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OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

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OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Phrama scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

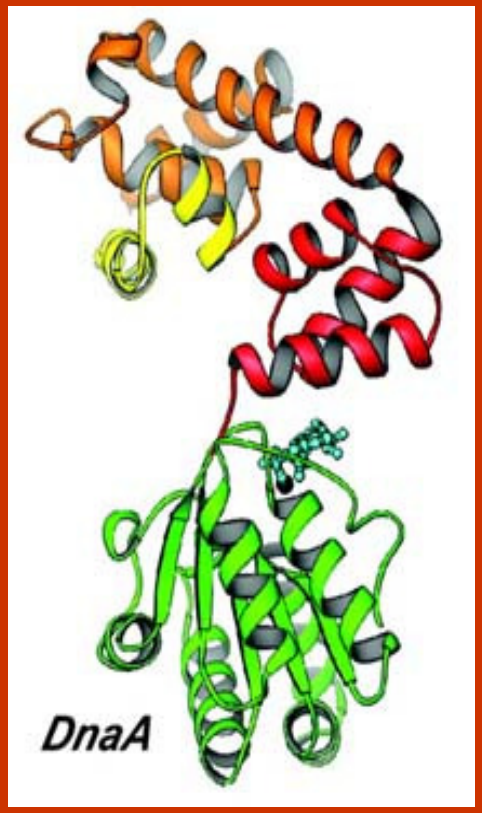
OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

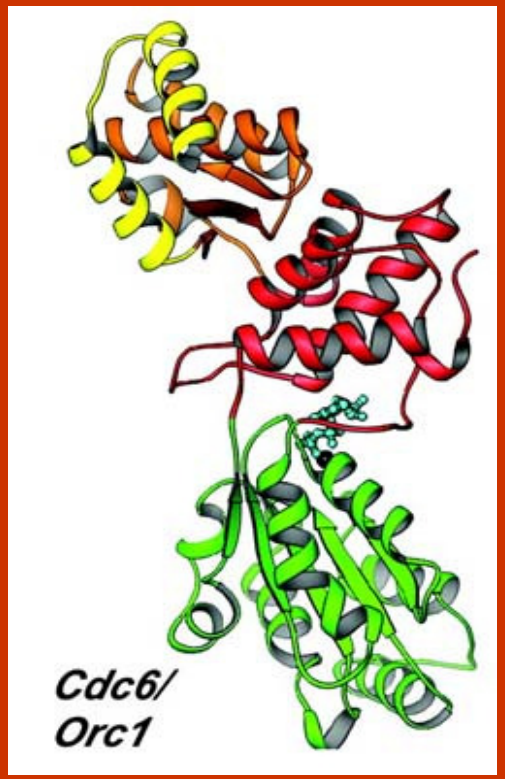
Crosstalk between acidic phospholipids present in bacterial membranes and DnaA, the initiator of Escherichia coli chromosomal replication

Rahul Saxena, Ph.D.
Department of Biochemistry and Molecular & Cellular Biology

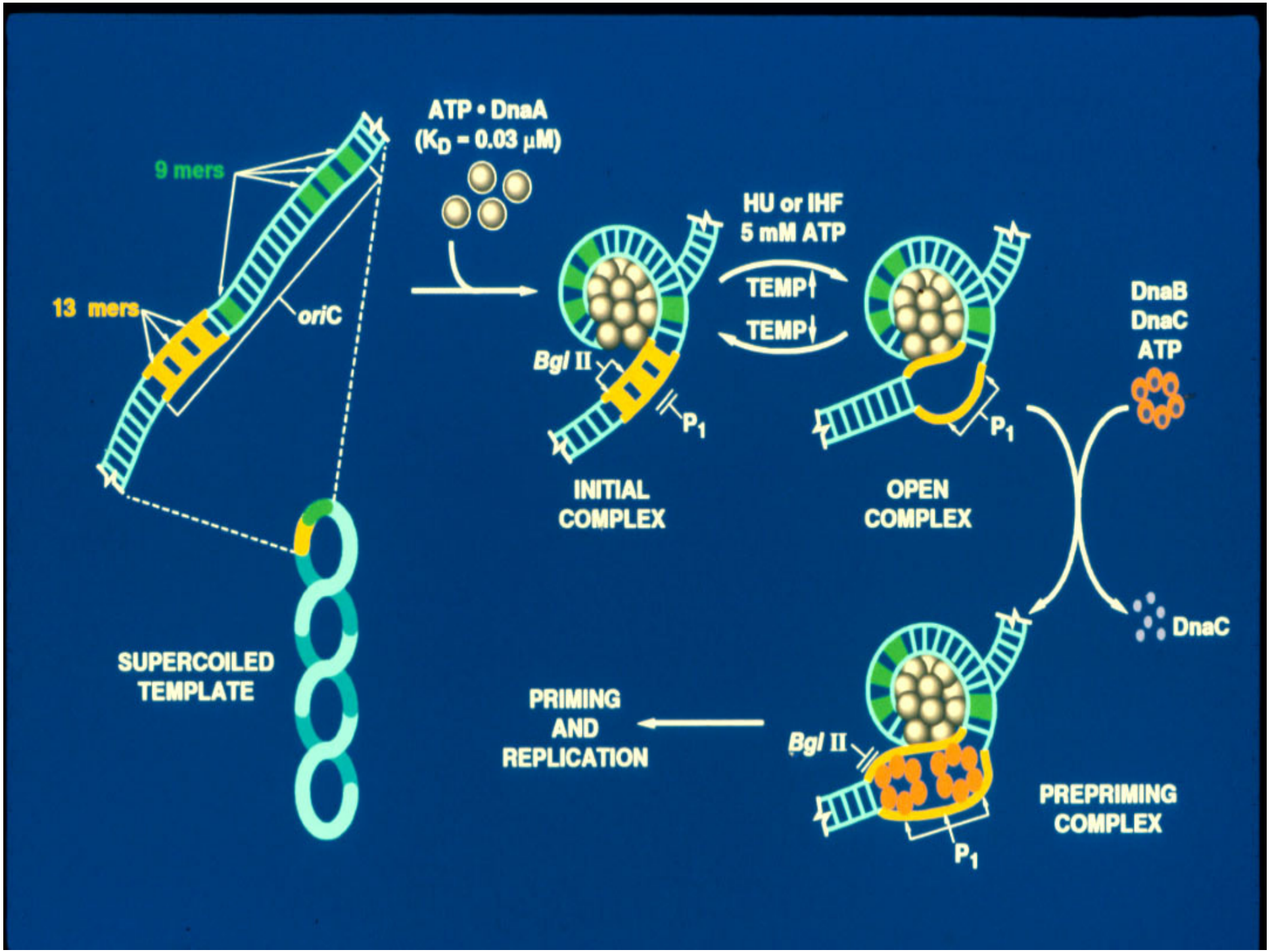
Georgetown University Medical Center
Washington, DC

- Both in prokaryotes and eukaryotes mother cell passes the exact same genetic information to its daughter cell.
- Moreover chromosomal origin of replication initiates DNA synthesis, once and only once per cell cycle.
- Interestingly a striking similarity exists between prokaryotic and eukaryotic initiator protein, which are considered to be the structural homologue.

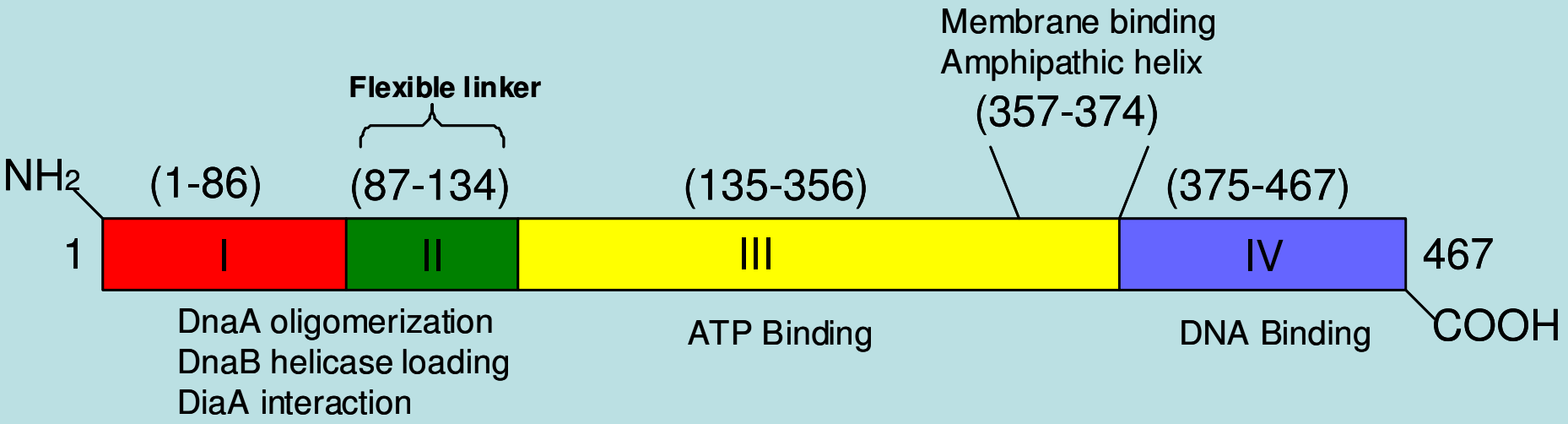




Erzberger et al EMBO J 21, 4763-4773

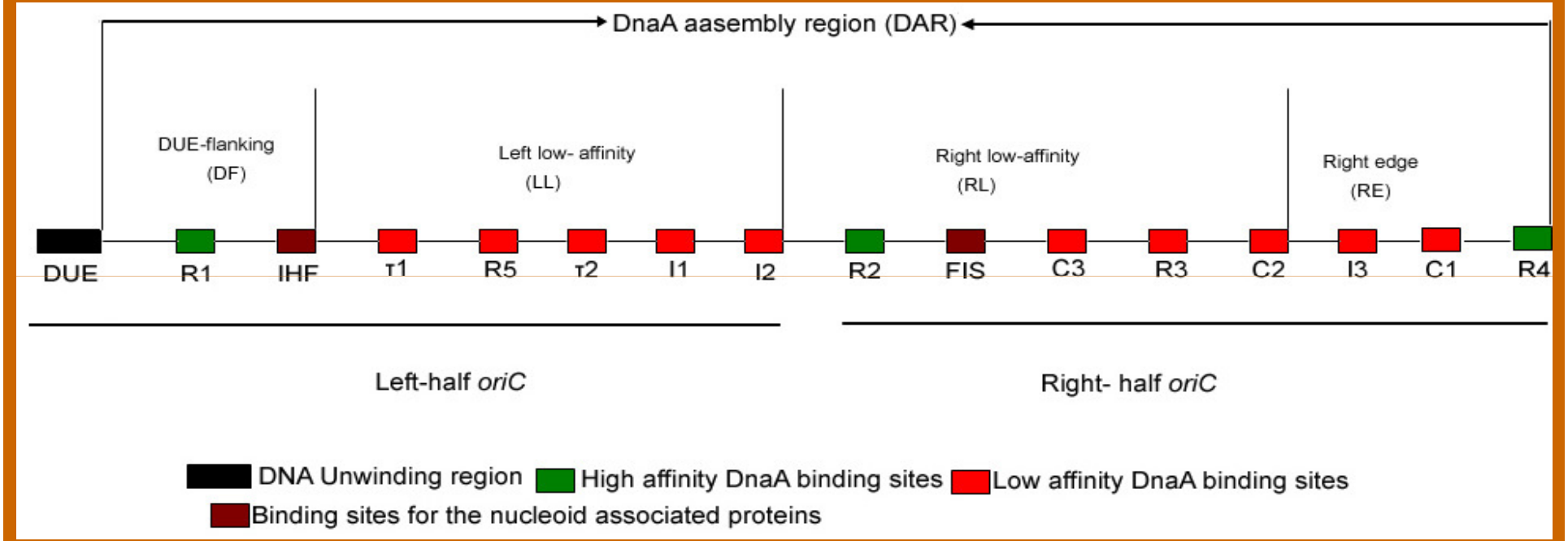

9 mers
13 mers
oriC
SUPERCOILED TEMPLATE
ATP • DnaA
(K_D = 0.03 μM)
Bgl II
P1
INITIAL COMPLEX
HU or IHF
5 mM ATP
TEMP
TEMP
OPEN COMPLEX
DnaB
DnaC
ATP
DnaC
PRIMING AND REPLICATION
Bgl II
P1
PREPRIMING COMPLEX

