Figures and figure supplements

Transcription factor clusters regulate genes in eukaryotic cells

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Figure 1. Single-molecule Slimfield microscopy of live cells reveals Mig1 clusters. (A) Dual-color fluorescence microscopy assay. Mig1-GFP localization change (cyan, right panels) depending on glucose availability. (B) Example Slimfield micrographs of change of Mig1-GFP localization (green) with glucose for three cells, nuclear Nrd1-mCherry indicated (red, left), mean and SEM error bounds of total cytoplasmic (yellow) and nuclear (blue) contributions shown (lower panel), n = 15 cells. Display scale fixed throughout each time course to show pool and foci fluorescence. (C) Example Slimfield micrographs of cells showing nuclear (left), trans-nuclear (center) and cytoplasmic (right) Mig1-GFP localization (green, distinct foci white arrows), Nrd1-mCherry (red) and segmented cell body (yellow) and nuclear envelope (blue) indicated. Display scales adjusted to only show foci. (D) Kernel density estimations (KDE) for Mig1-GFP content in pool and foci for cytoplasm and nucleus at glucose (+/-), n = 30 cells.

DOI: https://doi.org/10.7554/eLife.27451.002
Figure 1—figure supplement 1. Brightfield and fluorescence micrographs of key strains and glucose conditions. Representative Slimfield fluorescence images obtained from the strains and different extracellular glucose conditions used in this study. Brightfield non-fluorescence images, segmentation.
Figure 1—figure supplement 1 continued

perimeter indicated for cell body (orange) and nucleus (cyan), and fluorescence images are indicated, the latter showing both green and red channels obtained as the frame average from the first five consecutive Slimfield images. Fluorescence images are of the first illuminated frame and are all normalized by total pixel intensity. Snf1AS represents an ATP analog sensitive version of Snf1, Snf1-I132G. For the Mig1-mEos2 strain (inset, bottom right) this shows the brightfield image (left panel), a 300 consecutive frame average from the red channel after photoconversion (middle panel) and super-resolution false color heat map reconstruction, 40 nm lateral resolution, >2000 localizations (right panel).

DOI: https://doi.org/10.7554/eLife.27451.003
Fluorescent reporter strains have similar viability to wild type, with relatively fast maturation of fluorescent protein, and no evidence for GFP-mediated oligomerization. (A) (left panel) Mean doubling time ± SEM, number of cultures n = 6, (right panel) relative expression of Mig1 to constitutive ACT1 using qPCR in the wild type and Mig1-mGFP in cells pre-grown in elevated (4%) and depleted (0.2%) glucose, SD error bars, n = 3 repeats for each. (B) ‘Monomeric’ mGFP (red) vs Standard enhanced eGFP (blue) in vitro intensity KDE distributions. GFP/mCherry maturation. n = 1000 foci (C) After continuous illumination images were taken at subsequent time intervals. To prevent appearance of newly synthesized fluorescent proteins, 100 µg/ml cycloheximide was added 1 hr prior to photobleaching. Upper panels represent autofluorescence appearance in green and red channels in BY4741 wild type cells. Standard epifluorescence images (green/red) overlaid on brightfield (gray). (D) GFP and mCherry maturation in minimal YNB media with complete amino acid supplement and 4% glucose. The background-corrected total cellular fluorescence intensity for the wild type (autofluorescence) and Mig1-GFP:Nrd1-mCherry strain was quantified at each time point for each cell in ImageJ. Error bars indicate SEM. (E) and (F) In vivo Mig1-GFP vs Mig1-mGFP stoichiometry KDE distributions compared in glucose (+) and glucose (−) respectively (χ² = 0.28, p=1×10⁻¹³² and χ² = 0.011, p=3×10⁻²¹⁶). n = 30 cells.

