Interaktionen und Modifikationen von RNAs und Proteinen

(Modul 10-202-2208; Spezialvorlesung)

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Central Dogma of Gene Expression

$$\text{DNA} \rightarrow \text{RNA} \rightarrow \text{protein}$$

- only about 2% of human DNA is protein coding
  about 80% is transcribed

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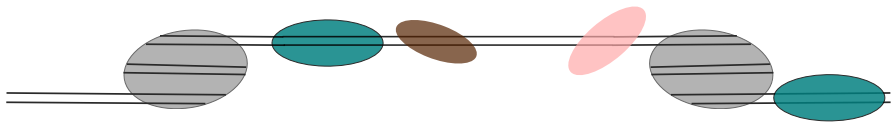
With growing complexity of organisms, so grows the complexity of regulatory mechanisms

Gene Expression Regulation Simple

Transcriptional control

Histones, DNA and DNA binding proteins



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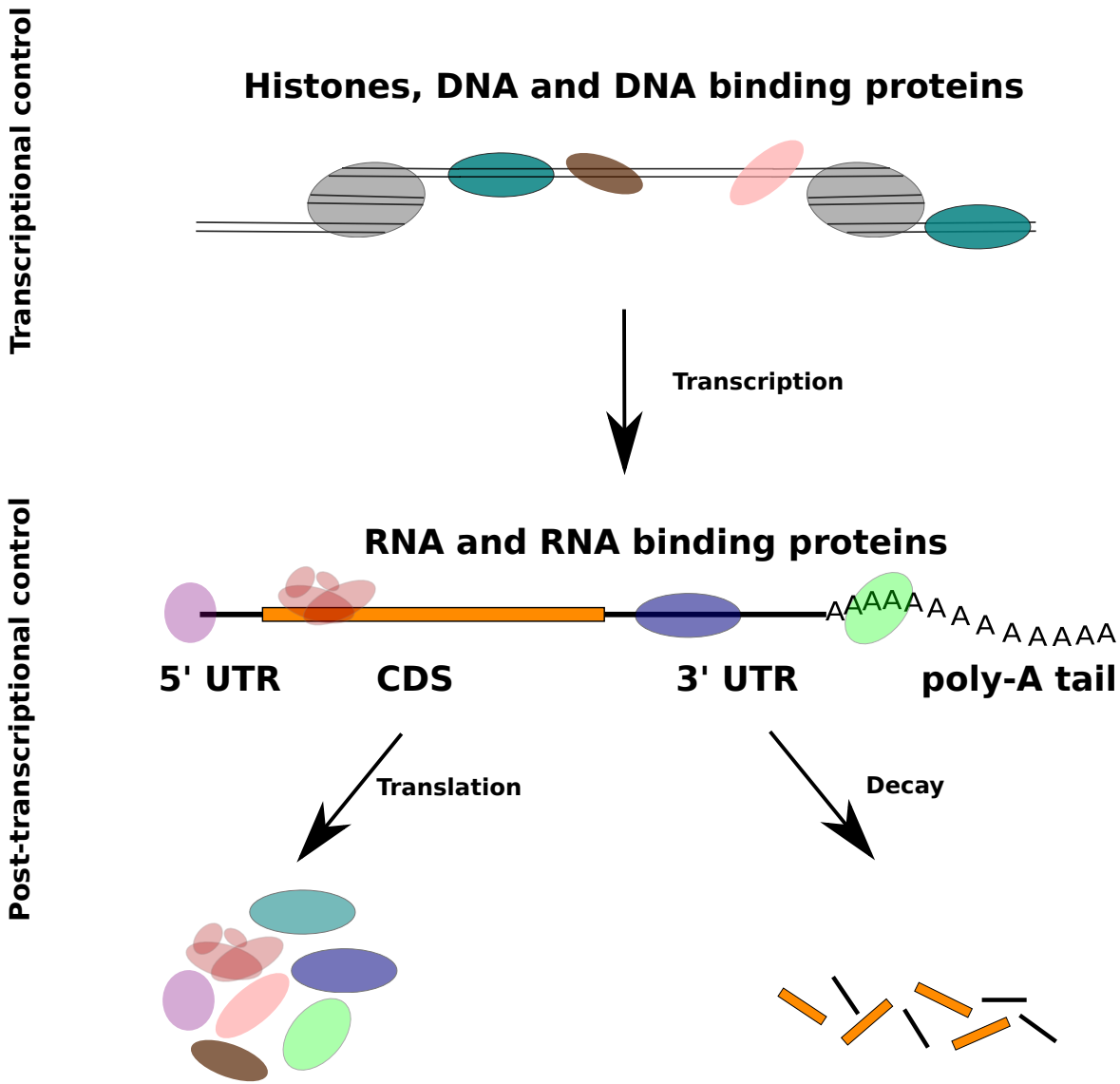

RNA cycle of life

Synthesis

RNA-polymerase transcription from DNA template

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From here on post-transcriptional

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Processing

Via various complexes

de-/capping, de-/adenylation, splicing, modification, ...

