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APRIN is a unique Pds5 paralog with features of a chromatin regulator in hormonal differentiation

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Abstract

Activation of steroid receptors results in global changes of gene expression patterns. Recent studies showed that steroid receptors control only a portion of their target genes directly, by promoter binding. The majority of the changes are indirect, through chromatin rearrangements. The mediators that relay the hormonal signals to large-scale chromatin changes are, however, unknown. We report here that APRIN, a novel hormone-induced nuclear phosphoprotein has the characteristics of a chromatin regulator and may link endocrine pathways to chromatin. We showed earlier that APRIN is involved in the hormonal regulation of proliferative arrest in cancer cells. To investigate its function we cloned and characterized APRIN orthologs and performed homology and expression studies. APRIN is a paralog of the cohesin-associated Pds5 gene lineage and arose by gene-duplication in early vertebrates. The conservation and domain differences we found suggest, however, that APRIN acquired novel chromatin-related functions (e.g. the HMG-like domains in APRIN, the hallmarks of chromatin regulators, are absent in the Pds5 family). Our results suggest that in interphase nuclei APRIN localizes in the euchromatin/heterochromatin interface and we also identified its DNA-binding and nuclear import signal domains. The results indicate that APRIN, in addition to its Pds5 similarity, has the features and localization of a hormone-induced chromatin regulator.

Keywords: APRIN; Hormonal regulation; Proliferative arrest; Chromatin; Cancer; HMG domain; AT-hook; Pds5

1. Introduction

The ability of cells to undergo proliferative quiescence is critical in development and it emerged in multicellular organisms [1]. A major component of its regulation is hormonal [2,3] and it is part of the differentiation program in reproductive tissues [4,5]. Steroid hormones play a critical role in differentiation and induce massive changes in gene expression patterns. Microarray analysis showed that approximately 3.2–4.3% of the genes tested were hormone regulated, both *in vitro* [6] and *in vivo* [7]; these changes were also observed at the protein level [8]. Consequently, androgen regulation may affect a total of ∼1200–4000 genes in the human genome, on the basis of ∼28,000–30,000 coding and ∼100,000 non-coding genes by recent estimates [9]. In contrast, analyses of receptor–promoter complexes and confocal microscopy detected only 250–300 androgen receptor sites per nucleus [10] and only a handful has been characterized [7].

These data together with recent chromatin immune-crosslinking analyses strongly suggest that steroid receptors control only a portion of the target genes directly [11]. The global changes in gene expression patterns are mostly the results of indirect mechanisms [12] including non-genomic membrane receptor effects and chromatin changes. The long-term, differentiation-related hormonal changes, however, argue against the participation of plasma membrane-based mechanisms because molecules involved in these pathways reach a peak in a few minutes and their effects last only for a couple of hours [13]. Chromatin involvement, on the other hand, was confirmed by receptor localization studies [14,15], receptor interaction assays [16] and by detection of global rearrangements of the chromatin architecture [17]. Hormone-induced chromatin changes, in turn, can regulate the expression of large sets of genes [18]. The mediators

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and the mechanisms that translate the hormonal signals into large-scale chromatin changes are mostly unknown.

To explore these mechanisms, we used the hormone-sensitive LNCaP cell line [19–21] in which the transition to hormone-induced proliferative arrest [22,23] has been shown to coincide with chromatin changes [17]. We isolated proliferation arrest-specific genes [23] and showed that a newly identified gene, APRIN (formerly AS3) has a critical role in proliferative arrest in the G0/G1 phase of cell cycle [24,25]. We found that loss of heterozygosity (LOH) in the D13S171 marker within the APRIN genomic sequence [26,27] linked this protein to a variety of cancers, including prostate cancer [28–31]. The molecular mechanisms of the APRIN-mediated proliferative arrest and its association with cancer, however, remain unknown.

