Molecular Biology (7)

Measurement of transcriptional activity

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Resources

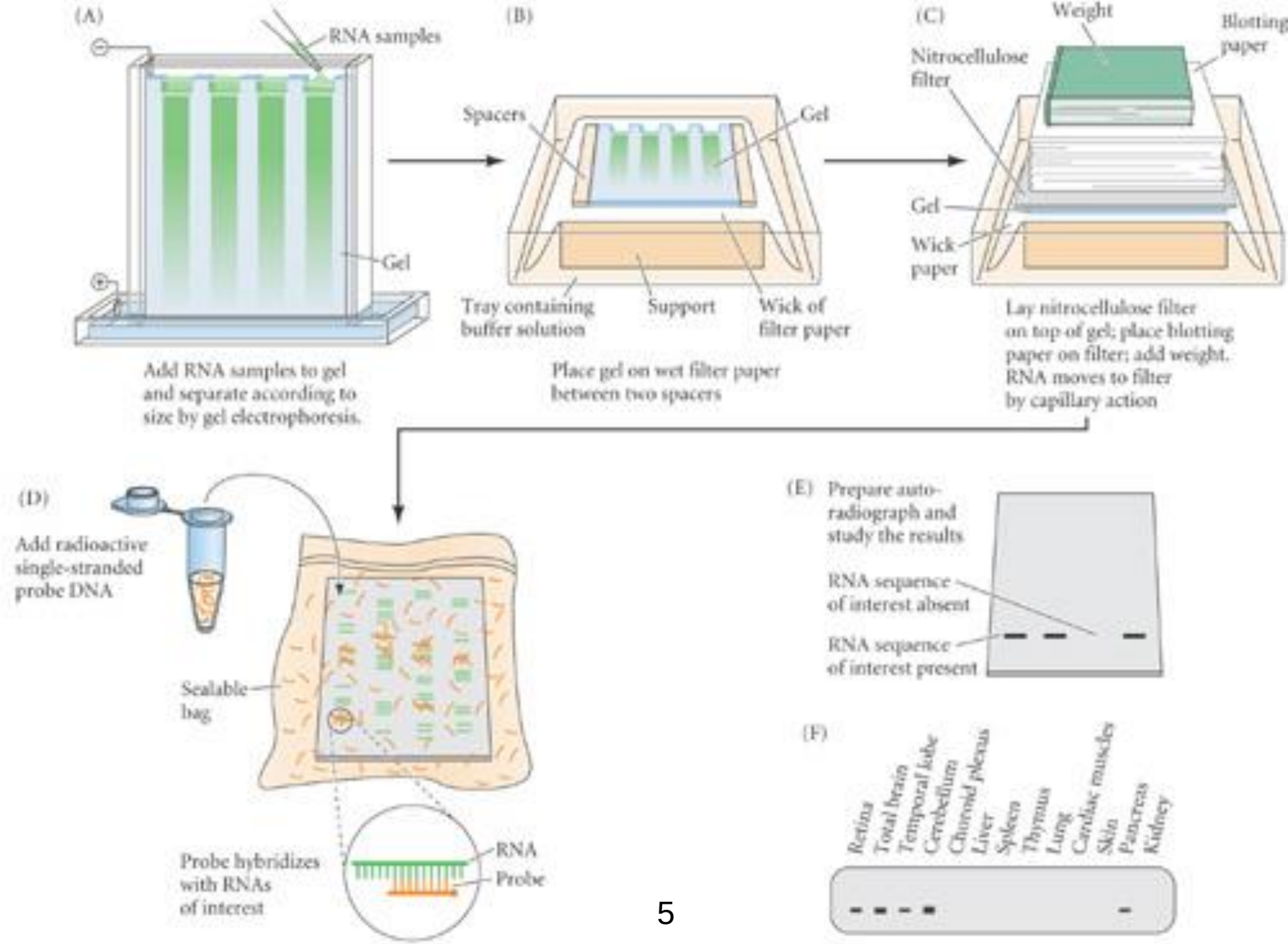
- This lecture
- Cooper, Chapter 8

How can we measure RNA levels and site of expression?

