Published in final edited form as:

Biochem Biophys Res Commun. 2011 May 6; 408(2): 230–235. doi:10.1016/j.bbrc.2011.03.131.

Osmotic stress alters chromatin condensation and nucleocytoplasmic transport

John D. Finan, Holly A. Leddy, and Farshid Guilak

Departments of Orthopaedic Surgery and Biomedical Engineering Duke University Medical Center, Durham, NC

Abstract

Osmotic stress is a potent regulator of biological function in many cell types, but its mechanism of action is only partially understood. In this study, we examined whether changes in extracellular osmolality can alter chromatin condensation and the rate of nucleocytoplasmic transport, as potential mechanisms by which osmotic stress can act. Transport of 10 kDa dextran was measured both within and between the nucleus and the cytoplasm using two different photobleaching methods. A mathematical model was developed to describe fluorescence recovery via nucleocytoplasmic transport. As osmolality increased, the diffusion coefficient of dextran decreased in the cytoplasm, but not the nucleus. Hyper-osmotic stress decreased nuclear size and increased nuclear lacunarity, indicating that while the nucleus was getting smaller, the pores and channels interdigitating the chromatin had expanded. The rate of nucleocytoplasmic transport was increased under hyper-osmotic stress but was insensitive to hypo-osmotic stress, consistent with the nonlinear osmotic properties of the nucleus. The mechanism of this osmotic sensitivity appears to be a change in the size and geometry of the nucleus, resulting in a shorter effective diffusion distance for the nucleus. These results may explain physical mechanisms by which osmotic stress can influence intracellular signaling pathways that rely on nucleocytoplasmic transport.

Keywords

chondrocyte; cartilage; cell mechanics; chromatin; diffusion; photobleaching

Introduction

Osmotic stress exerts a potent influence on cell physiology. While most cells are maintained in a relatively constant osmotic environment, a number of cell types, such as epithelial cells in the bronchial tubes [1], urinary tract [2] and intestines [3] are exposed to a dynamic osmotic environment and possess complex osmoregulatory mechanisms that are essential for normal function. Osmotic stress changes cell volume, disrupts the actin cytoskeleton and activates second messenger pathways, including calcium signaling [4] and inositol phosphate signaling [5]. On a longer time scale, most cells attempt to reverse these changes by manufacturing or expelling neutral osmolytes such as taurine to maintain osmotic equilibrium while restoring normal ion concentrations [6].

Osmotic stresses also arise in the body as a result of electromechanical coupling phenomena [7]. The extracellular matrix in articular cartilage and intervertebral disc contains a high concentration of proteoglycans [8]. The fixed negative charges on these molecules draw ions

into the tissue. Compression of the tissue results in exudation of the water [9] but not ions, creating a hyper-osmotic stress in the cell environment [7] that induces cell and nuclear deformation [10,11]. Mounting evidence suggests that this osmotic environment serves as a regulator of chondrocyte physiology, influencing signaling pathways [12], gene expression [13] and protein synthesis [14].

The mechanisms by which osmotic stress affects biological activity are only partially understood and may include membrane-based as well as intracellular signaling pathways [12]. Osmotic stress induces calcium signaling in a variety of cell types, which may be mediated by the cation channel TRPV4 [15]. An alternative mechanism by which extracellular stresses may influence cell physiology is through a direct physical effect on the nucleus, altering nuclear shape and size [16,17,18,19,20,21,22]. Under hyper-osmotic stress, the nucleus shrinks and assumes a more convoluted shape, and this geometric change may have functional consequences for transcription, translation, or nucleocytoplasmic transport.

While it is clear that osmolality alters cell and nuclear structure and morphology [16], the effects of these changes on the transport properties of the cell and nucleus are unknown. In this regard, the osmotic sensitivity of nucleocytoplasmic transport is potentially a novel means by which osmotic stress can modulate gene expression. The goal of this study was to measure transport within and between the nucleus and the cytoplasm at a range of osmolalities to examine the hypothesis that osmotic stress can alter the rate of nucleocytoplasmic transport. Isolated articular chondrocytes were exposed to a range of osmolalities, and the diffusion properties within and between the cytoplasm and nucleus were measured using fluorescent photobleaching techniques.

