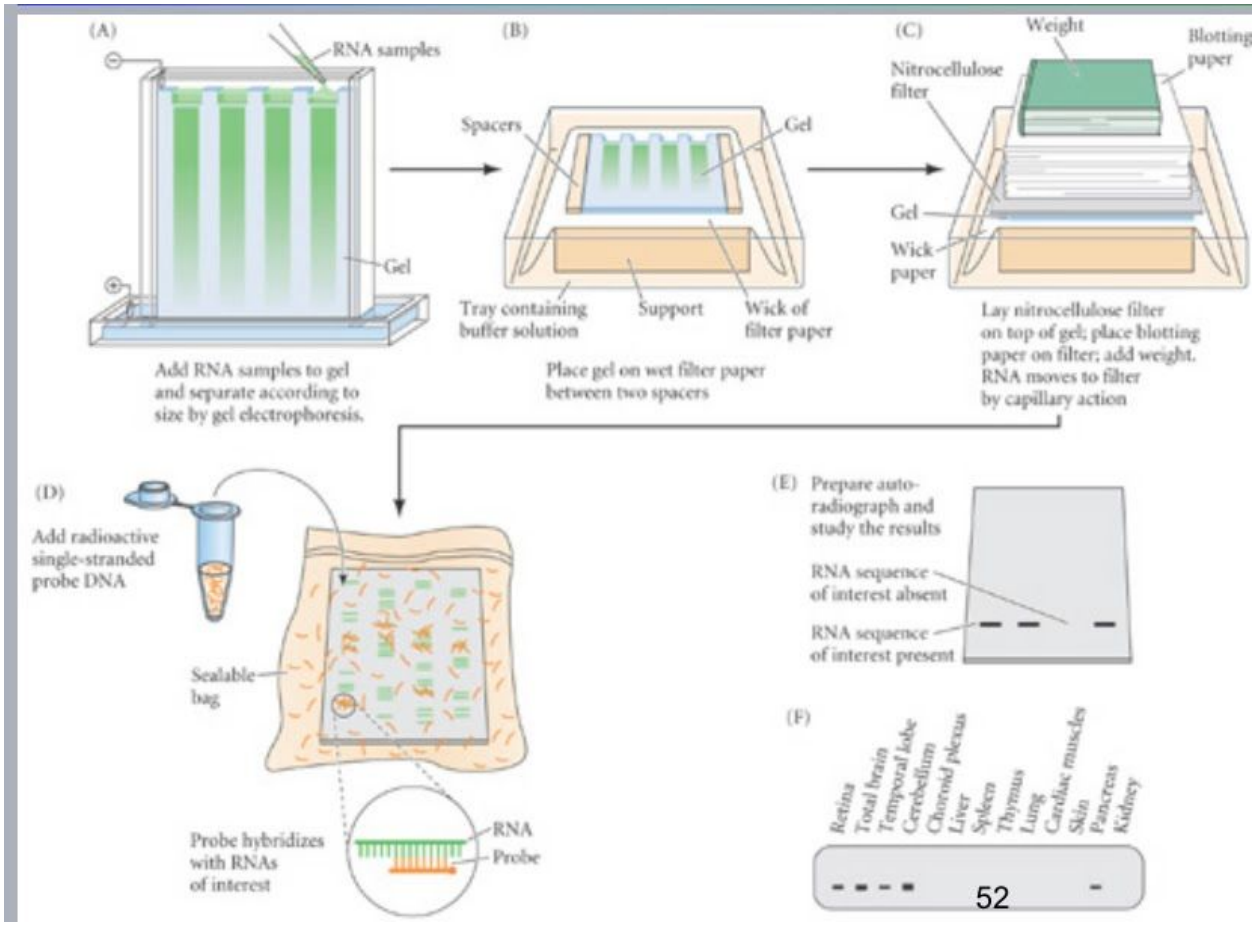
2/ Samples are placed in the wells of the gell
(samples runs from the negative end to the positive end)
In the top we have large RNA molecules while small RNA molecules are found in the bottom as they migrate fast relative to larger RNA molecules
Note : RNA bands can't be distinguished individually because we have a lot of RNA molecules of different sizes