DOI: https://doi.org/10.7554/eLife.27451.004

Figure 1—figure supplement 2. Fluorescent reporter strains have similar viability to wild type, with relatively fast maturation of fluorescent protein, and no evidence for GFP-mediated oligomerization. (A) (left panel) Mean doubling time ± SEM, number of cultures n = 6, (right panel) relative expression of Mig1 to constitutive ACT1 using qPCR in the wild type and Mig1-mGFP in cells pre-grown in elevated (4%) and depleted (0.2%) glucose, SD error bars, n = 3 repeats for each. (B) ‘Monomeric’ mGFP (red) vs Standard enhanced eGFP (blue) in vitro intensity KDE distributions. GFP/mCherry maturation. n = 1000 foci (C) After continuous illumination images were taken at subsequent time intervals. To prevent appearance of newly synthesized fluorescent proteins, 100 µg/ml cycloheximide was added 1 hr prior to photobleaching. Upper panels represent autofluorescence appearance in green and red channels in BY4741 wild type cells. Standard epifluorescence images (green/red) overlaid on brightfield (gray). (D) GFP and mCherry maturation in minimal YNB media with complete amino acid supplement and 4% glucose. The background-corrected total cellular fluorescence intensity for the wild type (autofluorescence) and Mig1-GFP:Nrd1-mCherry strain was quantified at each time point for each cell in ImageJ. Error bars indicate SEM. (E) and (F) In vivo Mig1-GFP vs Mig1-mGFP stoichiometry KDE distributions compared in glucose (+) and glucose (−) respectively (χ² = 0.28, p=1×10⁻¹³² and χ² = 0.011, p=3×10⁻²¹⁶). n = 30 cells.

DOI: https://doi.org/10.7554/eLife.27451.004
Figure 1—figure supplement 3. In vivo Mig1-GFP foci intensity traces as a function of time. Showing the raw tracked intensity (blue line) and filtered (red squares) using an edge preserving Chung-Kennedy filter (Leake et al., 2004; Leake et al., 2003) (15 ms window) for (A) nuclear foci (B) cytoplasmic foci and (C) overtracked foci from > 0.5 s into the photobleach with steps at single GFP intensity. (D) The intensity distribution of Mig1-GFP in vivo at glucose (+/-) using kernel density estimate. Mode values are identical within error: 5200 ± 1500, 4600 ± 700 respectively.

DOI: https://doi.org/10.7554/eLife.27451.005
Figure 2. Mig1 foci stoichiometry, mobility and localization depend on glucose. Heat map showing dependence of stoichiometry of detected GFP-labeled Mig1 foci with D under (A) glucose (+) and (B) glucose (−) extracellular conditions. Mean values for glucose (+) nuclear and glucose (−) cytoplasmic foci indicated (arrows). n = 30 cells. Heat maps generated using 1000 square pixel grid and 15 pixel width Gaussians at each foci, using variable color scales specified by colorbar on the right.

DOI: https://doi.org/10.7554/eLife.27451.011
Figure 2—figure supplement 1. Mig1 phosphorylation does not affect clustering but regulates localization. Heat maps showing dependence of stoichiometry of detected GFP-labeled Mig1 foci with D in (A,B) SNF1 deletion strain, (C) strain with ATP analog sensitive variant of Snf1, Snf1-I132G, in presence of 1NM-PP1, and (D, E) strain with four serine phosphorylation sites of Mig1 mutated to alanine.
DOI: https://doi.org/10.7554/eLife.27451.012
Figure 2—figure supplement 2. Wild type Snf1 and analog sensitive have similar effect on Mig1. (A) Representative Slimfield fluorescence images showing expected Mig1 localization for a strain carrying an analog sensitive Snf1 and upon treatment with DMSO. (B,C) Heat maps showing dependence of stoichiometry of detected GFP-labeled Mig1 foci with microscopic diffusion coefficient $D$ in analog sensitive Snf1.

DOI: https://doi.org/10.7554/eLife.27451.013
Figure 2—figure supplement 3. Boxplot summary of wild type and mutant Mig1 stoichiometry and microscopic diffusion coefficient. Boxplots for stoichiometry (top) and diffusion coefficient (bottom). Median in red, quartiles in blue. Black dotted lines mark the extrema, limited to 4x and 2x upper quartile value for stoichiometry and microscopic diffusion coefficient $D$ respectively with outliers marked with red crosses.