Functional domains of DnaA protein



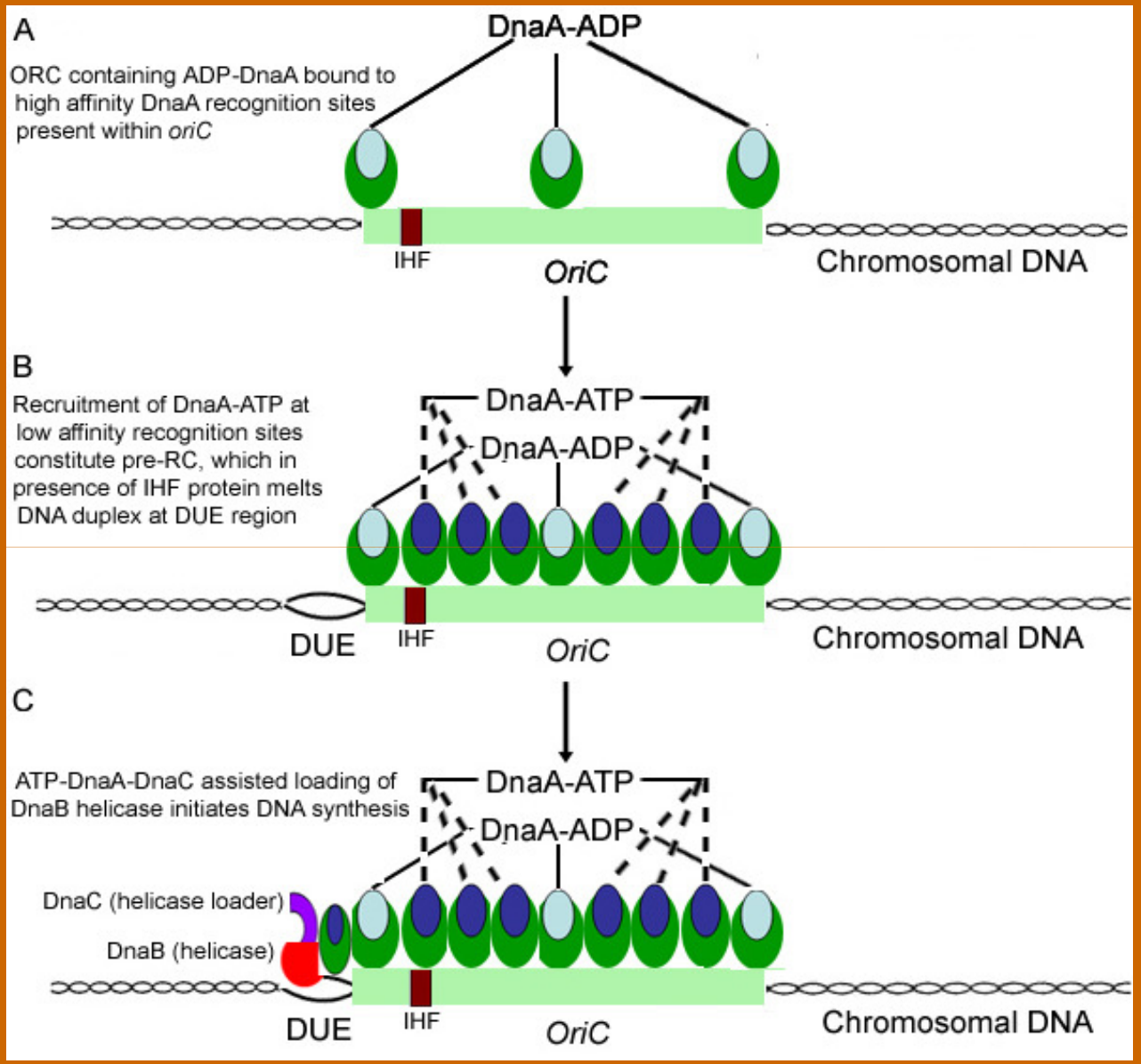
(R. Saxena *et al., Int J Mol Sci. 2013:* 14: 8517-37)

Architecture of E. coli chromosomal origin



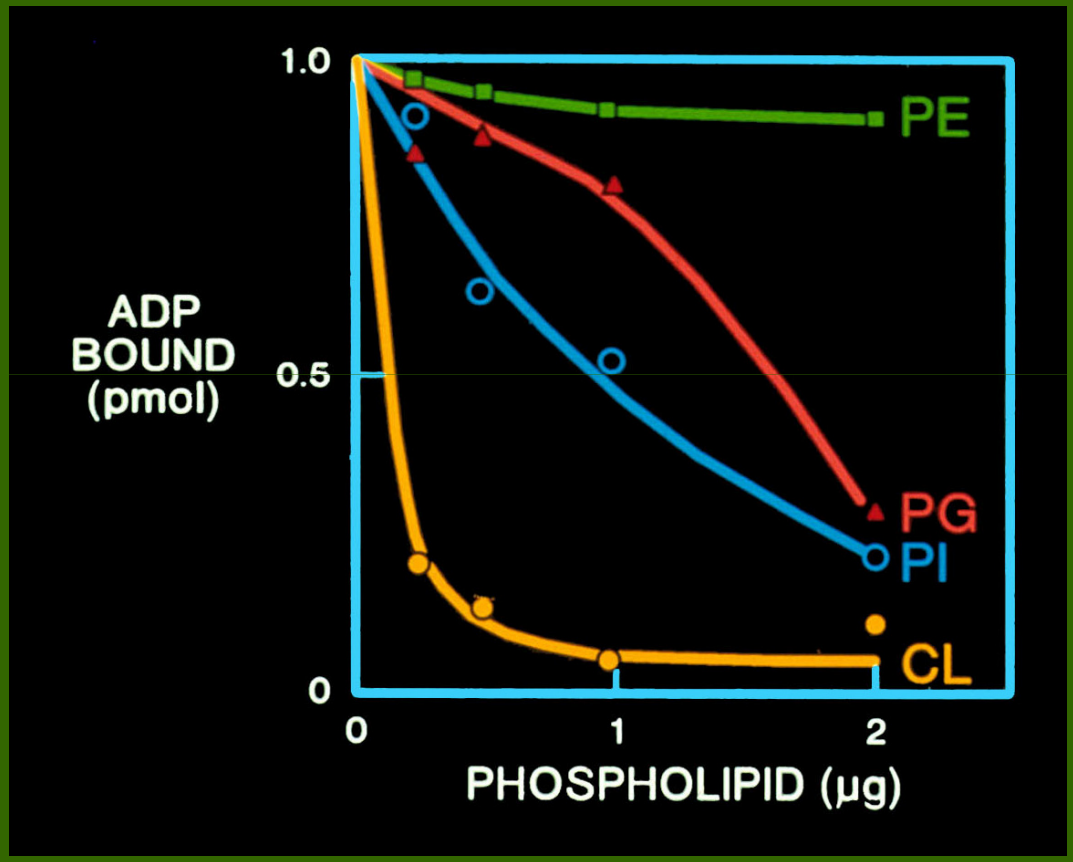
(R. Saxena, *OA Biochemistry 2013: 1(2):13*)

Architecture of E. coli chromosomal origin



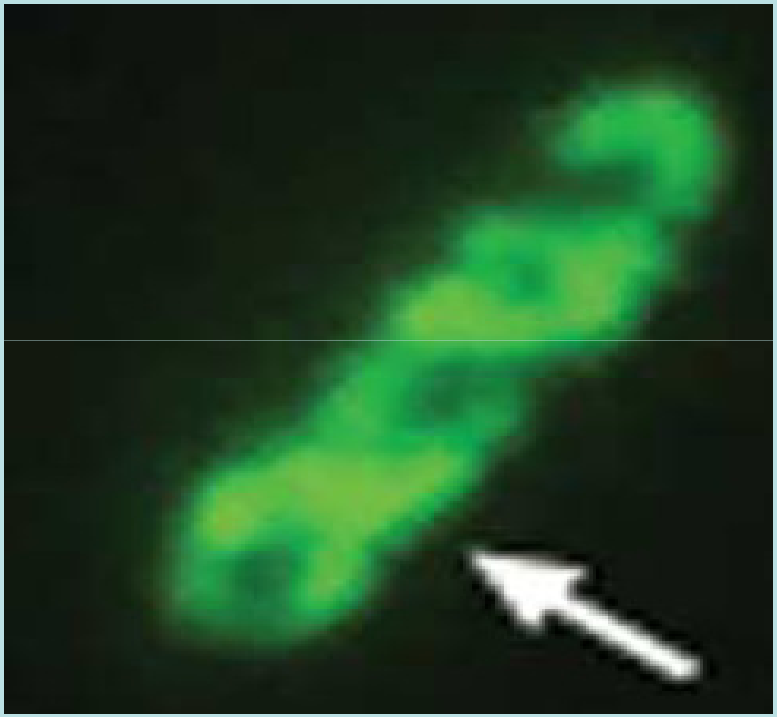
(R. Saxena, *OA Biochemistry 2013: 1(2):13*)

Acidic lipids in a fluid bilayer promote release of DnaA bound nucleotide .



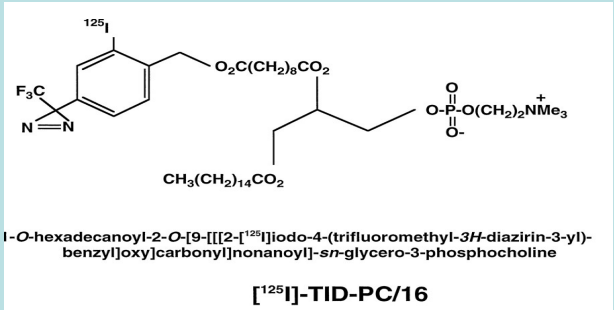
Sekimizu and Korenberg *JBC* 263:7131-35

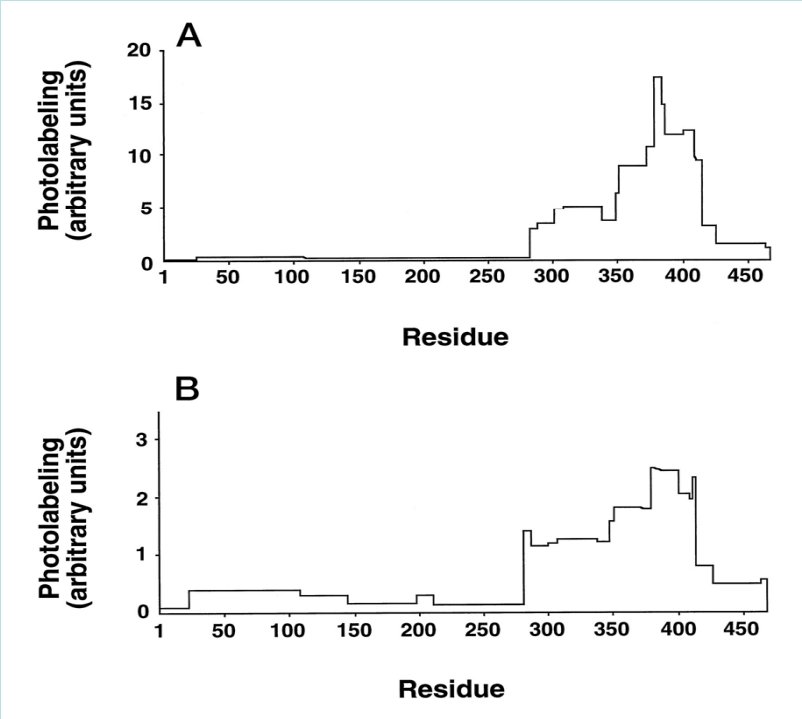
Cell fractionation and microscopic studies reveal that DnaA protein resides on the membrane



