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Role of linker histone in chromatin structure and function: H1 stoichiometry and nucleosome repeat length

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Abstract

Despite a great deal of attention over many years, the structural and functional roles of the linker histone H1 remain enigmatic. The earlier concepts of H1 as a general transcriptional inhibitor have had to be reconsidered in the light of experiments demonstrating a minor effect of H1 deletion in unicellular organisms. More recent work analysing the results of depleting H1 in mammals through genetic knockouts of selected H1 subtypes in the mouse has shown that cells and tissues can tolerate a surprisingly low H1 content. One common feature of H1-depleted nuclei is a reduction in nucleosome repeat length (NRL). Moreover, there is a robust linear relationship between H1 stoichiometry and NRL, suggesting an inherent homeostatic mechanism that maintains intranuclear electrostatic balance. It is also clear that the 1 H1 per nucleosome paradigm for higher eukaryotes is the exception rather than the rule. This, together with the high mobility of H1 within the nucleus, prompts a reappraisal of the role of linker histone as an obligatory chromatin architectural protein.

Introduction

The abundance of the H1 class of histones in typical eukaryotic nuclei approaches that of the nucleosome core histones, suggesting that it performs one or more vital functions in the nucleus, and a great deal of effort has been spent elucidating these functions. In addition to the huge body of primary research literature, some 25 review articles relating to various aspects of H1 function have appeared in the last 10 years alone (e.g. Widom 1998, Vignali & Workman 1998, Thomas 1999, Travers 1999, Zlatanova *et al.* 2000, Georgel & Hansen 2001, Brown 2003, Bustin *et al.* 2005), and it is perhaps a measure of the complexity of the problem that the titles of several reviews end with a question mark (e.g. Wolffe *et al.* 1997, Crane-Robinson 1997, 1999, Ausio 2000, Harvey & Downs 2004).

Until a few years ago, the evidence for interrelated dual roles of H1 in chromatin structure and transcriptional regulation seemed well established, but subsequent work on the effects of H1 depletion in a number of organisms has required a reconsideration, especially of the regulatory aspects. More recent studies of the effects of H1 depletion *in vivo* on chromatin structure and function (Fan *et al.* 2003, 2005) have raised additional questions. Here, some of this recent work is discussed and its implications considered. Due to space limitations, many aspects of H1 biology had to be omitted, and, for discussion of other topics, the reader is referred to the recent reviews cited above.

Evidence from *in-vitro* studies

Much of the earlier work on H1 was conducted on soluble chromatin released from nuclei after micrococcal nuclease digestion. The dissociation of H1 from chromatin at a lower ionic strength than the core histones makes it possible to compare H1-containing and H1-depleted chromatin. In a related approach, chromatin may be reconstituted from DNA with the core histones only, or with core histones and H1. These strategies have allowed careful examination of the physical properties of chromatin in the presence and absence of H1. Loss of H1 leads to decondensation of chromatin, a decrease in sedimentation velocity (Bates *et al.* 1981), and, at low ionic strengths, a corresponding change in conformation from a zigzag arrangement of nucleosomes and linker DNA to a more open and randomly folded beads-on-a-string form (Thoma *et al.* 1979, Bednar *et al.* 1998, Carruthers *et al.* 1998). In H1-containing chromatin, an extra ~20 bp of DNA beyond the ~146-bp nucleosome core is partially protected from nuclease digestion (Simpson 1978). This observation, together with studies showing that the globular domain of H1 is located close to the entry–exit sites of linker DNA on the nucleosome (Allan *et al.* 1980, Pruss *et al.* 1996, Goytisolo *et al.* 1996, reviewed in Crane-Robinson 1997, Zhou *et al.* 1998, Widom 1998, Thomas 1999, Travers 1999) provided a structural rationale for the role of H1 in 'sealing' two complete turns of nucleosomal DNA, bringing the entering and exiting linker DNA segments together, a phenomenon that could be directly observed (Hamiche *et al.* 1996, Bednar *et al.* 1998). Since linker histones are rich in lysine and arginine (typically ~60 K+R per molecule residues in mammals), their presence in chromatin will strongly influence the balance of charges in the nucleus. Based on changes in chromatin conformation observed at different mono- and divalent ion concentrations, Clark & Kimura (1990) demonstrated a close fit of observations by Widom (1986) on chromatin compaction *in vitro* to predictions based on the polyelectrolyte theory of Manning (1978), and concluded that the mechanism of chromatin compaction is primarily electrostatic, with H1 playing a key role. Of the three domains present in the linker histones of higher eukaryotes, the central globular region is located on the nucleosome core close to the entry–exit site of linker DNA (Wolffe *et al.* 1997, Thomas 1999, Travers 1999), while the extended C-terminus, which contains a high concentration of lysines, is essential for chromatin compaction *in vitro* (e.g. Allan *et al.* 1986, Carruthers & Hansen 2000, Georgel & Hansen 2001, Lu & Hansen 2003, 2004) and its absence leads to greatly reduced chromatin binding *in vivo* (Misteli *et al.* 2000).