Materials and Methods

Cell Isolation and Culture

All experiments were performed on articular chondrocytes isolated from knees of skeletally mature pigs [23]. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1.5% HEPES, 10% fetal bovine serum and 1% non-essential amino acids (Gibco, Grand Island, NY). The media was adjusted to pH 7.4 and the osmolality was adjusted to 380 mOsm by addition of sucrose, to mimic physiologic osmolality in cartilage [24]. Cells were cultured overnight before testing.

Fluorescent labeling of cells

Fluorescein-labeled 10 kDa dextrans (Invitrogen, Carlsbad, CA), which are small enough to cross the nuclear envelope by passive diffusion, were loaded into the cells by electroporation using a custom-built electroporation apparatus [25]. Cells were incubated for 10 minutes and then nuclei were labeled with 1 μ M Syto 82 (Invitrogen) for 10 minutes at 37 °C.

SCAMP experiments

The diffusion coefficient of 10kDa dextran was measured within both the cytoplasm and the nucleus using scanning microphotolysis (SCAMP). Cells were mounted in a perfusion chamber on the stage of a confocal laser scanning microscope (LSM 510, Zeiss) and equilibrated to osmolalities ranging from 255 mOsm to 657 mOsm. Media osmolality was adjusted by addition of NaCl or deionized water. The nuclear images were used to position the scanning region of interest (ROI) close to the center of the nucleus for nuclear experiments and outside the nucleus for cytoplasmic experiments. The ROI consisted of a line 10 pixels (0.12 μ m/pixel). This line was scanned continuously 45 times at a rate of 1 scan every 767 μ s at high laser power. Each experiment was performed twice to eliminate the effect of bound, immobile fluorophore in the ROI. The change in intensity of the ROI

was simulated using a computational model to determine the diffusion coefficient [26]. In instances where the R^2 statistic of the fit was less than 0.9, the determined coefficients were rejected as unreliable.

FRAP experiments

Transport between the nuclear and cytoplasmic compartments was investigated using fluorescent recovery after photobleaching (FRAP) [27]. A model for FRAP as it applies to the particular case of nucleocytoplasmic transport is presented in the next section.

Cultured cells were prepared as described above for SCAMP experiments. Experimental media were prepared at 280 mOsm (hypo-osmotic), 380 mOsm (iso-osmotic) and 480 mOsm (hyperosmotic). Osmotic stress was applied as for SCAMP experiments. The ROI used to bleach the nucleus was a circle 2.8 μm in diameter located at the center of the nucleus (Figure 1). The ROI used to measure recovery of fluorescence was the outline of the nucleus.

Mathematical Modeling of FRAP Experiments

This mathematical model relates fluorescent bleaching data to nucleocytoplasmic transport parameters [28]. The model consists of two compartments representing the nucleus and the cytoplasm that are separated by a permeable membrane (Figure 1F). At time $t=0$ (the instant after the bleaching pulse), the concentration of fluorescent dextran in the nucleus is zero. The concentration in the cytoplasm is equal to a finite value C_0 . In practice, the bleaching pulse does not eliminate all fluorescence in the nucleus and C_0 is equal to the difference between the fluorescent intensity in the nucleus and the fluorescent intensity in the cytoplasm immediately after the bleaching pulse. The volume of each compartment is assumed to remain constant throughout the experiment. It is assumed that both compartments are sufficiently well mixed at all times that spatial gradients of concentration can be neglected. The validity of this assumption was assessed based on experimental results.

