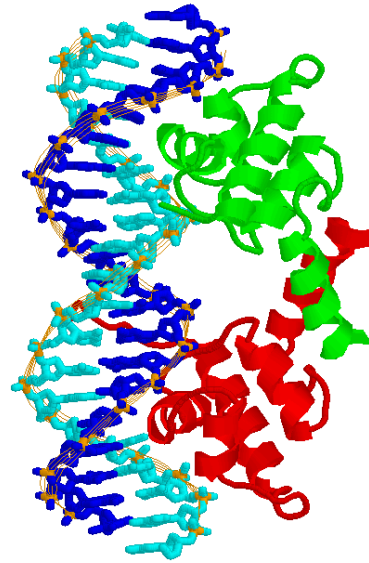


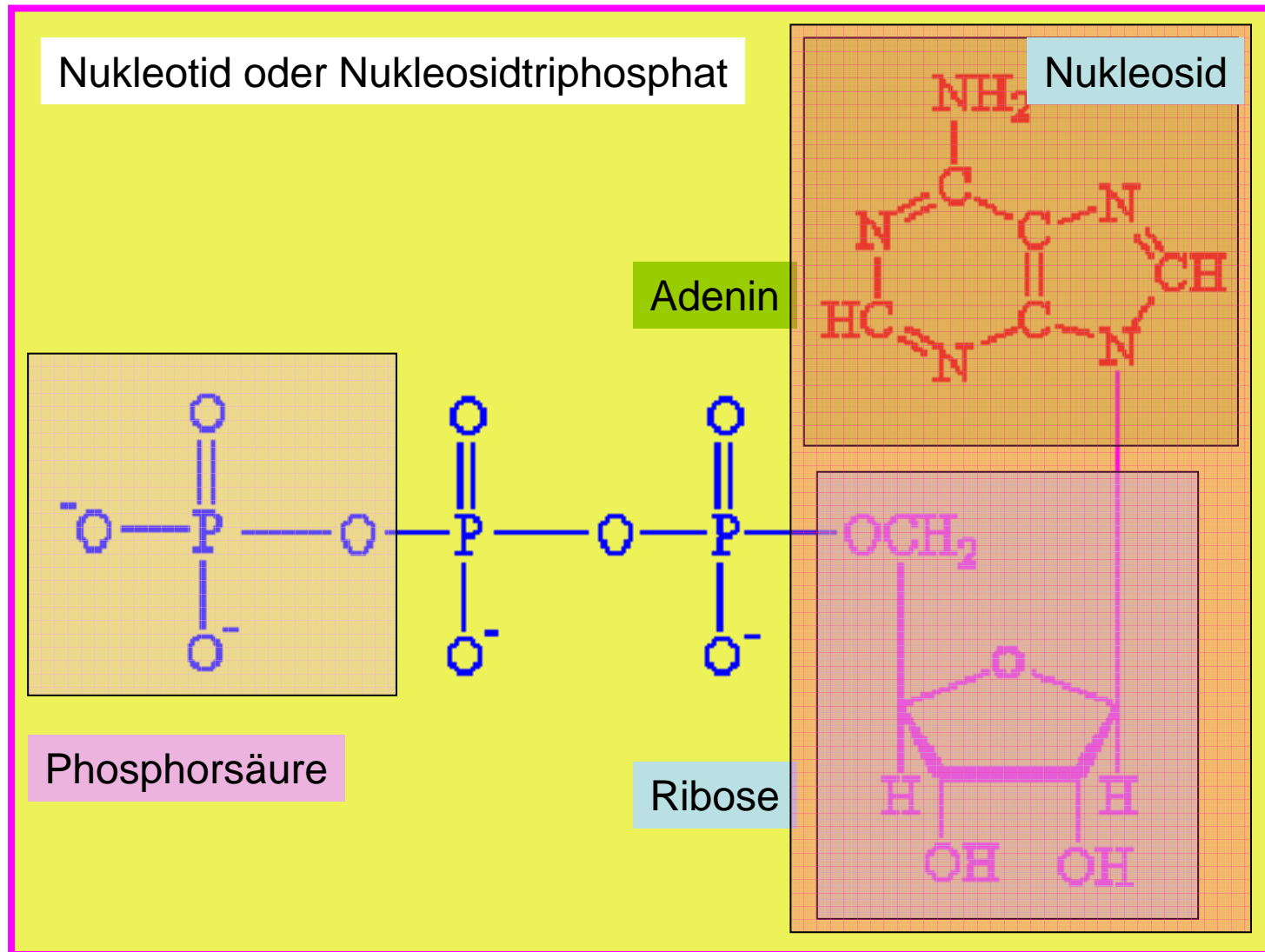
Molekulargenetik der Eukaryoten

WS 2006/7, VL2

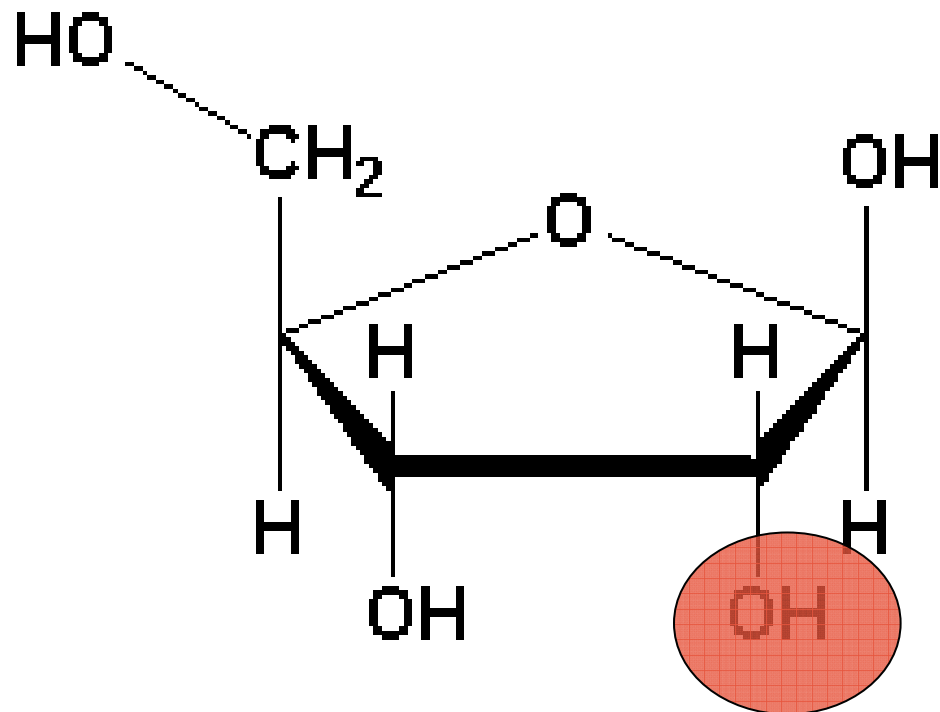


Erwin R. Schmidt
Institut für Molekulargenetik

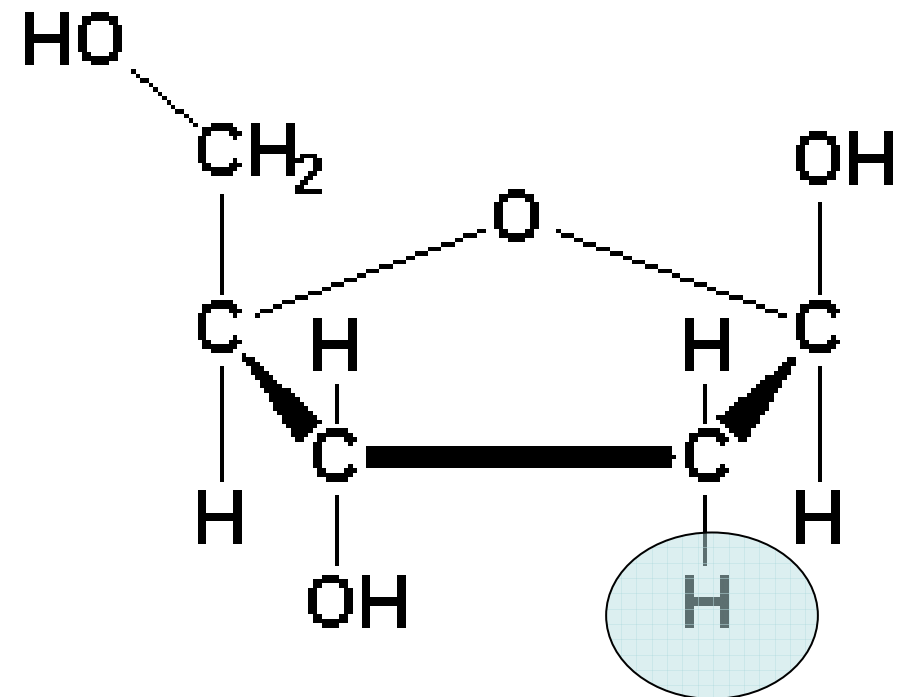
z. B. Adenosintriphosphat (ATP)



Ribose und 2-Deoxy-Ribose

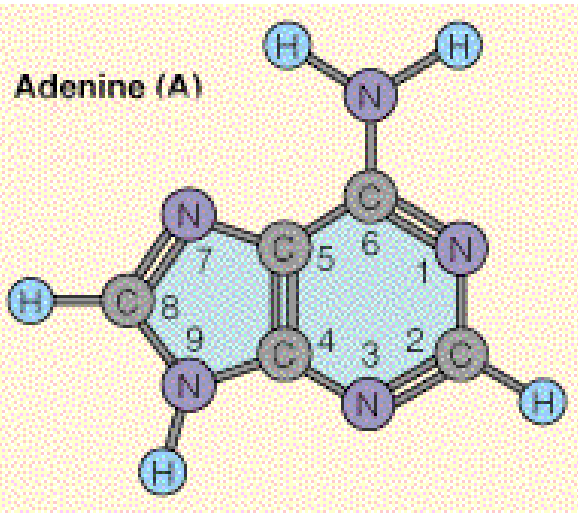


D-Ribose

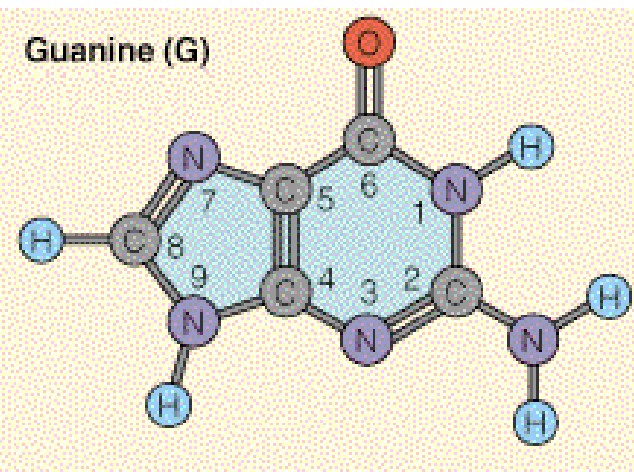
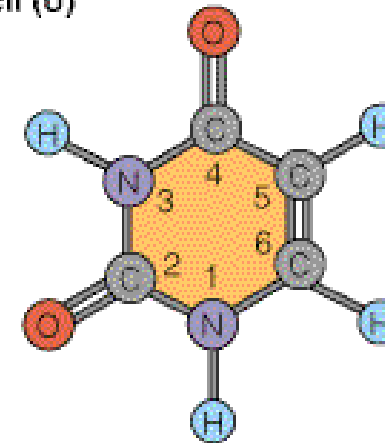


Deoxyribose

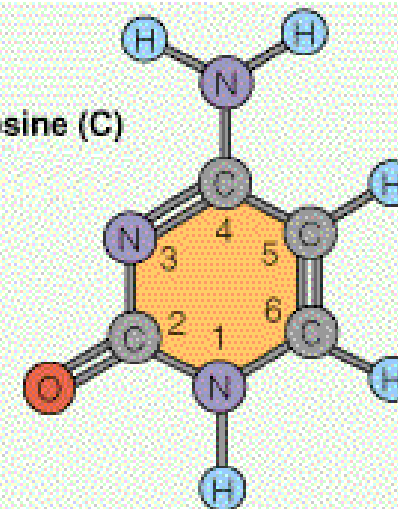
Die Nukleobasen der RNA



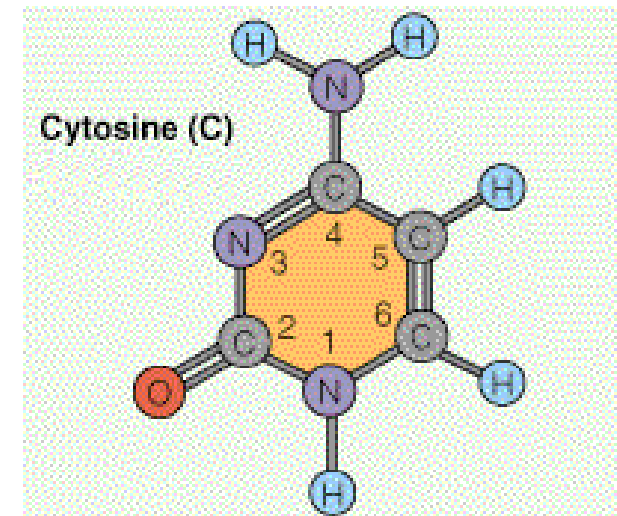
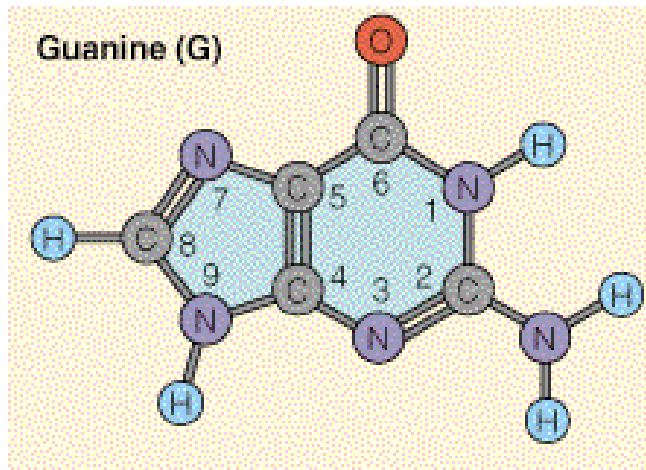
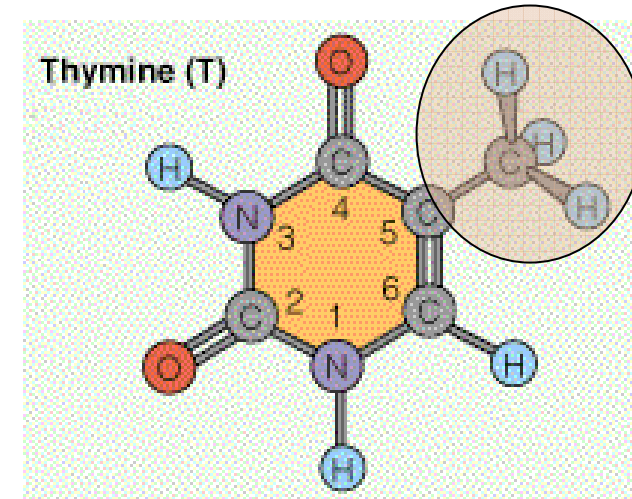
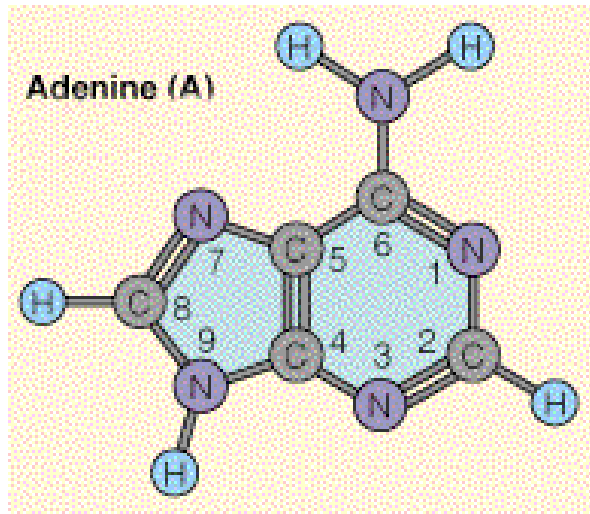
Uracil (U)