Also relevant to H1 function is a large body of data showing that, at least *in vitro*, H1-containing chromatin shows strong inhibition of transcription (e.g. Shimamura *et al.* 1989, O'Neill *et al.* 1995) and nucleosome sliding (Pennings *et al.* 1994) in comparison with H1-depleted chromatin. Remodelling by SWI/SNF is also modulated by linker histone (Hill 2001, Horn *et al.* 2002, Ramachandran *et al.* 2003). A further connection between H1 and transcription has come from experiments in which chromatin is fractionated into transcriptionally active and inactive components. Transcriptionally active chromatin is typically depleted in H1 compared with inactive chromatin (e.g. Weintraub 1985, Bresnick *et al.* 1992, Smith & Hager 1997) and a recent chromatin immunoprecipitation study has confirmed the phenomenon *in situ* (Kim & Dean 2003). Although there are cases where H1 appears to stimulate transcription of specific genes (reviewed in Brown 2003), these results have led to the concept of H1 as a general repressor of chromatin 'activity' through its contributions to chromatin architecture and compaction, much of which can be attributed to electrostatic interactions.

In-vivo studies

The concept of H1 as a general repressor of chromatin activity has been challenged by *in-vivo* experiments in which H1 has been eliminated or its level dramatically reduced. These experiments were initially focussed on lower eukaryotes where there is typically a single linker histone gene. Surprisingly, knockouts of the macronuclear H1 in Tetrahymena and the H1 analogue Hho1 in budding yeast were viable, and showed relatively minor phenotypic changes (Shen *et al.* 1995, Shen & Gorovsky 1996, Hellauer *et al.* 2001). Rather than the massive transcriptional upregulation that might be anticipated, the levels of the different RNA categories in Tetrahymena were not measurably different, and, in *S. cerevisiae*, a small subset of genes was down-

regulated. However, the H1s of primitive unicellular eukaryotes are typically rather different from their vertebrate counterparts. Hho1 consists of two structured domains rather than the tripartite domain structure of higher eukaryotic linker histones (Ali & Thomas 2004), and its abundance is low (Freidkin & Katcoff 2001, Downs *et al.* 2003). Tetrahymena H1 also has a very different domain structure from metazoan linker histones in that it lacks the most conserved globular domain found in multicellular organisms (Wu *et al.* 1986, Wolffe *et al.* 1997). Interestingly, its loss was accompanied by an increase in nuclear volume (Shen *et al.* 1995), consistent with expectations based on disruption of the electrostatic balance in the nucleus (Clark & Kimura 1990).

Embryogenesis in *Xenopus* continued after depletion of the embryonic H1 subtype B4 (Dasso *et al.* 1994), and, in a cell-free system derived from *Xenopus* eggs, the formation of chromosomes from exogenously added sperm occurred in the absence of H1 (Ohsumi *et al.* 1993). This latter phenomenon has recently been revisited under conditions where the exogenous DNA completed a full round of replication and chromosome segregation (Maresca *et al.* 2005). In this case, H1 depletion resulted in a dramatic lengthening of chromosomes, suggesting an important role of H1 for at least one aspect of mitotic chromosome condensation.