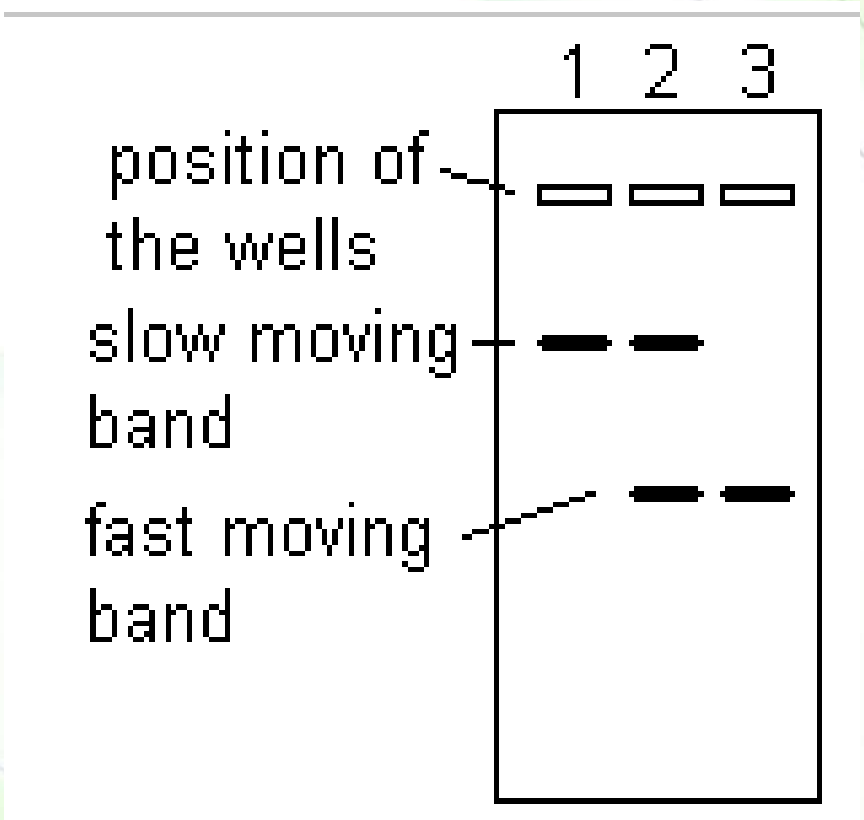
Basic methods: Northern blotting, in situ hybridization

