The NuRD architecture

Hillary F. Allen[1,2], **Paul A. Wade**[3], and **Tatiana G. Kutateladze**[1,2]

[1]Department of Pharmacology, University of Colorado School of Medicine, Aurora, CO 80045, USA

[2]Neuroscience Program, University of Colorado School of Medicine, Aurora, CO 80045, USA

[3]Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA

Abstract

The nucleosome remodeling and deacetylase (NuRD) complex regulates chromatin organization, gene transcription, genomic stability and developmental signaling. NuRD has a unique dual enzymatic activity, containing an ATPase and a histone deacetylase (HDAC) among its six core subunits. Recent studies indicate that NuRD composition and the interplay between subunits may dictate the diverse functions of the complex. In this review, we examine the structures and biological roles of the NuRD subunits and discuss new avenues of research to advance our understanding of the NuRD-mediated signaling network.

Keywords

NuRD complex; chromatin

Introduction

The eukaryotic genome is tightly regulated through mechanisms involving chromatin reorganization and the installation or removal of epigenetic marks on DNA and histones. These mechanisms provide accessibility of DNA to nuclear proteins and protein complexes that mediate fundamental DNA-templated processes, including gene transcription and DNA repair, recombination and replication. To date, most known regulatory complexes utilize a single mechanism; however NuRD complex constitutes a notable exception coupling two. NuRD was identified in 1998, and it remains the only chromatin remodeler with ATPase and histone deacetylase functions[1; 2; 3; 4]. One of the catalytic subunits of the complex, CHD3/4 ATPase, utilizes energy derived from hydrolysis of ATP for DNA sliding and repositioning of nucleosomes. The second catalytic subunit, HDAC1/2 (histone deacetylase), deacetylates acetylated lysine residues of histone and non-histone proteins. This dual enzymatic activity is proposed to be important for the efficient formation of heterochromatin with densely packed hypoacetylated nucleosomes and the rapid termination of gene transcription[5; 6]. In addition to the catalytic subunits, other components of the complex include the non-enzymatic proteins MBD2/3 (methyl-CpG-binding domain), RBBP7/4 (retinoblastoma-binding proteins), MTA1/2/3 (metastasis-associated) and p66 α/β (Fig. 1).

The NuRD complex has a multifaceted biological role. It has been implicated in a wide variety of nuclear processes including gene transcription, DNA damage repair, maintenance of genome stability, and chromatin assembly[5; 7]. The subunit composition of the complex

Correspondence to Tatiana.Kutateladze@ucdenver.edu.

varies, reflecting alterations in the activity and localization of NuRD and influencing downstream signaling events[8]. NuRD functions can further be modulated through changes in the local epigenetic environment. Aberrant activity of NuRD is associated with human diseases including cancer and aging, and therefore NuRD components represent potential targets for therapeutic interventions[7; 9]. In this review, we detail the molecular mechanisms underlying biological activities of each component of the NuRD complex, discuss cross-talk between the subunits, and summarize functional properties of the complex in various biological pathways.

CHD3/4 ATPase

The human chromodomain helicase DNA-binding protein 3 (CHD3) and CHD4, also known as Mi-2 α and Mi-2 β respectively, were discovered as autoantigens in an autoimmune disease dermatomyositis[10; 11]. CHD3 and CHD4 belong to the class II subfamily of CHD ATPases, and each is present as a core catalytic subunit in separate NuRD complexes. CHD3 and CHD4 are ~200 kDa proteins, conserved in vertebrate animals and plants. They contain tandem plant homeodomain fingers (PHDs), two chromodomains (CDs) and an ATPase/helicase module (Fig. 1b). Additionally, two domains of unknown function (DUF) have been identified at the C-terminus of these proteins. The PHD fingers of CHD4 have been shown to recognize histone H3 tails[12; 13]. The PHD fingers are followed by CDs which bind to DNA[14], and the SNF2-like ATPase/helicase domain that hydrolyzes ATP, providing the energy necessary for histone displacement and sliding during nucleosome remodeling[15].