DOI: https://doi.org/10.7554/eLife.27451.014
Figure 3. Repressor clusters have heterogeneous mobility depending on localization. (A) Cumulative probability, glucose (+) nuclear tracks (blue) and two component exponential fit (red), with dual Gamma fit to D (inset) with similar parameters. (B) Mean MSD vs τ (i.e. time interval tau) from cytoplasmic (yellow), small (blue, stoichiometry ≤ 20 Mig1-GFP molecules) and large nuclear (purple, stoichiometry > 20 Mig1-GFP molecules) foci, SEM indicated, on log-log axes, n = 30 cells for glucose (+) and (−). Anomalous diffusion model fits to time intervals ≤ 30 ms (dashed black line), anomalous coefficient α = 0.4–0.8. (C) Heat map of trans-nuclear track localizations normalized to crossover point, generated using 1000 square pixel grid and 10 pixel width Gaussians at each localization (D) distance parallel (left) and perpendicular (right) to nuclear envelope with time, normalized to crossover point for Mig1-GFP foci entering (blue) and leaving the nucleus (red), (E) dwell times at nuclear envelope and single exponential fits (dotted). (F) Example glucose (+) single cell FRAP Slimfield images, fixed display scale (G) mean and SEM nuclear intensity after bleaching, n = 5 and 7 cells for glucose (+)/−, respectively.

DOI: https://doi.org/10.7554/eLife.27451.016
Figure 3—figure supplement 1. Cumulative probability distance analysis reveals a single mobile population in the cytoplasm at glucose (+/-) and in the nucleus and glucose (−/−). Cumulative density functions of first displacement in trajectories (blue) with appropriate fits (red). Bottom right panel indicates Mig1 mutant for which the Zn finger domain has been deleted.

DOI: https://doi.org/10.7554/eLife.27451.017
Figure 4. Mig1 clusters are stabilized by depletion forces and bind to promoter targets. (A) Zoom-in on pairwise difference distribution for stoichiometry of Mig1-GFP foci, 7-mer intervals (dashed) and power spectrum (inset), mean and Gaussian sigma error (arrow). (B) Stoichiometry for Mig1-GFP clusters in vitro in PEG absence (blue)/presence (red). n = 1000 foci. Inset shows the full range while outer zooms in on cluster stoichiometry. (C) 3C model of chromosomal DNA (blue shaded differently for each chromosome) with overlaid Mig1 promoter binding sites from bioinformatics (red), simulated image based on model with realistic signal and noise added (inset). (D) Cluster (red) and monomer (dark blue) model (goodness-of-fit $R^2 < 0$) for Mig1-GFP stoichiometry (10 replicates) compared against experimental data (cyan, $R^2 = 0.75$). DOI: https://doi.org/10.7554/eLife.27451.018
Figure 4—figure supplement 1. Additional Mig1 cluster investigations. (A) Zoom-in on pairwise difference distribution for stoichiometry of GFP-labeled Mig1 foci detected during FRAP, -8 mer intervals (dashed lines) and power spectrum (inset) shown, mean and Gaussian sigma error (arrow). (B) GFP-labeled Mig1 cluster size as a function of stoichiometry with power law fit indicated. (C) Immuno-gold transmission electron microscopy for negatively stained 90 nm cryosections of (upper panel) two different Mig1-GFP cells and (lower panel) two different Msn2-GFP cells, with zoom in (inset).
DOI: https://doi.org/10.7554/eLife.27451.019
**Figure 4—figure supplement 2.** In vitro cluster characterization. (A) Native PAGE of total cell protein extracts obtained from cells grown in 4% glucose (+) and 0.05% glucose (−) conditions followed by western blotting and probing with anti-GFP antibodies. (B) Coomassie staining of purified Mig1-mGFP fraction indicates a single band that corresponds to the size of a Mig1-GFP monomer (molecular weight 83.4 kDa). (C) Mig1 phosphorylation status is detected by SDS-PAGE on total cell protein extracts obtained from cells grown in different glucose conditions followed by western blotting. De/phosphorylation of Mig1 in glucose (+/-) respectively is not affected by the SNF1-I132G mutation. (D) Slimfield images of Mig1-GFP clusters in vitro in the absence (left) and presence of PEG (right), display intensity scaled in units of GFP per 5 pixel circular region of interest (ROI). (E) Distribution of stoichiometry for mGFP clusters in vitro in absence (blue)/presence (red) of PEG and the expected distribution of overlapping mGFP monomers (yellow).