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Final un-/stable RNA molecule

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Via various complexes
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Final un-/stable RNA molecule

Available RNA depending on ratio $\frac{synthesis}{decay}$

Affects all sort of RNA (mRNA, tRNA, miRNA, lncRNA, ...)

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proteins or **protein-complexes**

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Or the lack thereof

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Remember the many structures for single molecule of RNA

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A manually curated collection of more than 1.500 RBPs in human highlights their vast number and potential for interaction and regulation

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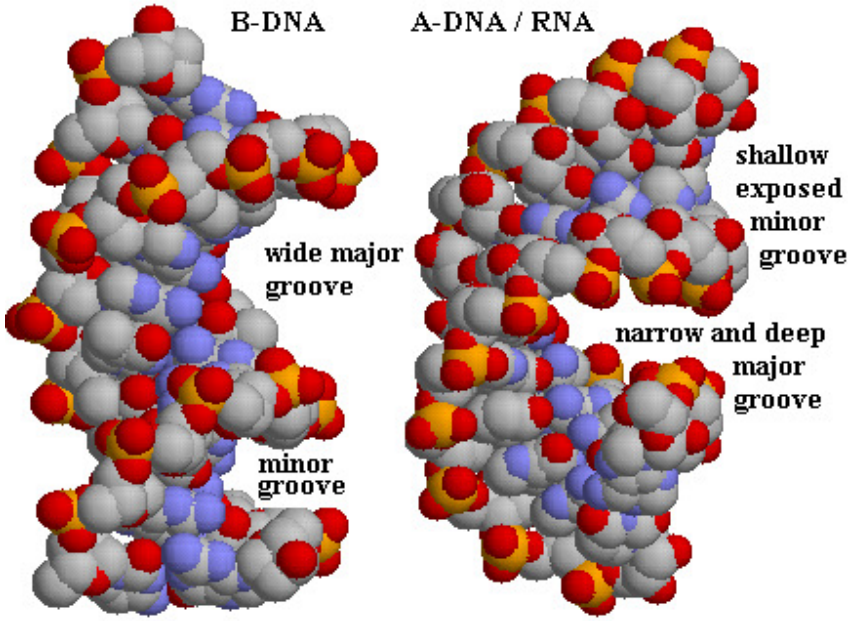
Many of the known RBPs seem to prefer single stranded binding regions, although some have been shown to interact with structured RNA sites

Binding RNA vs. binding DNA

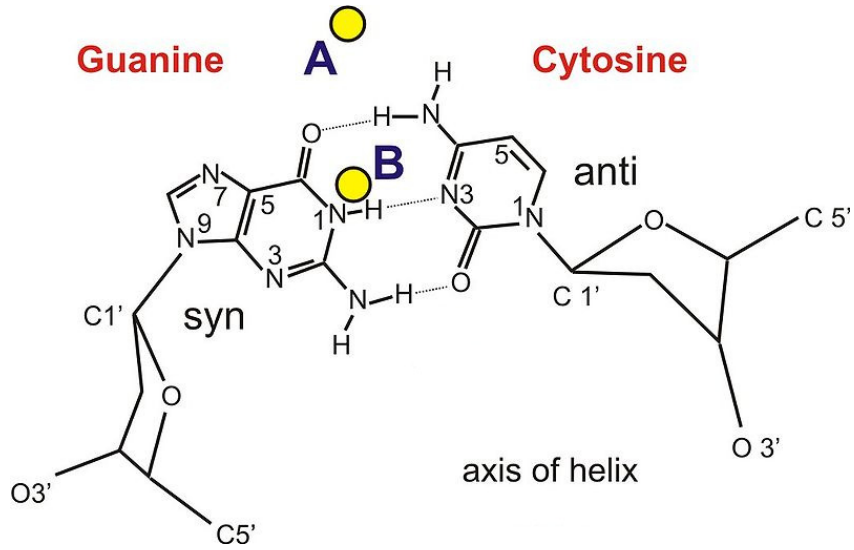
Feature	**RNA**	**DNA**
chemical nature	2'-OH	2'-desoxy
sequence	U	T
base paring rule	U-A & U-G	only T-A
structure	stem-loop structures	long double helix
helix geometry	A-form	usually B-form
function	information processing	information storage