Separated by a short linker, the PHDs of CHD4 associate with nucleosomes in a bivalent manner, concomitantly recognizing two histone H3 tails[16]. Posttranslational modifications (PTMs) of histone H3 fine-tune this association in a functionally relevant way. Methylation of Lys9 (H3K9me3) enhances binding of PHDs, whereas methylation of Lys4 (H3K4me3) abolishes it[12]. The synergistic interactions of PHDs displace HP1 β from pericentric sites, inducing changes in chromatin structure and leading to the dispersion of the pericentric heterochromatin mark H3K9me3 [16]. The bivalent engagement is also required for the repressive activity of NuRD[16]. Histone binding of PHDs augments the ATPase and chromatin remodeling functions of CHD4 [17]. Likewise, the ATPase/helicase domain promotes the association of the PHDs/CDs-containing region of CHD4 with histones[18].

The solution structure of the second PHD2 finger of CHD4 in complex with H3K9me3 peptide provides insight into the molecular mechanism of histone recognition[13]. The PHD2 finger folds into a double-stranded antiparallel β sheet and three loops, stabilized by two zinc-binding clusters. The H3K9me3 peptide adopts an extended conformation and forms an additional antiparallel β strand, pairing with the β sheet of the protein (Fig. 2a). Intermolecular hydrogen bonds restrain the N-amino group of Ala1 and the side chain of Lys4 of the peptide. The trimethylammonium group of Lys9 makes a cation- π contact with a phenylalanine of the PHD2 finger. The structures of the PHD1 and PHD2 fingers of CHD4 in the unbound states superimpose well[13; 19], suggesting a similar mode of histone recognition for PHD1.

The NMR structure of the second chromodomain (CD2) of CHD4 shows that it consists of three β strands and an α helix (PDB 2EE1, unpublished). Unlike other CDs that recognize methylated histone marks, for example H3K9me3 and H3K27me3 [20], the CHD4 chromodomains do not associate with histone tails and instead bind DNA[14]. It has been proposed that interaction with DNA facilitates the movement of CHD4 along DNA, since higher rates of ATP hydrolysis are observed for the ATPase/helicase domain linked to CDs as compared to the activity of the ATPase/helicase domain alone[17]. Both CDs are necessary for the repressive activity of NuRD[21].

Small-angle X-ray scattering (SAXS) studies reveal intricate contacts within the individual domains of CHD4 and suggest the existence of regulatory mechanisms[17; 18]. In the SAXS models, CDs interact robustly with the ATPase/helicase domain and additional contacts are seen between the ATPase/helicase domain and DUF1, between CDs and DUF1, and between PHDs and CDs. The PHDs-CDs interaction is found to limit the association of CHD4 with nucleosomes[17]. As suggested by Morra et al., PHDs can modulate binding of CDs to DNA[18]. Particularly, an acidic linker between the PHD fingers can mask one of the DNA-binding sites of the CDs, precluding the necessary interaction with the DNA[18].

A considerable interface formed between CDs and the ATPase/helicase domain suggests another regulatory mechanism, in which the ATP- or DNA-binding sites in the inactive ATPase/helicase domain are sterically blocked by CDs[17; 18]. A structural rearrangement in CHD4 in which PHDs and CDs release the ATPase/helicase domain, allowing access to ATP or DNA, would then stimulate the enzymatic activity[17]. These results are in line with the reports on autoinhibition of the ATPase motor by CDs in the homologous protein CHD1 [22]. The crystal structure of CHD1 demonstrates that an acidic helix connecting two CDs packs against the DNA-binding surface in both lobes of the ATPase motor, regulating access to the motor and allowing CHD1 to distinguish between nucleosomes and naked DNA[22].

The C-terminal region of CHD3/4, containing at least two DUFs, possesses repressive transcriptional activity. CHD3 and/or CHD4 from different species have been shown to associate with a number of co-repressors through this region. Interaction with hunchback, Tramtrack69, KAP1, NAB2 and RFP facilitates NuRD recruitment to genomic sites which are down-regulated by these co-repressors[23; 24; 25; 26; 27]. Other reported ligands of CHD3/4 include the E7 oncoprotein, the nuclear receptor ROR Ikaros, and proteins of unknown function Ki-1/57 and CGI-55 [28; 29; 30; 31]. Additionally, CHD3/4 is in direct contact with HDAC1 [4; 25], and thus represents a hub molecule that mediates multiple interactions of NuRD. It is involved in the inter-subunit assembly, bridging the HDAC1 subunit, as well as in the association with histone H3 and DNA and in tethering non-NuRD proteins to the complex. It may also link these functions to its catalytic ATPase activity.

