DNA polymerase Summary

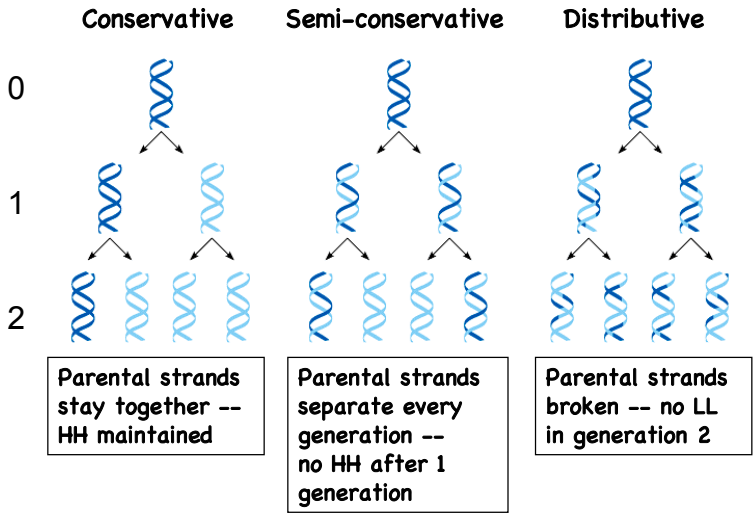
1. DNA replication is semi-conservative.
2. DNA polymerase enzymes are specialized for different functions.
3. DNA pol I has 3 activities: polymerase, 3'-->5' exonuclease & 5'-->3' exonuclease.
4. DNA polymerase structures are conserved.
5. But: Pol can't start and only synthesizes DNA 5'-->3'!
6. Editing (proofreading) by 3'-->5' exo reduces errors.
7. High fidelity is due to the race between addition and editing.
8. Mismatches disfavor addition by DNA pol I at 5 successive positions. The error rate is ~$1/10^9$.

DNA replication is semi-conservative

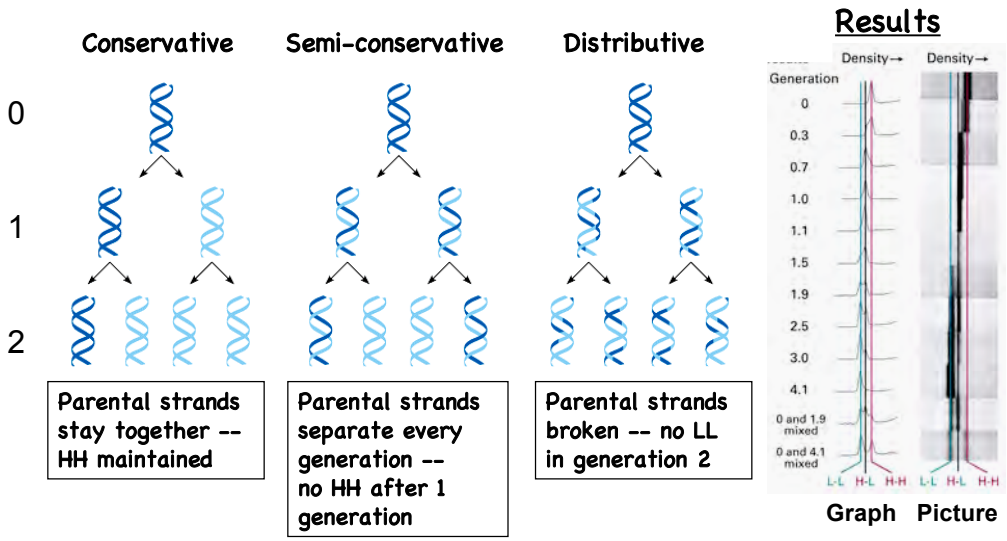
Meselson-Stahl experiment

1. Grow E. coli on ^{15}N ("heavy") ammonia
2. Switch to ^{14}N (normal, "light") ammonia
3. Harvest aliquots as a function of time
4. Isolate DNA
5. Separate on the basis of DNA density using density gradient centrifugation
   A. Pour $CsCl_2$ gradient into a tube
   B. Layer DNA on top
   C. Centrifuge until DNA stops moving (DNA floats when the density matches that of the salt solution)