Stoichiometry of H1

A careful quantitation of the stoichiometry of H1 showed that lymphocyte chromatin had almost exactly 1 H1 per nucleosome (Bates & Thomas 1981). These authors also examined chicken erythrocytes, which contain two types of linker histone (H1 and H5) and found, on average, 0.9 H5 and 0.4 H1 per nucleosome, for a total of 1.3 molecules per nucleosome. In contrast, liver nuclei had only 0.7 H1 per nucleosome. Since then, there has been little interest in the accurate quantitation of linker histones, and the generalization of 1 H1 per nucleosome has persisted. However, this topic has now been examined in detail in a number of mouse tissues. In higher organisms, studies on H1 are complicated by the presence of several subtypes, encoded by separate genes. In mice, there are six somatic subtypes (H1a-e and $H1^0$), which differ in primary sequence and in relative abundance from tissue to tissue. In the context of genetic experiments designed to investigate H1 function by knocking out successive H1 subtypes, Fan *et al.* (2003, 2005) quantitated the amounts of each H1 subtype in normal hepatocytes, spleenocytes, thymocytes and embryonic stem (ES) cells. These studies revealed the following cell-specific total levels of H1 per nucleosome: spleenocytes 0.79, thymocytes 0.83, adult hepatocytes 0.79, neonatal hepatocytes 0.76, ES cells 0.5.

Nucleosome repeat length and H1

Eukaryotes exhibit a wide range of nucleosome repeat lengths (NRL), and, in higher organisms, variations occur between species and between cell types within an organism (van Holde 1989). The lowest recorded values (~165 bp) are from budding yeast, and the highest (~220 bp) from echinoderm sperm. For most vertebrate cells and tissues, nucleus-wide NRL values fall between 175 bp and 190 bp, although it should be stressed that these are average values. Certain regions of chromatin, especially gene promoter regions may contain precisely positioned nucleosomes as well as nucleosome-free regions (Yuan *et al.* 2005). In a study comparing rat neuronal and glial chromatin, Pearson *et al.* (1984) reported striking differences in both NRL and H1 content for these neural cell types. While neuronal chromatin had a NRL of 162 bp and an H1 ratio of 0.45 molecules per nucleosomes, the values for glia were 201 bp and 1.04, respectively.

Recent work on several mouse cell types that have been genetically manipulated to have a reduced total H1 content (Fan *et al.* 2003, 2005) has allowed the relationship between NRL and H1/nucleosome to be explored further. The mouse has 6 somatic H1 variants (Parseghian & Hamkalo 2001) as well as testis- and oocyte-specific subtypes, and the systematic knockout of up to three somatic variants has provided animals and embryonic stem (ES) cells with a range of total H1 levels (Fan *et al.* 2003, 2005). For each cell type, the content of each H1 subtype was quantitated by HPLC, and NRL values were determined from micrococcal nuclease digests. These data, together with values from the earlier literature are summarized in Table 1. The mouse values range from wild-type thymus (0.83 H1/nucleosome, NRL 196 bp) to ES cells with deletion of H1c, H1d and

Table 1. Values and sources of data used in Figure 1.

System	NRL (bp)	H1/ Nucleosome	Reference
Wild-type mouse thymus	196	0.83	Fan *et al.* 2003
$H1^0$, H1c, H1e null mouse thymus	189	0.47	Fan *et al.* 2003
Wild-type mouse liver	195	0.79	Fan *et al.* 2003
$H1^0$, H1c, H1e null mouse liver	191	0.64	Fan *et al.* 2005
Wild-type mouse ES cells	189	0.5	Fan *et al.* 2005
H1c, H1d, H1e null ES cells	174	0.25	Fan *et al.* 2005
Chicken erythrocyte	212[a]	1.3	Bates & Thomas 1981
Rat neuron	162	0.45	Pearson *et al.* 1984
Rat glia	201	1.04	Pearson *et al.* 1984
S. cerevisiae	165[a]	0.03[b]	Friedkin & Katcoff 2001
S. cerevisiae	165[a]	0.25[b]	Downs *et al.* 2003

[a]From van Holde, 1989
[b]Values for Hho1.

