Structural Systems Biology using Future Coherent Light Sources

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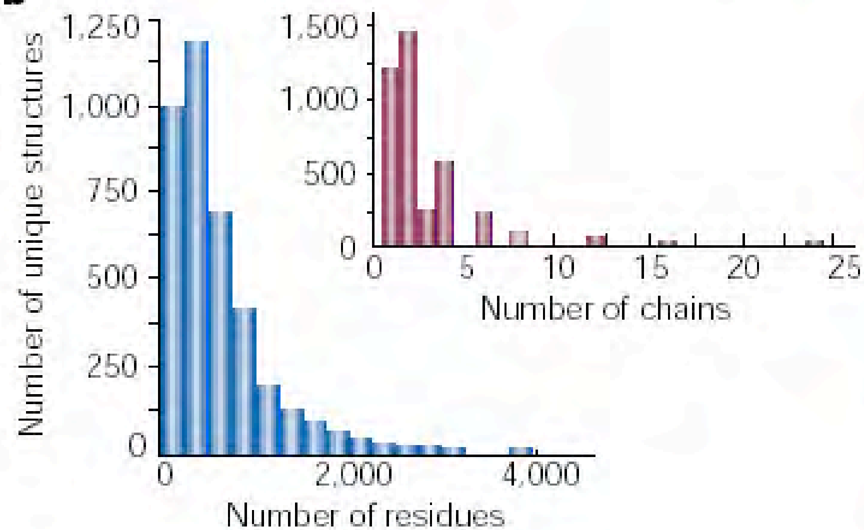
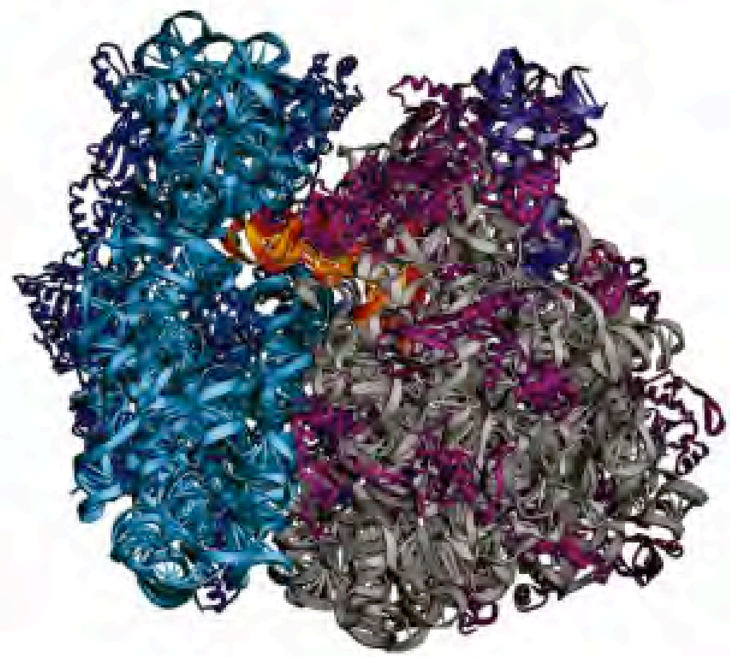
ERL Bio Workshop / Jun06

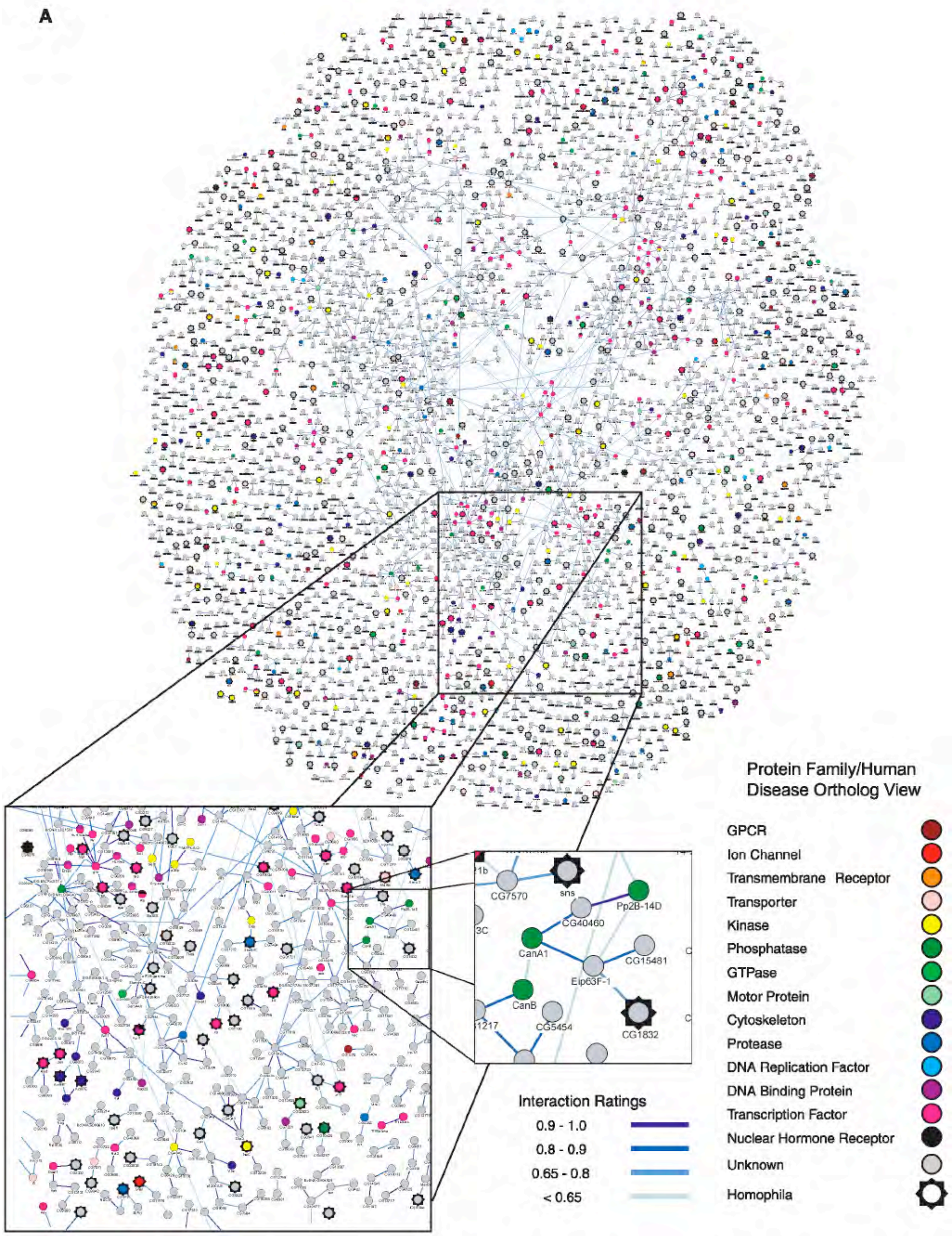
a

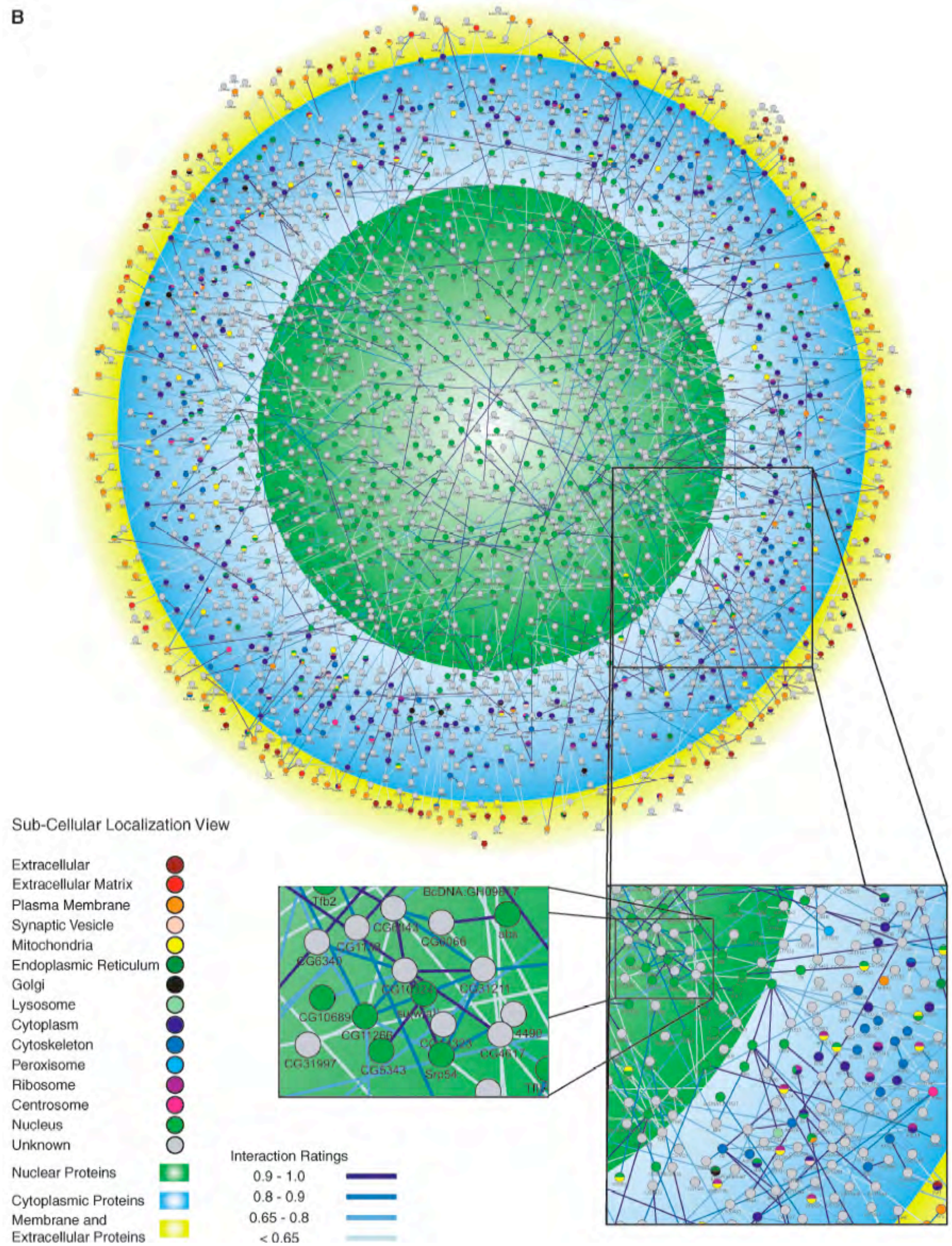
b



Number of unique structures
1,250
1,000
750
500
250
0
0
2,000
4,000
Number of residues
1,500
1,000
500
0
0
5
10
15
20
25
Number of chains




A
CG7570
sns
Pp2B-14D
CG40460
CanA1
CG15481
Eip63F-1
CanB
CG5454
CG1832
Interaction Ratings
0.9 - 1.0
0.8 - 0.9
0.65 - 0.8
< 0.65
Protein Family/Human
Disease Ortholog View
GPCR
Ion Channel
Transmembrane Receptor
Transporter
Kinase
Phosphatase
GTPase
Motor Protein
Cytoskeleton
Protease
DNA Replication Factor
DNA Binding Protein
Transcription Factor
Nuclear Hormone Receptor
Unknown
Homophila



