

# Competition between histone H1 and HMGN proteins for chromatin binding sites

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The ability of regulatory factors to access their nucleosomal targets is modulated by nuclear proteins such as histone H1 and HMGN (previously named HMG-14/-17 family) that bind to nucleosomes and either stabilize or destabilize the higher-order chromatin structure. We tested whether HMGN proteins affect the interaction of histone H1 with chromatin. Using microinjection into living cells expressing H1-GFP and photobleaching techniques, we found that wild-type HMGN, but not HMGN point mutants that do not bind to nucleosomes, inhibits the binding of H1 to nucleosomes. HMGN proteins compete with H1 for nucleosome sites but do not displace statically bound H1 from chromatin. Our results provide evidence for *in vivo* competition among chromosomal proteins for binding sites on chromatin and suggest that the local structure of the chromatin fiber is modulated by a dynamic interplay between nucleosomal binding proteins.

## INTRODUCTION

The structure and activity of the chromatin fiber are modulated by numerous nuclear proteins, including the linker histone H1 and the HMGN non-histones (the nomenclature of which has been modified recently and reviewed in Bustin, 2001a,b), proteins known to affect its compactness. Several types of studies suggest that histone H1 and HMGN proteins have opposite effects on the structure and activity of the chromatin fiber. Histone H1 stabilizes the higher-order chromatin structure (Thomas, 1999), hinders the access of transcriptional coactivators to DNA (Herrera *et al.*, 2000; Cheung *et al.*, 2002; Horn *et al.*, 2002) and acts as a general repressor of transcription (Laybourn and Kadonaga, 1991; Wolffe *et al.*, 1997). In contrast, HMGN proteins are nucleosome binding proteins that reduce the compaction of the chromatin fiber and enhance transcription from chromatin templates (Bustin, 2001b). In the chicken globin gene cluster, transcriptional activation is associated with

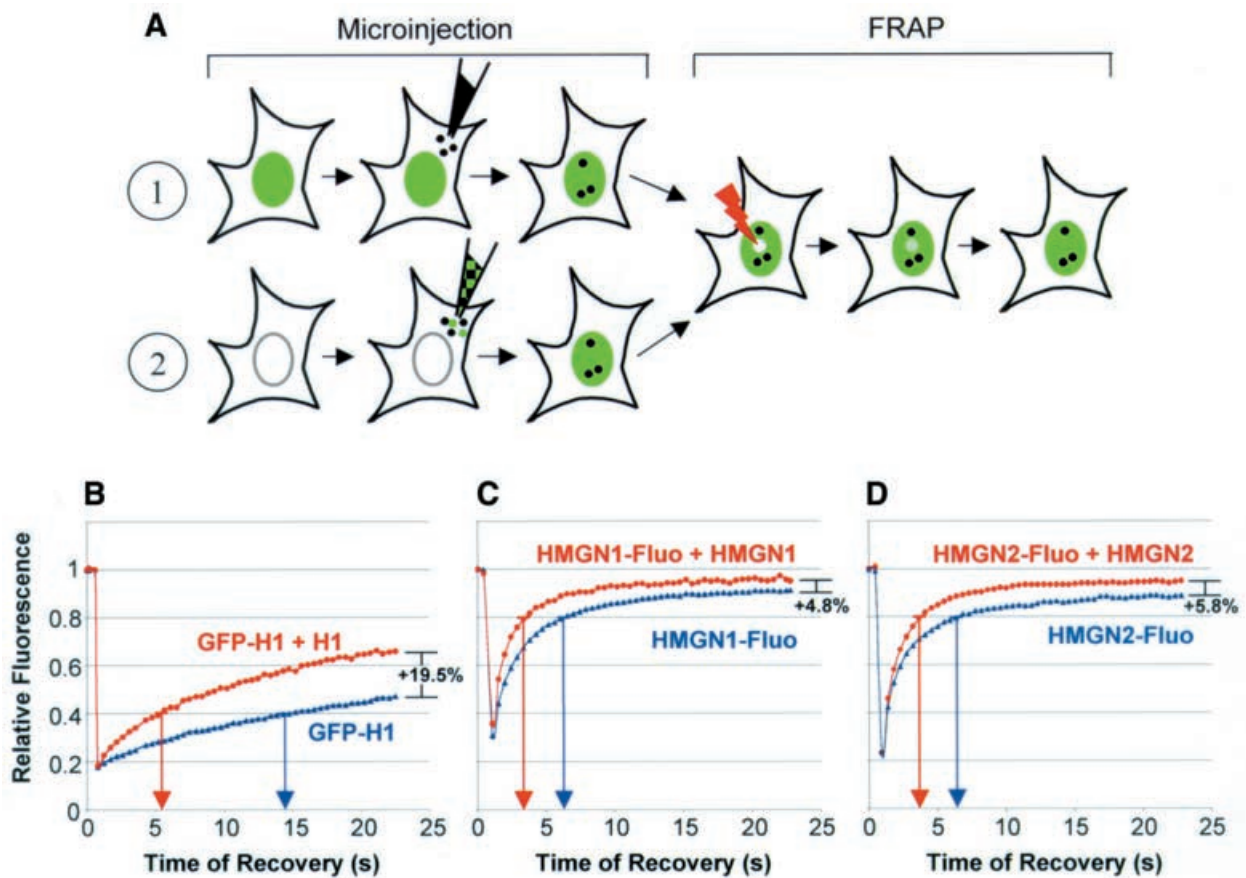
depletion of H1 and enrichment in HMGN proteins (Postnikov *et al.*, 1991). In SV40 minichromosomes, HMGN1 (HMG-14) alleviates both the chromatin compaction and the transcriptional inhibition induced by histone H1 (Ding *et al.*, 1997). The chromatin binding sites of H1 partially overlap with those of HMGN (Alfonso *et al.*, 1994), and contact between these proteins has been detected (Ring and Cole, 1979; Boulikas *et al.*, 1980). The intranuclear organization of both H1 (Lever *et al.*, 2000; Misteli *et al.*, 2000) and HMGN (Phair and Misteli, 2000) proteins is dynamic, and their binding to any specific site on chromatin is transient. Taken together, these observations raise the possibility that these proteins compete for chromatin binding sites and that a dynamic interplay between them may play a role in modulating the structure of specific regions in the chromatin fiber.