3/ The RNA is transferred to a thin membrane (nitrocellulose filter / same as nylon paper in southern blotting) forming a replica

4/ Probes are added (DNA probes)
Radioactively / fluorescently labeled

Note : if a certain sequence of RNA is complementary to the probe they hybridize (hybrid as we are talking about the binding between DNA & mRNA)
And this hybridization happens normally in the cell when transcription takes place

5/ the membrane is exposed to the x-ray film (bands bound to the probes are detected)



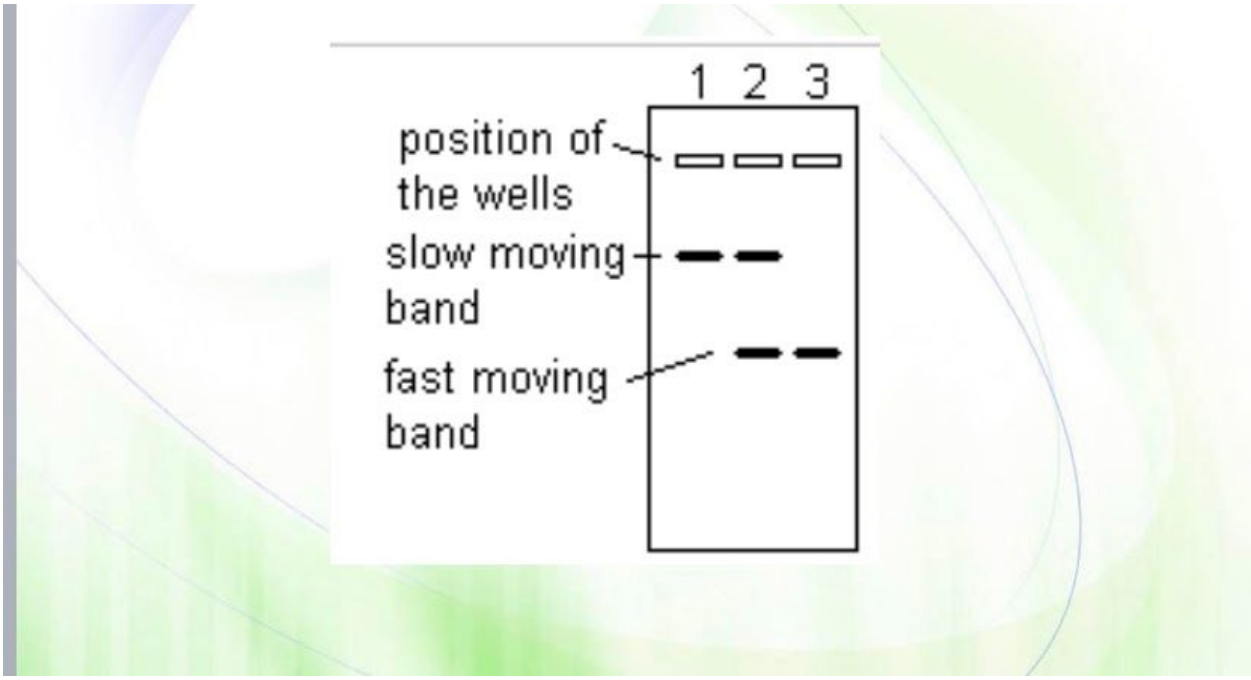
What do we know at the end of this whole process (specifically in this example) ?

[A] In 1, 2, 4 , the gene which produces the mRNA which binds to the specific probe is expressed
On the other side , and as no signal from 3 is detected , this particular gene isn't expressed
[B] We know the sizes of the mRNA molecules
[C] How much RNA is there in the sample ? How much a gene is expressed ?
The thickness / intensity of the band
The thicker the band (more intense) the more the gene is expressed

Note : what we see in northern blotting is exons (mature mRNA) while we don't see introns (pre-mRNA) as processing of mRNA happens very quickly

What are your interpretations ?

