The Hmgn family of chromatin binding proteins regulate stem cell pluripotency and neuronal differentiation

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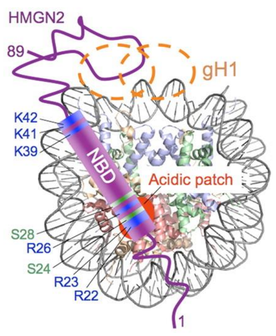
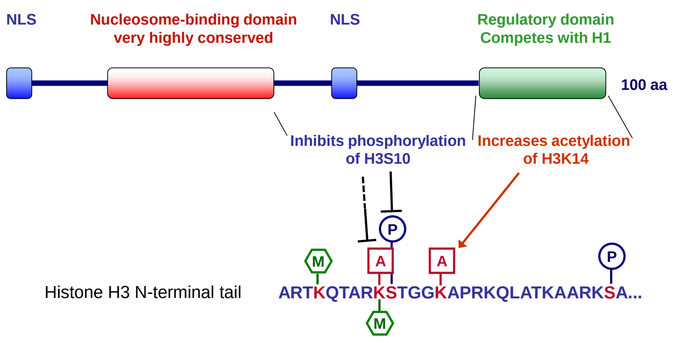
Hmgn proteins

- Bind as dimers to nucleosomes
- Regulate the deposition of histone modifications
- Compete with linker histones
- Interact with several transcription factors.



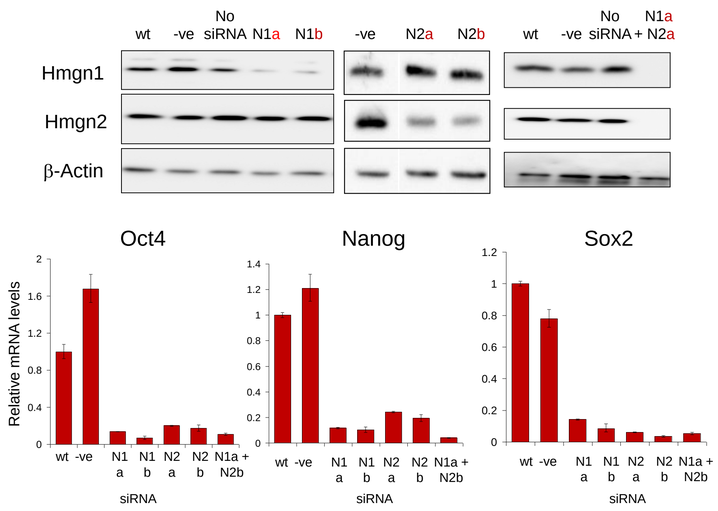
Kato H et al. PNAS 2011;108:12283-12288



Summary: *Hmgn1* and *Hmgn2* are ubiquitously highly expressed in embryonic tissue, and are progressively downregulated during embryogenesis. Using RNAi, we have shown that Hmgn proteins are essential for the expression of key pluripotency genes, including *Oct4* and *Nanog*, in embryonal carcinoma cells. ChIP experiments show that levels of H3K4me3 at the promoters of *Oct4* and *Nanog* are decreased in Hmgn2 knockdown cells, whereas levels of H3K27me3 are increased. This provides a molecular basis for the observations that an *Hmgn2* knockout is embryonic lethal, and that manipulating the *Hmgn* content of early *Xenopus* embryos causes major developmental defects.

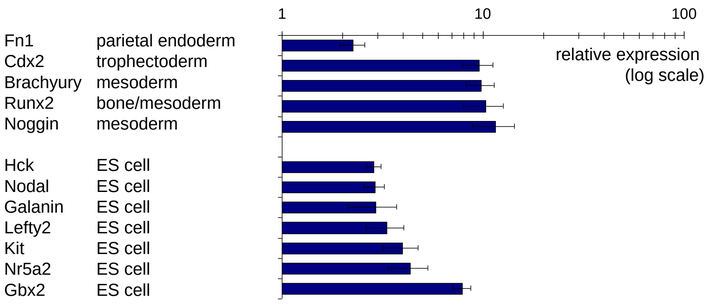
We have also shown that Hmgn proteins are very highly expressed in neural stem cells, both during embryogenesis and in the adult mouse brain. We studied the effects of *Hmgn* knockdown on the neuronal differentiation of embryonal carcinoma cells *in vitro*. The expression levels of various neural stem cell and neuronal genes were altered following *Hmgn* knockdown, showing that Hmgn proteins are important for accurate neuronal differentiation and function.