To test whether *in vivo* HMGN proteins affect the interaction of H1 with chromatin, we microinjected HMGN1 and HMGN2 (HMG-17) into living cells and used fluorescence recovery after photobleaching (FRAP) to monitor the binding of H1 to chromatin (Misteli *et al.*, 2000). The rate of FRAP is inversely related to the time that a molecule resides at its binding site. We reasoned that, if the injected HMGN and the fluorescently labeled H1 compete for the same binding sites, a fraction of the fluorescent H1 protein would have a shorter chromatin residence time, a change that will lead to an increase in the rate of recovery of fluorescence and could be detected in a FRAP experiment.

## RESULTS AND DISCUSSION

To test whether competition between nuclear proteins for chromatin binding sites *in vivo* can be monitored by FRAP, we probed whether competition for identical binding sites between a fluorescently labeled protein and its non-labeled counterpart can be detected (Figure 1A). We injected purified, unlabeled

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**Fig. 1.** FRAP analysis of competition between proteins for binding sites in living cells. (A) Experimental approach. (1) A nuclear protein is injected into the cytoplasm of cells expressing a GFP-fusion protein. (2) Either fluorescent protein alone or a mixture of fluorescent and non-fluorescent proteins is microinjected into the cytoplasm. After the microinjected proteins enter the nucleus, the mobility of the fluorescent proteins (either as GFP-fusion products or chemically labeled) is analyzed by FRAP. (B–D) Quantitative analysis of FRAP experiments after bleaching cells containing either fluorescent protein alone (blue) or a mixture of fluorescent and non-fluorescent protein (red). The proteins analyzed are indicated in each panel. The values are the mean  $\pm$  SD from at least eight cells of a typical experiment. Arrows on the abscissa point to the time required to reach either 40% (B) or 80% (C and D) of the pre-bleach fluorescence intensity. The percentages on the right of the curves indicate the difference in the percentage recovery between the curves within 23 s after photobleaching.

histone H1 (Figure 2B) into the cytoplasm of cells stably expressing H1<sup>0</sup>-GFP and compared the intranuclear mobility of the H1<sup>0</sup>-GFP in the injected cells with that in control, non-injected cells using FRAP (Figure 1B). We have already demonstrated that H1-GFP is functionally indistinguishable from the endogenous H1 by several criteria (Misteli *et al.*, 2000). In the conditions used, H1<sup>0</sup>-GFP represents <5% of the total H1, and therefore the cellular amount of H1 is not significantly changed. In the control, non-injected cells, the time required to reach 40% (t<sub>40</sub>) of the pre-bleach fluorescence intensity was 14 s. In the H1-injected cells, the t<sub>40</sub> was significantly reduced and was 6 s. Within 23 s after photobleaching, 47% of the signal was recovered in the control cells, whereas 66% of the signal was recovered in the injected cells (Table I). Thus, as a consequence of competition for the same binding sites, the exogenously introduced H1 increased the intranuclear mobility of the stably expressed H1<sup>0</sup>-GFP.

In a modification of this approach (Figure 1A, path 2), we microinjected into the cytoplasm of mouse embryonic fibroblasts either fluorescently labeled HMGN protein alone or the same amount of labeled HMGN mixed with an 8-fold excess of

unlabeled HMGN. Fluorescence microscopy on living cells revealed that the protein entered the nucleus within 15 min (data not shown). Because the chromatin residence time of HMGN is significantly shorter than that of H1<sup>0</sup> (Lever *et al.*, 2000; Misteli *et al.*, 2000; Phair and Misteli, 2000), we measured the differences at t<sub>80</sub>, i.e. the time necessary to recover 80% of the pre-bleach fluorescence intensity. FRAP measurements indicate that the presence of unlabeled HMGN1 decreased the mean t<sub>80</sub> of the labeled protein from 6.3 to 3.8 s and that the presence of unlabeled HMGN2 decreased the t<sub>80</sub> of HMGN2 from 6.2 to 4.0 s (Figure 1C and D). For both HMGN proteins, an 8-fold increase in the amount of injected nuclear protein reproducibly increased the amount of fluorescence recovered after 23 s from ~90 to 95% (Figure 1C and D), indicating that the excess HMGN decreased the relative amount of the HMGN that is immobile. These experiments demonstrate that FRAP can be used to assess competition between chromatin binding proteins for common binding sites in living cells.