The governing equation is a mass balance stating that the flux of dextran across the membrane is equal to the flux of dextran entering the nucleus. This leads to a first order differential equation in two variables,

$$V_n \frac{dC_n}{dt} = PS(C_c - C_n) \quad (1)$$

where V_n is the volume of the nucleus, C_n is the concentration in the nucleus, P is the permeability of the nuclear envelope, S is the surface area of the nuclear envelope and C_c is the concentration in the cytoplasm. A second mass balance states that the flux of dextran into the nucleus equals the flux of dextran out of the cytoplasm. Applying the initial conditions and substituting into Eq. 1 leads to a first order differential equation in one variable,

$$\frac{dC_n}{dt} = \frac{PS}{V_n} [C_0 - QC_n - C_n] \quad (2)$$

where Q is the ratio of nuclear volume to cytoplasmic volume, V_n/V_c . Solution of this equation yields the following expression

$$C_n(t) = \frac{C_0}{Q+1} [1 - e^{-kt}] \quad \text{where} \quad k = \frac{PS(Q+1)}{V_n} \quad (3)$$

A similar model exists in the literature that assumes infinite cytoplasmic volume [29]. Equation 3 approaches the analogous expression in that model as V_c approaches infinity. Eq. 3 has a single rate constant k that describes the transport between the two compartments. However, as in other similar studies [29], the experimental data showed two rate constants: a fast rate constant associated with transport of dextrans within the cytoplasm and a slow time constant associated with transport between the cytoplasm and the nucleus (Figure 1). To account for this phenomenon, Eq. 3 was extended as follows

$$C_n(t) = A[1 - e^{-kt}] + B[1 - e^{-ct}] \quad (4)$$

Where $A = \frac{C_0}{Q+1}$, k is as defined in Eq. 3 and B and c are constants describing intracytoplasmic transport. Eq. 4 was fit for A , B , c and k against experimental data.

Cells were culled from the study when Q was less than 1 (this led to the exclusion of 2 cells out of 70).

The volume ratio was estimated from two-dimensional cross sectional images acquired during the experiment using the following expression:

$$\frac{V_n}{V_c} = \left(\frac{A_n}{A_c} \right)^{\frac{3}{2}} \quad (5)$$

where A_n is the cross sectional area of the nucleus and A_c is the cross sectional area of the cell. Since chondrocytes maintain a rounded morphology in short-term culture, this equation represents the assumption that the area ratio of the nucleus and the cytoplasm in the imaging plane is not different, on average, from the same ratio out of plane. Three-dimensional imaging of experimental cells to determine the surface area to volume ratio of the nucleus was impractical due to photobleaching. Instead, a separate set of high resolution, three dimensional images of chondrocyte nuclei under similar conditions was generated and used to develop an empirical expression relating the surface to volume ratio to the cross sectional area to perimeter length ratio, yielding the following expression:

$$\frac{S}{V} = 0.4059 \frac{L^2}{A^{\frac{3}{2}}} \quad (6)$$

This expression was validated against three-dimensional images separate from those used to develop it.

The rate of nucleocytoplasmic transport, k , was determined by fitting the fluorescence time history of the nucleus from the FRAP experiments to Eq. 4 using a trust-region-reflective least squares algorithm in MATLAB (The Mathworks, Natick, MA). The surface to volume ratio was computed from cross sections of the nucleus as described above and the permeability of the nuclear envelope was determined from the second half of Eq. 4.

Lacunarity Measurements

Lacunarity is a measure of the texture of an image, representative of the distribution of voids in an image [30]. In this study, lacunarity of nuclei at different osmolalities was measured to quantify chromatin organization. As chromatin condenses under hyper-osmotic stress, it shifts from a homogeneous distribution to an uneven distribution punctuated by dark, chromatin-free pores [31] (Figure 3).

Experimental solutions were prepared at 380 (iso-osmotic) and 580 (hyper-osmotic) mOsm as described previously. Chondrocytes were incubated in 5 μ g/ml solution of Hoechst 33342 (Sigma Aldrich, St. Louis, MO) for at least 20 minutes before testing. The cells were imaged in three dimensions with a pixel size of 48.1 nm and a slice thickness of 130 nm. Osmotic stress was applied and three-dimensional imaging was repeated.