siRNA knockdown of Hmgn1 or Hmgn2 reduces the expression of pluripotency markers in pluripotent embryonal carcinoma cells.

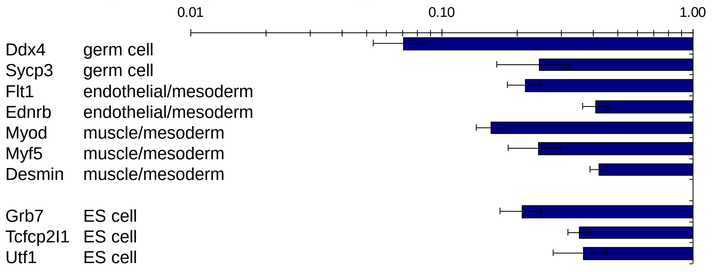


PCR array analyses reveal that several markers of early differentiation are upregulated in Hmgn-knockdown EC cells. The expression of several genes required for ES self renewal and pluripotency is also disrupted.

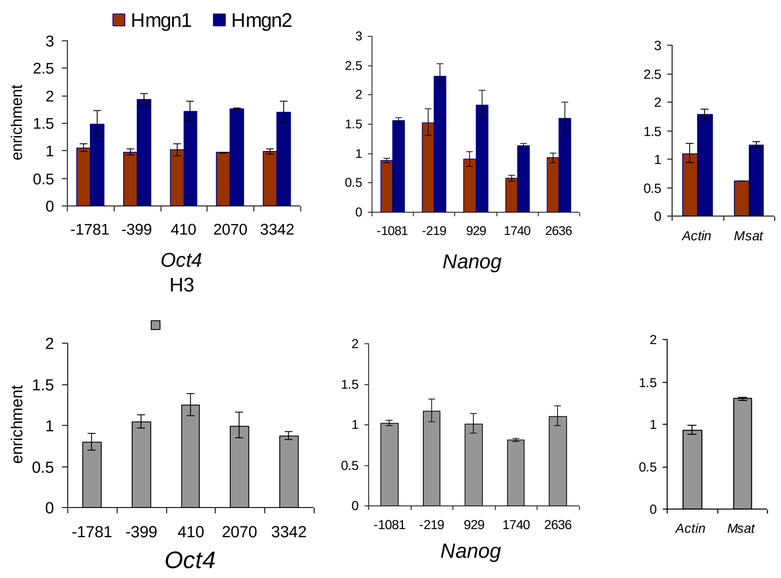
Upregulated genes in Hmgn knockdown cells



