# PcrA helicase

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MCB 290

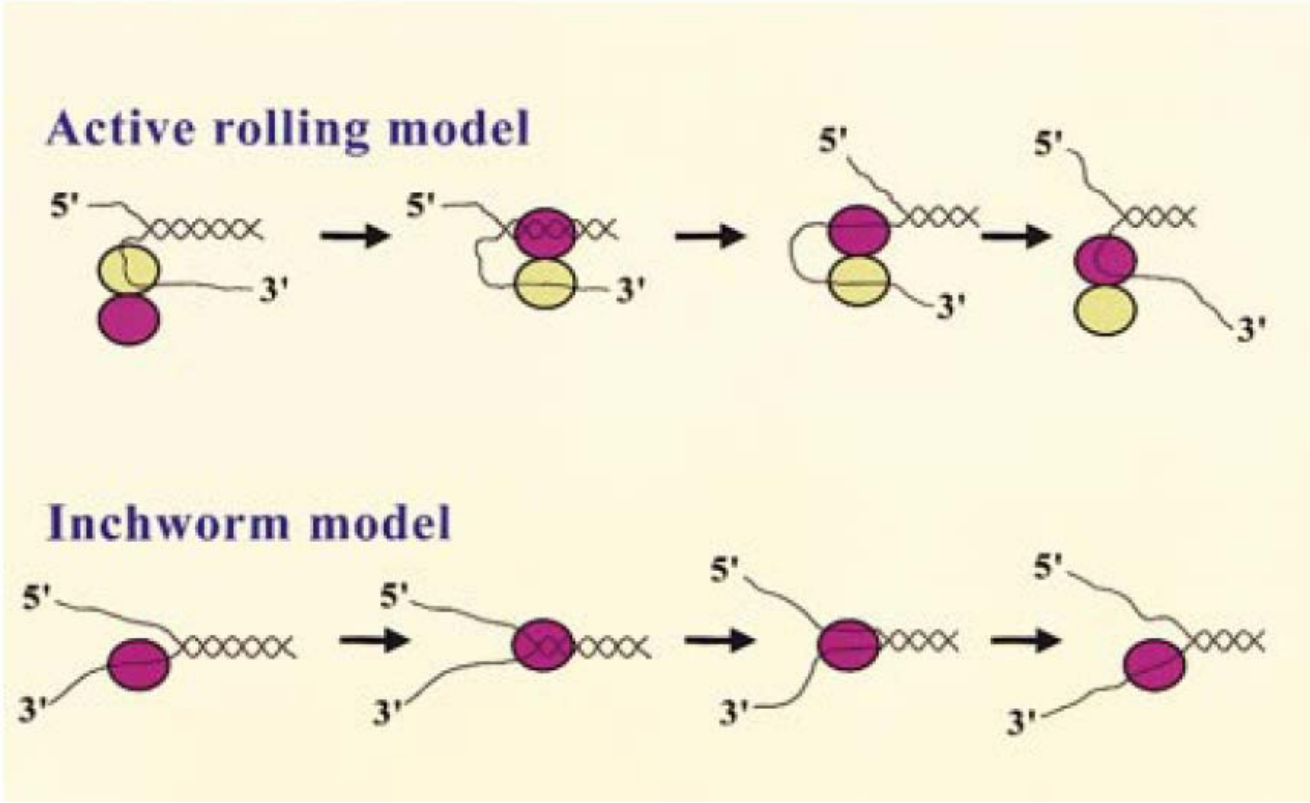
# Outline

- A few facts about helicases
- PcrA crystal structure
- Directionality, step size, elegant techniques
- PcrA: active helicase, not only translocase
- Translocation detailed model

# About DNA helicases

- Molecular motors that use ATP to open double stranded DNA
- Necessary for replication, recombination, repair and transcription (helicase defects in humans associated with cancer)
- Can be classified according to polarity – feed them dsDNA with either 3’ or 5’ overhang and see which one they unwind.
- Can be hexamers, tetramers, dimers or monomers.
- Classified according to primary structure –resulting in mechanistic debates like in the PcrA case