Northern blotting

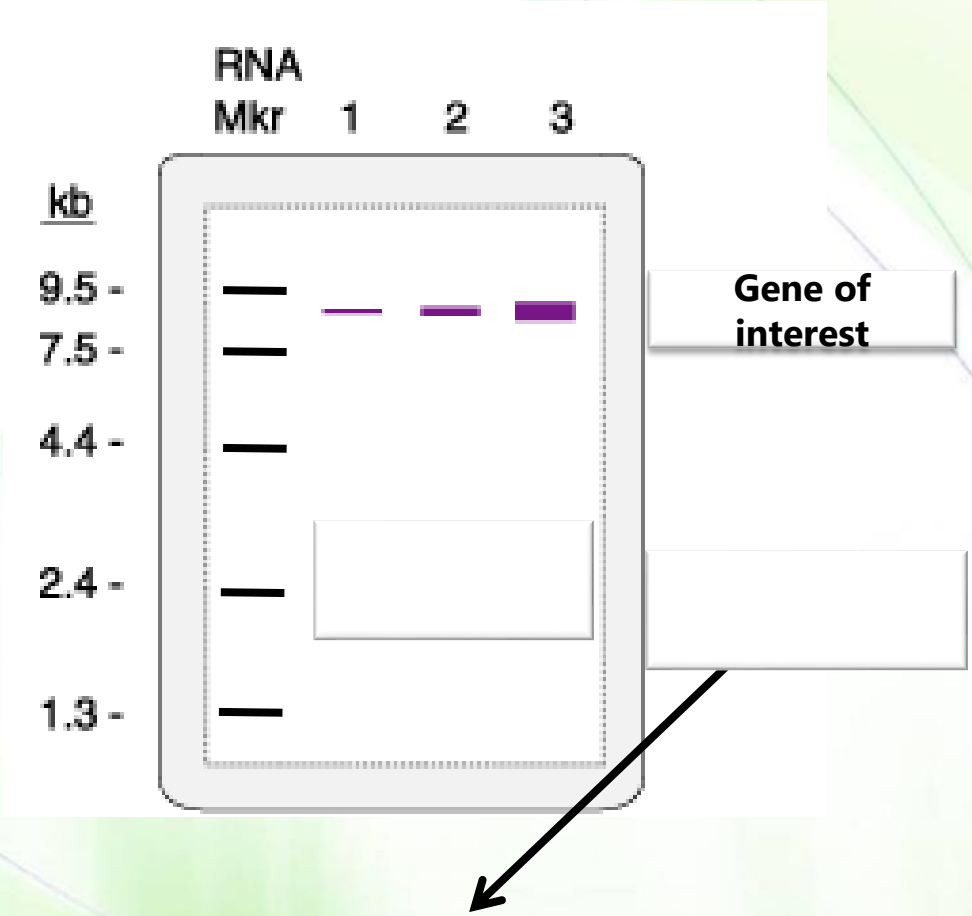
- This is done exactly like Southern blotting except that:
  - RNA from cells is isolated instead of DNA.
  - RNA molecules are fractionated based on size by gel electrophoresis.
  - The fractionated RNA molecules are transferred onto a membrane.
  - RNA molecules are targeted by a labeled DNA probe with sequence that is complementary to a specific RNA molecule.
- 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
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
(F)
Retina
Total brain
Temporal lobe
Cerebellum
Choroid plexus
Liver
Spleen
Thymus
Lung
Cardiac muscles
Skin
Pancreas
Kidney

What are your interpretations?

