

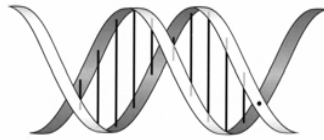
Multiple levels of chromatin structure compact DNA

10,000 fold

Packing ratio

Diameter

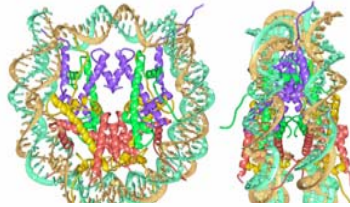
1nm



Double helical DNA

2nm

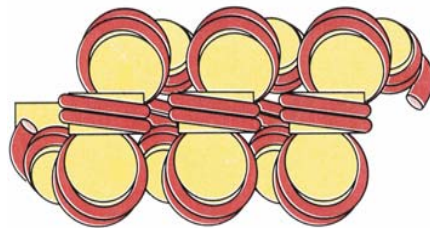
5



DNA is wrapped around histones to form nucleosomes

11nm

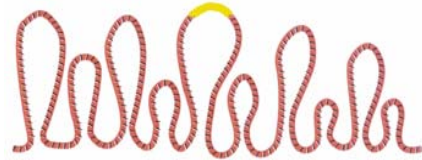
5



Nucleosomes condense to form chromatin fibres

30nm

80



30nm fibres are arranged in loops.

300nm

5



These loops are may be arranged in another spiral to form chromosomes

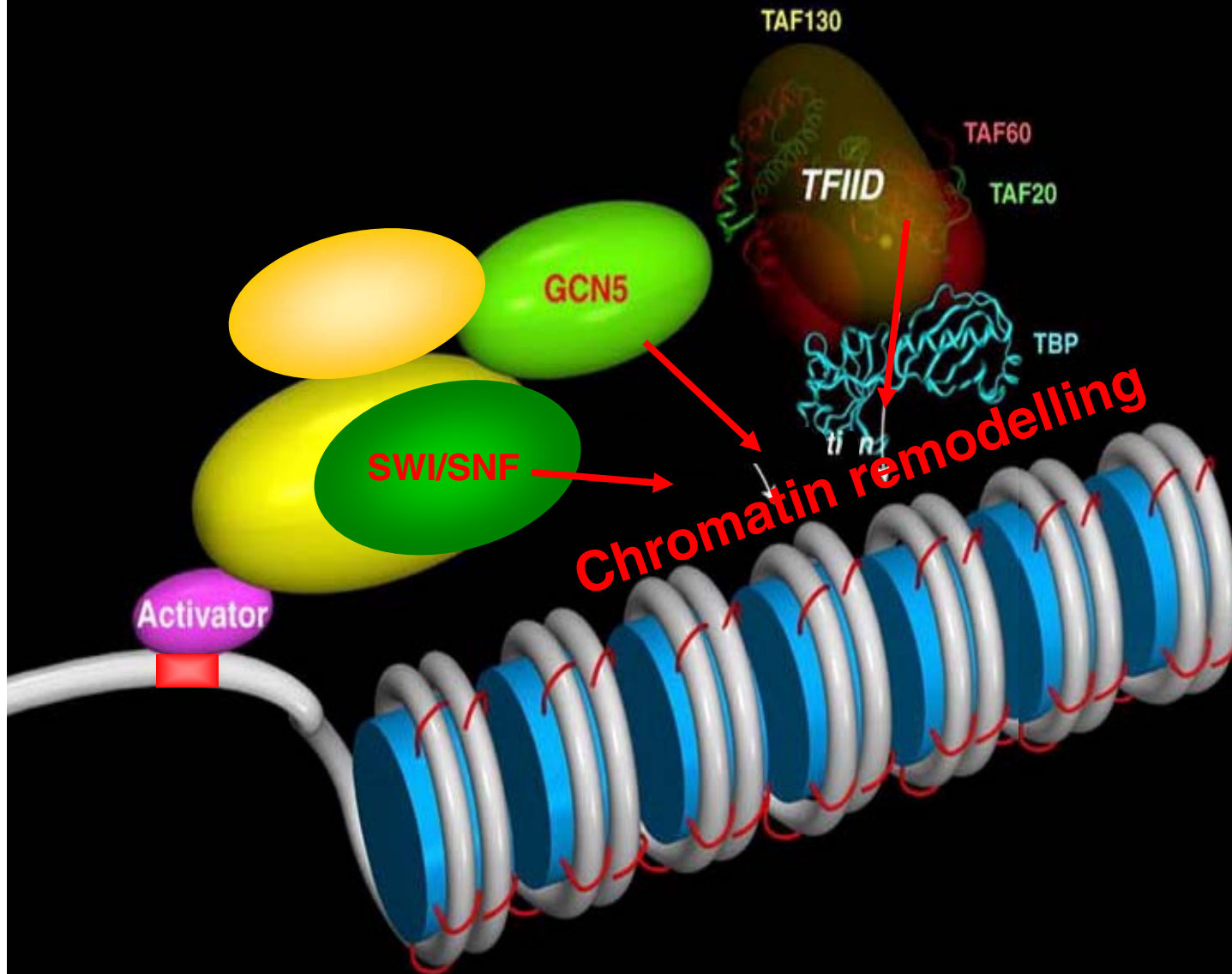
700nm

2

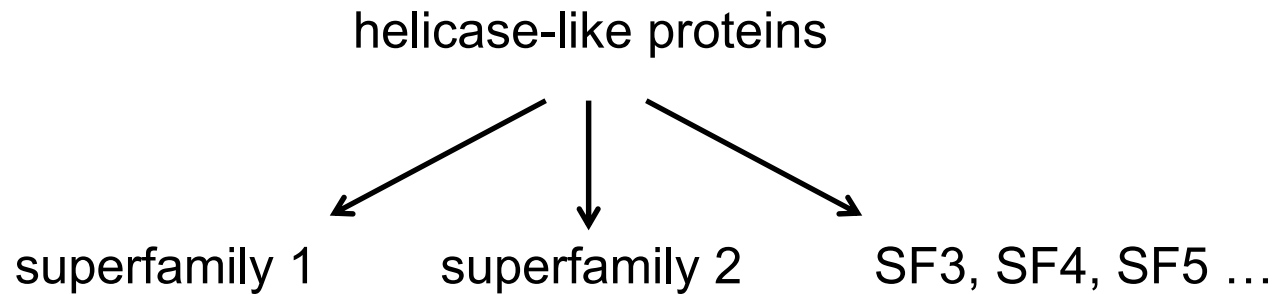


Each individual has 23 pairs of chromosomes

Chromatin remodelling is an important step during gene regulation.

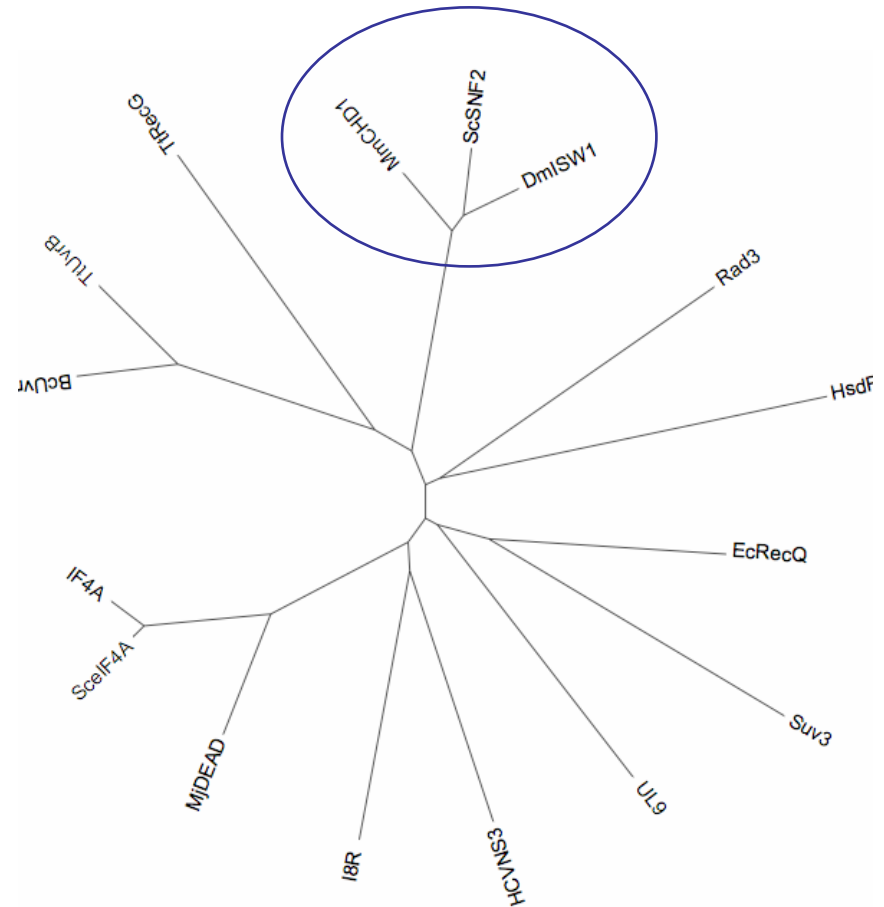


superfamily 2 is part of the helicase-like proteins

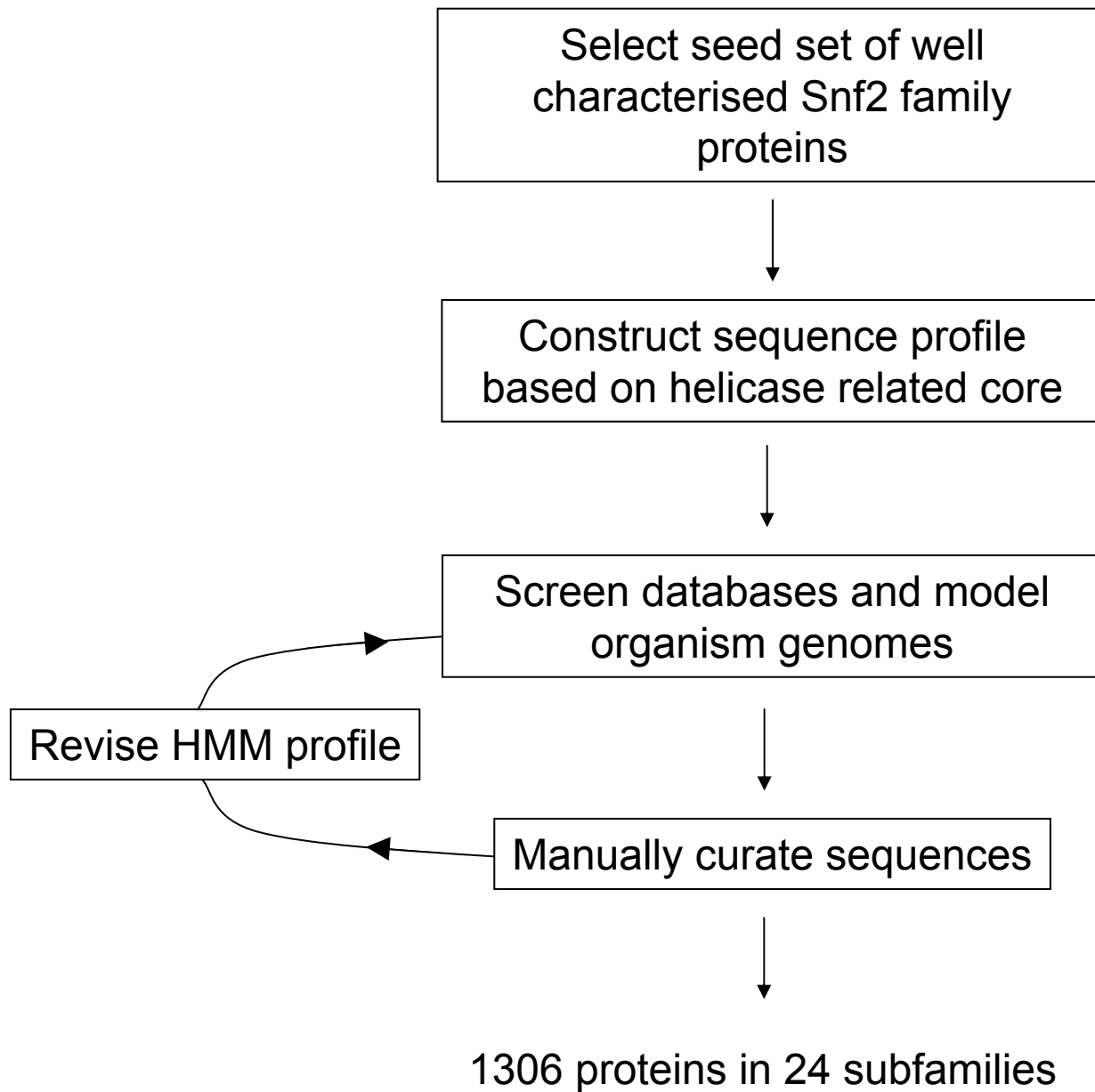


SF2 is diverse

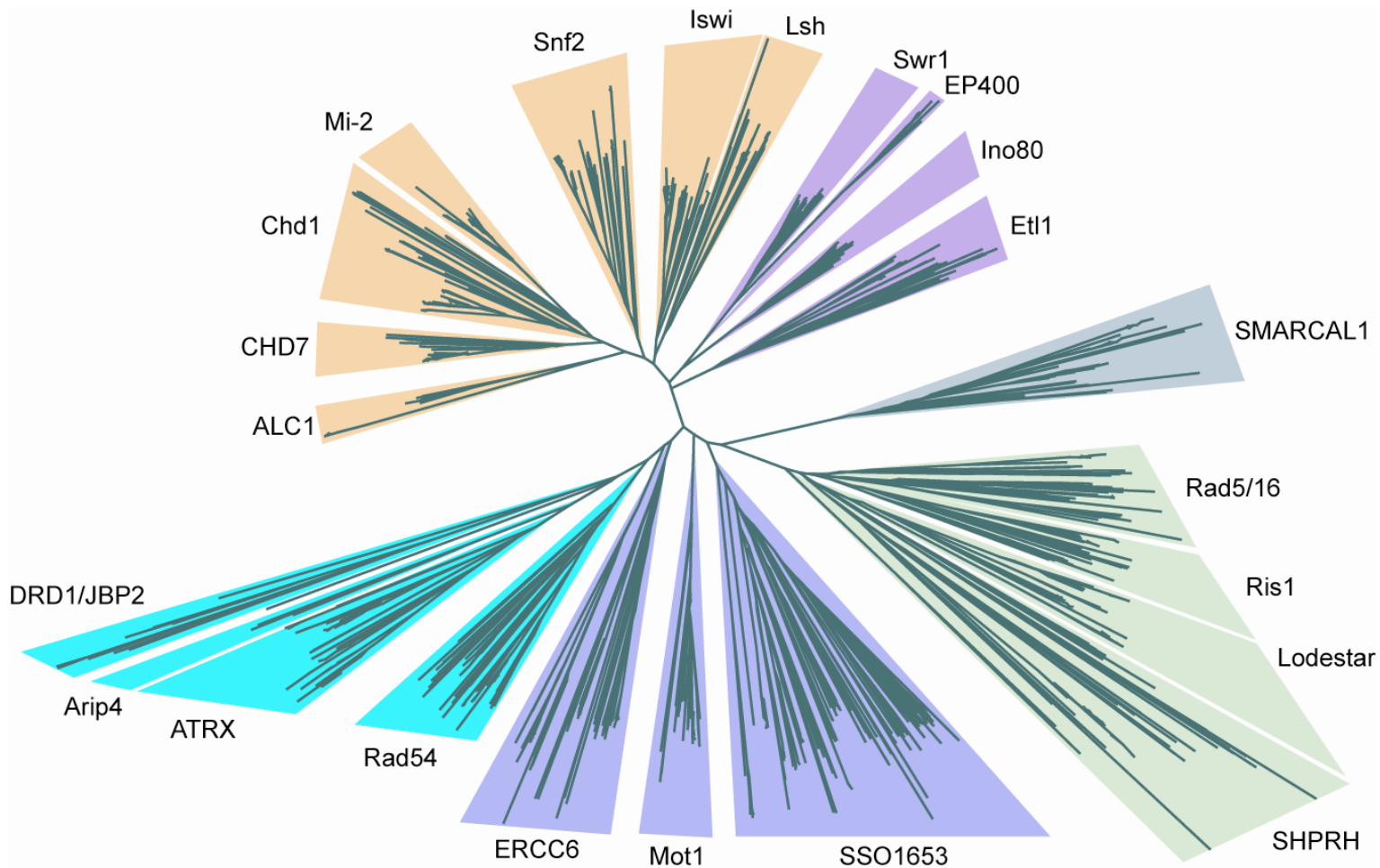
- suv3 = mitoch. DEAD box helicases
- rad3 = XPB helicases
- ul9 = Herpes virus helicases
- hsdR = Type III restriction enzymes
- i8r = NPH-II viral RNA helicases
- if4a = eIF4a family helicases
- TtRecG = Thermotoga RecG helicase
- HCV NS3 = Herpes NS3 helicase
- EcRecQ = Ecoli recQ helicase
- Tt, BcUvrB = Thermotoga, Bacillus UvrB helicases
- MiDEAD = Methanococcus DEAD box helicase