# The debate around PcrA

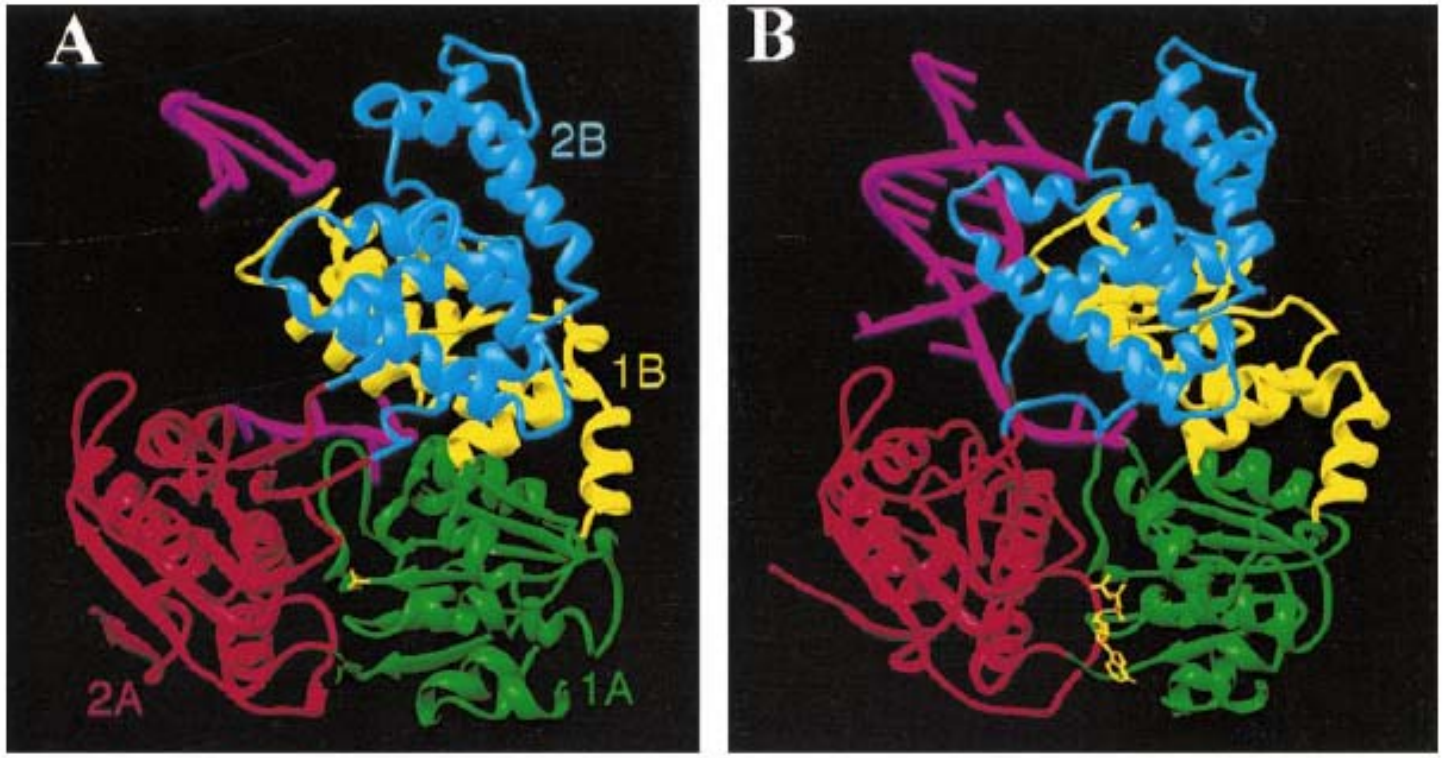



-**rolling model: step size has to be at least equal to the binding site size on the product single strand. Rep is believed to work this way.**

-**inchworm model: consistent with any step size.**

Velankar, S., P. Soultanas, M. Dillingham, H. Subramanya, and D. Wigley. Cell. 1999 Apr 2;97(1):75-84.

# Crystal structures with DNA




**A. PcrA monomer with sulphate -analog of phosphate ion (the product complex)**

**B. PcrA monomer with ATP analog: ADPNP (the substrate complex)**

**The bound DNA is colored magenta, with ADPNP and in gold**

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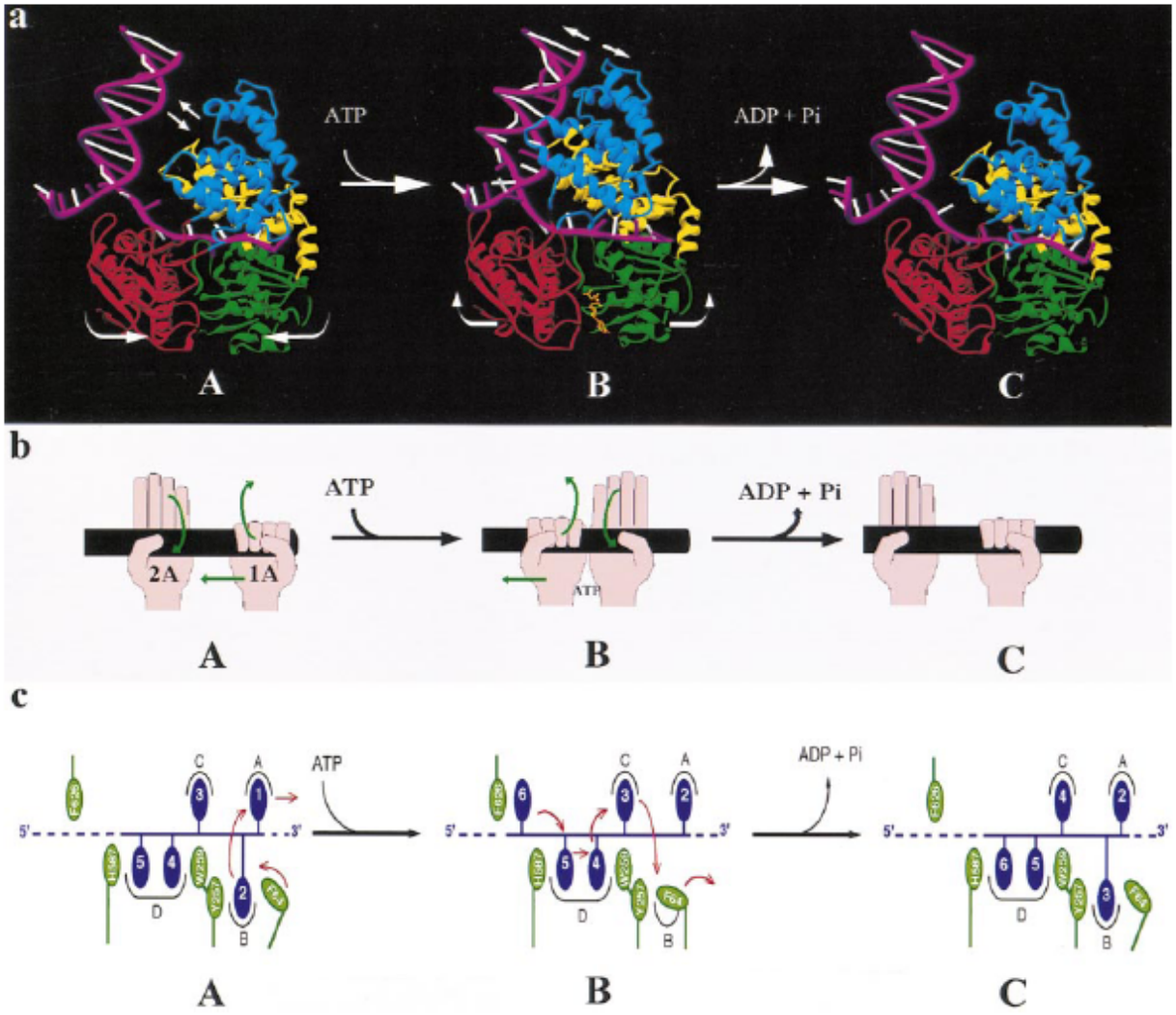
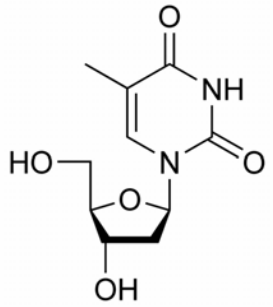
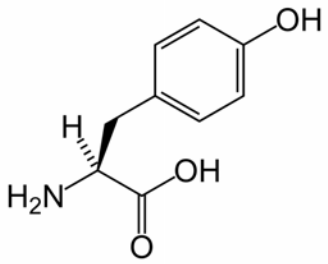
# Proposed mechanism




