

# Interaktionen von RNAs und Proteinen

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## RNA-protein binding vs. DNA-protein binding

“Is binding of proteins to **RNA** the same as binding of proteins to **DNA**?”

“Will the same rules apply?”

```
listen_to_lecture = undef;

if (above_statement == true) {
    I_know_already = true;
    goto elsewhere;
}
else {
    listen_to_lecture = "yes";
}
```

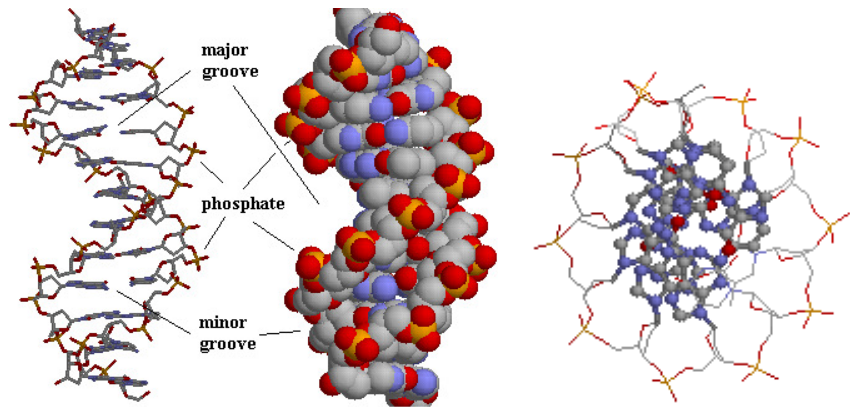
# RNA vs. DNA – similar or what?

- ▶ **chemical nature:**
- ▶ **sequence:**
- ▶ **base pairing rule:**
- ▶ **structure:**
- ▶ **helix geometry:**
- ▶ **function:**

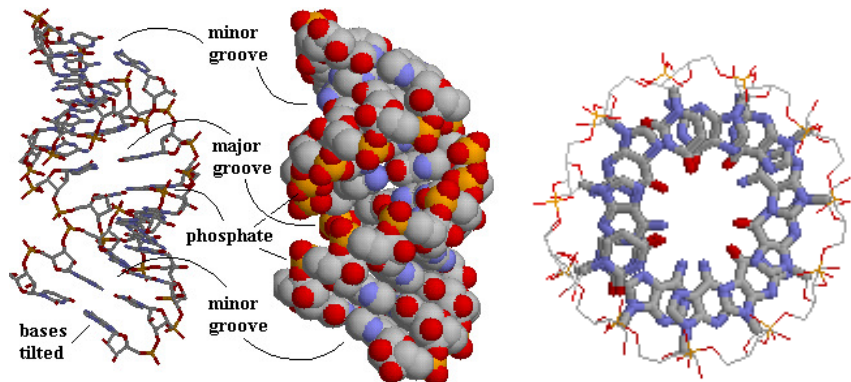
## RNA vs. DNA – similar or what?

- ▶ **chemical nature:** 2'-OH – 2'-desoxy
- ▶ **sequence:** U – T
- ▶ **base pairing 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

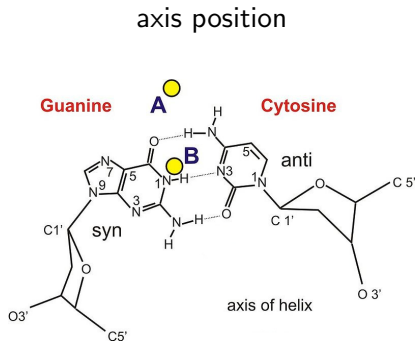
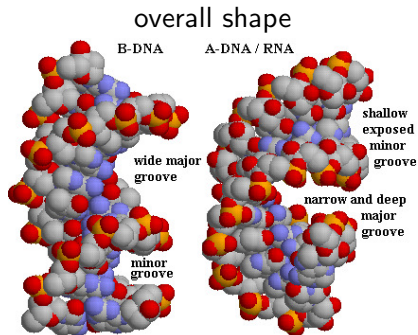
## DNA commonly in B-form



## DNA/RNA in A-form



# comparison of B-form and A-form



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

# Conclusion

If DNA and RNA are different in so many (particularly molecular) aspect, it is likely that the mode of interaction between DNA and protein differs from the mode of interaction between RNA and protein.





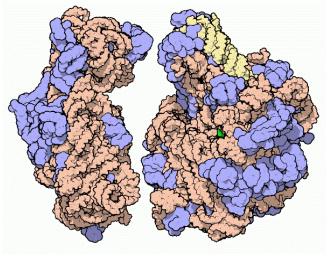
# RNA-Protein Interactions

Every RNA is in complex with unspecifically or specifically binding proteins. Together they form **RiboNucleoProtein** Complex (RNPs).