Sequences of structures or for consensus of 100 aligned helicase region Blast hits to Koonin archetype (<e-40) were anchored at motifs and each intermotif region was aligned independently . Aligned blocks were reassembled for NJ tree calculation



The Snf2 protein is one member of an extended family of related proteins



Many subfamilies are widely distributed amongst eukaryotes

		genes encoding predicted proteins																								
		Snf2	Iswi	Lsh	ALC1	Chd1	Mi-2	CHD7	Swr1	EP400	Ino80	Ett1	Rad54	ATRX	Arip4	DRD1	JBP2	Rad5/16	Ris1	Lodestar	SHPRH	Mot1	ERCC6	SSO1653	SMARCAL1	rapA
fungi	<i>S cerevisiae</i>	2	2	1	0	1	0	0	1	0	1	1	2	0	0	0	0	2	1	0	1	1	1	0	0	0
	<i>S pombe</i>	2	0	0	0	2	1	0	1	0	1	3	2	0	0	0	0	2	3	0	1	1	1	0	0	0
	<i>N crassa</i>	1	1	1	0	1	1	0	1	0	1	1	1	0	1	0	0	3	3	3	3	1	2	0	0	0
pl	<i>A thaliana</i>	4	2	1	1	1	3	0	1	0	1	1	1	1	0	6	0	5	5	0	2	1	3	0	2	0
	<i>C intestinalis</i>	1	1	2	0	1	1	1	1	0	0	1	1	1	1	0	0	0	0	0	0	1	2	0	1	0
	<i>C elegans</i>	2	1	0	0	1	2	1	1	0	0	1	2	1	1	0	0	0	0	4	1	1	1	0	1	0
invertebrates	<i>A mellifera</i>	1	1	1	0	1	1	1	0	0	1	1	1	1	1	0	0	0	0	1	0	1	0	0	1	0
	<i>A gambiae</i>	1	1	1	0	1	1	1	1	0	1	1	2	2	1	0	0	0	0	1	1	1	0	0	1	0
	<i>D melanogaster</i>	1	1	0	0	1	2	1	1	0	1	1	1	1	1	0	0	0	0	2	1	1	0	0	1	0
vertebrates	<i>D rerio</i>	5	1	0	2	1	4	4	1	0	3	2	1	0	1	0	0	1	0	1	1	1	3	0	1	0
	<i>T nigroviridis</i>	3	1	1	1	2	4	3	1	2	1	1	3	2	2	0	0	1	0	1	1	2	1	0	1	0
	<i>F rubripes</i>	3	1	1	1	2	4	4	1	1	1	1	2	2	2	0	0	1	0	1	1	1	3	0	2	0
mammals	<i>X tropicalis</i>	2	2	1	0	2	4	4	1	1	1	1	2	1	1	0	0	1	0	1	1	1	3	1	2	0
	<i>G gallus</i>	1	2	1	1	3	2	3	0	1	1	1	2	1	1	0	0	0	0	1	1	1	3	0	2	0
	<i>M domestica</i>	2	2	1	1	2	3	4	1	2	1	1	2	1	1	0	0	1	0	1	1	1	4	0	2	0
	<i>C familiaris</i>	2	2	1	1	2	3	4	1	1	1	1	2	1	1	0	0	1	0	1	1	1	3	0	2	0
	<i>B taurus</i>	2	2	1	1	2	3	4	1	1	1	1	2	1	1	0	0	1	0	1	1	1	2	0	2	0
	<i>R norvegicus</i>	2	3	1	1	2	2	2	1	0	1	1	1	1	1	0	0	1	0	1	1	1	2	0	2	0
	<i>M musculus</i>	2	4	1	1	2	2	3	0	1	1	1	2	1	1	0	0	1	0	1	1	1	3	0	2	0
	<i>P troglodytes</i>	2	2	1	1	2	3	4	1	1	1	1	2	1	1	0	0	1	0	1	1	1	3	0	2	0
	<i>H sapiens</i>	2	2	1	1	2	3	4	1	1	1	1	2	2	1	0	0	1	0	1	1	1	3	0	2	0

snf2.net: proteins by subfamily/organism for eukaryotes - Microsoft Internet Explorer

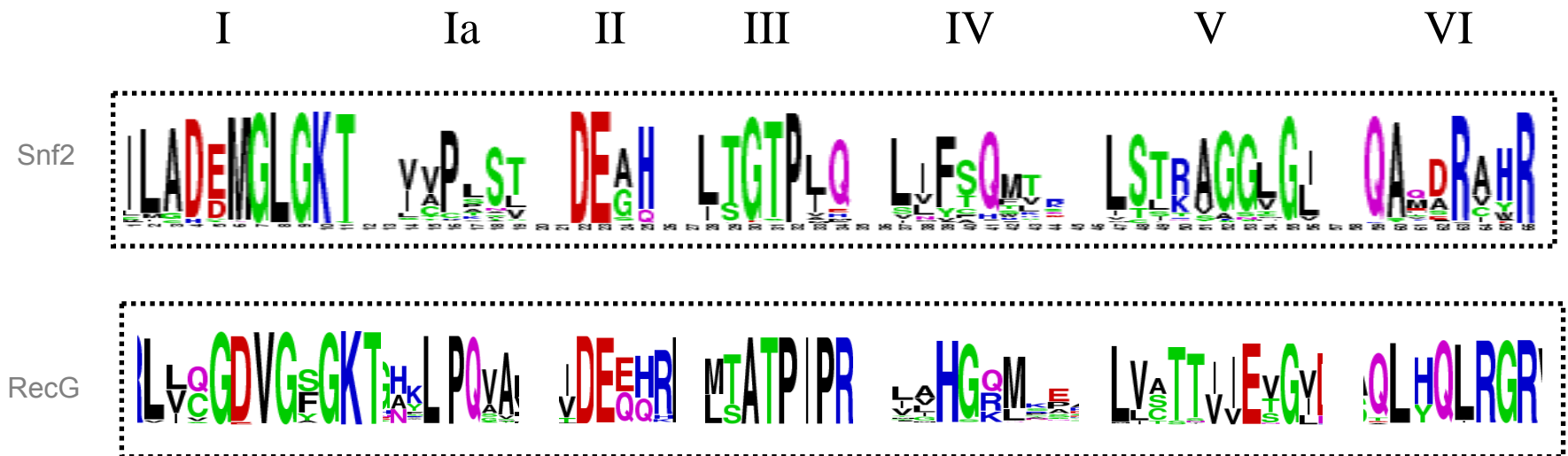
Address: http://toh.juststuf.co.uk/db1/db1.html

snf2.net
snf2 family protein database

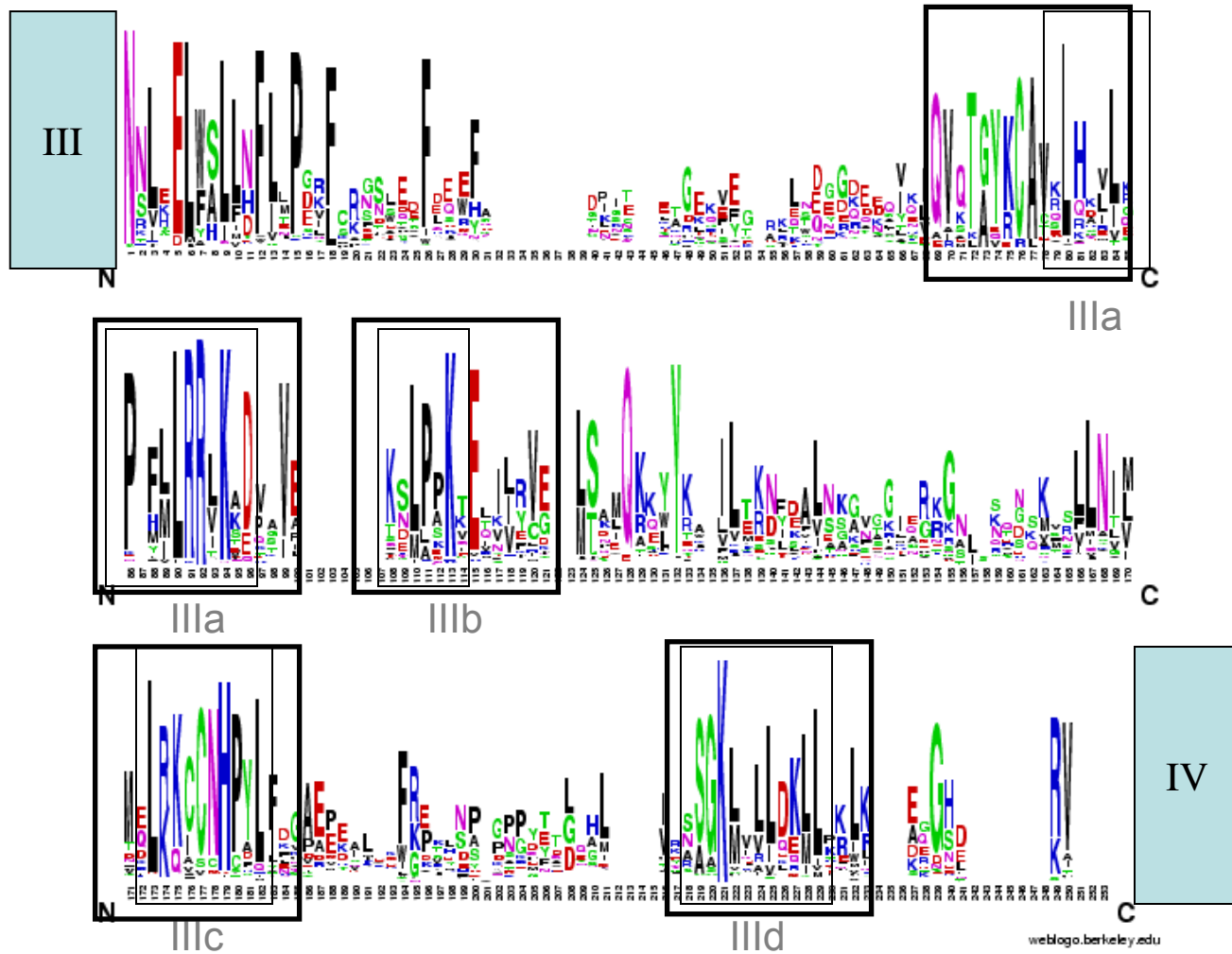
Proteins by subfamily and organism for eukaryotes

species phylogroup	Snf2	Iswi	Chd1	Mi-2	Chd7	Lsh	ALC1	Swi1	EP400	Ino80	Est1	Rad54	ATRX	Arip4	DRD1	JBP2	Rad5/16	Ris1	Lodestar	SHPRH	Mot1	ERCC6	SSO1653	SMARCAL1	rapA	combined		
<i>G lamblia</i> protist		1	2							1												2				6		
<i>E histolytica</i> protist		1	1		2							2										1			2		11	
<i>L major</i> protist						3		1				1		1		2	3					2		2	1		16	
<i>T Brucei</i> protist						2		1				1		1		2	2					2					12	
<i>T cruzi</i> protist						3		1				2		2		3	2					4			3		20	
<i>P falciparum</i> protist	1	1	1			1		1			1	1					2							1			10	
<i>P vivax</i> protist	1	1	1			1		1			1	1					2								1		10	
<i>P yoelii</i> protist	1	1	1			1		1			1	1					2								1		10	
<i>T parva</i> protist	1	1	1			1		1			1	1											1		1		9	
<i>T annulata</i> protist	1	1	1			1		1			1	1											1		1		9	
<i>C reinhardtii</i> protist	1	2	1		1	1						3	1				1				1		2	1			16	
<i>C merolae</i> protist	2	1			1	1		1									1		1	1	1	1			1		12	
<i>D discoideum</i> protist	2	1	1		2			1		1	1	2				1	1		1	3	1	2		2	2		22	
<i>E cuniculi</i> fungi		1			1																		1			1		6
<i>C neoformans</i> fungi	1	1	1	1		1		1		1	1	2					5	1		3	1	1					21	
<i>S cerevisiae</i> fungi	2	2	1			1		1		1	1	2					2	1		2	1	1					17	
<i>S bayanus</i> fungi	2	2	1			1		1		1	1	2					2	1		1	1	1					17	
<i>S castelli</i> fungi	2	3	1			1		1		1	1	2					2			1	1	1					16	
<i>S mikatae</i> fungi	2	2	1			1		1		1	1	2					2	1		1	1	1					17	
<i>S paradoxus</i> fungi	2	2	1			1		1		1	1	2					2			1	1	1					16	
<i>Y lipolytica</i> fungi	2	2	1			1		1		1	1	2					2	2		2	1	1					19	
<i>D hansenii</i> fungi	2	2	1			1		1		1	1	2					2	2		2	1	1					19	
<i>A gossypii</i> fungi	2	2	1			1		1		1	1	2					2	1		1	1	1					20	
<i>S pombe</i> fungi	2	2	1			1		1		1	3	2					2	3		1	1	1					20	
<i>C albicans</i> fungi	4	3	2			2		2		2	2	4					4	2		2	2	2					33	
<i>C glabrata</i> fungi	2	2	1			1		1		1	1	2					2	1		1	1	1					17	
<i>K lactis</i> fungi	2	2	1			1		1		1	1	2					2	1		1	1	1					17	
<i>K waltii</i> fungi	2	2	1			1		1		1	1	2					2	1		1	1	1					16	
<i>S nodorum</i> fungi	2	1	1	1		2		1		1	1	2					4	3		2	2	1	2				26	
<i>U maydis</i> fungi	1	1	1			1		1		1	1	2					3	1		2	1	1					17	
<i>A nidulans</i> fungi	1	2	1	1		1		1		1	1	2					4	2		2	1	2					23	
<i>N crassa</i> fungi	1	1	1	1		1		1		1	1	1					3	3		3	1	2					25	
<i>C globosum</i> fungi	1	1	1	1		1		1		1	1	1					3	4		2	2	1	2				24	
<i>M grisea</i> fungi	1	1	1	1		1		1		1	1	1					4	1		2	3	1	1				21	
<i>A thaliana</i> plant	6	3	1	3		1	2	1		1	1	1	1			6	5	5		2	1	3		2			45	
<i>O sativa</i> plant	3	3	2	2		2	1	1		1	1	1	1			8	4	5		1	1	4		2			43	
<i>C intestinalis</i> invertebrate	10	10	7	5	2	6		2			2	3	2	1							2	4			5		61	
<i>C elegans</i> invertebrate	3	1	1	2	1			1			1	2	1	1						6	2	1	1				28	
<i>B mori</i> invertebrate	1	1	1								1	1	2	1								1	1		1		12	
<i>A mellifera</i> invertebrate	5	2	2	5	1	1				1	1	3	3	2						4		4				6	45	
<i>A gambiae</i> invertebrate	1	1	1	1	1	1				1	1	2	3	2						2	1	1					21	
<i>D melanogaster</i> invertebrate	4	3	1	3	1			3			1	1	2	1						2	1	1					26	
<i>D rerio</i> vertebrate	6	5	7	16	13		2	1		6	4	1	1				2			1	1	1	6				74	
<i>T nigroviridis</i> vertebrate	3	1	2	4	3	1	1		2	1	1	3	2	2				1		1	2	1					34	
<i>F rubripes</i> vertebrate	16	2	9	15	10	1	1	4	3	3	1	3	7	2				3		2	1	2	4		3		90	
<i>X tropicalis</i> vertebrate	14	11	15	13	11	4		1	3	5	1	6	4	2				4		2	3	2	4	1	4		110	
<i>G gallus</i> vertebrate	2	4	7	6	11	3	1		3	2	2	2	2	1						2	1	1	5				60	