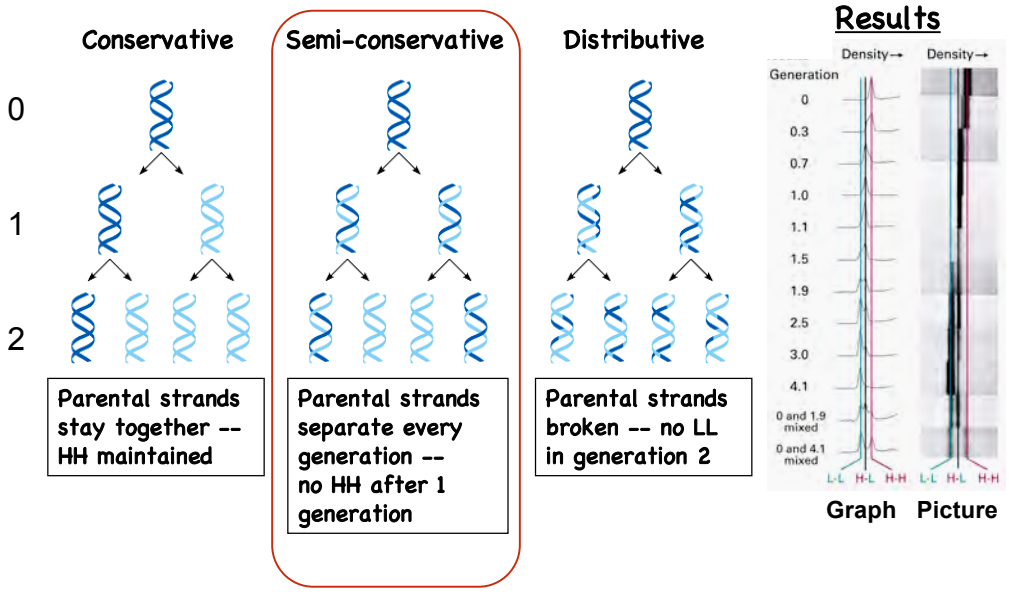
Predictions of Meselson-Stahl experiment



Results of Meselson-Stahl experiment



DNA replication is semi-conservative



Arthur Kornberg discovered DNA dependent DNA polymerase

Used an "*in vitro*" system: the classic biochemical approach

1. Grow *E. coli*
2. Lyse cells
3. Prepare extract
4. Fractionate extract
5. Search for DNA polymerase activity using an ASSAY
   (Incorporate radioactive building blocks,
   Precipitate DNA chains (nucleotides soluble),
   Quantify radioactivity.)

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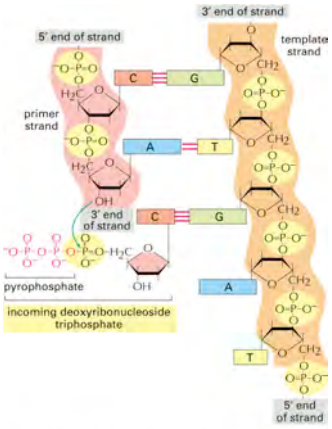
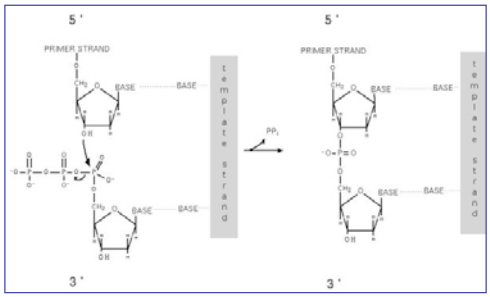
Requirements for DNA polymerase activity

Template	[Basis for heredity]
dNTPs (not ATP, not NDPs, not NMPs)	[Building blocks]
Mg^{2+}	[Promotes reaction]
Primer - (complementary bases at 3' end, removed by fractionation and added back)	[DNA pol can't start!]