K. Boeneman et al., *Mol. Microbiol.* 72:645-57.

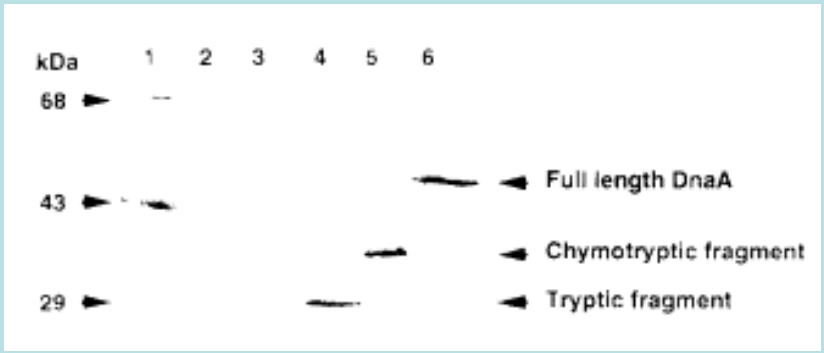
A distinct region of DnaA protein is preferentially photolabeled

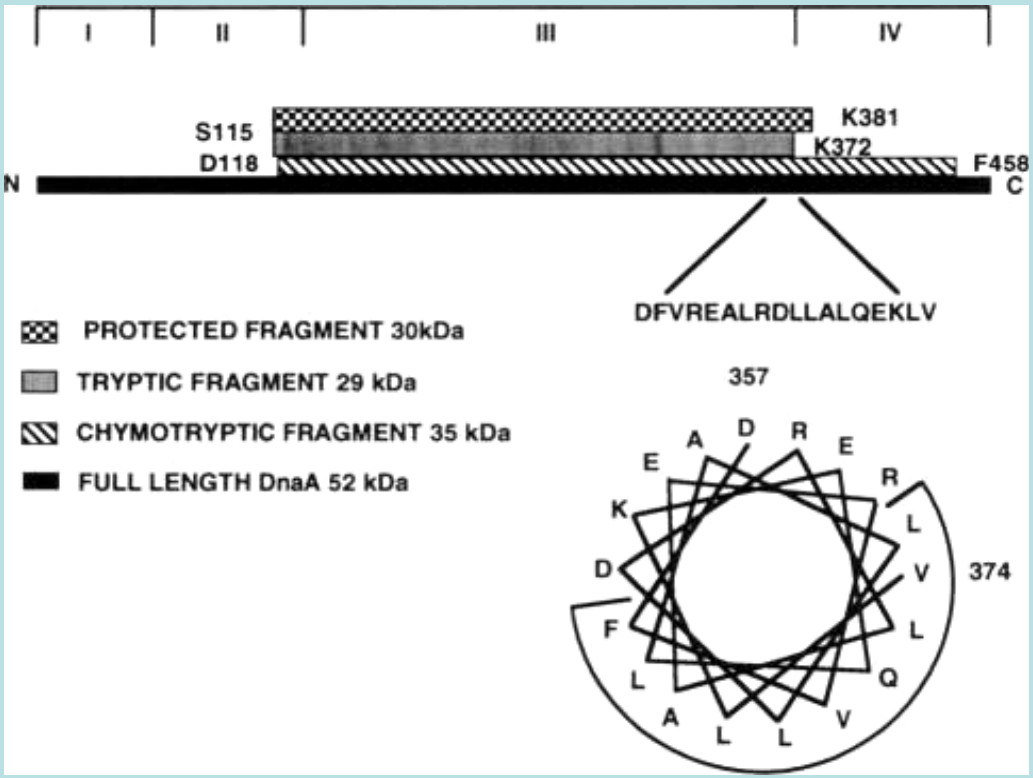




Garner J et al., *JBC* 273:5167-73

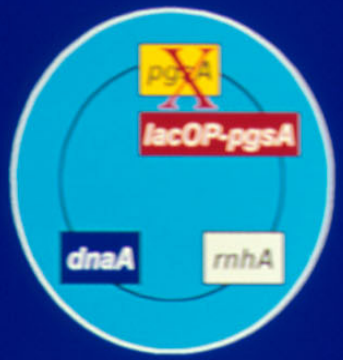
Functional Fragments of DnaA Protein Reveal the Membrane-binding Domain

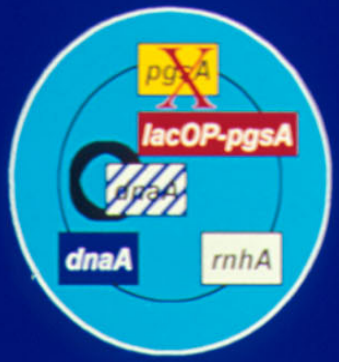




Garner and Crooke, EMBO J. 15, 3477- 85.

Screen for Mutations in *dnaA* That Can Suppress the Growth Arrest Phenotype of Acidic Phospholipid-deficient Cells





IPTG	Acidic PL levels	Growth
+	normal	+
–	low	–
–	low	–
+ Arabinose		

W. Zheng et al., *EMBO J* 20, 1164-72.

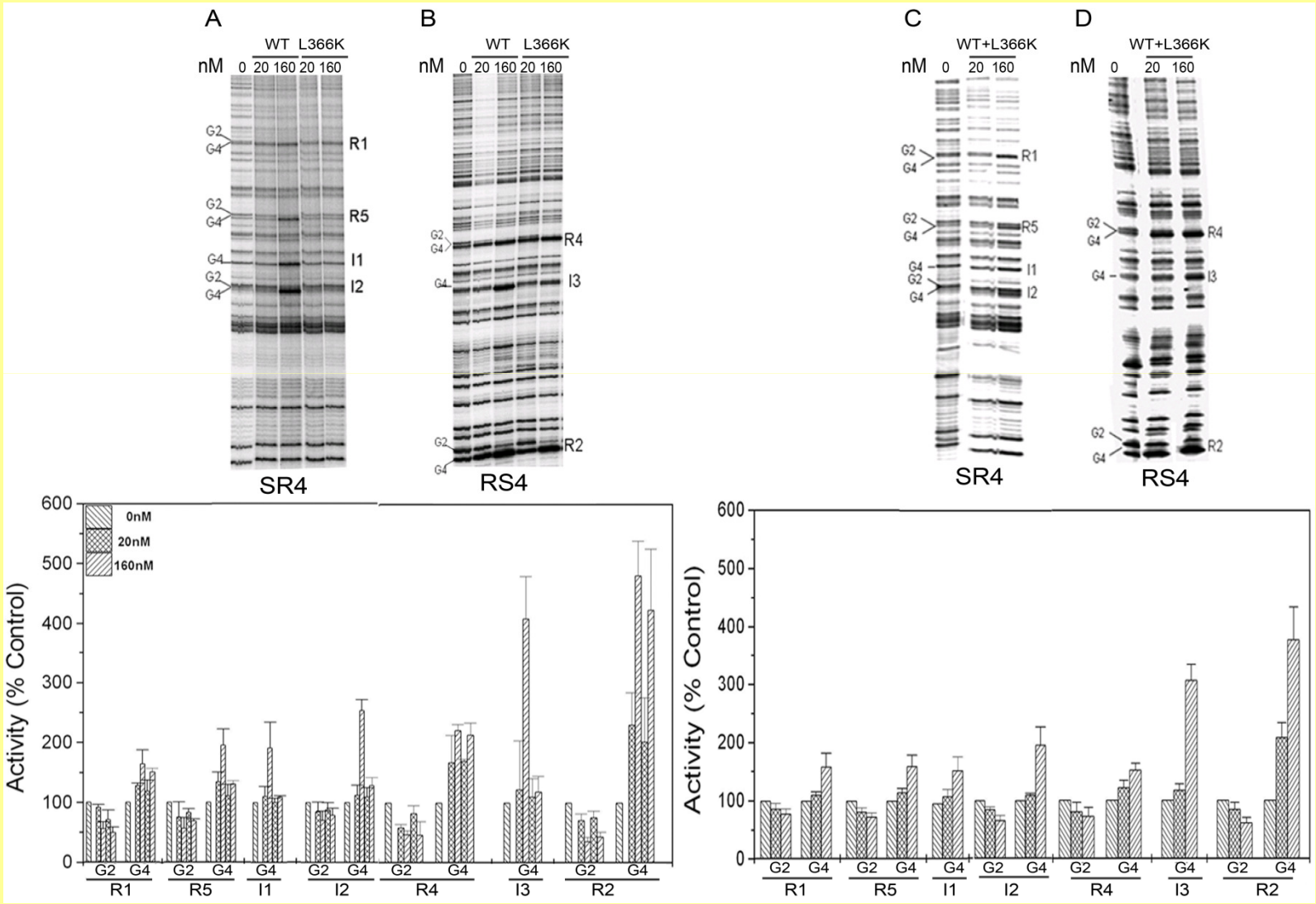
Growth arrested phenotype of phospholipid deficient cells can be restored by over-producing DnaA mutant present in membrane binding amphipathic region

HD L1001 transformed with plasmid expressing	Number of Colonies[1] +IPTG +Glucose	-IPTG +Glucose	-IPTG +Arabinose
DnaA (L363R, L366E, L367E, L369K)	1000	0	506
DnaA (L363K, L373R)	1000	0	594
DnaA (L363E)	1000	0	0
DnaA (L363K)	1000	0	0
DnaA (L366E)	1000	0	0
DnaA (L366K)	1000	0	615
DnaA (L367E)	1000	0	0
DnaA (L367K)	1000	0	0
DnaA (L373R)	1000	0	0

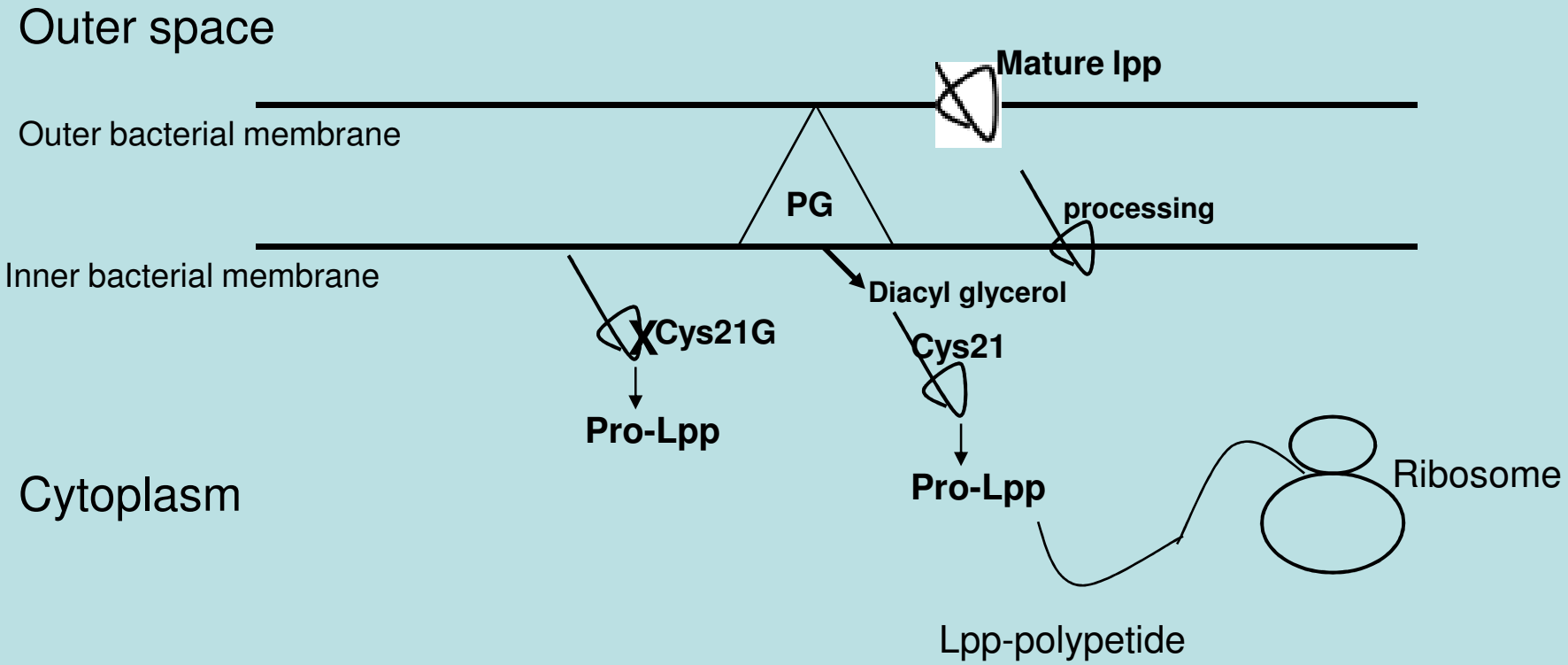
[1]Values are normalized to 1000 colonies for growth in the presence of IPTG and are an average from two or three independent transformations.