**Deoxythymidine**




**Tyrosine**

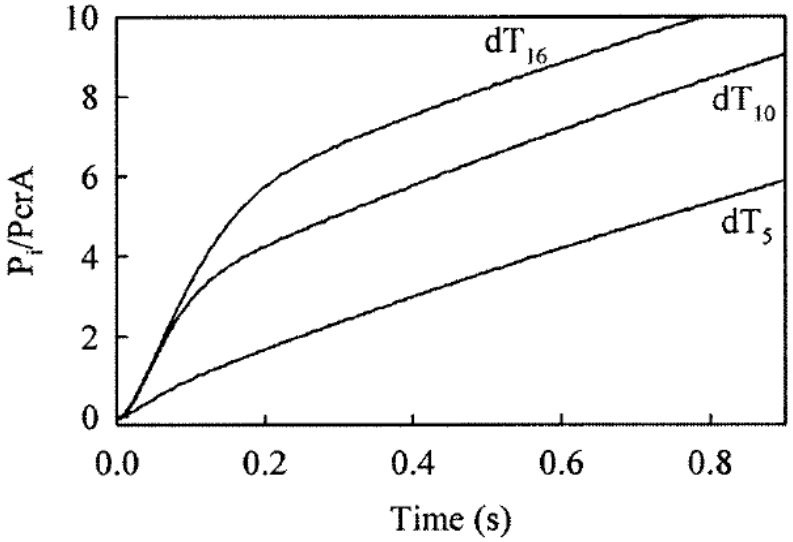
Velankar, S., P. Soultanas, M. Dillingham, H. Subramanya, and D. Wigley. Cell. 1999 Apr 2;97(1):75-84.

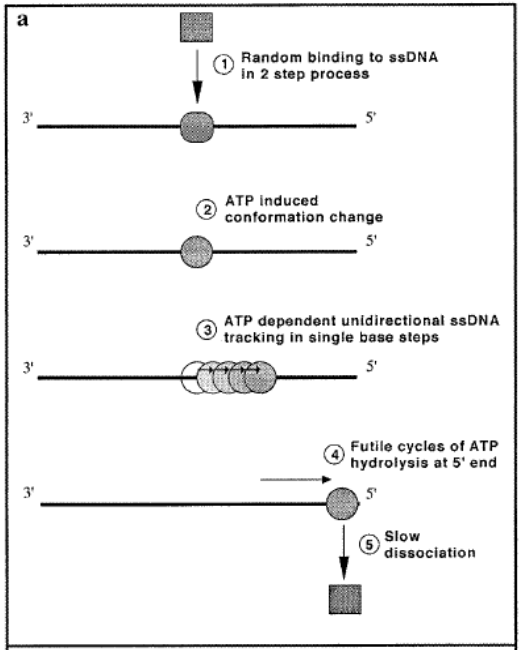
# Step size and translocation speed

-use fluorescent sensor for inorganic phosphate (MDCC-PBP)

-monitor fluorescence ⟷ watch kinetics of ATP hydrolysis by PcrA

-repeat in the presence of ssDNA (polyT) of various lengths.



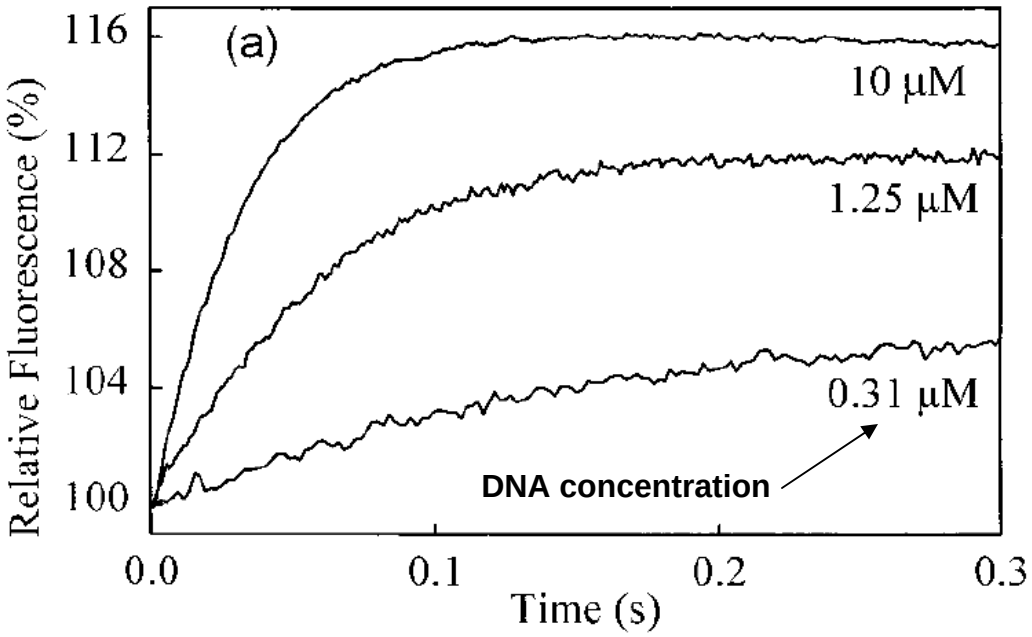



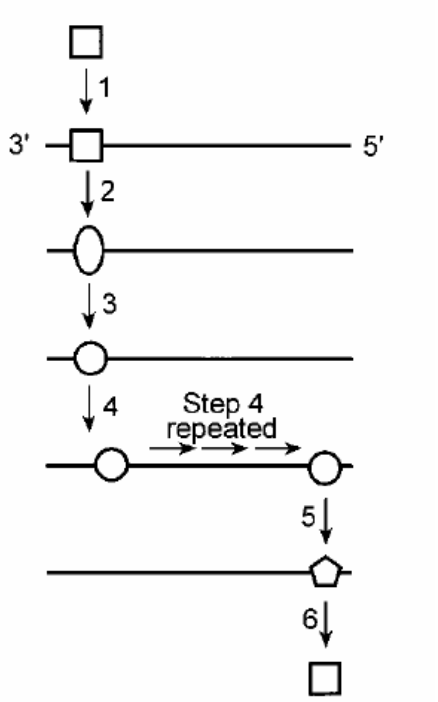

Dillingham MS, Wigley DB, Webb MR. Biochemistry. 2000 Jan 11;39(1):205-12.

