Single RNA polymerases in single bacteria: a PALM-driven journey

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Imaging CDT, Oxford, 14th Jan 2015

Gene expression: the path from gene to protein

DNA
Transcription
RNA
Translation
Protein

RNA Polymerase (RNAP)
RNA
Ribosome
Transcription
1 μm

Bacterial transcription cycle

RNAP
σ
Promoter DNA
Holoenzyme
Closed Complex
DNA
Isomerizations
RNAP core
Open Complex
NTPs
RNA<9 nt
RNA>9 nt
Abortive RNA
RNA (mature)
Elongation Complex
Initial Transcribing Complexes

The *in vitro* world: are we missing the big picture?

presence of other biomolecules
crosstalk with other processes
macromolecular crowding
supporting substructure and compartments
stochasticity and noise for low-copy molecules

Spatial organization of transcription in bacteria?

Are RNA polymerases spatially organized in bacteria?

Functional units?

Re-organization at different cellular states?

Mechanism and kinetics of re-organization?

Sub-cellular RNAP distribution in fixed *E.coli*

Ding Jin lab (NIH)

DIC | β'-GFP (RNAP) | DAPI | β'-GFP+ DAPI | DIC+ DAPI

Cabrera & Jin, Mol Micro (2003) 50, 1493

- RNA polymerases form "transcription foci"
- differences for different growth media
- study limited by spatial resolution and sensitivity

Subcellular localization of RNAP in live bacteria

Mat Stracy

Federico Garza de Leon
Stephan Uphoff

Diffraction limits the resolution of optical microscopy

- Image of single fluorophore: point-spread function (PSF)
- ~250 nm wide (x-y plane) due to diffraction:

$$\Delta x \sim \frac{0.61\lambda}{NA}$$

Ernst Abbe

Diffraction limits resolution (Rayleigh criterion)

Δx=200-300 nm

(just resolved)

E. Abbe, *Arch Mikrosk Anat* 1873, 9, 413.

High-precision localisation

But can localise single object with high precision (<<λ/2)

- determine center of emission pattern by fitting a 2D Gaussian to a PSF

$$I(x,y) = I_0 + A \cdot e^{-\frac{(x-x_c)^2}{2s_x^2}} \cdot e^{-\frac{(y-y_c)^2}{2s_y^2}}$$

Intensity
Y-Axis (Pixel)
X-Axis (Pixel)

- measure of precision: standard error of the mean σ_μ of Gaussian fit

Standard deviation of Gaussian fit along x or y

$$\sigma_\mu \sim \frac{s_i}{\sqrt{N}}$$ (mainly Poissonian noise)

for 10^4 photons, precision of 125nm/100 ~ 1.25 nm (!)

Super-resolution imaging by single-molecule localization

(F)PALM (photoactivated localization microscopy) – Betzig et al 2006, Hess et al, 2006
(d)STORM (stochastic optical reconstruction microscopy) – Rust et al 2006, Heilemann et al 2006

Standard deviation of Gaussian along x or y

$$\sigma_\mu \sim \frac{s_i}{\sqrt{N}}$$ (mainly Poissonian noise)

COS7 cells, microtubulin network (immunostained)

Switch off | Switch on subset | Localize

Georges Seurat: "La parade"

PALM with photoactivated fluorescent proteins

PA-FP: photoactivatable GFP variant

405 nm → 561 nm → 600 nm

off | photo-activated | bleached

PA-FP (Kaede), TIRF, 561exc

PAmCherry, mEos, PA-GFP, etc

Betzig et al *Science* 2006

PALM of RNA polymerase in fixed *E.coli*

Mike Heilemann (Frankfurt)

Diffraction-limited image

Super-resolution image (PALM, β'-PAmCherry)

15 nm localiz. precision 35 nm resolution, wide-field imaging

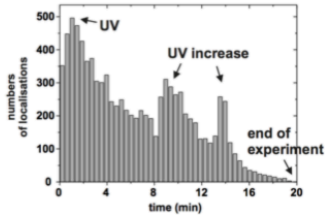
Image with molecular clusters

(cluster size, shape, location, stoichiometry; intercluster distances)

band
large cluster
single molecule

Endesfelder et al, *Biophysical Journal* 2013

Photoactivation is quantitative



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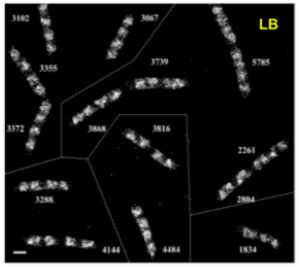
RNAP distribution in rich media (LB)

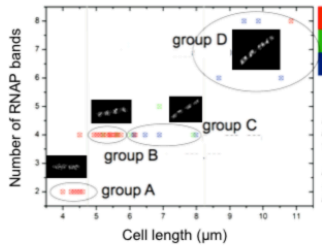


- 3,685 ±1,275 (s.d.) localizations per cell
- no. of localizations: lower bound for copy number (detection efficiency 50-90%)
- RNAP in 2-8 nucleoids depending on cell size
- within bands, RNAP distribution is non-uniform

Endelsfelder et al, *Biophysical Journal* 2013

14 01:16

RNAP bands as a function of cell cycle



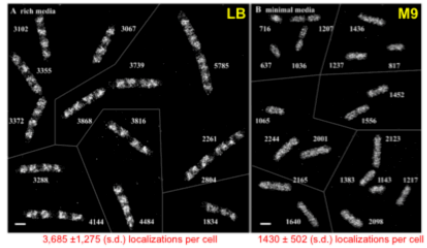
not dividing
septum initiated
very clear septum