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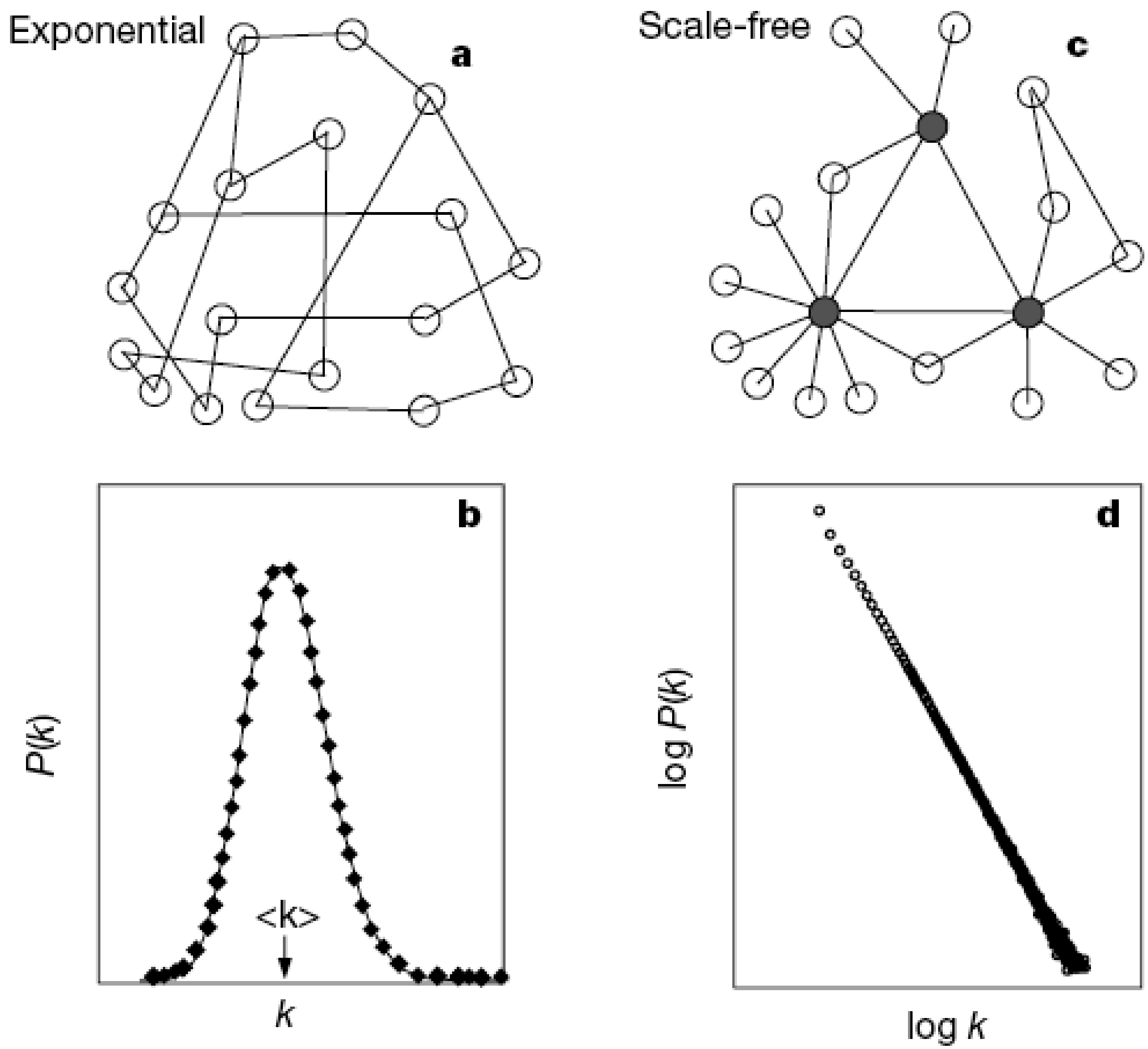
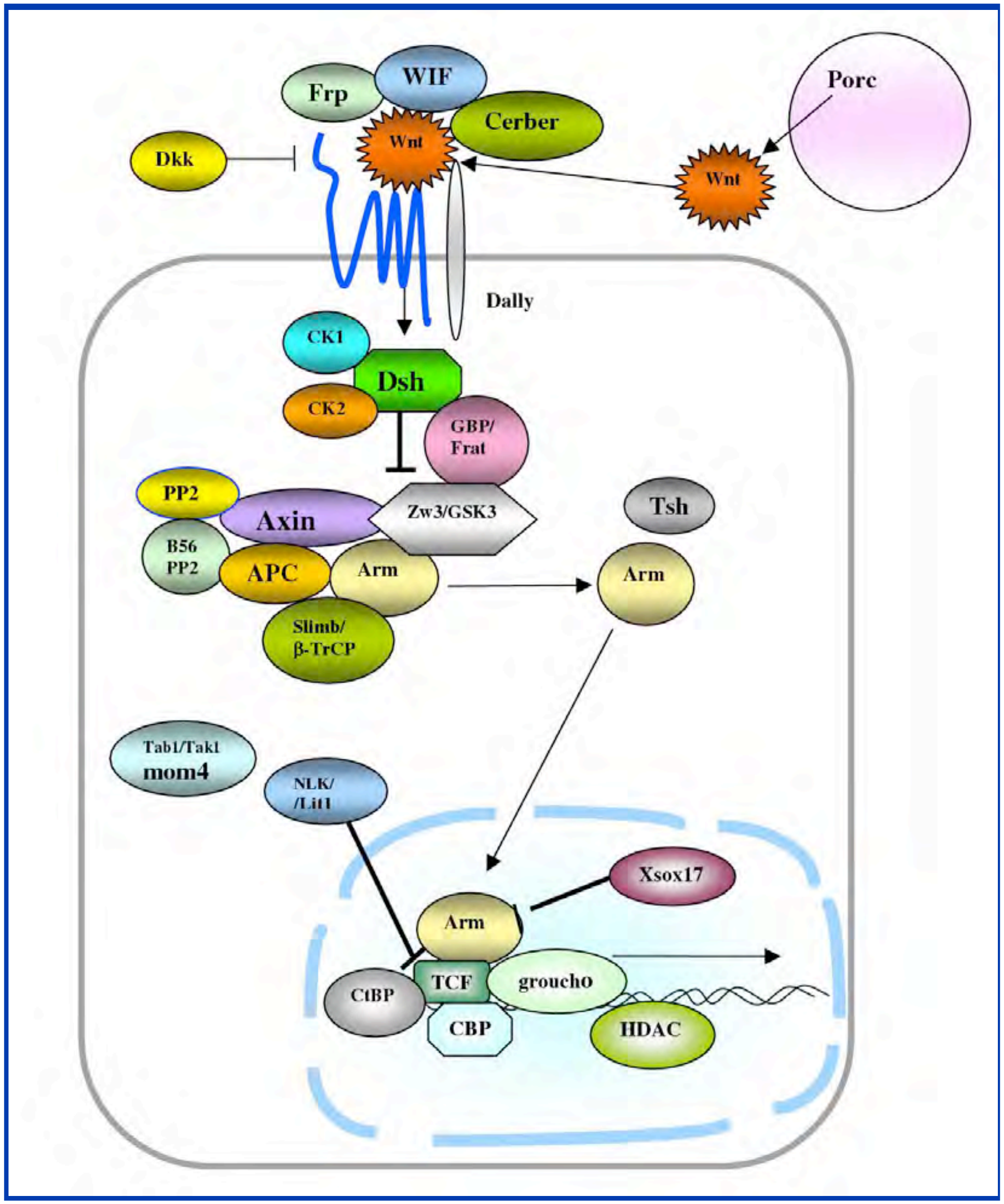
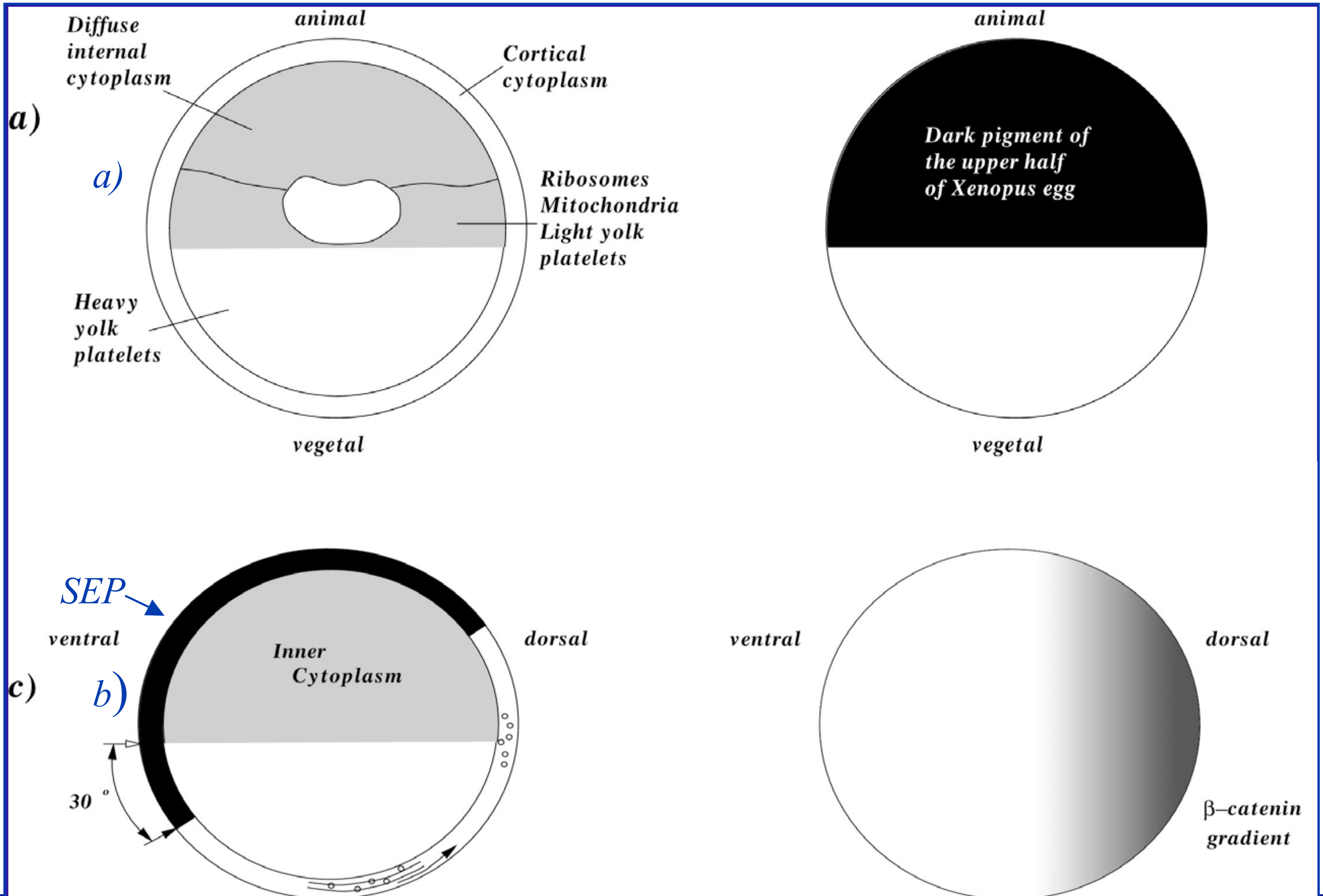
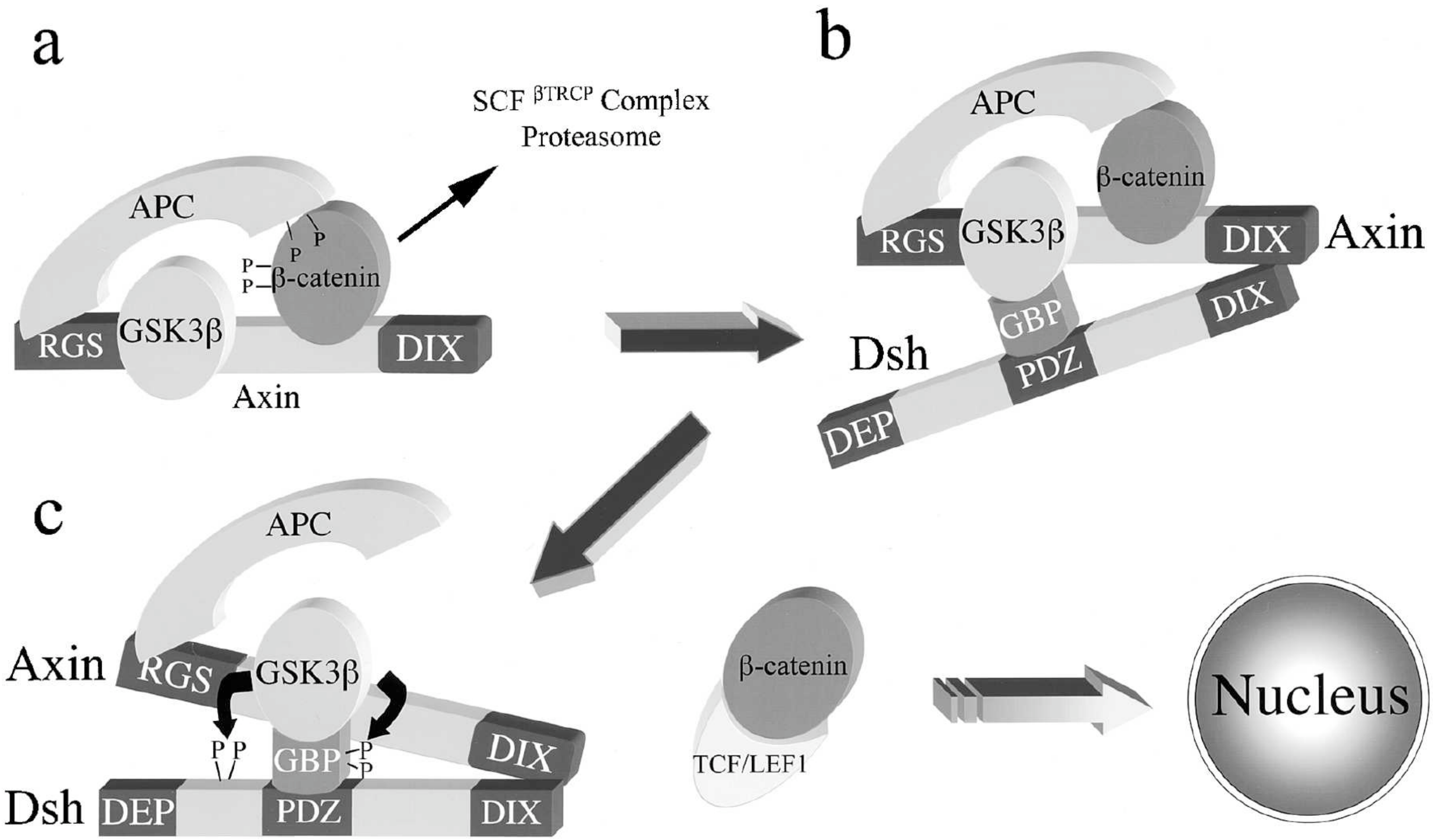