Lacunarity was measured using the gliding box algorithm [32]. This algorithm involves comparing the local average intensity within a box of a defined size to the overall average intensity of the image and then averaging this difference for every possible location of the box within the image. The lacunarity yields an estimate of feature size relative to the size of the box used to probe the image. The images were deconvolved (SVI Huygens 3D Deconvolution software, Scientific Volume Imaging B. V., Hilversum, the Netherlands) and then the lacunarity of the deconvolved image was calculated in three dimensions in a Matlab code [33] that had been modified to operate on irregularly shaped three dimensional images with a box of dimension $0.43 \times 0.43 \times 0.38 \mu\text{m}$. Images that were partially saturated were excluded from the analysis.

Results

There was a statistically significant inverse correlation between extracellular osmolality and the diffusivity of 10kDa dextran in the cytoplasm (Figure 2A). The mean diffusion coefficient in the cytoplasm was $13.41 \mu\text{m}^2\text{s}^{-1}$ at 380 mOsm and decreased by $2.58 \mu\text{m}^2\text{s}^{-1}$ for every 100mOsm increase in osmolality. The mean diffusivity in the nucleus was $13.67 \mu\text{m}^2\text{s}^{-1}$ and was not correlated with osmolality (Figure 2B). The lacunarity of the nucleus significantly increased under hyper-osmotic stress, indicating that the pores and channels interdigitating the chromatin within the nucleus expanded (Figure 3).

The model of nucleocytoplasmic transport provided excellent fits to the experimental data, yielding a mean R^2 value of 0.998 and the fast-mixing assumption was upheld according to the following criterion [34]:

$$D \geq 10rP \quad (6)$$

where D is the diffusion coefficient in the nucleus, r is the radius of the nucleus (mean $r = 3.09 \mu\text{m}$), and P is the permeability of the nuclear envelope (mean $P = 13.67 \mu\text{m}^2/\text{s}$).

The rate constant for nucleocytoplasmic transport of 10 kDa dextrans increased significantly under hyper-osmotic stress but was not significantly affected by hypo-osmotic stress (Figure 4A). The surface to volume ratio of the nucleus fell as the osmolality increased (Figure 4B). According to Eq. 3, the permeability of the nuclear envelope and the volume of the nucleus relative to the cytoplasm also influence the rate constant of nucleocytoplasmic transport. Neither quantity was sensitive to osmolality in a statistically significant manner although the sensitivity of relative nuclear volume, Q (which trended downwards with increasing osmolality), approached statistical significance (ANOVA $p = 0.07$). The average value of

the permeability was $0.0521 \mu\text{m/s}$ and the average value of the volume of the nucleus relative to that of the cytoplasm was 0.365.

Discussion

The findings of this study indicate that changes in nuclear volume under hyper-osmotic stress can have a significant effect on the rate constant of nucleocytoplasmic transport. While the overall rate of nucleocytoplasmic transport is a function of several different parameters, including nuclear permeability, nuclear surface area to volume ratio, and nuclear to cytoplasmic volume ratio, we found that the surface area to volume ratio of the nucleus was only parameter sensitive to osmolality. These findings suggest that the osmotically-induced change in nucleocytoplasmic transport is due primarily to a change in geometry under hyper-osmotic stress. Furthermore, our findings demonstrated that despite large reductions in nuclear size, the accompanying alterations in chromatin structure resulted in an increase in nuclear lacunarity. Thus, the diffusion coefficient within the nucleus was insensitive to the osmotic environment, whereas the diffusion coefficient in the cytoplasm was inversely proportional to osmotic stress.