H1e (0.25 H1/nucleosome, NRL 175 bp). A plot of NRL versus H1 per nucleosomes for the mouse data (Figure 1, red squares) gives a striking fit to a straight line (linear regression $r^2 > 0.95$), indicating a robust linear relationship between these two parameters. A possible rationale for this is that as the amount of linker histones per nucleosome is reduced, charge homeostasis tends to be restored by a reduction in nucleosome spacing. From the slope of the regression line, it can be calculated that the loss of 1 H1 molecule per nucleosome is equivalent to a ~37 bp reduction in NRL. Since a single H1 molecule has ~60 positive charges and 37 bp of linker DNA has 74 negative charges, to a first approximation the variation in NRL with H1 content is consistent with the maintenance of electrostatic charge homeostasis.

Figure 1 also includes values for rat neuronal and glial chromatin (filled circles), (Pearson *et al.* 1984). The glial values closely fit the extrapolated regression line mouse data, while neuronal chromatin follows the trend but falls below the regression line. The NRL (212 bp) and H1 stoichiometry (1.3 linker histone/nucleosome) for chicken erythrocyte nuclei (Bates & Thomas 1981) is very close to the position predicted by the murine regression line (Figure 1, filled diamond).

Extrapolating the regression line to zero linker histone predicts a baseline NRL of ~167 bp. The assembly of new chromatin following DNA replication begins with the formation of core histone octamers, and is followed by the addition of H1. Measurements of the NRL of nascent chromatin reveal that newly replicated HeLa chromatin has an NRL of ~165 bp (Annunziato & Seale 1982), close to the value predicted for H1-free chromatin (Figure 1). Whether this reflects a true reduction in NRL or the high mobility of nascent nucleosomes (Smith *et al.* 1984) is uncertain. However, it is clear that newly replicated chromatin is in a state of rapid flux, and rapidly changes to the mature NRL and H1 content (Annunziato *et al.* 1981, Bavykin *et al.* 1993). Interestingly, in a study of replication in synchronized CHO cells in the presence of hydroxyurea, D'Anna *et al.* (1986) reported that the newly synthesized chromatin had a shortened NRL and was depleted in H1. A partially purified extract from *Xenopus* oocytes showed an ATP-dependent nucleosome positioning activity, with a NRL of ~165 bp (Tremethick & Frommer 1992). Importantly, when exogenous H1 was included in the reaction, the NRL increased to ~190 bp.

A case of a naturally short NRL of ~165 bp is provided by budding yeast (Thomas & Furber 1976, van Holde 1989). As noted above, Hho1, the yeast analogue of H1 (Patterton *et al.* 1998), has a different domain structure (Ali & Thomas 2004) from the canonical H1, and its abundance has been variously reported as 1 per 4 nucleosomes (Downs *et al.* 2003) and 1 per 37 nucleosomes (Friedkin & Katcoff 2001). Both values are consistent with the correlation between NRL and H1/nucleosome (Figure 1, triangles), the lower value falling close to that predicted by the murine data.

The lack of accurate H1 stoichiometries for most other systems precludes quantitative comparisons, but several studies reporting qualitative data are pertinent. The relationship between NRL and H1 levels predicts that the artificial elevation of H1 in a nucleus should be accompanied by an increase in NRL. This effect has been reported by Gunjan *et al.* (1999) who over-expressed $H1^0$ and H1c in cultured mouse fibroblasts. The resulting 1.2–1.4-fold increase in total H1 level was accompanied by an increase in NRL of 15–16 bp. Much earlier, Noll