Part 1



After the addition of the same probe which binds to the sequence , why are such differences present (binding of the same probe to mRNA molecules of different lengths) ?

[1] Dealing with non specific probes

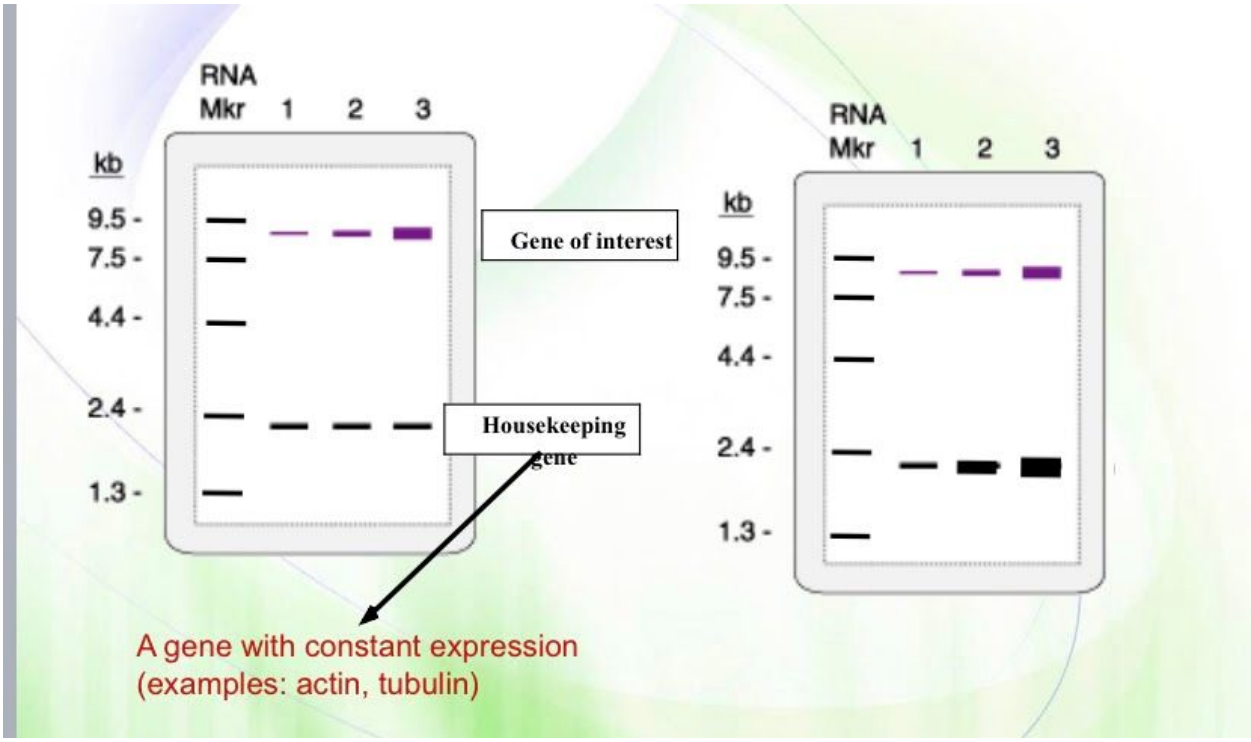
These bind to one mRNA molecule in one sample and to two mRNA molecules in other sample so one could be the real / wanted mRNA molecule (indicates the expression of the gene of interest) and the other represents an artifact because of non specific hybridization

[2] alternative splicing of mRNA
The same gene is expressed but the pre-mRNA is spliced differently (producing two different mRNA molecules of different lengths both have a complementary sequence to the probe)
Note :Greater length of the band indicates including more exons and vice versa

[3] Expression of isoforms , different genes but they do share the similar sequences of the probe
E.g : histones
What is the difference between H2A & B?
Hardly anything ... there are differences but they are homologous (they are highly similar in sequence (DNA/ mRNA))
So if we use a probe for H2A it also may hybridize to the probe H2B because they have highly similar sequences .