The inverse relationship between osmolality and cytoplasmic diffusivity is consistent with a previous report in other cell types [35] and is likely due to increased macromolecular crowding. However, no correlation between diffusivity and osmolality was observed in the nucleus (Figure 2B). This finding may be due to osmotically-induced changes in the microstructure of the nucleus that do not occur in the cytoplasm. Chromatin condenses under hyper-osmotic stress [22,31], causing the pores of the interchromatin domain to enlarge (Figure 3). This may facilitate transport of macromolecules since nuclear particles have been observed to move preferentially through chromatin poor regions [36]. This phenomenon may offset the decrease in the diffusion coefficient caused by increased macromolecular crowding. However, it is important to note that one limitation of this study is the fact that we examined one specific size of an inert molecule (10kDa dextran), and molecules size or shape could influence these phenomena. For example, Guigas [37] found that hyper-osmotic stress significantly decreased the diffusivity of 5nm gold nanoparticles in the nucleus, in contrast to the present study. The most likely explanation for this difference is the difference in the shape and size of the particles used. Furthermore, the previous study used fluorescence correlation spectroscopy (FCS), which examines a volume about one order of magnitude smaller than that probed by SCAMP. Also, different theoretical models are employed. The SCAMP method assumes ideal diffusion whereas the FCS method incorporates and quantifies anomalous diffusion.

Several properties of the nucleus can influence the rate of nucleocytoplasmic transport according to Eq. 3: surface-to-volume ratio, nuclear to cytoplasmic volume ratio and permeability. The only property to exhibit a statistically significant sensitivity to osmolality was surface to volume ratio. The nuclear to cytoplasmic volume ratio showed a trend with osmolality (ANOVA $p = 0.07$) while the permeability showed no such trend (ANOVA $p = 0.54$). A geometric mechanism for osmotic sensitivity of nucleocytoplasmic transport has significant implications. The osmotic sensitivity of nucleus geometry plateaus in the hypo-osmotic range [16], presumably due to the tensile properties of the nuclear lamina [38]. This property is consistent with the finding that the rate constant of nucleocytoplasmic transport is insensitive to hypo-osmotic stress (Figure 4A). It is also important to note that the mechanism of osmotic sensitivity is not a change in nuclear permeability. This implies that similar sensitivity will be observed regardless of whether a molecule crosses the envelope by passive diffusion or active transport. Previous work has shown that changes in nuclear surface area to volume ratio significantly affect nucleocytoplasmic transport of calcium. This result is consistent with the findings of this study, despite the fact that calcium

molecules are orders of magnitude smaller than the dextran molecules used in these experiments [39].

Osmotically-sensitive transport may represent a novel mechanism for modulating gene transcription because many gene transcription factors activate their targets by trafficking from the cytoplasm into the nucleus. For example, this mechanism is directly consistent with a recent report showing that hyper-osmotic stress upregulates SOX9 expression in chondrocytes via a p38 dependent mechanism [12]. As p38 activation of SOX9 is dependent on its translocation from the cytoplasm to the nucleus [40], our results suggest that this activation would proceed more quickly under hyper-osmotic stress. This modulating effect would constitute a potential mechanotransduction mechanism in cells such as intervertebral disc cells that are subjected to hyper-osmotic stress as fluid is exuded from the tissue under load [9].

Acknowledgments

This work was supported in part by NIH grants AG15768, AR48182, AR50245, AR48852, and T32 AI07217.