To test whether HMGN proteins compete with H1<sup>0</sup> for shared binding sites, we injected either HMGN1 or HMGN2 into the cytoplasm of cells stably expressing H1<sup>0</sup>-GFP. Confocal

**Table I.** HMGN proteins increase the intranuclear mobility of H1<sup>0</sup>-GFP

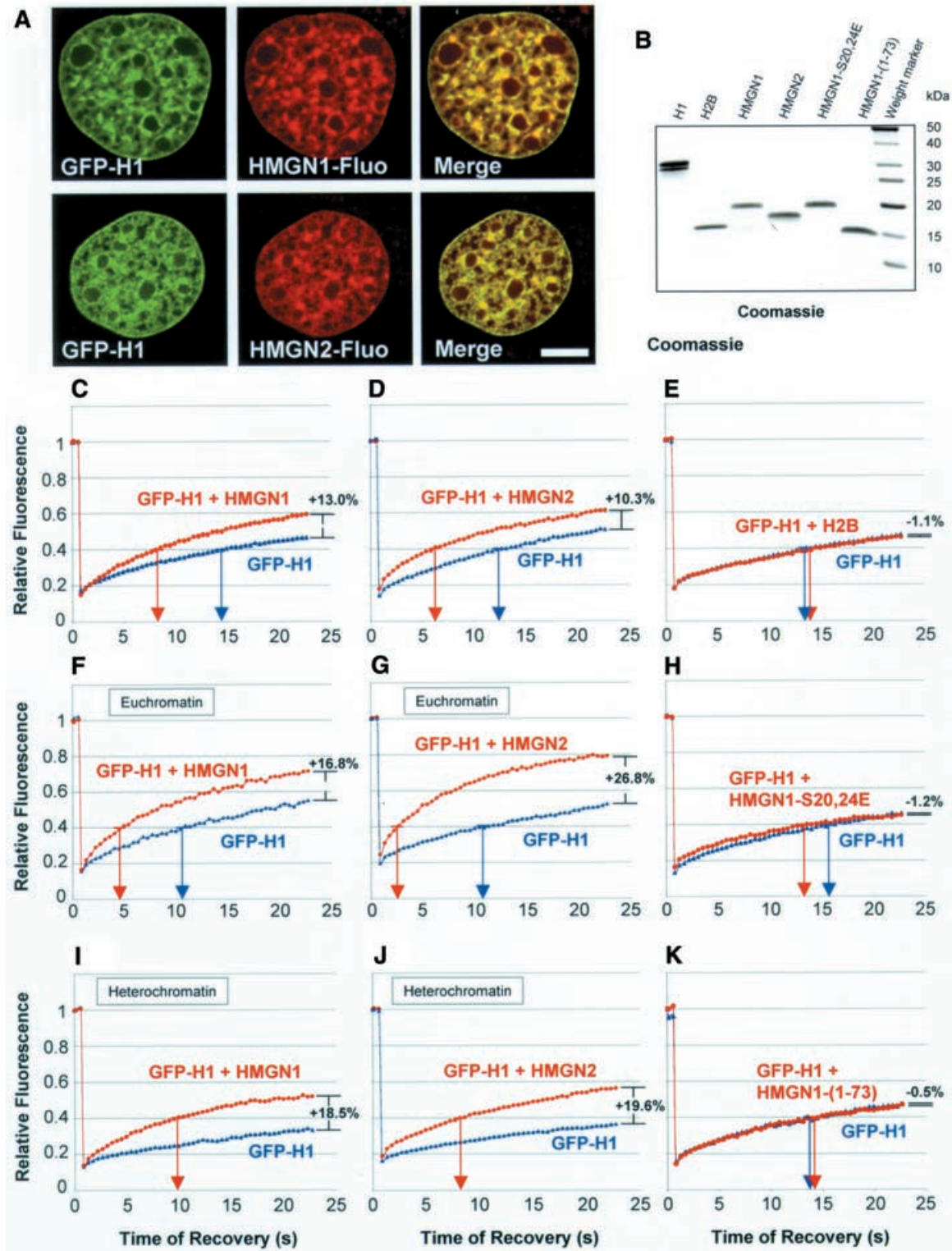
Experiment		t40		Recovery after 23 s	
		Time (s) ± SD	<i>t</i> -test	% ± SD	<i>t</i> -test
H1 <sup>0</sup> -GFP	Mixed chromatin domains	14.7 ± 4.1	<i>P</i> < 0.005	46.7 ± 5.6	<i>P</i> < 0.005
H1 <sup>0</sup> -GFP + H1		5.7 ± 2.1		66.3 ± 8.5	
H1 <sup>0</sup> -GFP	Mixed chromatin domains	13.7 ± 4.1	<i>P</i> < 0.06	46.7 ± 5.6	<i>P</i> < 0.02
H1 <sup>0</sup> -GFP + HMGN1		9.3 ± 5.1		58.9 ± 10.1	
H1 <sup>0</sup> -GFP	Mixed chromatin domains	12.8 ± 3.1	<i>P</i> < 0.005	50.7 ± 6.1	<i>P</i> < 0.005
H1 <sup>0</sup> -GFP + HMGN2		6.0 ± 1.5		61.0 ± 3.6	
H1 <sup>0</sup> -GFP	Mixed chromatin domains	12.5 ± 1.8	<i>P</i> > 0.6	46.9 ± 5.0	<i>P</i> > 0.6
H1 <sup>0</sup> -GFP + H2B		13.2 ± 3.8		45.8 ± 3.8	
H1 <sup>0</sup> -GFP	Mixed chromatin domains	15.4 ± 3.0	<i>P</i> > 0.2	46.0 ± 3.9	<i>P</i> > 0.6
H1 <sup>0</sup> -GFP + HMGN1-S20,24E		13.6 ± 3.3		47.1 ± 6.5	
H1 <sup>0</sup> -GFP	Mixed chromatin domains	12.3 ± 3.8	<i>P</i> > 0.6	46.9 ± 6.5	<i>P</i> > 0.8
H1 <sup>0</sup> -GFP + HMGN1 (1–73)		13.2 ± 3.4		46.4 ± 4.7	
H1 <sup>0</sup> -GFP	Euchromatin	10.2 ± 3.5	<i>P</i> < 0.005	55.9 ± 7.3	<i>P</i> < 0.005
H1 <sup>0</sup> -GFP + HMGN1		4.2 ± 1.4		69.0 ± 9.9	
H1 <sup>0</sup> -GFP	Euchromatin	10.6 ± 2.7	<i>P</i> < 0.005	52.2 ± 3.4	<i>P</i> < 0.005
H1 <sup>0</sup> -GFP + HMGN2		2.6 ± 0.6		79.0 ± 4.1	
H1 <sup>0</sup> -GFP	Heterochromatin	> 23	n.a.	35.1 ± 5.8	<i>P</i> < 0.005
H1 <sup>0</sup> -GFP + HMGN1		10.5 ± 3.8		53.7 ± 8.2	
H1 <sup>0</sup> -GFP	Heterochromatin	> 23	n.a.	36.3 ± 3.2	<i>P</i> < 0.005
H1 <sup>0</sup> -GFP + HMGN2		8.2 ± 1.8		55.9 ± 6.3	