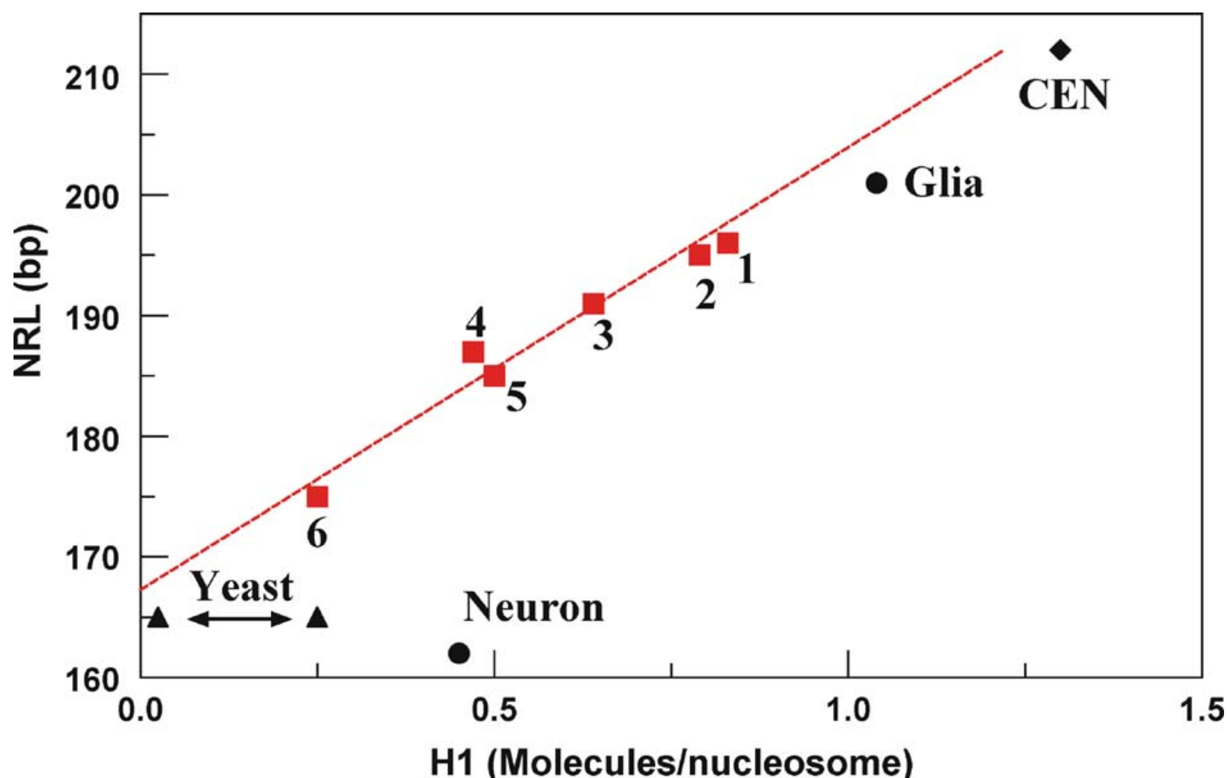

Figure 1. Plot of NRL versus H1 per nucleosome ratio. Squares-points from wild-type and H1-depleted mouse tissues (Fan *et al.* 2003) and ES cells (Fan *et al.* 2005): 1. Wild-type thymus; 2. Wild-type liver; 3. H1-depleted liver; 4. H1-depleted thymus; 5. Wild-type ES cell; 6. H1-depleted ES cell. Dashed line is linear regression for the mouse data, with extrapolation. Circles – Neuron and glia data from Pearson *et al.* (1984). Diamond – Chicken erythrocyte chromatin (CEN). Triangles – *S. cerevisiae* data from Freidkin & Katcoff (2001) and Downs *et al.* (2003).

(1976) and Morris (1976) noted correlations between the low NRL values for *Neurosopora crassa* (170 bp) and *Aspergillus nidulans* (154 bp) and the reduced positive charge of the corresponding linker histones.

Using an *in-vitro* chromatin assembly system based on *Drosophila* egg extracts, Blank & Becker (1995) showed that the NRL of the assembled nucleosome arrays was strongly dependent on the level of cations in the reaction. NRL values increased with increasing concentrations of K^+, Mg^{++}, polyamines, and H1, supporting the importance of electrostatic charge balance, and demonstrating that the phenomenon can occur in a cell-free environment, and is thus in a sense, 'self-organizing.' Furthermore, in a fully defined *in-vitro* chromatin reconstitution system, Stein & Bina (1984) showed that linker histone was necessary to create polynucleosomes with the native NRL; in the absence of H1, nucleosome spacing was shorter and more variable.

