Communicative & Integrative Biology 6:5, e25153; September/October 2013; © 2013 Landes Bioscience

BRG1, a SWI/SNF chromatin remodeling enzyme ATPase, is required for maintenance of nuclear shape and integrity

Anthony N. Imbalzano,* Karen M. Imbalzano and Jeffrey A. Nickerson*
Department of Cell and Developmental Biology; University of Massachusetts Medical School; Worcester, MA USA

We recently reported that reducing the levels of BRG1, the catalytic subunit of mammalian SWI/SNF chromatin remodeling enzymes, induces alterations in nuclear shape in a breast epithelial cell line. Immunostaining the BRG1 knockdown cells with nuclear lamina antibodies revealed a significantly increased frequency of grooves, or invaginations, in the nuclei. Disruption of each of the major cytoplasmic filament systems (actin, tubulin and cytokeratins) had no impact on the BRG1-dependent changes in nuclear shape, indicating that the observed changes in nuclear morphology are unlikely to be a result of alterations in the integrity of the nuclear-cytoplamic contacts in the cell. We propose that the BRG1-dependent nuclear shape changes reflect a role for the chromatin remodeling enzyme in maintaining the structural integrity of the nucleus via global regulation of chromatin structure and dynamics within the nucleus.

Keywords: BRG1, SWI/SNF, chromatin remodeling, chromatin, lamina, nuclear shape, breast epithelia

Submitted: 05/22/13

Accepted: 05/23/13

Citation: Imbalzano AN. Imbalzano KM, Nickerson JA. BRG1, a SWI/SNF chromatin remodeling enzyme ATPase, is required for maintenance of nuclear shape and integrity. Commun Integr Biol 2013; 6: e25153; http://dx.doi.org/10.4161/cib.25153
*Correspondence to: Anthony N. Imbalzano and Jeffrey A. Nickerson; Email: Anthony.imbalzano@umassmed.edu and Jeffrey.nickerson@umassmed.edu

Addendum to: Imbalzano KM, Cohet N, Wu Q, Underwood JM, Imbalzano AN, Nickerson JA. Nuclear shape changes are induced by knockdown of the SWI/SNF ATPase BRG1 and are independent of cytoskeletal connections. PLoS ONE 2013; 8:e55628; PMID:23405182; http://dx.doi.org/10.1371/journal.pone.0055628

Contiguous physical connections between the cell surface proteins that communicate with the extra-cellular environment, the cytoskeleton and the nucleus make possible signal transduction mechanisms that are critical to processing nearly every signaling cue to which a cell can be exposed. However, these components play an equally critical role in maintaining the structural integrity of the cell and the nucleus. Previous work suggested that changes in nuclear shape could be induced by either external forces transduced from the cytoskeleton or by internal nuclear forces. Either mechanism would require linkages between the structures at the nuclear periphery, the nuclear lamina and the nuclear envelope, and either the cytoskeleton or internal nuclear structures. The LINC (Linker of Nucleoskeleton and Cytoskeleton) complex at the nuclear periphery, as well as the associated SUN and nesprin proteins, likely serve this purpose since they tie together peripheral chromatin, the nuclear lamins and the cytoskeleton.[1-3] The importance of maintaining the appropriate structural integrity in both the nucleus and in the cell as a whole is reflected in the correlation between alterations to cell and nuclear structure and the presence of disease, which most notably include laminopathies and cancers. Indeed, the presence of nuclear and/or cytoplasmic structural changes in patient biopsy samples can be the primary means of diagnosis.[4] Yet the molecular bases for changes in nuclear and/or cytoplasmic structure are often poorly or not at all understood.

We previously observed that ectopic expression of a dominant negative, ATPase-deficient BRG1 protein in fibroblasts caused an increase in nuclear size of ~10%, though no changes in nuclear shape were seen.[5] As in our latest study,[6] we hypothesized that the chromatin remodeling enzyme, directly or indirectly, was affecting the gene expression levels of one or more structural components of cytoskeletal or nuclear architecture, thereby compromising the integrity of nuclear size and/or shape. However, microarray analysis failed to identify any possible candidate gene, and, as detailed in our published report, a candidate approach using immunofluorescence failed to identify changes in levels or distribution for a handful of

nuclear lamina proteins while disruption of cytoskeletal filaments did not alter the appearance or the frequency of altered nuclei upon BRG1 knockdown.[6]

Though it certainly remains possible that the cause of the observed changes in nuclear morphology is a BRG1-dependent change in the expression of a structural protein or in a regulatory factor controlling the expression of such proteins, we wish to put forth the hypothesis that BRG1, by virtue of its chromatin remodeling activity, may be globally controlling chromatin organization, especially as it relates to chromatin interactions with the nuclear lamina, and may therefore be controlling nuclear shape. Multiple lamina proteins mediate connections to genomic chromatin,[7,8] but how chromatin comes to be in a position to be bridged to the nuclear lamina, and how that positioning is maintained and regulated, is just beginning to be elucidated.[9,10] We propose that BRG1, and therefore, BRG1-based SWI/SNF enzymes, contribute to higher order genome organization, and consequently to the regulation of nuclear shape.