The time required to reach 40% (t40) of the pre-bleach fluorescence intensity was determined from each curve. The recoveries at 23 s are means of the last five images of each recovery curve (last 0.6 s). The results are from recovery curves of at least eight cells. The statistical significance of the differences of t40 and total recovery relative to controls was determined by Student's *t*-test.

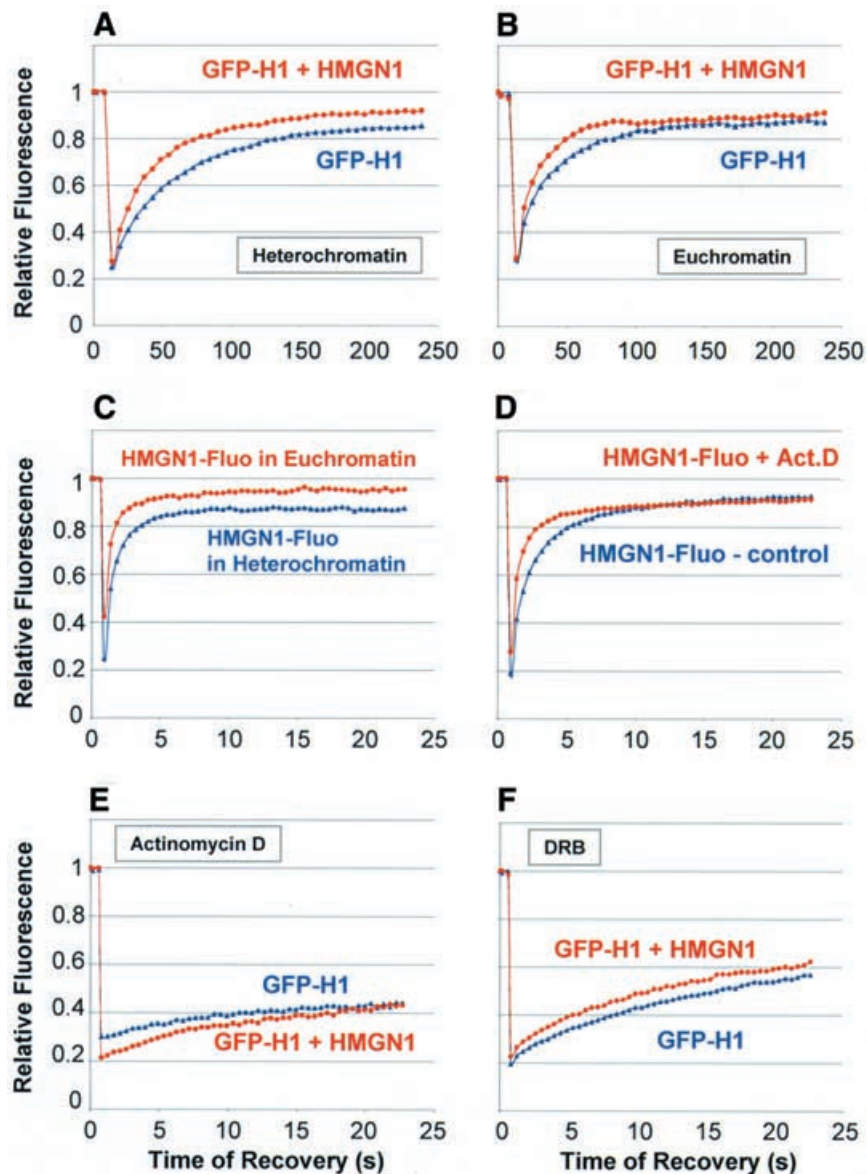
fluorescence microscopy of living cells revealed that the injected HMGN proteins colocalize with the stably expressed H1<sup>0</sup>-GFP (Figure 2A). Because the recovery kinetics of H1 relate to the degree of chromatin compaction (Lever *et al.*, 2000; Misteli *et al.*, 2000), we photobleached H1<sup>0</sup>-GFP in either euchromatin, heterochromatin or in regions containing both euchromatin and heterochromatin (Figure 2; Table I). We defined heterochromatin as regions that were strongly labeled by H1<sup>0</sup>-GFP. These regions were also strongly stained by antibody against methylated lysine 9 of H3 (data not shown, but see Jenuwein and Allis, 2001), Hoechst and HP1 antibody (Misteli *et al.*, 2000). Euchromatin regions were defined as weakly stained by H1<sup>0</sup>-GFP and Hoechst (Misteli *et al.*, 2000). These regions were strongly stained by antibody against acetylated lysine 9 of H3 (data not shown, but see Jenuwein and Allis, 2001). HMGN proteins affected the mobility of H1<sup>0</sup> in all chromatin regions to a significant degree (Table I), suggesting that the two proteins compete for binding sites in chromatin. In the regions containing both condensed and de-condensed chromatin, microinjection of HMGN proteins reduced the t40 of H1<sup>0</sup>-GFP from 13–14 to 6–9 s and the signal recovered after 23 s increased from 46–50 to >60% (Figure 2C and D). In euchromatin, microinjection of HMGN reduced the t40 of H1<sup>0</sup> from 11–13 to 3–4 s (Figure 2F and G). In heterochromatin, where the t40 of H1<sup>0</sup> is ~28 s (data not shown; Misteli *et al.*,

