SUPPLEMENTAL FIGURES

Figure S1. Localization and Pol II occupancy of myogenic genes in C2C12 cells. (A) Representative images of DNA Immuno-FISH assay of *MyoD* gene locus in myoblasts. (B) Representative images of DNA Immuno-FISH assay of *Myogenin* Gene locus in myoblasts. (Red) FISH signal. (Green) anti-LaminB. (C) Chromatin immunoprecipitation of RNA Pol II in MyoD and Myogenin promoters in myoblasts and myotubes. An antibody against Ser5-phosphorylated Pol II was used.
Figure S2. Representative images and radial intensity plots of immunostaining C2C12 myoblasts. (A) Pol II (4H8). (B) Pol II (Ser5P). (C) TBP. (D) TAF4. (E) TAF11. Left: Representative images. (green) Antibodies, (red) Anti-LaminB, (blue) Hoechst33342. Right: Corresponding radial intensity plots of nuclear lamin signals (red), Hoechst 33342 staining (blue) and antibody stain signals (black).
**Figure S3. Immunostaining with anti-TAF3 and anti-TRF3 antibodies in C2C12 myoblasts.** (A) Anti-TAF3 immunofluorescence on C2C12 cells untreated, blocked with TAF3 antigen or transfected with TAF3 shRNA. (B) Knockdown of ectopically expressed mouse TAF3 and TRF3 protein in HEK293 cells by shRNAs assayed by western blot. (C) Representative images and intensity plots of anti-TAF3 immunostaining. (D) Anti-TRF3 immunofluorescence on cells untreated or blocked with TRF3 antigen. (E) Anti-TRF3 immunofluorescence on cells untreated or transfected with TRF3 shRNA or scrambled shRNA. (F) Representative images and intensity plots of anti-TRF3 immunostaining.
Figure S4. Distributions of H3K4Me3 and H3K9Me3 in myoblasts and myotubes. (Green) Corresponding antibodies, (Red) Anti-LaminB, (Blue) Hoechst33342. Images and intensity line profiles are shown.
**Figure S5. Imaging transcription factors in living C2C12 myoblasts.** (A) Live cell images of tagged transcription factors (green) and coexpressed LaminA tagged with a second color as a nuclear periphery marker (red) in C2C12 myoblasts: (i) GFP-Rpb9, (ii) GFP-TAF11, (iii) GFP-hTAF1, (iv) GFP-TAF3. Cells in i—iv are coexpressed with mCherry-LaminA. (v) mEos2-TAF3 and PSCFP2-Lamin A. All scale bars are 5 µm. (B) More representative images to compare localizations of TAF3 WT versus TAF3 M882A and D877A mutants in living cells. TAF3 are labeled green and Lamin A are labeled red.
**Figure S6. Localizing genes, chromatin mark and transcription components in primary myoblasts.** (A) DNA FISH of *MyoD* gene (red), (B) DNA FISH of *Myogenin* gene (red), Lamin B as green. (C, D) RNA Pol II (4H8), (E, F) RNA Pol II (Ser5P), (G, H) H3K9Me3. (I, J) TAF3. (K, L, M) are the respective intensity plots for Pol II (4H8), H3K9me3 and TAF3 immunostaining in primary cells (n = 5, 3, 4, respectively). (N) Comparing intensity plots of TAF3 signals in C2C12 myoblasts (Figure 2) and in primary myoblasts. One way ANOVA test of intensity values: P = 0.93. All scale bars are 5 µm.
Figure S7. Immunofluorescence of Pol II and H3K4Me3 upon inhibition of transcription activity. C2C12 myoblasts were immunostained with Pol II (4H8) and H3K4Me3 antibodies without and with treatment with α-amanitin (50 µg/ml) overnight.
Figure S8. Tethering MyoD promoter to the nuclear lamina. Red fluorescence signals are from LacI-mCherry or LacI-mCherry-Lamin B1 (Kumaran and Spector, J. Cell Biol. 2008). Transgene loci are labeled with a strong mCherry signal and indicated by the white arrows. Green fluorescence signals indicate the expression of EGFP protein driven by MyoD promoter.
SUPPLEMENTAL RESULTS

Our preliminary attempts to tether a MyoD promoter transgene to the NL suggest that tethering may lead to a decrease in the expression of a GFP reporter driven by the MyoD promoter (Figure S8). A close examination also suggests that artificially tethered MyoD promoters, often seen as a diffraction-limited loci, are constrained within a diffraction-limited radial distance from the NL (Figure S8), while native MyoD genes detected by FISH are often resolved from the NL through diffraction-limited confocal microscopy (Figure 1A, B, Figure S1). These intriguing findings suggest that a finer dissection of the spatial organization of the nuclear periphery will be necessary to further unravel the relationships between gene positioning and gene activation.

SUPPLEMENTAL MATERIALS AND METHODS

Fluorescence In Situ Hybridization (FISH)

Mouse C2C12 myoblasts and myotubes were fixed with 4% paraformaldehyde in 1X PBS, and permeabilized with 1X PBS + 0.5% Triton X-100. Genomic DNA was denatured by placing the slide in an 80 °C dry bath for 5 minutes. Bacterial Artificial Chromosomes containing MyoD or Myogenin gene loci were used as probes for DNA FISH. Expression vectors containing MyoD or Myogenin cDNAs were used as probes for RNA FISH and an empty vector was used as the control. Hybridization was performed in a humidified chamber overnight 37 °C in the dark. Slides were washed with pre-hybridization buffer (2X SSC, 50% Formamide) before and after hybridization at 37 °C for 1 hour. Slides were then washed three times with PBST (1X PBS + 0.05% Tween-20), blocked with 1% BSA in PBST for 1 hour, and then treated with antibodies for detection in the following order: anti-Lamin B (Santa Cruz) at 1:200 dilution for 1 hour at room temperature (RT), anti-digoxigenin-Rhodamine
(Roche) at 1:50 dilution for 1 hour at RT, donkey-anti-goat-Alexa488 (Invitrogen) at 1:500 dilution for 1 hour at RT. Slides were washed three times between each antibody incubation with PBST (1X PBS + 0.05% Tween-20). Slides were mounted with Vectashield (Vectorlabs) and visualized with a LSM 510 Confocal Laser Scanning Microscope. Confocal laser scanning images were shown and thus 1 or 2 FISH signals appear per nuclear optical section.