Downregulated genes in Hmgn knockdown cells

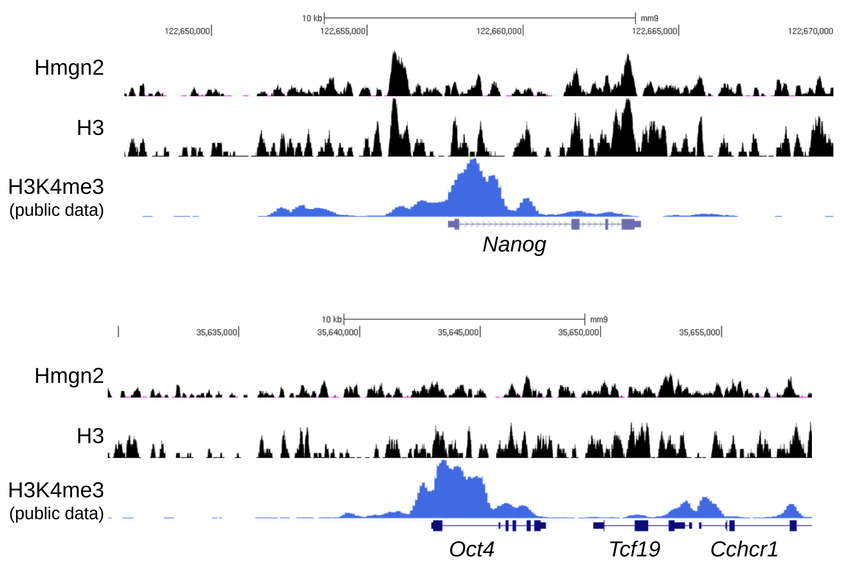


Hmgn proteins are enriched across the *Oct4* and *Nanog* gene loci

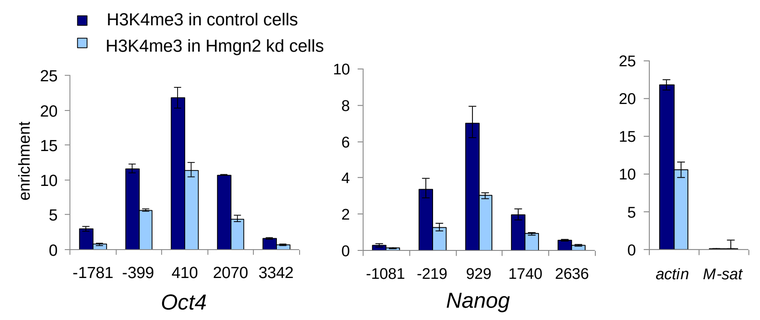


Hmgn1 and Hmgn2 binding profiles in control cells. Enrichment at each PCR set was normalised to input and then normalised to average H3 enrichment across all primer sets to control for differences between chromatin preps.

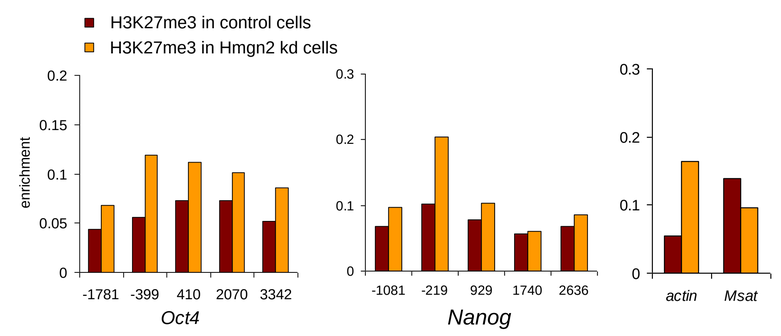
ChIP-seq reveals peaks of Hmgn2 and H3 upstream of *Nanog*



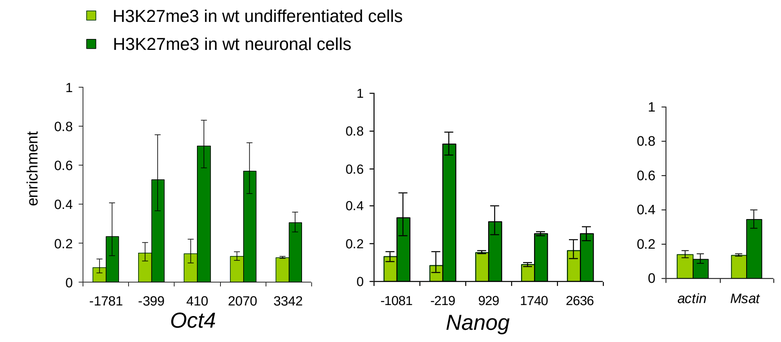
Knockdown of Hmgn2 reduces H3K4me3 on *Oct4* and *Nanog*