W. Zheng et al., *EMBO J* 20, 1164-72.

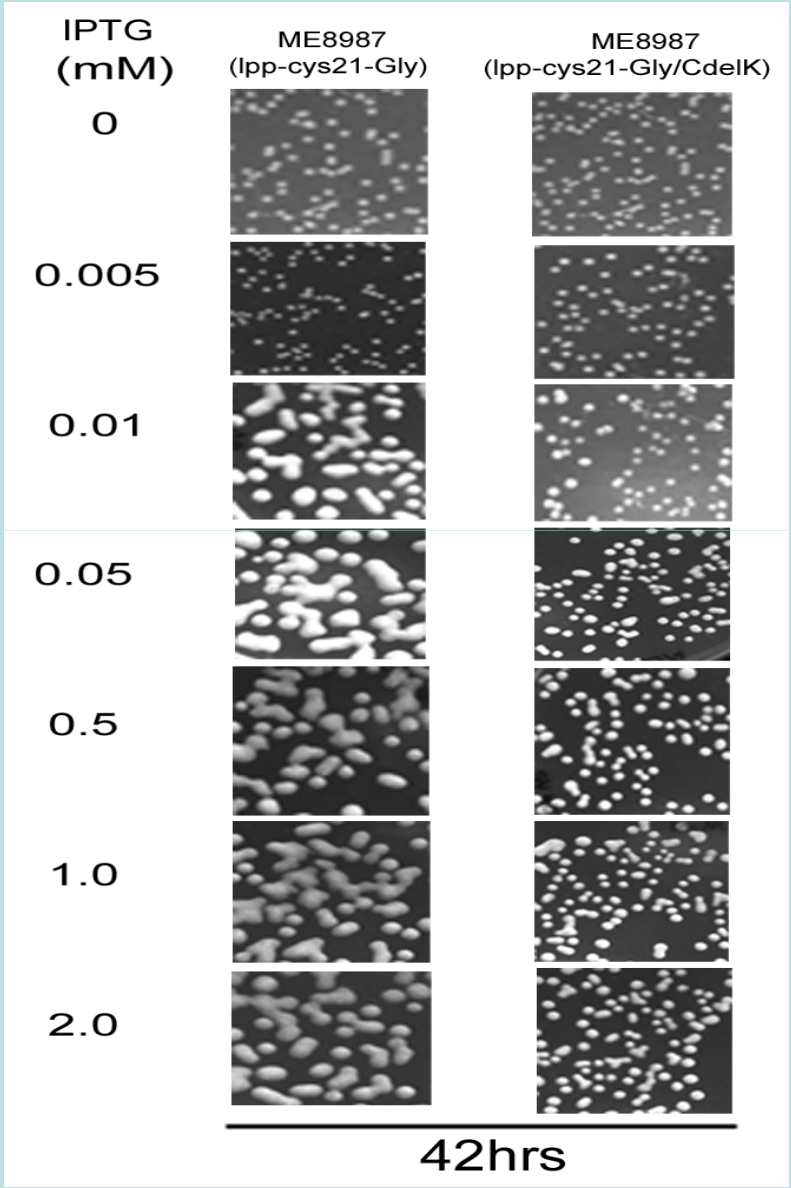
DnaA(L366K) is feeble in binding to low affinity recognitionn sequences but when augmented with low amount of DnaA can convert ORC to pre-RC

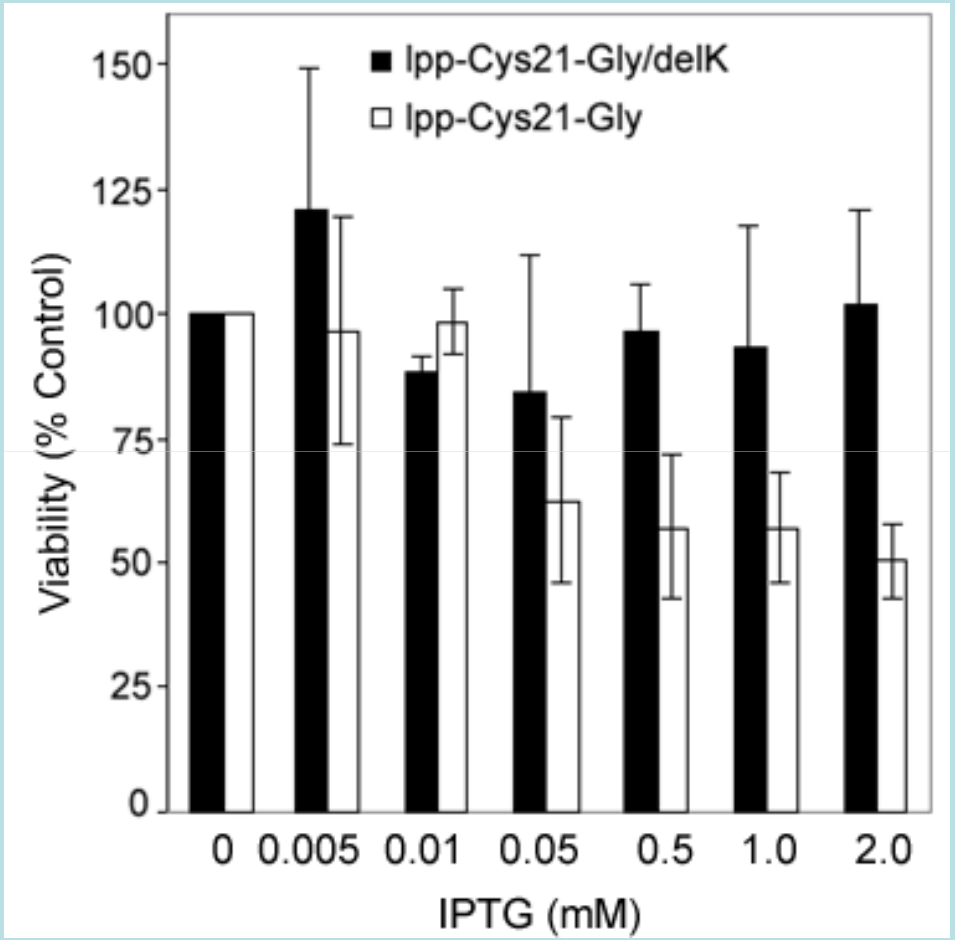


R. Saxena *et al.*, *JBC* 286:33770-77

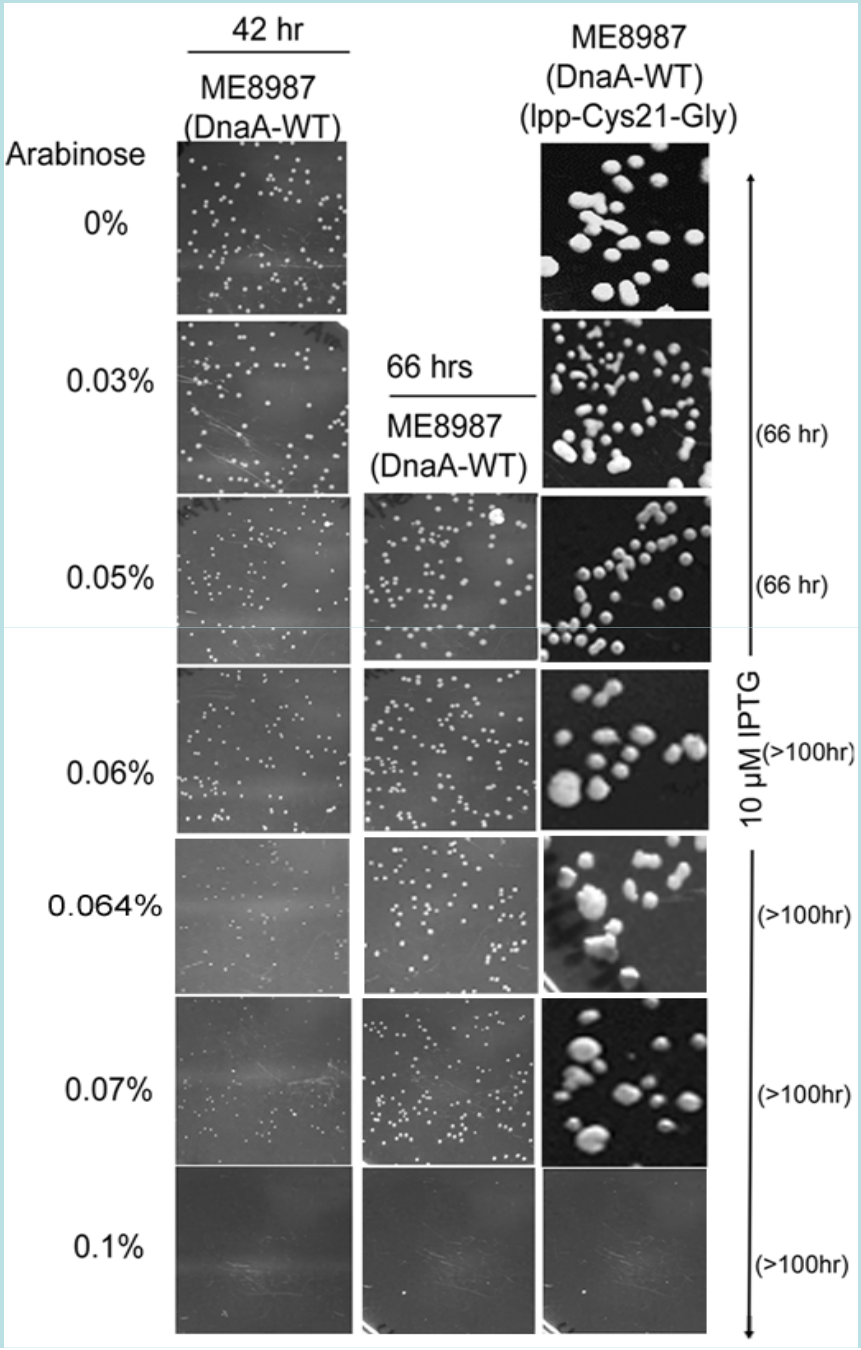

Outer space
Mature lpp
Outer bacterial membrane
PG
processing
Inner bacterial membrane
Diacyl glycerol
Cys21G
Cys21
Pro-Lpp
Pro-Lpp
Cytoplasm
Ribosome
Lpp-polypetide

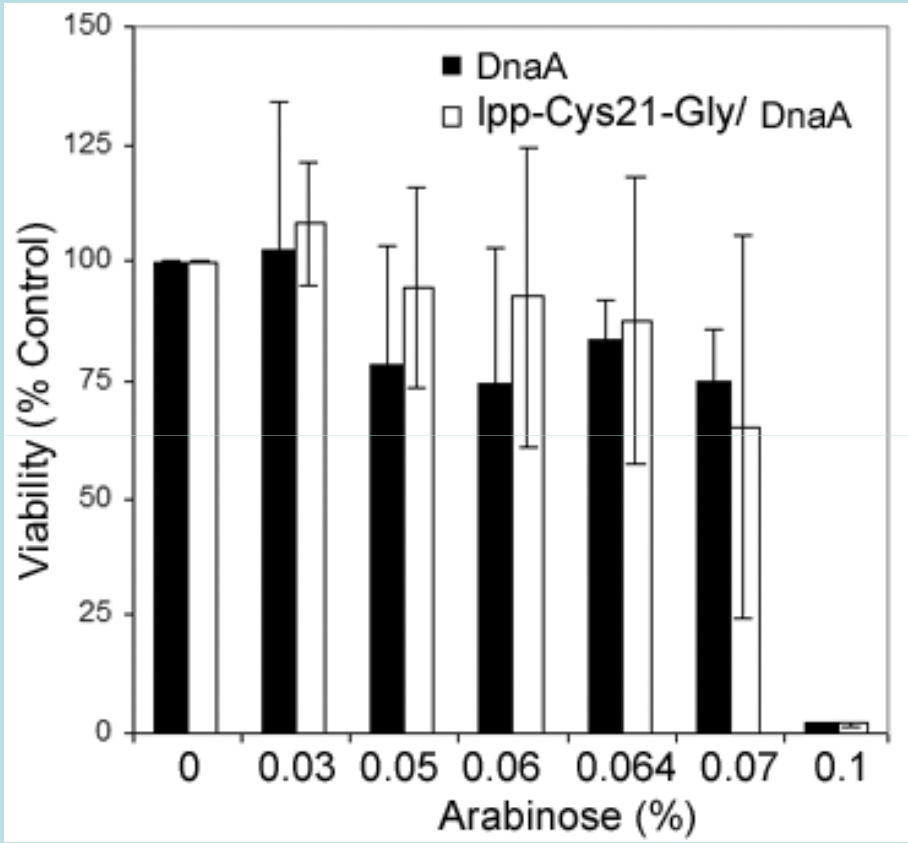
Accumulation of immature Murein lipoprotein on inner membrane of E. coli Cells causes mucoidy