The helicase motifs present in Snf2 proteins are distinct from those of other SF2 members



Snf2 proteins have additional regions of homology

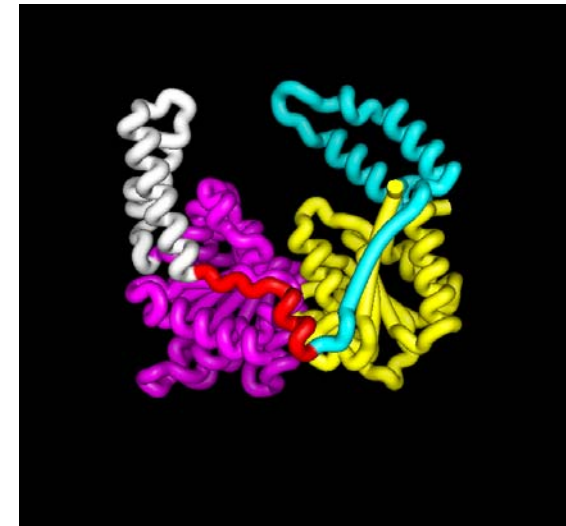
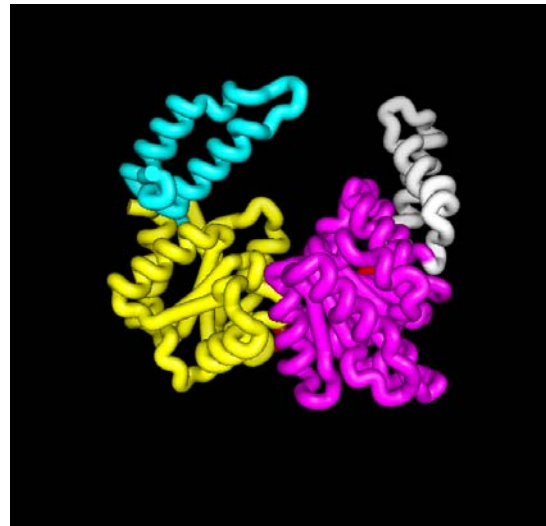
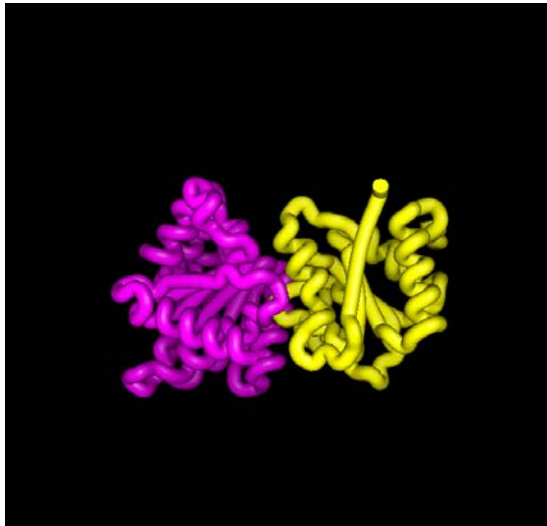


The region between motifs II and IV forms an extension to the RecA domains

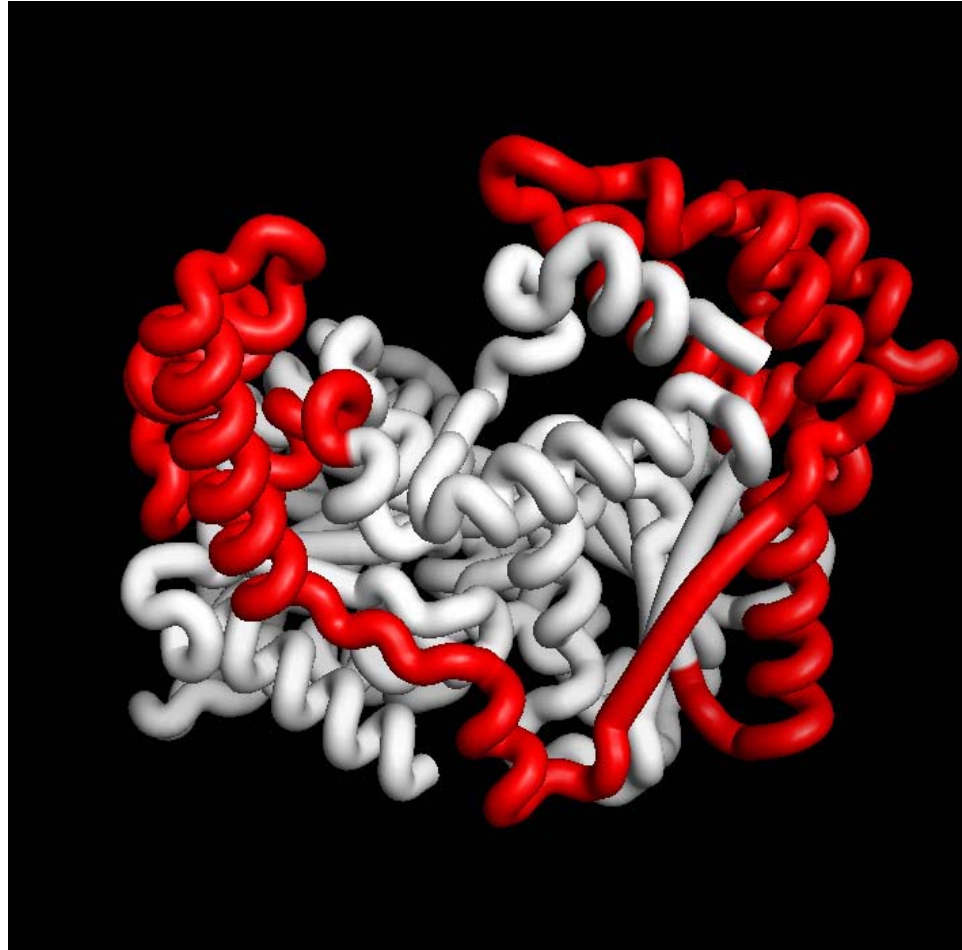
core RecA-like domain 1 (magenta) and domain 2 (yellow) lobes

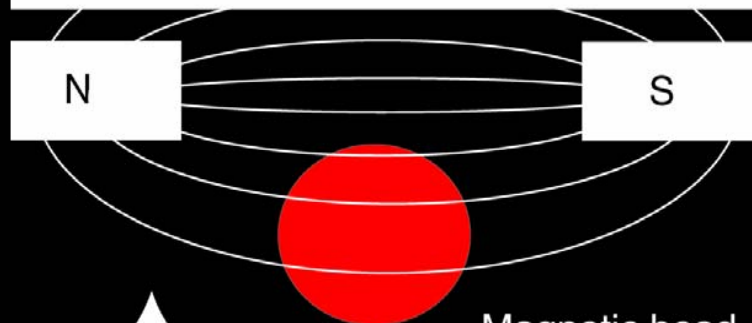
alpha helical projections HD1 (white) from domain 1 and HD2 (pink) from domain 2

conserved linking chain (red) comprises alpha helix and beta strand which extends domain 2 sheet



What do Snf2 family motors do?