Implications of low H1 stoichiometry

Under conditions where the stoichiometry of H1 is less than 1 per nucleosome, there will be some nucleosomes with bound H1 and some without. If the linear relationship between H1 content and NRL also applies to individual nucleosomes, then nucleosomes with H1 should be associated with much longer linkers than those without. Such a mixture of long and short linkers in a nucleus would result in a wide variation in length of DNA extracted from oligonucleosomes, resulting in a significant broadening or smearing of bands in nucleosome ladders. This is clearly not observed, nucleosome ladders from nuclei with high and low H1 content being equally sharp up to $N = 8$ in all cases (Fan *et al.* 2003, 2005). Thus, it appears that, as H1 levels are gradually changed, there is a corresponding gradual rather than quantized change in both local and global NRL, an

observation also made by Blank & Becker (1995) based on their *in-vitro* assembly system.

The question also arises as to whether, in nuclei with less than 1 H1/nucleosome, linker histone is clustered or randomly distributed. It is possible, for example, that compact chromatin such as pericentromeric heterochromatin is able to compete more effectively for H1. However, immunofluorescence (IF) with an anti-H1 antibody showed no large-scale difference in H1 distribution between wild-type and H1-depleted mouse ES cells either in interphase nuclei or metaphase chromosomes (Fan *et al.* 2005). In contrast, electron microscopic examination of individual polynucleosomes ($N = 10$–30) supported very local changes in compaction, with H1-depletion resulting in polynucleosomes being significantly less compact (Fan *et al.* 2005).

Since protein coding sequences comprise a minority of total nuclear DNA, and of this minority only a subset will be primed for transcription in a given cell type, it was likely that global NRL values were representative only of non-coding chromatin, and that transcribable DNA and its associated control sequences could differ. However, Southern blotting showed that the NRL of the adenine deaminase (ADA) 'housekeeping' gene was similar to the global value, and that both the local ADA and global NRL values were reduced to the same extent in H1-depleted nuclei (Fan *et al.* 2005). This result suggests that the relationship between NRL and H1 per nucleosome ratio is valid both for coding and non-coding chromatin.

Cellular response to changes in H1 level

In the mouse, deletion of one or two H1 subtypes results in a compensatory upregulation of other subtypes, resulting in the normal level of H1/nucleosome, and no apparent phenotype (Sirotkin *et al.* 1995, Fan *et al.* 2001, 2003). Knocking out additional subtypes apparently creates a situation in which the upregulation process fails to fully compensate, at least in some tissues. Under these circumstances, a reduction in NRL occurs.

The loss of macronuclear H1 in *Tetrahymena* resulted in an increase in nuclear size (Shen *et al.* 1995) consistent with an expansion of chromatin in response to an electrostatic imbalance. That response does not appear to occur in H1-depleted mouse cells. Thymocyte nuclei are spherical and extremely regular in size, making it possible to detect quite small changes in volume. However, there was no significant change in nuclear volume between thymocytes from animals in which the total H1 per nucleosome was reduced almost 50% (Fan *et al.* 2003). Simple calculation suggests that only ~80% of the electrostatic effect of this reduction in H1 would be compensated by the average 8-bp reduction in NRL observed. It is therefore possible that other compensatory mechanisms such as changes in core histone modifications or an elevation of intranuclear cations or polyamines may occur. In this respect, Fan *et al.* (2005) noted a decrease in H4 acetylation in H1-depleted ES cells.

Life with low H1

Unlike the situation in *Tetrahymena* and budding yeast where cells are viable in the absence of H1, mice show a variety of developmental defects and are embryonic lethal in triple null mutants where total H1 loss approaches 50% (Fan *et al.* 2003). However, ES cells can be propagated from early embryos of both wild-type and mutant animals. The wild-type ES cells are already comparatively low in H1 with 0.5 molecules per nucleosome, and in the triple null mutant cells, this is halved, giving the surprisingly low value of 0.25 H1 per nucleosome (Fan *et al.* 2005). Yet, the mutant ES cells show no obvious changes in morphology or growth characteristics. These results suggest that in the mouse, certain developmental programs require more H1 than do the housekeeping functions needed for cell cycling, consistent with a role for H1 in epigenetic regulation (see below). It will be interesting to determine whether ES cells continue to be viable with even lower H1 levels.

