Supplementary Information for

Clusters of bacterial RNA polymerase are biomolecular condensates that assemble through liquid-liquid phase separation

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Supplementary Materials and Methods

**Bacterial strains.** Strains used in this study are listed in Table S1. New strains were constructed by P1 transduction (1) or lambda red recombination (2), as appropriate.

- WLBS100: P1 transduction from RRL265 into MG1655
- WLBS112: P1 transduction from JW3229 into WLBS100
- WLBS119: P1 transduction from JW0406 into WLBS100
- WLBS120: P1 transduction from JW0872 into WLBS100
- WLBS152: PCR from pROD93 using primers aml-15, 16; Lambda red into MG1655/pKD46
- WLBS168: PCR from pROD93 using primers aml-58, 59; Lambda red into MG1655/pKD46

| Strain  | Genotype                                      | Source                  |
|---------|-----------------------------------------------|-------------------------|
| MG1655  | F-, λ-, rph-1                                  | CGSC                    |
| SS6282  | JC13509; hupA-mCherry frt-kan, sulAp-gfp::ΔattB | (3)                     |
| JW3229  | AG1; pCA24N-Fis                                | NBRP; (4)               |
| JW3138  | AG1; pCA24N-NusA                                | NBRP; (4)               |
| JW3229  | BW25113; Δfis-779::kan                         | CGSC; (5)               |
| JW0872  | BW25113; Δlrp-787::kan                         | CGSC; (5)               |
| JW0406  | BW25113; ΔnusB-776::kan                        | CGSC; (5)               |
| RRL50   | AB1157; lacI-mCherry frt-cat::ΔleuB, [lacO240-Hyg]2268kb, [tetO240-Gm]852kb | This study              |
| RRL265  | AB1157; rpoC-mCherry frt-cat                   | This study              |
| WLBS100 | MG1655; rpoC-mCherry frt-cat                   | This study              |
| WLBS112 | MG1655; rpoC-mCherry, Δfis-779::frt-kan        | This study              |
| WLBS119 | MG1655; rpoC-mCherry cat, ΔnusB-776::frt-kan   | This study              |
| WLBS120 | MG1655; rpoC-mCherry cat, Δlrp-787::frt-kan    | This study              |
| WLBS152 | MG1655; rpoC-mMaple3 frt-kan                   | This study              |
| WLBS168 | MG1655; nusA-mMaple3 frt-kan                   | This study              |
| TB54    | MG1655; lacI-mMaple, [lacO240-hyg]2735kb::ApheA| (6)                     |
Table S2. Oligonucleotides used in this study.

| Primer | Sequence                                                                 | Purpose                                                                 |
|--------|--------------------------------------------------------------------------|------------------------------------------------------------------------|
| aml-15 | CCAGCCTGGCAGAAGTCTGGAGTGGCAGGGTGGCTGCTGCTGGTTCTGGCTGATAACGAGTCGGCTGGCTCCGCTGCTGGTTCTGG | Amplify mMaple3 from pRod93 and integrate downstream of rpoC          |
| aml-16 | CCCCCCTAAAAAAACCCGCCGAAGGCGTTTTTTACGTTATTGCGGATTTAATCCTCCTATTCC          |                                                                        |
| aml-58 | GCCAGCGTTTTAATCGTTACATCTGTTCATGCTTGGTCTTCCATCTCCAATCGTTACATCTGTTCATGCTTGGTCTTCC | Amplify mMaple3 from pRod93 and integrate downstream of nusA          |
| aml-59 | GCCAGCGTTTTAATCGTTACATCTGTTCATGCTTGGTCTTCCATCTCCAATCGTTACATCTGTTCATGCTTGGTCTTCC |                                                                        |

Table S3. Plasmids used in this study.

| Plasmid | Features                                                                 | Source |
|---------|--------------------------------------------------------------------------|--------|
| pKD46   | Lambda red genes                                                         | (2)    |
| pROD93  | mMaple Kan R6K gamma ori, for C-terminal insertions                      | (6)    |

Growth conditions. Liquid cultures were inoculated from a single colony and grown over night at 37°C in LB. Cultures were diluted into fresh LB to an OD600 of ~0.01 and grown for another 16 hrs. Cells were diluted again into fresh medium (LB, M9 or EZ), as indicated, for imaging. M9 minimal medium and EZ Rich Defined Medium (Teknova) were supplemented with 0.2% (w/v) glucose.