2000), microinjection of HMGN1 or HMGN2 reduced the t40 to <10 s (Figure 2I and J). Control microinjections indicate that histone H<sub>2</sub>B, which binds to chromatin non-specifically, did not affect the mobility of H1<sup>0</sup> (Figure 2E). More significantly, an HMGN1 double point mutant in which two serines in the nucleosomal binding domain were mutated to glutamic acid (HMGN1-S20,24E), which enters the nucleus but does not bind to nucleosomes (Prymakowska-Bosak *et al.*, 2001), did not affect the mobility of H1<sup>0</sup> (Figure 2H). Likewise, an HMGN1 deletion mutant that lacks the C-terminal region that interacts with the N-terminus of histone H3 (Ding *et al.*, 1997; Trieschmann *et al.*, 1998) also failed to affect the mobility of H1<sup>0</sup> (Figure 2K). Thus, the increase in H1<sup>0</sup> mobility is specific to HMGN and is contingent on the ability of HMGN to bind to nucleosomes and interact with the tail of histone H3.

Previous FRAP analysis indicated that, while most of the nuclear H1 is mobile and continuously exchanging, a small fraction is more tightly bound and is significantly less mobile (Lever *et al.*, 2000; Misteli *et al.*, 2000). When we performed FRAP analysis over a period of 4 min, we noticed that HMGN1 has a relatively small effect on the mobility of H1<sup>0</sup>-GFP in heterochromatin (Figure 3A) and no effect on the mobility of H1<sup>0</sup>-GFP in euchromatin (Figure 3B). The HMGN1 effect reflects its stronger binding to heterochromatin (Figure 3C). The similarity in the recovery plateau reached in both control and



**Fig. 2.** Competition between H1<sup>0</sup>-GFP and HMGN proteins for chromatin binding sites. (A) Colocalization of HMGN1 and HMGN2 with H1<sup>0</sup>-GFP. Confocal images of living cells expressing H1<sup>0</sup>-GFP and microinjected with fluorescently labeled HMGN proteins. Scale bar = 5  $\mu$ m. (B) SDS-PAGE analysis of competitors microinjected into the H1<sup>0</sup>-GFP expressing cells. (C–K) Quantitative analysis of FRAP experiments of cells expressing H1<sup>0</sup>-GFP that either were not (blue) or were (red) injected with the proteins indicated in each panel. The values are the mean  $\pm$  SD from at least eight cells of a typical experiment. Arrows on the abscissa point to the time required to reach 40% of the pre-bleach fluorescence intensity. The percentages on the right of the curves indicate the difference in the percentage recovery between the curves within 23 s after photobleaching. Note the differences between euchromatin and heterochromatin and that the controls in (E) and (H) did not affect the mobility of H1<sup>0</sup>-GFP.