B-form and A-form double-helix

overall shape



axis position



Same nucleotide sequences and **Same** cWW (cis-Watson-Crick/Watson-Crick) base pairs can form very different helical structures

RNA-Protein Interactions

Every RNA is in complex with unspecifically or specifically binding proteins. Togehter they form **R**ibo**N**ucleo**P**rotein Complex (RNPs).

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paired regions of stems
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RBPs contain RNA-binding domains (RBDs)

Although these domains are very specific and employ different interaction mechanisms, they can share some features that enable RNA-protein interactions

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Some RBPs do not contain a canonical RBD

Sequence-specific vs. sequence-unspecific binding

Sequence-specificity can be achieved via two strategies

H-bonds between protein backbone and RNA bases
$\rightarrow$ highly dependent of protein fold (hydrophobic sidechains looking towards RNA, almost no intramolecular stacking of RNA bases, instead intermolecular with sidechains, RNA bases not exposed to solvent, very rigid and specific scaffold)

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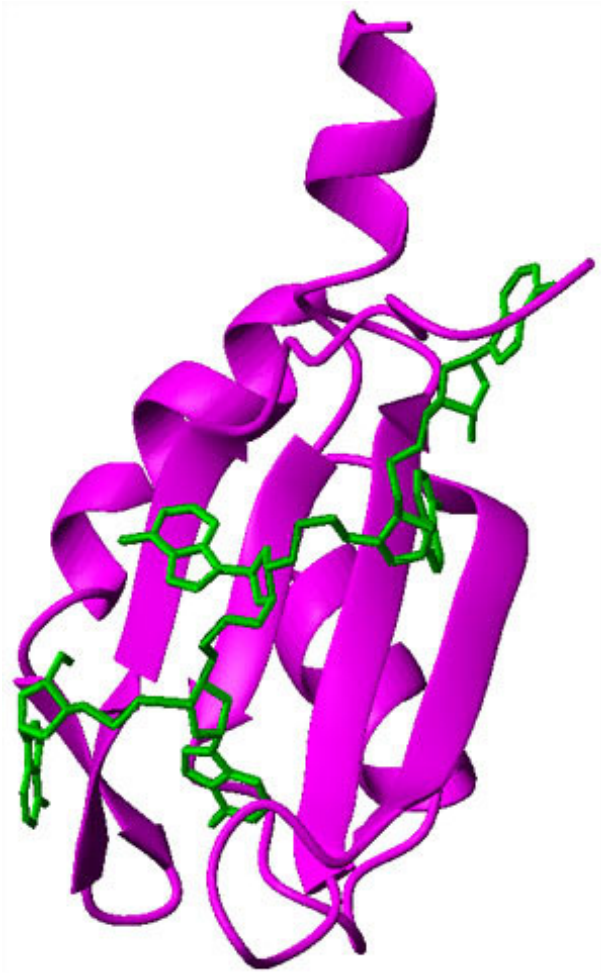
RBPs often employ a mix of strategies to bind their targets
$\rightarrow$ makes prediction of target sequences challenging

For some RBPs specific sequence preferences are known, for others they can be guessed from the available RBDs or do not exist