References

1. Fedan JS, Yuan LX, Chang VC, Viola JO, Cutler D, Pettit LL. Osmotic regulation of airway reactivity by epithelium. *J Pharmacol Exp Ther*. 1999; 289:901–910. [PubMed: 10215669]
2. Miyakawa H, Woo SK, Chen CP, Dahl SC, Handler JS, Kwon HM. Cis- and transacting factors regulating transcription of the BGT1 gene in response to hypertonicity. *Am J Physiol*. 1998; 274:F753–F761. [PubMed: 9575900]
3. Madara JL. Increases in guinea pig small intestinal transepithelial resistance induced by osmotic loads are accompanied by rapid alterations in absorptive-cell tight-junction structure. *J Cell Biol*. 1983; 97:125–136. [PubMed: 6863387]
4. Erickson GR, Northrup DL, Guilak F. Hypo-osmotic stress induces calcium-dependent actin reorganization in articular chondrocytes. *Osteoarthritis Cartilage*. 2003; 11:187–197. [PubMed: 12623290]
5. Erickson GR, Alexopoulos LG, Guilak F. Hyper-osmotic stress induces volume change and calcium transients in chondrocytes by transmembrane, phospholipid, and G-protein pathways. *J Biomech*. 2001; 34:1527–1535. [PubMed: 11716854]
6. Hall AC, Bush PG. The role of a swelling-activated taurine transport pathway in the regulation of articular chondrocyte volume. *Pflugers Arch*. 2001; 442:771–781. [PubMed: 11512034]
7. Lai WM, Hou JS, Mow VC. A triphasic theory for the swelling and deformation behaviors of articular cartilage. *J Biomech Eng*. 1991; 113:245–258. [PubMed: 1921350]
8. Maroudas A. Physicochemical Properties of Articular Cartilage. In: Freeman, MAR., editor. *Adult Articular Cartilage*. Kent, England: Pitman Medical Publishing Co. Ltd.; 1979.
9. Sivan S, Neidlinger-Wilke C, Wurtz K, Maroudas A, Urban JP. Diurnal fluid expression and activity of intervertebral disc cells. *Biorheology*. 2006; 43:283–291. [PubMed: 16912401]
10. Lee DA, Knight MM, Bolton JF, Idowu BD, Kayser MV, Bader DL. Chondrocyte deformation within compressed agarose constructs at the cellular and sub-cellular levels. *J Biomech*. 2000; 33:81–95. [PubMed: 10609521]
11. Guilak F. Compression-induced changes in the shape and volume of the chondrocyte nucleus. *J Biomech*. 1995; 28:1529–1541. [PubMed: 8666592]
12. Tew SR, Peffers MJ, McKay TR, Lowe ET, Khan WS, Hardingham TE, Clegg PD. Hyperosmolarity regulates SOX9 mRNA posttranscriptionally in human articular chondrocytes. *Am J Physiol Cell Physiol*. 2009; 297:C898–C906. [PubMed: 19657054]
13. Palmer GD, Chao Ph PH, Raia F, Mauck RL, Valhmu WB, Hung CT. Time-dependent aggrecan gene expression of articular chondrocytes in response to hyperosmotic loading. *Osteoarthritis Cartilage*. 2001; 9:761–770. [PubMed: 11795996]

