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Experimental Cell Research 304 (2005) 593–603
Experimental Cell Research
www.elsevier.com/locate/yexcr

FoxA1 binding to the MMTV LTR modulates chromatin structure and transcription

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Received 4 October 2004, revised version received 3 December 2004
Available online 30 December 2004

Abstract

Novel binding sites for the forkhead transcription factor family member Forkhead box A (FoxA), previously referred to as Hepatocyte Nuclear Factor 3 (HNF3), were found within the mouse mammary tumor virus long terminal repeat (MMTV LTR). The effect of FoxA1 on MMTV LTR chromatin structure, and expression was evaluated in *Xenopus laevis* oocytes. Mutagenesis of either of the two main FoxA binding sites showed that the distal site, −232/−221, conferred FoxA1-dependent partial inhibition of glucocorticoid receptor (GR) driven MMTV transcription. The proximal FoxA binding segment consisted of two individual FoxA sites at −57/−46 and −45/−34, respectively, that mediated an increased basal MMTV transcription. FoxA1 binding altered the chromatin structure of both the inactive- and the hormone-activated MMTV LTR. Hydroxyl radical foot printing revealed FoxA1-mediated changes in the nucleosome arrangement. Micrococcal nuclease digestion showed the hormone-dependent sub-nucleosome complex, containing ~120 bp of DNA, to be expanded by FoxA1 binding to the proximal segment into a larger complex containing ~200 bp. The potential function of the FoxA1-mediated expression of the MMTV provirus for maintenance of expression in different tissues is discussed.

Keywords: FoxA1; HNF3-α; Mouse mammary tumor virus; MMTV; Gene regulation; Chromatin; Glucocorticoid receptor; Pioneer protein; *Xenopus* oocyte; Retrovirus

Introduction

The intranuclear DNA of all eukaryotic cells is packed into chromatin, where the smallest structural unit is one nucleosome. The nucleosome core particle consists of a histone octamer, and organizes 147 bp of the DNA [1]. However, the chromatin is not a static structure. Remodeling of the local chromatin structure is a vital process since nucleosomes may obscure the DNA, making it partly inaccessible for many DNA binding proteins [2]. All processes involving DNA, such as transcription, replication, recombination and DNA repair, therefore require that the histone-DNA contacts become at least partially disrupted over the stretch of DNA where these processes are to take place. When it comes to transcription, packing of DNA into chromatin disables access of the basal transcription machinery, such as TATA-box binding protein and RNA polymerases, thus preventing genes from being expressed at random [3,4]. A critical step in gene activation is therefore the targeting of chromatin structural changes to the correct site. Binding of so-called pioneer transcription factor(s), i.e., proteins capable of binding to its cognate DNA segments even when organized into chromatin [5], are considered to trigger a stepwise chromatin opening and switching from inactive to the active chromatin.

In the mouse mammary tumor virus (MMTV) promoter, the hormone-activated glucocorticoid receptor (GR) binds to a cluster of glucocorticoid response elements (GREs), and triggers nucleosomal rearrangement [6], probably by recruitment of co-activators. The histone acetyltransferases p300 and pCAF, as well as Brg1, a subunit of the chromatin remodeling complex SWI/SNF, has been shown to be

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doi:10.1016/j.yexcr.2004.12.002

recruited to a GR induced MMTV promoter in T47D cells [7]. In tissue culture cells, the 1.2-kb MMTV LTR is organized into six translationally positioned nucleosomes termed A–F, where A is located at the transcriptional start site [8]. Hormone activated chromatin-remodeling results in increased accessibility of the GRE containing B-nucleosome located approximately at position −210/−60. This decondensation of the activated B-nucleosome is revealed as a DNase I hypersensitive site [8,9] as well as a hydroxyl radical hypercutting region [6,8,10]. When an MMTV LTR-driven reporter gene is microinjected into *Xenopus laevis* oocytes, the nucleosomes covering the MMTV LTR remain randomly positioned in the inactive promoter and a translational nucleosome positioning is induced by the hormone receptor-mediated activation [6].