Structural role of H1

What are the implications for chromatin fibre formation of one H1 per four nucleosomes? Clearly, the concept of a 30-nm chromatin fibre structure driven exclusively by the architectural properties of H1 needs to be reconsidered. Although polynucleosomes from both wild-type (0.5 H1 per nucleosome) and H1-depleted (0.25 H1 per nucleosome) ES cells appear much more irregular than their counterparts

from rat liver or chicken erythrocytes, they nevertheless adopt a fibre conformation at about the same salt concentrations as rat liver and chicken erythrocyte chromatin (Fan *et al.* 2005). These fibres are much more organized and compact than chromatin from which H1 has been totally depleted *in vitro* (e.g. Thoma *et al.* 1979). Recent work with highly uniform reconstituted arrays of nucleosomes has demonstrated that under certain conditions, most notably a high divalent cation concentration, arrays of nucleosomes can adopt a zigzag conformation in the absence of H1 (Dorigo *et al.* 2004, Schalch *et al.* 2005). At present, very little is known about the concentrations of di- or polyvalent cations in the nucleus, but it is conceivable that locally high levels could be involved in organizing chromatin.

The role of H1 in the formation and stability of mitotic chromosomes remains unclear. In the absence of H1, chromosomes assembled and replicated in a *Xenopus* egg extract failed to compact properly, leading to severe segregation anomalies (Maresca *et al.* 2005). However, ES cells lacking three H1 subtypes and containing only one H1 per four nucleosomes showed no overt cytological abnormalities at mitosis, and had a division time comparable to the wild-type (Fan *et al.* 2005). In normal cycling cells, H1 undergoes phosphorylation which culminates at cell division, and it has been postulated that this weakens its binding to chromatin, allowing the profound structural changes that accompany chromosome formation (Roth & Allis 1992). H1 phosphorylation has also been implicated in the regulation of chromatin remodelling (Horn *et al.* 2002). It will be interesting to see whether H1 phosphorylation continues to cycle in the same manner in H1-depleted cells.

H1 mobility and stoichiometry

The finding that H1 in living nuclei was highly mobile, with a residence time of about 1 min (Misteli *et al.* 2000, Lever *et al.* 2000) confirmed earlier *in-vitro* studies (reviewed in Widom 1998) indicating that H1 was in a state of free exchange. These findings suggested that modulations in the transcriptional competence of a gene could be achieved by the binding of activator molecules to control sequences during the temporary dissociation of H1 molecules (reviewed in Brown 2003, Bustin *et al.* 2003). H1 mobility, combined with an H1 content of less than one per nucleosome is likely to increase the potential for chromatin modulation and access to chromatin by regulatory complexes. Further, chromatin structures influenced by H1, such as the 30-nm fibre, will probably also be in a highly dynamic state. Whether the mobility and 'sharing' of H1 among nucleosomes also contributes to the lack of quantized nucleosome spacings discussed above remains to be determined.

H1 and transcriptional regulation

As noted above, despite its action as a powerful inhibitor of transcription from chromatin templates *in vitro*, H1 removal has only a minor effect on mRNA synthesis in *Tetrahymena* and *S. cerevisiae*. DNA chip comparison of mRNA species and abundance in wild-type and triple H1 null ES cells revealed a similar subtle effect (Fan *et al.* 2005). Interestingly, a disproportionate number of the genes with altered expression in the mutant cells are regulated epigenetically via DNA methylation, and also showed aberrant DNA methylation in their control regions. These results suggest a new and unexpected aspect to H1 function, unrelated to chromatin structure. Whether this function causes the embryonic lethal developmental failures in H1-depleted mice (Fan *et al.* 2003) remains to be seen. One concept related to transcriptional control that continues to hold is that H1 content increases during terminal differentiation of cells, a period of progressive shut down of groups of genes. Thus, at least for vertebrate systems, the maximum level of total linker histone is found in the fully differentiated and transcriptionally inert avian erythrocyte, and the minimum in pluripotent ES cells (Table 1).

Conclusions

One of the surprising, and perhaps sobering, conclusions to emerge from recent work on H1 is the extent to which the results from earlier *in vitro* experiments have been misleading. Subsequent *in-vivo* work has required a reappraisal both of the concept of H1 as a general transcriptional repressor, and its role as a chromatin architectural protein. The ability to create cell lines with increasingly low levels of total H1 will probably lead to more insights

in the future – it will be especially interesting if viable mammalian cells can be created with no H1 at all.

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