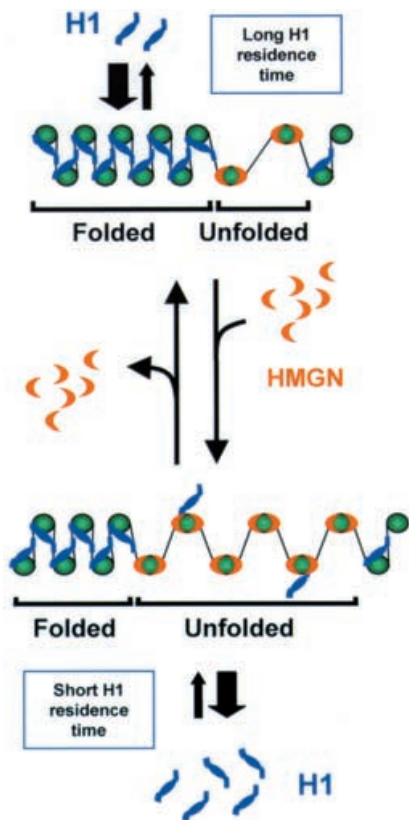


**Fig. 3.** Loss of competition between HMGN1 and H1<sup>0</sup>-GFP in cells treated with compounds that affect the interaction of these proteins with chromatin. (A) FRAP analysis of the effect of HMGN1 on the mobility of H1<sup>0</sup>-GFP in heterochromatin. (B) FRAP analysis of the effect of HMGN1 on the mobility of H1<sup>0</sup>-GFP in euchromatin. Note that HMGN does not affect the immobile fraction of H1<sup>0</sup>-GFP. (C) FRAP analysis of HMGN1 in euchromatin and heterochromatin. (D) FRAP analysis indicating that Act-D treatment decreases the residence time of HMGN proteins on chromatin. (E) FRAP analysis indicating that Act-D treatment abolishes the ability of HMGN proteins to reduce the residence time of H1<sup>0</sup>-GFP on chromatin. (F) FRAP analysis indicating that DRB abolishes the ability of HMGN proteins to reduce the residence time of H1<sup>0</sup>-GFP on chromatin.

injected cells (Figure 3A and B) indicates that HMGN did not affect significantly the mobility of the H1<sup>0</sup> fraction that is more statically bound to chromatin (cf. Figure 2C). Our finding that HMGN proteins enhance the mobility of the rapidly exchanging H1<sup>0</sup> to a significantly larger extent than that of the less mobile H1<sup>0</sup> fraction suggests that HMGN proteins affect the mobility of H1<sup>0</sup> by dynamically competing for binding sites on nucleosomes rather than by displacing tightly bound H1<sup>0</sup> from its chromatin binding sites.

To further confirm that HMGN proteins compete with H1 for binding sites on chromatin, we performed FRAP analysis on cells treated with either 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole

(DRB), a kinase inhibitor that decreases the mobility of H1 (Lever *et al.*, 2000), or actinomycin D (Act-D), a DNA intercalator that displaces HMGN proteins from chromatin (Schroter *et al.*, 1985). Act-D treatment significantly increased the mobility of HMGN1, and the t80 decreased from 5.3 to 3 s (Figure 3D). It also abolished the effect of HMGN1 on the mobility of H1<sup>0</sup>-GFP (Figure 3E; cf. Figure 2C), an expected result since this DNA intercalator releases the H1 and HMGN proteins from chromatin. Likewise, consistent with the expected effects of the interaction of H1<sup>0</sup> with nucleosomes, treatment of cells with DRB also abolished the effect of the injected HMGN on the intranuclear mobility of H1<sup>0</sup> (Figure 3F; cf. Figure 2C). Thus,



**Fig. 4.** Dynamic interplay between HMGN proteins and histone H1 on the surface of the nucleosome. The interaction of H1 with nucleosomes stabilizes the higher-order chromatin structure and promotes the formation of a compacted, folded chromatin fiber. An increase in the local concentration of HMGN proteins, which compete with H1 for shared binding sites on the nucleosome (Alfonso *et al.*, 1994), decreases the H1 residence time at selected chromatin loci and promotes the unfolding of the chromatin fiber.

treatments that interfere with the ability of HMGN proteins to bind to nucleosomes or enhance the interaction of H1<sup>0</sup> with chromatin diminish the ability of HMGN proteins to compete with H1<sup>0</sup> for nucleosome binding sites.