Forkhead box A1 (FoxA1), previously referred to as Hepatocyte Nuclear Factor 3-α (HNF3-α), is a member of the forkhead transcription factor family that is involved in several aspects of metazoan development [11]. FoxA1 is expressed from gastrulation and during the development of neural tube, brain, intestine, and liver [12–14]. Furthermore, FoxA1 is expressed in adult liver, from which it was first purified [15], as well as adult lung and in the small intestine, albeit at low level [16,17]. Fox proteins are structurally similar to the linker histones H1 and H5 [18,19]. However, in contrast to linker histones, DNA binding of FoxA1 is sequence specific (reviewed by Kaufmann and Knochel [20]). In addition, the C-terminal domain of FoxA1 binds to core histones [5]. It has been speculated that FoxA, and probably also other Fox proteins, replace linker histones in order to open chromatin and anchor nucleosomes to specific sites, leading to stabilization of specific nucleosomal arrays and translational positioning. It has indeed been found that FoxA1 position nucleosomes in the mouse serum albumin enhancer in vivo [21] and that it invades and remodels compacted chromatin in vitro [5].

Here, we show that FoxA1 binds to specific sites within the MMTV LTR in vivo and by doing so alters the chromatin structure and affects transcription. FoxA1 binding sites are found at position −225, −51, and −39 relative to transcription start site, thus within the regions flanking the nucleosome B DNA region. We find that binding of FoxA1 fails to induce nucleosome positioning in the absence of hormone-activated GR. However, the hormone- and receptor-dependent nucleosome array of the MMTV LTR shows distinct FoxA1-dependent changes involving a more open chromatin structure over the C-nucleosome. Furthermore, the previously reported hormone-dependent sub-nucleosomal B-particle, seen by micrococcal nuclease (MNase) treatment [6,22], is transformed into a larger complex, probably encompassing the proximal FoxA site, indicating binding of FoxA1 to a hormone and receptor organized enhanceosome structure. We also discuss the potential role of forkhead proteins in maintenance of MMTV expression in different tissues.

Materials and methods

Plasmids

The construction of pMMTV:M13, harboring the 1.2-kb MMTV LTR fused to the herpes simplex thymidine kinase gene at position +137 of the MMTV promoter, was previously described [6].

Construction of pMMTVΔ^{-428}:M13 deleted at the −428 position, i.e., in the linker between nucleosome C and D [6] was done by a PCR reaction with oligos containing an *Xho*I site in the 5′ end and containing the −428/−407 MMTV LTR coding strand (5′-GATCTCGAGCCAGAGCTAGACCTCCTTGGT-3′) and the polylinker oligo (5′GATCTCGAGGTACCCAATTCGCCCTATAGT-3′) priming in the opposite direction of the pBSMTV:Tk(−Xho1), i.e., the same MMTV:Tk construct as above but moved into the pBluescript® vector (Stratagene). After trimming with *Xho*I and ligation, the so obtained construct was transferred into M13 mp18 by usage of the *Kpn*I and *Xba*I restriction sites. The M13 mp18 construct was used for preparation of ssDNA.

A ΔFoxA^{-225} MMTV LTR mutant, lacking the upstream FoxA-site, was constructed by the guidance of a mutant unable to bind FoxA1 described before [23]. Primers 5′CTTTGCTAGCTACCAACCTTGCGGTTCCC and 5′CTTCCTAGGAGGCTAATCATAATAACTCATACCATAAGT were used in a PCR with pMMTVΔ^{-428}:M13 as template. The PCR product was trimmed with *Nhe*I and *Avr*II, purified from a 0.7% agarose gel, and ligated.

As the downstream MMTV LTR FoxA-site mutant, ΔFoxA$^{-51/-39}$, a previously constructed Oct1-site mutant, pMMTVΔOctdΔOctp:M13 [24] was used. This mutant was used to knockout Oct1 binding, and thereby also mutated the overlapping FoxA site.

The FoxA1/RN3P vector for FoxA1 mRNA production was generated by PCR-amplified FoxA1-cDNA of pET28b-mHNF3α [23] with primers 5′GTCAGAATTCACAGCCACCATGTTAGGGACTGTGAAGATGG and 5′GTCAGCGGCCGCTCAGGAAGTATTTAGCACGGGTCTG. The PCR product was trimmed with *Eco*RI and *Not*I and ligated into the RN3P vector [25] that had been cleaved with the same two enzymes. The plasmid was linearized with *Asp 718* and in vitro transcribed by mMESSAGE mMACHINE kit (Ambion).

The construction of the pβGR/RN3P for production of mRNA for ratGR was described before [6].

Oocyte injections

DNA and mRNA injections into *X. laevis* oocytes where described previously [6]. Here 3 ng ssDNA was injected into the nucleus and 3 ng GR mRNA and 1.5 ng FoxA1 mRNA was injected into the cytoplasm where indicated. Also, injection of reporter construct AdenoMajorLate (AdML) as a control was omitted.