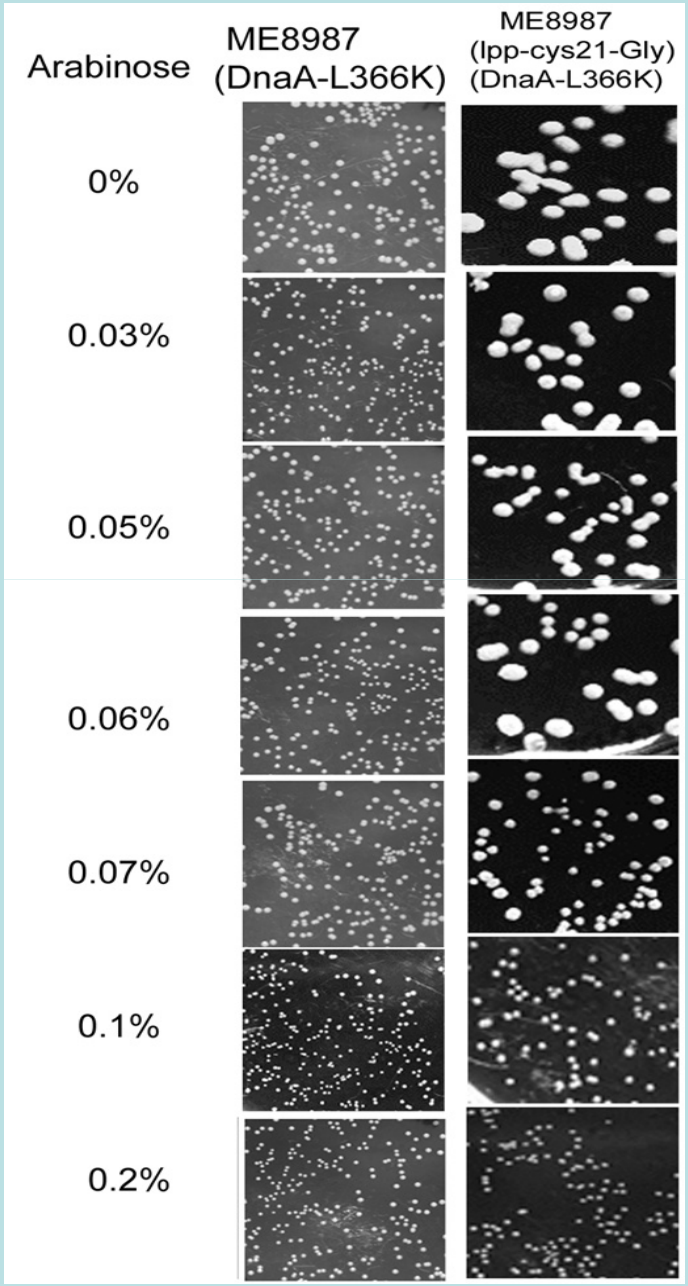


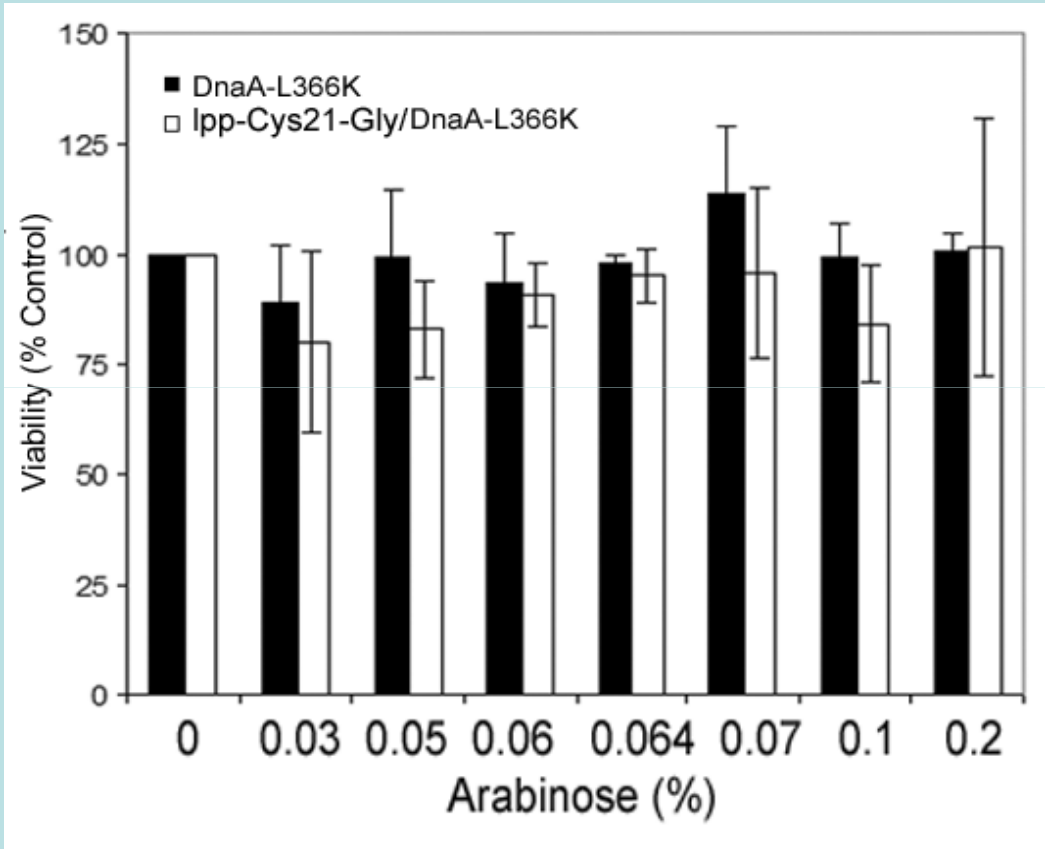
E. Coli cannot tolerate the overproduction of DnaA





DnaA(L366K) can restore the altered morphology and cell viability of E. coli overproducing Murein lipoprotein





Future prospective:

- Whether the immature pro-lipoprotein accumulated on the inner membrane is causing toxicity for conversion of replication inefficient ORC to replication efficient pre-RC

- How DnaA-L366K bypasses this toxic affect

- A better understanding of how membrane domains of acidic phospholipids influence nucleotide exchange

- Studying the mutant could be an approach to know that if there is any cross talk between the acidic phospholipids present in bacterial membranes and initiation of chromosomal replication.

Acknowledgement

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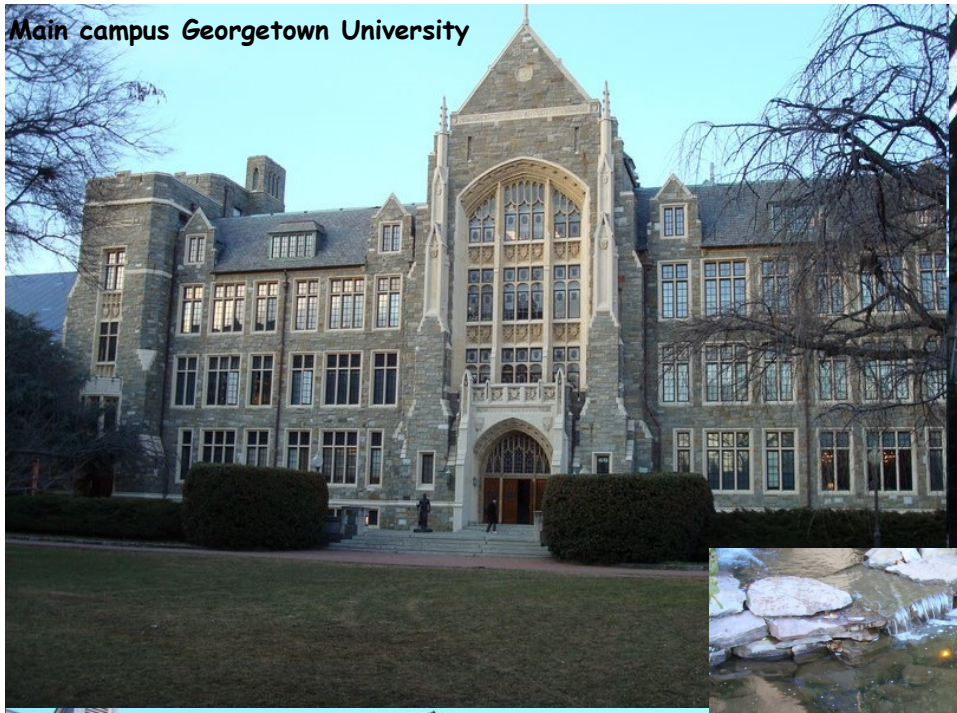
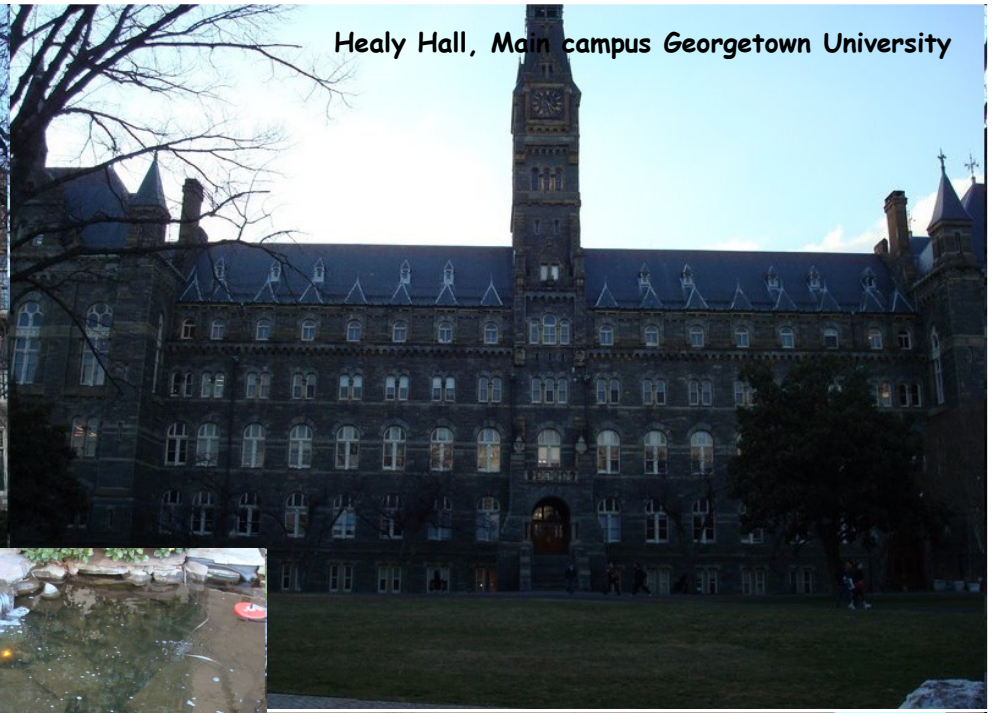
Dr. Zhenya (Jenny) Li
Dr. Yanyua Yang
Dr. Weidong Zheng
Dr. Nicholas Fingland



Main campus Georgetown University



Healy Hall, Main campus Georgetown University

Georgetown University

Medical-Dental building

Lombardi Cancer Research center

Biochemistry of DnaA

- DnaA, the initiator protein initiates chromosomal replication by binding to high affinity and low affinity recognition elements present within *oriC*.