14. Borghetti P, Della Salda L, De Angelis E, Maltarello MC, Petronini PG, Cabassi E, Marcato PS, Maraldi NM, Borghetti AF. Adaptive cellular response to osmotic stress in pig articular chondrocytes. *Tissue Cell*. 1995; 27:173–183. [PubMed: 7778094]
15. Liedtke W, Choe Y, Marti-Renom MA, Bell AM, Denis CS, Sali A, Hudspeth AJ, Friedman JM, Heller S. Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell*. 2000; 103:525–535. [PubMed: 11081638]
16. Finan JD, Chalut KJ, Wax A, Guilak F. Nonlinear osmotic properties of the cell nucleus. *Ann Biomed Eng*. 2009; 37:477–491. [PubMed: 19107599]
17. Finan JD, Guilak F. The effects of osmotic stress on the structure and function of the cell nucleus. *J Cell Biochem*. 2010; 109:460–467. [PubMed: 20024954]
18. Lammerding J, Schulze PC, Takahashi T, Kozlov S, Sullivan T, Kamm RD, Stewart CL, Lee RT. Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. *J Clin Invest*. 2004; 113:370–378. [PubMed: 14755334]
19. Rowat AC, Lammerding J, Ipsen JH. Mechanical properties of the cell nucleus and the effect of emerin deficiency. *Biophys J*. 2006; 91:4649–4664. [PubMed: 16997877]
20. Dahl KN, Scaffidi P, Islam MF, Yodh AG, Wilson KL, Misteli T. Distinct structural and mechanical properties of the nuclear lamina in Hutchinson-Gilford progeria syndrome. *Proc Natl Acad Sci U S A*. 2006; 103:10271–10276. [PubMed: 16801550]
21. Maniotis AJ, Chen CS, Ingber DE. Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc Natl Acad Sci U S A*. 1997; 94:849–854. [PubMed: 9023345]
22. Irianto, JK.; Martins, MM.; Lee, RP.; A, D. Osmotic Challenge Influences the Morphology and Levels of Chromatin Condensation in the Chondrocyte Nucleus. ORS 2011 Annual Meeting; Long Beach, California. 2011. p. 1947
23. Pritchard S, Erickson GR, Guilak F. Hyperosmotically induced volume change and calcium signaling in intervertebral disk cells: the role of the actin cytoskeleton. *Biophys J*. 2002; 83:2502–2510. [PubMed: 12414684]
24. Urban JP, Hall AC, Gehl KA. Regulation of matrix synthesis rates by the ionic and osmotic environment of articular chondrocytes. *J Cell Physiol*. 1993; 154:262–270. [PubMed: 8425907]
25. Teruel MN, Meyer T. Electroporation-induced formation of individual calcium entry sites in the cell body and processes of adherent cells. *Biophys J*. 1997; 73:1785–1796. [PubMed: 9336174]
26. Leddy HA, Christensen SE, Guilak F. Microscale diffusion properties of the cartilage pericellular matrix measured using 3D scanning microphotolysis. *J Biomech Eng*. 2008; 130:061002. [PubMed: 19045531]
27. Axelrod D, Ravdin P, Koppel DE, Schlessinger J, Webb WW, Elson EL, Podleski TR. Lateral motion of fluorescently labeled acetylcholine receptors in membranes of developing muscle fibers. *Proc Natl Acad Sci U S A*. 1976; 73:4594–4598. [PubMed: 1070010]
28. Truskey, GA.; Yuan, F.; Katz, DF. Transport Phenomena in Biological Systems. Upper Saddle River, New Jersey: Pearson Prentice Hall; 2004.
29. Peters R. Nucleocytoplasmic flux and intracellular mobility in single hepatocytes measured by fluorescence microphotolysis. *Embo Journal*. 1984; 3:1831–1836. [PubMed: 6207019]
30. Beg AA, Finco TS, Nantermet PV, Baldwin AS Jr. Tumor necrosis factor and interleukin-1 lead to phosphorylation and loss of I kappa B alpha: a mechanism for NF-kappa B activation. *Mol Cell Biol*. 1993; 13:3301–3310. [PubMed: 8497253]
31. Albiez H, Cremer M, Tiberi C, Vecchio L, Schermelleh L, Dittrich S, Kupper K, Joffe B, Thormeyer T, von Hase J, Yang S, Rohr K, Leonhardt H, Solovei I, Cremer C, Fakan S, Cremer T. Chromatin domains and the interchromatin compartment form structurally defined and functionally interacting nuclear networks. *Chromosome Res*. 2006; 14:707–733. [PubMed: 17115328]
32. Mandelbrot, BB. The Fractal Geometry of Nature. W. H. Freeman; 1983.
33. Allain C, Cloitre M. Characterizing the lacunarity of random and deterministic fractal sets. *Phys Rev A*. 1991; 44:3552–3558. [PubMed: 9906372]
34. Fenichel, IR.; Horowitz, SB. Biological Membranes. Boston: Little, Brown and Comp.; 1969.