DNA polymerase mechanism





Each dNTP provides the nucleophile (3'-OH) for the next round
PPi hydrolyzed to 2 $PO_4^{=}$

Nobel Prize for DNA polymerase I

Comparison of DNA Polymerases of *E. coli*

	DNA polymerase I
Structural gene*	*polA*
Subunits (number of different types)	1
M_r	103,000
3′→5′ Exonuclease (proofreading)	Yes
5′→3′ Exonuclease	Yes
Polymerization rate (nucleotides/sec)	16–20
Processivity (nucleotides added before polymerase dissociates)	3–200

Mutant viable? Yes!

Nobel Prize for DNA polymerase I

Comparison of DNA Polymerases of *E. coli*

	DNA polymerase I	II
Structural gene*	*polA*	*polB*
Subunits (number of different types)	1	≥4
M_r	103,000	88,000†
3′→5′ Exonuclease (proofreading)	Yes	Yes
5′→3′ Exonuclease	Yes	No
Polymerization rate (nucleotides/sec)	16–20	40
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500

Mutant viable? Yes! Yes!

Nobel Prize for DNA polymerase I

Comparison of DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	*pol*A	*pol*B	*pol*C (*dna*E)
Subunits (number of different types)	1	≥4	≥10
M_r	103,000	88,000†	830,000
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/sec)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

Mutant viable? Yes! Yes! No

Nobel Prize for DNA polymerase I

Comparison of DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	*pol*A	*pol*B	*pol*C (*dna*E)
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M_r	103,000	88,000†	830,000
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
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Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

Mutant viable? Yes! Yes! No
Function repair replication

+ DNA pol IV: mutagenesis
+ DNA pol V: error-prone repair

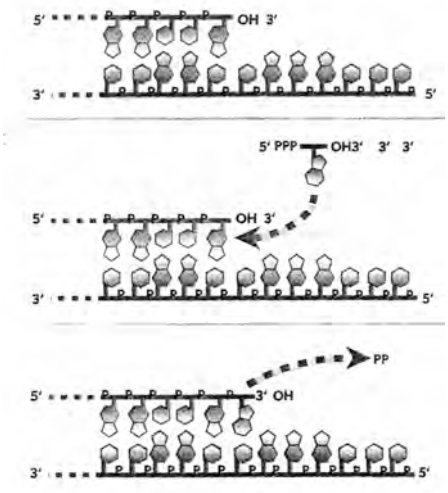
Examples of eukaryotic DNA polymerases

Greek name	HUGO name	Class	Other names	Proposed main function
α (alpha)	POLA	B	*POL1*	DNA replication
β (beta)	POLB	X		Base excision repair
γ (gamma)	POLG	A	*MIP1*	Mitochondrial replication
δ (delta)	POLD1	B	*POL3*	DNA replication
ε (epsilon)	POLE	B	*POL2*	DNA replication
ζ (zeta)	POLZ	B	*REV3*	Bypass synthesis
η (eta)	POLH	Y	*RAD30*, XPV	Bypass synthesis
θ (theta)	POLQ	A	mus308, eta	DNA repair
ι (iota)	POLI	Y	RAD30B	Bypass synthesis
κ (kappa)	POLK	Y	DinB1, theta	Bypass synthesis
λ (lambda)	POLL	X	*POL4*, beta2	Base excision repair
μ (mu)	POLM	X		Non-homologous end joining
σ (sigma)	POLS	X	*TRF4*, kappa	Sister chromatid cohesion
	REV1L	Y	*REV1*	Bypass synthesis
	TDT	X		Antigen receptor diversity

plus many more

Pol	α	β	δ	ε	γ (mitochondrial)
Mass	**300,000**	**40,000**	**170-230,000**	**250,000**	**180-300,000**