- ▶ RNAs fold into stem-loop structures  
→ many different types of interactions
- ▶ proteins with **RNA binding domains** bind
  - ▶ **single stranded RNA** (ssRNA)  
short motifs in unpaired regions  
commonly in **sequence specific** manner
  - ▶ **double stranded RNA** (dsRNA)  
paired regions of stems  
commonly in **sequence unspecific** manner
- ▶ RNAs can interact with complex structural motifs  
e.g. PKR (protein kinase RNA-activated)
- ▶ RNAs can form **large complexes** with proteins  
e.g. ribosome, spliceosome  
RNA aptamers (synthetic, highly specific binders)

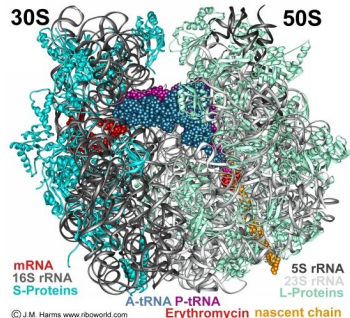
# The Ribosome: Ribosomal RNAs and Proteins

- ▶ small subunit (30S): 16S rRNA, 21 proteins
- ▶ large subunit (50S): 5S rRNA, 23S rRNA, 31 proteins
- ▶ contact surfaces rather than binding sites



small subunit (left), large subunit (right)

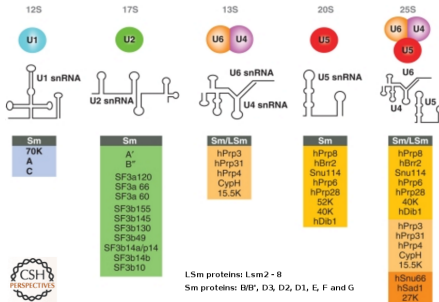
proteins – lila; RNAs – orange, yellow



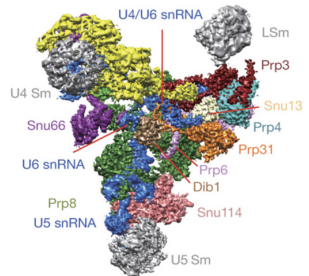
bacterial 70S ribosome (www.riboworld.com)

# The Spliceosome: spliceosomal RNAs and Proteins

- ▶ highly dynamic, transient complex composition
- ▶ 5 small nuclear RNAs (snRNAs): U1, U2, U4/U6, U5
- ▶ more than 100 associated proteins



major human spliceosomal snRNPs

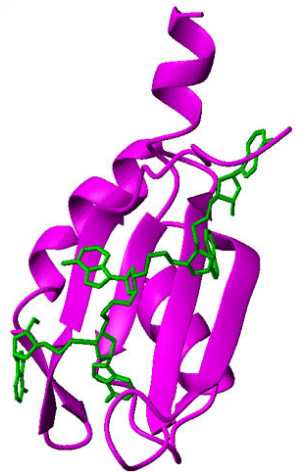


yeast U4/U6.U5 tri-snRNP

# RRM – RNA-recognition motif

- ▶ most common
- ▶ binds ssRNA
- ▶ sequence specific
- ▶ 4 anti-parallel  $\beta$ -sheets

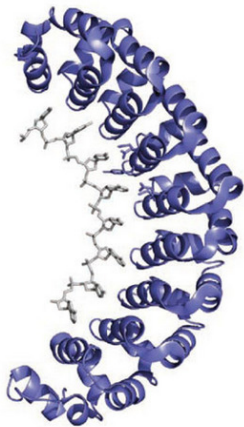
RRM in pink, RNA (5nt) green



## zinc finger (CCHH-type)

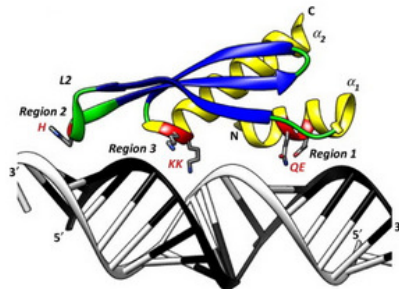
- ▶ unspecific binding to dsRNA
- ▶ specific binding to ssRNA
- ▶ RNA bases bulged out
- ▶ one finger per nucleotide