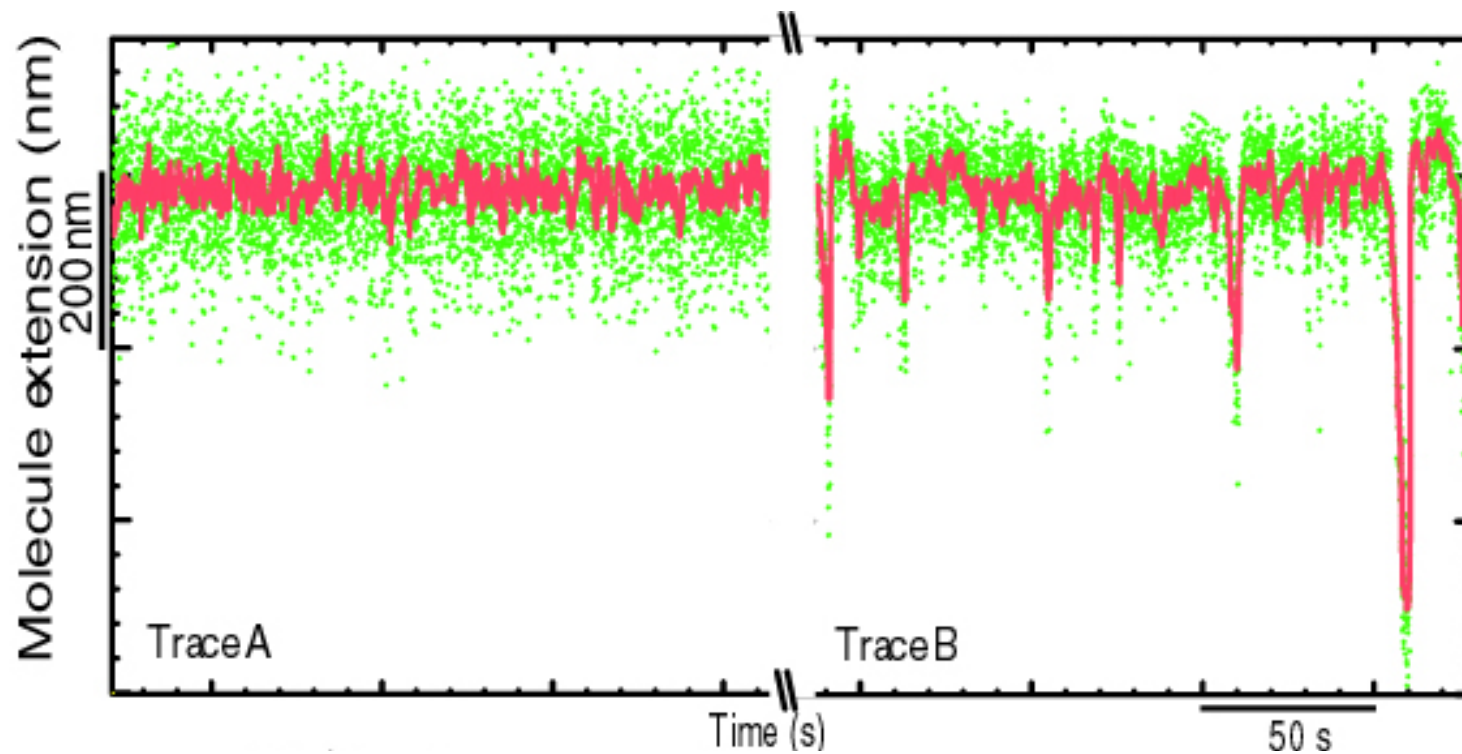
Magnetic bead



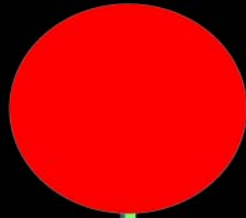
DNA



RSC and ATP cause transient shortenings in DNA length



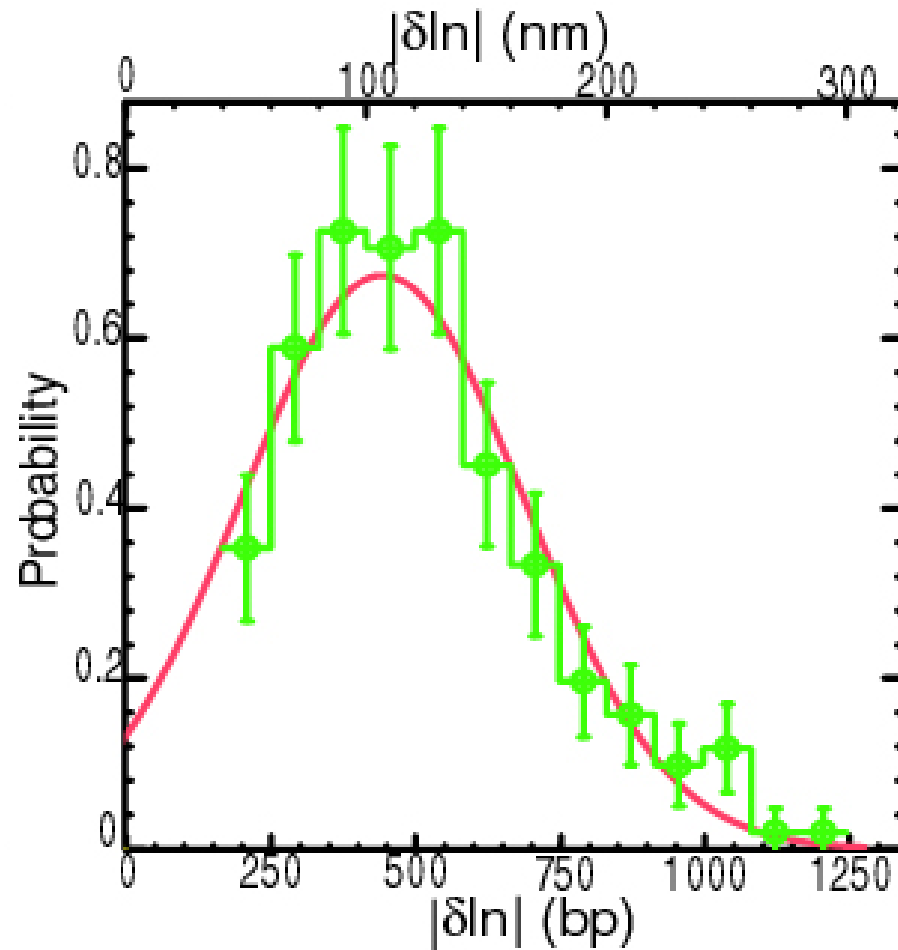
Bead Moves
Down



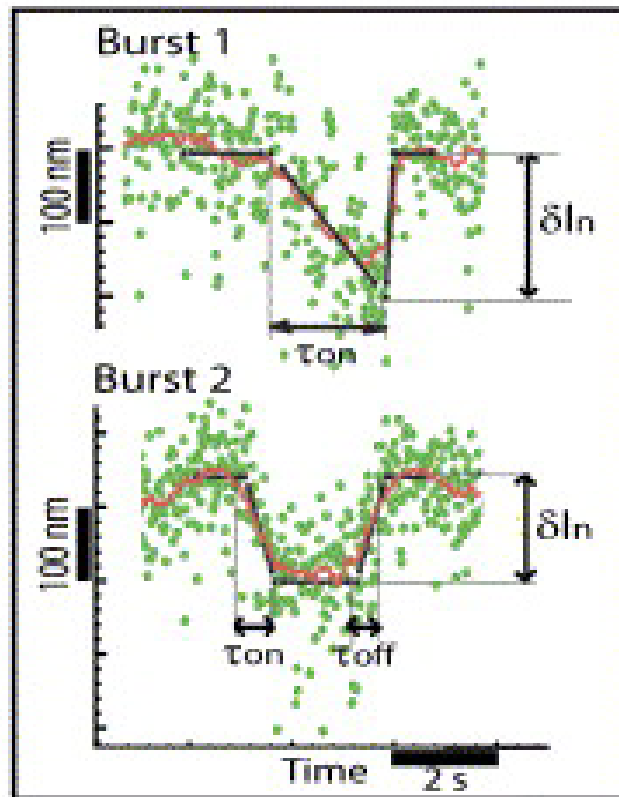
DNA Translocation



The average shortening is 106 nm or 440 bp



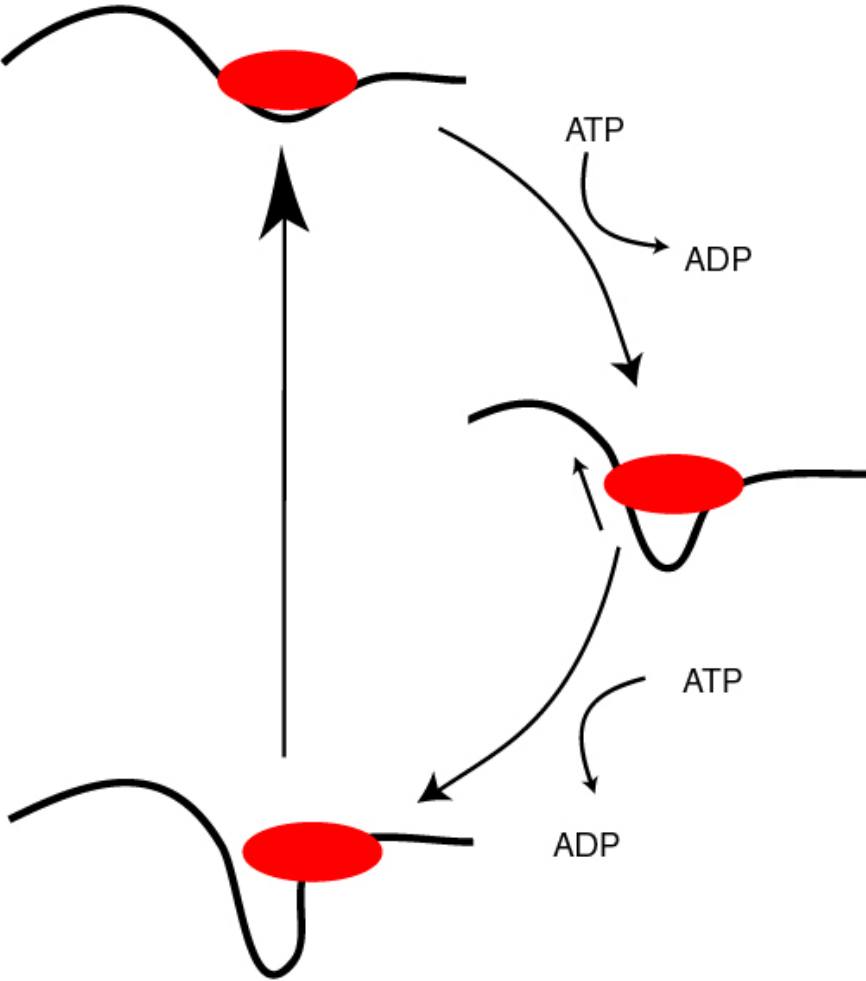
Two classes of shortening event



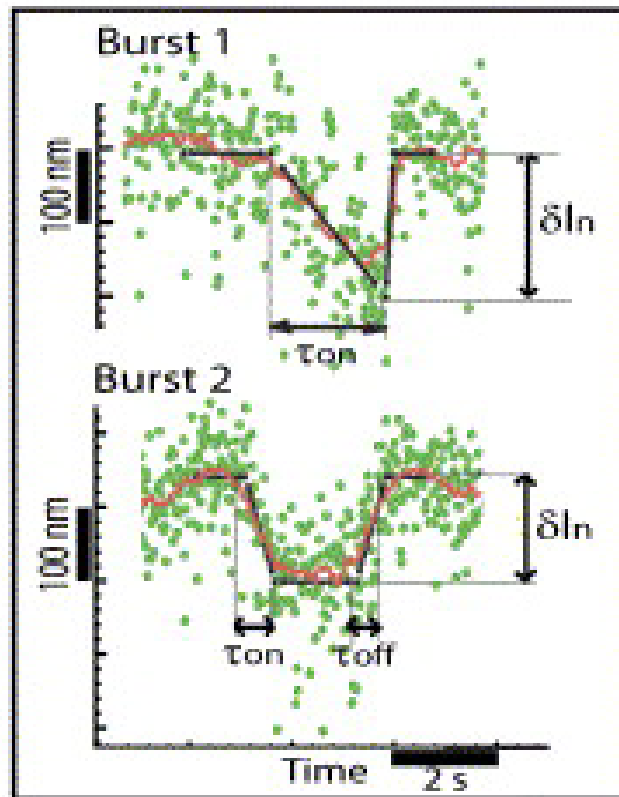
40%

60%

Loss of one DNA contact would cause the collapse of a loop



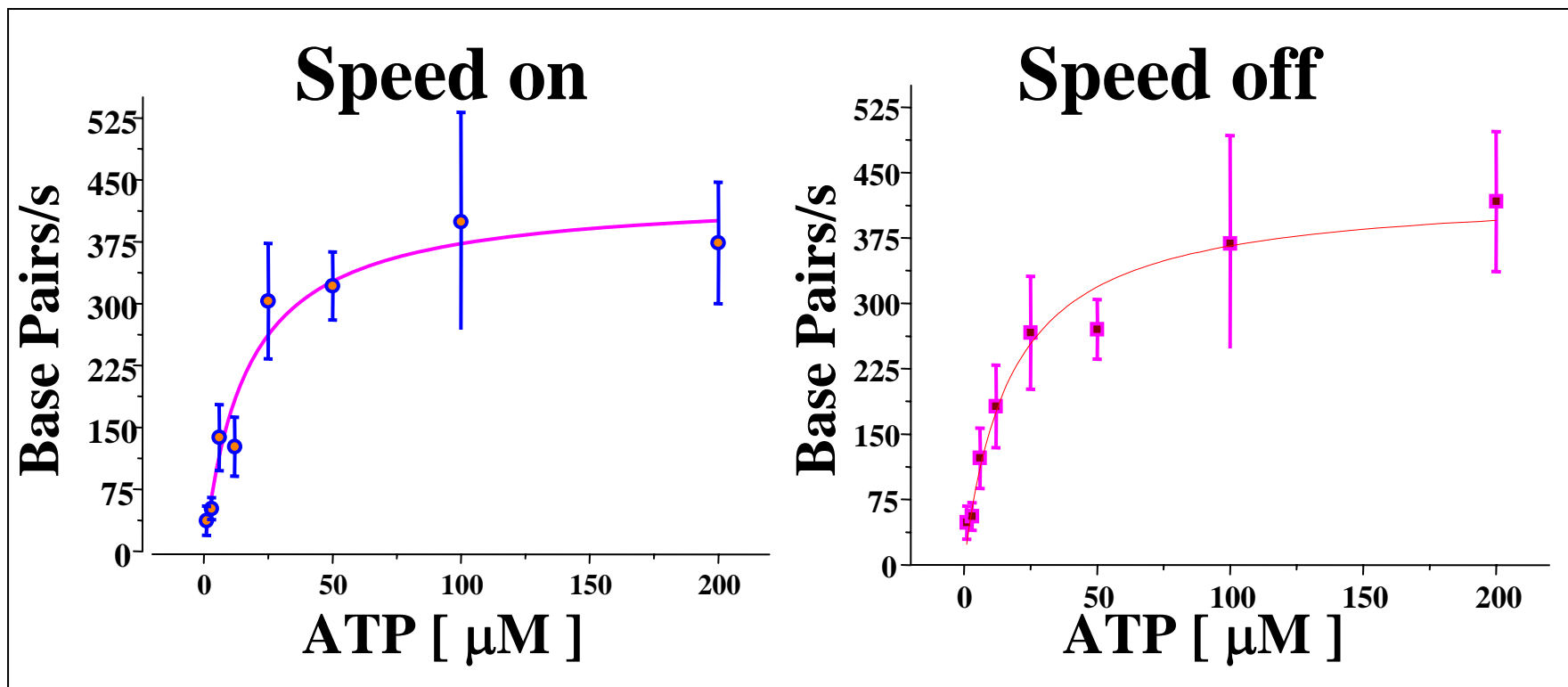
Two classes of shortening event

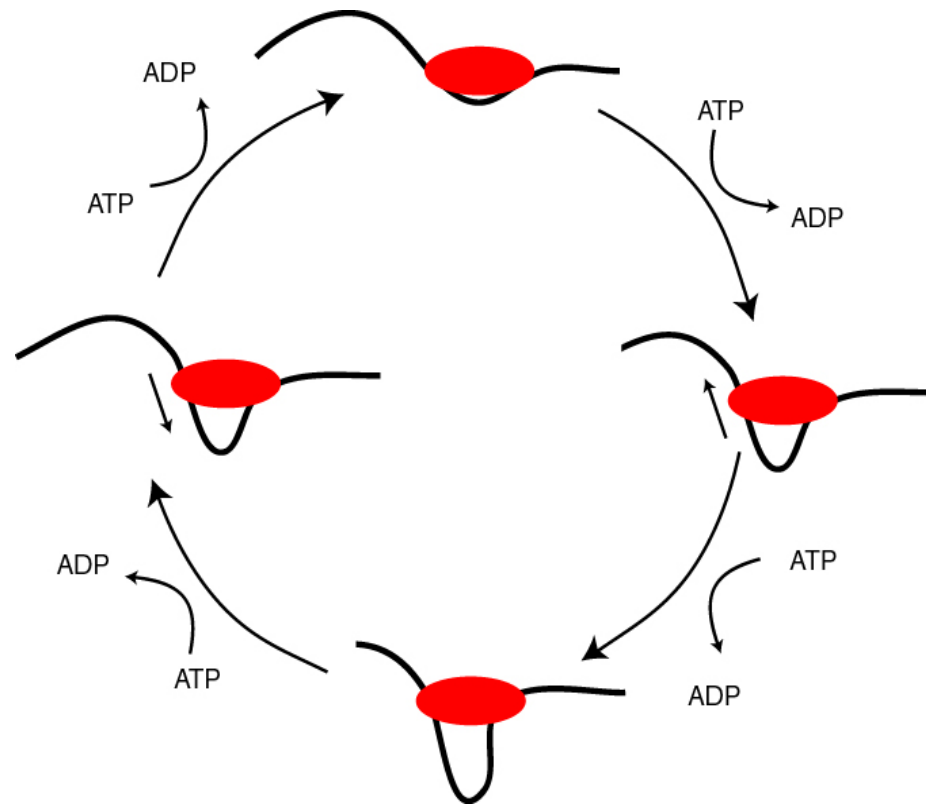


40%

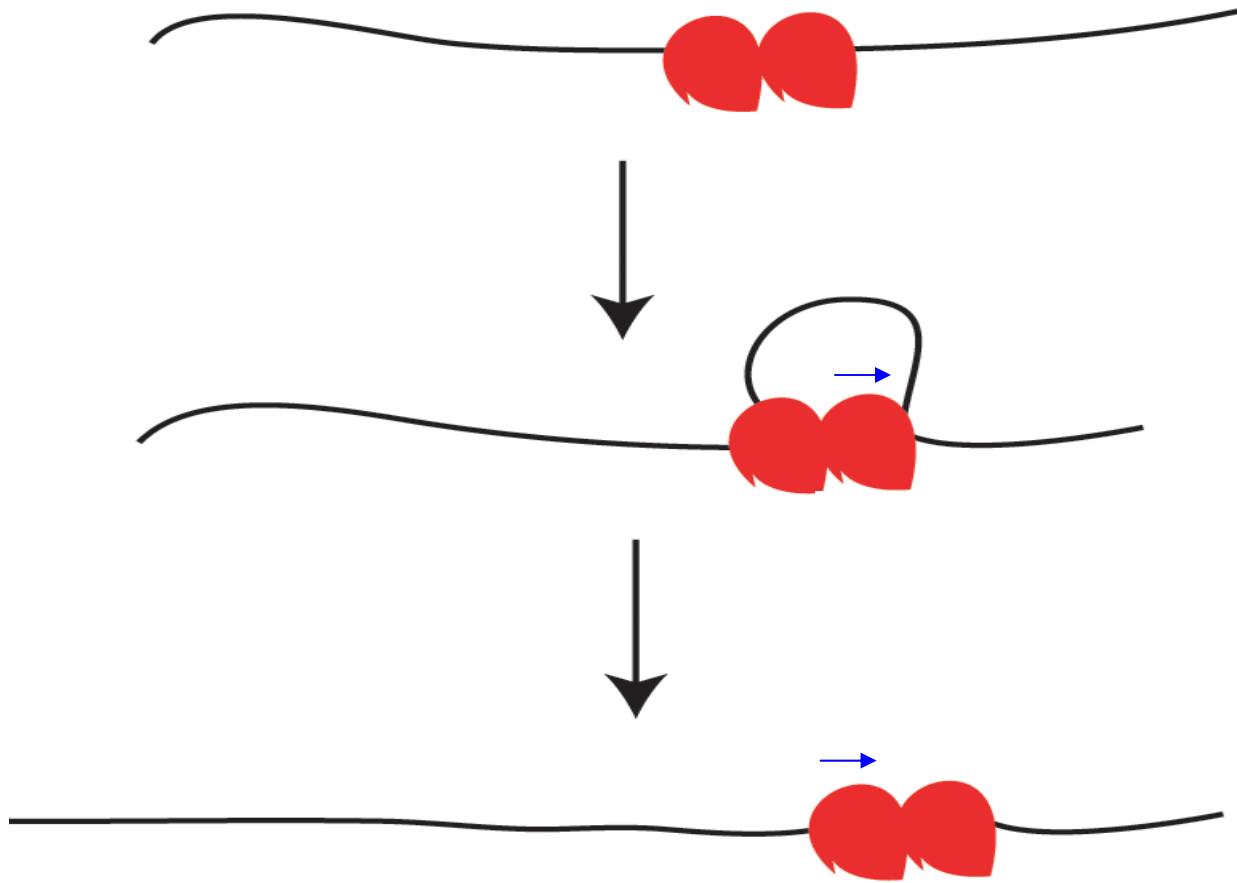
60%

Both the rate of shortening and extension are ATP-dependent

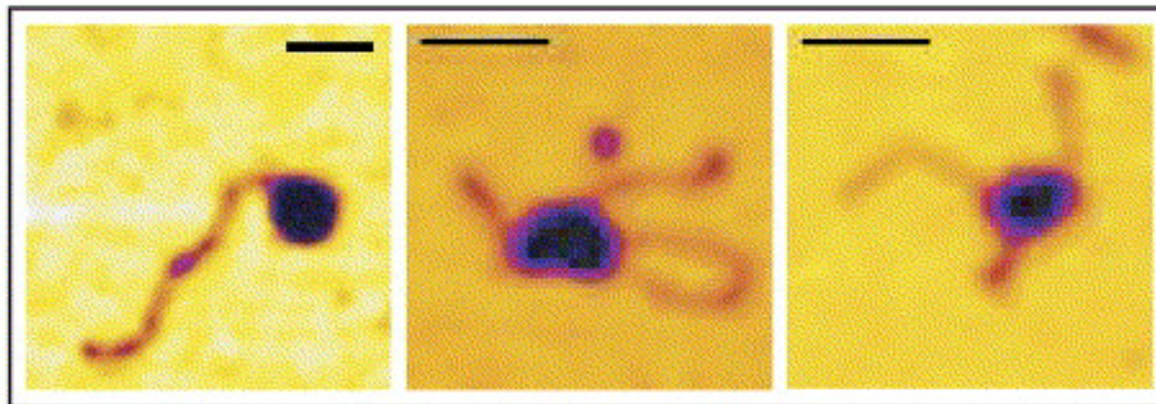
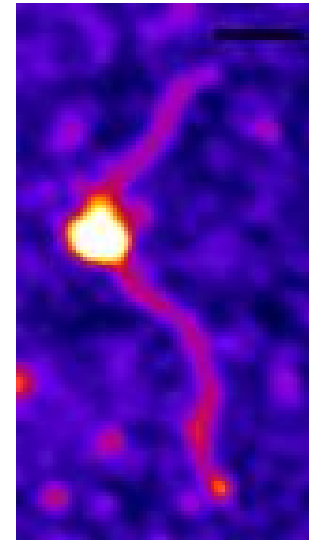
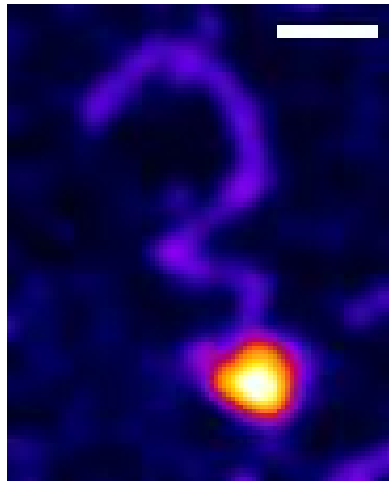