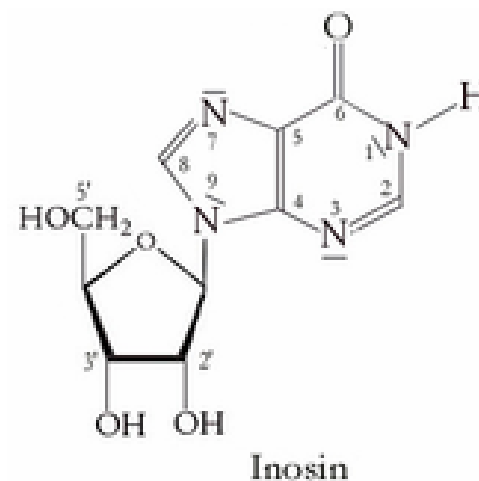
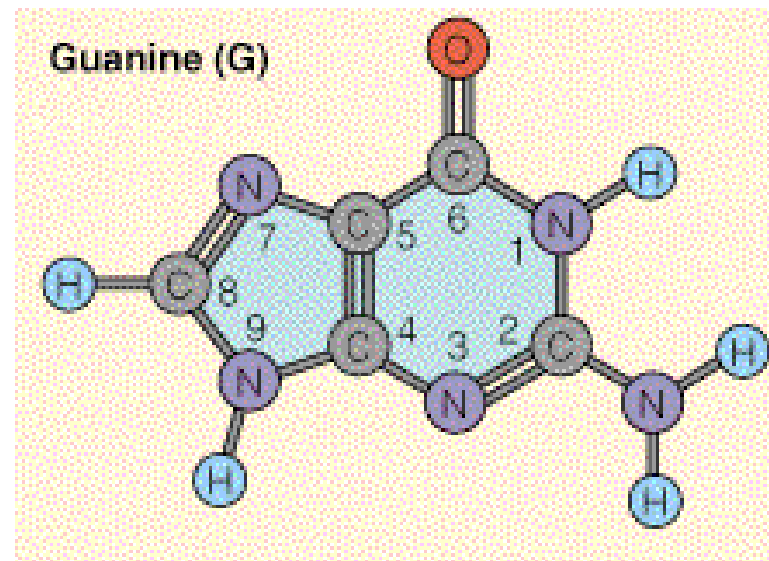
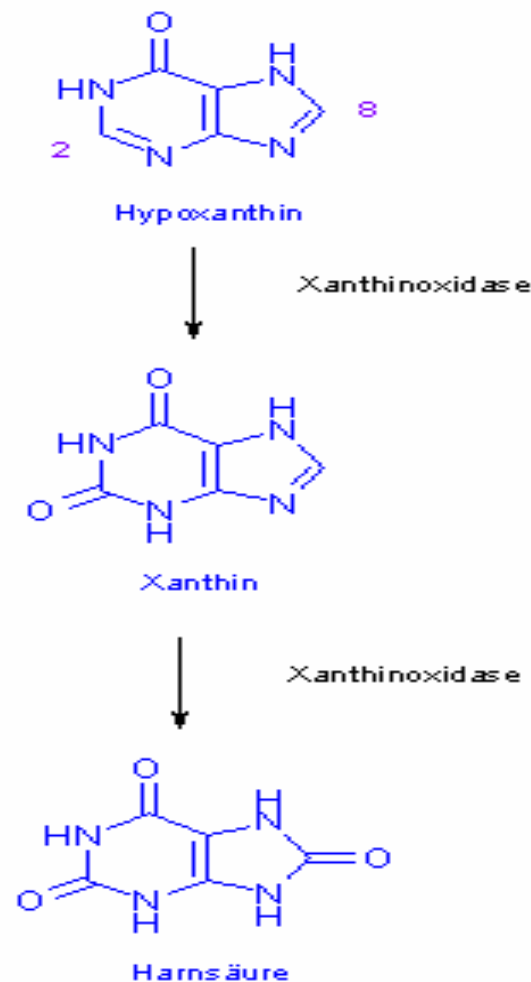
Cytosine (C)



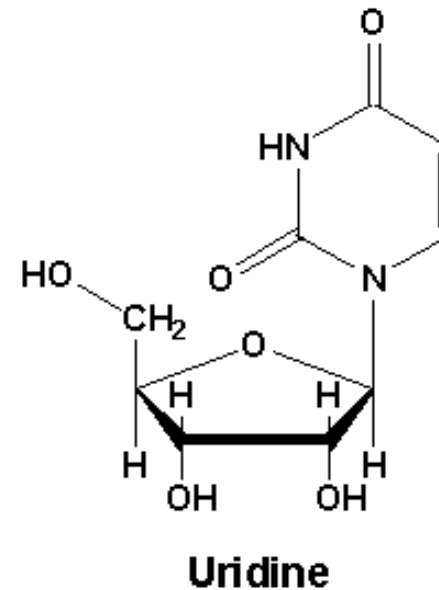
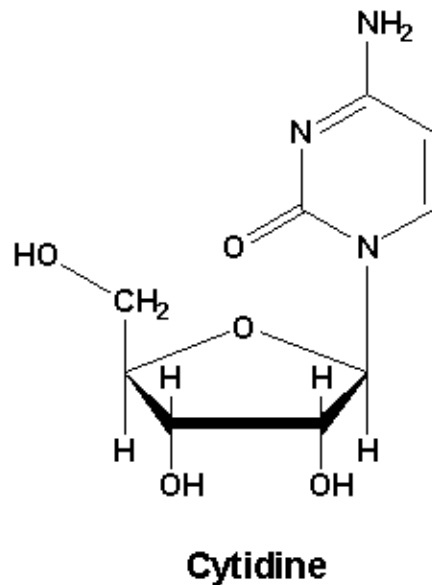
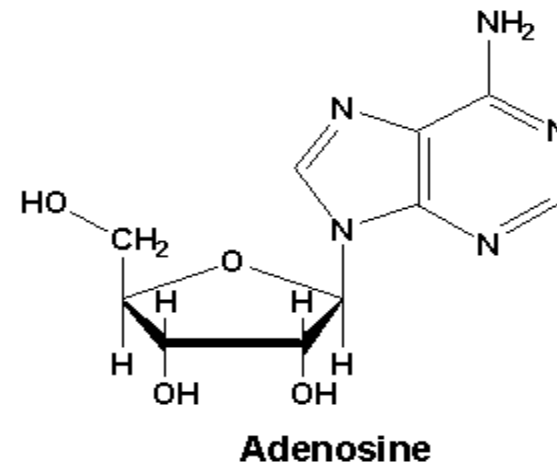
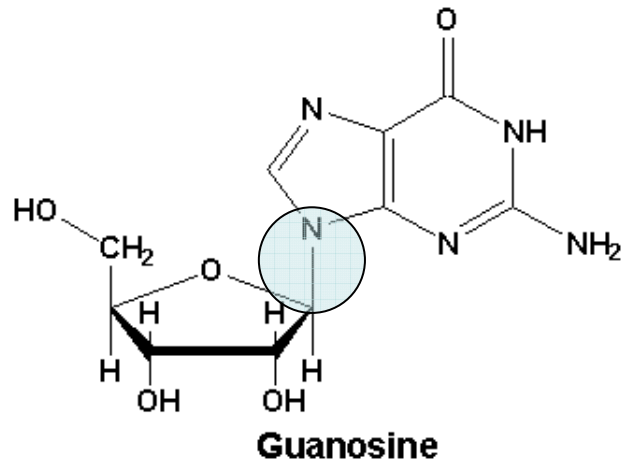
Die Nukleobasen der DNA



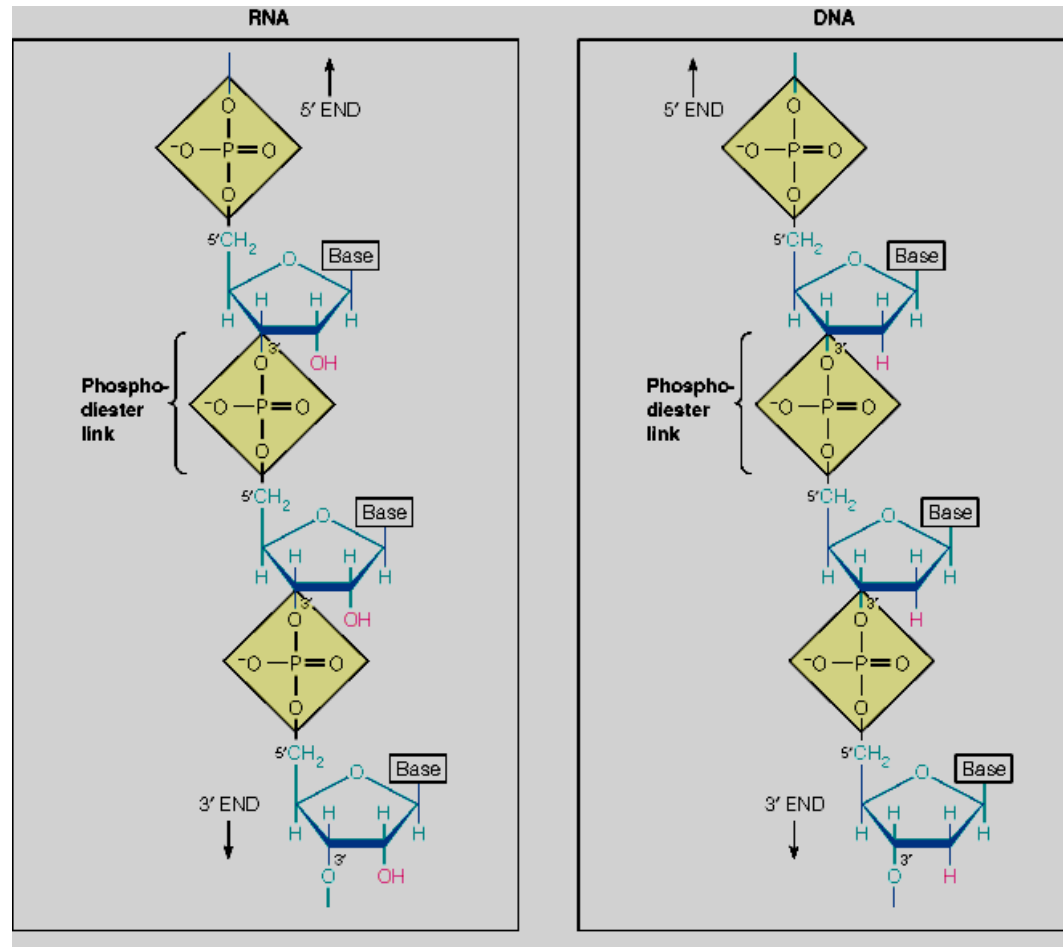
Seltene Basen in der RNA/DNA



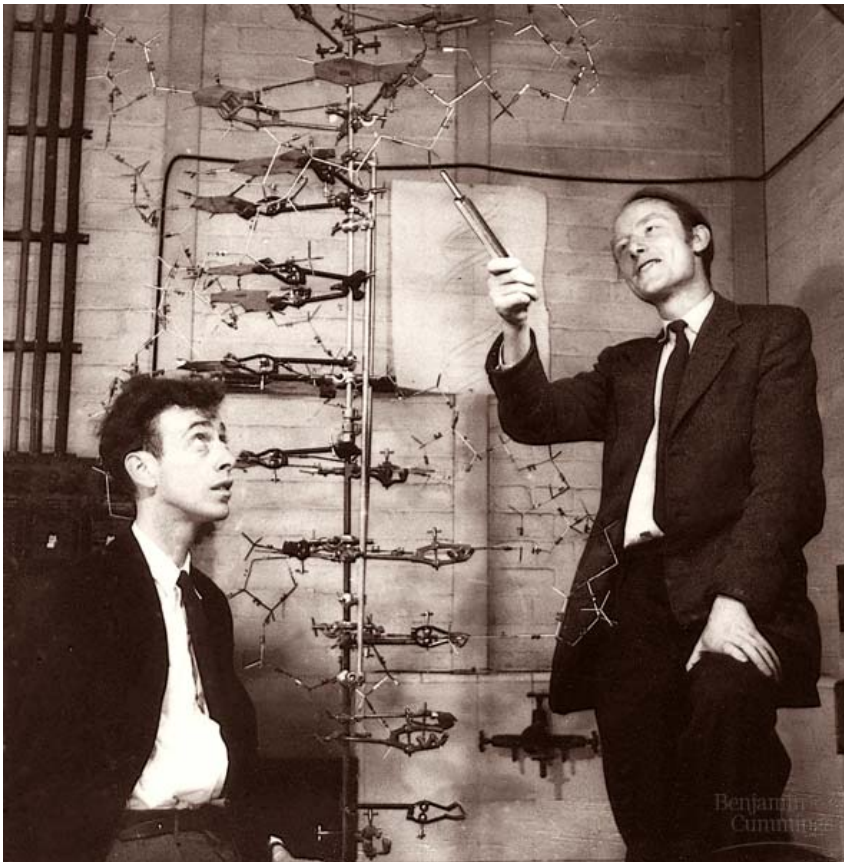
Die Nukleobasen werden mit der Pentose über N-glycosidische Bindungen zu „Nucleosiden“ verknüpft



Polynukleotide entstehen durch Bildung von Phosphodiesterbindungen zwischen Zucker und Phosphat