# Stalling at the end

fluorescent base analogue 2-aminopurine :

- is a close analogue of adenine
- is capable of forming base pairs with thymine
- fluorescence increases by 80% when bound to a protein

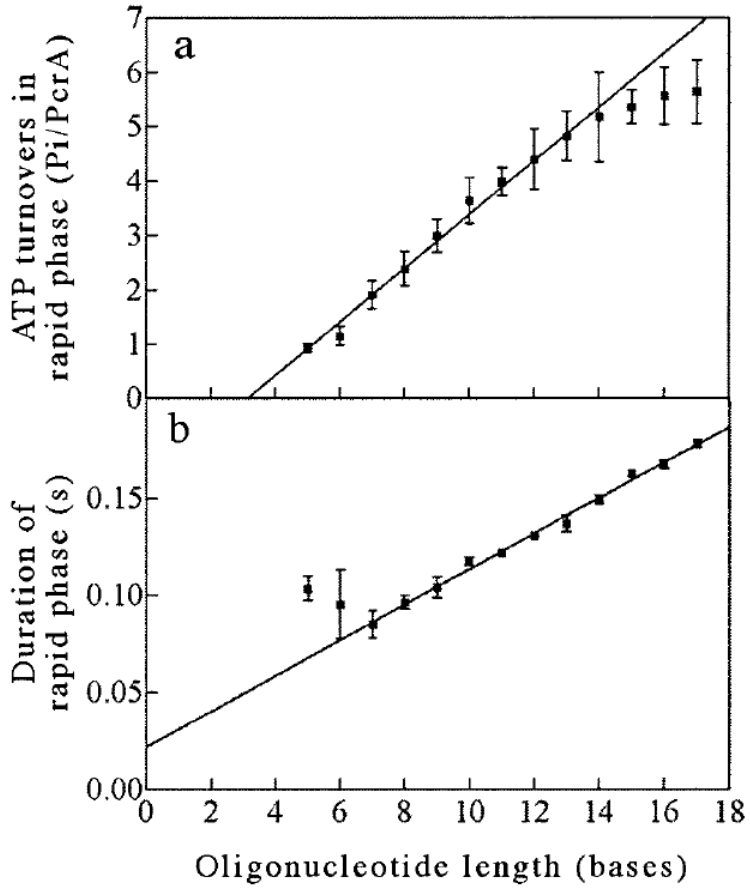


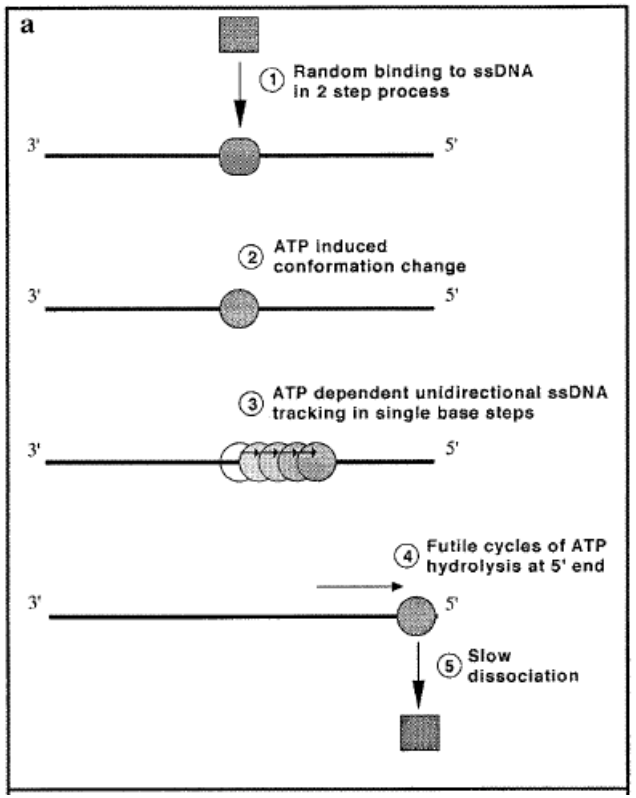




**Dillingham MS, Wigley DB, Webb MR. Biochemistry. 2002 Jan 15;41(2):643-51.**

# Step Size and Translocation Speed

**Slope of ATP/base=0.5 suggests step size of 1 base per ATP, if initiation on DNA is at random sites, and not at 3' end**

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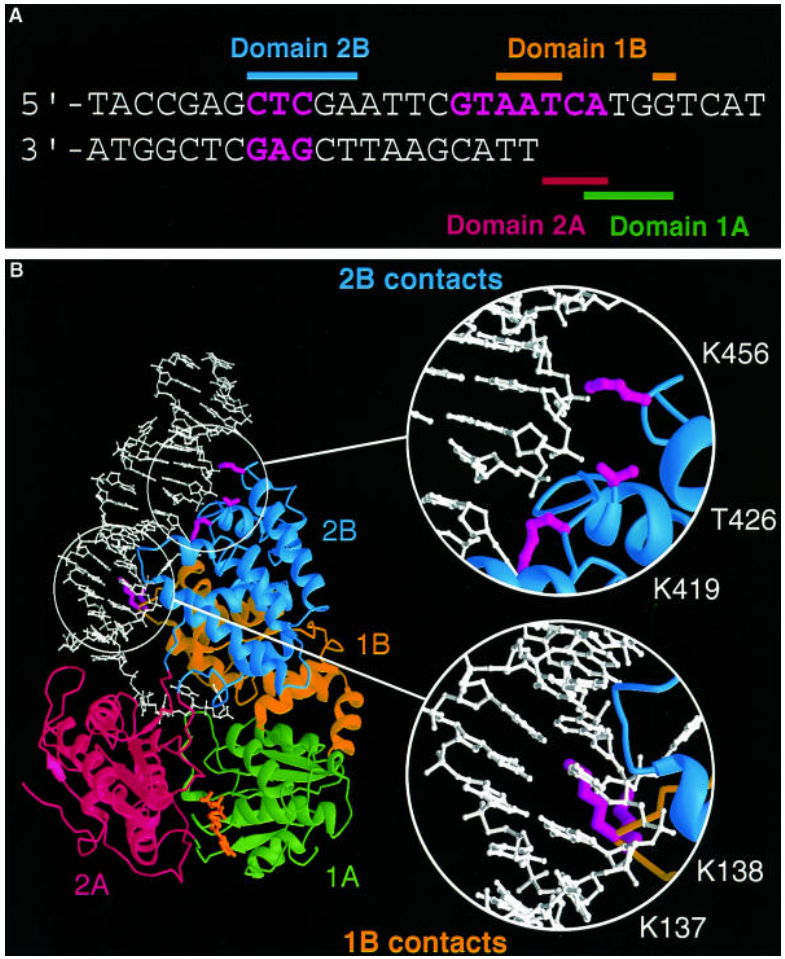
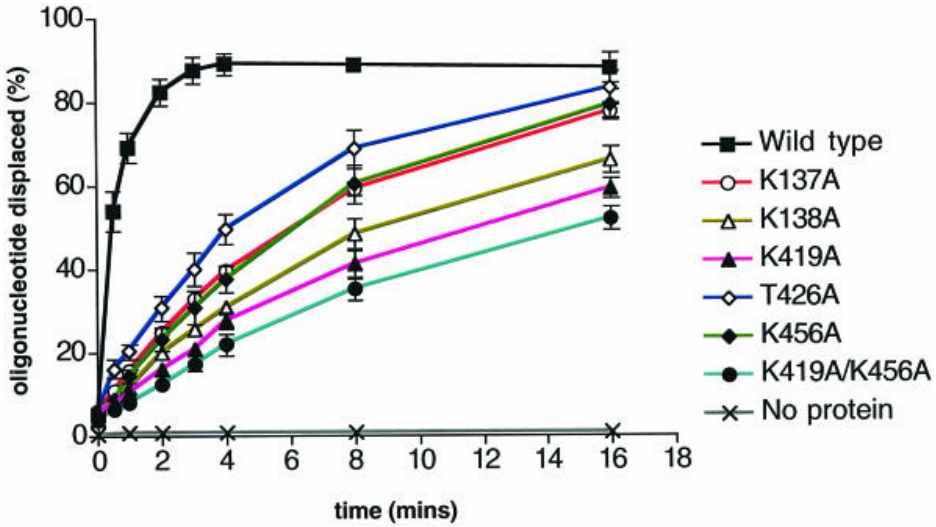
# Uncoupling DNA translocation and helicase activity in PcrA