HDAC1/2

The first histone deacetylase, HDAC1, was purified from cow protein extracts in 1996 [32]. Since then, over a dozen HDACs have been discovered and grouped into three classes (I, II and IV), according to sequence homology, phylogenetic analysis, and catalytic mechanisms[33]. HDAC1 and HDAC2 comprise class I of histone deacetylases (together with HDAC3 and HDAC8). They are conserved in all eukaryotes and are ubiquitously expressed in many tissues. HDAC1/2 catalyzes the removal of the acetyl moiety from the amino group of lysine residues in histones and non-histone proteins. In addition to being a major component of the NuRD complex, HDAC1/2 is present in a number of multisubunit complexes, including Sin3A, CoREST, NCoR/SMRT and ES-specific NODE[34; 35; 36; 37].

HDAC1 and HDAC2 are ~50 kDa proteins, which share 85% sequence identity. They contain a central catalytic deacetylase domain and a C-terminal, slightly more variable tail, which can be posttranslationally modified, i.e. phosphorylated at serine residues, or acetylated, sumoylated and ubiquitinylated at lysine residues[38]. The C-terminal tail may be involved in protein-protein interactions (Fig. 1b) [39].

The crystal structure of the HDAC2 catalytic domain in complex with a small molecule inhibitor N-(4-aminobiphenyl-3-yl)benzamide shows the characteristic / deacetylase fold[40; 41]. It contains an eight-stranded parallel sheet, sandwiched between 17 helices (Fig. 2b). The active site of HDAC2 consists of a deep, narrow, lipophilic pocket or a "tube"

and an internal cavity immediately adjacent to the tube. Hydrophobic and aromatic residues line up the walls of the tube and the cavity. The zinc ion is positioned at the bottom of the tube and is chelated by two aspartate residues and a histidine. The zinc ion is required for catalysis, in which the carbonyl of an acetyllysine substrate, polarized by zinc coordination, facilitates the general base-promoted nucleophilic attack of a water molecule[24]. The structure of the benzamide-bound HDAC2 provides invaluable information for the design of new generation of HDAC inhibitors because the biphenyl derivative occupies not only the tube, but also partially the internal cavity[40].

MBD2/3

MBD2 and MBD3 are members of the methyl cytosine-guanosine (CpG)-binding domain (MBD) family of proteins[42; 43]. Of the five MBD members, only MBD2 and MBD3 associate with NuRD and are required for the complex formation and gene repression. MBD2 and MBD3 reside within distinct NuRD complexes[44] and display some differences in their activities. MBD2 binds robustly to methylated DNA[45; 46], whereas binding of mouse MBD3 to methylated DNA is ~22-fold weaker[47]. Both MBD2 and MBD3 associate with hydroxymethylated DNA, albeit weakly, in the μM range[47]. MBD3 is the smallest subunit of NuRD (30 kDa), and MBD2 is ~40 kDa. MBD2 contains the N-terminal glycine-arginine repeats (GR), MBD, a transcriptional repression domain (TRD), and a C-terminal coiled-coil motif (Fig. 1b). Although the role of GR is unclear, binding of MBD to cytosine (methylated at position 5) within a CpG dinucleotide (mCpG) is well documented[45; 46; 48; 49]. TRD of *Drosophila* MBD2 is involved in the interaction with p55, a *Drosophila* homolog of RBBP7/4 [50]. The coiled-coil motif associates with p66 α (see p66 α below)[51; 52]. MBD3 consists of an MBD domain that binds to methylated DNA with a low affinity, a coiled-coil motif, and a C-terminal poly-glutamate (E) region (Fig. 1b).