Exponential
a
b
P(k)
<k>
k
Scale-free
c
d
log P(k)
log k



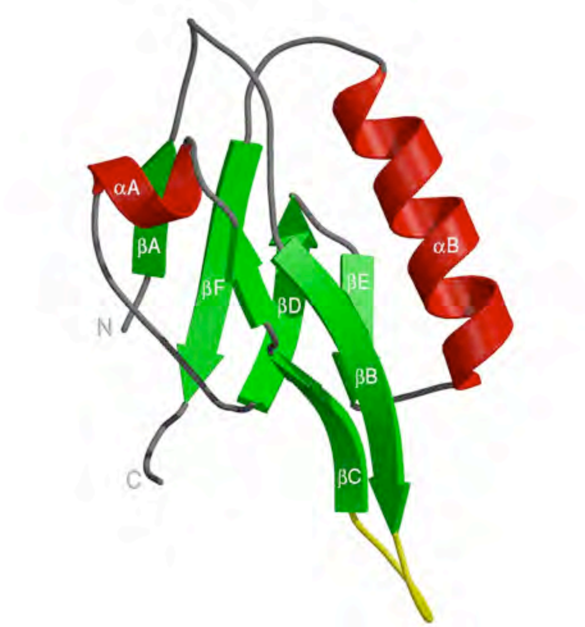
Moon lab
HHMI/Washington

Xenopus *axis specification*



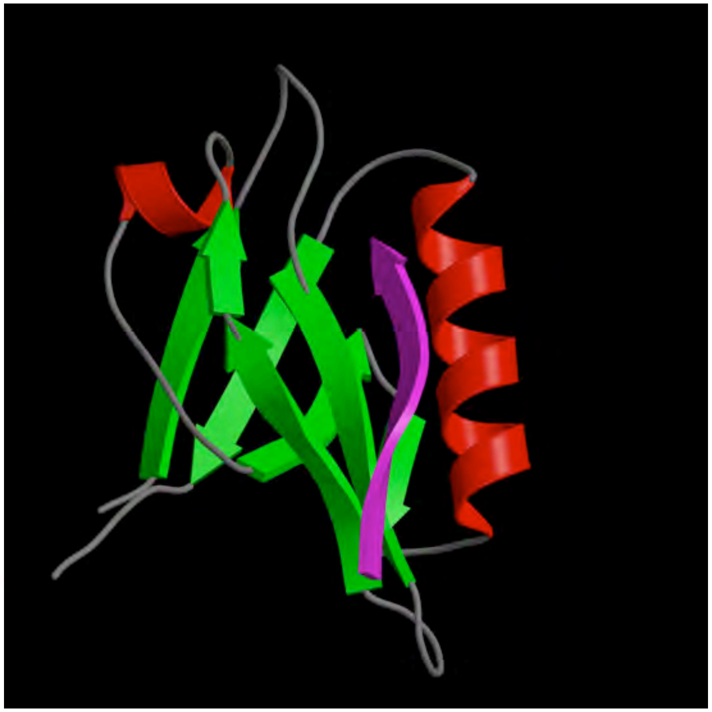

a
SCF βTRCP Complex
Proteasome
APC
P
P
P
P
β-catenin
RGS
GSK3β
DIX
Axin
b
APC
β-catenin
RGS
GSK3β
DIX
Axin
GBP
DIX
Dsh
PDZ
DEP
c
APC
Axin
RGS
GSK3β
P P
GBP
P
P
DIX
Dsh
DEP
PDZ
DIX
β-catenin
TCF/LEF1
Nucleus

Xenopus dishevelled PDZ domain



monomer

homodimer



XDpr peptide complex

Dishevelled / Dapper Interactions

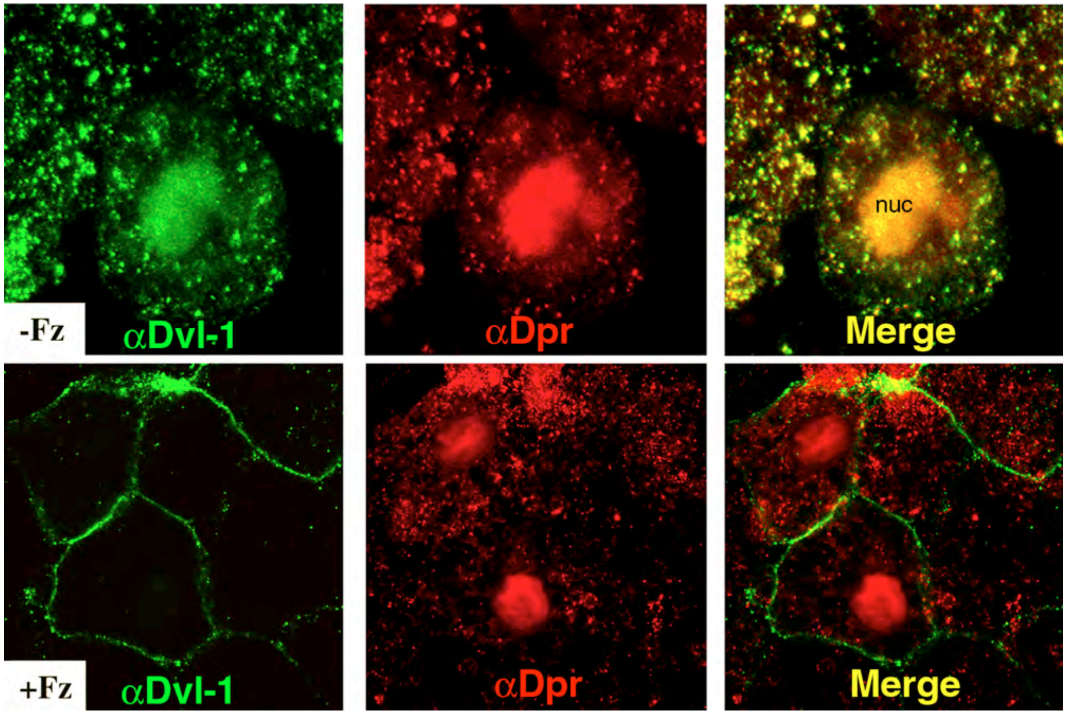
Dapper (Dpr) discovered by Two-hybrid with dsh PDZ as bait

Dpr associates with dsh as shown by GST-pulldown

Deletion of –MTTV abolishes binding

Dpr antagonizes response to Wnt in reporter assay

Ectopic expression of Fz effects localization of Dsh and but not Dpr



XDpr is differentially expressed during development

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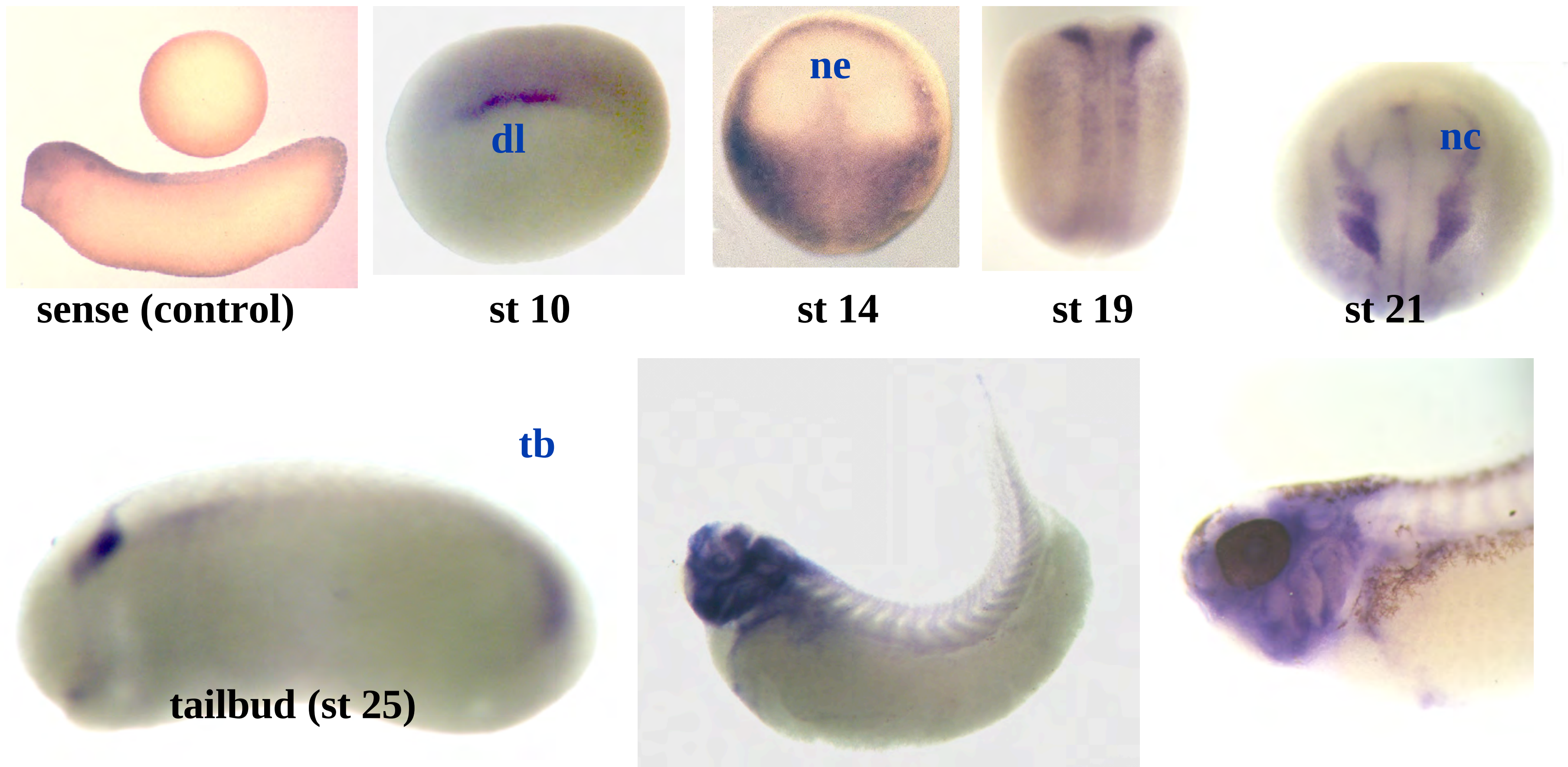
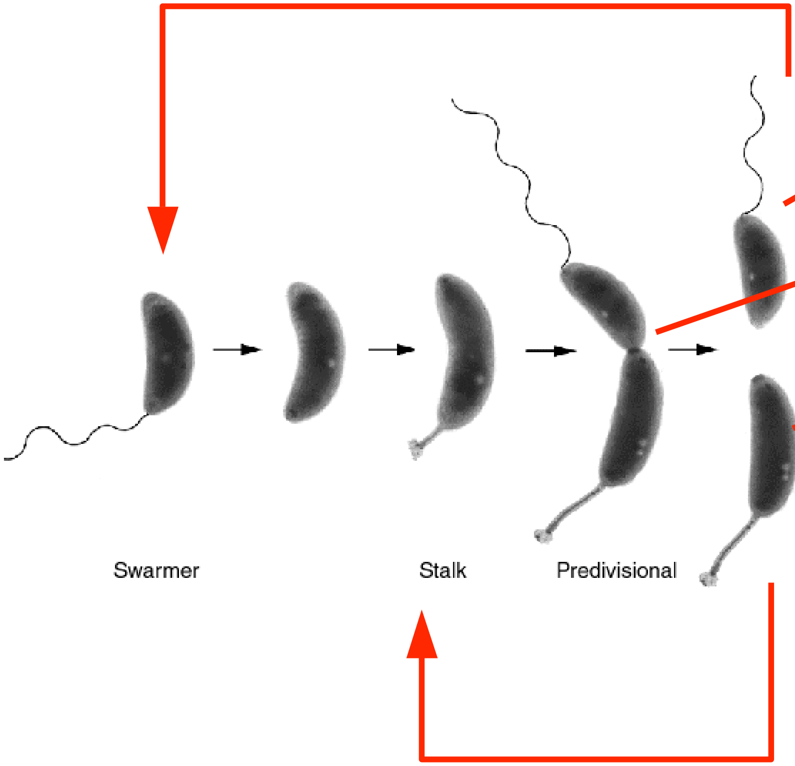


sense (control) st 10 st 14 st 19 st 21

tadpole (st 40)

dl = dorsal lip ne = anterior neurectoderm
nc = migrating neural crest tb = tailbud

Caulobacter crescentus



Motile swarmer progeny

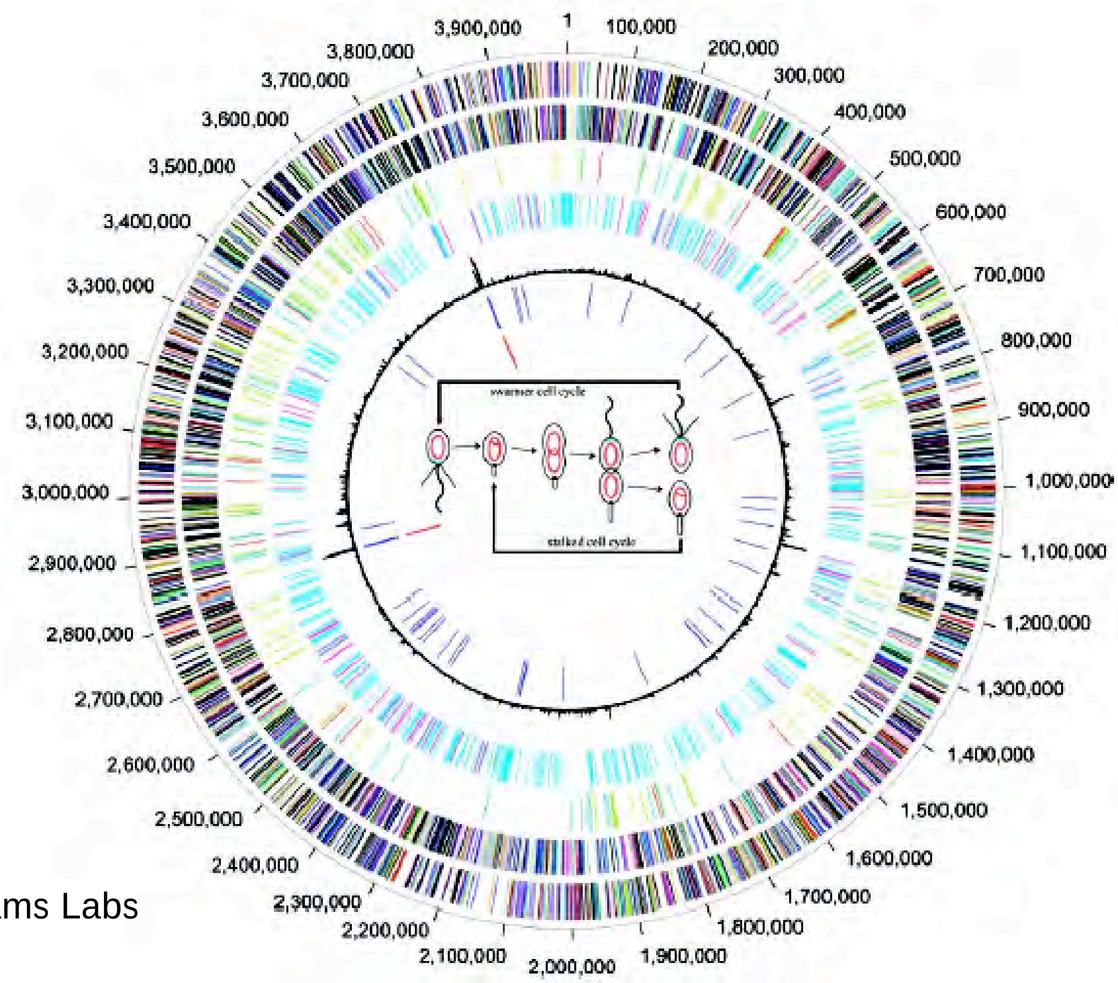
Asymmetric cell division

Sessile stalk progeny

Swarmer and stalk progeny have distinct morphologies, cell cycles, and behaviors . . .