RRM – RNA-recognition motif

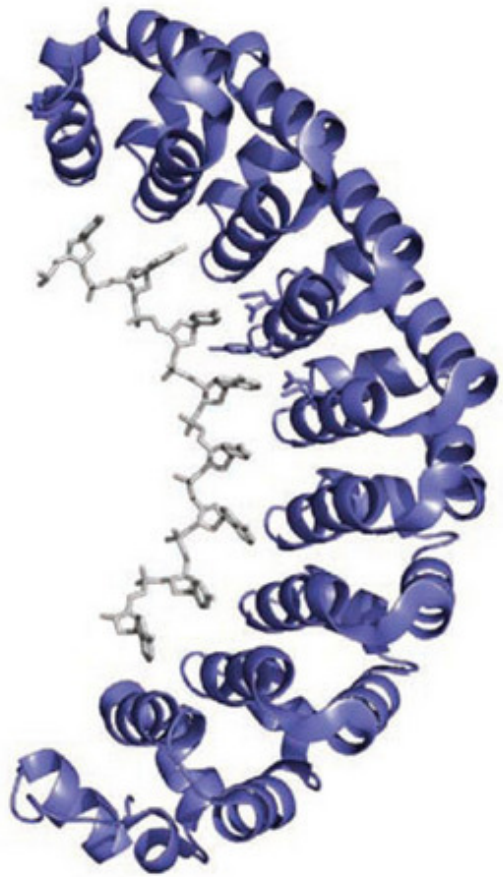
- most common
- binds ssRNA
- sequence specific
- 4 anti-parallel β-sheets packed against 2 α-helices
- 4-6nt interaction
- often in tandems/triplets
- very versatile

RRM in pink, RNA (5nt) green



zinc finger (CCCH/CCHC/C2H2)

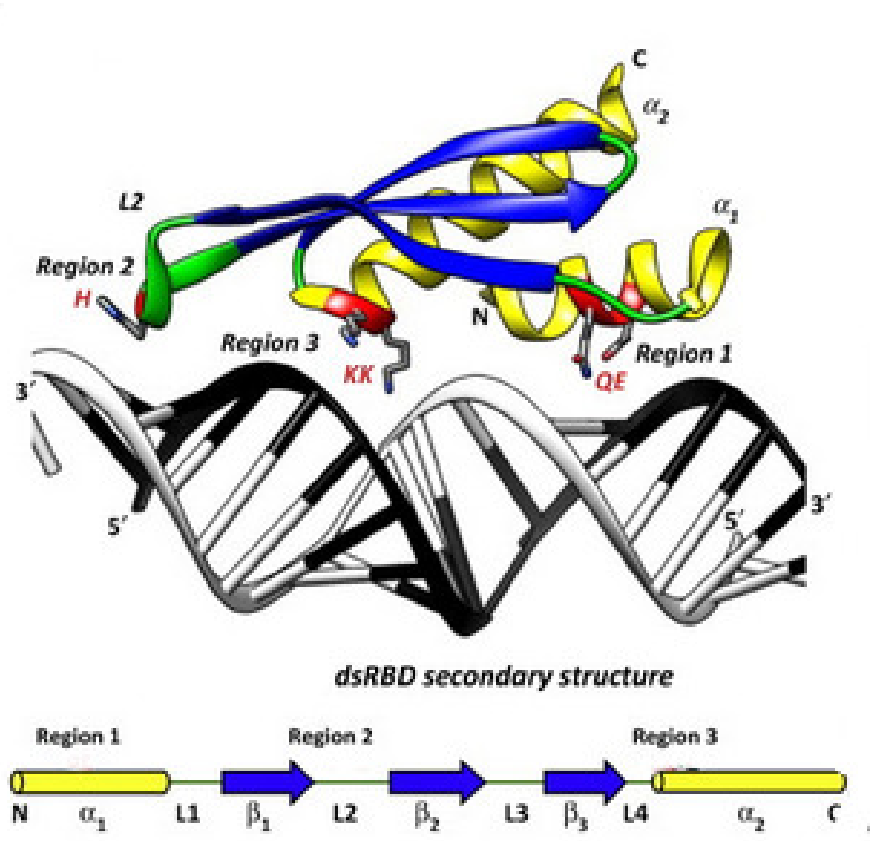
- C2H2 bind DNA, CCCH RNA, CCHC viral RNA
- coordinate zinc as common property
- unspecific binding to dsRNA
- specific binding to ssRNA
- RNA bases bulged out
- Intermolecular stacking and H-bond between RNA and backbone



9 zinc fingers (blue), RNA (8nt) grey

dsRBD – double-stranded RNA Binding Domain

- 3 anti-parallel β-sheets
- α-helices on N- and C-terminus
- unspecific binding to dsRNA via α-helices and loop between β-sheets
- contacts backbone
- sequence-specific binding of minor groove (ADAR2)



β-sheets (blue), A-form RNA

Protein interaction elements

Interaction between RNA and proteins depends on both, a protein domain that recognizes the target RNA, as well as RNA elements that are recognizable by the protein domain.

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While protein domains have been studied and characterized in detail, RNA elements crucial for interaction are in general understudied

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Of course there are others (*e. g.* splice sites, poly-A, ...)