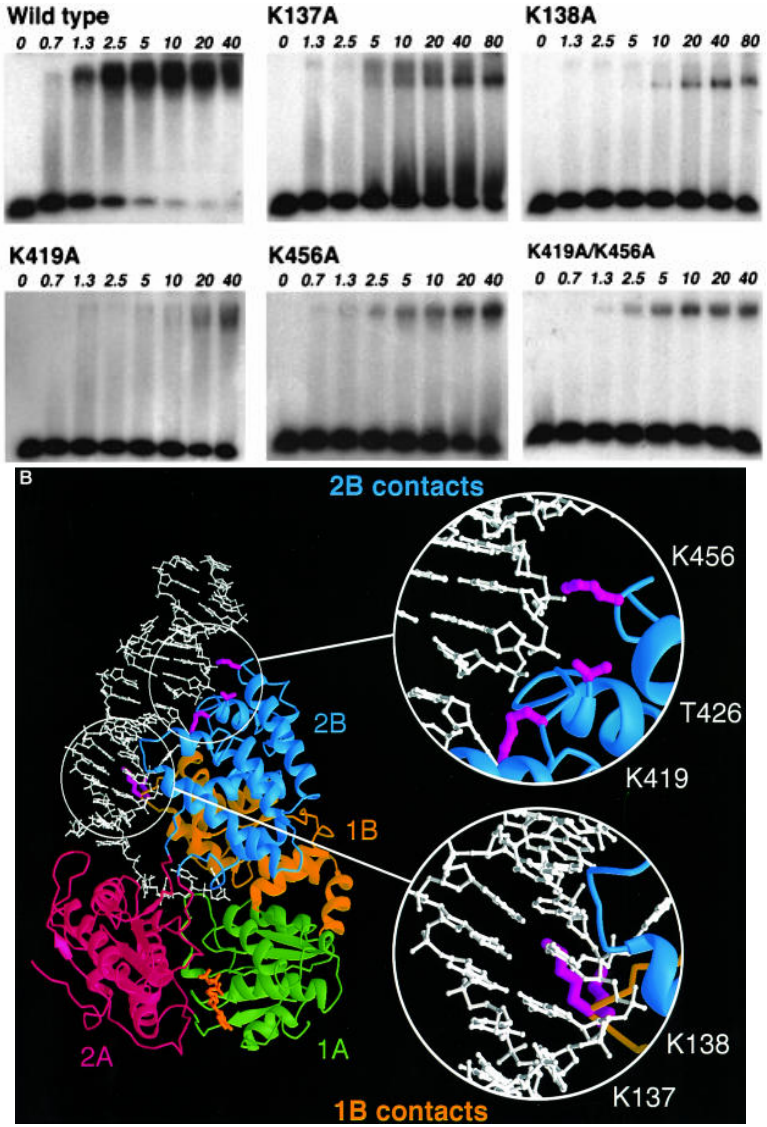


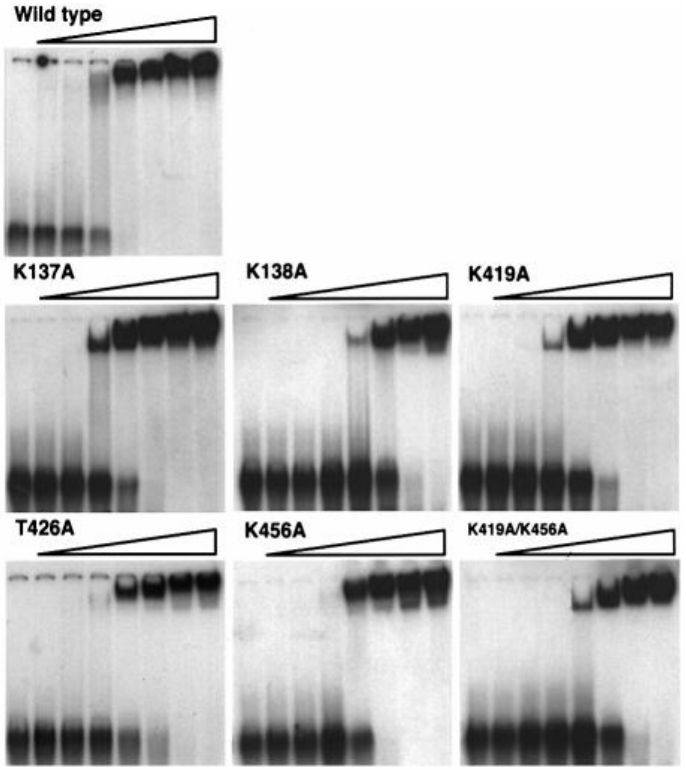

Soultanas P, Dillingham MS, Wiley P, Webb MR, Wigley DB. EMBO J. 2000 Jul 17;19(14):3799-810.