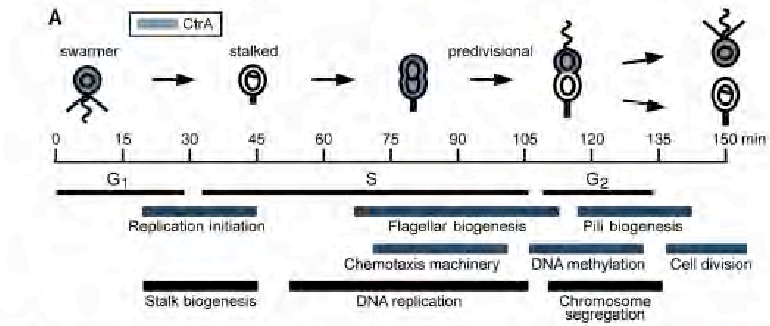
therefore the genetic circuitry must be differentially initialized at some point.

Caulobacter crescentus
~3000 genes



Shapiro and McAdams Labs
Stanford

Caulobacter microarray analysis



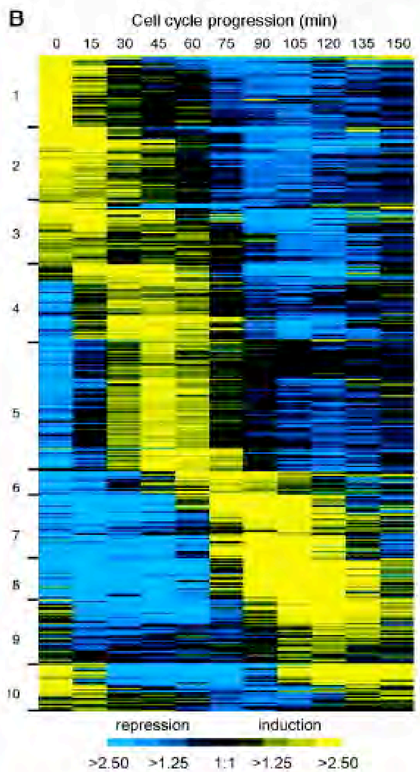
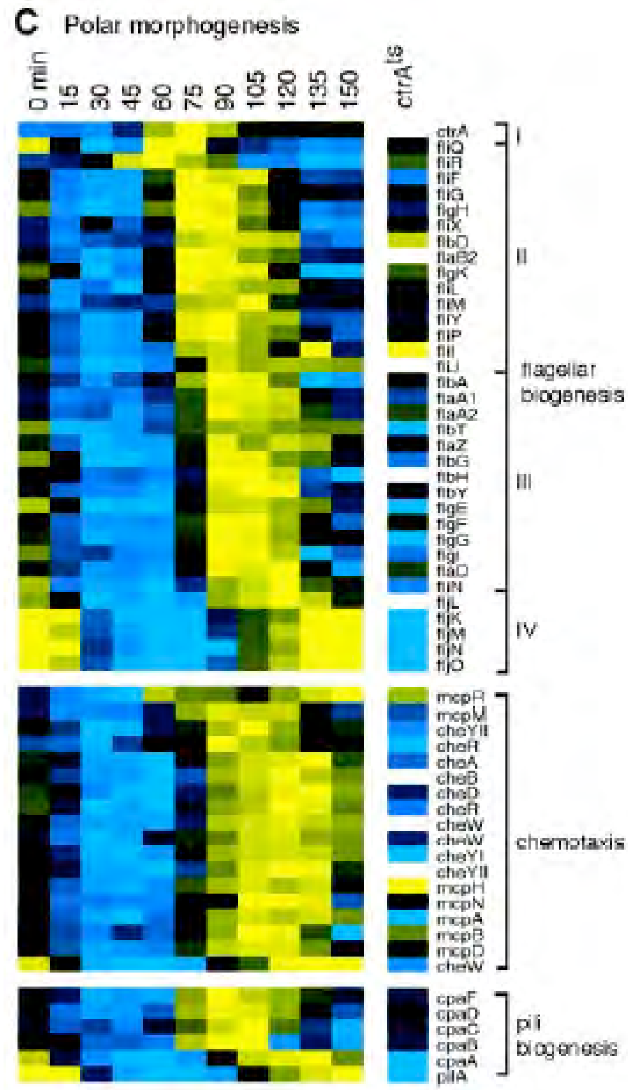
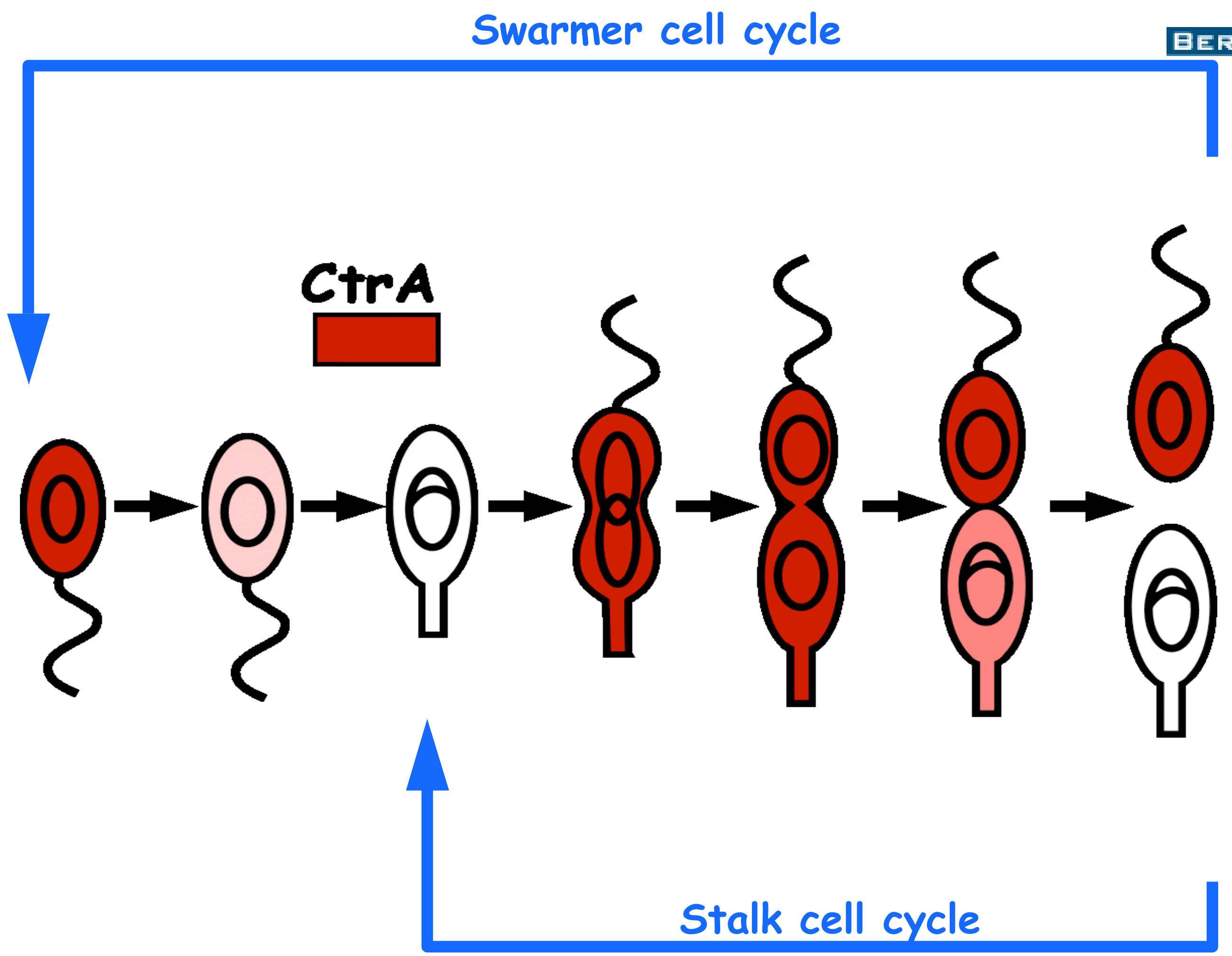


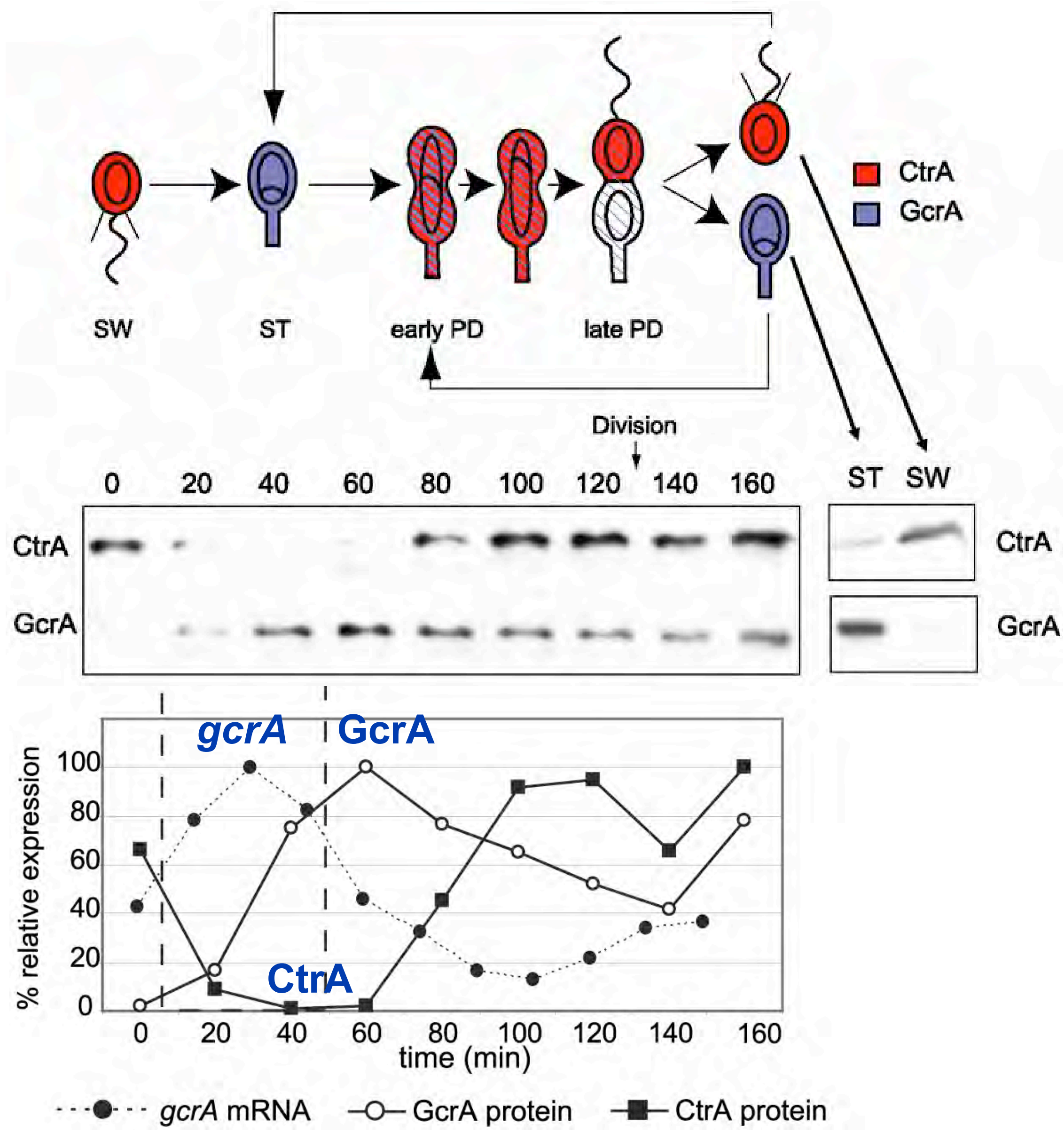
Fig. 1. (A) Temporally coordinated events of the *Caulobacter* cell cycle. Motile, piliated swarmer cells differentiate into stalked cells at the G_1-S transition by shedding their polar flagellum, growing a stalk at that site, losing the polar pili, and initiating DNA replication. Circles and "theta" structures in the cells represent quiescent and replicating chromosomes, respectively. CtrA is present in the shaded cells, where it represses DNA replication initiation and is cleared by proteolysis during the swarmer cell–stalked cell (G_1-S) transition. Cell division yields distinct progeny, a swarmer cell and a stalked cell. Bars below indicate timing of cell cycle functions (gray indicates a function controlled by CtrA). (B) Clustered expression profiles for the 553 identified cell cycle–regulated transcripts are organized by time of peak expression. Expression profiles for genes are in rows with temporal progression from left to right, as indicated at the top. Ratios are represented using the color scale at the bottom. Expression profiles were clustered using the self-organizing map analysis of the GeneCluster software and plotted using TreeView software. Each cluster is numbered; for an expanded, annotated view of these clusters, see (5).