BRG1 has been shown to catalyze local changes in nucleosome position and structure on target genes as part of its role in regulating gene expression.[11-13] BRG1 interacts with transcriptional regulatory proteins, interacts with target gene chromatin, and alters chromatin accessibility. In vitro studies suggest that its chromatin remodeling properties can be recapitulated by recombinant BRG1.[14] However, the SWI/SNF enzyme is nearly two million Daltons and has at least 11 subunits, many of which are variant, which allows for a great diversity in the subunit composition of BRG1-based enzymes.[15] While there is evidence that the additional subunits can help mediate interactions with transcriptional regulatory proteins, the functions of the additional subunits are poorly characterized. Why is BRG1 exclusively found as part of a large complex when its ATP-dependent functions seems to be sufficient for the biochemical activities associated with chromatin remodeling at target loci?

Perhaps the answer lies in the idea that BRG1-based SWI/SNF enzymes have additional functions beyond nucleosome remodeling at promoter and enhancer sequences. ChIP-seq data from multiple studies in different cell line backgrounds reveal that BRG1, as expected, binds to regulatory sequences at target genes but also binds elsewhere throughout the genome, with a significant proportion of the total binding sites being intergenic.[16-19] This raises a question as to the relevance of these binding sites. Do they represent distant regulatory sequences affecting gene expression or is there another purpose to BRG1 binding in intergenic regions, such as organizing higher order chromatin structure? Intriguing studies of the α-globin and β-globin loci in the K562 erythroleukemia cell line document BRG1-dependent looping between promoters and distal regulatory sequences,[20,21] providing evidence that BRG1 can organize chromatin in three-dimensional space along a specific region of a chromosome. Similarly, BRG1 organizes chromatin looping and higher order chromatin structure that promotes gene expression at the T-helper 2 (Th2) cytokine locus and at the CIITA locus.[22,23] Recently, Lan et al., integrated K562 cell ChIP-seq and DNase-seq data with data from a genome-wide approach to mapping loci that interact with each other in K562 cells.[24] They identified 12 different clusters of interacting genomic loci that could be distinguished by the epigenetic status of the loci. Of note, one of the clusters was marked by the binding of BRG1 and INI1, an invariant subunit of all SWI/SNF enzymes. While this data does not demonstrate function, it is nevertheless provocative that some three-dimensional physical interactions between genomic loci correlate with the presence of BRG1 and presumably BRG1-based SWI/SNF enzyme. Finally, another study documents inter-chromosomal interactions between ER responsive genes following stimulation of cells by estrogen; these interactions were lost when a BRG1-associated subunit called BAF53 was knocked down by siRNA techniques.[25] This result gives credence to the idea that SWI/SNF enzymes can mediate chromatin organization between specific loci on different chromosomes as well as regulate chromatin organization in response to external stimuli.

Collectively, these studies support the idea that BRG1 organizes chromatin structure both within the confines of a specific gene locus as well as at greater distances and even between loci on different chromosomes. We extend this concept to

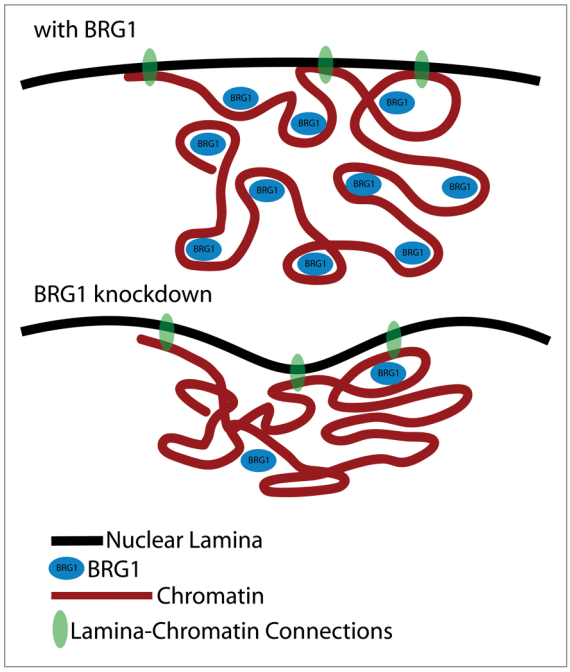

Figure 1. Model for BRG1-dependent nuclear shape change. Upon knockdown of BRG1, chromatin condenses, collapsing the chromosome territory and resulting in the formation of grooves, or invaginations, in the nuclear lamina.