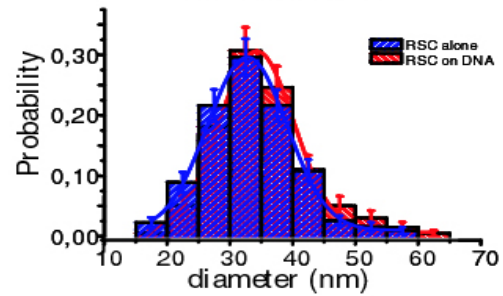
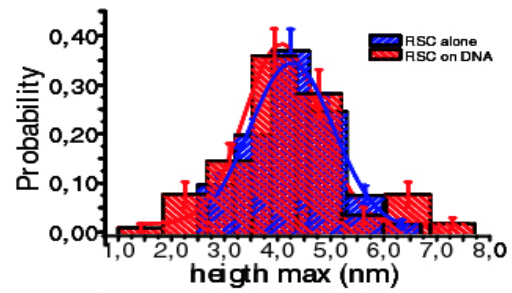
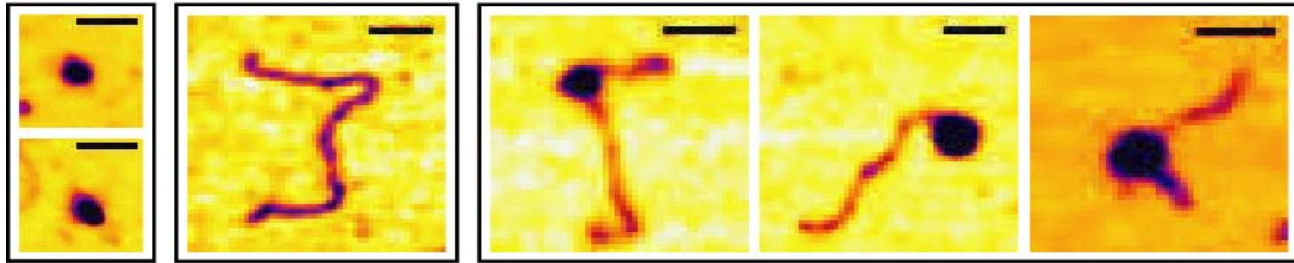