Die typische Struktur ist die „Doppelhelix“

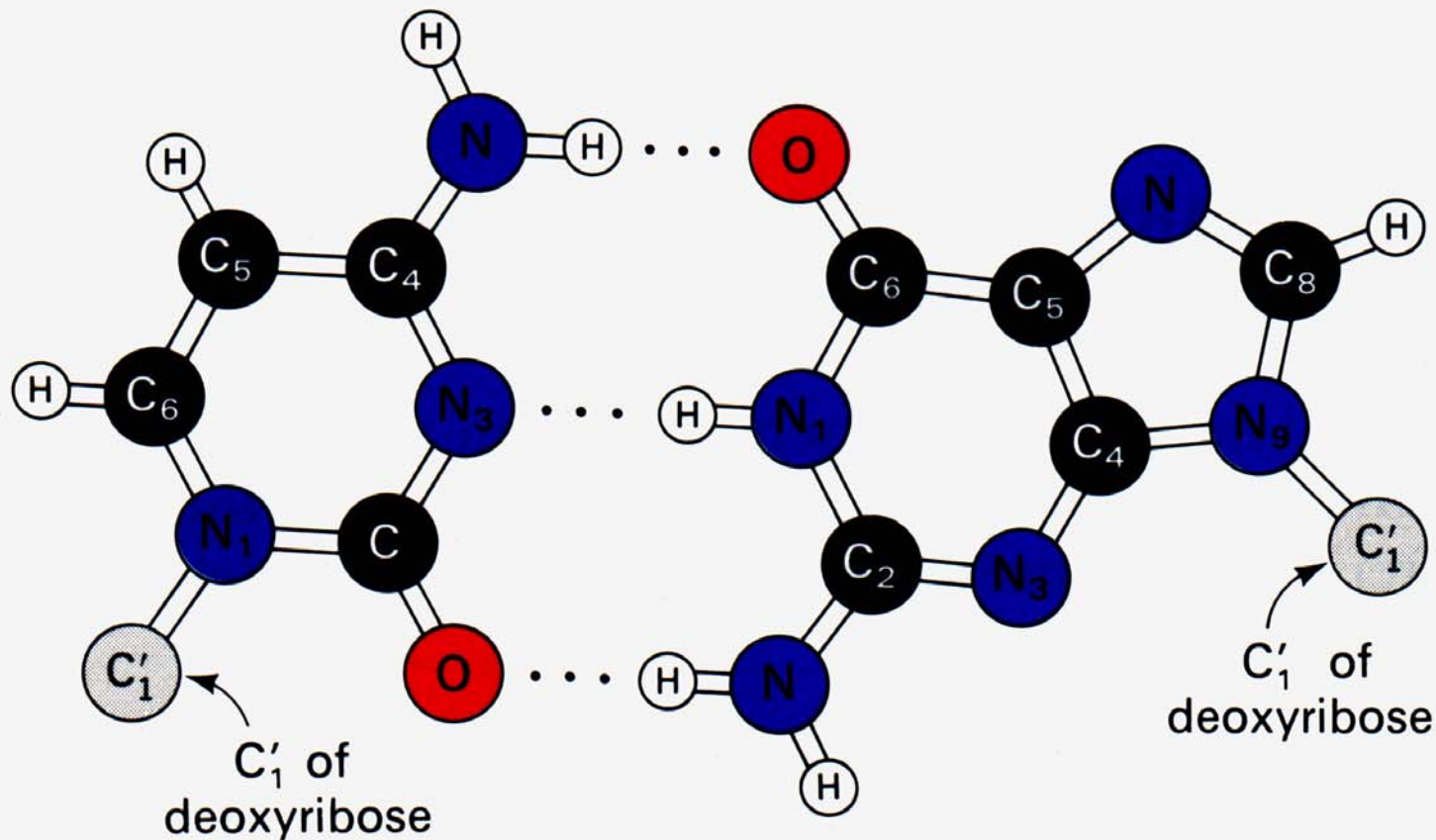


Watson und Crick, DNA-Modell 1953



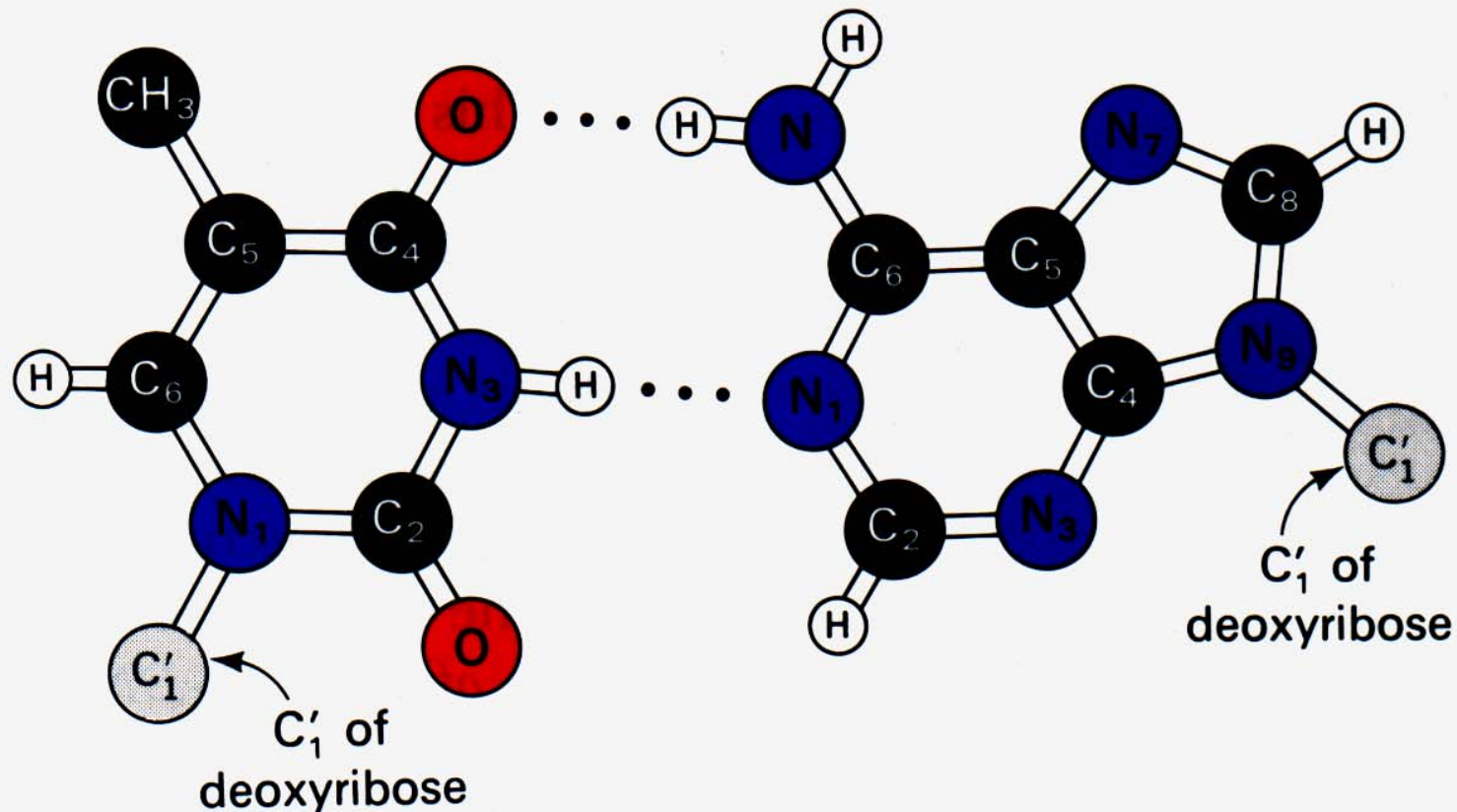
B-DNA

Die Sekundärstruktur wird über Wasserstoff-
Brückenbindungen stabilisiert



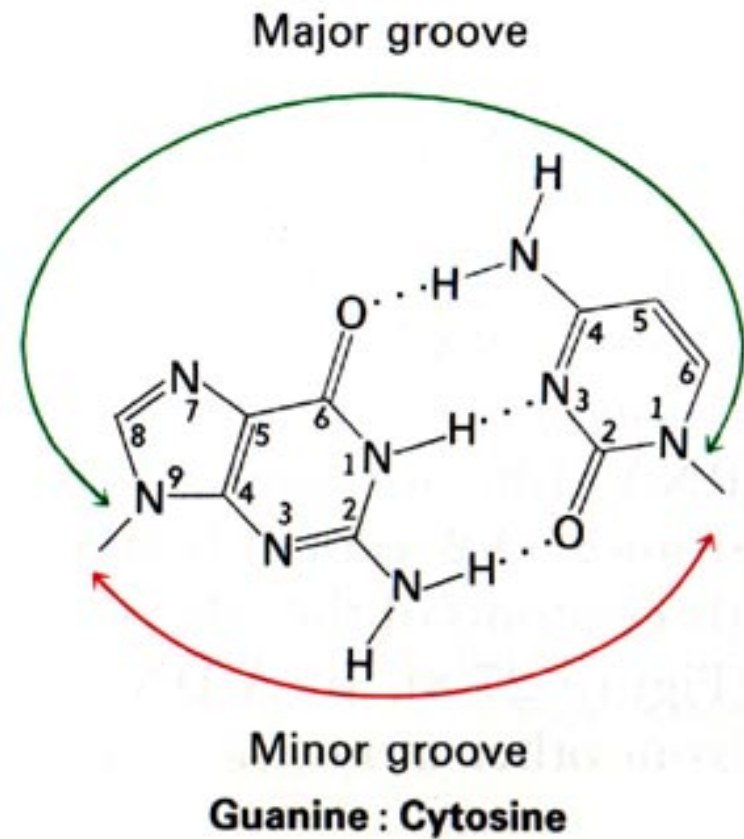
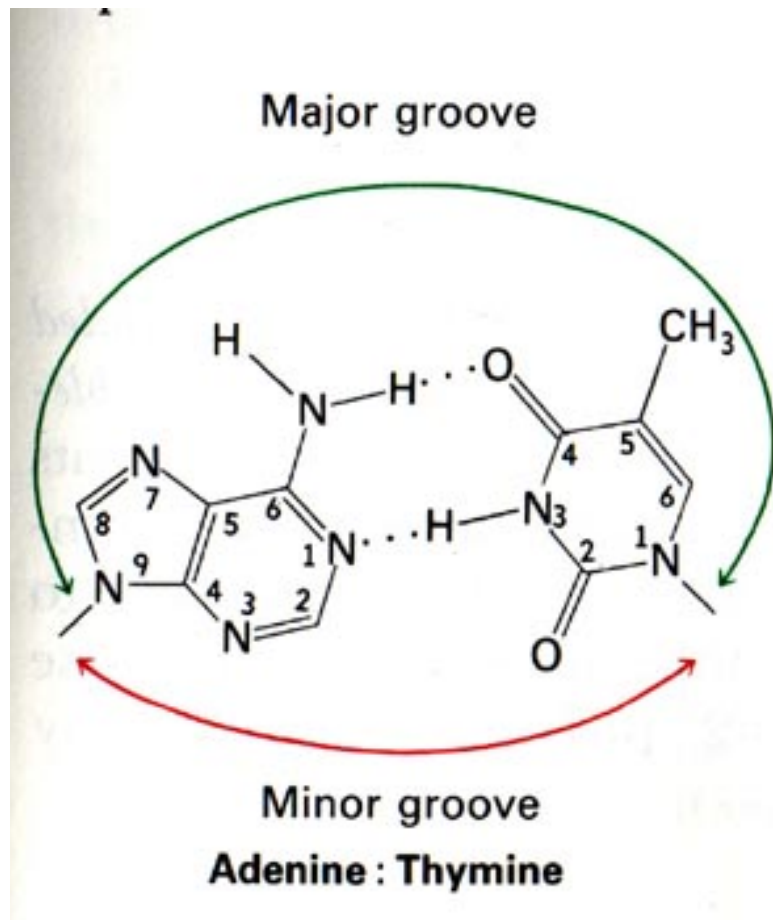
Cytosin-Guanin