- Acidic lipids in a fluid bilayer promote release of bound nucleotide.
- Electrostatic interactions between anionic lipid head-groups and DnaA may stabilizes DnaA-membrane association.

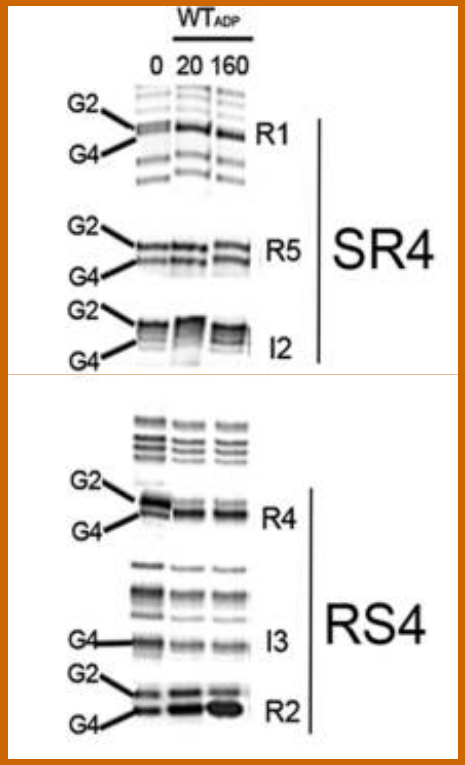
- A discrete domain of DnaA protein that inserts into the hydrophobic region of acidic bilayers is essential for functional membrane interaction.

- Expression of DnaA protein with mutations in its membrane-binding domain suppresses the arrested growth of acidic phospholipid- deficient cells.

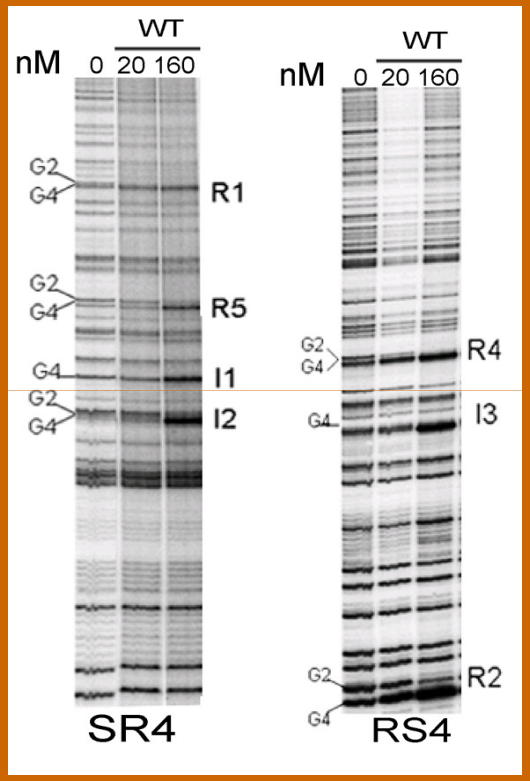
- ADP-DnaA binds to only high affinity recognition sequences to produce replication inefficient origin recoginition complex.

- ATP-DnaA binds to both high and low affinity recognition sequences and convert ORC to replication efficient pre-Replication Complex.

Architecture of E. coli chromosomal origin



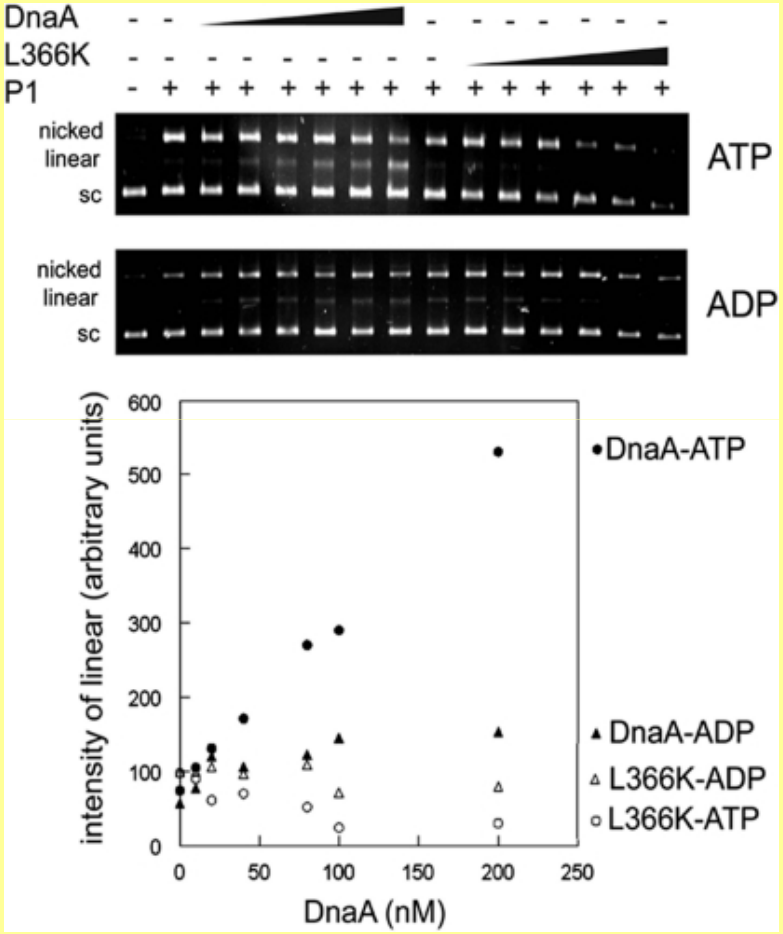
ADP-DnaA forms an origin recognition complex by binding to *oriC* sites R1, R2, and R4.



ATP-DnaA can bind within both low affinity sites (such as I1, I2, I3, R1, R5M) and high affinity DnaA boxes (R1, R2, and R4) to form a pre-RC

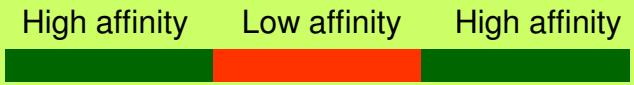
R. Saxena *et al.*, *JBC* 2011

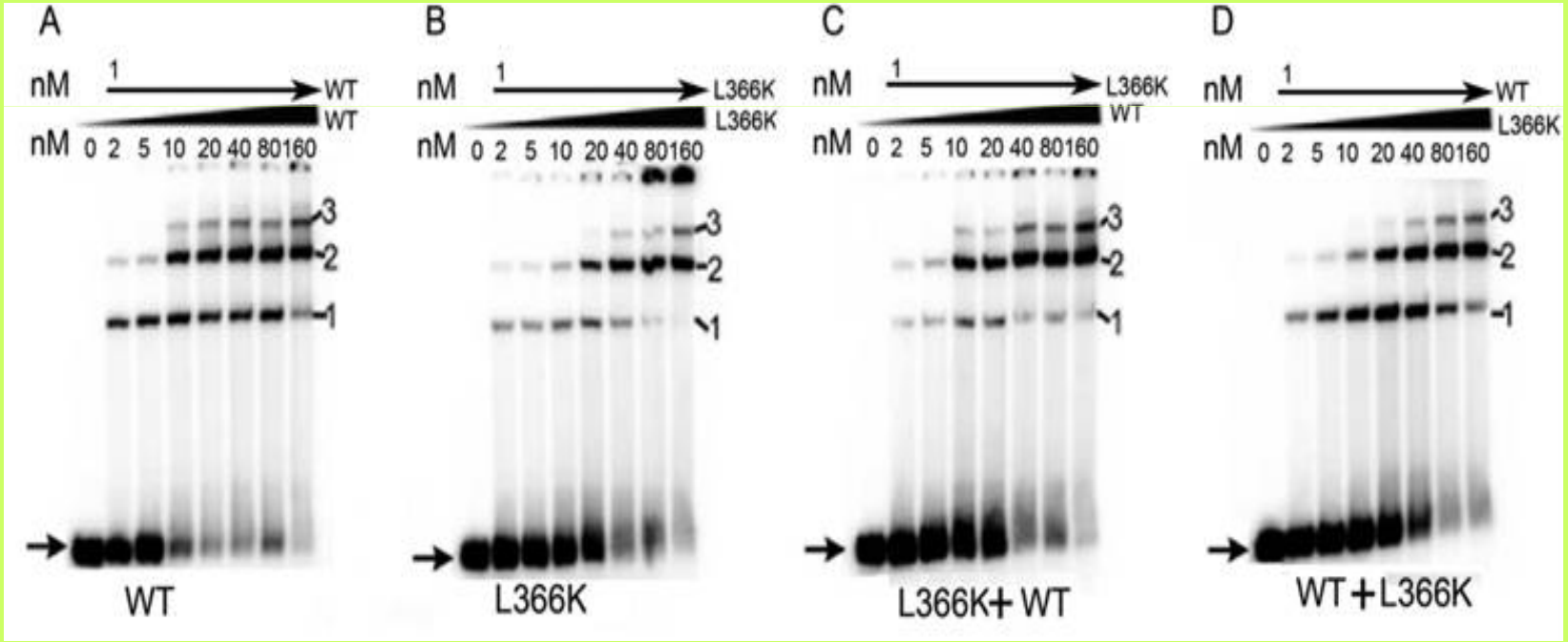
Neither ATP- or ADP-DnaA(L366K) can unwind *oriC*



R. Saxena *et al.*, *JBC* 2011

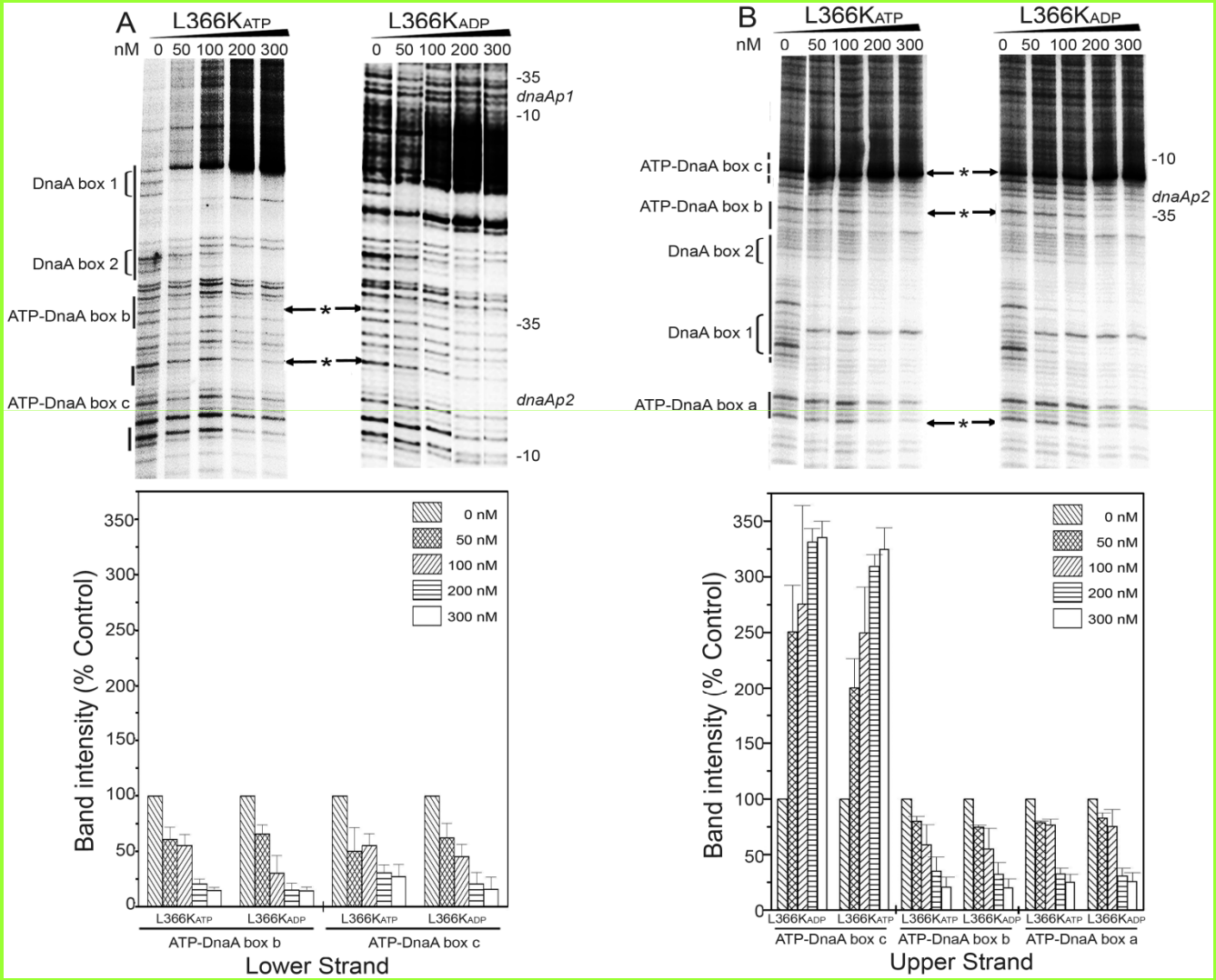
DnaA(L366K) cannot bind low affinity sites but when bound to a strong site can assist loading of wild-type DnaA to low affinity site