DNA shortening could result from the action of two complexes

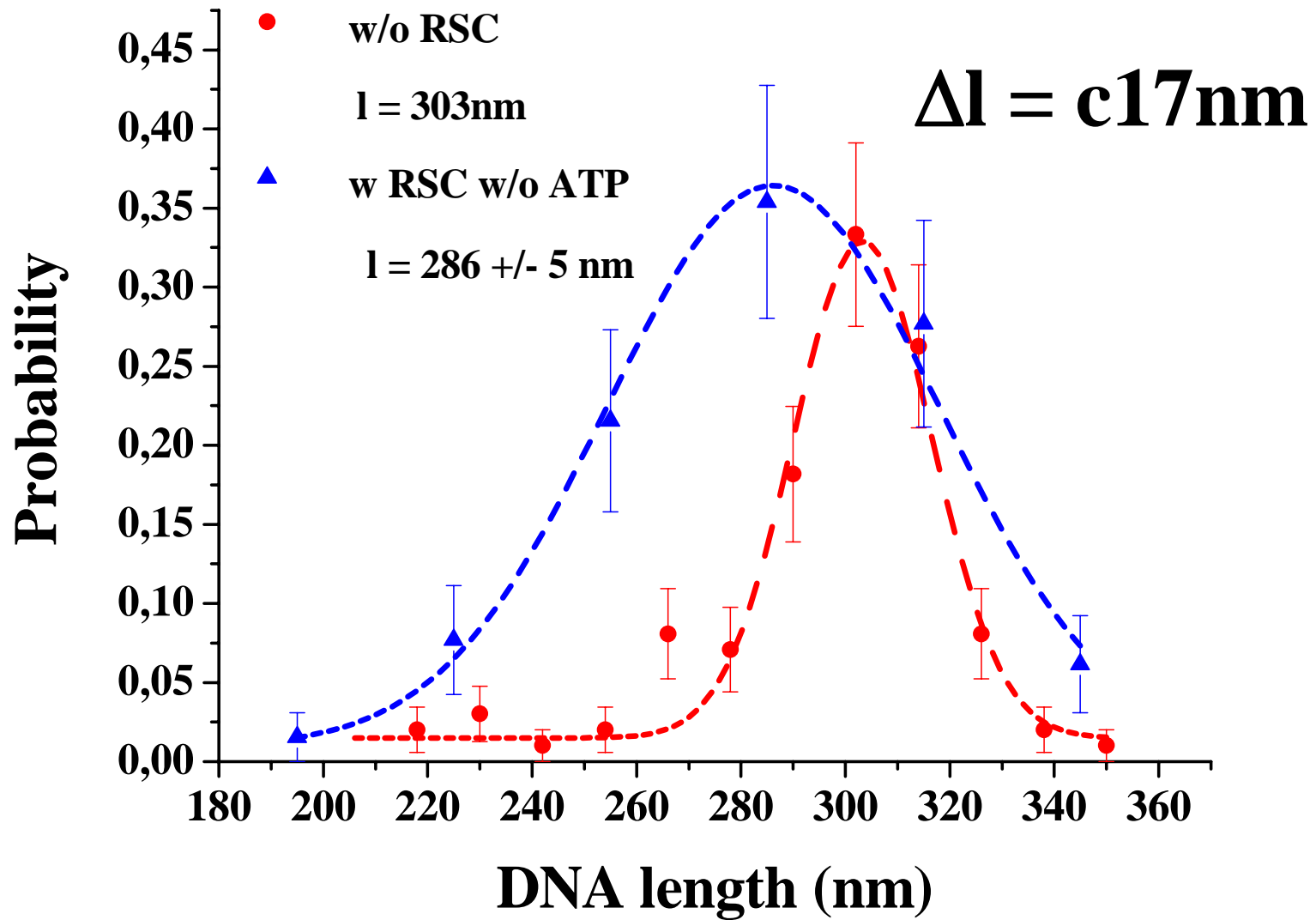


Imaging RSC complexes by AFM

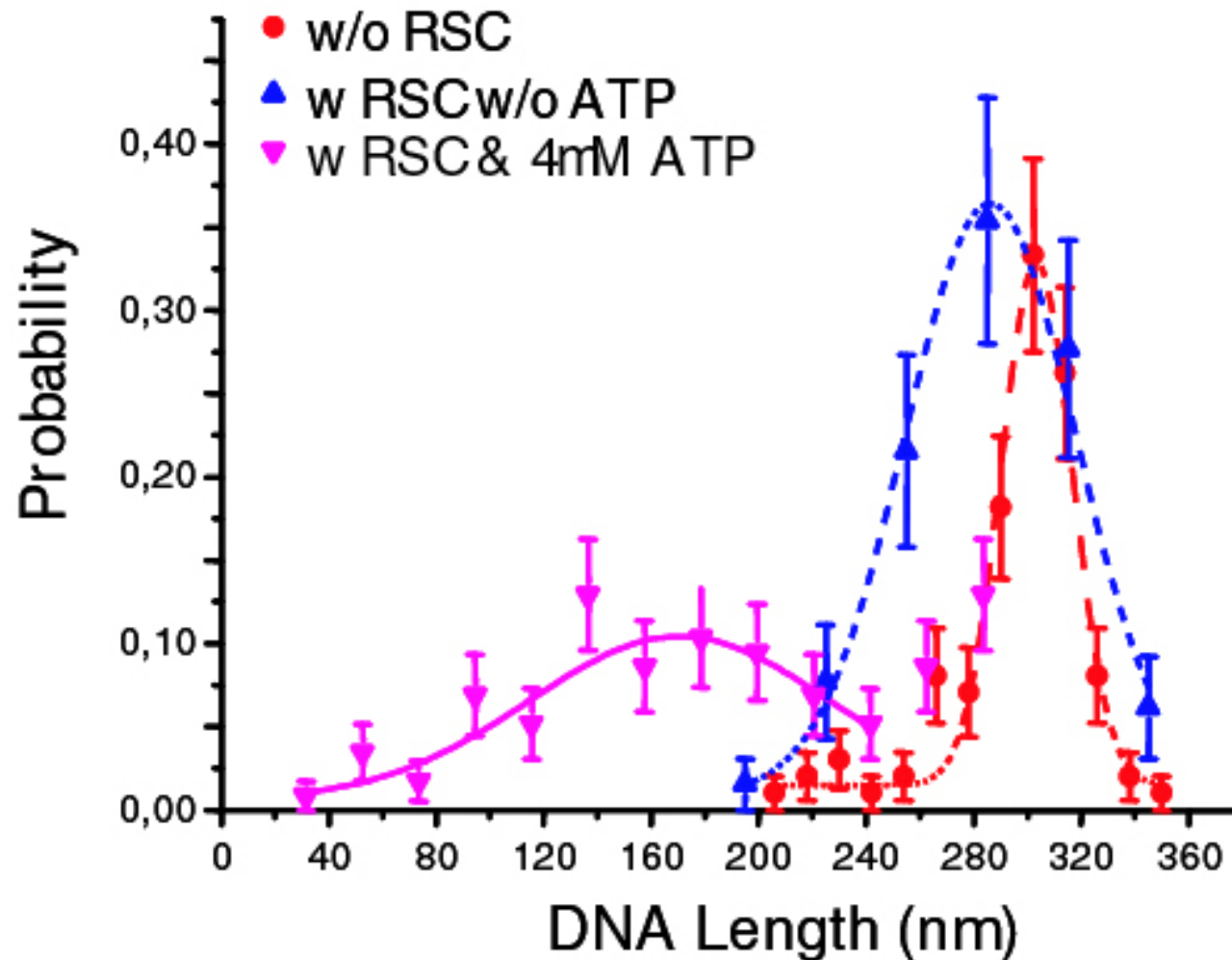


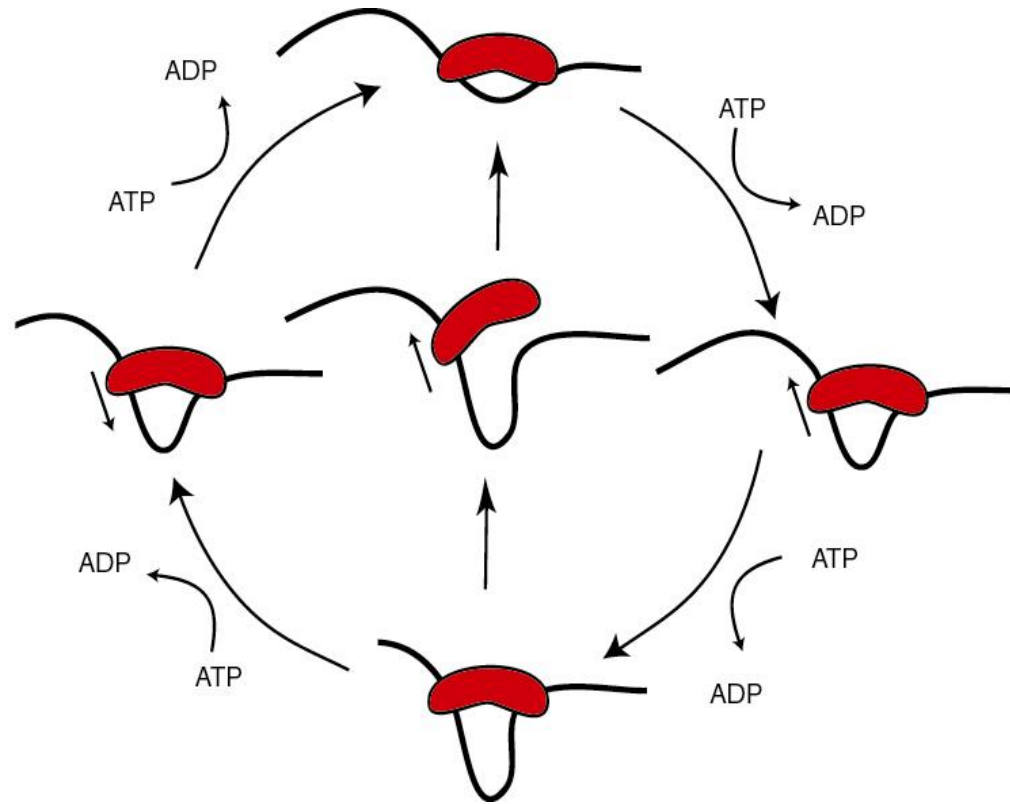


RSC causes a small DNA shortening in the absence of ATP

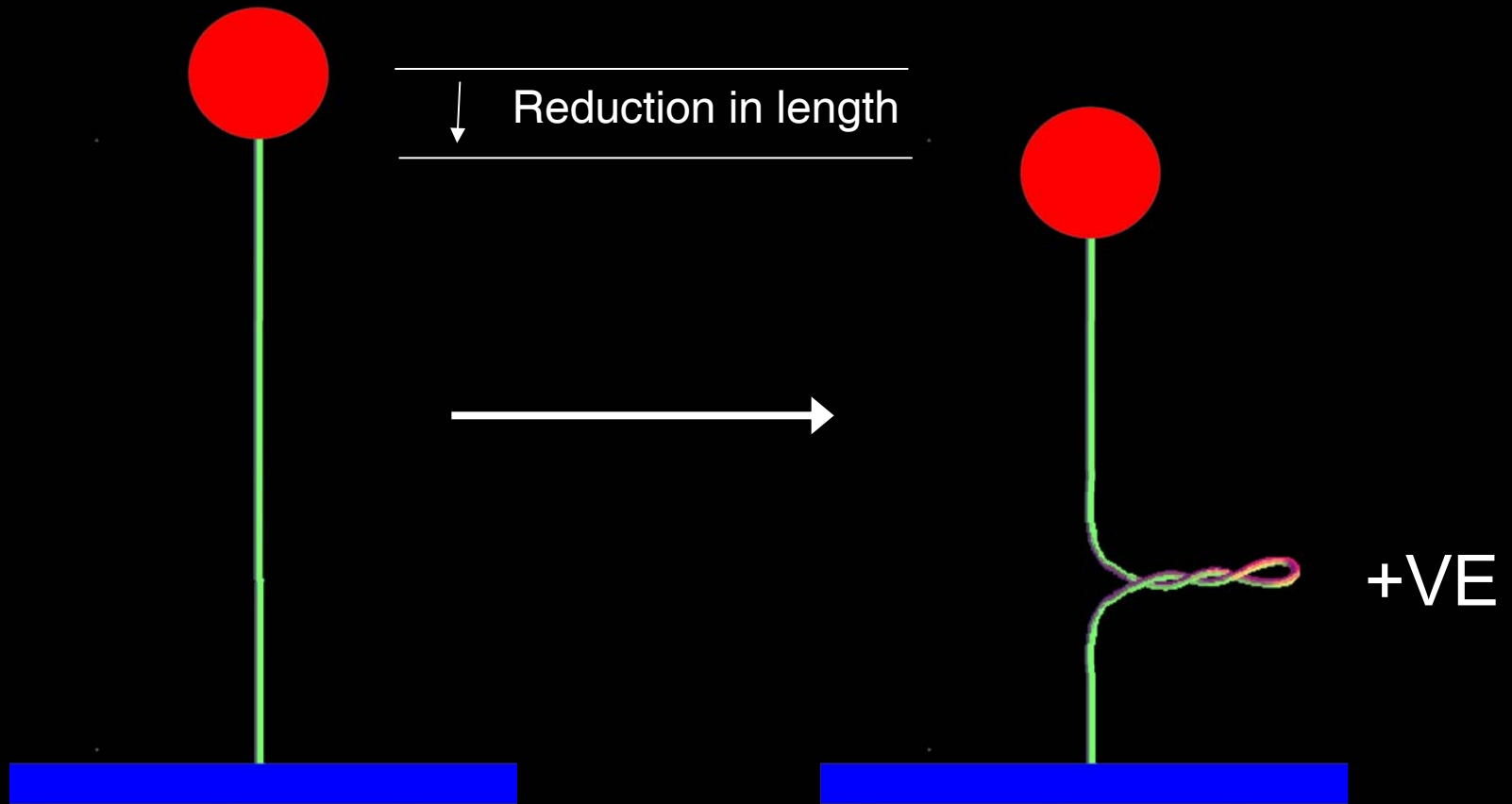


An average reduction in contour length of 130nm is detected by AFM

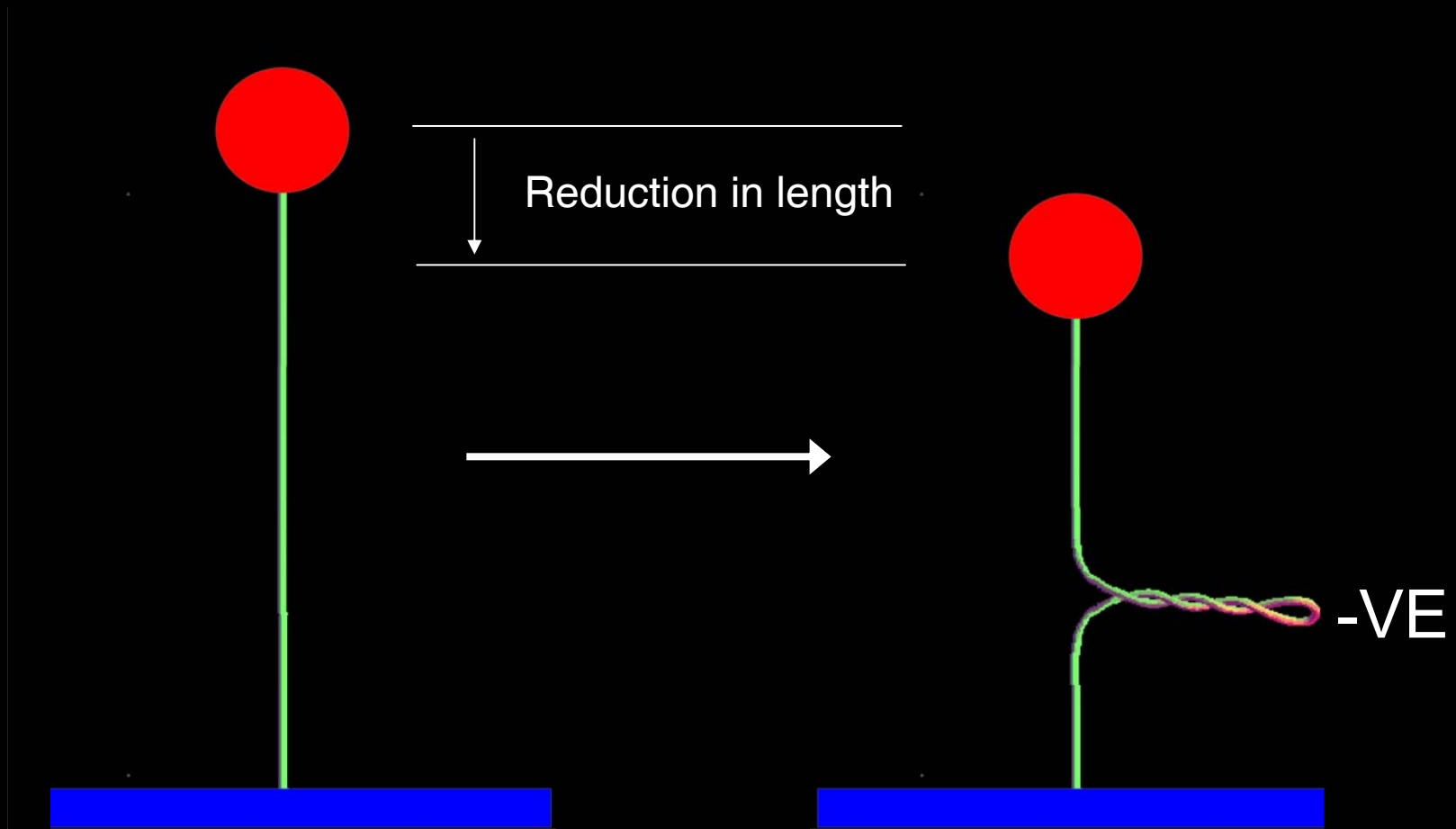




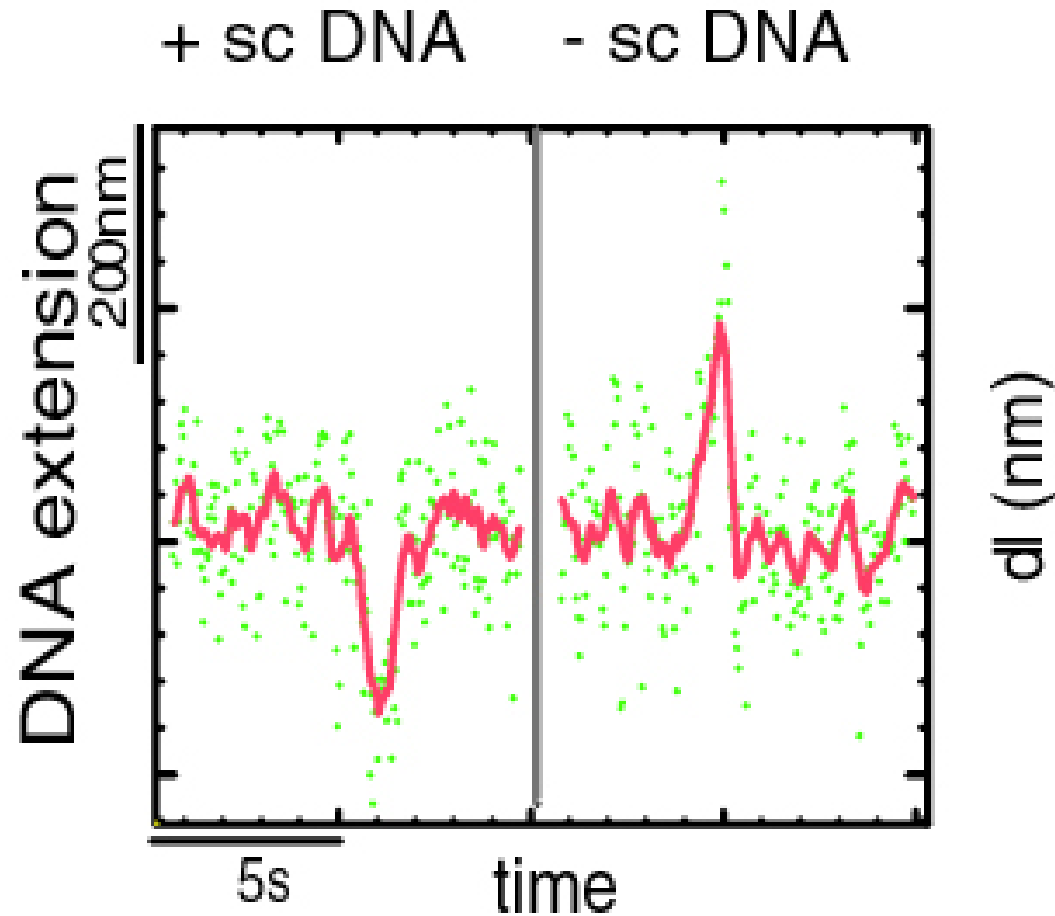
Rotation on at constant force results in the formation of plectomeres that cause contraction of DNA



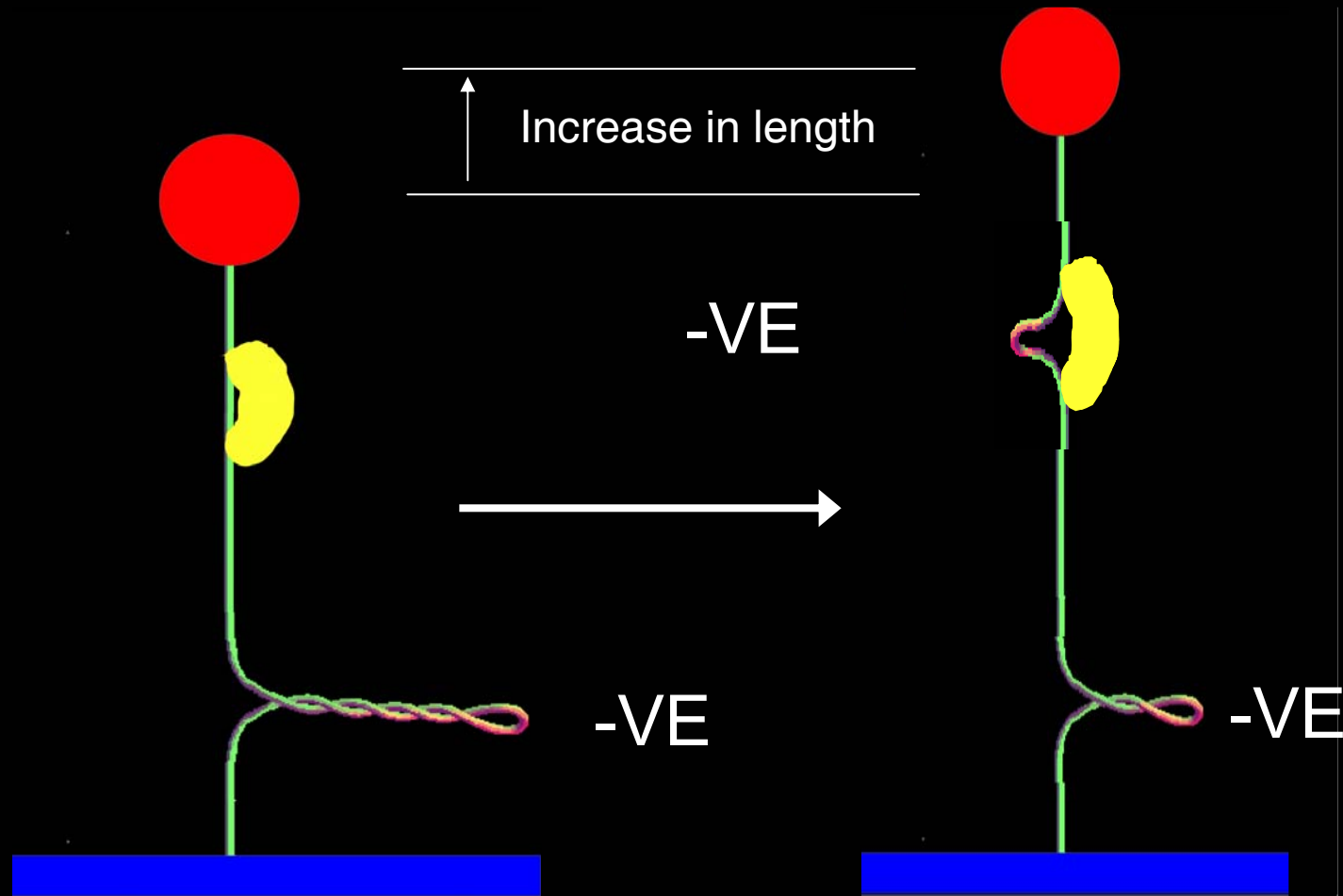
Rotation on at constant force results in the formation of plectomeres that cause contraction of DNA



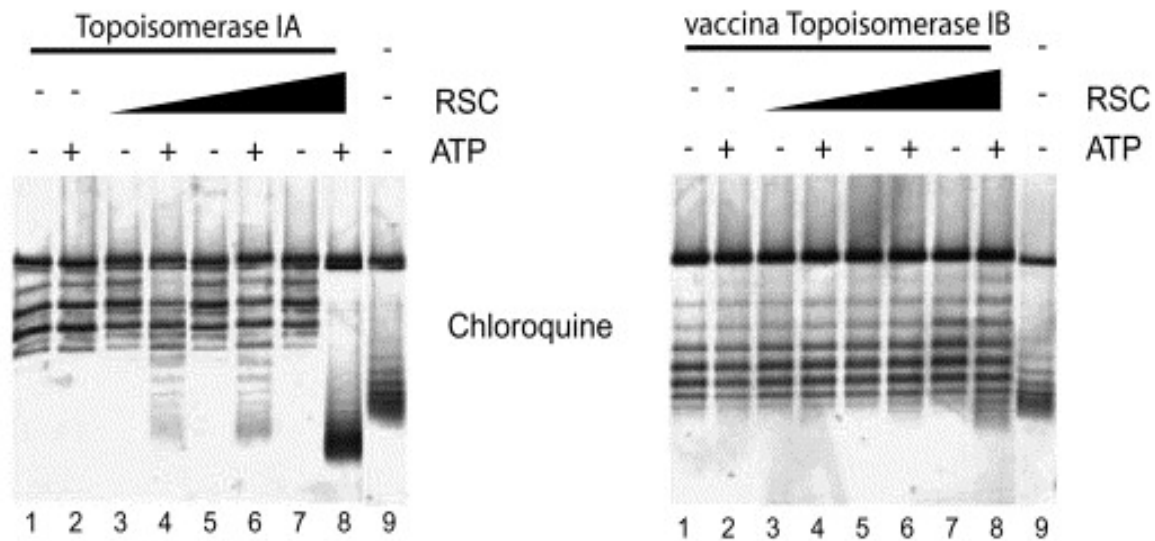
DNA is transiently extended on negatively supercoiled DNA