DOI: https://doi.org/10.7554/eLife.27451.020
Figure 4—figure supplement 3. Additional 3C modelling. (A) 3C model (blue) with overlaid bound Mig1 clusters to promoter binding sites from bioinformatics (red), and Mig1 clusters near the NE (green); (B) predicted stoichiometry distributions for GFP-labeled Mig1 foci in the nucleus at elevated extracellular glucose for a range of different binding models, including: a model which simulates both nuclear envelope (NE) translocating clusters and cluster binding to promoter targets (yellow), a model which simulates both nuclear envelope (NE) translocating monomers and monomer binding to promoter targets and DNA (blue), and a model which simulates just cluster binding to promoter targets but excludes any effects from translocating clusters (purple). These models are optimized to the experimentally determined stoichiometry distribution (cyan); (C) predicted Mig1 monomer stoichiometry distributions for Mig1 bound to promoter sites in three different orientations ~10° apart.

DOI: https://doi.org/10.7554/eLife.27451.021
Figure 5. Msn2 and Mig1 forms functional clusters colocalized to transcribed mRNA from their target genes. (A) Kernel density estimations for Msn2-GFP in pool and foci for cytoplasm and nucleus at glucose (+/−). (B) Heat maps showing dependence of stoichiometry and D of detected Msn2-GFP.
foci, n = 30 cells. (C) Slimfield imaging on the same cell in which microfluidics is used to switch from glucose (+) to glucose (−) indicating the emergence of PP7-GFP foci at glucose (−) which are coincident with Mig1-mCherry foci at glucose (+), dependent on the Mig1 Zn finger (same intensity display scales throughout). These Mig1 and PP7 foci have a high level of colocalization as seen from (D) the distribution of the numerical overlap integral between foci in red and green channels at glucose (+) and glucose (−) respectively, peaking at ~0.95. n = 21 cells. (E) Two example cells showing at glucose (−) Msn2-mKO2 foci colocalize with PP7-GFP foci. PP7-2xGFP and Msn2-mKO2 images are frame averages of ~1000 frames, Mig1-mCherry is a Slimfield image.

DOI: https://doi.org/10.7554/eLife.27451.023
Figure 6. Mig1 and Msn2 contain disordered sequences which mediate cluster formation. (A) Structural prediction for Mig1; Zn finger motif (cyan), disordered sections (red) from PyMOL, beta sheet (gray), phosphorylation sites (yellow); zoom-in indicates structure of conserved Zn finger from PSI-BLAST to PDB ID: 4R2E (Wilms tumor protein, WT1). (B) DISOPRED prediction for Mig1 and Msn2; disordered regions (red), Zn finger regions (cyan). (C) Circular dichroism of GFP labeled Mig1 in PEG absence (blue)/presence (orange). (D) Distribution of nearest neighbor distances for Mig1 sites within promoters on same (blue) or different (red) chromosome. (E) Schematic of depletion-stabilized Mig1 cluster bound to multiple promoter targets (Zn finger PDB ID: 4R2E). (F) Amino acid residue electrostatic charge plots for Mig1 and Msn2 from EMBOSS (Rice et al., 2000). Residues ‘D’ and ‘E’ are Figure 6 continued on next page.
assigned a charge of $-1$, 'K' and 'R' a charge of $+1$, and the residue 'H' is assigned a charge of $+0.5$, then a rolling 75 amino acid residue window is used. Figures and Tables.

DOI: https://doi.org/10.7554/eLife.27451.024