CtrA, the first *Caulobacter* cell cycle master regulator identified, is dynamically regulated in time and space

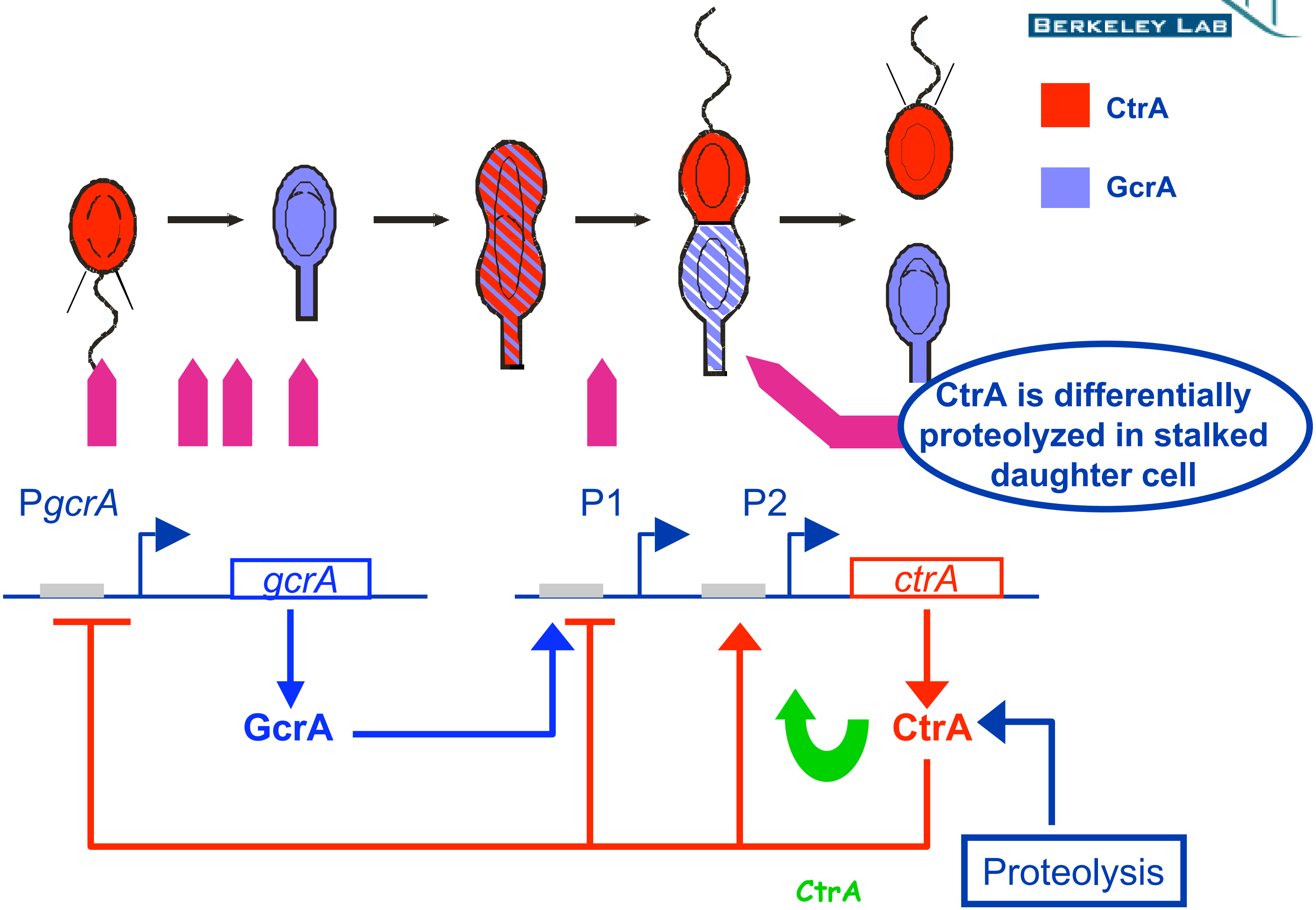


GcrA, a second master regulator, oscillates out of phase with CtrA

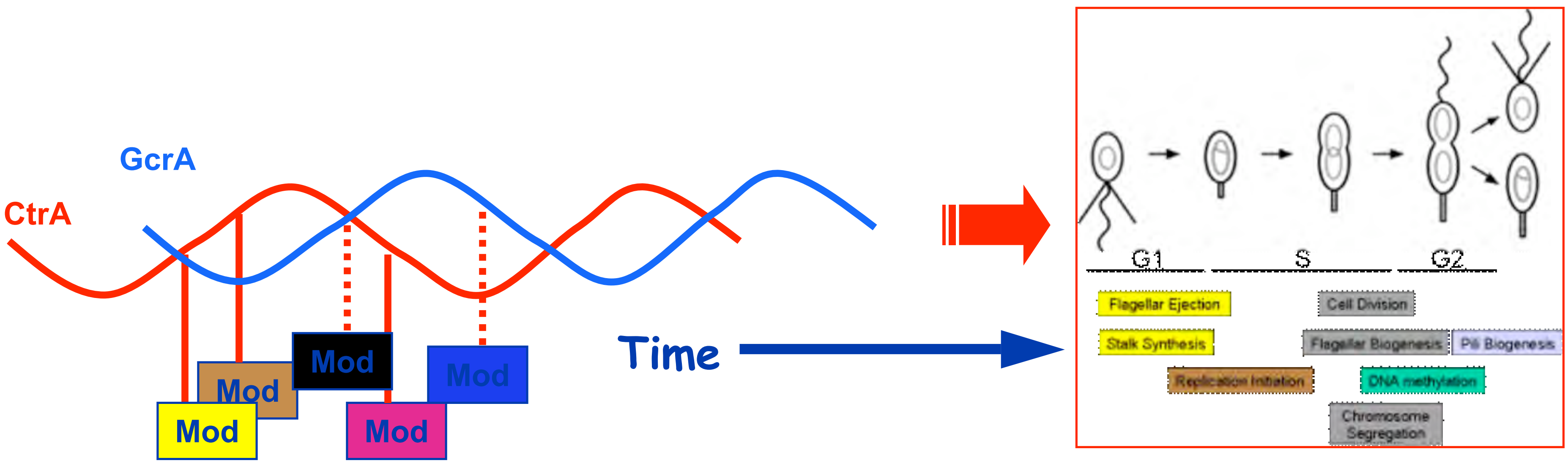


Holtzendorff, Hung, Brende, Reisenauer, Viollier, McAdams, Shapiro. *Science*, April, 2004

GcrA acts with CtrA to create an oscillator that drives the cell cycle



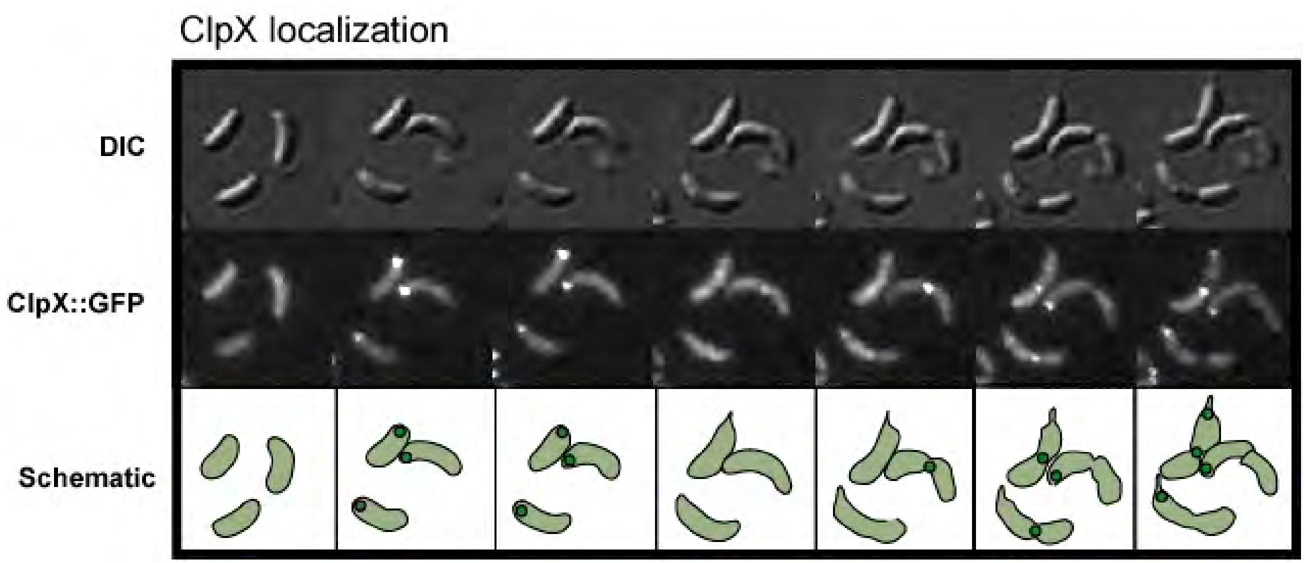
Changing concentrations of the master regulators initiate cell cycle functions

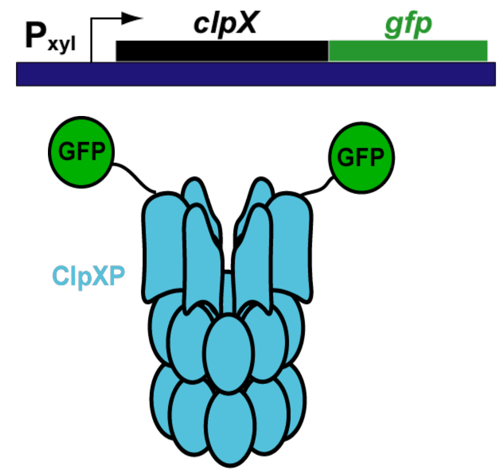


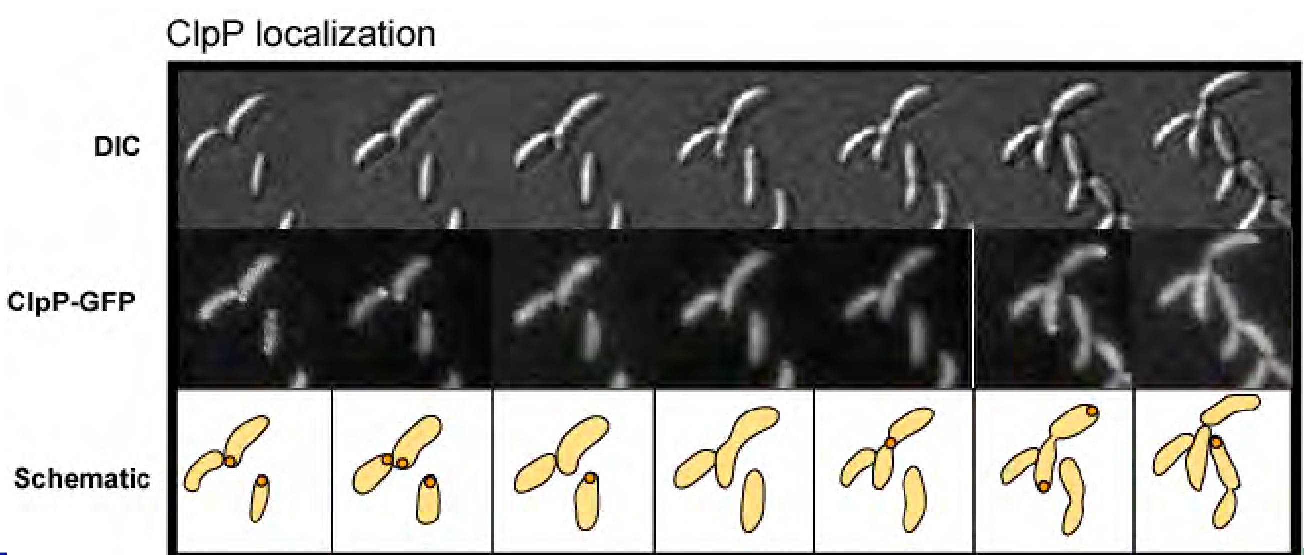
Regulons

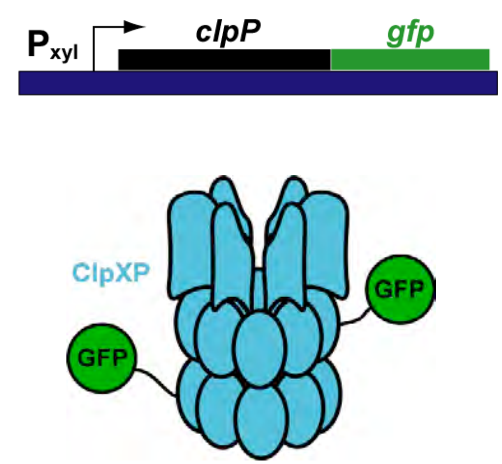
CtrA	~95 genes
GcrA	~50 genes

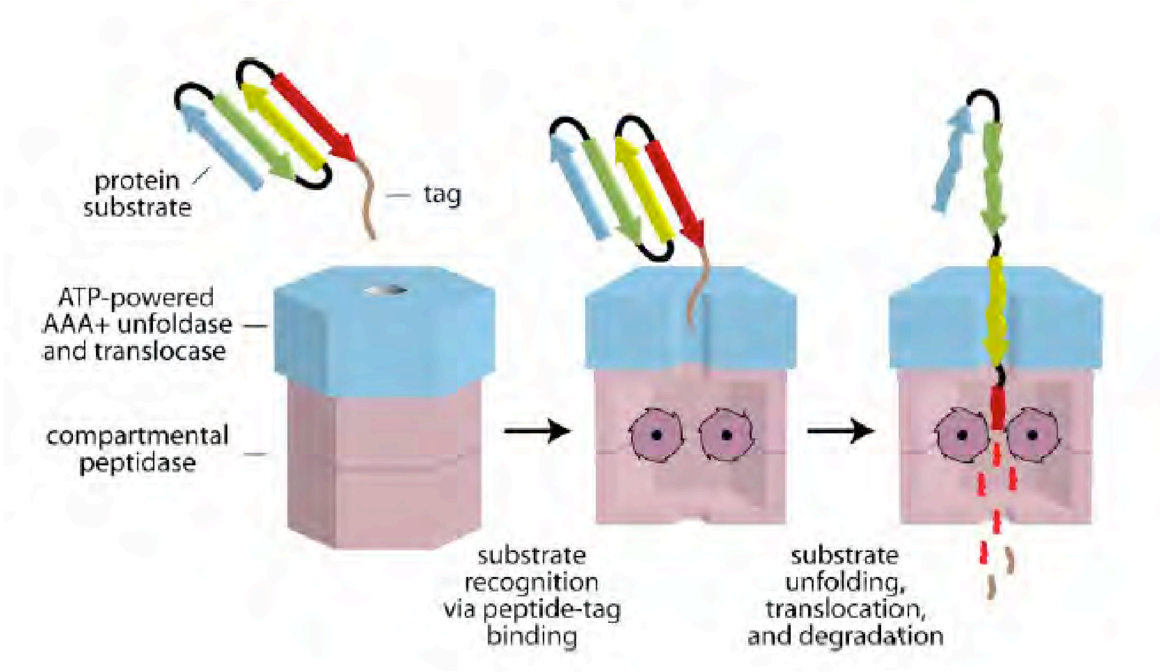
ClpXP dynamically localizes through the cell cycle