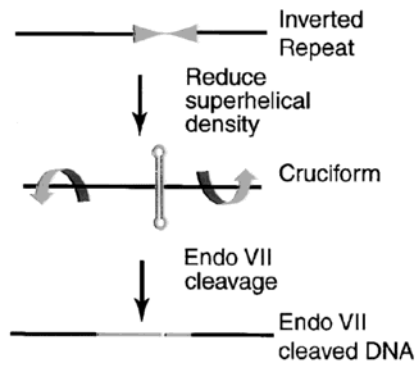
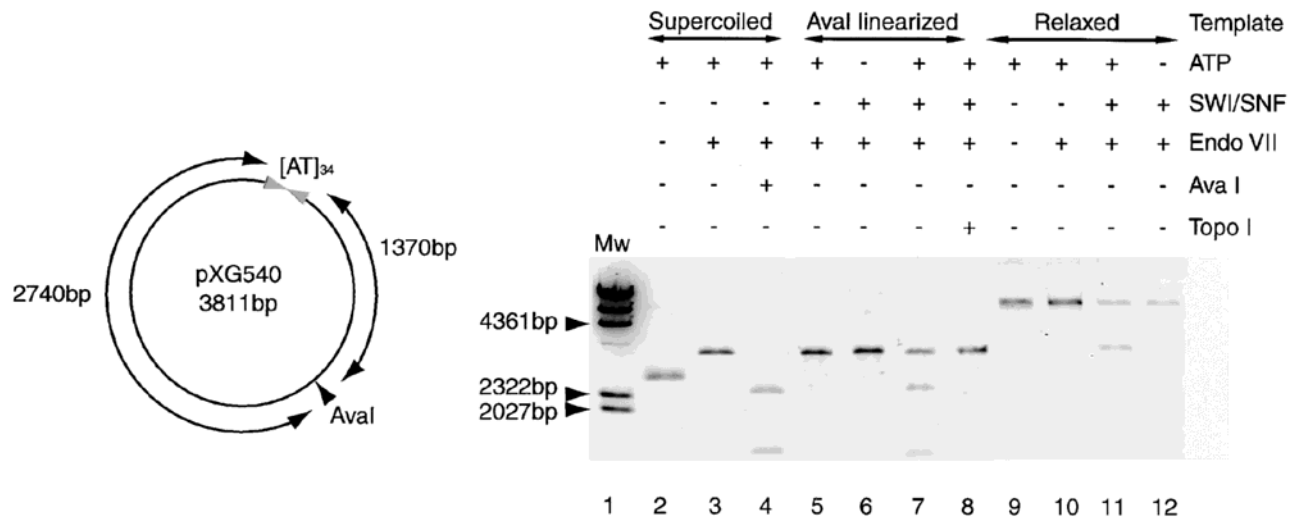


On negatively pre-twisted DNA reduction in the size of plectomeres could cause extension



RSC generates unconstrained negative supercoils in plasmid DNA





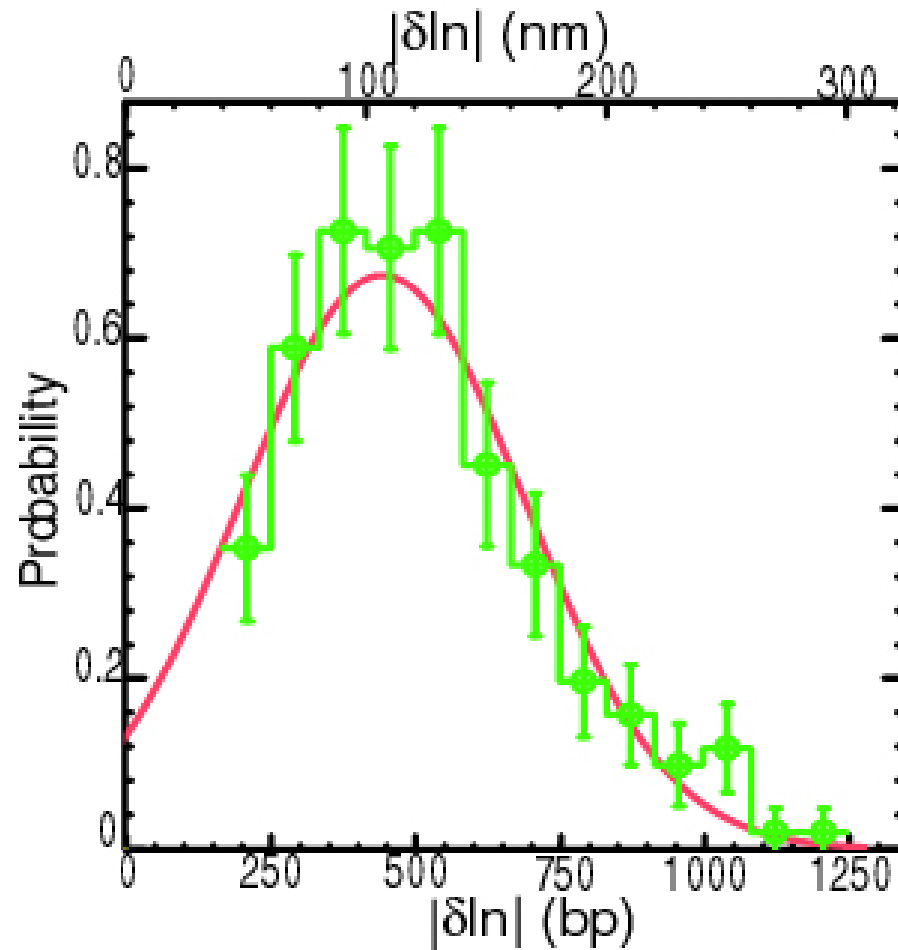
Differences in the generation of torsion on positive and negatively supercoiled DNA

$$\bullet \delta l_n = l-t = -106 \text{ nm on nicked DNA}$$

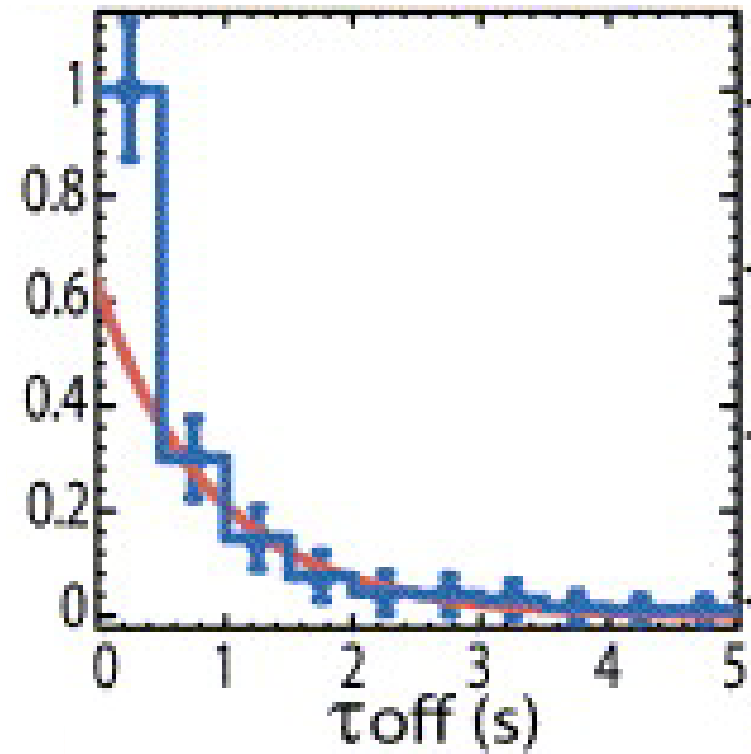
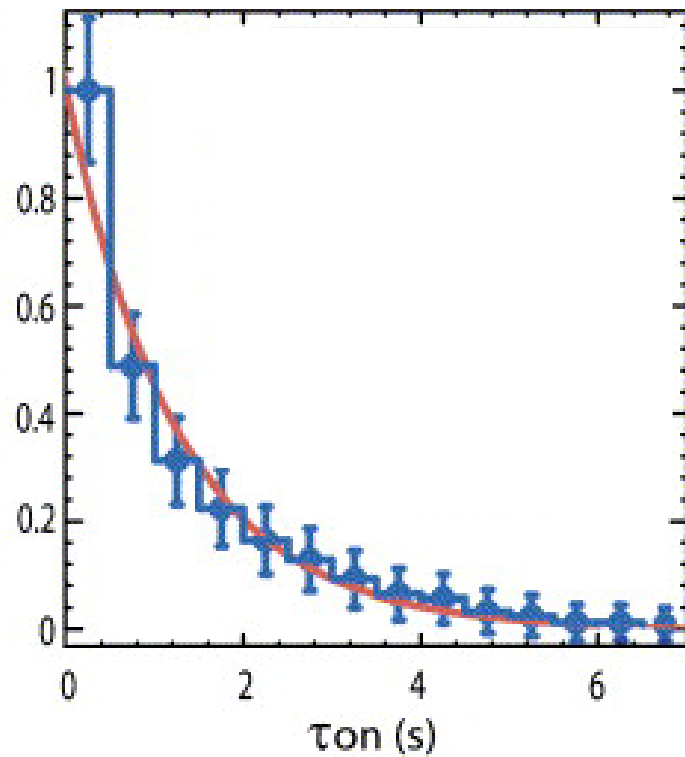
- on -ve supercoiled DNA
- $\delta l^- = l-t + n-lp = 259 \text{ nm}$
- $n = 6.1$
- on +ve supercoiled DNA
- $\delta l^+ = l+t - n+lp = -138 \text{ nm}$
- $n = 0.6$

superhelical density generated is always low

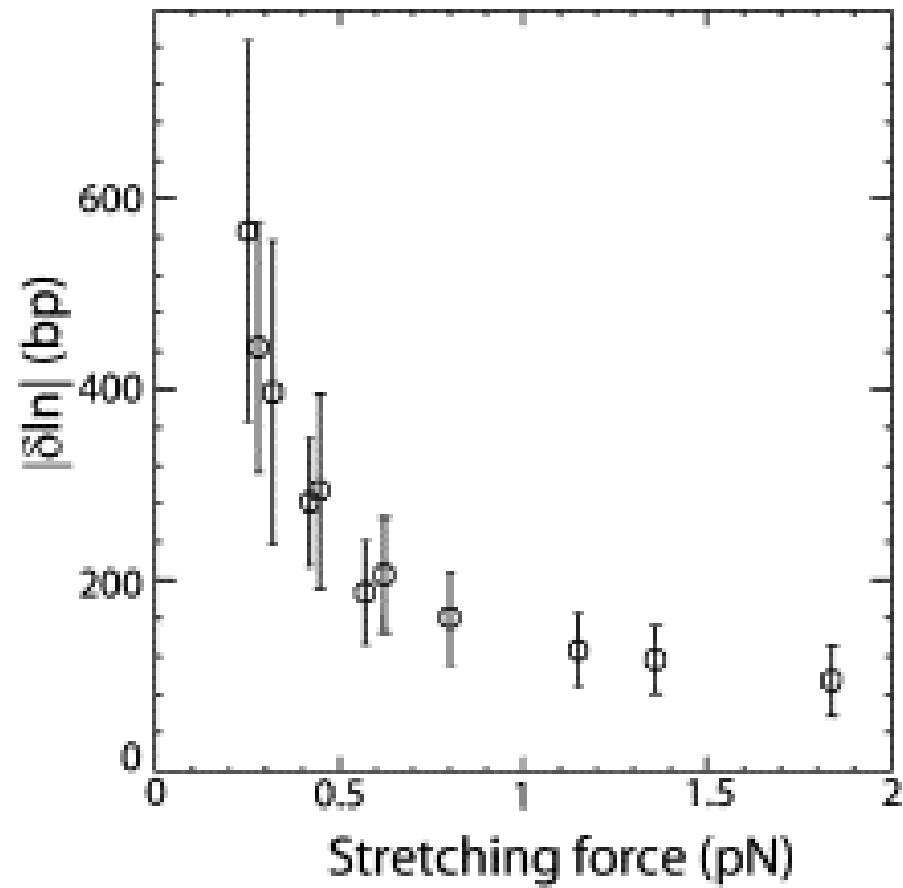
The change in length has a Gaussian distribution



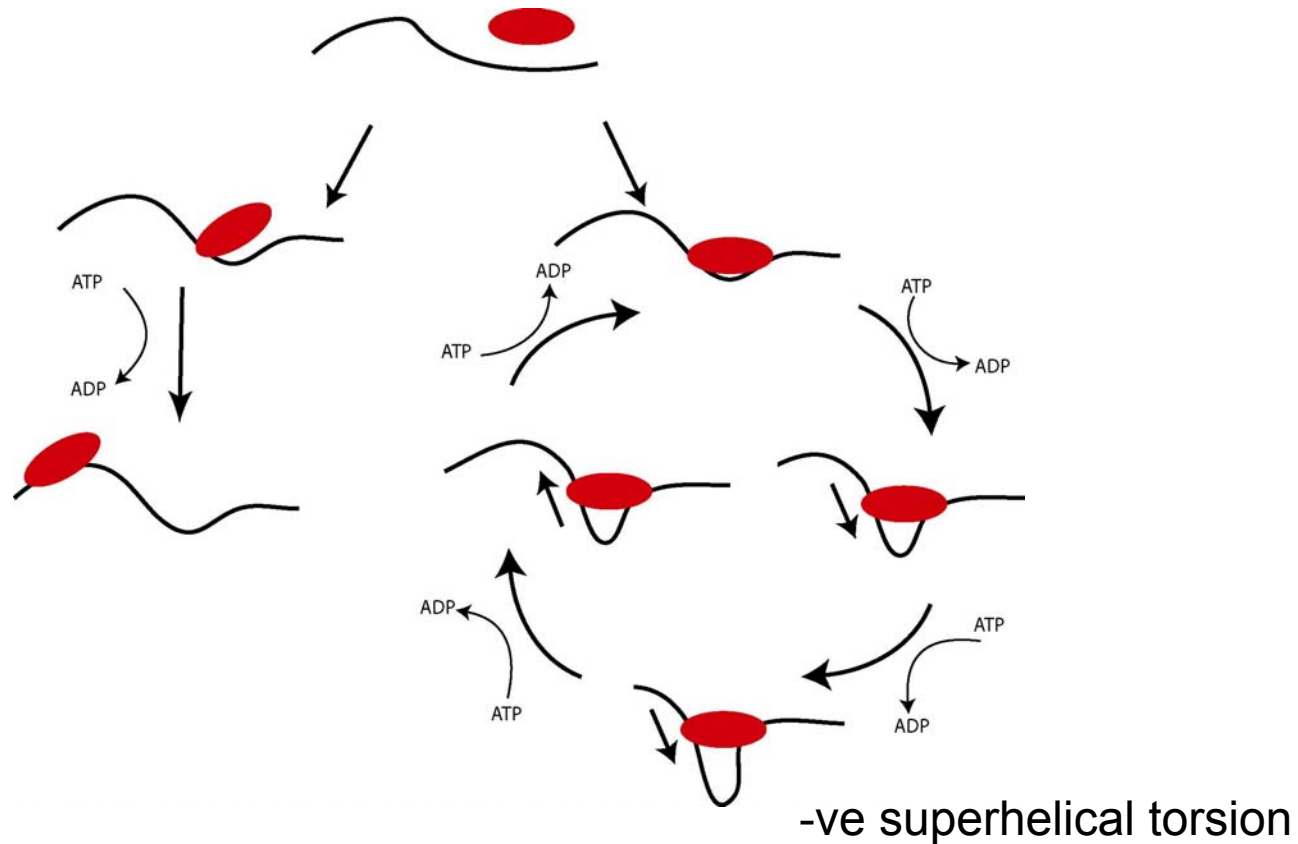
The times for shortening and extension are distributed exponentially