The structure of MBD from chicken MBD2 bound to a 10 bp DNA fragment, derived from the β-*globin* promoter and containing a mCpG sequence, has been determined by NMR spectroscopy[53]. The MBD module consists of a four-stranded β-sheet and an α-helix (Fig. 2c). The finger-like loop between β2 and β3 strands extends into the major groove of DNA. The structure shows that MBD selectively recognizes mCpG through conserved Arg and Tyr residues that make base-specific interactions with the DNA. Two Arg residues, Arg24 and Arg46, form hydrogen bonds with symmetrically related guanine bases (Gua106 and Gua116), which allow for the aliphatic side chain of each arginine to pack against the methyl groups of mCyt105 and mCyt115. The aromatic side chain of Tyr36 interacts with the methyl group of mCyt105. Unlike other MBDs, the MBD2 MBD module binds its target methylated DNA sequence robustly and in a single orientation. A substantial enhancement in both specificity and affinity is achieved through recognition of the base pairs immediately flanking mCpG, particularly the following guanine. NMR relaxation studies demonstrate that DNA binding stabilizes the DNA-contacting regions, i.e the central β-sheet and the finger-like loop, whereas the N- and C-terminal parts of MBD undergo internal dynamic motions[53].

MBD2 has been shown to associate with HDAC1/2 and MTA2 and target NuRD to a set of genes[43; 54] It also interacts with MIZF[55], focal adhesion kinase (FAK)[56], and can form heterodimers with MBD3 [57; 58]. MBD3 binds HDAC1, MTA2 and RBB7/4 [43; 48]. Taken together, MBD2/3 has been proposed to mediate gene silencing by recruiting NuRD and associating macromolecules to the sites of methylated DNA.

RBBP7/4

The retinoblastoma-binding proteins RBBP7 and RBBP4, historically named retinoblastoma-associated proteins RbAp46 and RbAp48, were isolated through their

interaction with the Rb tumor suppressor[59]. These proteins act as histone chaperones and associate with various chromatin-remodeling and chromatin-modifying complexes, including Sin3A, PRC2 and NuRD. The amino acid sequences of RBBP7 and RBBP4 are 92% identical, and although occasionally these proteins impart distinct functions, they both can be found as core subunits of the NuRD complexes.

RBBP7/4 are ~50 kDa proteins composed of a central module, the WD40 repeat, flanked by short N-terminal and C-terminal helices (Fig. 1b). The WD40 repeat binds to the first α-helix of histone H4 [60] and to the N-terminal region of the GATA-1 cofactor FOG-1[61]. The crystal structures of RBBP7 and RBBP4 show that the WD40 repeat has a seven-bladed β-propeller architecture and utilizes different sides for intra- and intermolecular interactions (Fig. 3a, b) [60; 61]. The N-terminal and C-terminal helices of RBBP7/4 are packed against the first and seventh blades of the propeller. The α-helix of histone H4 is bound between the N-terminal α-helix and an extended loop, a so-called PP loop, of RBBP7 blade six (Fig. 3a) [60]. Binding is driven primarily by electrostatic interactions between the negatively charged residues of the PP loop and Arg36, Arg39 and Arg40 of H4, as well as hydrophobic contacts between the hydrophobic residues of the N-terminal α-helix and Ile34 and Leu37 of H4. Notably, in the RBBP7-H4 complex, histone H4 is in the conformation, which differs from its conformation in an intact nucleosome[60]. The authors propose that in the context of chromatin, histone H4 undergoes conformational changes or unfolding to be accessible to RBBP7. This interaction may be essential in the processes of ATP-dependent chromatin remodeling and nucleosome assembly and disassembly.