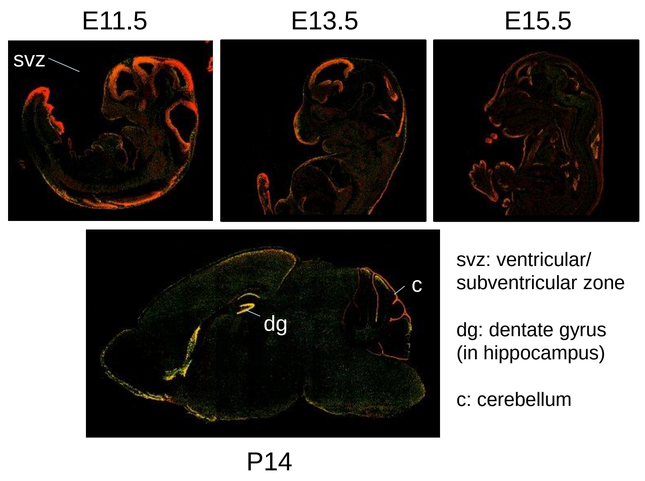
Knockdown of Hmgn2 increases H3K27me3 on *Oct4* and *Nanog*



H3K27me3 on *Oct4* and *Nanog increases in day 3 neuronal cells*



Hmgn2 is expressed in neural progenitor cells during embryogenesis

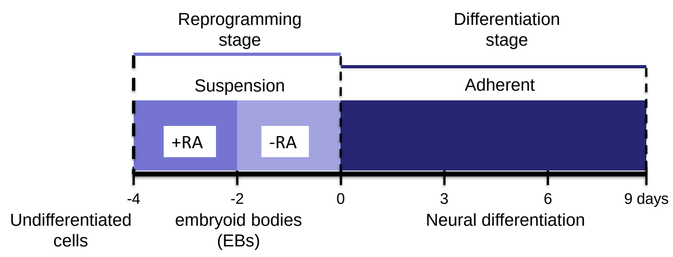


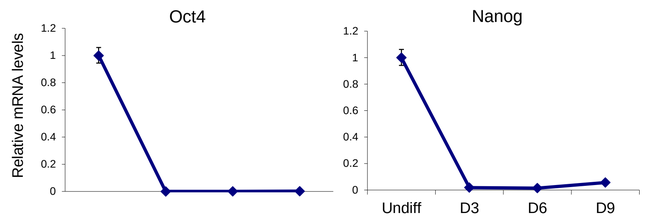
svz: ventricular/ subventricular zone

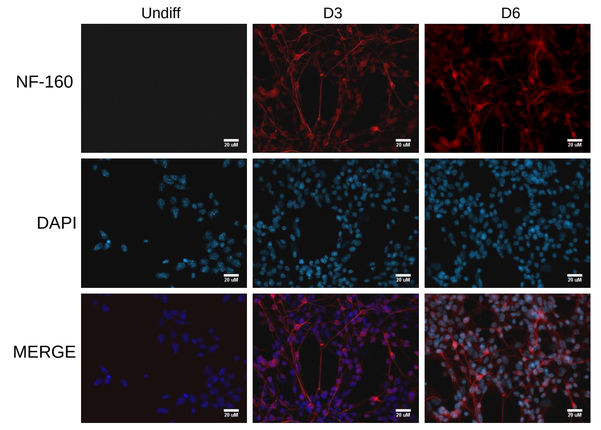
dg: dentate gyrus (in hippocampus)

c: cerebellum

Retinoic acid-induced differentiation of EC cells down the neuronal lineage.

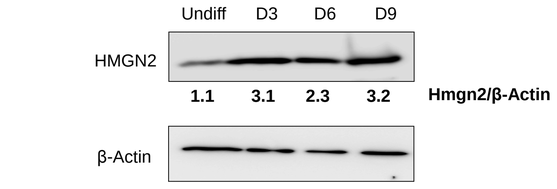






- Pluripotency markers Oct4, Sox2 and Nanog are repressed during differentiation.
- Induction of neuronal markers Map2, NF-160, nestin, NSE and NMDAR-2 is observed from day 3.
- Induction of glial marker GFAP from day 9.

Hmgn2 levels increase during neuronal differentiation of EC cells



Expression of several neuronal genes is significantly altered in day 3 immature neuronal cells following *Hmgn1* and/or *Hmgn2* knockdown.