Number of RNAP bands scales linearly with cell age

Bands: ***nucleoids*** *(copies of condensed bacterial chromosome)*

15 ☆

RNAP distribution in rich vs. minimal media



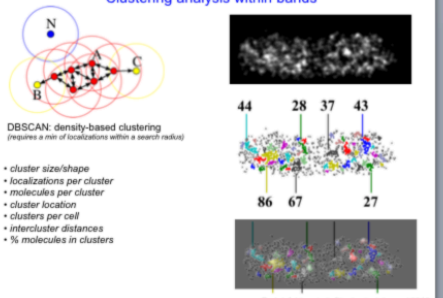
3,685 ±1,275 (s.d.) localizations per cell

1430 ± 502 (s.d.) localizations per cell

in M9:
molecular density per cell similar (~700 RNAPs per µm²)
RNAP found in 1-2 nucleoids
RNAP distribution more uniform than in LB

16 :30

Clustering analysis within bands

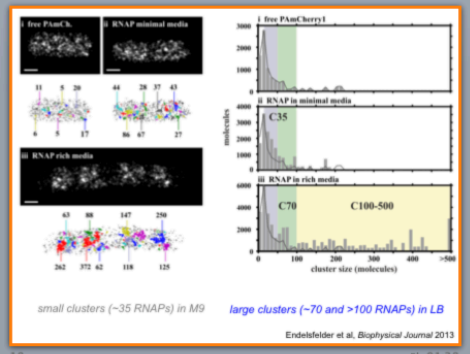


DBSCAN: density-based clustering
(requires a min of localizations within a search radius)

- *cluster size/shape*
- *localizations per cluster*
- *molecules per cluster*
- *cluster location*
- *clusters per cell*
- *intercluster distances*
- *% molecules in clusters*

Endelsfelder et al, *Biophysical Journal* 2013

17 ☆ 02:17



small clusters (~35 RNAPs) in M9

large clusters (~70 and >100 RNAPs) in LB

Endelsfelder et al, *Biophysical Journal* 2013

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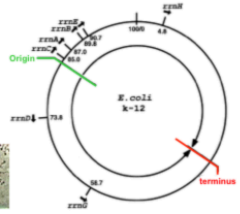
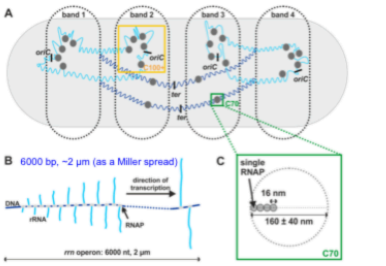
Origin of clusters in LB: RNAPs on ribosomal RNA operons?

- 80% of active RNAPs are on rRNA operons (6000 bp each)
- *rrn* operons heavily occupied; 80-90 RNAPs per operon (EM)



19 :31

rrn operons: highly compact regions ~100 RNAPs each



Endelsfelder et al, *Biophysical Journal* 2013

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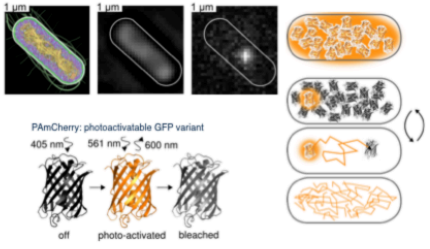
but…

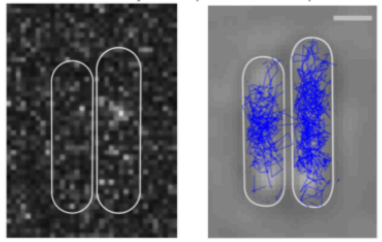
what about the RNAP distribution in live cells?

Tracking PALM: photoactivation, localization, tracking

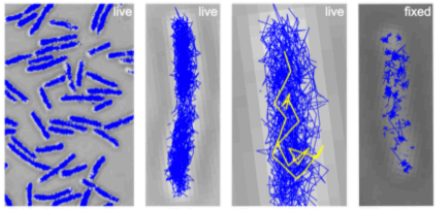


Imaging movement of single DNA Pol I molecules in live *E.coli*

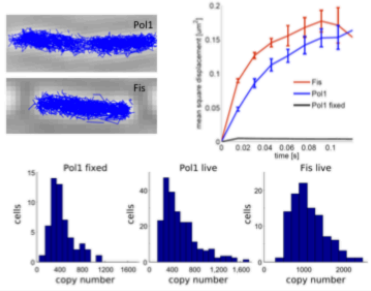
Pol1 – PAmCherry fusion expressed from native promoter



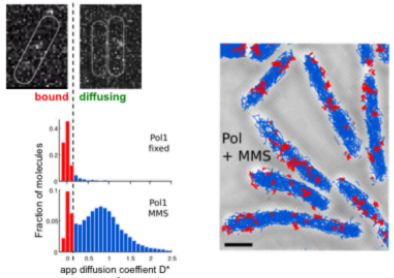
Tracking DNA Pol I in live and fixed *E.coli*



Copy numbers and diffusion analysis for DNA Pol I



Sort polymerases as bound or diffusing



Uphoff et al, PNAS 2013