Die Sekundärstruktur wird über Wasserstoff-
Brückenbindungen stabilisiert



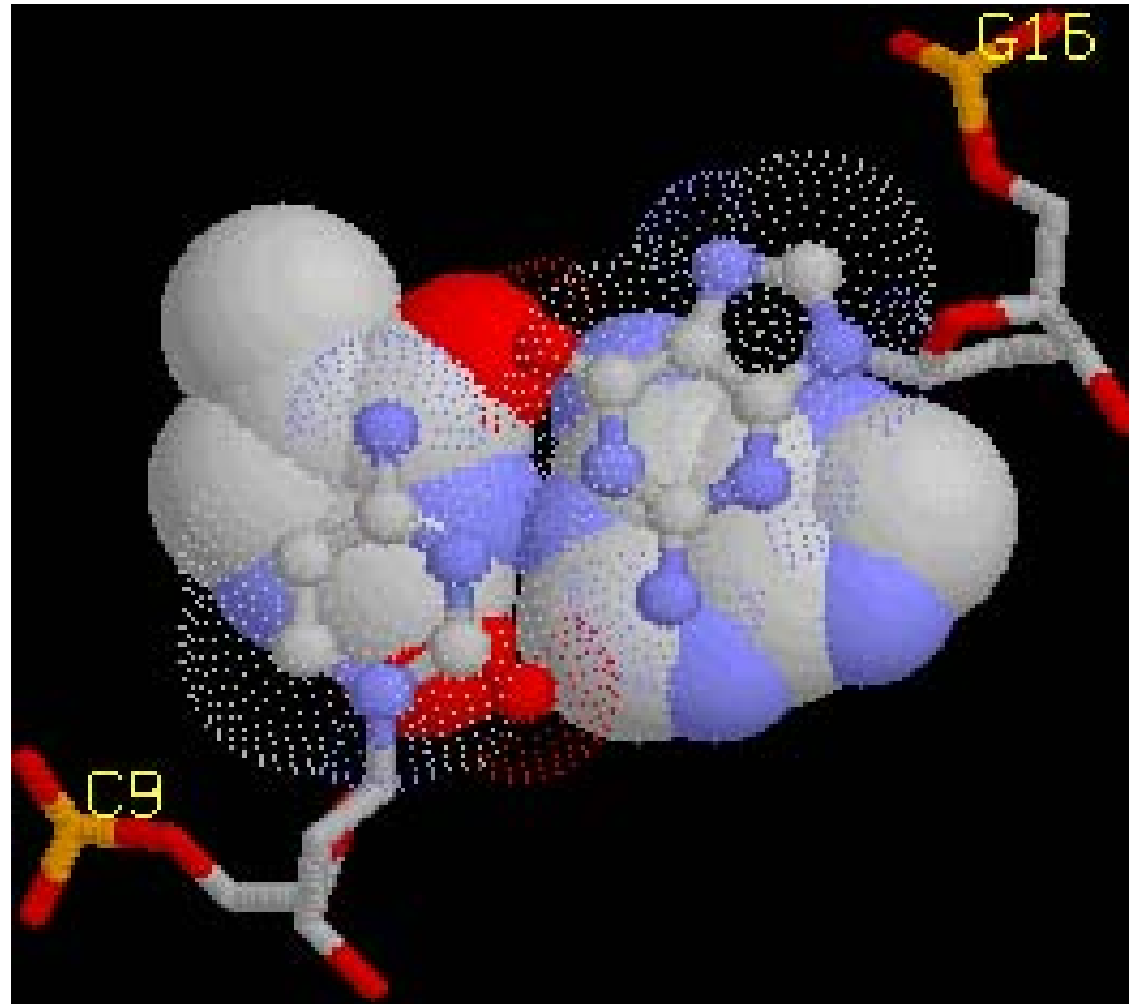
Thymin-Adenin

Die Basenpaare haben „zwei Seiten“



„stacking forces“ – Stapelkräfte

Beispiel AT-GC stacking

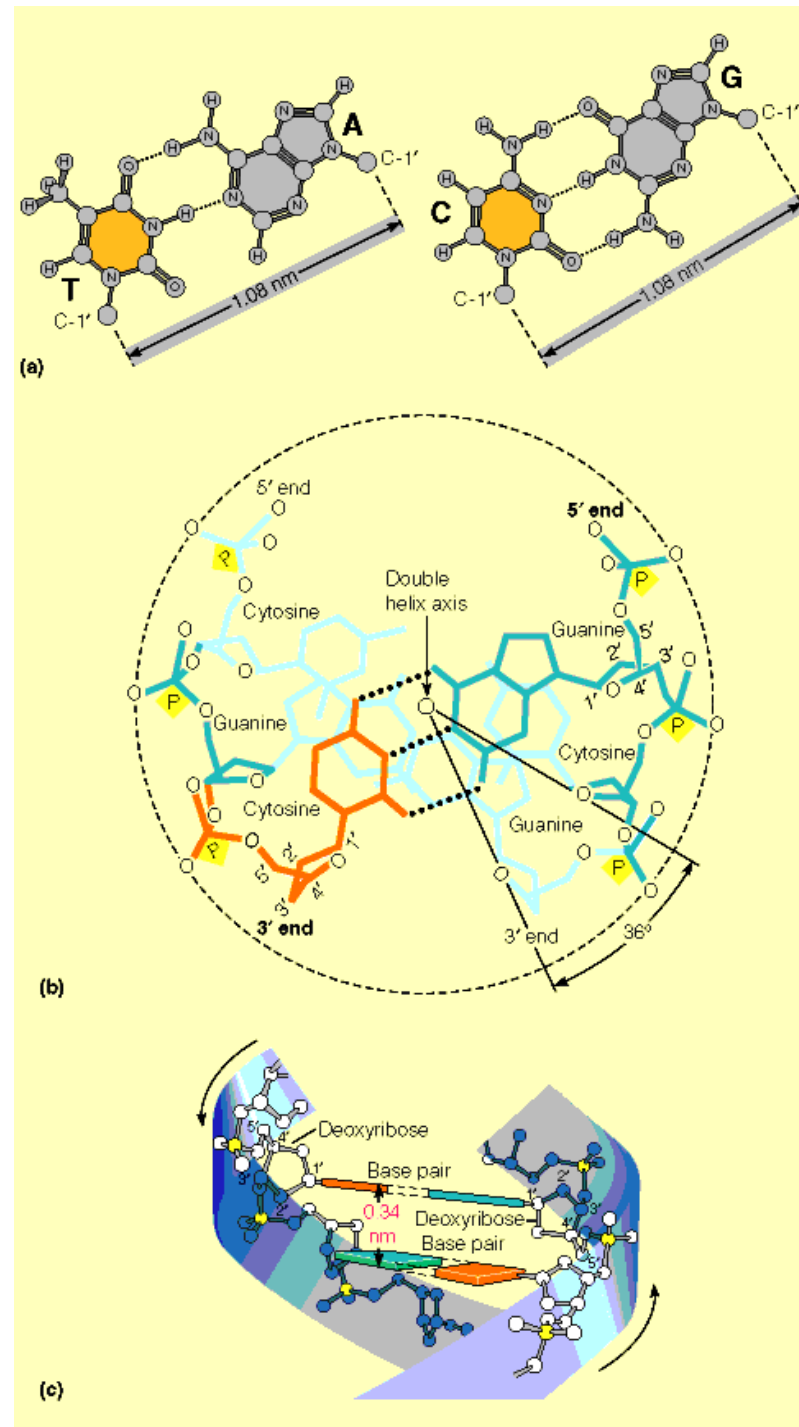


Stapelkräfte stabilisieren die DNA-Helix erheblich

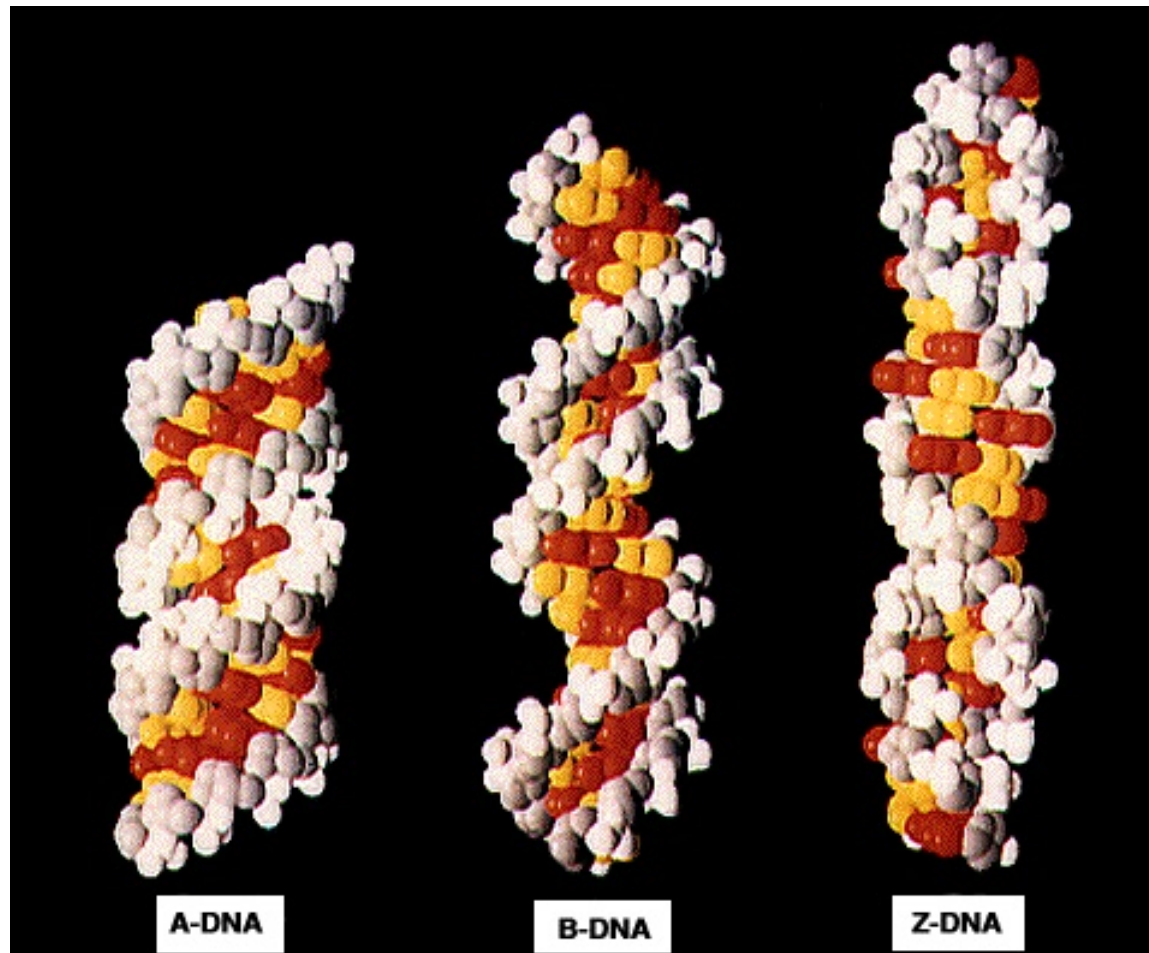
Properties of individual dinucleotide base pairs

Dinucleotide base pairs	Stacking energies (Kcal/mol bp)	twist angle	bp/turn
(GC).(GC)	-14.59	40.0	9.0
(AC).(GT)	-10.51	34.4	10.5
(TC).(GA)	-9.81	36.9	9.8
(CG).(CG)	-9.61	29.8	12.1
(GG).(CC)	-8.26	33.7	10.7
(AT).(AT)	-6.57	32.1	11.4
(TG).(CA)	-6.57	34.5	10.<
(AG).(CT)	-6.78	27.9	13.0
(AA).(TT)	-5.37	35.6	10.1
(TA).(TA)	-3.82	36.0	10.0

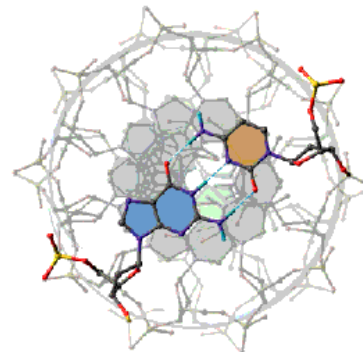
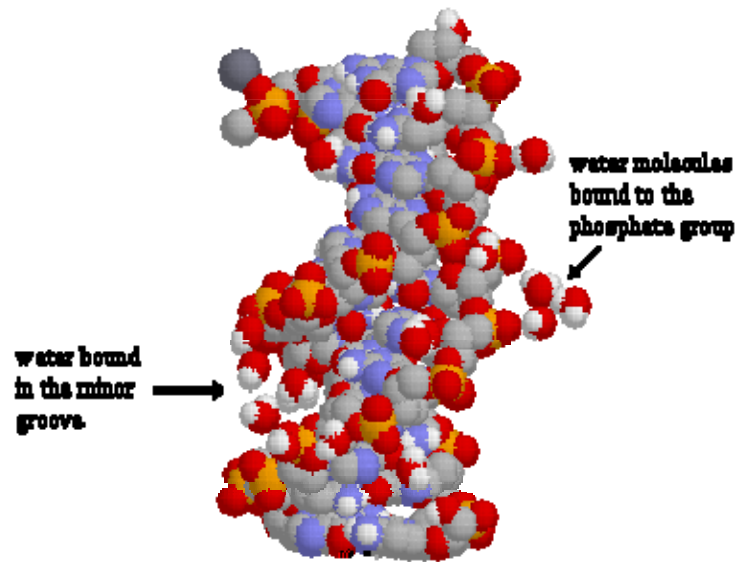
Die
Sekundärstruktur
wird über
Wasserstoff-
Brückenbindungen
stabilisiert



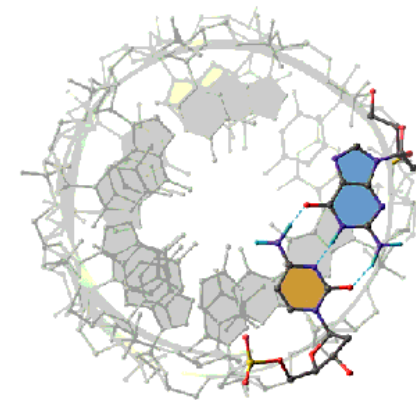
Neben der B-DNA gibt es weitere Helixformen



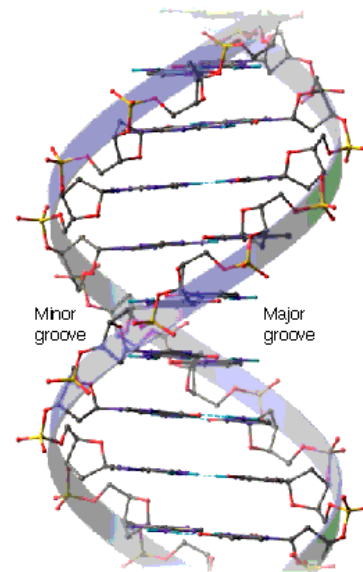
A- und B-Form unterscheiden sich deutlich



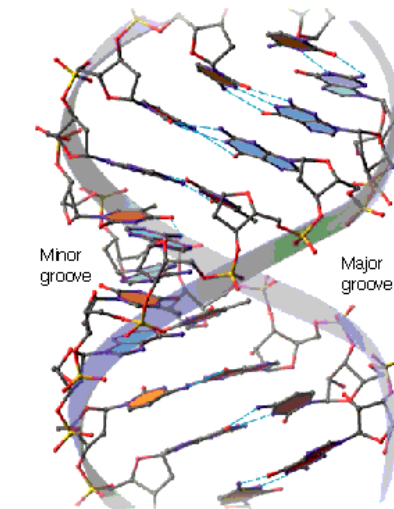
(a) B-DNA, end-on view



(c) A-DNA, end-on view

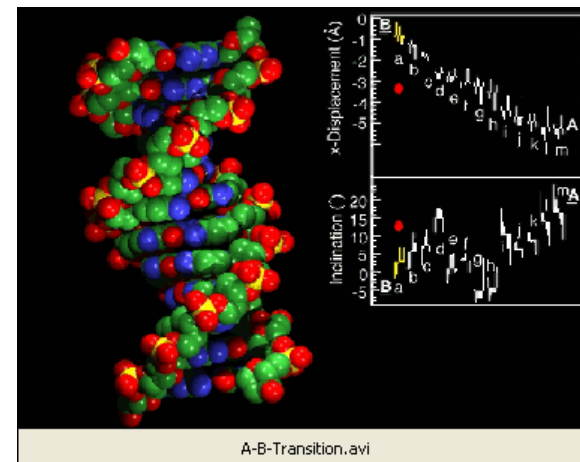
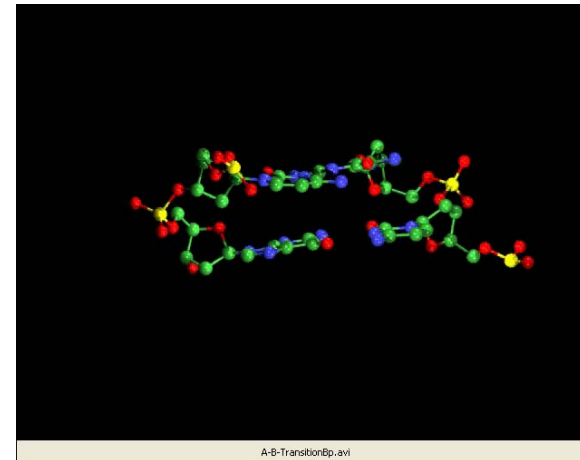
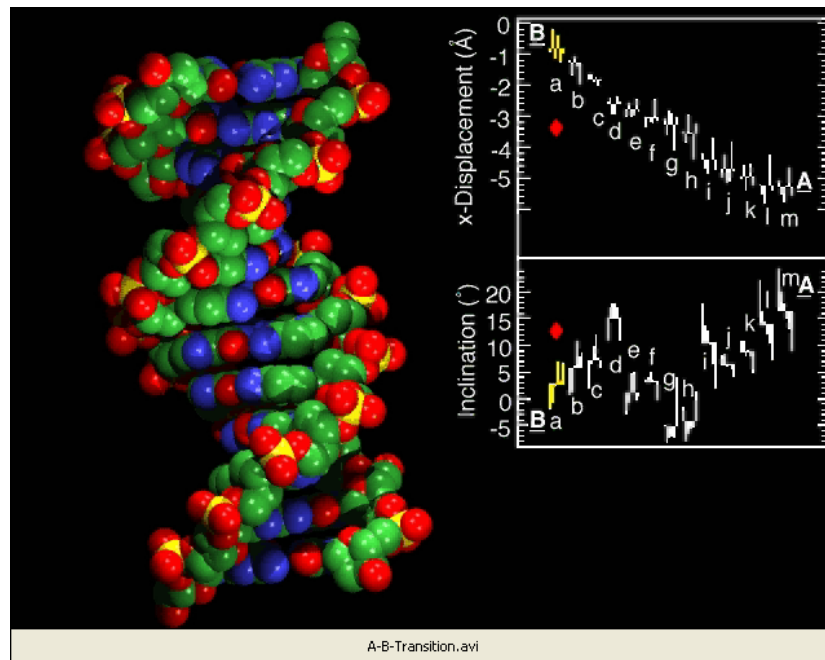


(b) B-DNA, side view

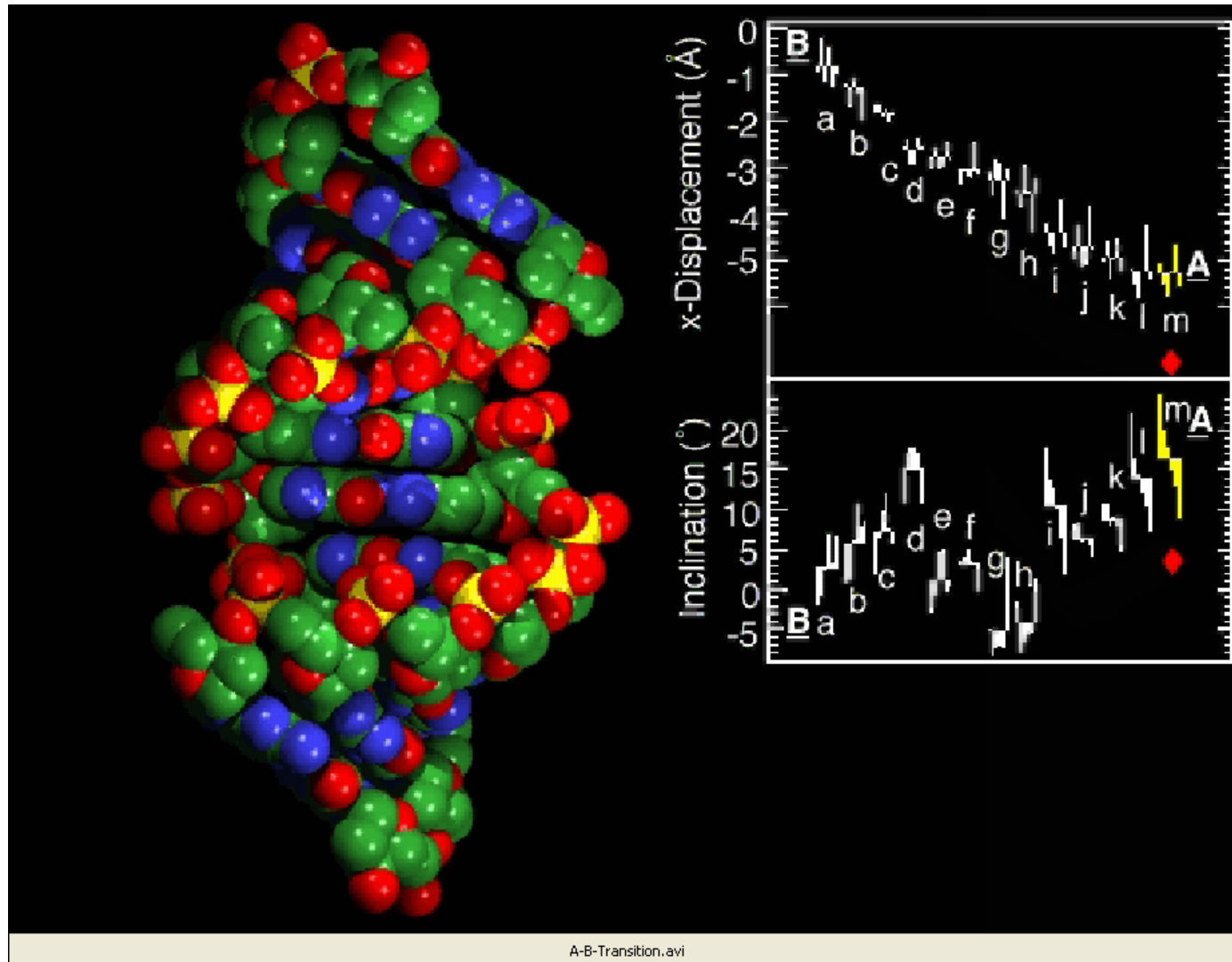


(d) A-DNA, side view

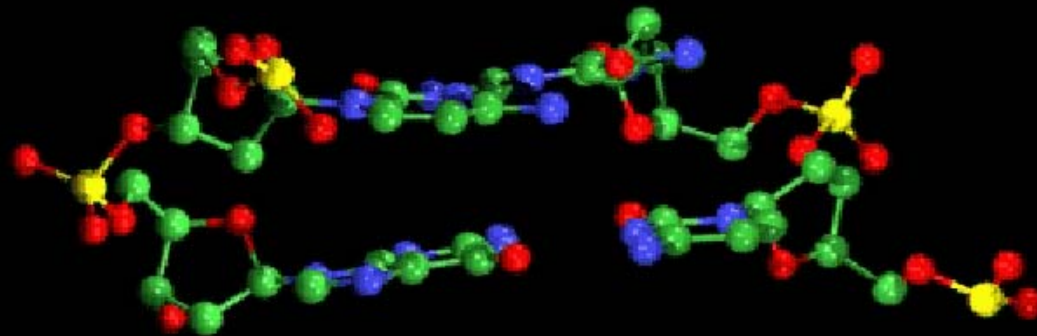
A- und B-Formen können sich ineinander umwandeln