Time-course imaging of fixed cells. Samples were collected every 60 min; fixed in 4% formaldehyde with constant mixing for 30 min at 37°C; washed three times in M9 at room temperature; and stored at 4°C. Fixed cells were mounted on 1% M9-agarose pads and imaged on an inverted Leica DMI 6000B equipped with a 100X 1.46 NA objective lens, a spinning disk confocal head (Yokogawa CSU10) and an EM-CCD camera (Hamamatsu ImagEM). Five to ten fields of view were randomly acquired for each time point.

Image analysis was done using custom scripts in Matlab (Mathworks). Cells were segmented from bright field images and fluorescence intensity values of all pixels within a cell were extracted. Clustering was calculated as described in Maddox 2006 (7).

Protein purification. 6xHis-tagged GFP-Fis and GFP-NusA were expressed from pCA24N and purified using Ni-NTA resin (Qiagen) in a gravity-flow column at 4°C as described (Kitagawa 2005). Proteins were stored in 300 mM NaCl at -80°C and buffer-exchanged in spin columns (Amicon) just prior to condensation assays.

Single-molecule imaging in live cells. Cells were grown in fresh medium (EZ or M9) for 90 min before mounting on 1% agarose pads, made with the same media. Pads were assembled in Gene Frames (1.0 x 1.0 cm, Thermo Fisher) with clean cover slips. Briefly, cover slips were soaked in Versa-Clean (Fisher) overnight; washed in methanol and acetone; sonicated for 30 min; baked in
a plasma oven for 15 min; and flamed prior to mounting. Samples were maintained at 37°C throughout imaging.

Images were acquired on an inverted Olympus IX83 with a 100X 1.4 NA objective lens, an Orca-Flash 4.0 sCMOS camera (Hamamatsu) and an iChrome Multi-Laser Engine (Toptica Photonics). The target area was photobleached by continuous excitation at 561 nm and 65% laser power prior to acquisition. 5000 frames at 20 ms intervals were acquired on the bleached field of view with excitation at 561 nm and 25% laser power and continuous activation at 405 nm and 1% laser power for RpoC or 5% laser power for NusA and LacI.

To compare with RpoC-mCherry in fixed cells, images of non-converted RpoC-mMaple3 molecules were acquired for 500 ms with excitation at 488 nm and 15% laser power (Fig. S1A).

Single-molecule tracking and data analysis. Bright field images were used to segment cells prior to tracking. Fluorescent spots were detected by a Laplacian of Gaussian (LoG) method in TrackMate (8) and subsequently filtered based on intensity, using an estimated blob diameter of 4 pixels (520 nm) and a quality threshold of 5.0. Both the median filtering and subpixel localization options were selected. Spots were then linked into tracks using a simple nearest neighbour search with a max linking distance of 5 pixels (650 nm), a max gap-closing distance of 8 pixels (1040 nm) and a max gap of 1 frame (20 ms). Tracks shorter than 10 frames (86.9% of total) were discarded.

Maximum intensity projections were made across 1000 frames, generating 5 projections per movie. Putative clusters were identified in each projection using the LoG method in TrackMate. Individual tracks were assigned to putative clusters if their trajectories overlapped with the cluster area. A threshold of three tracks per cluster was set to remove putative clusters that were defined by a single track. Each molecule was then classified as “In” if its track was localized inside a cluster for its entire duration or “In/Out” if its track was inside a cluster for 50-99% of its duration. All other tracks were classified as “Out”. This classification scheme results in an accurate estimate of the mobility of “In” molecules, but at the expense of “Out” molecules. Since the “Out” class is the default, many slow-moving molecules are falsely assigned to this class, skewing its mean $D_{app}$ down (Fig. S4).