35. Seksek O, Biwersi J, Verkman AS. Translational diffusion of macromolecule-sized solutes in cytoplasm and nucleus. *J Cell Biol.* 1997; 138:131–142. [PubMed: 9214387]
36. Bacher CP, Reichenzeller M, Athale C, Herrmann H, Eils R. 4-D single particle tracking of synthetic and proteinaceous microspheres reveals preferential movement of nuclear particles along chromatin - poor tracks. *BMC Cell Biol.* 2004; 5:45. [PubMed: 15560848]
37. Guigas G, Kalla C, Weiss M. Probing the nanoscale viscoelasticity of intracellular fluids in living cells. *Biophys J.* 2007; 93:316–323. [PubMed: 17416631]
38. Dahl KN, Kahn SM, Wilson KL, Discher DE. The nuclear envelope lamina network has elasticity and a compressibility limit suggestive of a molecular shock absorber. *J Cell Sci.* 2004; 117:4779–4786. [PubMed: 15331638]
39. Wittmann M, Queisser G, Eder A, Wiegert JS, Bengtson CP, Hellwig A, Wittum G, Bading H. Synaptic activity induces dramatic changes in the geometry of the cell nucleus: interplay between nuclear structure, histone H3 phosphorylation, and nuclear calcium signaling. *J Neurosci.* 2009; 29:14687–14700. [PubMed: 19940164]
40. Nielsen MB, Christensen ST, Hoffmann EK. Effects of osmotic stress on the activity of MAPKs and PDGFR-beta-mediated signal transduction in NIH-3T3 fibroblasts. *Am J Physiol Cell Physiol.* 2008; 294:C1046–C1055. [PubMed: 18272822]

Figure 1.

(A) Chondrocytes were loaded with dextran before the bleaching pulse. (B) Syto-82 was used to image the nucleus and to set the bounds of the bleaching beam (white circle) within the nucleus. (C) Immediately after the bleaching pulse, fluorescence is absent from the nucleus but present in the cytoplasm. (D) By the end of the experiment, fluorescence is again equally distributed due to nucleocytoplasmic transport. (E) Recovery exhibits two rate constants because it is driven by two different transport processes. The recovery is tracked in the projected area of the nucleus so some of the recovered fluorescence is due to nucleocytoplasmic transport and some is due to intracytoplasmic transport of dextrans into the region outlined in green after the bleaching pulse. These two transport processes can be distinguished because they occur on different time scales. Nucleocytoplasmic transport corresponds to the slower time constant in Eq. (4). (F) Schematic of FRAP model. Two compartments representing the nucleus, with volume V_n and dextran concentration C_n , and the cytoplasm with volume V_c and dextran concentration C_c , are separated by a permeable membrane with surface area S and permeability P .

Figure 2.

Diffusion coefficients in the chondrocyte nucleus and cytoplasm as measured by scanning microphotolysis. (A) The diffusion coefficient in the cytoplasm decreased with increasing extracellular osmolality ($p < 0.001$, $R^2 = 0.40$). (B) The diffusion coefficient in the nucleus was not correlated with extracellular osmolality ($p > 0.1$)

Figure 3.

The lacunarity of the nucleus increased under hyper-osmotic stress. (A) Under isoosmotic conditions, a cross-sectional confocal image of a chondrocyte nucleus showed that Hoechst signal intensity was homogeneously distributed. Intensity is represented by a false color map to aid visualization. (B) Under hyper-osmotic conditions, the distribution of the Hoechst signal became heterogeneous, with dense regions of chromatin (black arrow) and large chromatin poor regions (white arrow) now visible. (C) The condensation of chromatin within the nucleus enlarges the pores of the inter-chromatin domain, as evidenced by an increase in the lacunarity of the nucleus under hyper-osmotic stress ($n \geq 8$, mean $\pm$ s.d., $*p < 0.05$). The mean initial value of lacunarity was 0.11.

Figure 4.

(A) The rate of nucleocytoplasmic transport increased under hyper-osmotic stress but was unaffected by hypo-osmotic stress. (B) The surface to volume ratio increased with increasing osmolality. ($n \geq 22$, mean $\pm$ s.d., * $p < 0.05$)