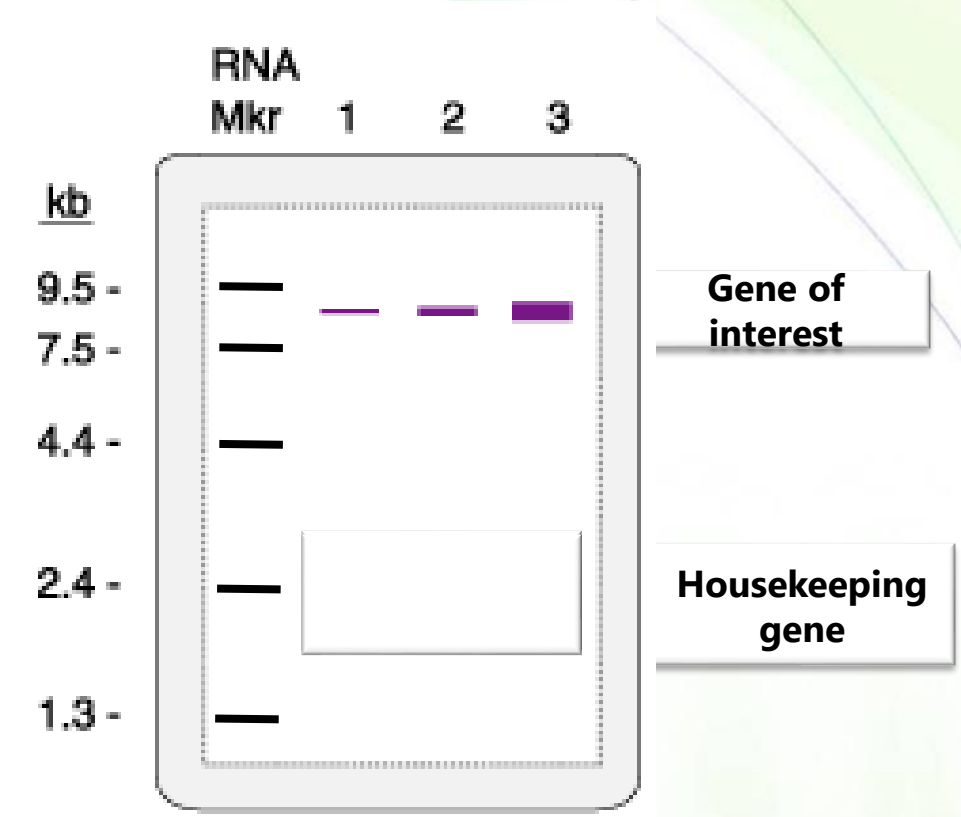


What are your interpretations?



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

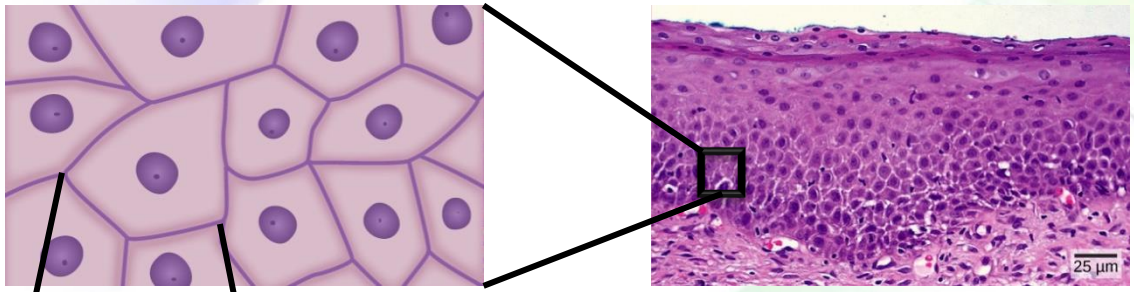
What are your interpretations?