DNA polymerase activities -- 5'-->3' nucleotide addition



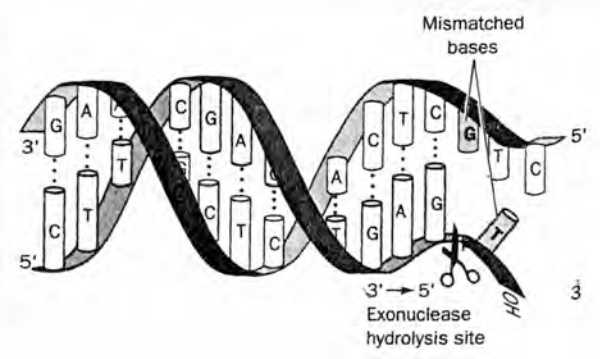
Primer has a free 3'-OH

Incoming dNTP has a 5' triphosphate

Pyrophosphate (PP) is lost when dNMP adds to the chain

DNA polymerase reactions -- editing

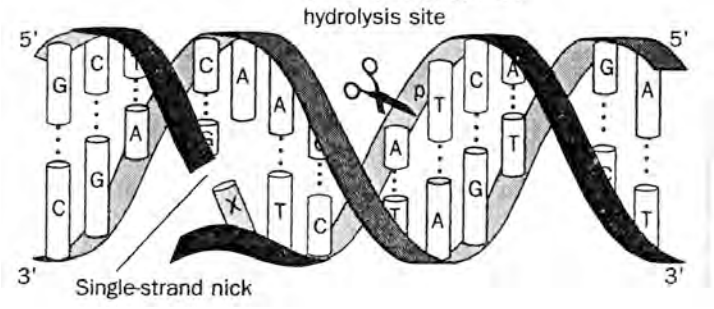
3'-->5' exonuclease



Opposite reaction compared to polymerase
(But no PPi used or dNTP made)

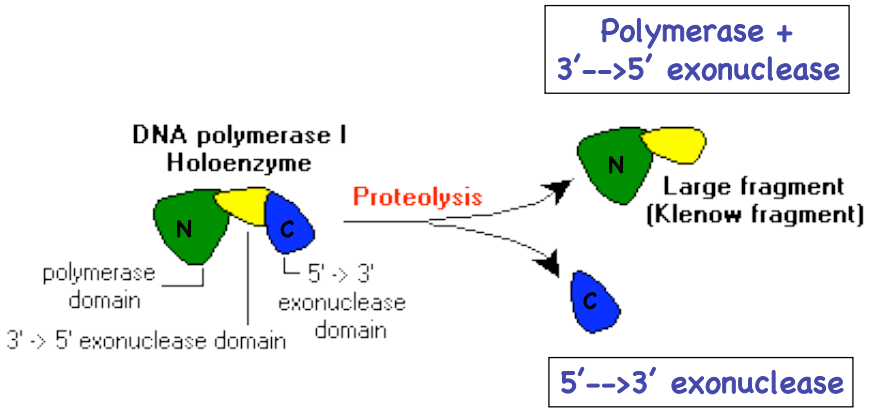
DNA polymerase reactions -- nick translation

5'-->3' exonuclease

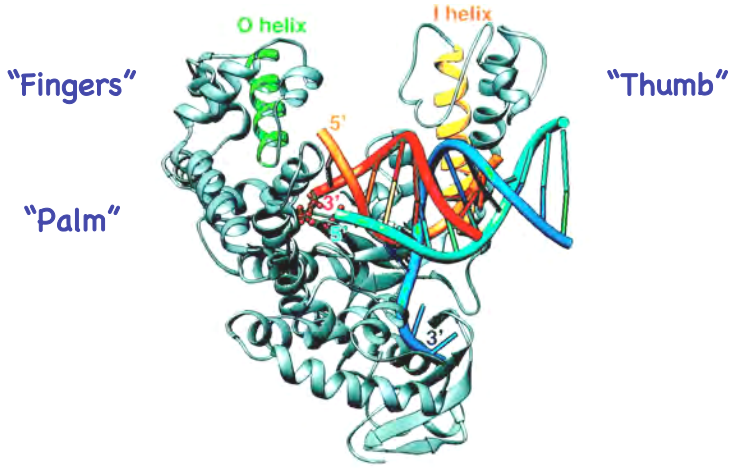


Creates single-stranded template in front for repair

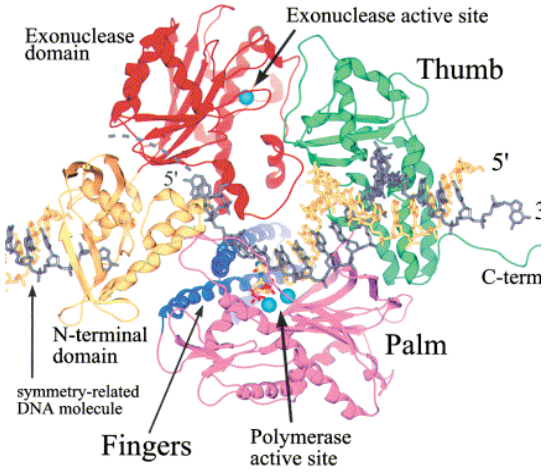
DNA pol I Klenow fragment lacks 5'-->3' exonuclease