For each track, we calculated the time-averaged mean square displacement:

$$\text{MSD}(\tau) = \langle [r(t + \tau) - r(t)]^2 \rangle = 2dD_{app}\tau^\alpha$$

where $r(t)$ is the position at time $t$, $d$ is the dimension of the system, $D_{app}$ is the apparent diffusion coefficient and $\alpha$ is the anomalous scaling exponent. We extracted $D_{app}$ assuming that $\alpha = 1$ because tracks were too short ($16 \pm 7$ frames) to detect confined or subdiffusive motion reliably (Fig. S4A).
**Figure S1.** A) Fluorescence images of a fixed cell expressing RpoC-mCherry and a live cell expressing RpoC-mMaple3, both grown in EZ at 37°C for 120 min. B) Normalized pixel intensity histograms for fixed and live cells. C) Fluorescence images of fixed cells grown in LB with cell outlines (white) and segmented RNAP (vermillion) overlaid. The normalized intensity threshold used for segmentation increases from top to bottom. A normalized intensity threshold of 0.50 was chosen because this value best distinguishes between clusters and bulk nucleoid at 120 min. D) Quantification of clustering (percent of pixels below threshold) over time for different threshold values.
Figure S2. Predicted disorder (by IUPred) for A) NusA, B) Fis, C) Lrp and D) NusB. E) Clustering as a function of doubling time for WT cells grown in LB, M9, EZ at 37°C or LB at 30°C, compared to deletion mutants in LB at 37°C. Dashed line represents a linear fit to all WT conditions.
Figure S3. A) GFP-Fis (70 μM) condenses into small droplets at 500 mM NaCl, in the absence of dextran. B) GFP-NusA (14.5 μM) is soluble at 125 mM NaCl in the absence of dextran, but condenses into droplets when 10% dextran is added. C) GFP-NusA droplets (14.5 μM protein, 125 mM NaCl) dissolve in 10% 1,6-hexanediol.
Figure S4. A) (top) Raw time-averaged mean square displacement curves for single molecules of RpoC in cells grown in EZ at 37°C. (middle) Distribution of scaling exponent, $\alpha$, extracted from the slopes of MSD curves. (bottom) Distribution of single-molecule track lengths. Mean ± standard deviation for $\alpha$ and track length are given in the upper-right of respective plot. B) Distribution of $D_{app}$ for single molecules of LacI in cells grown in EZ at 37°C. (top) All tracks prior to classification. (bottom) Distribution for each class following classification: In (green), In/Out (red) and Out (blue). The “Out” class contains numerous slow-moving molecules that are likely misclassified “In” molecules. C) Results from SMAUG for RpoC and LacI single-molecule tracking data. D) Comparison between results from our classification scheme (SMT, described in main text) and a Gaussian mixture model (GMM).
Figure S5. Distribution of $D_{app}$ for LacI, plus each class of RpoC and NusA tracks, in cells grown in EZ, M9 and EZ + 5% 1,6-hexanediol (EZ + Hex).
SI References

1. Thomason LC, Costantino N, Court DL (2007) *E. coli* Genome Manipulation by P1 Transduction (John Wiley & Sons, Inc., Hoboken, NJ, USA), pp 1.17.1–1.17.8.

2. Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA* 97(12):6640–6645.

3. Marceau AH, et al. (2011) Structure of the SSB-DNA polymerase III interface and its role in DNA replication. *EMBO J* 30(20):4236–4247.

4. Kitagawa M, et al. (2005) Complete set of ORF clones of *Escherichia coli* ASKA library (a complete set of *E. coli* K-12 ORF archive): unique resources for biological research. *DNA Res* 12(5):291–299.

5. Baba T, et al. (2006) Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol Syst Biol* 2:2006.0008.

6. Beattie TR, et al. (2017) Frequent exchange of the DNA polymerase during bacterial chromosome replication. *eLife* 6:22635.

7. Maddox PS, Portier N, Desai A, Oegema K (2006) Molecular analysis of mitotic chromosome condensation using a quantitative time-resolved fluorescence microscopy assay. *Proc Natl Acad Sci USA* 103(41):15097–15102.

8. Tinevez J-Y, et al. (2017) TrackMate: An open and extensible platform for single-particle tracking. *Methods* 115:80–90.