The FOG-1-binding site of RBBP4 has no overlap with the H4-biding site of RBBP7. The FOG-1 peptide lies across the top of the β-propeller (Fig. 3b) [61]. Several loops connecting the propeller blades create a highly acidic surface, which accommodates 13 N-terminal residues of FOG-1. The positively charged Arg3, Arg4 and Lys5 residues of the FOG-1 peptide are particularly involved in the interaction and form hydrogen bonds and salt bridges with four glutamic residues and two asparagines of RBBP4. The side chain of Arg4 inserts deeply into the axial channel of the β-propeller and makes a cation-π interaction with a tyrosine. The RRKQXXP motif of FOG-1 is present in other co-repressors and transcription factors, including BCL11A/B and SALL1, and these proteins may associate with RBBP4 in a similar manner[61]. Indeed, BCL11B interacts directly with RBBP7/4 and aids in the recruitment of NuRD to promoters of its target genes[38]. All of the FOG-1-binding residues in RBBP4 are conserved in RBBP7, implying that both proteins recognize FOG-1, which is in line with the results observed in IP assays[62]. Furthermore, RBBP7/4 has been reported to bind a tumor suppressor BRCA1 and another subunit of NuRD, MTA1 [61; 63]. Thus, RBBP7/4 is involved in the NuRD assembly, the association with histone H4 when it is accessible, and can also link non-core proteins to the complex.

MTA1/2/3

Metastasis-associated gene (MTA) proteins comprise a family of ubiquitously expressed coregulators. *MTA1* was initially characterized as a gene involved in breast cancer invasion and metastasis[64]. Overexpression of *MTA1* is considered to be a hallmark of many carcinomas, and a high level of MTA1 has been shown to correlate with the invasive growth and poor prognosis. MTA1 and two other members, MTA2 and MTA3, are found in mutually exclusive NuRD complexes, which interact with distinct cofactors and are implicated in different signaling pathways[3]. MTAs are ~70–80 kDa proteins that share four conserved modules: a bromo adjacent homology (BAH) domain, an ELM2 domain, SANT, and a GATA-zinc finger (GATA-ZnF) (Fig 1b). Although neither of the MTA domains has yet been characterized structurally, the overall scaffolds of the BAH, SANT and the GATA-

ZnF domains are known. The structure and function of the ELM2 domain remains undefined; however it is postulated to have DNA- or protein-binding capabilities.

A typical BAH domain consists of several β-strands and α-helices and is involved in protein-protein interactions, including binding to nucleosomes[65]. SANT domains form a compact 3-helix bundle, which can associate with DNA or histones[66]. The GATA-ZnF domain has one or two small double stranded antiparallel β-sheets and an α-helix, which are connected by a zinc-binding cluster. GATA-Zn recognizes the T/AGATAA/G sequence of DNA but can also recruit proteins[67]. MTA proteins have been shown to interact directly with transcriptional repressors BCL-6 and BCL11B, as well as with the oestrogen receptor α and its coregulators NRIF3, MAT1, MICoA and LMO4, and FOG-1, though it remains undetermined which domains of MTAs mediate these interactions[61; 68; 69; 70; 71; 72; 73; 74].

p66α/β

The transcriptional repressor proteins p66α and p66β (human paralogs GATAD2A (GATA zinc finger domain containing D2A) and GATAD2B, respectively) comprise the sixth core component of the NuRD complex[42]. p66α and p66β are highly conserved throughout evolution and share a high degree of sequence similarity. They are 68 kDa and 66 kDa in size and contain at least two conserved regions[75]. The first conserved region (CR1) encompasses ~40 residues (amino acids 137–178 of p66α) in the N-terminal part of the protein[75]. CR1 folds into an α-helix and binds directly to the C-terminal α-helix (CC) of MBD2 [51]. The N-terminal part has also been implicated in the interactions with other components of NuRD. The C-terminal (CR2) region includes a GATA-Zn finger and is involved in binding to unmodified histone tails[51].

The solution structure of the p66α-MBD2 complex shows a canonical arrangement of two α-helices (Fig. 3c)[52]. The α-helices form an anti-parallel coiled-coil with unique ionic and hydrogen bonding interactions imparting specificity. The 'knobs' made of the bulky hydrophobic side chains of Ile, Val, and Leu of each α-helix fit into 'holes' in the opposing α-helix. Arg and Lys residues surrounding the hydrophobic core create ion pairs and hydrogen bonds with oppositely charged Glu and Asp residues in another coil.