A- und B-Formen können sich ineinander umwandeln



dabei ändert sich die Zuckerkonformation



Ribose „passt“ nicht in die B-Helix

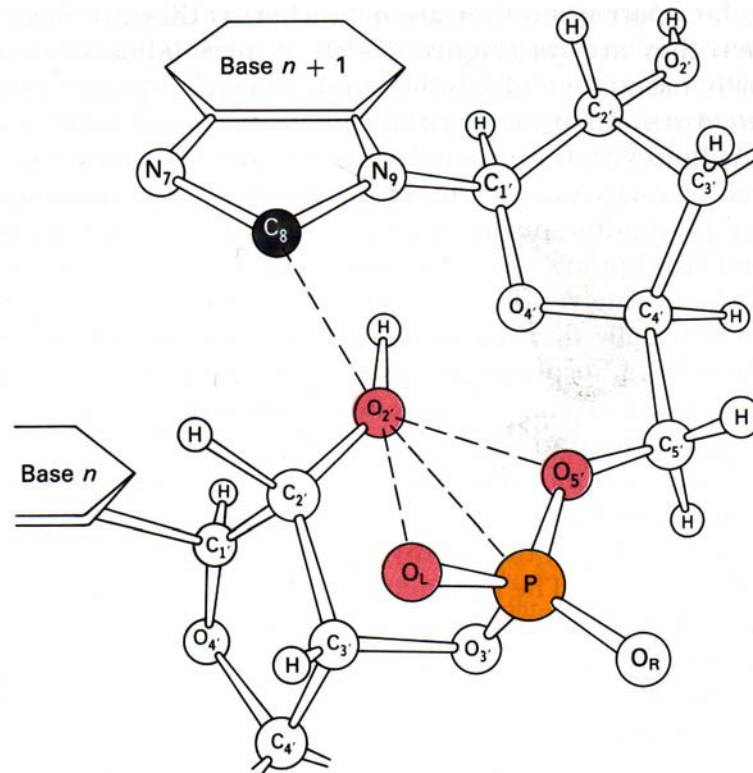
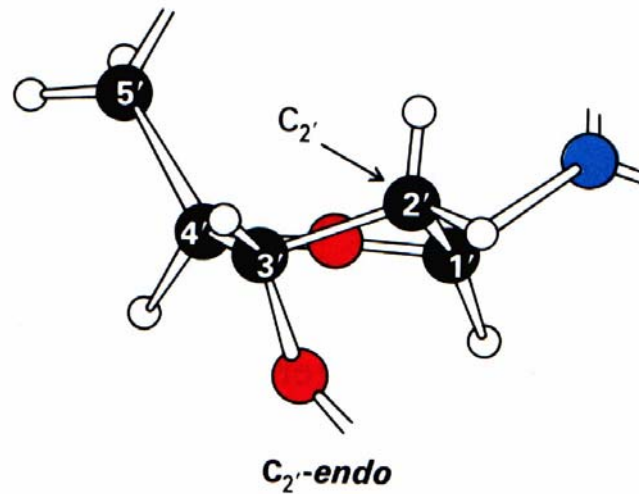


Figure 27-8

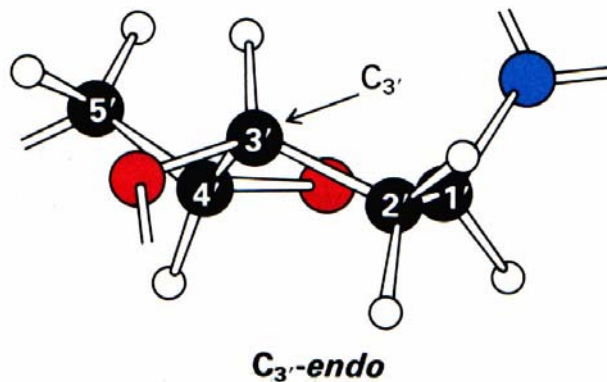
Ribose sugars do not fit into a helix of the B-DNA type because there is insufficient room for the 2'-oxygen atom. Four contacts would be closer than the allowed van der Waals distance. [After R. E. Dickerson. The DNA helix and how it is read. Copyright © 1983 by Scientific American, Inc. All rights reserved.]

In A- und B-Form ist die
Zuckerkonformation verschieden

B-Form



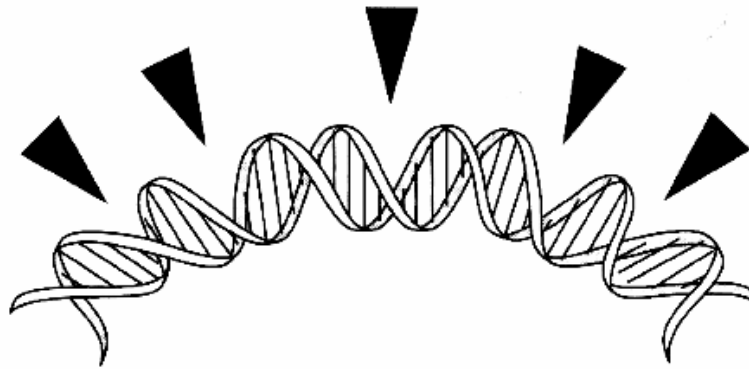
A-Form



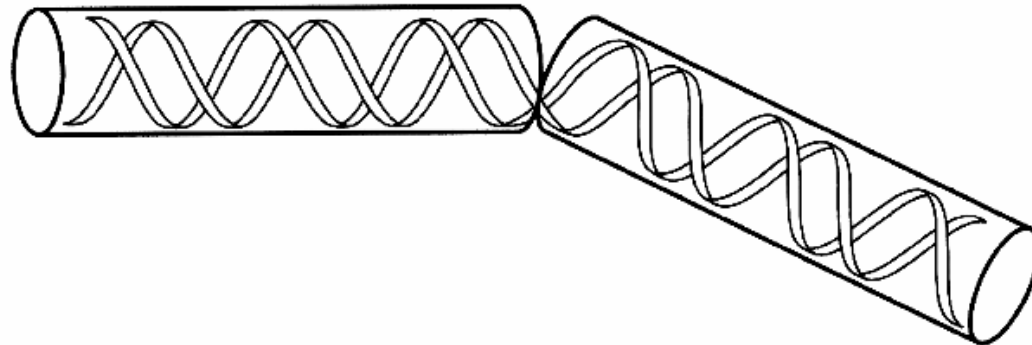
Die B-Helix kann Superstrukturen ausbilden

z. B. gekrümmte DNA

Wedge Model



Junction Model



Die B-Helix kann Superstrukturen ausbilden

z. B. gekrümmte DNA

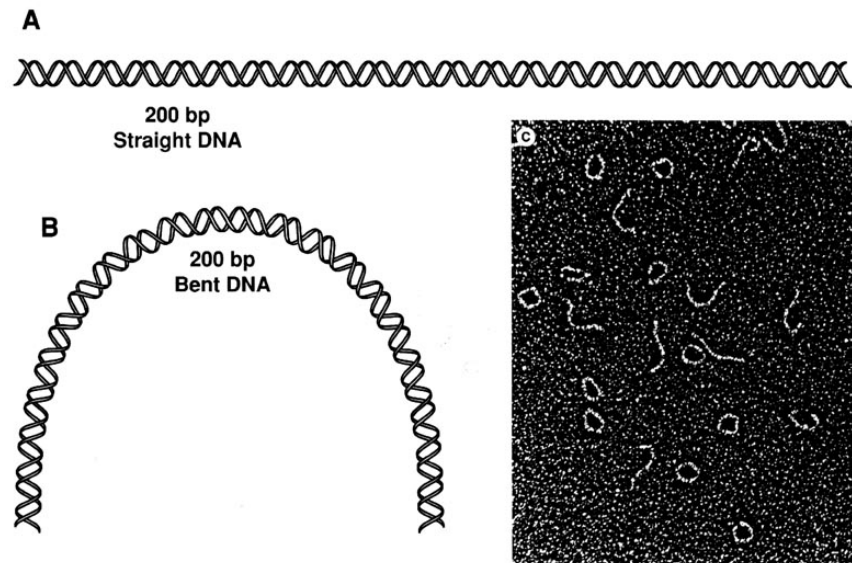
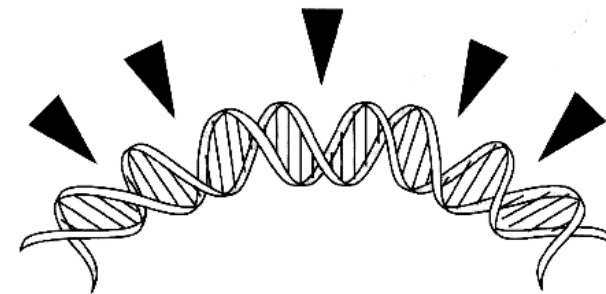
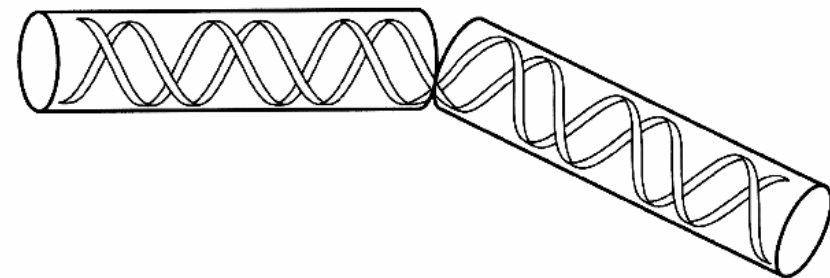


Figure 2.1 Straight DNA and bent DNA. (A) A representation of a 200-bp straight DNA molecule. The persistence length of DNA is 150–200 base pairs. Persistence length is the length of DNA that resists easy deformation, or a length that is not considered very flexible. DNA smaller than its persistence length cannot be easily bent into a circle. (B) A 200-bp piece of bent DNA is shown. Some DNAs, because of their primary base sequence, will adopt a stable bent or curved configuration. (C) The electron micrograph shows a 223-bp fragment of *Crithidia fasciculata* DNA containing 18 phased A₄ to A₆ tracts. Although the fragment is linear and is blunt ended, many molecules appear to be intact circles. Without the phased A tracts, the DNA would appear as linear, relatively straight molecules. Courtesy of Jack D. Griffith.

Wedge Model



Junction Model



Superstrukturen entstehen durch unplanare Basenstellungen



Tilt



Roll

Figure 2.5 Tilt and roll wedges. As discussed in Chapter 1, different angles of deformation are possible when one base stacks on another. One angle is the *tilt angle*, which is in the direction of hydrogen bonding. Another is the *roll angle*, which occurs at 90° to the direction of hydrogen bonding. The tilt angle opens in the direction of the phosphate backbone whereas roll can open toward either the major or the minor groove.

Superstrukturen entstehen durch unplanare Basenstellungen

Basen können einen „Propeller Twist“ zeigen

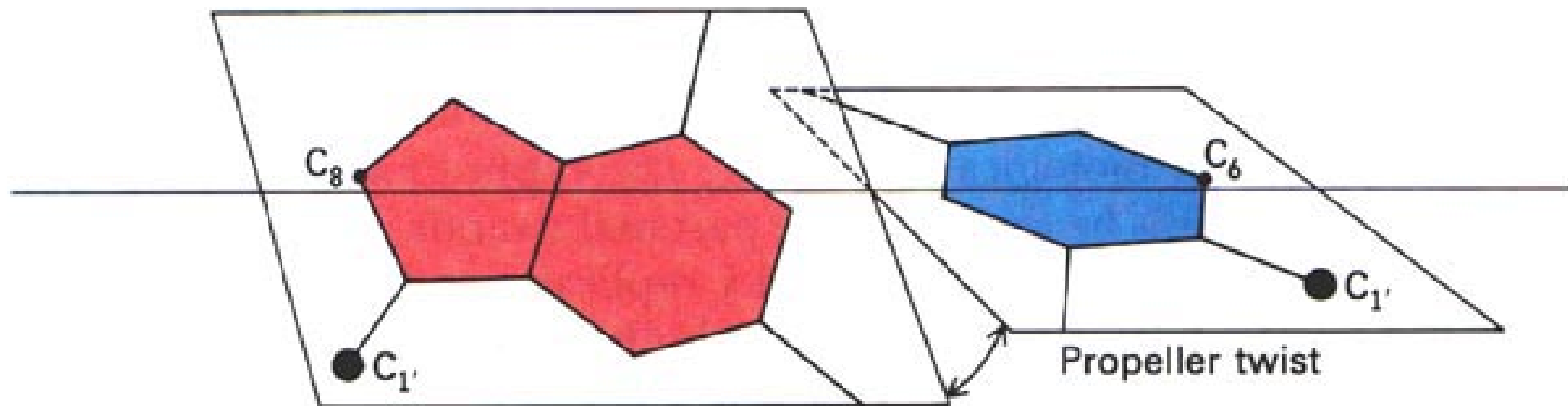


Figure 27-3

The bases of a DNA base pair are not perfectly coplanar. They are twisted with respect to each other, like the blades of a propeller.

Die Basen
können
relativ zum
Zucker in
Syn-oder
Anti-Stellung
sein

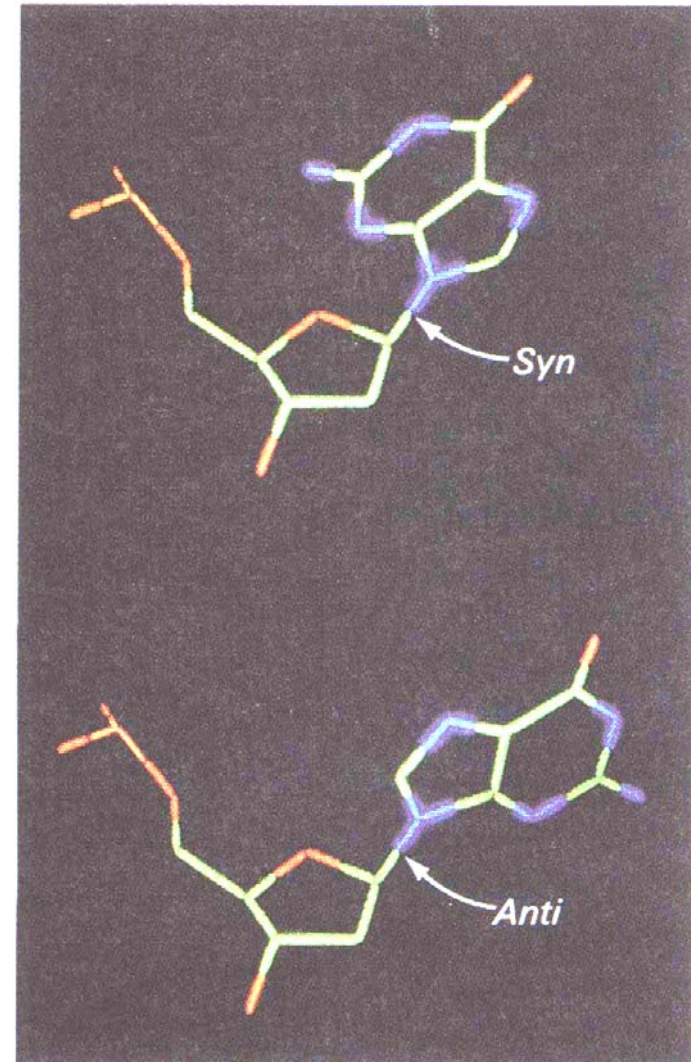
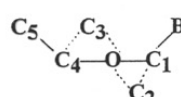


Figure 27-10

The glycosidic bond is *syn* when the base and sugar are on the same side, whereas it is *anti* when they are opposite one another.