In this study, we combined microinjection and FRAP techniques to study competition between proteins for common chromatin binding sites in living cells. This approach is suitable for studying competition between any proteins that share binding sites. Our findings provide evidence that in living cells HMGN proteins compete with histone H1 for binding sites on chromatin. HMGN mutants that do not bind to nucleosomes do not compete with H1, and drugs that prevent the binding of HMGN proteins to chromatin abolish the ability of HMGN proteins to displace H1. These findings argue strongly that the effects of HMGN proteins on the chromatin residence time of H1 is due to competition for binding sites on nucleosomes. The possible consequence of this competition is schematically illustrated in Figure 4. The scheme takes into account the well-established finding that histone H1 stabilizes the higher-order chromatin structure (Thoma and Koller, 1977; van Holde, 1988), whereas HMGN proteins destabilize this structure and reduce the compactness of the chromatin fiber (reviewed in Bustin,

2001b). Recent FRAP experiments also demonstrated that the residence time of H1 is limited (Lever *et al.*, 2000; Misteli *et al.*, 2000). As illustrated in the scheme, our results raise the possibility that a dynamic interplay between HMGN proteins and histone H1 on the surface of the nucleosome affects the chromatin structure at selected sites. The residence time of H1 on chromatin is significantly longer than that of HMGN proteins, and thus the binding of H1 to chromatin is stronger than that of HMGN proteins. A temporary increase in the local concentration of HMGN proteins could weaken the interaction of H1 at selected chromatin loci, thereby 'unfolding' chromatin and providing a window of opportunity for the binding of regulatory factors to their chromatin targets. Indeed, in the nucleus HMGN proteins are organized into distinct foci, and clusters of contiguous HMGN-containing nucleosomes have been detected in the chromatin fiber (Postnikov *et al.*, 1997).

H1 inhibits the accessibility of regulatory factors to their nucleosomal targets (Herrera *et al.*, 2000; Cheung *et al.*, 2002; Horn *et al.*, 2002) and therefore plays an important role in regulating gene expression in the context of chromatin. By decreasing the residence time of H1 on chromatin, HMGN proteins may reduce the inhibitory effects of the higher-order chromatin structure. Thus, the dynamic behavior of chromatin binding proteins in the cell nucleus plays a role in regulating the structure and activity of the chromatin fiber.

## METHODS

**Cell lines and purified proteins.** Experiments were performed with either a BALB/c 3T3 cell line expressing H1<sup>0</sup>-GFP (Misteli *et al.*, 2000) or in mouse embryonic fibroblasts. Histones, HMGN and HMGN mutants were prepared as described previously (Bustin, 1973; Bustin *et al.*, 1991; Ding *et al.*, 1997; Prymakowska-Bosak *et al.*, 2001). HMGN proteins were fluorescently labeled at a -SH group introduced at position 88 in HMGN1 and 82 in HMGN2 (Hock *et al.*, 1998). Protein concentrations were determined by the Bradford assay (Bio-Rad) and by Coomassie Blue staining of SDS-PAGE of the protein in the solutions used for microinjection.

**Microinjection in cultured cells.** Cells were plated 2 days prior to injection onto Labtek Chambered Coverglass II (Nalgen) in DMEM without phenol-red (Biofluids). Before injection, the medium was replaced with DMEM fortified with 5 mM HEPES pH 7.4. The final concentration of labeled proteins was 0.05 mM and of unlabeled proteins was 0.4 mM (except histone H1, at 0.2 mM) in 50 mM Tris-HCl pH 7.4 (injection buffer). Fluorescent Texas Red labeled high-molecular dextran (Molecular Probes) was added to the injection mixture to identify injected cells. Freshly prepared solutions were clarified by centrifugation at 10 000 g for 30 min at 4°C. In select experiments, the medium contained either Act-D (Sigma) at 5 µg/ml or DRB (Calbiochem) at 100 nM. Cells were microinjected at 37°C using Micromanipulator 5171 and Microinjector 5246 (Eppendorf). Microinjections were performed under air pressure using sterile glass capillaries (Femtotips, Eppendorf). After injection, cells were incubated at 37°C, 5% CO<sub>2</sub>, for at least 30 min before performing FRAP experiments.

**Microscopy and FRAP.** Microscopy and FRAP were performed with a Zeiss LSM 510 confocal microscope using the 488 nm line of an argon laser and the 543 nm line of an HeNe laser as

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described previously (Misteli *et al.*, 2000; Phair and Misteli, 2000). Typically, five pre-bleach images were acquired, followed by a single bleach pulse of 152 ms using a spot of 2  $\mu\text{m}$  in diameter. Single images were then collected with a 152 ms interval. For imaging, the laser power was set to 0.1% of a 25 mW argon laser (488 nm line); and for bleaching, the laser power was set to 100%. FRAP recovery curves were generated from background-subtracted images. The total fluorescence was determined for each image and compared to the initial total fluorescence to determine the amount of fluorescence lost during the bleach and imaging. The fluorescence intensity in the bleach area was normalized to the initial fluorescence in the bleach area. In a typical experiment, several spots, in 8–15 cells, were used for FRAP. Each experiment was repeated at least three times. Student's *t*-test was used to determine the statistical significance of the results.