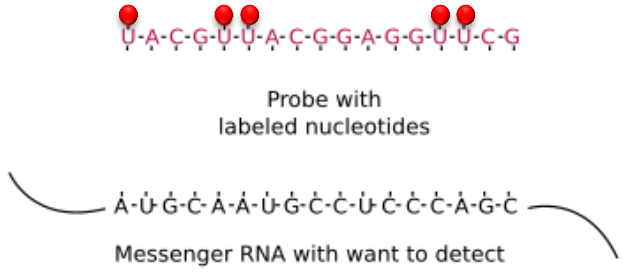


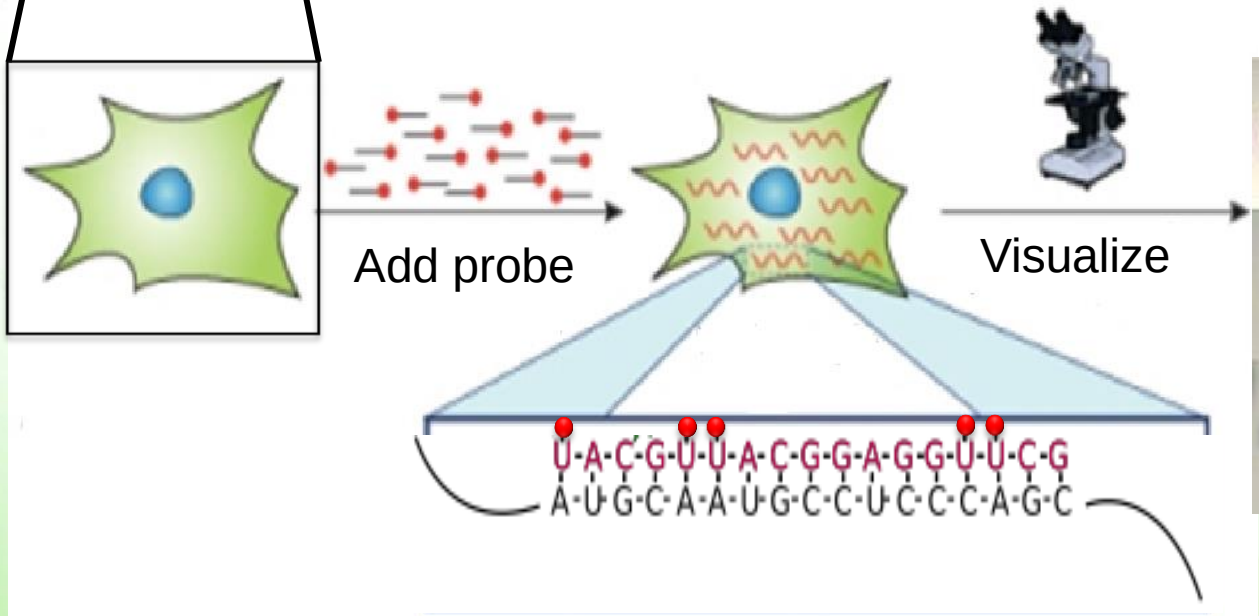
In situ hybridization

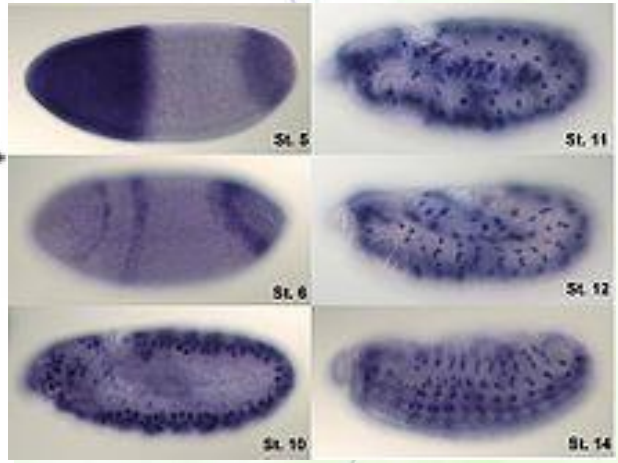
- In situ hybridization methods reveals the distribution of specific RNA molecules in cells in tissues.
- RNA molecules can hybridize when the tissue is incubated with a complementary DNA or RNA probe.
- In this way the patterns of differential gene expression can be observed in tissues, and the location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

Procedure of in situ hybridization

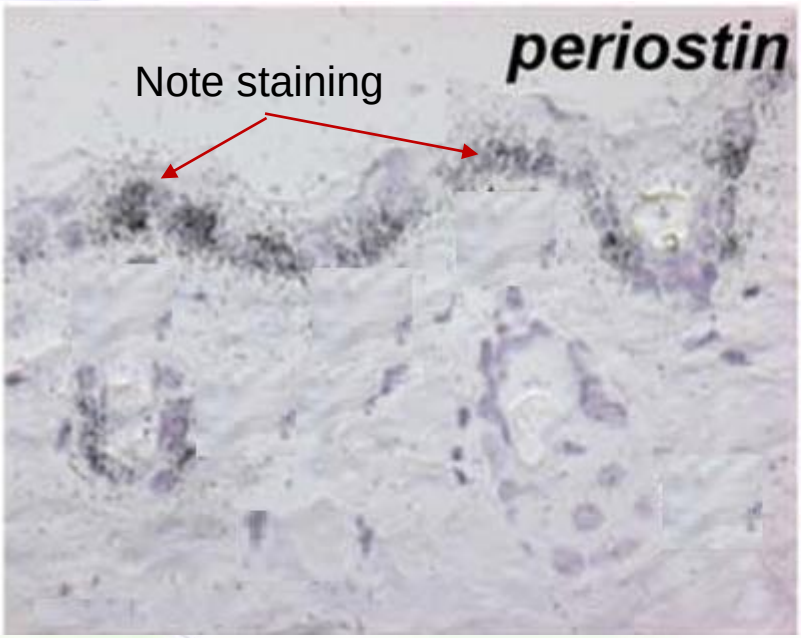








ISH (RNA)



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