**Immunofluorescence Staining**

C2C12 myoblasts and myotubes were fixed, permeabilized and washed as described in FISH, and incubated with primary antibodies overnight at 4 °C. After washing with PBST, secondary antibodies were applied to the slide and incubated for 1 hour at RT. Slides were stained with Hoechst33342 (0.5 µg/ml, Invitrogen) and mounted with VectaShield (Vectorlabs) and visualized with a LSM 510 confocal laser scanning microscope (Zeiss).

**RNA Pol II Inhibition and Generation of Stable Cell Lines**

Inhibition of RNA Pol II transcription was performed by incubating with 50 µg/ml α-amanitin diluted in growth media for overnight. Labeling of nascent transcripts with Bromo-UTP incorporation was performed as previously described (Jackson et al. 1993). Bromo-UTP was incorporated for 20 minutes. For generating the MyoD promoter transgene, MyoD::3xEGFP vector was linearized by digestion with ApaLI, purified and co-transfected with pSwitch vector (Invitrogen) which contains a Hygromycin resistance marker) into C2C12 myoblasts with Lipofectamine 2000 (Invitrogen). Stably-transfected cells were selected for 2 weeks with 1 mg/ml hygromycin. GFP expression was verified through a fluorescence microscope. The stable cell line containing MyoD::3xEGFP were amplified in presence of
Hygromycin, grown to ~10^8 cells and were fixed and collected for ChIP, and were also processed for DNA FISH with EGFP sequences as the hybridization probe.

**DNA Constructs**

Mouse TAF3, TAF11, Rpb9 cDNAs were cloned into the mEos2-C1 vector (McKinney et al. 2009). Mouse Lamin A cDNA was cloned into the PSCFP2-C vector (Evrogen). Mouse TAF3 and human TAF1 cDNAs were cloned into the pAcGFP-C1 vector (Clontech). TAF3 deletion mutants and point mutants were generated through PCR and site-directed mutagenesis, respectively. TAF3 (BHC80PHD) was kindly provided by Dr. H. Timmers, TAF3 (SMCXPHD1) was generated by cloning PHD1 from SMCX cDNA in frame fusion to TAF3ΔPHD. BAF, TAF3, and BAF-TAF3 cDNA was first cloned into pCMV10-3xFlag vector and then PCR cloned into in pAcGFP-C1 vector downstream of the GFP cassette. MyoD promoter (2.5kb) (Hu et al. 2008) was inserted in front of the 3xEGFP cassette of the pEGFP-13 vector (kindly provided by Dr. B. Glick). The 64x LacO operator sequence was inserted in front of the MyoD promoter sequence at the MyoD::3xEGFP vector through blunt-end ligation. LacI-mCherry and LacI-mCherry-Lamin B1 vectors were kindly provided by Drs. R. Kumaran and D. Spector (Kumaran and Spector 2008).

**Tethering MyoD Promoter to the Nuclear Lamina**

The 64xLacO-MyoD::3xEGFP vector was linearized by digestion with ApaLI, purified and co-transfected with pSwitch vector (Invitrogen) which contains a Hygromycin resistance marker) into C2C12 myoblasts with Lipofectamine 2000 (Invitrogen). Stably-transfected cells were selected for 2 weeks with 1 mg/ml hygromycin. GFP expression was verified through a fluorescence microscope. CMV-LacI-mCherry and CMV-LacI-mCherry-LaminB1 were transfected into the stable cell line with
64xLacO-MyoD::3xEGFP transgene. Two days later, cells were screened to visualize transgene loci with and without tethering, and at GFP channel to examine GFP expression.

Analysis of Immunofluorescence Staining Images
A custom-written Matlab (Mathworks, Natick, MA) script was used to measure the radial intensities. Briefly, measurement lines were drawn approximately orthogonal to the nuclear lamina and the fluorescent intensity profiles along these lines were measured in three color channels. Then the measurement lines were progressively moved along the entire contour of the lamina within the image to record about 200 line profiles in three color channels. The positions of the peak intensity in the Lamin B signal in each line profile were recorded to align the 200 line profile vectors in three channels. Then the aligned 200 line profile vectors were summed in three color channels, normalized to the peak signal in each channel, and plotted versus the distance to the lamina (in μm).

Analysis of PALM Images
Briefly, a series of 10 rectangles of 200 nm thickness were defined and progressed from the nuclear periphery into the nuclear interior. The molecule library generated through PALM analysis was searched for molecules located within each rectangle and the rectangle with the highest Lamin A molecule counts was identified as the location of the nuclear periphery. 20 of the above described rectangle series were generated to encircle the nuclear lamina of the PALM image. Molecule counts from the 20 rectangle series were summed with respect to the nuclear periphery to generate the molecule counting histograms, and were divided by the total molecule counts in this analysis to generate the percentage of molecular counts for comparing different proteins.
REFERENCE