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

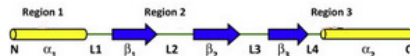


# dsRBD – double-stranded RNA Binding Domain

- ▶ unspecific binding to dsRNA
- ▶ contacts backbone
- ▶ basic residues
- ▶ 3 anti-parallel  $\beta$ -sheets
- ▶  $\alpha$ -helices on N- and C-terminus



dsRBD secondary structure



$\beta$ -sheets (blue), A-form RNA

# On the Function of Long Non-Coding RNAs (lncRNAs)

## Hypotheses

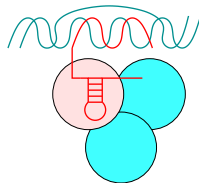
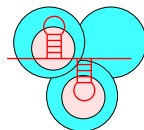
about the role of ncRNAs in protein complexes

### RNA as scaffold

- ▶ ncRNAs hold protein complexes together
- ▶ variants determine complex composition

### RNA as linker to DNA

- ▶ ncRNAs link protein complexes to DNA
- ▶ allows high binding specificity



# Functional RNA Classes and Their Protein Partners

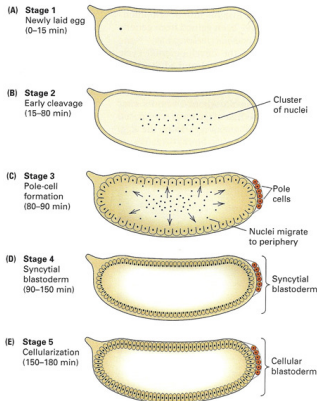
Which proteins are they interacting with?

- ▶ **mRNA:**
- ▶ **tRNA:**
- ▶ **rRNA:**
- ▶ **miRNA:**
- ▶ **snRNA:**
- ▶ **snoRNA:**
- ▶ **vaultRNA:**
- ▶ **lncRNA:**

homework



# RNA is needed for localization of Su(var)3-3 in Drosophila

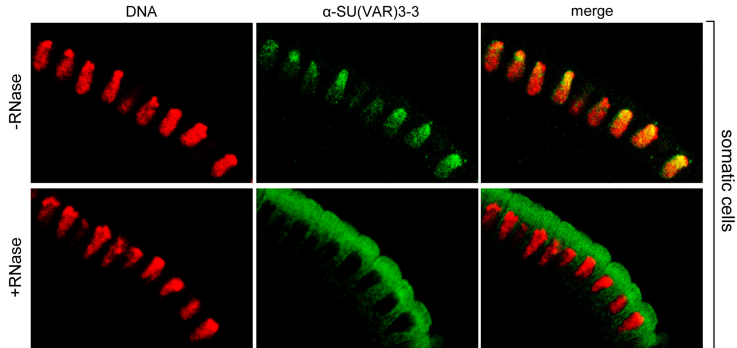


- ▶ drosophila
- ▶ development
- ▶ preblastoderm stage
- ▶ Su(var)3-3
- ▶ homolog of LSD1
- ▶ demethylates methylated H3K4
- ▶ localizes to nuclei
- ▶ H3K4 demethylation → heterochromatin formation

When RNA is degraded, Su(var)3-3 does not localize to nuclei.

Work on the next page was done by Prof. Gunter Reuter, Martin-Luther Universität, Halle-Wittenberg

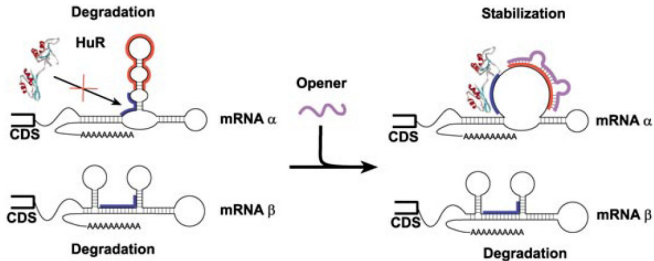
# Degradation of RNA with RNase at preblastoderm stage



**First row:** wild type localization of Su(var)3-3 to nuclei, in particular heterochromatin that is closer to the outer membrane (yellow spots in merge)

**Second row:** in the presence of RNase, the absence of RNA, Su(var)3-3 stays in the cytoplasm

# A synthetic “opener” sequence unblocks HuR binding



- ▶ HuR protects mRNAs from degradation
- ▶ it binds to the sequence motif NNUUNUUU if unpaired
- ▶ this motif is found in 3'UTRs
- ▶ a sequence specific, synthetic “opener” RNA can induce fold changes → freeing the HuR binding site



[Meisner, 2004] Nicole-Claudia Meisner, Jörg Hackermüller, Volker Uhl, András Aszódi, Markus Jaritz, and Manfred Auer. *mRNA Openers and Closers: Modulating AU-Rich Element-Controlled mRNA Stability by a Molecular Switch in mRNA Secondary Structure*. ChemBioChem (2004)