postulate that BRG1 organizes chromatin at the nuclear lamina. This hypothesis is supported by one ChiP-seq data set that indicates a large percentage of intergenic BRG1 locations are coincident with lamina-associated DNA sequences[18] and our functional observations that BRG1 knockdown in breast epithelial cells alters nuclear shape.[6] We propose that just as knockdown or interference with BRG1 or SWI/SNF enzyme compromises chromatin spatial organization at or between specific loci,[20,21,25] knockdown of BRG1 in breast epithelial cells compromises chromatin integrity at the lamina. The appearance of grooves, or invaginations, in the nuclear lamina[6] suggests that areas of cellular chromatin structure may have collapsed or become more condensed when BRG1 levels are reduced, while connections between the chromatin and lamina proteins have been maintained. Consequently, the lamina is pulled inward into the nucleus and the nuclear shape is altered (**Fig. 1**). Future work will seek to provide experimental support for this concept.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank A.R. Barutcu for critical comments on the manuscript. This work was supported by NIH P01 CA82834. A.N.I. is a member of the University of Massachusetts Diabetes Endocrinology Research Center, which is supported by NIH Grant 5P30DK32520. J.A.N. acknowledges additional support from NIH R01 EB014869.

References

1. Crisp M, Liu Q, Roux K, Rattner JB, Shanahan C, Burke B, et al. Coupling of the nucleus and cytoplasm: role of the LINC complex. J Cell Biol 2006; 172:41-53; PMID:16380439; http://dx.doi.org/10.1083/jcb.200509124
2. Starr DA, Fridolfsson HN. Interactions between nuclei and the cytoskeleton are mediated by SUN-KASH nuclear-envelope bridges. Annu Rev Cell Dev Biol 2010; 26:421-44; PMID:20507227; http://dx.doi.org/10.1146/annurev-cellbio-100109-104037
3. Mellad JA, Warren DT, Shanahan CM. Nesprins LINC the nucleus and cytoskeleton. Curr Opin Cell Biol 2011; 23:47-54; PMID:21177090; http://dx.doi.org/10.1016/j.ceb.2010.11.006
4. Zink D, Fischer AH, Nickerson JA. Nuclear structure in cancer cells. Nat Rev Cancer 2004; 4:677-87; PMID:15343274; http://dx.doi.org/10.1038/nrc1430
5. Hill DA, Chiosea S, Jamaluddin S, Roy K, Fischer AH, Boyd DD, et al. Inducible changes in cell size and attachment area due to expression of a mutant SWI/SNF chromatin remodeling enzyme. J Cell Sci 2004; 117:5847-54; PMID:15537831; http://dx.doi.org/10.1242/jcs.01502
6. Imbalzano KM, Cohet N, Wu Q, Underwood JM, Imbalzano AN, Nickerson JA. Nuclear shape changes are induced by knockdown of the SWI/SNF ATPase BRG1 and are independent of cytoskeletal connections. PLoS ONE 2013; 8:e55628; PMID:23405182; http://dx.doi.org/10.1371/journal.pone.0055628
7. Ho CY, Lammerding J. Lamins at a glance. J Cell Sci 2012; 125:2087-93; PMID:22669459; http://dx.doi.org/10.1242/jcs.087288
8. Bank EM, Gruenbaum Y. The nuclear lamina and heterochromatin: a complex relationship. Biochem Soc Trans 2011; 39:1705-9; PMID:22103511; http://dx.doi.org/10.1042/BST20110603
9. Kind J, Pagie L, Ortabozkoyun H, Boyle S, de Vries SS, Janssen H, et al. Single-cell dynamics of genome-nuclear lamina interactions. Cell 2013; 153:178-92; PMID:23523135; http://dx.doi.org/10.1016/j.cell.2013.02.028
10. McCord RP, Nazario-Toole A, Zhang H, Chines PS, Zhan Y, Erdos MR, et al. Correlated alterations in genome organization, histone methylation, and DNA-lamin A/C interactions in Hutchinson-Gilford progeria syndrome. Genome Res 2013; 23:260-9; PMID:23152449; http://dx.doi.org/10.1101/gr.138032.112