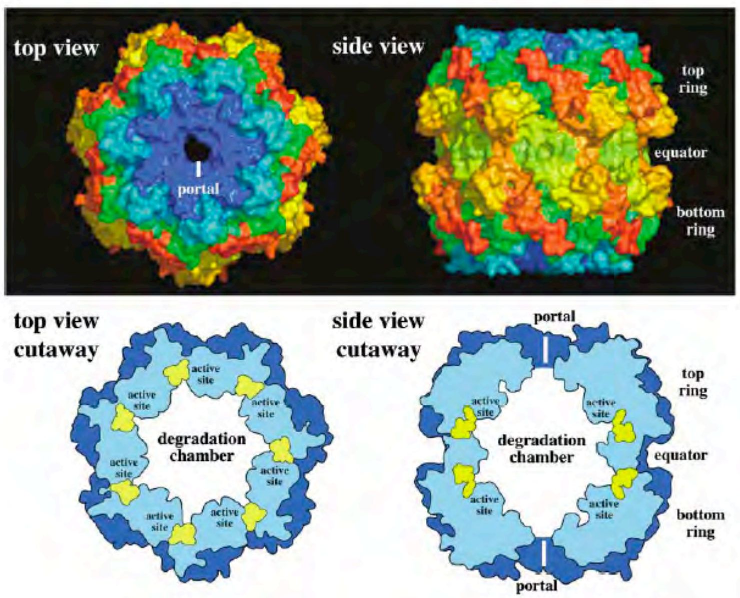




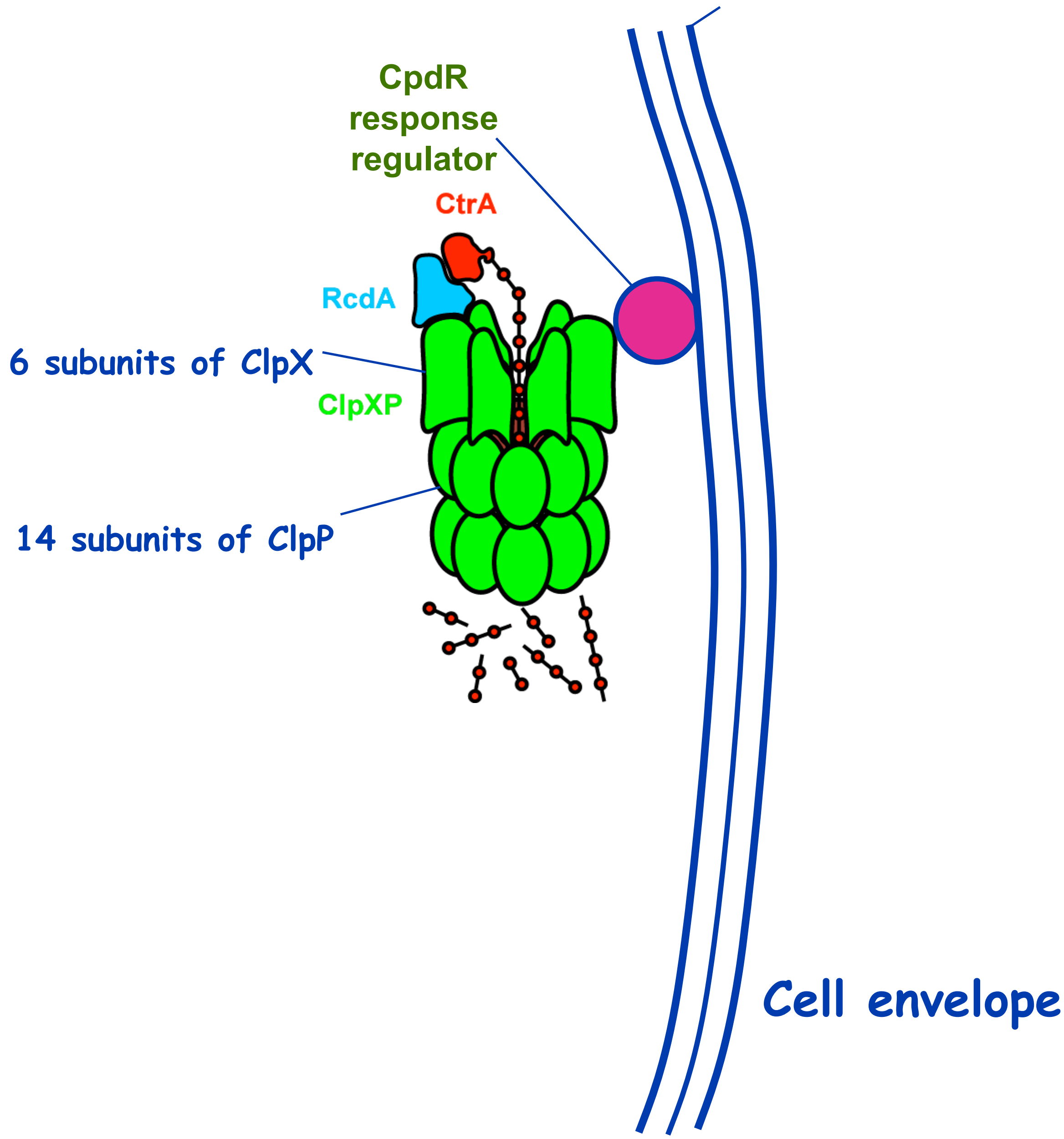




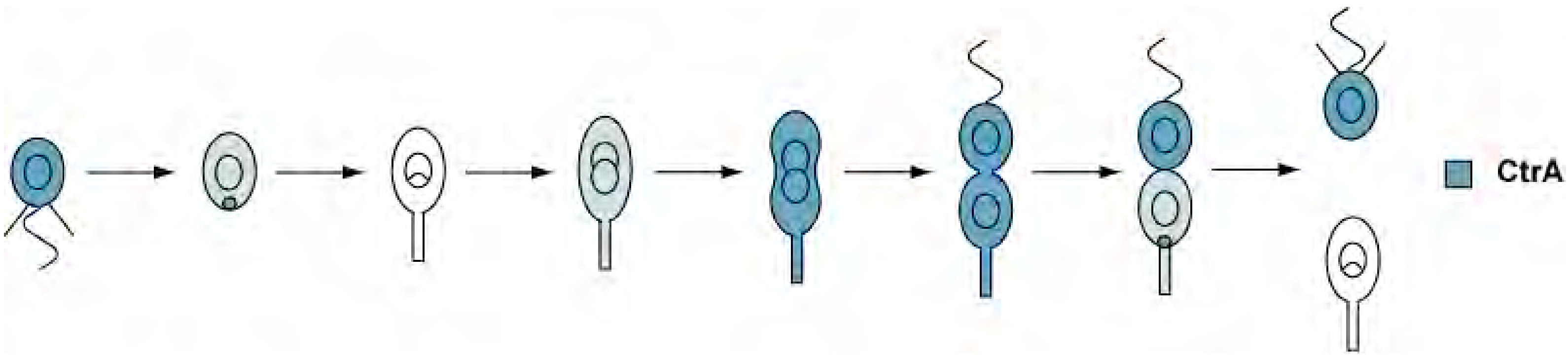

protein substrate
tag
ATP-powered AAA+ unfoldase and translocase
compartmental peptidase
substrate recognition via peptide-tag binding
substrate unfolding, translocation, and degradation

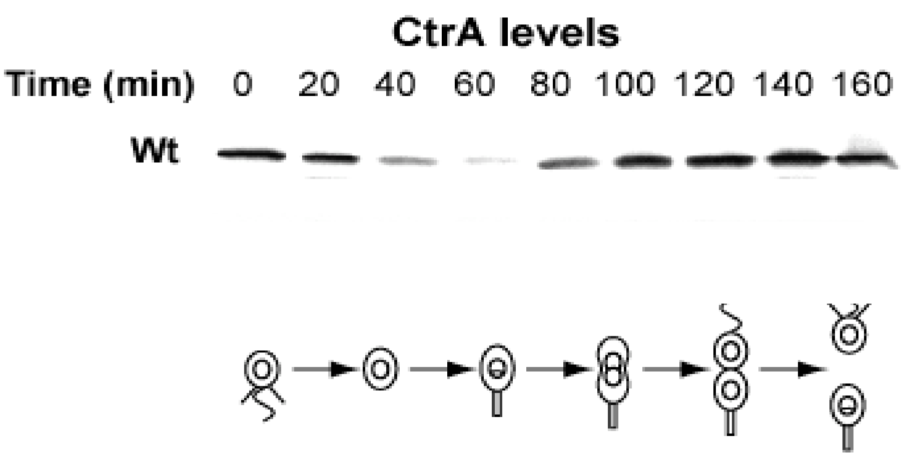

top view
portal
side view
top ring
equator
bottom ring
top view cutaway
active site
degradation chamber
side view cutaway
portal

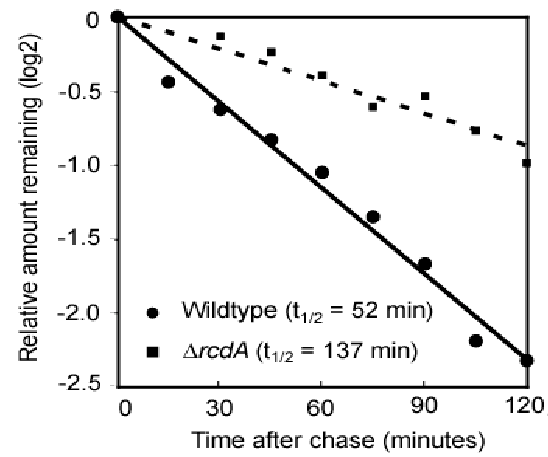
CtrA proteolysis complex at cell pole



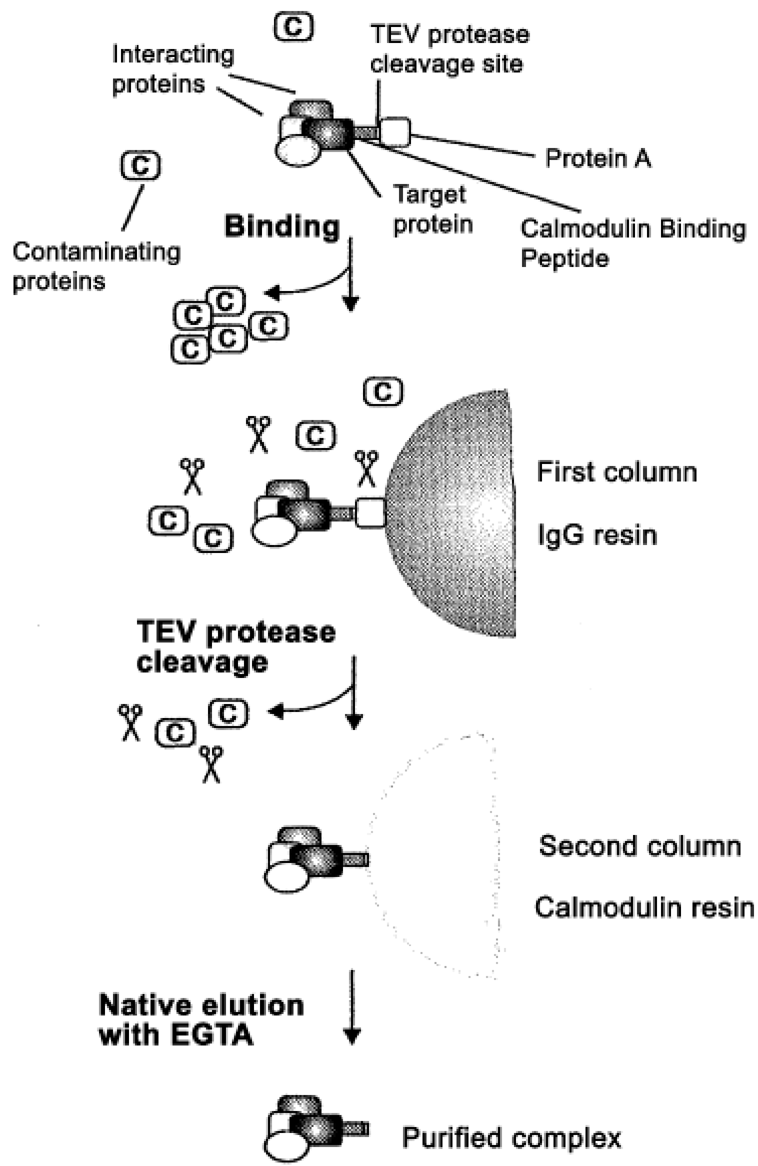
RcdA is involved in the specific degradation of CtrA