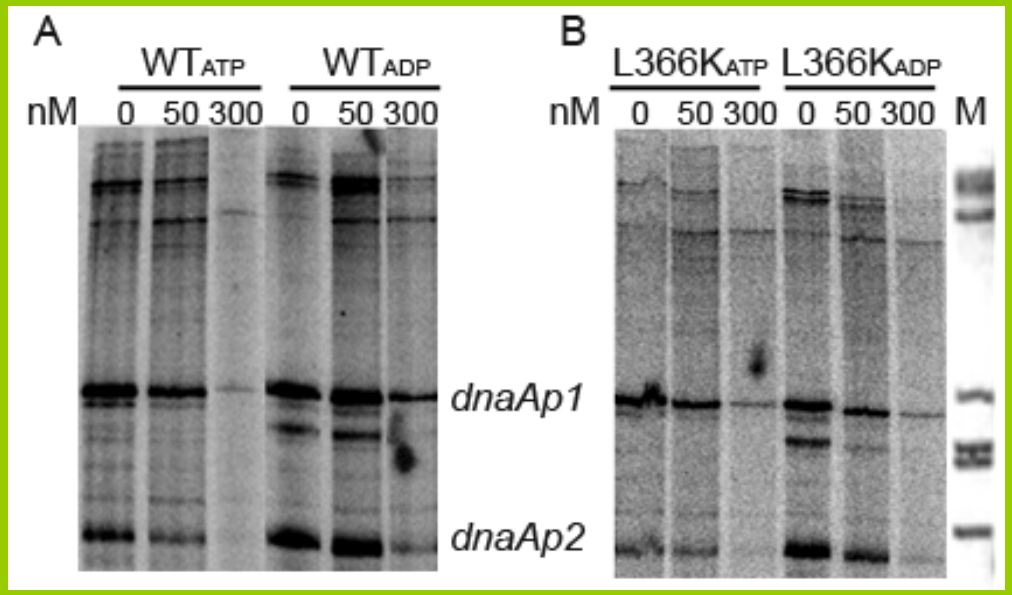
R. Saxena *et al.*, *JBC* 2011

ATP-DnaA(L366K) and ADP-DnaA(L366K) confer the same DNase I protection to *dnaA* promoter as ATP-DnaA



R. Saxena *et al.*, *JBC* 2011

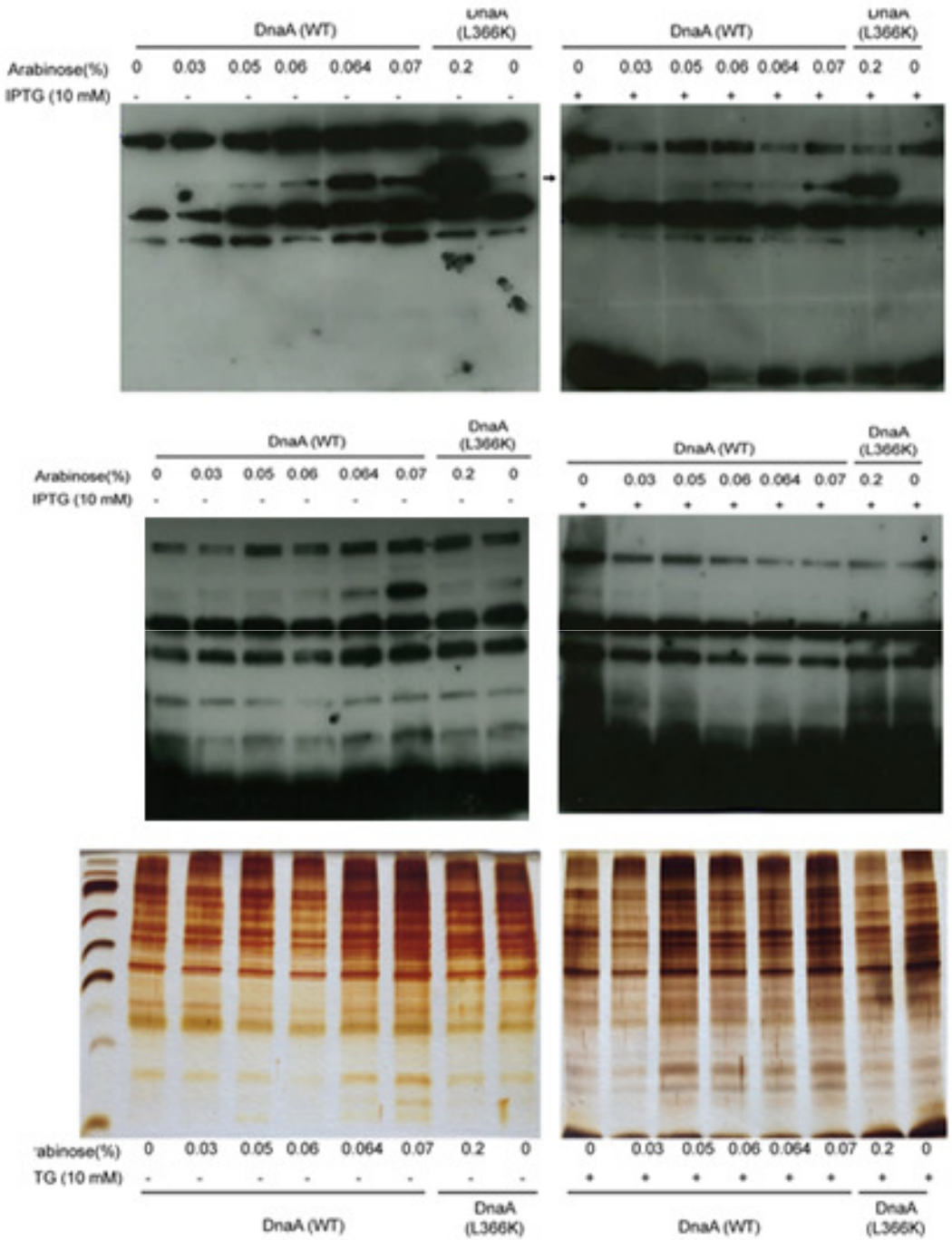
DnaA(L366K) in either nucleotide form represses *in vitro* transcription from *dnaA* promoter similar to ATP-DnaA

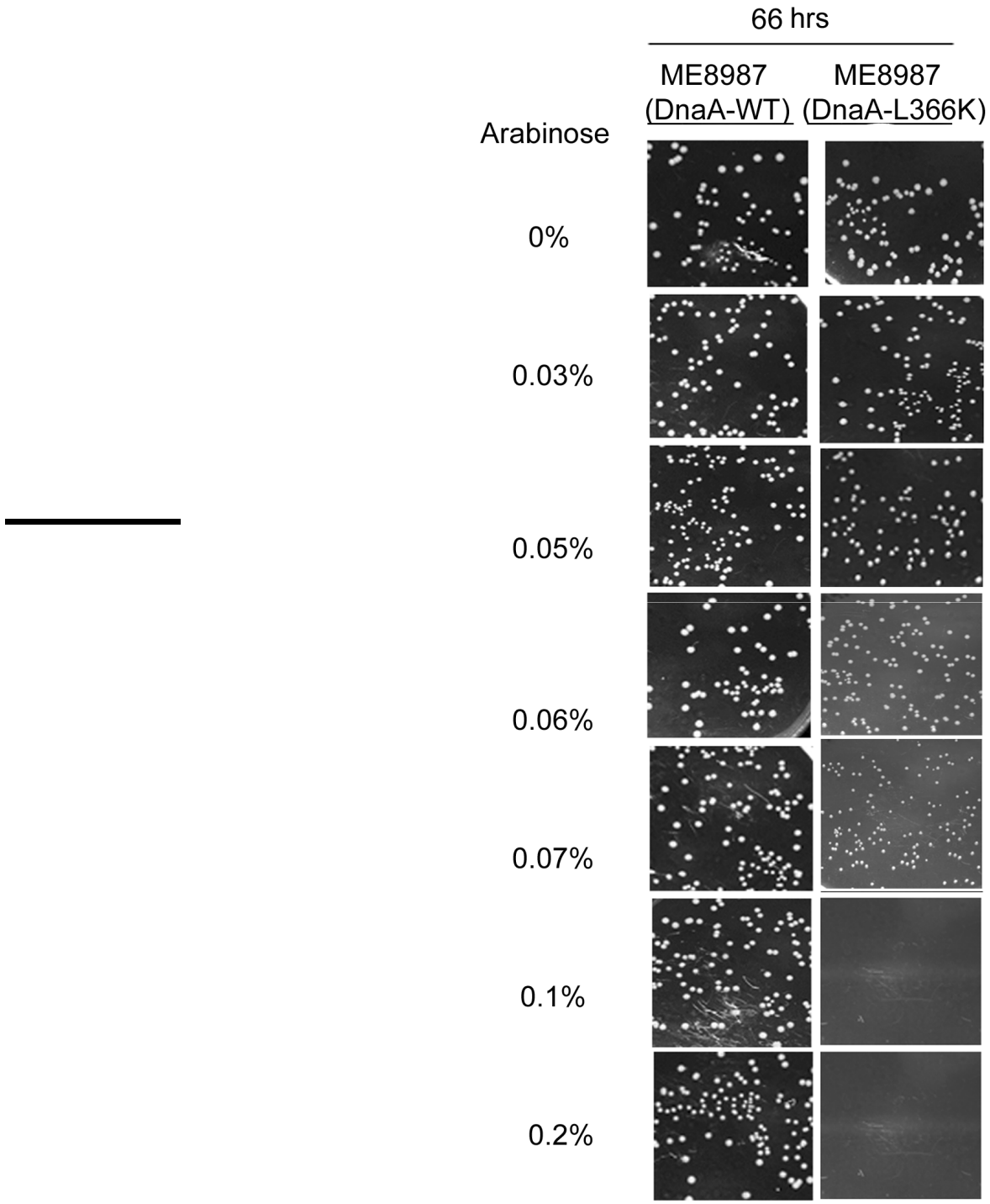


	WT_{ATP}		WT_{ADP}		$L366K_{ATP}$		$L366K_{ADP}$	
	(% expression)				(% expression)			
nM protein	50	300	50	300	50	300	50	300
dnaAp1	60±5.8	10±3.2	98±2.8	52±13.0	75±7.6	21±3.2	73±4.7	20±2.1
dnaAp2	40±9.0	6±2.0	90±7.0	40±11.0	68±5.0	12±2.0	71±3.6	14±3.6