Structure of the DNA complex of the Klenow fragment of DNA pol I

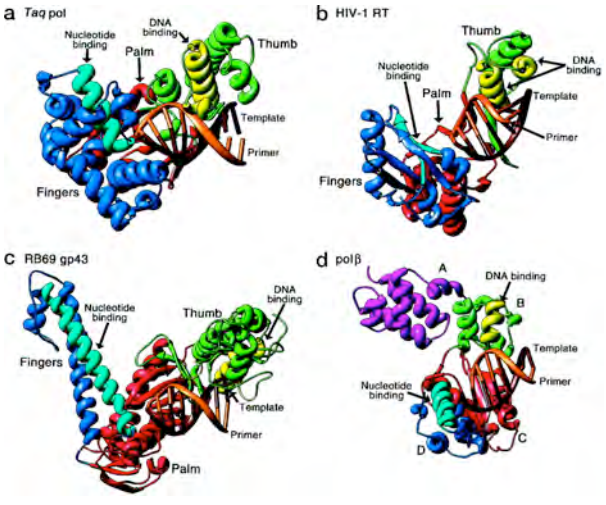


Functional sites in RB69 DNA polymerase + primer-template + dTTP

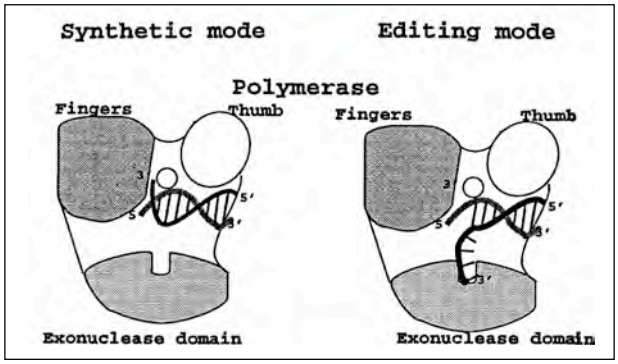


Fold conserved in DNA polymerases

"Fingers" "Palm" "Thumb"

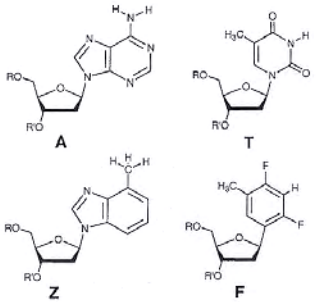


Two different active sites for nucleotide addition and 3'--> 5" exonuclease



Polymerization is a race against the 3'-->5' exonuclease
Relative rates of addition and exonuclease control net reaction
Fidelity is due to the race between the polymerase and exonuclease
Model: Rate of nucleotide addition dominates the net reaction.

DNA pol I reads shape (and polarity) of the incoming dNTP



Nonpolar A & T analogs add and direct synthesis efficiently!

Fig. 2 Autoradiogram of denaturing polyacrylamide gels showing single-nucleotide insertions with *a*, A or Z in the template strand and *b*, using dATP or dZTP as the nucleotide substrate. The data were taken at 37 °C using Klenow fragment (exo-) 0.2 units μl^{-1}, 5 μM primer–template duplex, 20 μM dNTP and the reactions were stopped after 2 min.

How does the polymerase "sense" mismatches?

Mismatches distort the polymerase active site

Experiment

1. Crystallize Klenow fragment plus primer-template
2. Add one Mg-dNTP
3. Wait for nucleotide addition in the crystal
4. In different crystals, create all 12 possible mismatches
5. Determine all 12 crystal structures
6. For G-T mismatch, add the next dNTP to move the mismatch
7. Solve structure
8. Repeat 6 & 7 four times to move mismatch away from the entry site.
9. Compare structures with correctly paired primer.