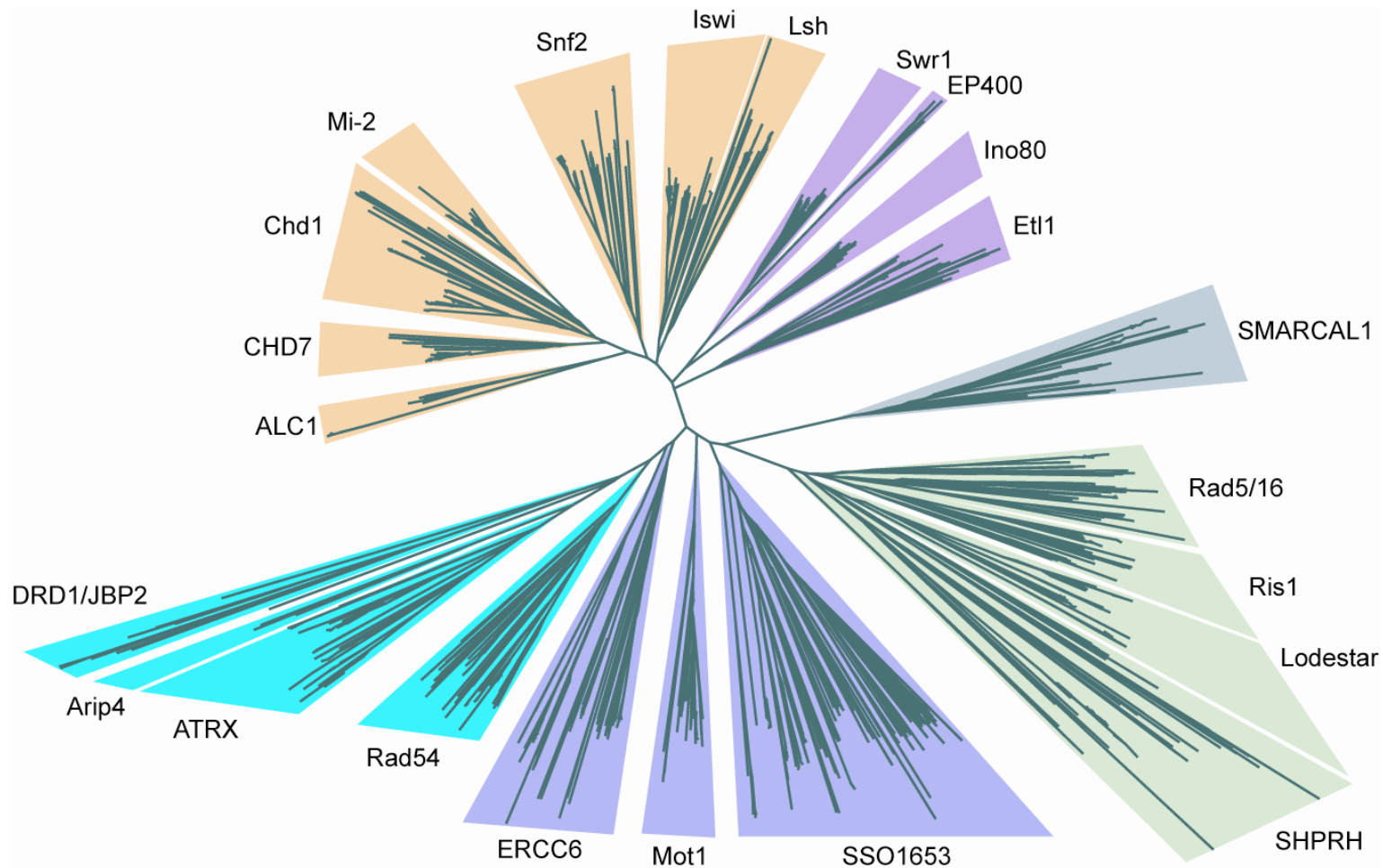
The distance translocated is sensitive to the force applied



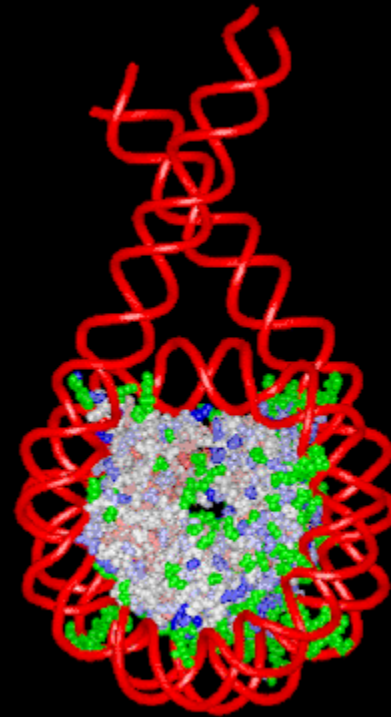
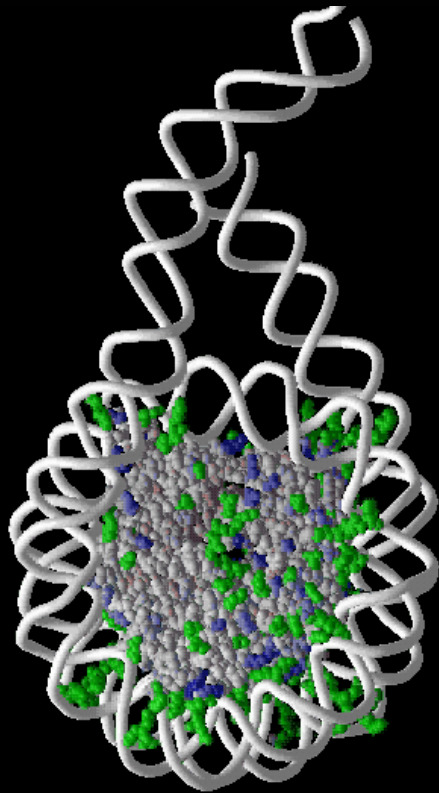
Translocation by RSC may be associated with a constrained loop



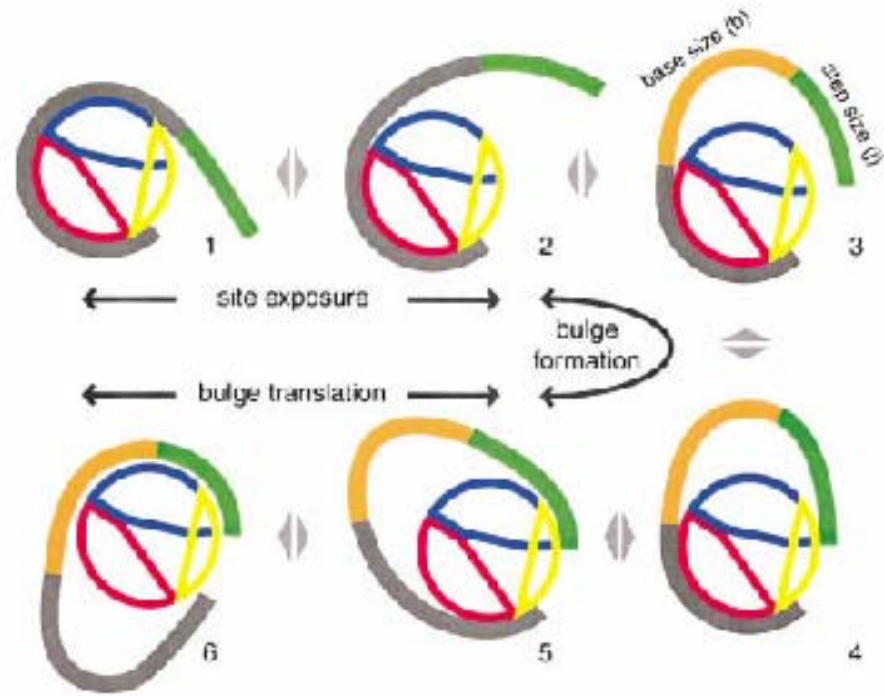
A subset of Snf2 family proteins have been observed to reposition nucleosomes



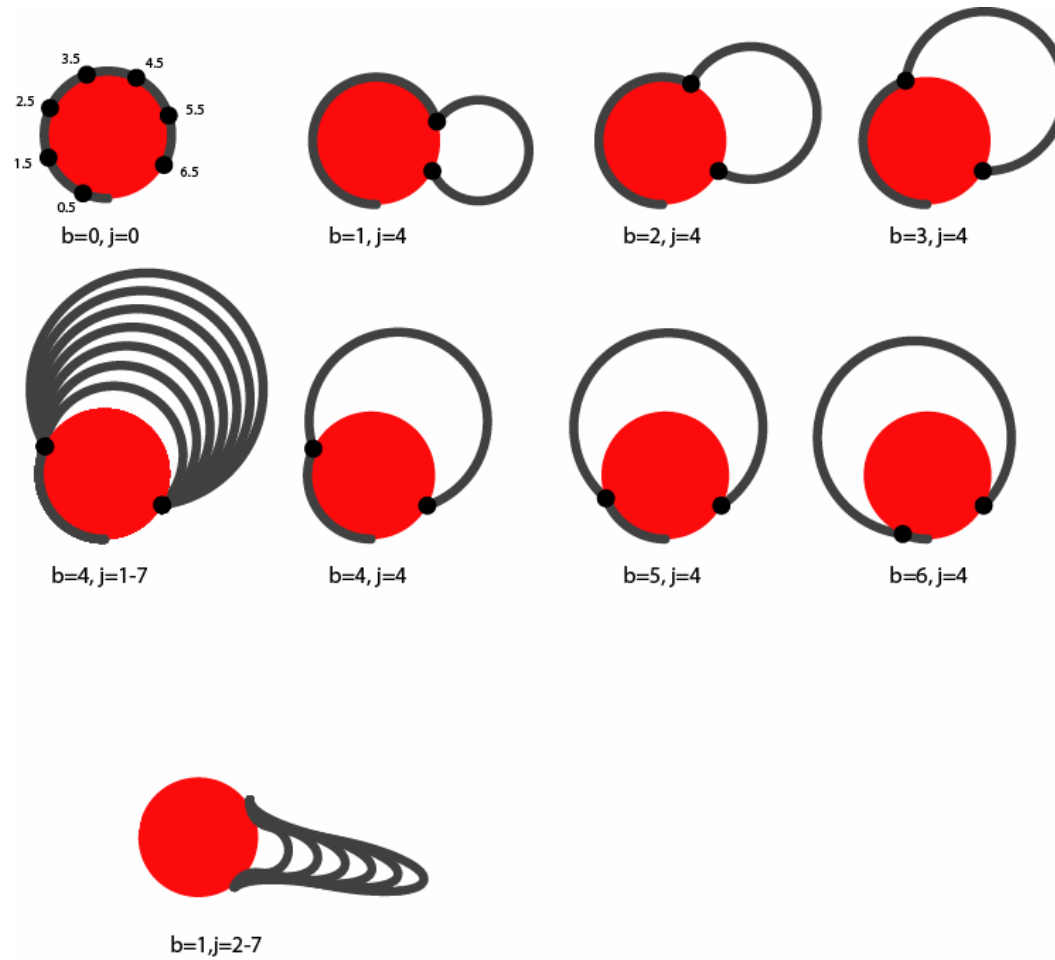
Possible mechanisms for nucleosome redistribution



Animations illustrating a couple of potential mechanisms for nucleosome repositioning can be viewed at: <http://www.dundee.ac.uk/biocentre/toh/mech1.html>



Different types of planar bulge



The elastic properties of DNA are likely to influence the type of bulge formed

$$\Delta G_{\text{bend}} = \frac{1}{2} B \left(\frac{\theta^2}{L} \right) \quad (1)$$

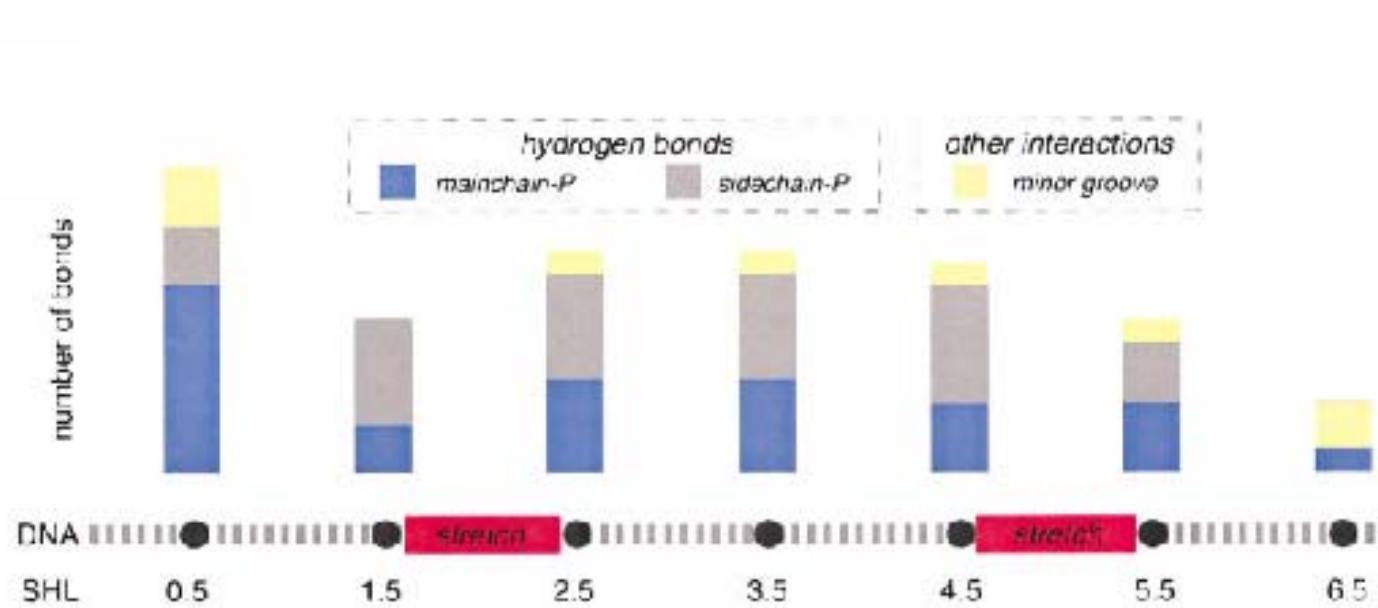
$$\Delta G_{\text{twist}} = \frac{1}{2} C \left(\frac{\phi}{L} \right)^2 L \quad (2)$$

Energy for deformation
Of DNA to form nucleosome = 14.5 kcal/mol = experimentally observed value 12-14

Net relative energy for bulging after compensating for loss of histone contacts.

	DNA Helical Turns Input (Step Size, j)							
	0	1	2	3	4	5	6	7
Bulge (b) from SHL6.5 to								
SHL5.5 (1)	1.0	1.80	1.76	1.65	1.56	1.49	1.43	1.38
SHL4.5 (2)	1.0	1.36	1.40	1.39	1.36	1.33	1.30	1.27
SHL3.5 (3)	1.0	1.26	1.28	1.28	1.26	1.25	1.23	1.21
SHL2.5 (4)	1.0	1.25	1.25	1.24	1.23	1.20	1.18	1.17
SHL1.5 (5)	1.0	1.27	1.26	1.24	1.22	1.20	1.19	1.17
SHL0.5 (6)	1.0	1.31	1.28	1.25	1.23	1.21	1.19	1.17

The binding of DNA at each superhelical location varies.



Are small deformations kinetically feasible?