What are your interpretations ?

Part 2



As we notice

mRNA band is thicker in 3 compared to 2 & 1 which means transcription activity varies in these three samples

E.g:

Let's suppose we're talking about lac operon

In sample 1 we have high concentration of glucose

Expression is inhibited therefore fewer amounts of mRNA are produced

In sample 2 we have lower amounts of glucose and great amounts of lactose

Greater gene expression consequently greater amounts of mRNA are produced

In sample 3 no glucose is present in the cell and we have great amounts of lactose

Greater gene expression consequently greater amounts of mRNA are produced

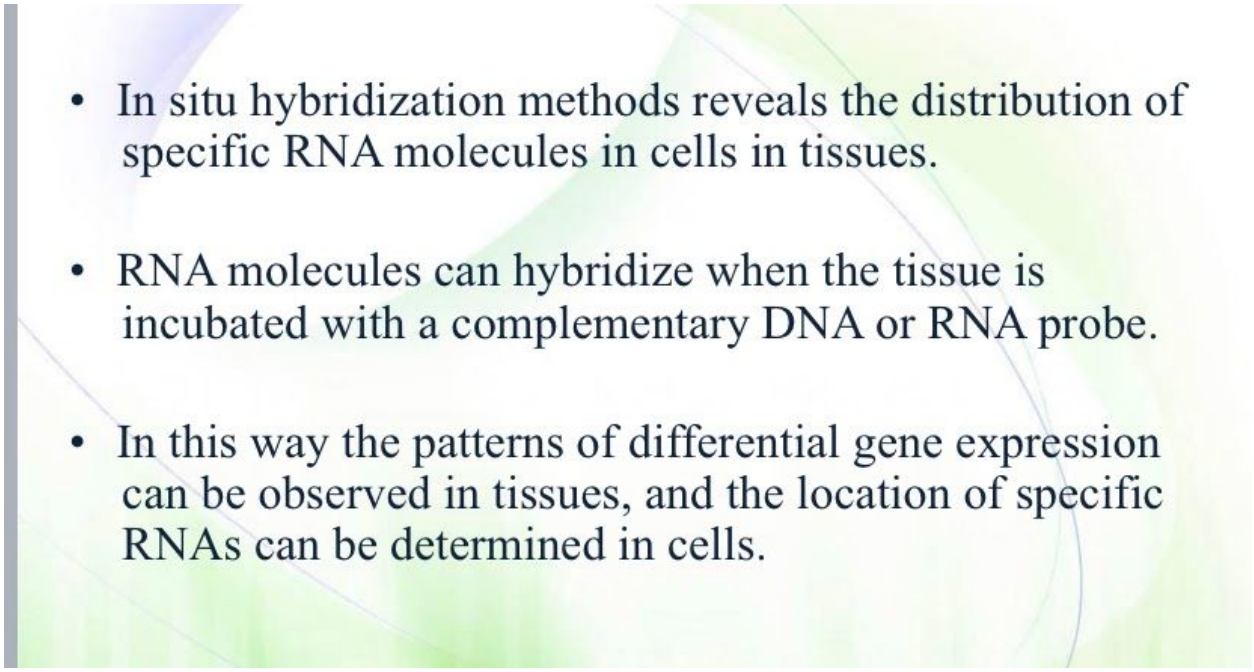
***But how can we make sure that the differences in the mRNA bands thickness is due to the difference of expression activity and not due to the total concentration of mRNA molecules for each sample ?

