HMGN proteins play roles in DNA repair and gene expression in mammalian cells

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Abstract

HMGN (high-mobility-group N) family members are vertebrate proteins that unfold chromatin and promote transcription and replication of chromatin templates in vitro. However, their precise roles in vivo have been elusive until recently. This paper summarizes recent advances from studies of Hmgn1 knockout mice and genetically engineered cell lines that are beginning to reveal the diverse roles that HMGN proteins play in DNA repair and transcription within mammalian cells.

HMGN (high-mobility-group N) proteins and chromatin structure

The DNA in eukaryotic cells is organized by histones and other proteins into chromatin which is comprised of arrays of nucleosomes. Significant advances have been made in recent years in understanding how the modification and remodelling of individual nucleosomes contributes to chromatin structure and transcription. Architectural proteins such as linker histones and HMGN are also important for the non-enzymic regulation of chromatin structure.

HMGN (formerly HMG-14/-17) proteins alter chromatin architecture by decompacting the nucleosomal array (reviewed in [1,2]). Studies on minichromosomes have revealed that two HMGN molecules bind to each nucleosome via their highly conserved nucleosome-binding domains [1]. The negatively charged C-terminal domains induce chromatin unfolding such that the minichromosomes have a decreased sedimentation rate in a sucrose gradient and are more accessible to nucleases [3,4]. The functional consequences of this change in architecture are increases in the rates of transcription and replication [3–5]. Functional links between HMGN proteins and transcription have also been suggested by studies on intact cells. Various reports have shown that HMGNs may be preferentially associated with actively transcribed genes [1,2], and intracellular localization experiments have shown that HMGNs co-localize with nascent transcripts [6].

The role of HMGN proteins in vivo

Although there is strong evidence that HMGNs can modulate transcription, further details about their precise roles in vivo have remained elusive. This can be partly attributed to the fact that HMGN proteins are only found in higher eukaryotes; hence genetic studies in organisms such as yeast and Drosophila have been unable to provide clues regarding their interaction partners or gene targets. The recent generation of Hmgn1 knockout mice has provided the first opportunity to investigate the roles of HMGN proteins in whole organisms [8], and should lead to a significant advancement in our knowledge of how this family of chromatin proteins contribute to growth and development.

Hmgn1−/− mice appear normal, although their fertility is somewhat reduced and the expression of some genes is altered [8]. On further investigation, Birger and co-workers discovered that the mice and cells derived from them have significantly increased sensitivity to UV irradiation. It was shown that Hmgn1−/− cells repair UV-damaged DNA at a lower rate than wild-type cells do, and the evidence supports a model whereby HMGN1 opens up chromatin at the sites of DNA damage, allowing the DNA repair machinery greater access to repair the lesion [8].

The mild phenotype of the Hmgn1−/− mice raises the question of whether the other HMGN family members can compensate for the loss of Hmgn1. There are currently four known members of the HMGN family. HMGN1 and HMGN2 have been studied for more than 30 years, whereas HMGN3 and HMGN4 were discovered more recently [9,10]. HMGN1 and HMGN2 have indistinguishable functions in vitro, but do not co-localize within the nuclei of living cells [11], indicating that they may perform distinct roles in vivo. Furthermore, the tissue-specific expression patterns of all four family members are different [9,10]. In situ hybridization studies have shown that HMGN2 is highly expressed in layers of the developing kidney that are undergoing differentiation [12,13]. This has given rise to the suggestion that HMGN proteins may act as general transcription facilitators, whose expression is induced in tissues that require many genes to be activated at one time, e.g., during differentiation. In this scenario, it might be predicted that the expression of any HMGN family member could fulfill the required function. An alternative hypothesis is that each HMGN protein can modulate the expression of a distinct subset of genes, through targeting by specific protein-interaction partners.

Key words: chromatin, glycine transporter, HMGN, microarray, repair, transcription

Abbreviation used: HMG-14/-17, high-mobility-group N

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Regulation of gene expression by HMGN proteins

To investigate whether HMGN proteins can modulate the expression of specific genes, we chose to study one of the newer HMGN family members, HMGN3 [14]. HMGN3 is unique in the HMGN family as it is expressed as two splice forms, HMGN3a and HMGN3b [10]. HMGN3b lacks much of the C-terminal chromatin unfolding domain, and so may not be able to unfold chromatin in the same way as the full-length HMGN proteins. However, HMGN3b does have the ability to modulate transcription, as it can interact with the thyroid hormone receptor and promote thyroid hormone-dependent transcription from a chromatin template [15].

HMGN3a or HMGN3b were overexpressed in Hepa 1 cells, a hepatoma cell line that has very low endogenous levels of HMGN3. Microarrays were used to analyse the gene expression profiles of several independent clones to determine whether HMGN3a or HMGN3b could alter patterns of gene expression [14]. We found that the expression of approx. 0.8% of genes was altered in the presence of HMGN3a, with HMGN3b modulating the expression of a distinct, but overlapping set of genes. Of particular interest was the glycine transporter Glyt1, as both GLYT1 and HMGN3 are reported to be expressed in glia cells in the brain and in the eye. Reverse transcriptase-PCR confirmed that Glyt1 expression was increased in cells stably expressing HMGN3a, and also in cells transiently expressing either HMGN3a or HMGN3b. Immunohistochemistry confirmed that HMGN3 and GLYT1 are co-expressed in the mouse retina, supporting the suggestion that HMGN3 may be a regulator of Glyt1 expression in its natural context [14].

These studies do not reveal whether HMGN3 is a direct regulator of Glyt1 expression, or whether it acts via an intermediary. To clarify this, we performed chromatin immunoprecipitation assays to investigate whether HMGN3 is bound at the endogenous Glyt1 gene. We found that HMGN3 was enriched in a 3.5 kb region encompassing the transcriptional start site and 3 kb of the downstream transcribed sequence (Figure 1). This supports a direct role for HMGN3 in Glyt1 expression, and suggests that HMGN3 may function at the level of transcription initiation, or the initial stages of elongation [14].

Summary

In conclusion, recent studies on Hmgn1−/− knockout mice and on cells engineered to express HMGN3 are beginning to reveal the diverse functions that HMGN proteins play within mammalian cells. They have general roles, such as increasing access to DNA lesions by DNA repair complexes, and more specific functions, such as the activation of Glyt1 transcription. Further studies on knockout mice and genetically engineered cell lines will enable us to probe further into the complexities of HMGN function, allowing us to ask questions about overlapping roles of different family members, and how they are targeted to specific functions within the cell.

References

7. Reference deleted

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