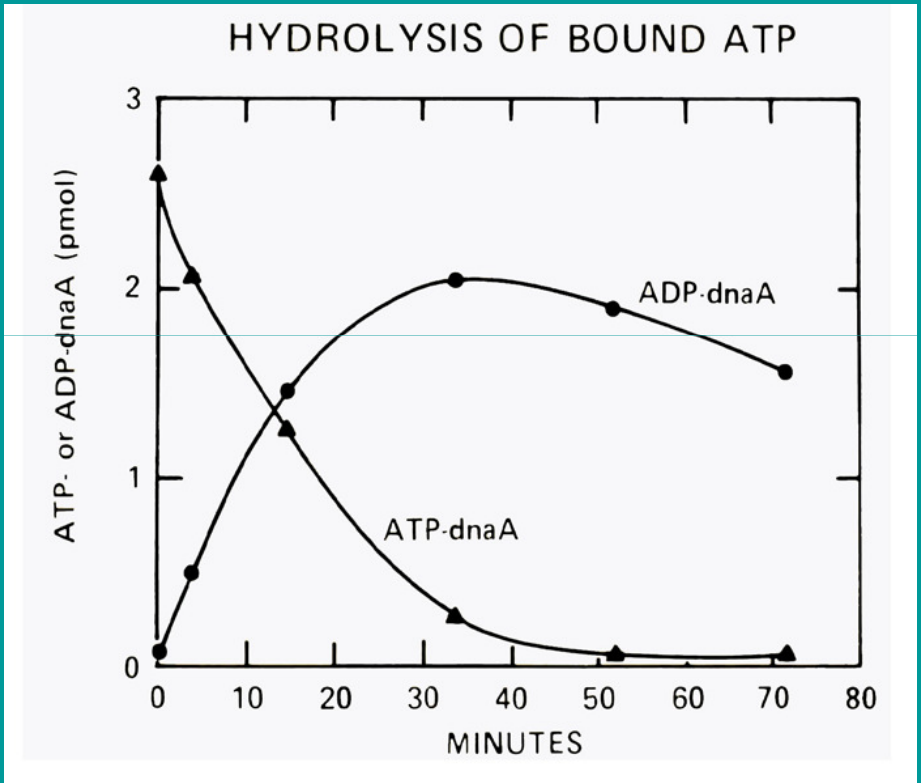
Each value is calculated as relative percentage of intensity obtained with no protein control (0 nM)

R. Saxena *et al.*, *JBC* 2011


DnaA (WT)
DnaA (L366K)
Arabinose(%)
0 0.03 0.05 0.06 0.064 0.07 0.2 0
IPTG (10 mM)
- - - - - - - -
+ + + + + + + +


66 hrs
ME8987 (DnaA-WT)
ME8987 (DnaA-L366K)
Arabinose
0%
0.03%
0.05%
0.06%
0.07%
0.1%
0.2%

Hydrolysis of ATP bound to DnaA, is a slow process by itself

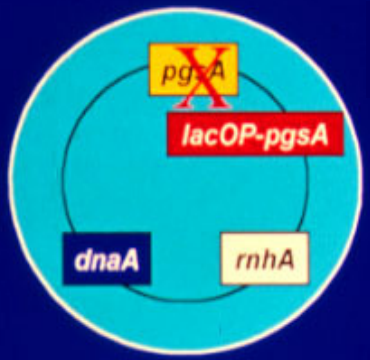


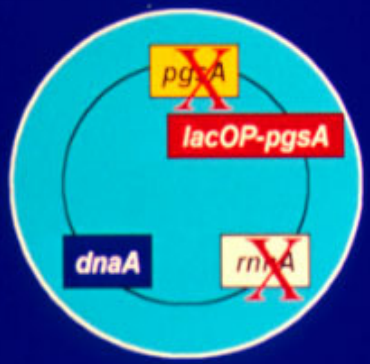
Colony Formation of HDL1001 Cells Transformed With Plasmids Harboring Deletion Mutant *dnaA* Genes

HDL1001 transformed with plasmids expressing	Number of Colonies +IPTG +Glucose	-IPTG +Glucose	-IPTG +Arabinose
Wild-type DnaA	1000	0	9
DnaAΔ317-322	1000	0	9
DnaAΔ322-329	1000	0	6
DnaAΔ329-333	1000	0	0
DnaAΔ332-337	1000	0	16
DnaAΔ336-340	1000	0	2
DnaAΔ340-345	1000	0	0
DnaAΔ346-356	1000	0	161*
DnaAΔ357-368	1000	0	517
DnaAΔ363-367	1000	0	700
DnaAΔ369-376	1000	0	442
DnaAΔ373-381	1000	0	577
DnaAΔ377-386	1000	0	407
DnaAΔ387-396	1000	0	764
DnaAΔ397-404	1000	0	227*
DnaAΔ405-417	1000	0	9
DnaAΔ418-422	1000	0	407
DnaAΔ432-427	1000	0	262
DnaAΔ428-435	1000	0	362*
DnaAΔ436-445	1000	0	667
DnaAΔ446-455	1000	0	714*
DnaAΔ456-464	1000	0	577
(Vector)	1000	0	0

Zheng et al, EMBO J 20, 1164-1172.

Acidic Phospholipids Are Involved in Initiation of Replication at *oriC In Vivo*



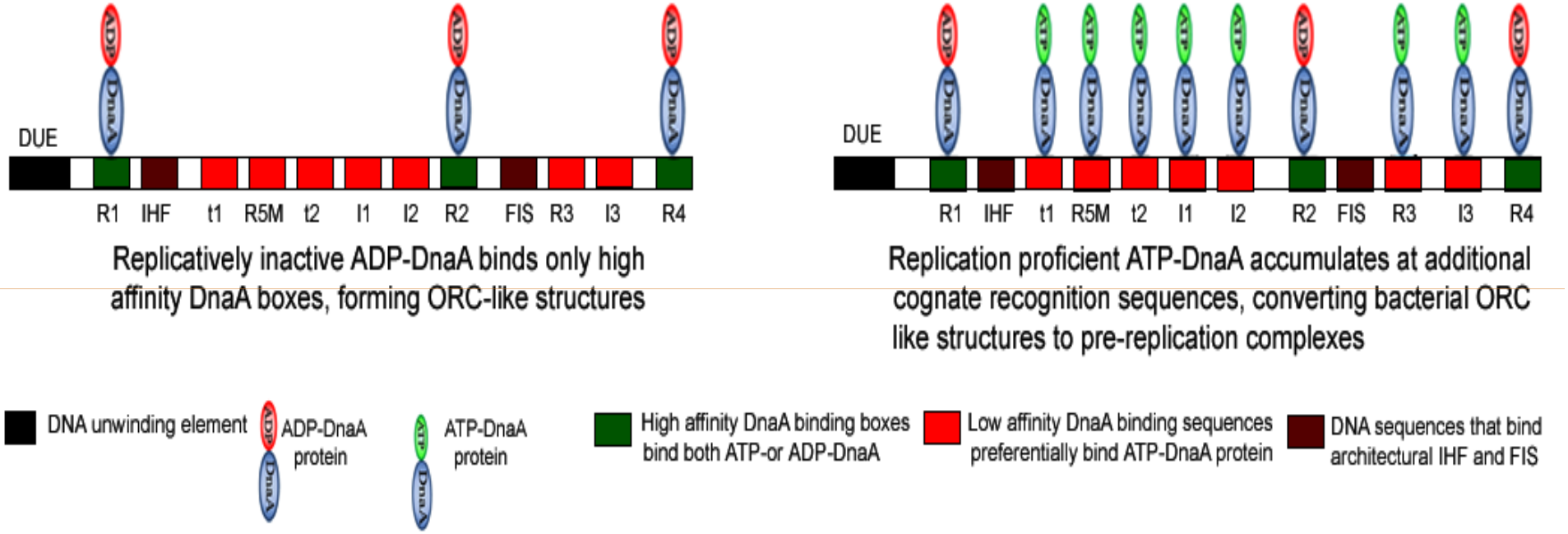


IPTG	Acidic PL levels	Growth
+	normal	+
–	low	–
–	low	+

Heacock and Dowhan *JBC 264, 14972 (1989)*

Xia and Dowhan *PNAS 92, 783 (1995)*

Architecture of E. coli chromosomal origin



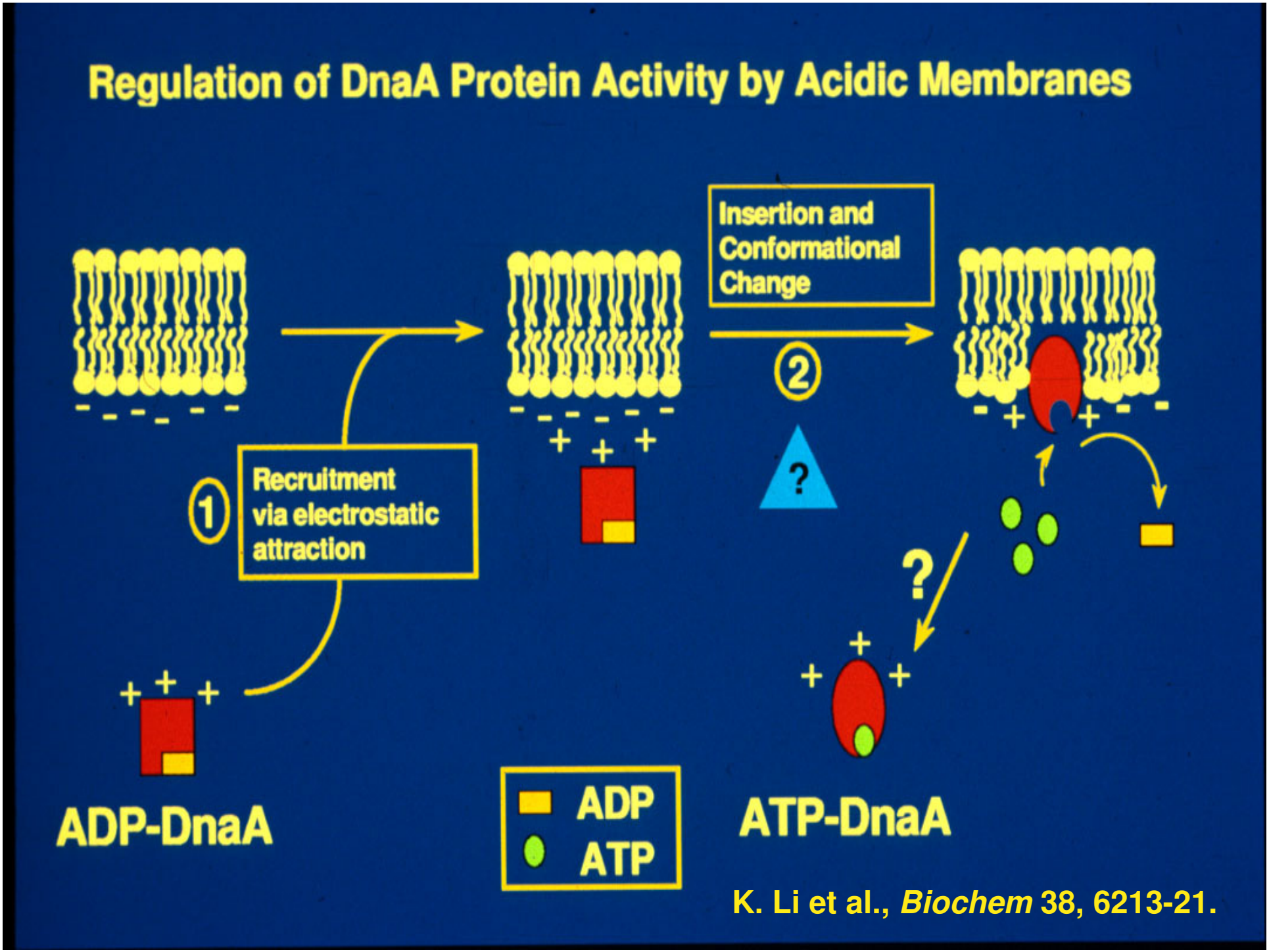
(R. Saxena *et al., Int J Mol Sci. 2013:* 14: 8517-37)

DnaA(L366K) is competent at repressing the dnaA

TABLE I
DnaA(L366K) is competent at repressing the *dnaA* promoter *in vivo*

E. coli strain	protein expressed	β-galatosidase activity (in Miller units)
EH2827/pBAD	none	250
EH2827pZL606	DnaA	130
EH2827pZL606 (L366K)	DnaA(L366K)	125
M182/pBAD	none	207
M182/pZL606	DnaA	100
M182/pZL606 (L336K)	DnaA(L366K)	80

R. Saxena *et al.*, *JBC* 2011


Regulation of DnaA Protein Activity by Acidic Membranes
Insertion and Conformational Change
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ADP-DnaA
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K. Li et al., *Biochem* 38, 6213-21.

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