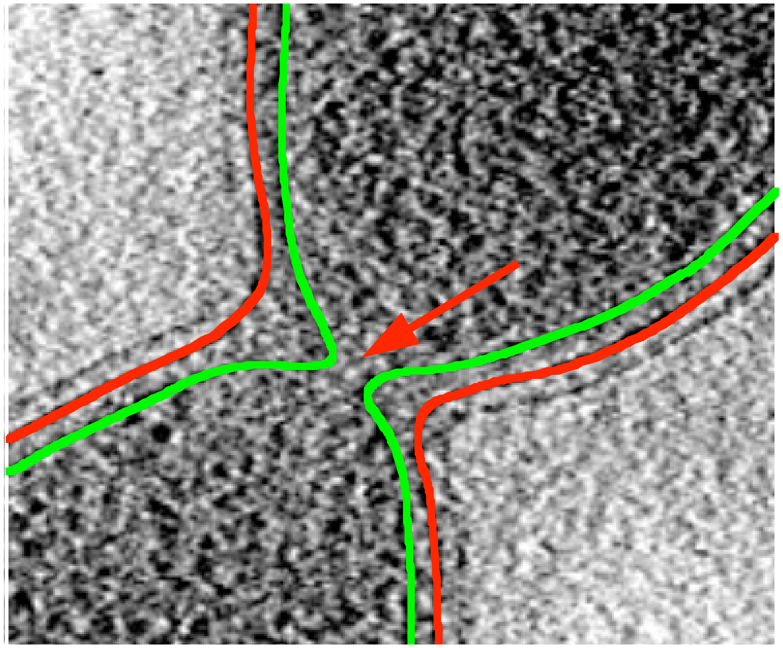




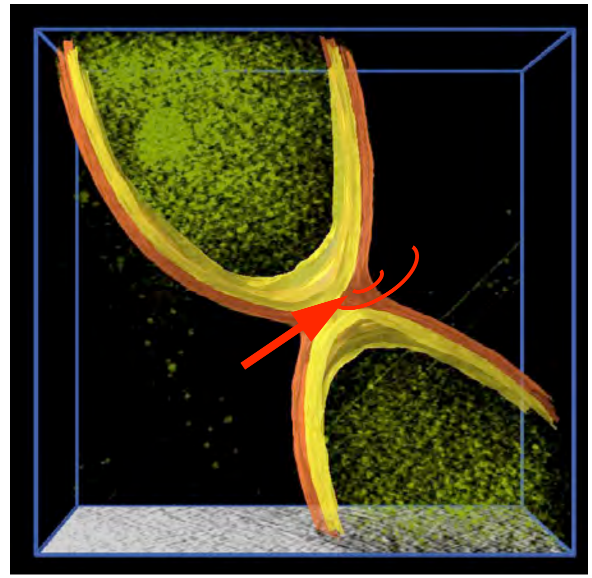
The TAP procedure overview

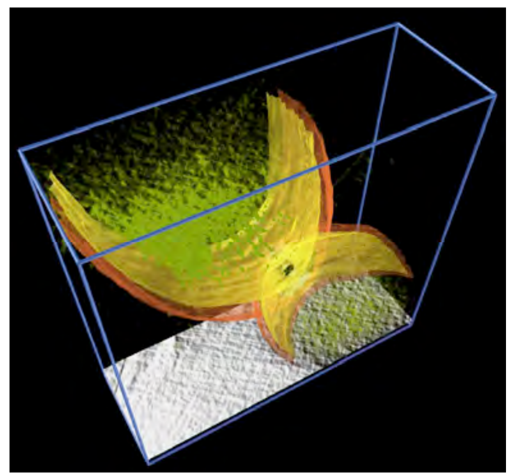


CryoEM tomographic images of cytoplasmic compartmentalization



100 nm





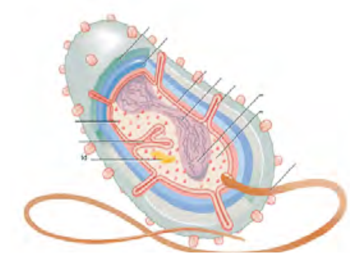
Observations

- 60 nm lipid bilayer "tether" structures never before observed
- Two spatially and temporally distinct constriction processes

cryoEM tomographs by Luis Comolli and Ken Downing at LBNL

Important concepts

- Many critically important regulatory proteins and multiprotein complexes perform their functions at a specific time and place in the cell

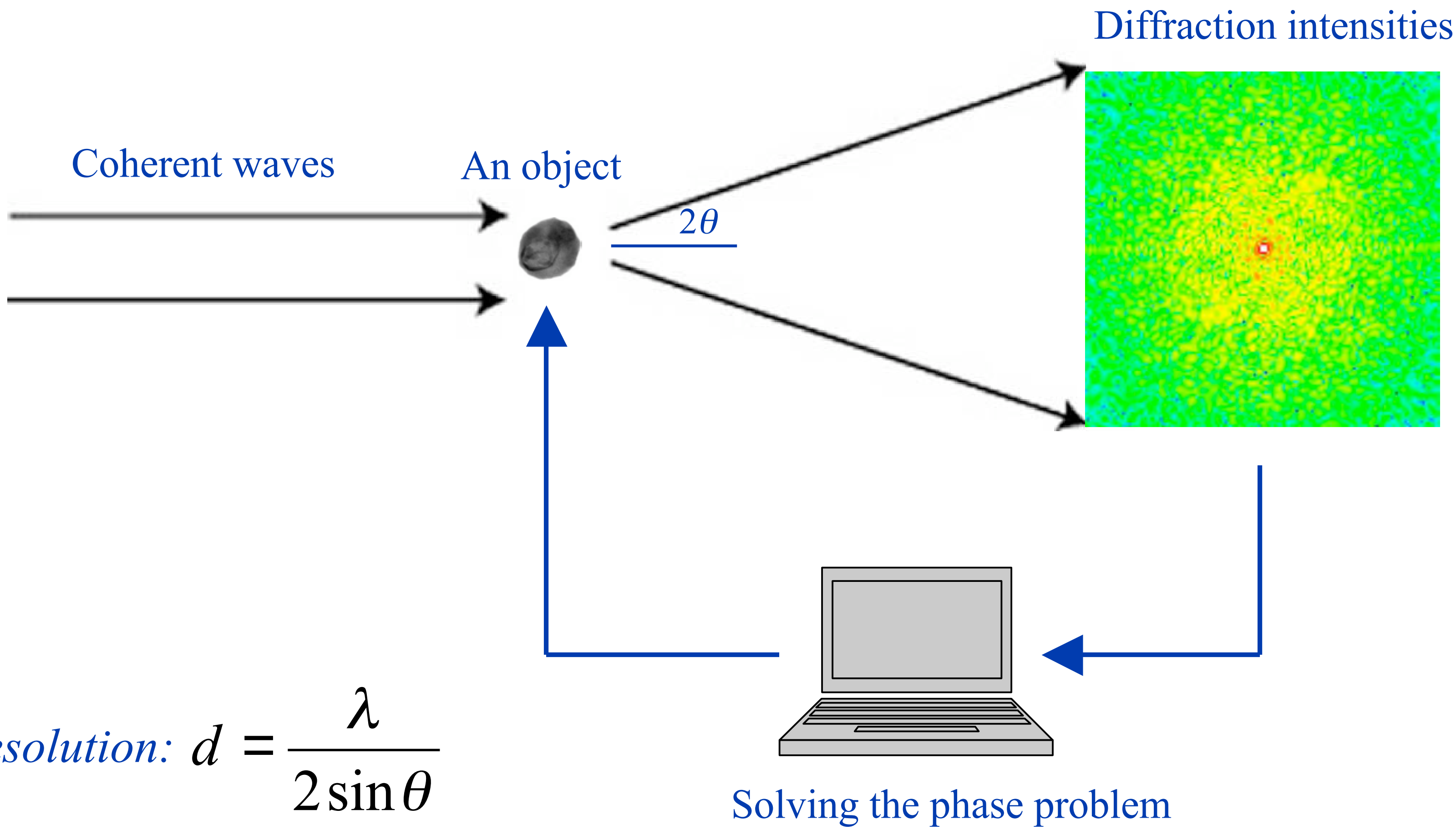


- The tiny bacterial cell is highly structured
  - Core mechanisms that implement chromosome replication, separation and organization of newly replicated chromosomes, positioning of polar structures, and cell division operate together as a tightly integrated dynamical machine
  - Composition, specificity, and function of the protein complexes implementing these functions must be examined both *in vitro* and in the context of the intact cell
  - Cell disruption frequently destroys structure, context, and function of the complexes

"Grand Challenge":

Mapping the dynamic interacteome -
understanding the large-scale organizing principles of the cell in space and time at the molecular to cellular level

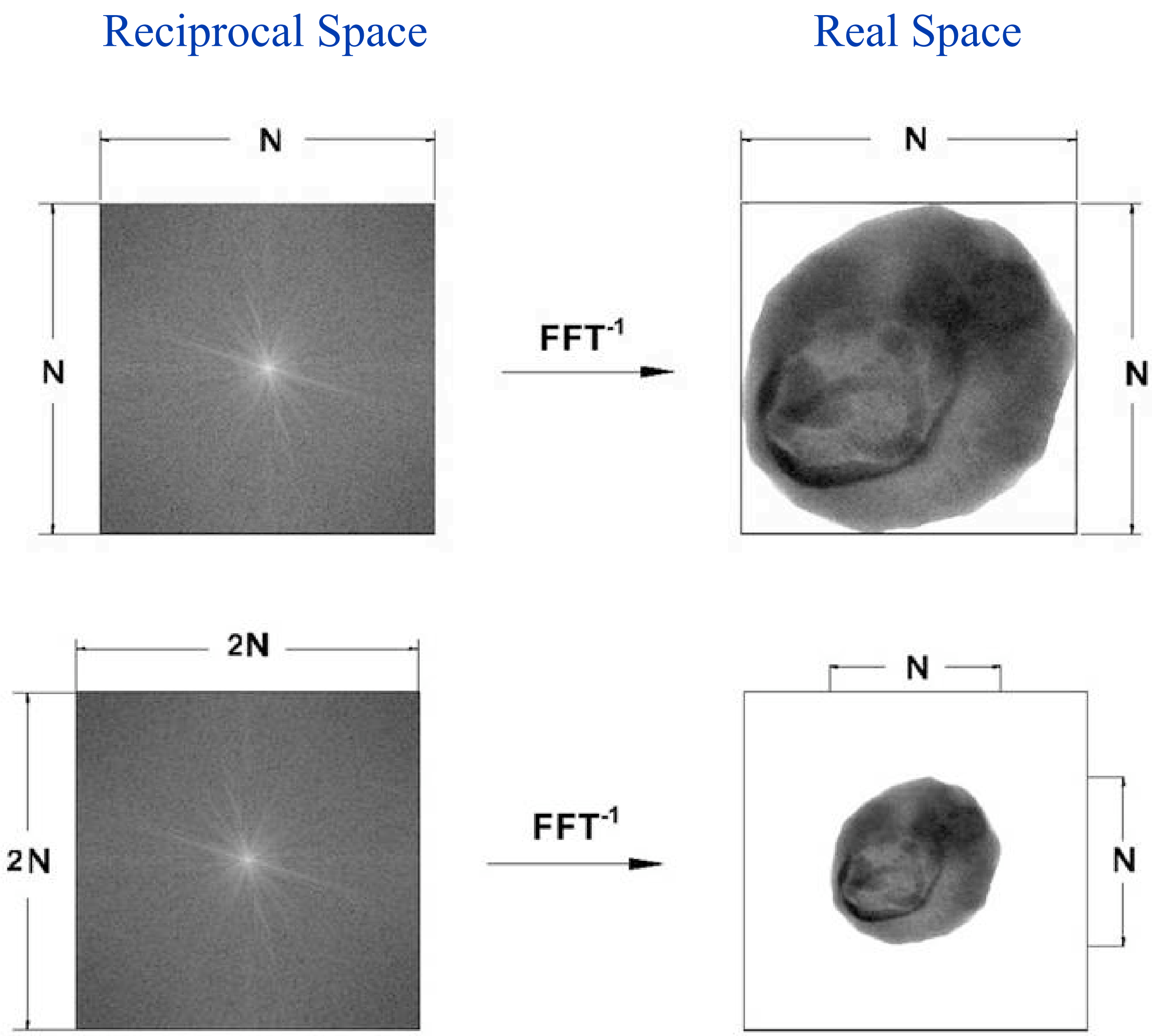
Crystallography	atomic resolution structures of biomolecules and their complexes
Solution x-ray scattering	molecular envelopes; supramolecular structure changes; HT characterization
Spectroscopy	chemical analysis
Coherent x-ray imaging experiments	subcellular localization; cellular architecture