# DNA translocation and helicase activity

Binding to double stranded DNA impaired




Binding to single stranded DNA unaffected

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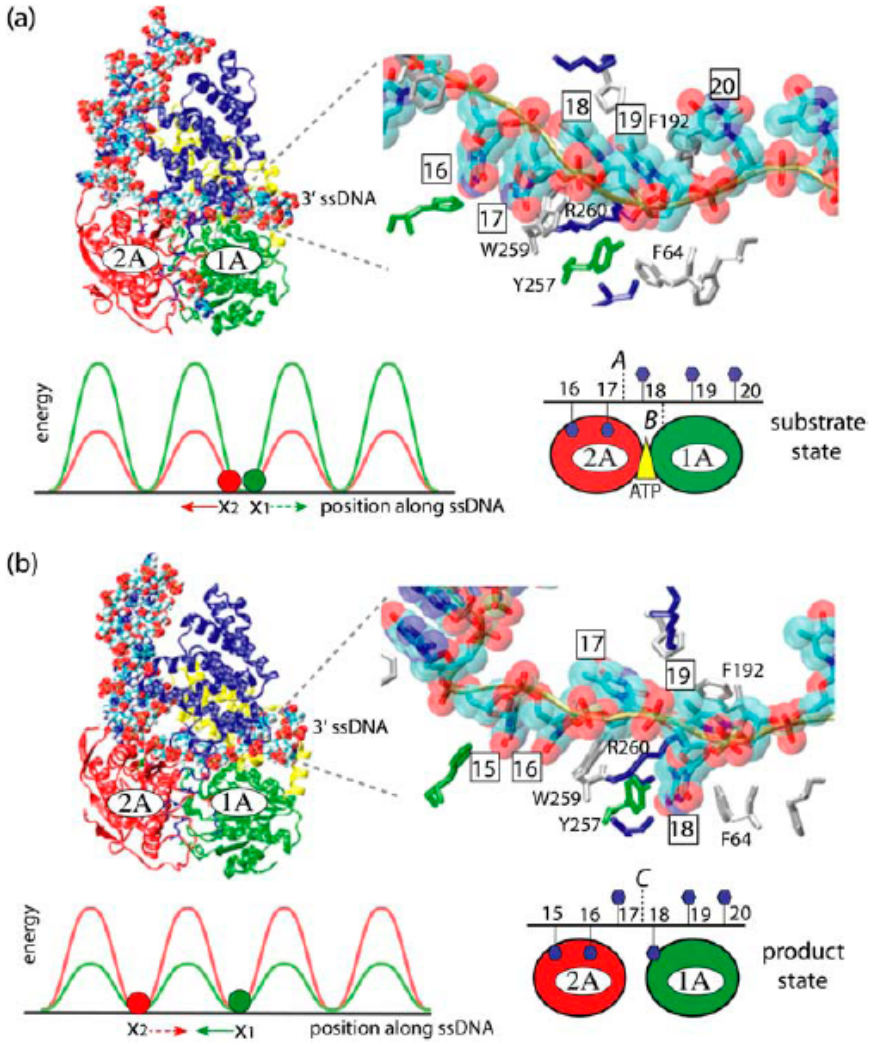
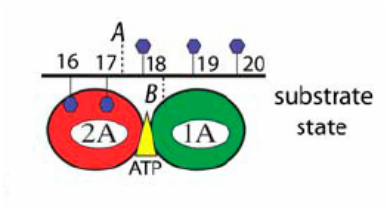
# Structure-based model of stepping

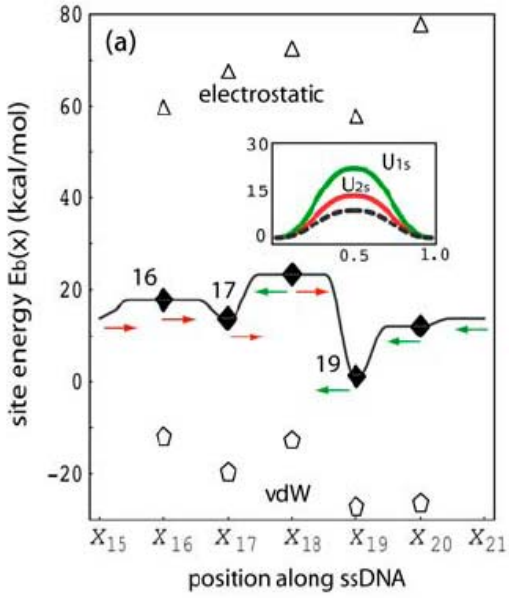


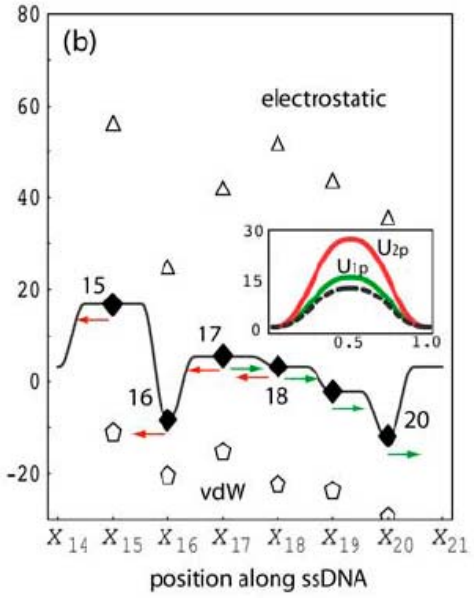

Yu J, Ha T, Schulten K. Biophys J. 2006 Sep 15;91(6):2097-114

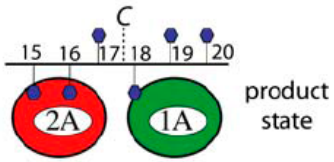
# Structure-based model of stepping

**ATP bound state** **$P_i$ bound state**




**Strategy:**

**-compute the binding energy of PcrA to individual nucleotides (Eb) using MD simulation**

**-use these energies and previous experimental data to estimate the potential of the domains in different states (U1s, U2s, U1p, U2p)**