Using housekeeping genes
Which aren't affected by surrounding cellular conditions
(constant expression)
E.g : actin / tubulin

When the thickness of the housekeeping gene band is the same in the three samples then the difference in the thickness of the other bands is due to the difference of expression activity

When the thickness of this band in the three samples isn't the same then the difference in the other bands represents an artifact and is due to the difference of the total concentration of mRNA molecules placed in each well

In situ hybridization (FISH)



In situ > in position / in place

So in this technique we aim to know where expression takes place

Tissues are made of many types of cells (stem cells / epithelial cells / fibroblasts / adipocytes /cancer cells / ....)

So in a tissue section , we aim to know not only how much mRNA is expressed in a tissue section but also where it is expressed

*** Mechanism :

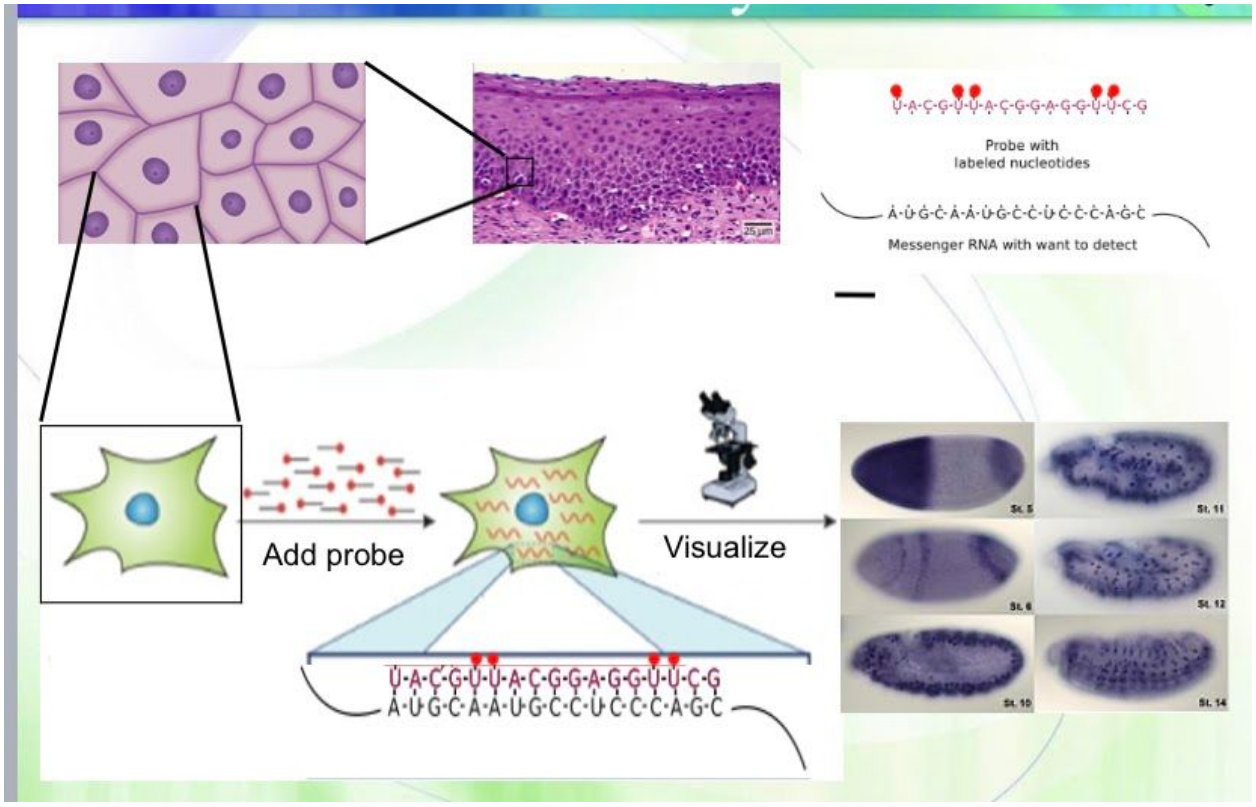
We add a labeled probe complementary to a specific sequence of nucleotides in the mRNA we're interested in، to the whole tissue section