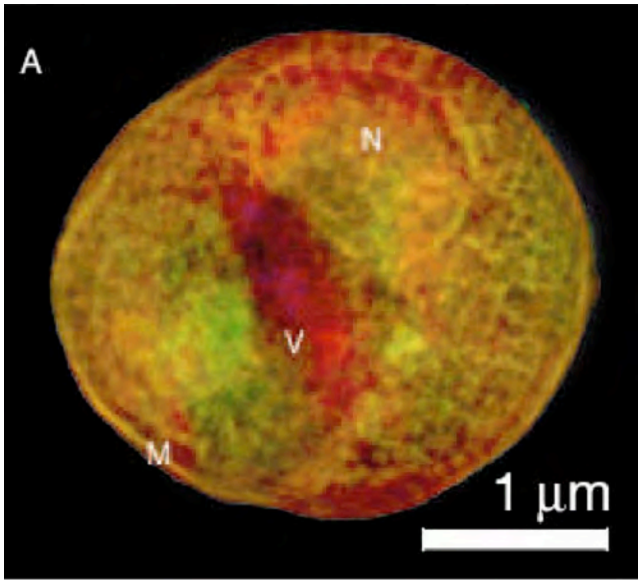


Resolution: $d = \dfrac{\lambda}{2\sin\theta}$

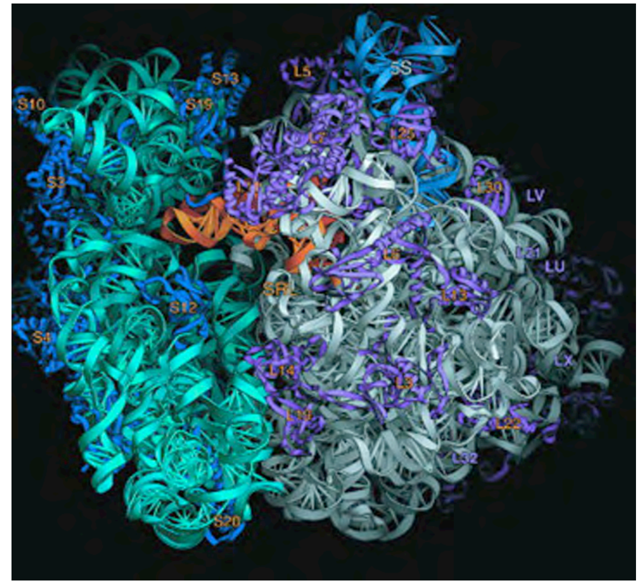
Miao lab
UCLA

A Pictorial Representation of the Oversampling Method

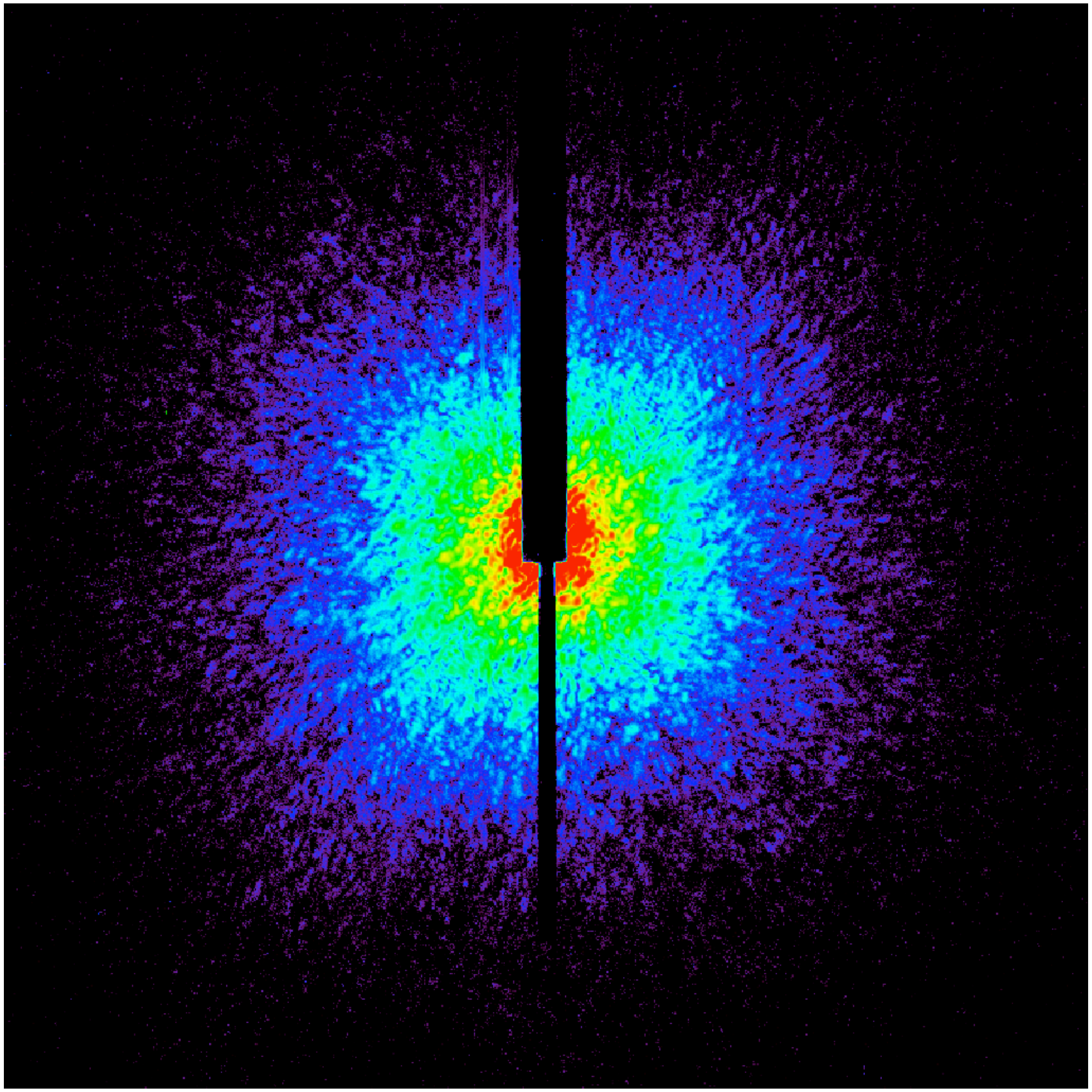




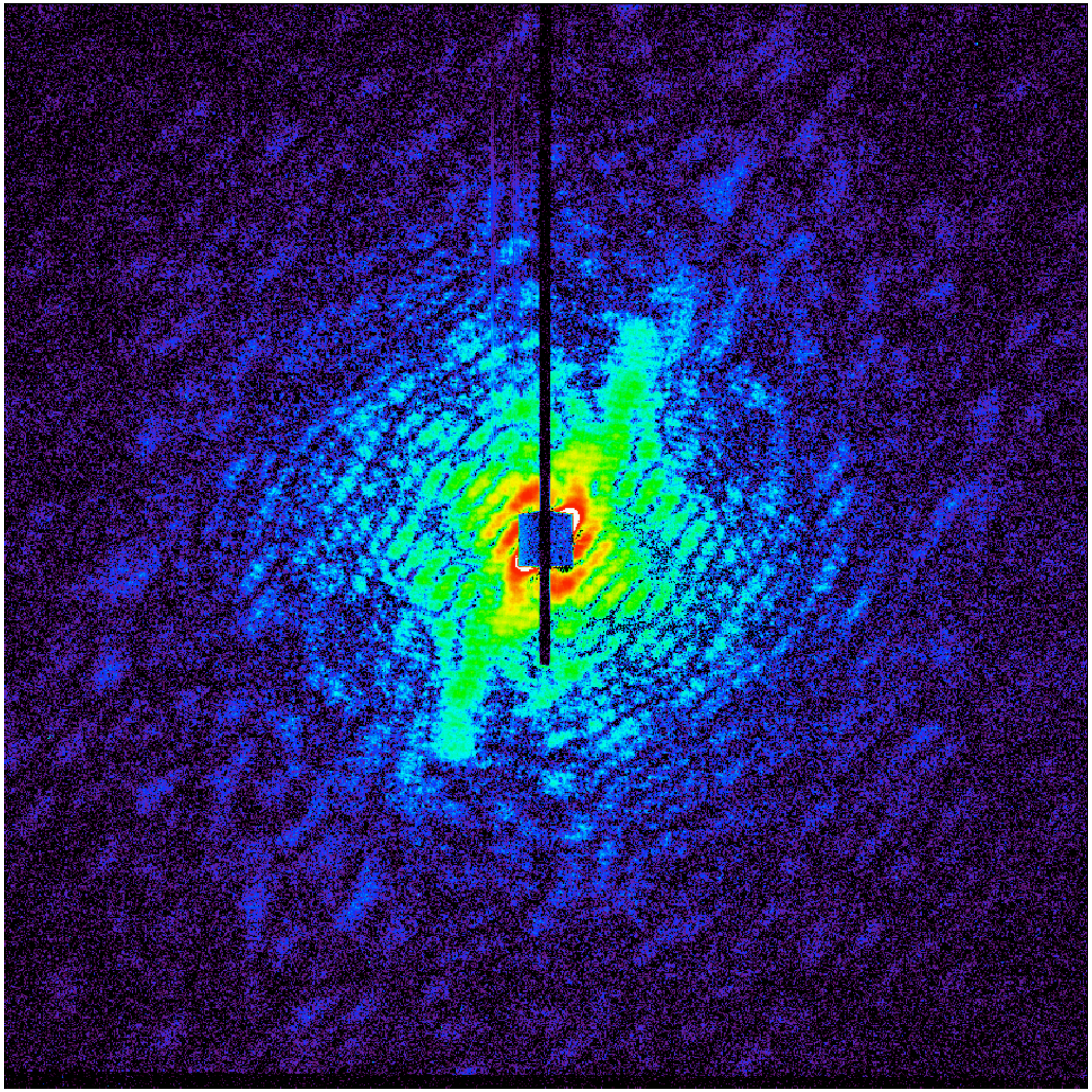
(Shapiro *et al*., PNAS **102**, 15343 (2005))



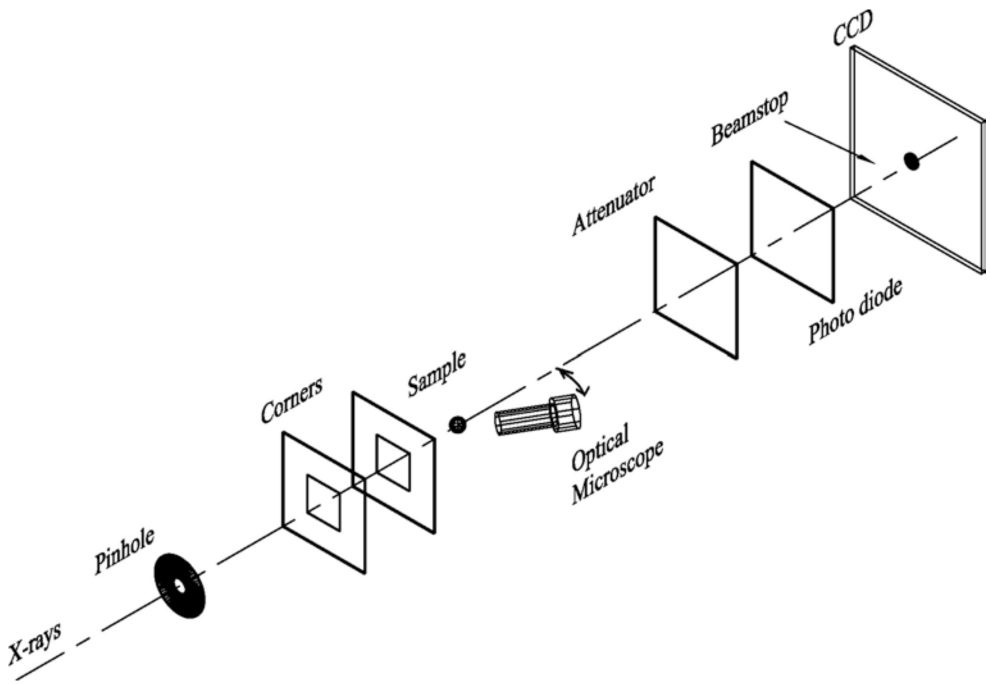
Pseudo-atomic maps of cellular organelles



Coherent x-ray pattern from *S. cerevisiae*

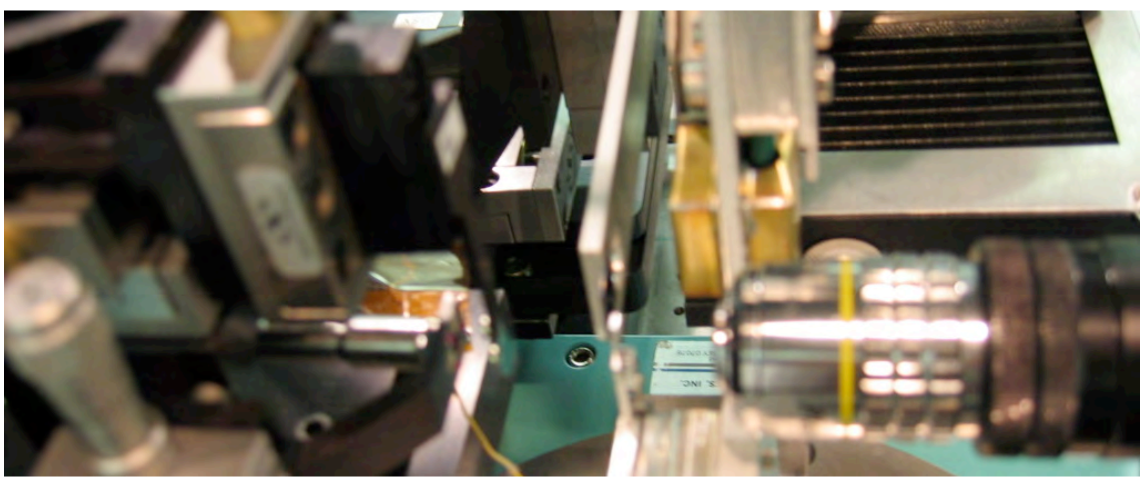


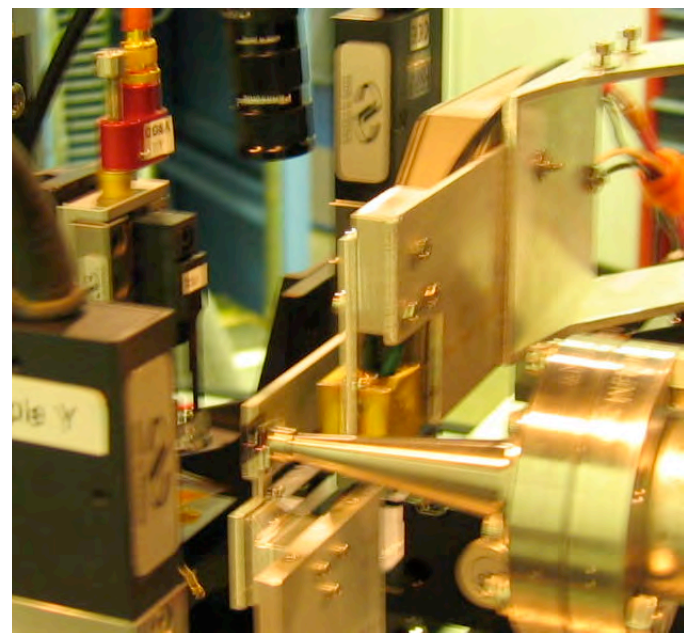
Coherent x-ray pattern from *Caulobacter*



APS 2ID-B

Martin de Jonge
Ian McNulty
David Paterson





Bright, coherent x-ray source

Sample preparation and handling

Cryogenic temperatures

Rotation for 3D tomographic analysis

Improved detectors - small pixels, fast readout

Better methods for analyzing data and integrating with larger body of knowledge

Understanding biological systems at a structural
as well as functional level -
molecular to cellular (and multi-cellular)

Benefits to health and environment

Biologically-inspired nano-machines

Exploiting cellular machinery; synthetic biology

Acknowledgments

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Zemer Gitai	*Princeton*
Natasha Friedland	*LBNL*
Li-wei Hung	*LANL*
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Martin de Jonge	*APS*
Ian McNulty	
David Paterson	

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DOE Genomics:GTL

NIH/NIGMS