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## REFERENCES

- Alfonso, P.J., Crippa, M.P., Hayes, J.J. and Bustin, M. (1994) The footprint of chromosomal proteins HMG-14 and HMG-17 on chromatin subunits. *J. Mol. Biol.*, **236**, 189–198.
- Boulikas, T., Wiseman, J.M. and Garrard, W.T. (1980) Points of contact between histone H1 and the histone octamer. *Proc. Natl Acad. Sci. USA*, **77**, 127–131.
- Bustin, M. (1973) Arrangement of histones in chromatin. *Nat. New Biol.*, **245**, 207–209.
- Bustin, M. (2001a) Revised nomenclature for high mobility group (HMG) chromosomal proteins. *Trends Biochem. Sci.*, **26**, 152–153.
- Bustin, M. (2001b) Chromatin unfolding and activation by HMGN(\*) chromosomal proteins. *Trends Biochem. Sci.*, **26**, 431–437.
- Bustin, M., Becerra, P.S., Crippa, M.P., Lehn, D.A., Pash, J.M. and Shiloach, J. (1991) Recombinant human chromosomal proteins HMG-14 and HMG-17. *Nucleic Acids Res.*, **19**, 3115–3121.
- Cheung, E., Zarifyan, A.S. and Kraus, W.L. (2002) Histone H1 represses estrogen receptor  $\alpha$  transcriptional activity by selectively inhibiting receptor-mediated transcription initiation. *Mol. Cell. Biol.*, **22**, 2463–2471.
- Ding, H.F., Bustin, M. and Hansen, U. (1997) Alleviation of histone H1-mediated transcriptional repression and chromatin compaction by the acidic activation region of chromosomal protein HMG-14. *Mol. Cell. Biol.*, **17**, 5843–5855.
- Herrera, J.E., West, K.L., Schiltz, R.L., Nakatani, Y. and Bustin, M. (2000) Histone H1 is a specific repressor of core histone acetylation in chromatin. *Mol. Cell. Biol.*, **20**, 523–529.
- Hock, R., Scheer, U. and Bustin, M. (1998) Chromosomal proteins HMG-14 and HMG-17 are released from mitotic chromosomes and imported into the nucleus by active transport. *J. Cell Biol.*, **143**, 1427–1436.
- Horn, P.J., Carruthers, L.M., Logie, C., Hill, D.A., Solomon, M.J., Wade, P.A., Imbalzano, A.N., Hansen, J.C. and Peterson, C.L. (2002) Phosphorylation of linker histones regulates ATP-dependent chromatin remodeling enzymes. *Nat. Struct. Biol.*, **9**, 263–267.
- Jenuwein, T. and Allis, D.C. (2001) Translating the histone code. *Science*, **293**, 1074–1080.
- Laybourn, P.J. and Kadonaga, J.T. (1991) Role of nucleosomal cores and histone H1 in regulation of transcription by RNA polymerase II. *Science*, **254**, 238–245.
- Lever, M.A., Th'ng, J.P., Sun, X. and Hendzel, M.J. (2000) Rapid exchange of histone H1.1 on chromatin in living human cells. *Nature*, **408**, 873–876.
- Misteli, T., Gunjan, A., Hock, R., Bustin, M. and Brown, D. (2000) Dynamic binding of histone H1 to chromatin in living cells. *Nature*, **408**, 877–881.
- Phair, R.D. and Misteli, T. (2000) High mobility of proteins in the mammalian cell nucleus. *Nature*, **404**, 604–609.
- Postnikov, Y.V., Shick, V.V., Belyavsky, A.V., Khrapko, K.R., Brodolin, K.L., Nikolskaya, T.A. and Mirzabekov, A.D. (1991) Distribution of high mobility group proteins 1/2, E and 14/17 and linker histones H1 and H5 on transcribed and non-transcribed regions of chicken erythrocyte chromatin. *Nucleic Acids Res.*, **19**, 717–725.
- Postnikov, Y.V., Herrera, J.E., Hock, R., Scheer, U. and Bustin, M. (1997) Clusters of nucleosomes containing chromosomal protein HMG-17 in chromatin. *J. Mol. Biol.*, **274**, 454–465.
- Prymakowska-Bosak, M., Misteli, T., Herrera, J.E., Shirakawa, H., Birger, Y., Garfield, S. and Bustin, M. (2001) Mitotic phosphorylation prevents the binding of HMGN proteins to chromatin. *Mol. Cell. Biol.*, **21**, 5169–5178.
- Ring, D. and Cole, R.D. (1979) Chemical cross-linking of H1 to the nucleosomal histones. *J. Biol. Chem.*, **254**, 11688–11695.
- Schroter, H., Maier, G., Ponstingl, H. and Nordheim, A. (1985) DNA intercalators induce specific release of HMG 14, HMG 17 and other DNA-binding proteins from chicken erythrocyte chromatin. *EMBO J.*, **4**, 3867–3872.
- Thoma, F. and Koller, T. (1977) Influence of histone H1 on chromatin structure. *Cell*, **12**, 101–107.
- Thomas, J.O. (1999) Histone H1: location and role. *Curr. Opin. Cell Biol.*, **11**, 312–317.
- Trieschmann, L., Martin, B. and Bustin, M. (1998) The chromatin unfolding domain of chromosomal protein HMG-14 targets the N-terminal tail of histone H3 in nucleosomes. *Proc. Natl Acad. Sci. USA*, **95**, 5468–5473.
- van Holde, K.E. (1988) *Chromatin*. Springer, New York, NY.
- Wolffe, A.P., Khochbin, S. and Dimitrov, S. (1997) What do linker histones do in chromatin? *BioEssays*, **19**, 249–255.

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