11. de la Serna IL, Ohkawa Y, Imbalzano AN. Chromatin remodelling in mammalian differentiation: lessons from ATP-dependent remodellers. Nat Rev Genet 2006; 7:461-73; PMID:16708073; http://dx.doi.org/10.1038/nrg1882
12. Ho L, Crabtree GR. Chromatin remodelling during development. Nature 2010; 463:474-84; PMID:20110991; http://dx.doi.org/10.1038/nature08911
13. Wu JI. Diverse functions of ATP-dependent chromatin remodeling complexes in development and cancer. Acta Biochim Biophys Sin (Shanghai) 2012; 44:54-69; PMID:22194014; http://dx.doi.org/10.1093/abbs/gmr099
14. Phelan ML, Sif S, Narlikar GJ, Kingston RE. Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. Mol Cell 1999; 3:247-53; PMID:10078207; http://dx.doi.org/10.1016/S1097-2765(00)80315-9
15. Hargreaves DC, Crabtree GR. ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. Cell Res 2011; 21:396-420; PMID:21358755; http://dx.doi.org/10.1038/cr.2011.32
16. De S, Wurster AL, Precht P, Wood WH 3rd, Becker KG, Pazin MJ. Dynamic BRG1 recruitment during T helper differentiation and activation reveals distal regulatory elements. Mol Cell Biol 2011; 31:1512-27; PMID:21262765; http://dx.doi.org/10.1128/MCB.00920-10
17. Yu Y, Chen Y, Kim B, Wang H, Zhao C, He X, et al. Olig2 targets chromatin remodelers to enhancers to initiate oligodendrocyte differentiation. Cell 2013; 152:248-61; PMID:23332759; http://dx.doi.org/10.1016/j.cell.2012.12.006
18. Euskirchen GM, Auerbach RK, Davidov E, Gianoulis TA, Zhong G, Rozowsky J, et al. Diverse roles and interactions of the SWI/SNF chromatin remodeling complex revealed using global approaches. PLoS Genet 2011; 7:e1002008; PMID:21408204; http://dx.doi.org/10.1371/journal.pgen.1002008
19. Ho L, Jothi R, Ronan JL, Cui K, Zhao K, Crabtree GR. An embryonic stem cell chromatin remodeling complex, esBAF, is an essential component of the core pluripotency transcriptional network. Proc Natl Acad Sci USA 2009; 106:5187-91; PMID:19279218; http://dx.doi.org/10.1073/pnas.0812888106
20. Kim SI, Bultman SJ, Kiefer CM, Dean A, Bresnick EH. BRG1 requirement for long-range interaction of a locus control region with a downstream promoter. Proc Natl Acad Sci USA 2009; 106:2259-64; PMID:19171905; http://dx.doi.org/10.1073/pnas.0806420106
21. Kim SI, Bresnick EH, Bultman SJ. BRG1 directly regulates nucleosome structure and chromatin looping of the alpha globin locus to activate transcription. Nucleic Acids Res 2009; 37:6019-27; PMID:19696073; http://dx.doi.org/10.1093/nar/gkp677
22. Cai S, Lee CC, Kohwi-Shigematsu T. SATB1 packages densely looped, transcriptionally active chromatin for coordinated expression of cytokine genes. Nat Genet 2006; 38:1278-88; PMID:17057718; http://dx.doi.org/10.1038/ng1913
23. Ni Z, Abou El Hassan M, Xu Z, Yu T, Bremner R. The chromatin-remodeling enzyme BRG1 coordinates CIITA induction through many interdependent distal enhancers. Nat Immunol 2008; 9:785-93; PMID:18500344; http://dx.doi.org/10.1038/ni.1619
24. Lan X, Witt H, Katsumura K, Ye Z, Wang Q, Bresnick EH, et al. Integration of Hi-C and ChIP-seq data reveals distinct types of chromatin linkages. Nucleic Acids Res 2012; 40:7690-704; PMID:22675074; http://dx.doi.org/10.1093/nar/gks501
25. Hu Q, Kwon YS, Nunez E, Cardamone MD, Hutt KR, Ohgi KA, et al. Enhancing nuclear receptor-induced transcription requires nuclear motor and LSD1-dependent gene networking in interchromatin granules. Proc Natl Acad Sci USA 2008; 105:19199-204; PMID:19052240; http://dx.doi.org/10.1073/pnas.0810634105