Verschiedene Helixformen unterscheiden sich in ihren räumlichen Dimensionen

	A	B	C	Z
Helixdrehung	rechts	→	→	links
Basen pro Windung	11	10,4	9,3	12
Höhe einer Windung	28 Å	34 Å	31 Å	45 Å
Ø Helix	23 Å	19 Å	19 Å	18 Å
Drehung zwischen Bp	33 °	36°	38°	- 30°
Neigung der Bp gegenüber Molekülachse	70°	90°		95°
Zuckerkonformation	C3'endo 	C2'endo	C2'endo	G : C 2'endo C : C 3'endo
Orientierung der Base um glykosid. Bindung	anti	anti	anti	G : syn (Base dem Zucker zugekehrt) C : anti
	<ul style="list-style-type: none"> • bei geringer Hydratisierung • in RNA- bzw. RNA/DNA-Helium • Basenpaare randständig • Bp-Anstellwinkel von 20° => G:U Paarung 	<ul style="list-style-type: none"> • <u>physiolog.</u> • Achse durch Bp 	<ul style="list-style-type: none"> • <u>nicht physiolog.!</u> 	<ul style="list-style-type: none"> • bei poly d (GC) (CG) und best. Salzgehalt • Bp randständig

Weitere Helixformen:

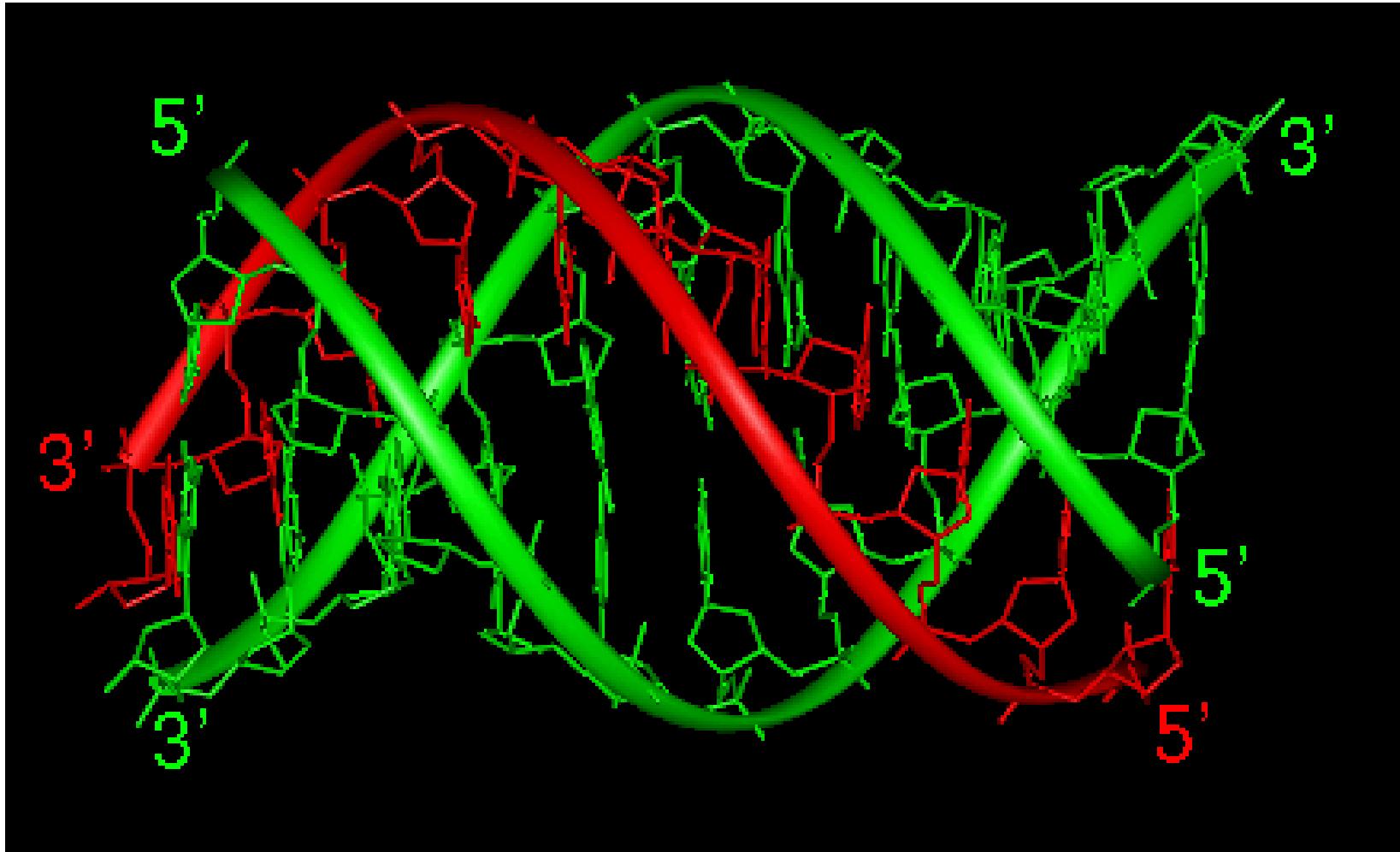


Triple helix



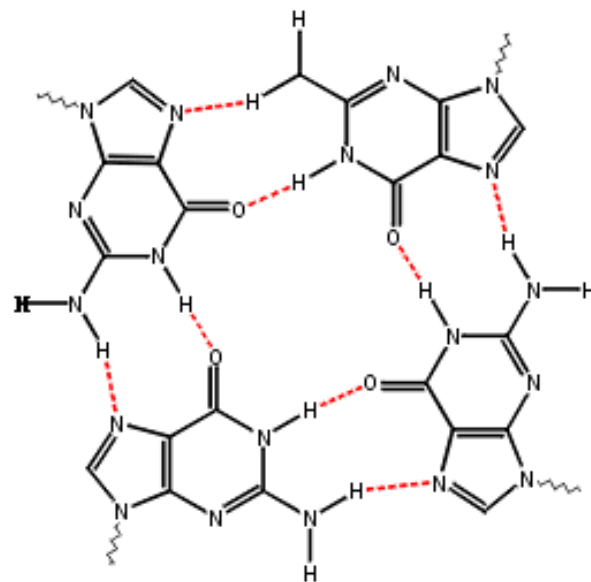
H-DNA. A sequence of alternating T and C residues can

Weitere Helixformen: Triple-Helix

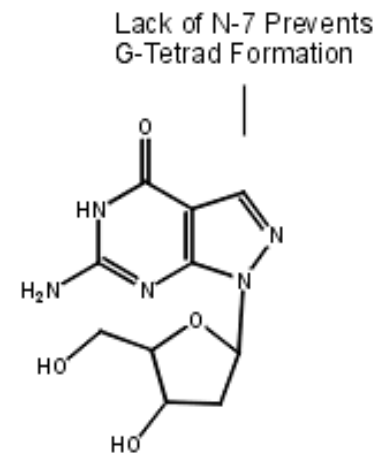


Weitere Helixformen: Quadruplex-Helix

Modified Bases – Super G™

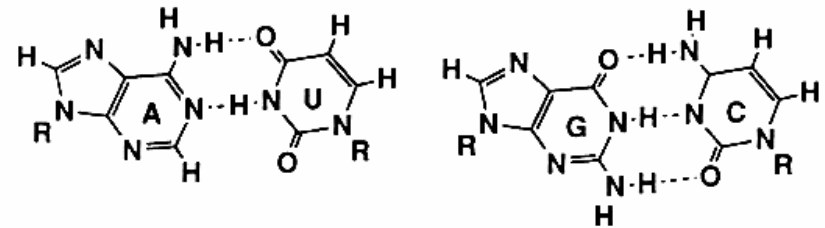


G-Tetrad

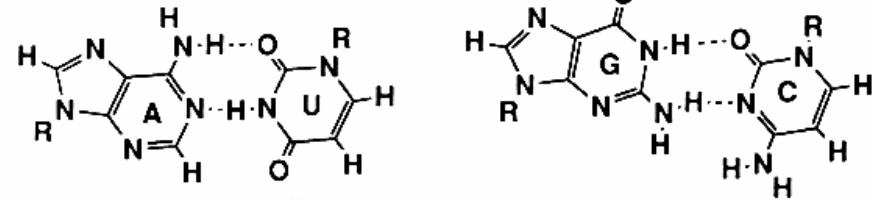


Super G™

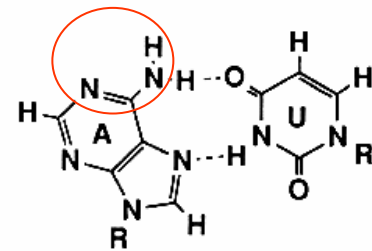
Watson/Crick und Hoogsteen- Basenpaarung



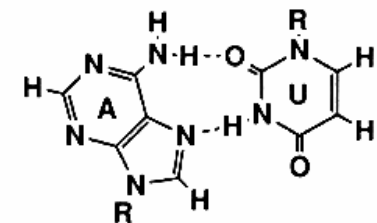
Watson-Crick



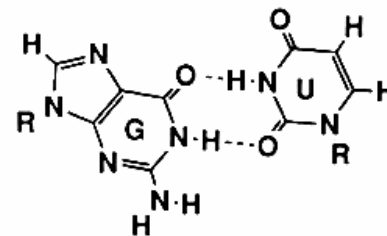
Reverse Watson-Crick



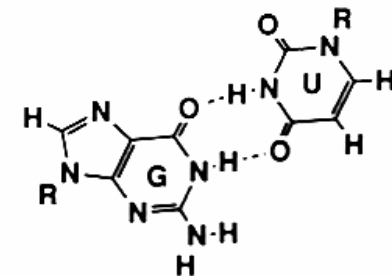
A-U Hoogsteen



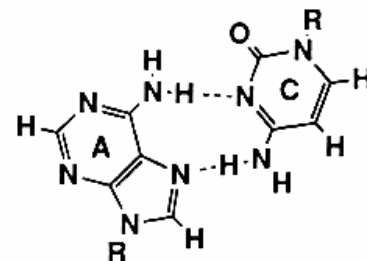
A-U Reverse Hoogsteen



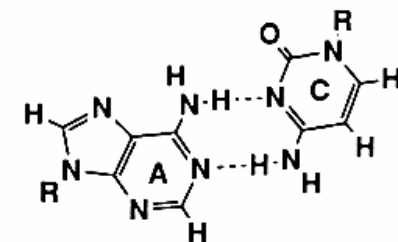
G-U Wobble



G-U Reverse Wobble

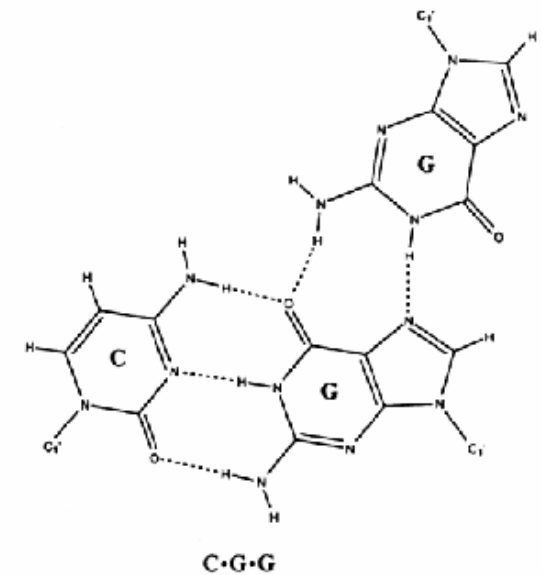
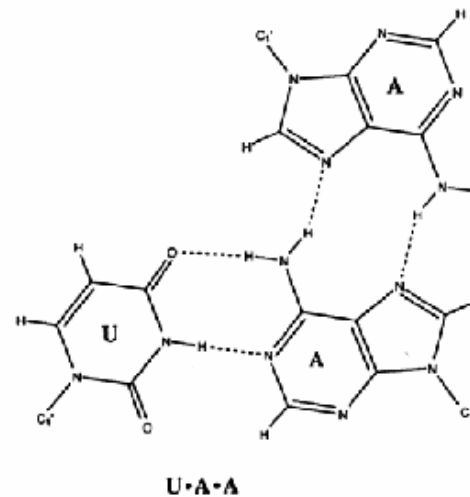
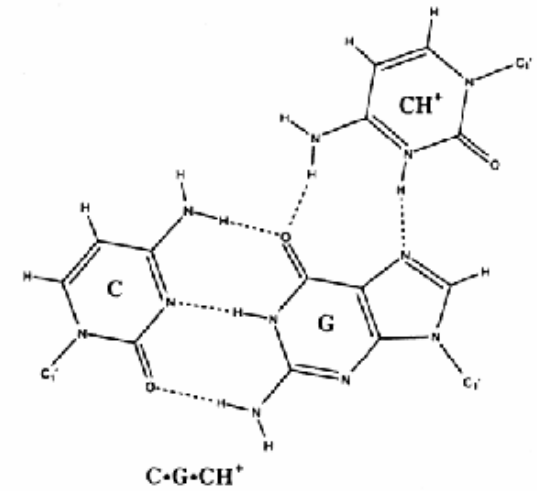
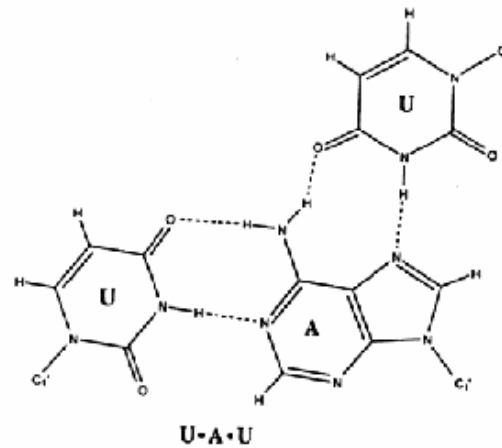