**- explore how movement depends on the interaction potential between domains (V)**

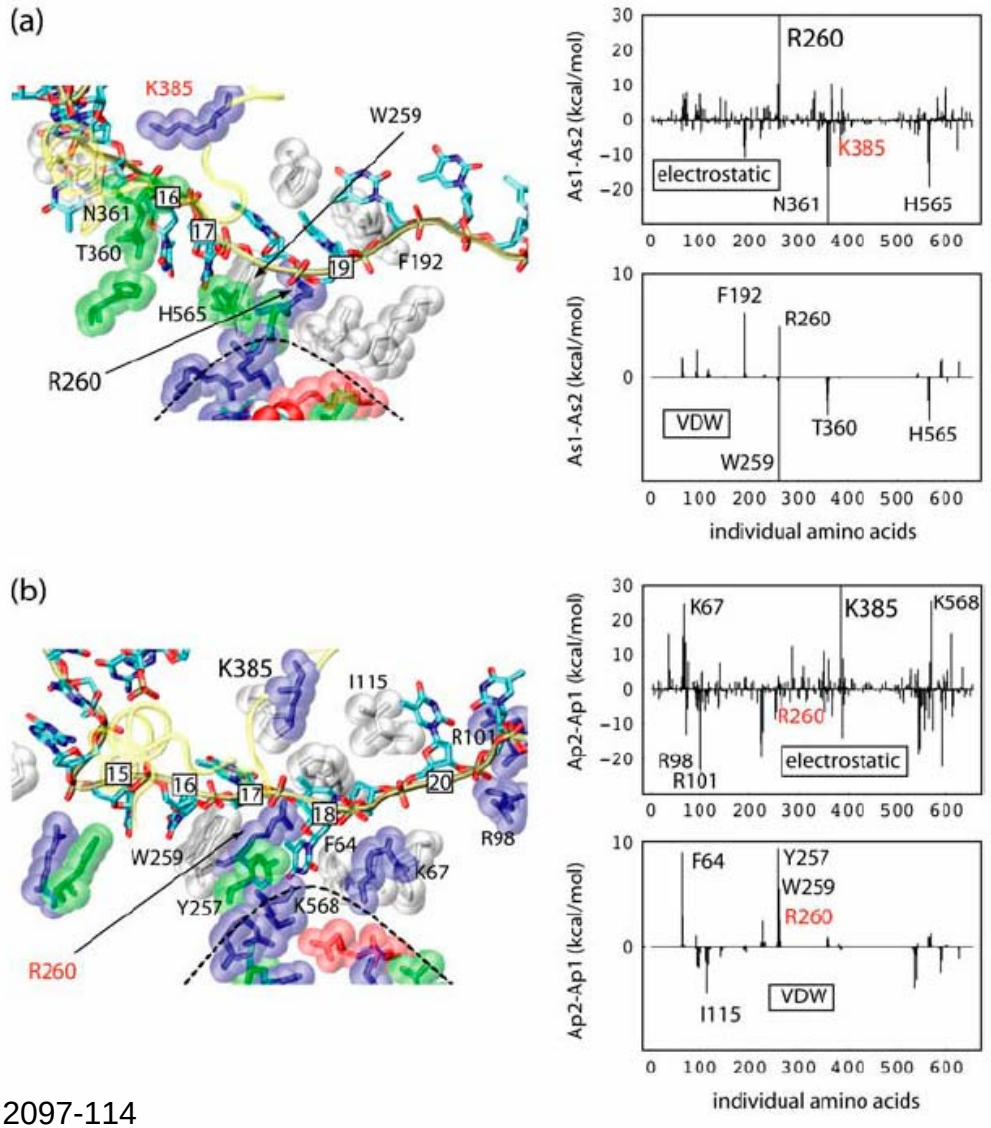
Yu J, Ha T, Schulten K. Biophys J. 2006 Sep 15;91(6):2097-114

# The amino-acids essential for direction

Contribution from individual amino-acid residues to the barrier difference |A2s–A1s| of domain motions of PcrA

(a) with ATP bound
(b) without ATP bound

Two main aminoacids:
-Arg-260 (agrees with mutations)
-Lys-385 (this model suggests that its mutation will disrupt helicase activity)

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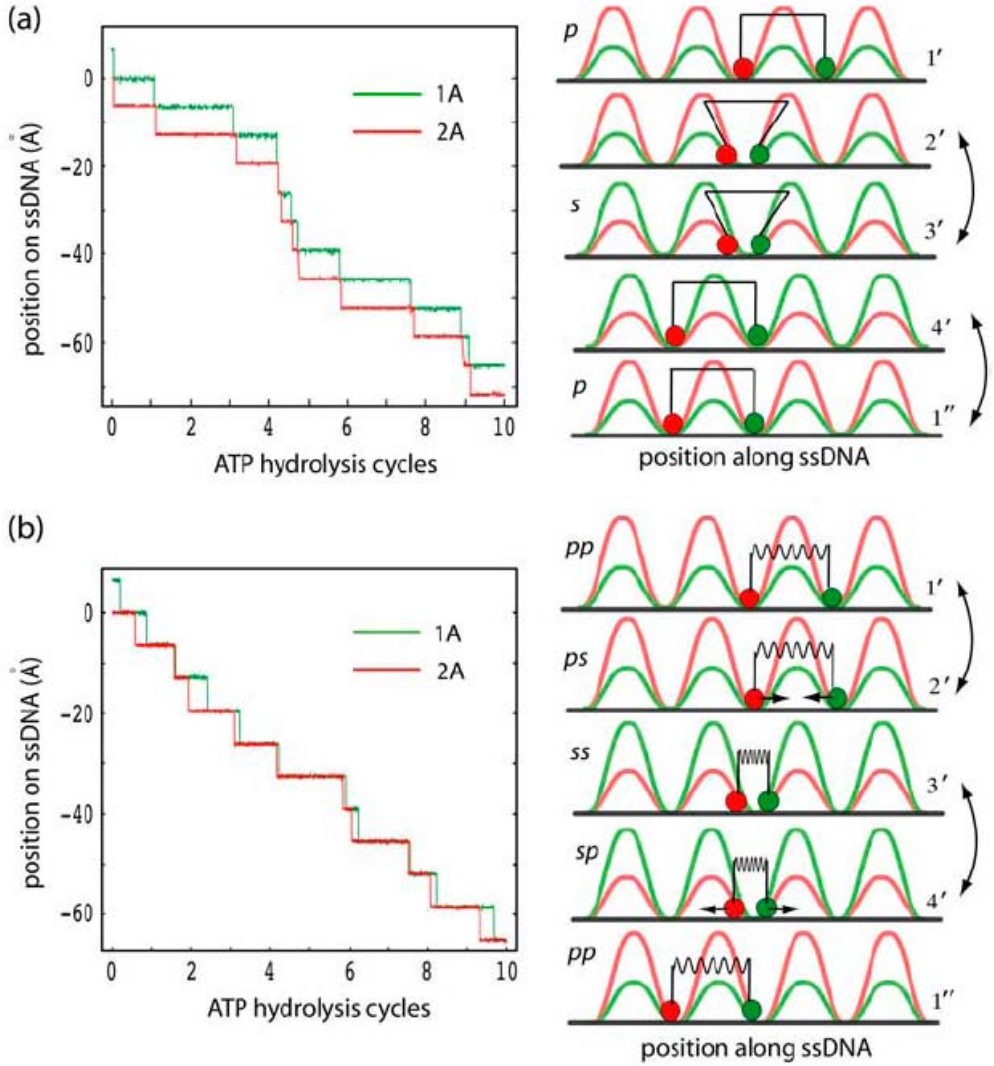
# Weak versus strong coupling