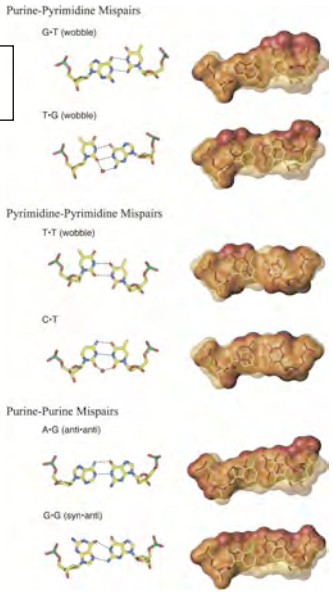
Results: Mismatches at n-1 to n-5 distort pol active site -- FIVE CHANCES TO CORRECT THE MISTAKE!

Johnson & Beese (2004) *Cell* **116**, 803-16.

Mismatched base pairs in the "entry site" distort the Pol I active site.

Left: Mismatch H-bonding pattern.

Johnson & Beese (2004) *Cell* **116**, 803-16.



Right: Molecular surface of the mismatch (red) compared to a cognate G-C pair (green) highlights the change in the structure of the primer terminus.

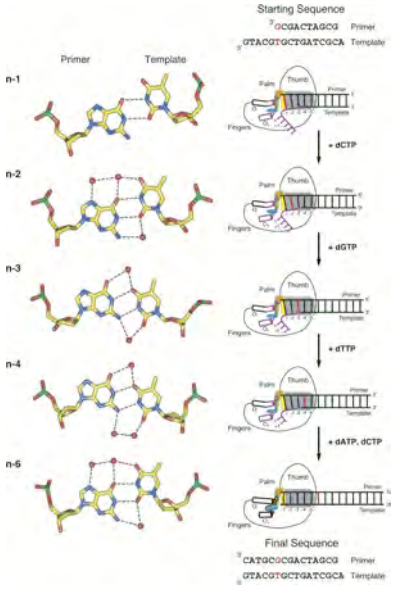
Four categories of distortions by insertion-site mismatches

1. Disruption of template strand and nucleotide binding site (G-T, G-G, A-C, T-C).
2. Disruption of primer strand arrangement and catalytic site (T-T, C-T).
3. Disruption of template and primer strands (A-G, T-G).
4. Fraying of added nucleotide (A-A, G-A, C-C).

Active site distortions continue for five succeeding additions!

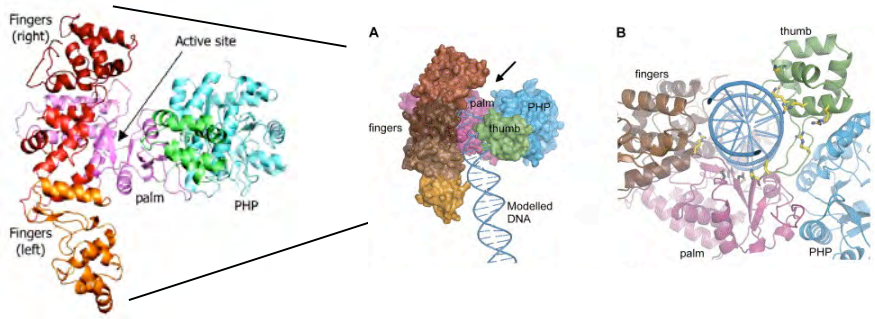
H-bonds to mismatched G-T pair (dashes) at each position after incorporation. Spheres are water molecules. N-3 and n-4 require base ionization or tautomerization of a base.

Mismatch slows polymerase for 5 successive additions, favoring the exonuclease reaction.



Bacterial DNA polymerase III: a distinct polymerase fold

Pol III structure --> Model for DNA complex



- Revealed conserved features of the DNA polymerase that copies bacterial genomes.
- Established a new model of the elongation complex including binding sites for DNA and interacting proteins.

Lamers et al. (2006) *Cell* **126**, 881-92; Bailey et al. (2006) *Cell* **126**, 893-904.

Summary

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