A-C Reverse Hoogsteen

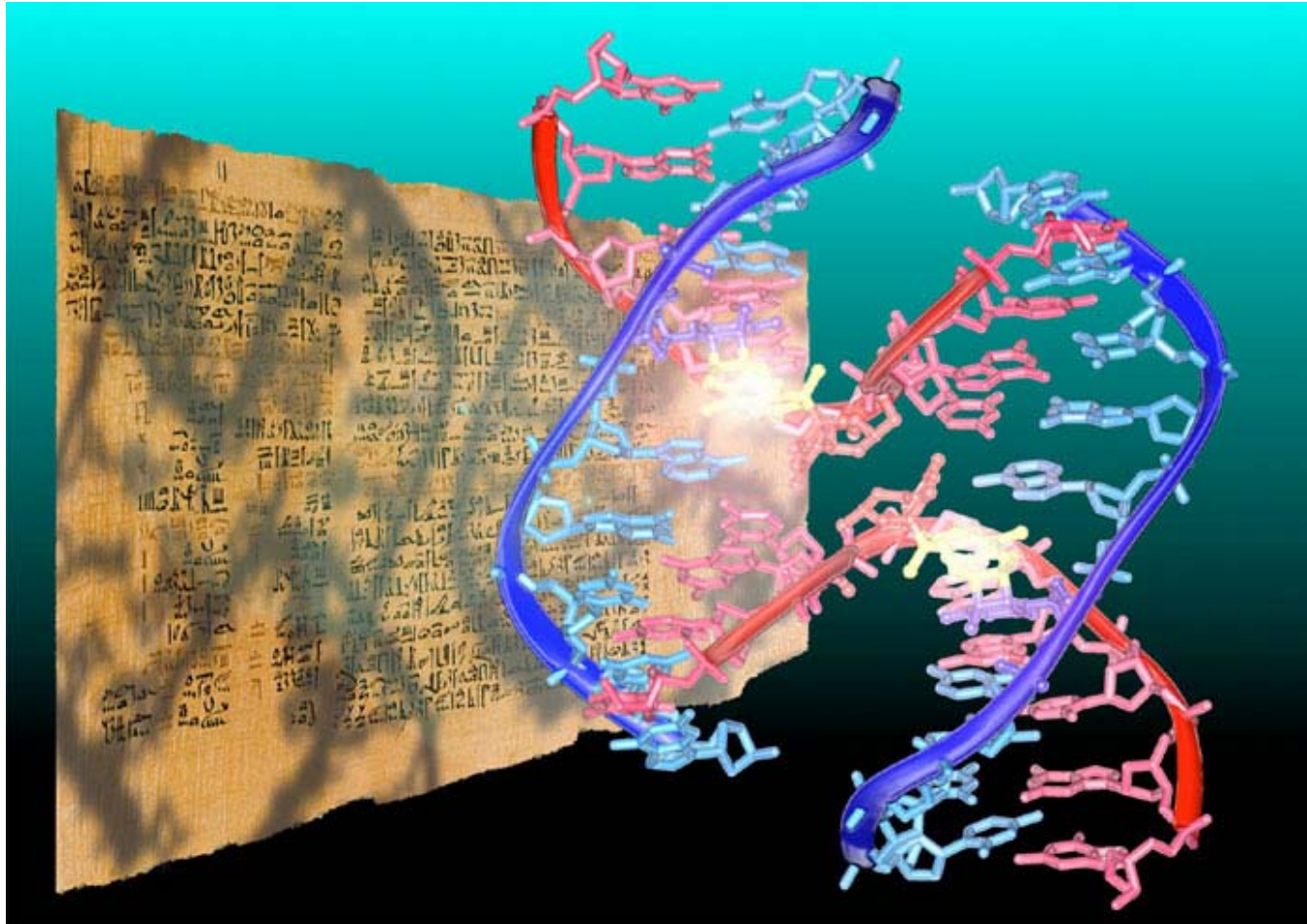


A-C Reverse Wobble

Watson/Crick und Hoogsteen- Basenpaarung

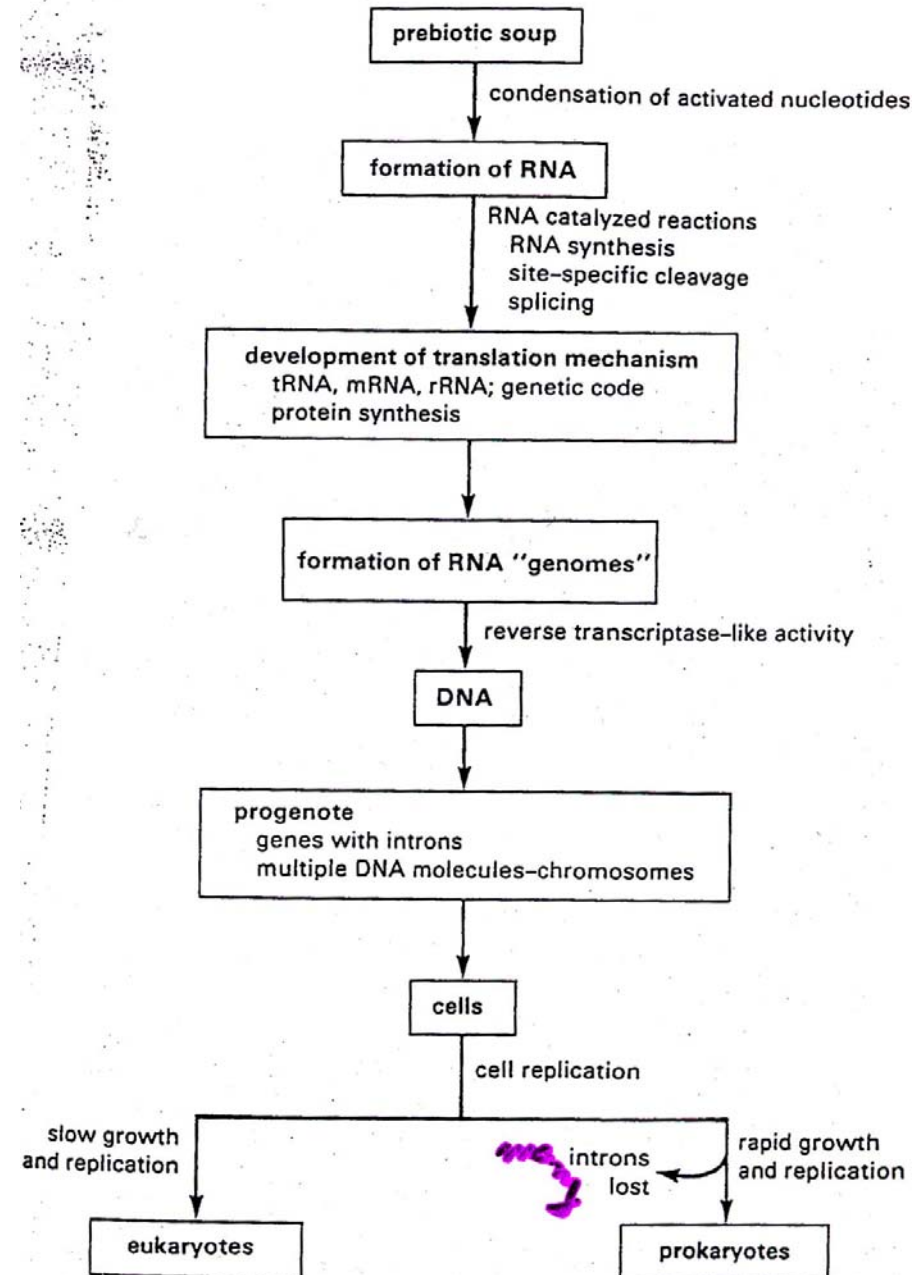


Weitere Helixformen: Vierstrang-DNA (Holliday-Junction)



Psoralen is one of the oldest known chemotherapeutic drugs--the use of a psoralen containing Nile weed to treat skin ailments was described in Ebers papyrus, an early Egyptian medical text published around 1550 BC (the front page is reproduced here). The drug photocrosslinks DNA and is shown in this figure to induce the formation of a four-stranded Holliday junction (reference, Eichman, *et al.*, *J. Mol. Biol.*, 2001, **308**: 15-26).

Von der Präbiotik über eine RNA-Welt zur DNA-Welt



Typische Unterschiede zwischen Pro- und Eukaryoten

- Kein Zellkern
- Keine Organellen
- Gene in Operons
- Polycistronische mRNA
- 5s, 13s + 18s rRNA-Gene geclustered
- Gene ohne Introns
- Keine 5,8s rRNA
- Nur eine RNA-Polymerase
- N-formyl-Methionin als Start-Aminosäure
- mRNA nicht polyadenyliert

- Zellkern mit Membran
- Mitochondrien/Plastiden
- Keine Operons
- monocistronische mRNA
- 5s und 18/28s RNA-Gene nicht gekoppelt
- Gene meist mit Introns
- 5,8 sRNA vorhanden
- 3 verschiedene RNA-Polymerasen
- Methionin als Start-Aminosäure
- mRNA fast immer polyadenyliert

Weitere Unterschiede zwischen Pro- und Eukaryoten

Trait	Eukaryotes	Eubacteria
genome	several linear chromosomes	one circular chromosome
operons	no	yes
rRNA genes	18S, 5.8S, 28S transcriptionally linked; 5S unlinked	16S, 23S, 5S transcriptionally linked
introns	yes, in protein rRNA and tRNA genes	not as far as we know
promoters	„TATA“ boxes for RNA polymerase II	-35 region and „Pribnow box“
RNA polymerases	three, for three gene classes	one, but developmentally variable

capped mRNAs	yes	no
5' mRNA leaders	usually present, no Shine Dalgarno sequence	present, Shine Dalgarno sequence
3' poly A tails	usually present and stable	absent or unstable
ribosome size	80S	70S
rRNA size	18S, 28S, 5.8S, 5S	16S, 23S, 5S
ribosome antibiotic sensitivity	cycloheximide and others	chloramphenicol and others
diphtheria toxin sensitivity	yes	no
initiator tRNA	methionine	formylmethionine

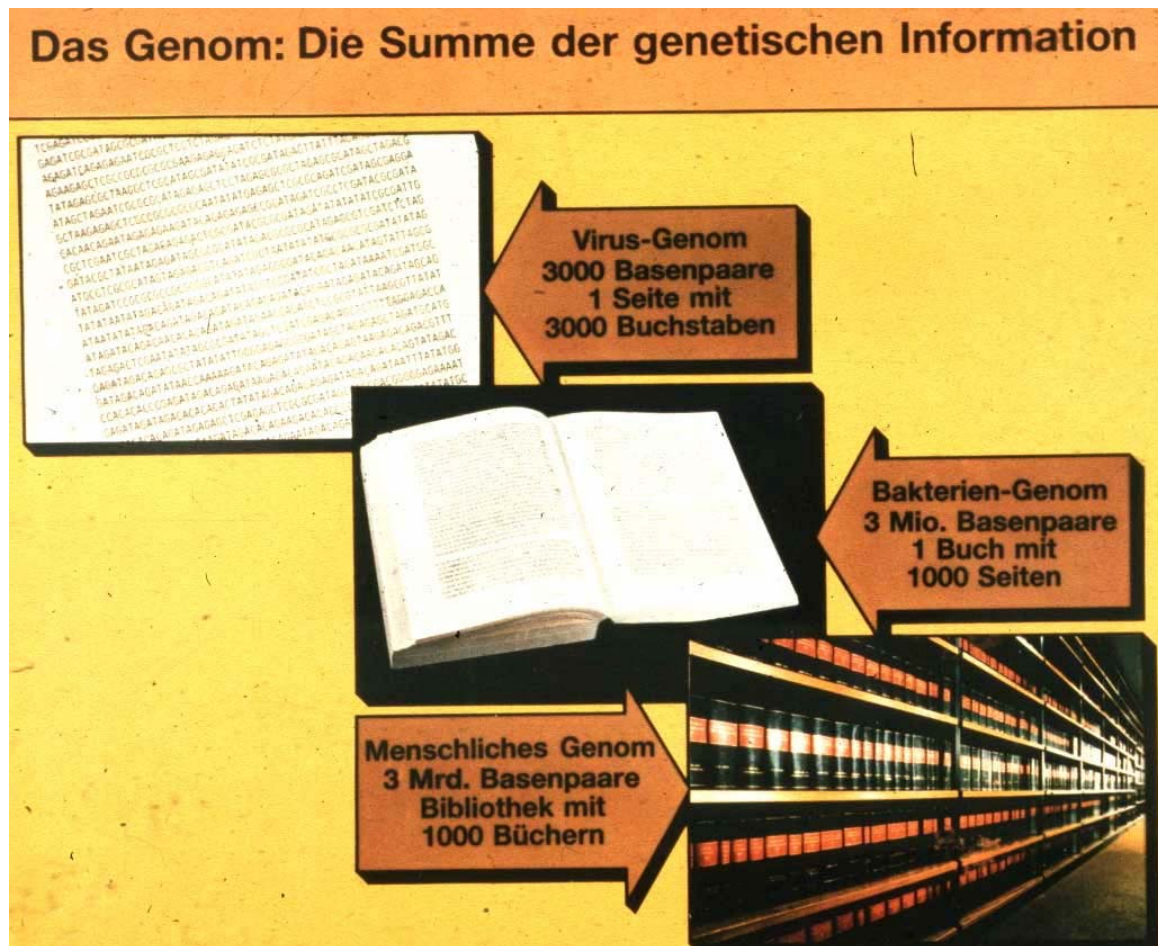
Weitere Unterschiede zwischen Pro- und Eukaryoten

Molecular Biological Differences between Eukaryotes and Prokaryotes

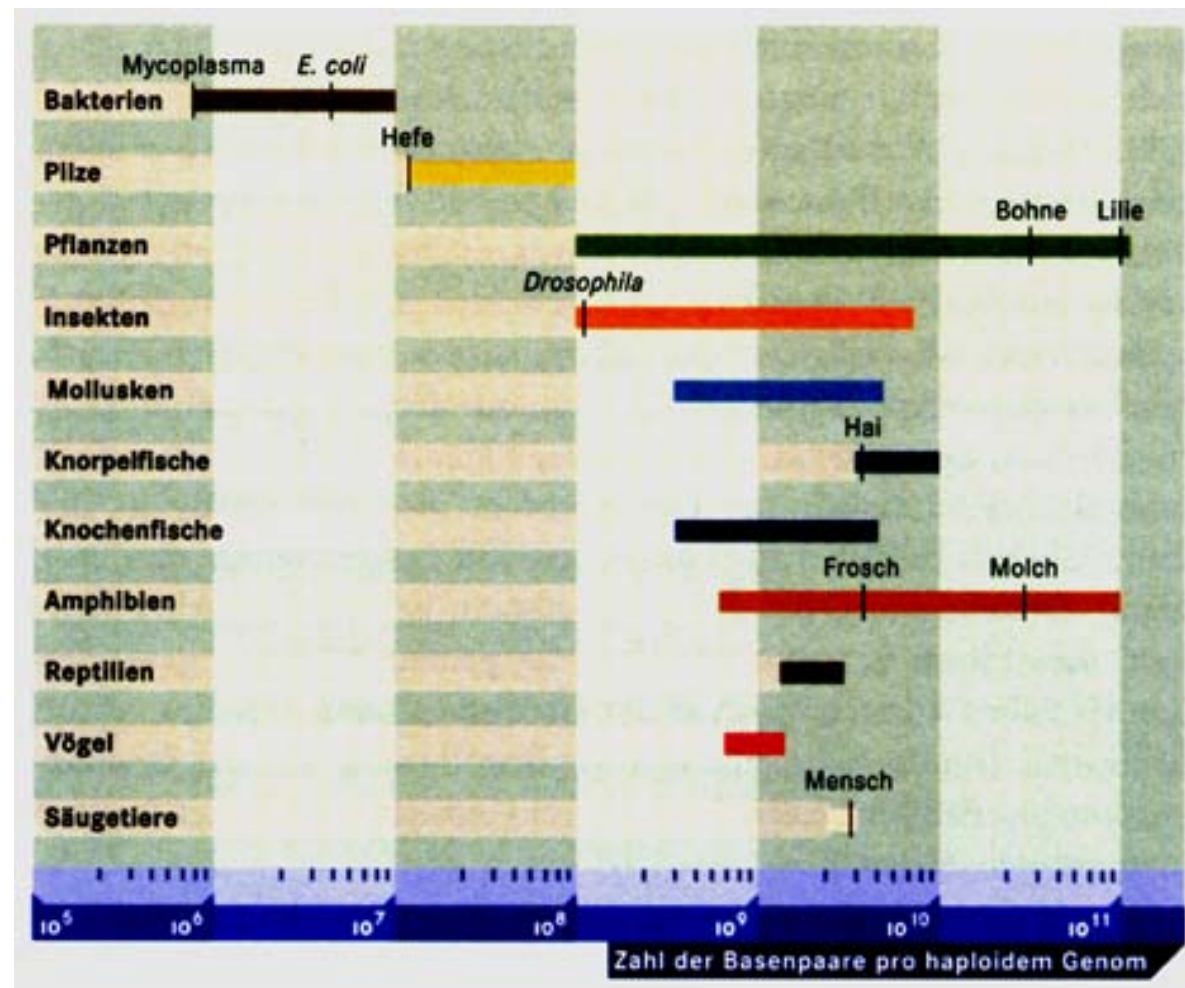
Trait	Eukaryotes	Eubacteria
genome	several linear chromosomes	one circular chromosome
operons	no	yes
rRNA genes	18S, 5.8S, 28S transcriptionally linked; 5S unlinked	16S, 23S, 5S transcriptionally linked
introns	yes, in protein rRNA and tRNA genes	not as far as we know
promoters	„TATA“ boxes for RNA polymerase II	-35 region and „Pribnow box“
RNA polymerases	three, for three gene classes	one, but developmentally variable

capped mRNAs	yes	no
5' mRNA leaders	usually present, no Shine Dalgarno sequence	present, Shine Dalgarno sequence
3' poly A tails	usually present and stable	absent or unstable
ribosome size	80S	70S
rRNA size	18S, 28S, 5.8S, 5S	16S, 23S, 5S
ribosome antibiotic sensitivity	cycloheximide and others	chloramphenicol and others
diphtheria toxin sensitivity	yes	no
initiator tRNA	methionine	formylmethionine

Ein weiterer wesentlicher Unterschied zwischen Pro- und Eukaryoten: die Genomgröße



Ein weiterer wesentlicher Unterschied zwischen Pro- und Eukaryoten: die Genomgröße



Die „Genomgröße“ entspricht der gesamten DNA einer haploiden Zelle

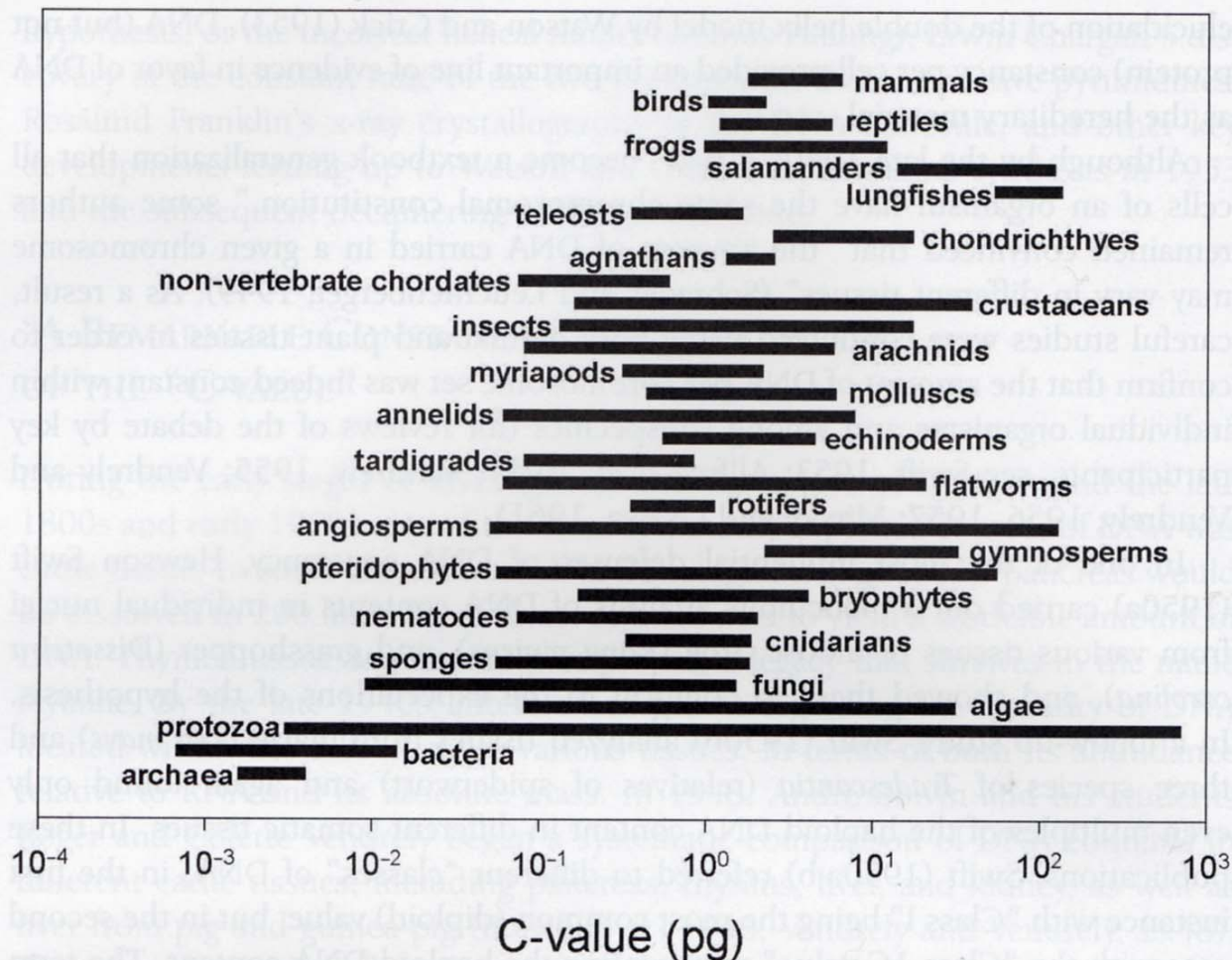
Die Genomgröße wird im Allgemeinen in Basenpaaren angegeben