**Weak coupling**

**(the barrier-crossing domain movements are rate limiting)**

**Strong coupling**

**(the arrival of ATP and ATP hydrolysis are rate limiting)**

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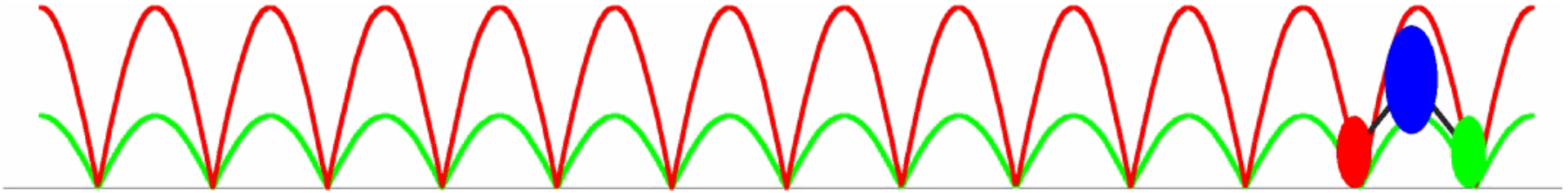
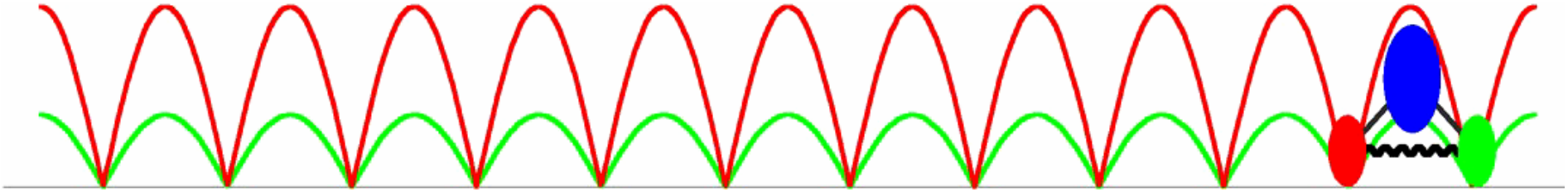
# Weak versus strong coupling

**Weak Coupling**

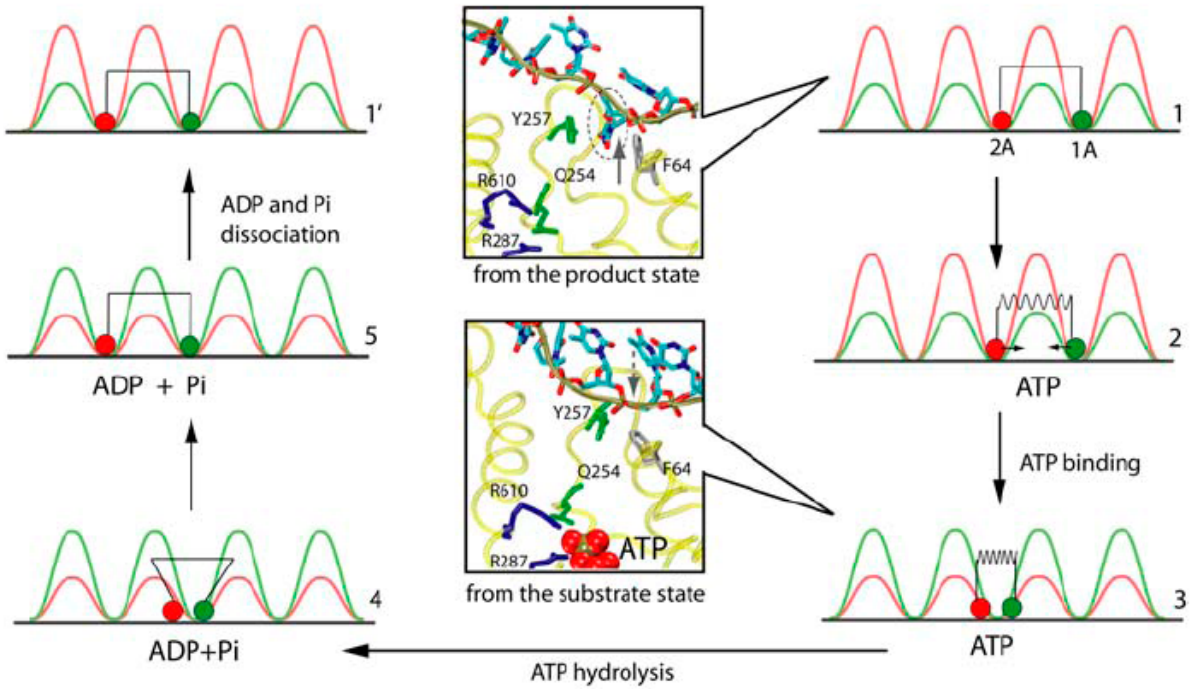


**Strong Coupling**



Yu J, Ha T, Schulten K. Biophys J. 2006 Sep 15;91(6):2097-114 **(movies from supplemental material)**

# PcrA mixes weak and strong coupling




**Mechanism proposed:**

-**structural homology with F1-ATPase implies power stroke on ATP binding – need strong coupling**

**- structural homology with Rep implies slow ADP release – need weak coupling**

Yu J, Ha T, Schulten K. Biophys J. 2006 Sep 15;91(6):2097-114

# Conclusions

- It’s good to know the structure!
- Model predicts a new mutation that should abolish helicase activity of PcrA (Lys-385).
- Model predicts a very precise kinetic mechanism that can be tested experimentally.
- New understanding of helicase mechanism allows engineering of a helicase that goes in the opposite direction on the same substrate.