A commonly used approach to assess the molecular mechanisms of proteins is to establish their functional domains [32,33]. Phylogenetic sequence analysis is a conservation-based method to identify functional domains and related proteins [34,35]. Conservation correlates with functional significance and identifies biologically relevant structural units [36]. We cloned and sequenced human and rodent ortholog APRIN cDNAs, computed the local conservation differences within small subdomains and established putative functional units. APRIN shares similarities with the ancient Pds5 (precocious dissociation of sister chromatids) gene lineage. Proteins of this family are involved in chromatid cohesion, so a cohesion-related function was proposed for APRIN [37]. The physical association of APRIN with chromatin has been demonstrated, but its cohesin association showed low affinity and a mechanistic role in cohesion remains to be confirmed [38].

The domain and structural differences we report here, however, turned the APRIN functional model to a new direction. We show that APRIN is only a paralog, and it diverged from the Pds5 lineage by gene duplication. The accumulated conservation differences, domain acquisitions in APRIN and its chromatin localization indicate a new functional entity that shares features with chromatin architectural regulators.

2. Materials and methods

2.1. Tissue culture and cell lines

The MCF7-AR1 cell line, a human androgen receptor-transfected MCF7 cell line was established in this laboratory [39]. This cell line and the LNCaP human prostate cancer cells were routinely maintained in DMEM (Dulbecco's modified Eagle's medium) medium supplemented with 5% fetal bovine serum (FBS). We kept the cells in 5% hormone-stripped FBS media for 3 days and treated them with 10 nM methyltrienolone (R1881) (NEN Life Science Products, Boston, MA), a synthetic androgen. For transfection experiments we used Lipofectamine 2000 following the manufacturer's instructions (Invitrogen, San Diego, CA).

2.2. Computer methodology

For similarity searches we used GenBank softwares (PairwiseBlast, Nucleotide Blast and Protein Blast) and the GCG program package (Genetics Computer Group, Madison, WI) including BestFit, GrowTree, Frames and Translate programs; for motif search, the Motifs, ProfileScan, CoilScan, and the HTHScan programs were used. In the alignment BLASTN program (version BLASTN 2.2.6), the default values were used in the score calculations: (a) 1 and −2 matrix values for match and mismatch, respectively; (b) for scoring gap penalties 5 and 2 were used for existence and extension; (c) the x_dropoff value was 15 and (d) for expect number and wordsize values we used 10 and 11, respectively. For domain search we used the HmmerPfam program and other web-based resources (www.sanger.ac.uk/Software/Pfam/).

2.3. Isolation and cloning of the mouse and rat APRIN cDNAs

We used RT-PCR methodology to isolate the full-length mouse and rat APRIN cDNA open reading frames. Two-step nested primers were designed using mouse-and rat-specific sequence information generated by our database searches. For amplification of the mouse APRIN, the following primers were used – mF1: 5′gaggggtacagacatttccatcatg; mF2: 5′atggctcattcaaagacaaggaccaac; mR1: 5′aatagaagttaatgacgtgttcatcg; mR2: 5′gttcatcgtctctctcgtttggag. For rat APRIN amplification the following primers were used – rF1 (same as mF1); rF2: 5′atggctcattcaaagacaagaaccaacg; rR1: 5′cataaagaaagttaatggcatgttcatc; rR2: 5′gcatgttcatcgtctctctcgtttgg. We used Elongase (Invitrogen) to amplify Marathon-Ready cDNA preparations from mouse and rat brains, respectively (Clontech, Palo Alto, CA). The PCR products (4340 bp for the mouse and 4334 bp for the rat) were cloned into the pCRII vector (Invitrogen) and sequenced in the Automatic Sequencing Core Facility at Tufts University.

2.4. Antibody reagents and expression studies

We generated two affinity purified polyclonal anti-APRIN antibody reagents. The first was raised against an oligopeptide between positions 1370–1387 (anti-APRIN-1370) [24]. Subsequent functional analyses revealed, however, that the epitope overlapped with the second HMG-like (High Mobility Group) DNA binding domain (AT-hook) and potentially interfered with APRIN expression studies and DNA binding assays. The recently developed second antibody targeted an epitope between positions 1434–1447 and was also affinity purified (anti-APRIN-1434). Both antibodies recognized their epitopes in both rat [25] and mouse APRIN proteins. Protein extractions and Western blots were performed according to standard procedures [25] using ventral and dorsal prostate lobes from 18-week-old CD-1 mice. For immunohistochemistry analyses, cell cultures were seeded