Ein Basenpaar hat ein durchschnittliche Molmasse von ca. 660 Dalton

Daraus folgt, ein Basenpaar wiegt ca. $1,1 \cdot 10^{-21} \text{g}$,
Oder 1 pg (picogramm) DNA = ca. 0,97 E9 Bp

Das C-Wert-Paradoxon: Organismen mit ähnlicher Komplexität haben oft stark verschieden große Genome

aus: R. Gregory/ The Evolution of the Genome



Das C-Wert-Paradoxon bei Pflanzen:

TABLE 2.2 Some well-known representative species showing the range of 1C DNA amounts in angiosperms

Species	Common name	Chromosome number (2n)	Ploidy level (x)	1C DNA amount	
				pg	Mb ^a
<i>Arabidopsis thaliana</i>	Thale cress	10	2	0.16	157
<i>Oryza sativa</i>	Rice	24	2	0.50	490
<i>Lycopersicon esculentum</i>	Tomato	24	2	1.00	980
<i>Glycine max</i>	Soybean	40	2	1.10	1078
<i>Zea mays</i>	Maize	20	2		
Seneca 60 line				2.50	2450
Zapalote Chico line				3.40	3332
<i>Hordeum vulgare</i>	Barley	14	2	5.55	5400
<i>Secale cereale</i>	Rye	14	2	8.28	8110
<i>Vicia faba</i>	Bean	12	2	13.33	13,060
<i>Allium cepa</i>	Onion	14	2	16.75	16,415
<i>Triticum aestivum</i>	Wheat	42	6	17.32	16,970
<i>Lilium longiflorum</i>	Easter lily	24	2	35.20	34,500
<i>Fritillaria assyriaca</i>	Fritillaria	48	4	127.40	124,850

^a1 pg \approx 980 Mb (see Chapter 1).

Das C-Wert-Paradoxon bei Tieren:

Taxon	C-value range (pg)	Mean C-value (pg)	Species studied
VERTEBRATES			
Jawless fishes	1.3–4.6	2.3	17 (16%)
Cartilaginous fishes	2.7–17.1	5.7	126 (12%)
Lungfishes	50–133	90.4	4 (66%)
Chondrostean fishes	1.5–6.5	3.3	17 (68%)
Teleost fishes	0.4–4.4	1.2	1050 (4%)
Amphibians	0.95–120	16.2	413 (7%)
Reptiles	1.1–5.4	2.3	303 (4%)
Birds	1.0–2.2	1.4	172 (2%)
Mammals	1.7–8.4	3.5	358 (7%)
INVERTEBRATES			
Insects	0.1–16.9	1.6	402 (0.05%)
Crustaceans	0.16–38.0	3.2	230 (0.6%)
Arachnids	0.08–5.7	2.4	118 (0.2%)
Molluscs	0.4–5.9	1.8	174 (0.2%)
Echinoderms	0.5–4.4	1.4	45 (0.9%)
Annelids	0.06–7.6	1.5	121 (1%)
Flatworms	0.06–20.5	2.2	61 (0.3%)
Nematodes	0.03–2.0	0.25	32 (0.3%)

aus: Gregory/ The Evolution of the Genome

Klassische Verfahren zur Analyse von Eukaryoten-DNA

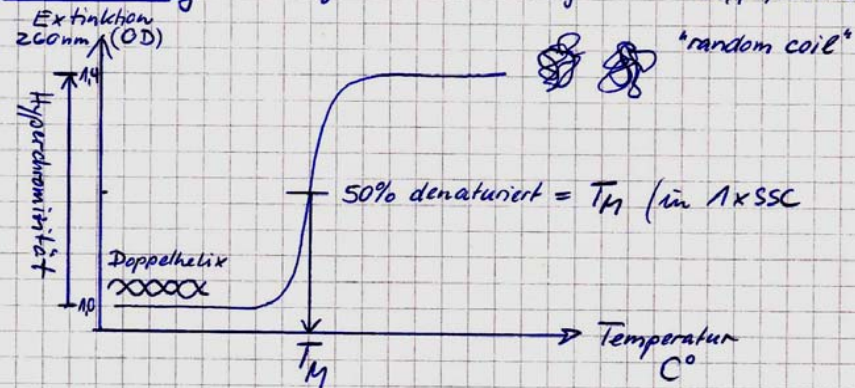
Die DNA-Schmelzanalyse:



Die DNA-Schmelzkurvenanalyse:

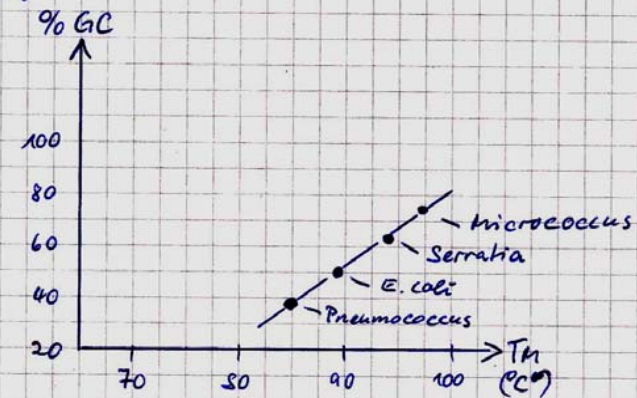
Denaturierung und Renaturierung von DNA

Denaturierung: Trennung der DNA-Einzelstränge durch Hitze, pH, Proteine u.a.



Die DNA-Schmelzkurvenanalyse:

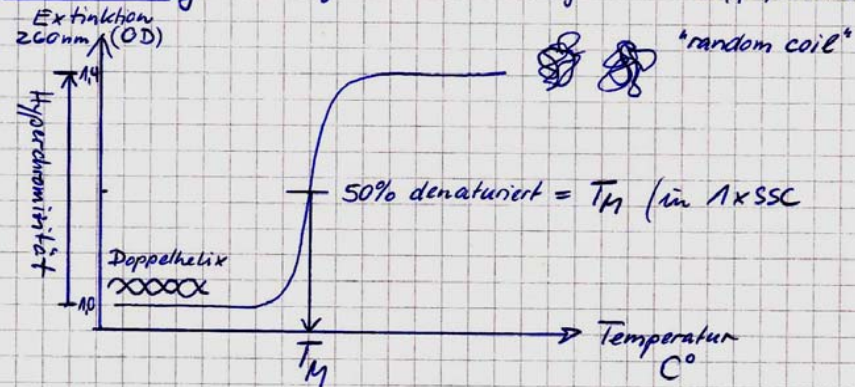
Abhängigkeit des T_m von der Basenzusammensetzung:



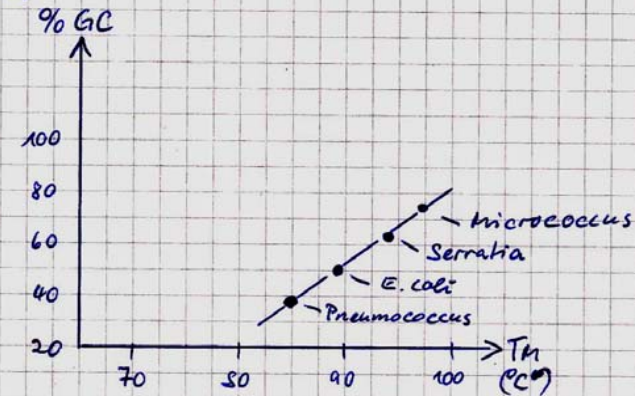
Die DNA-Schmelzkurvenanalyse:

Denaturierung und Renaturierung von DNA

Denaturierung: Trennung der DNA-Einzelstränge durch Hitze, pH, Proteine u.a.



Abhängigkeit des T_m von der Basenzusammensetzung:



$$T_m = 69,3 + 0,41 [G/C]$$

$$[G/C] = \frac{T_m - 69,3}{0,41}$$

} gilt für DNA in
SSC-Lösung

(Standard Salz-Citrat-Lösung
= 0,15M NaCl, 0,015M Na_3 Citrat)

Genome unterscheiden sich erheblich in der Basenzusammensetzung (GC-Gehalt)

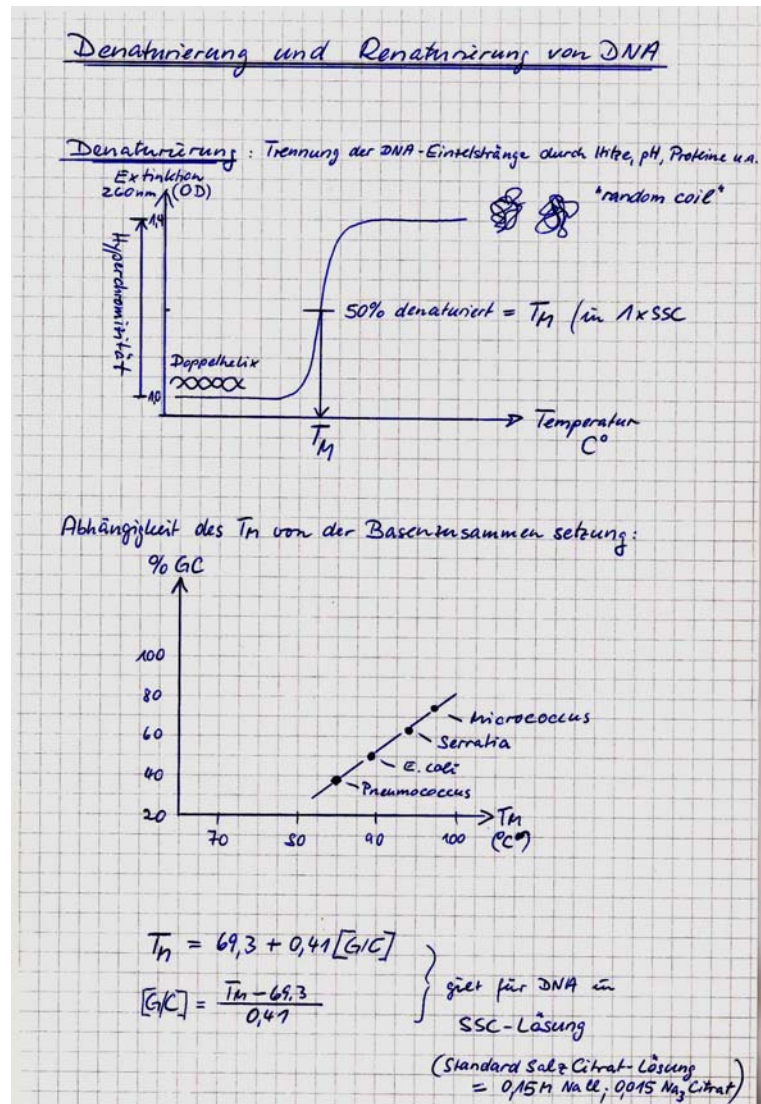
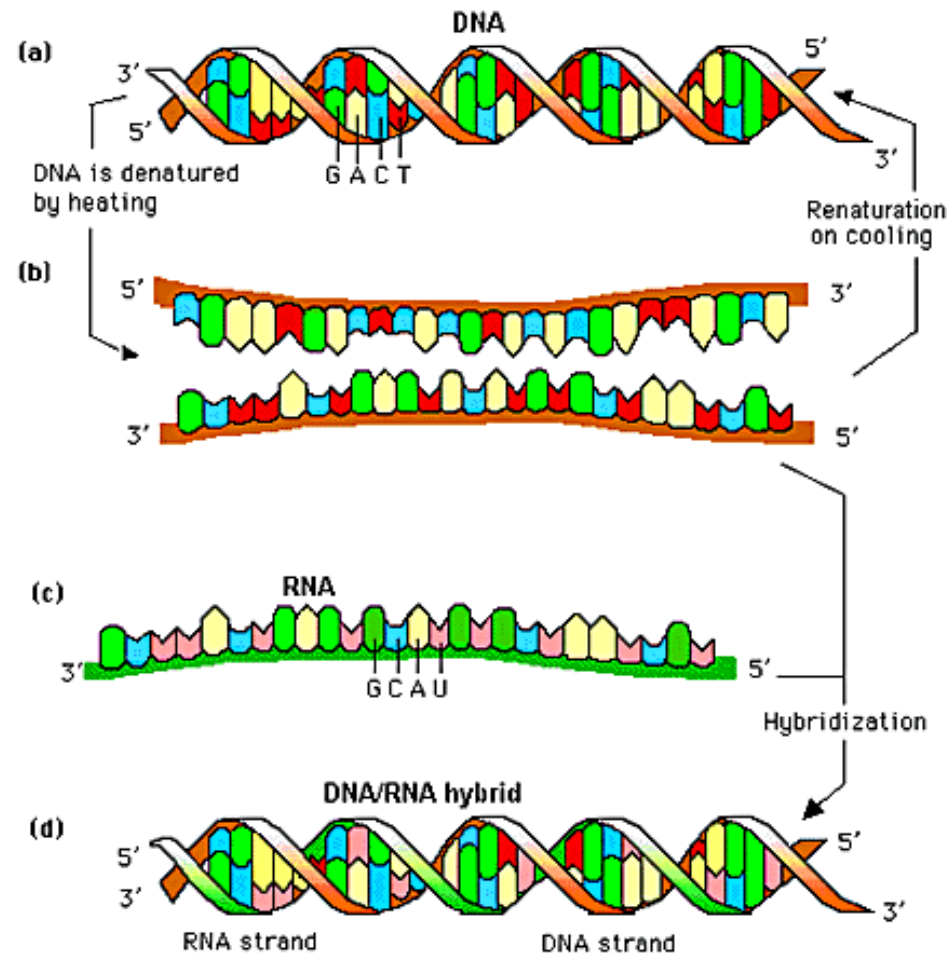


Table 6.3 Relative G + C Contents of Various DNAs

Sources of DNA	Percent (G + C)
Dictyostelium (slime mold)	22
Streptococcus pyogenes	34
Vaccinia virus	36
Bacillus cereus	37
B. megaterium	38
Hemophilus influenzae	39
Saccharomyces cerevisiae	39
Calf thymus	40
Rat liver	40
Bull sperm	41
Streptococcus pneumoniae	42
Wheat germ	43
Chicken liver	43
Mouse spleen	44
Salmon sperm	44
B. subtilis	44
T1 bacteriophage	46
Escherichia coli	51
T7 bacteriophage	51
T3 bacteriophage	53
Neurospora crassa	54
Pseudomonas aeruginosa	68
Sarcina lutea	72
Micrococcus lysodeikticus	72
Herpes simplex virus	72
Mycobacterium phlei	73

From Davidson, *The Biochemistry of the Nucleic Acids*, 8th ed., revised by Adams, et al.
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Denaturierte („geschmolzene“) DNA kann
„reassoziieren“ oder mit „hybridisieren“



Nucleic Acid Hybridization