So what about structure?

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Sequence elements important for single-stranded RNA binding proteins, but they require the target RNA sequence to be unpaired or in an accessible structural context like the loop section of a hairpin loop

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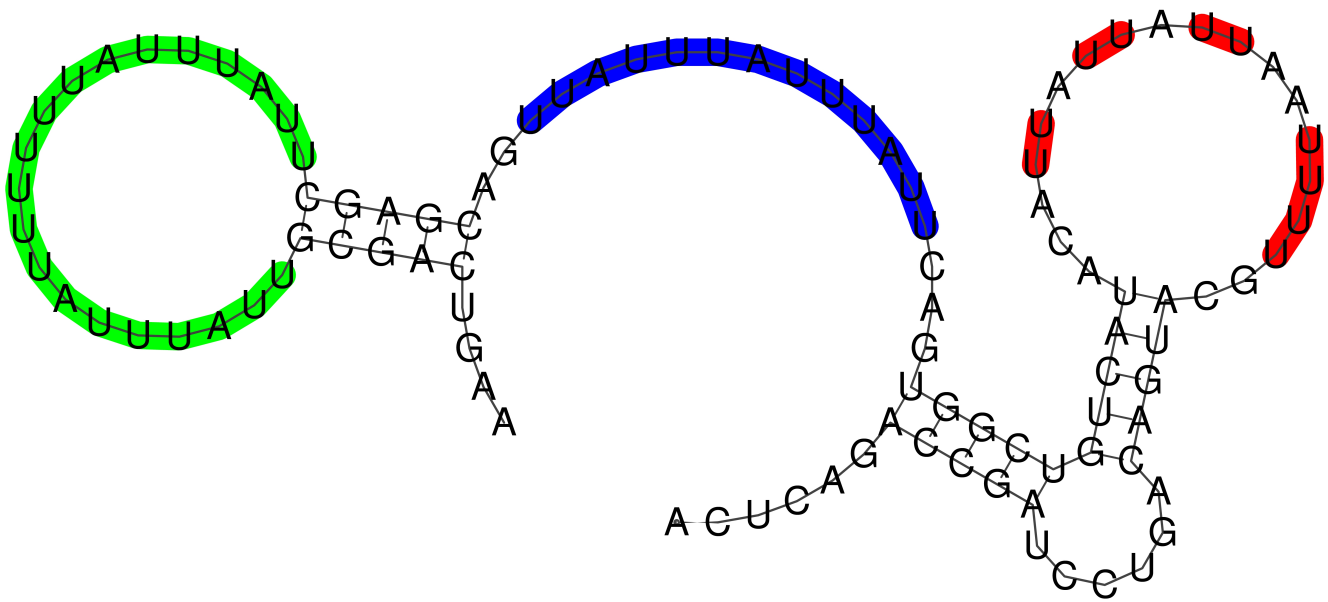

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Proteins can interact with their target not only at specific sites, but in a probing manner known as diffusional search $\rightarrow$ further complicating interaction analysis

Identifying RNA-protein interactions II

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Need for specific probes, antibodies, cell-types or substrates

Experimental Methods

Experimental methods for the characterization of RNA-RBP interactions can generally be broken down into

in vitro assays, which means free from other interacting factors and under experimental conditions

in vivo approaches which capture a snapshot of RBP binding to RNAs at natural expression levels or after induction

in vivo VS *in vitro* approaches

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in vivo approaches

technically more challenging but preserve the context of competing or assisting interactions

RNA vs protein centric methods

RNA-centric methods

Protein-centric methods

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RNA-centric methods $\rightarrow$ Pull down RNA

Protein-centric methods $\rightarrow$ Pull down protein

RNA vs protein centric methods

RNA-centric methods $\rightarrow$ Pull down RNA $\rightarrow$ analyze protein

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RNA vs protein centric methods

RNA-centric methods

- Mass spectrometry (MS) identify RBPs bound to RNA
- Allow the identification of novel RBP interactions
- RBPs for which antibodies are hard to come by
- Requires the purification of enough protein mass (hard)

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Protein-centric methods

- Co-IP of protein with crosslinked targets
- Require some knowledge about protein and specific antibodies for IP
- Can easily be applied in a high-throughput manner and require lower amounts of starting material (PCR)

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Purification of RNA-RBP complexes via target protein

Specific purification methods for protein *in vivo*
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- Sequencing

Protein centric assays