The probe hybridizes with the sequence of interest

Depending on the intensity of the signal , the amount of mRNA molecules is determined

And

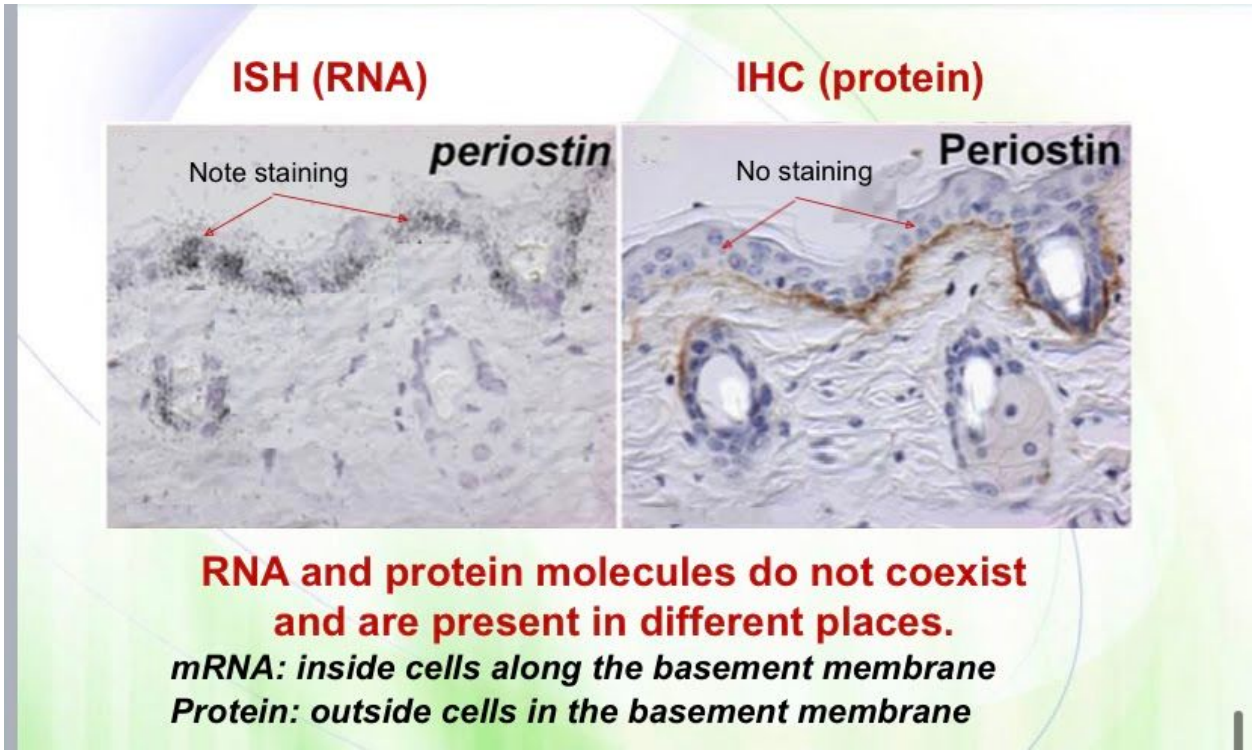
If no signal is detected from a specific area (made of specific type of cells) this means the gene responsible for the formation of the mRNA we're not interested in isn't expressed and by this we know what type of cells express this gene (where the gene is expressed)



E.g: Drosophila

A pattern of expression is shown because it's important for the development of organisms in addition to achieving polarity & symmetry

Due to the variation of gene expression between cells , these cells differentiate into different types (skin / bond / neurons) and therefore forming different body organs and parts.



Immunohistochemistry : a technique that we use to look where a protein is expressed in a tissue section using antibodies .

Using in situ hybridization we can visualize mRNA molecules to notice that they are located intracellularly along basement membrane

Using IHC we can visualize proteins translated from these mRNA molecules located extracellularly in the basement membrane

We wish you all the best of luck