The CR1 region is also implicated in binding to MBD3, MTA2, HDAC1/2 and RBBP7/4 [76]. Both p66α and p66β can be posttranslationally modified by SUMOylation[76]. Two SUMO sites in p66α (Lys30 and Lys487) and a single site in p66β (Lys33) have been identified. The association of p66α with HDAC1 depends on SUMOylation at Lys30, whereas SUMOylation of p66β at Lys33 is necessary for binding to RBBP7 [76].

NuRD complex and gene regulation–a changing of the guard?

The NuRD complex was initially uncovered in effort to identify histone-modifying enzymes. It was a surprising discovery that a single protein complex couples two distinct enzymatic functions – histone deacetylation and ATP-dependent chromatin remodeling. Based on predominant views at the time, a prevalent model predicted that NuRD would play a role in gene repression[77]. Further, it was largely thought that local genomic enrichment of NuRD would result from protein-DNA interactions (mediated by the MBD2/3 subunit interacting with methylated DNA) or by protein-protein interactions between the complex and transcriptional repressors.

Progress on testing the various predictions of this model was initially slow. Genetic model systems for *in vivo* functional analysis did not emerge rapidly; and biochemical and molecular tools took time to develop. Testing of the model required definition of target loci subject to regulation by NuRD. Thus, the earliest studies on NuRD function were grounded

in candidate approaches, where existing biological and expression data were interpreted as indication of a fundamental role for NuRD. Kumar and colleagues hypothesized that MTA1, a NuRD subunit known to be overexpressed in certain breast cancer cell lines[64], might be a major player in the transcriptional program of these cells. The authors went on to demonstrate that activation of the HER2 signaling pathway in breast cancer cells led to upregulation of MTA1, which was shown to directly interact with the estrogen receptor and repress ER driven transcription[78]. These data provided first direct confirmation of the prevalent models–NuRD was recruited to target genes through direct interaction with a sequence specific transcription factor where it acted to repress transcription.

This finding was echoed by a number of additional studies, which demonstrated that NuRD action opposed gene expression in a relatively static manner. Investigators linked NuRD activities to repression of genes important in breast cancer[79], prostate cancer[26], hematopoiesis[62; 80], lymphoma[68], immunological responses[81] and HIV[82]. In most cases, the NuRD complex was recruited through direct or indirect contacts of a specific subunit with a DNA binding protein. In at least one instance, local enrichment of NuRD was directed through interaction of MBD2 with methylated DNA[52]. The preponderance of evidence derived from candidate approaches, which largely flowed from the prevailing model, suggested that NuRD plays a critical role in gene silencing in a broad variety of biological contexts downstream of multiple, diverse signaling pathways.

Further evidence linking NuRD to silent chromatin states came through biochemical analyses performed with histone peptides. It was observed in two independent reports that NuRD complex could be enriched through binding to the histone H3 amino terminus. Further, this interaction was abrogated when the peptides were trimethylated at Lys4, a chromatin mark associated with gene activity[83; 84]. These findings suggested that NuRD targeting to genomic regions is more complicated, and might be influenced by interactions with DNA binding proteins or histones.

Initial inklings that these models could not explain all the biology relevant to NuRD complex came with the emergence of genetic systems. Georgopoulos and colleagues created a conditional mouse model in which the ATPase subunit CHD4 could be deleted in a cell type specific manner. They studied the role of NuRD in T lymphocyte development, finding a genetic requirement at several steps. Importantly, they also observed that CHD4 function was required for expression of the CD4 gene, where NuRD complex was noted to be present at an active enhancer[85]. This same group went on to utilize their genetic model to assess the requirement for CHD4 function in hematopoietic stem cells and their derivatives. NuRD function was found to be essential for the maintenance of stem cell populations; mutant cells skew their differentiation towards the erythroid lineage at the expense of lymphoid and myeloid cells. Notably, global gene expression analysis revealed that loss of CHD4 resulted in roughly equivalent numbers of downregulated and upregulated genes – implicating NuRD in both maintenance and repression of transcription[86].

A very recent spate of studies using murine embryonic stem cells has also indicated that NuRD function is more complicated than previous models predicted. MBD3 was demonstrated to be essential for differentiation, but not for maintenance of mouse ES cells[87]. Using a genetic system in ES cells permitting conditional rescue of the MBD3 null state, Hendrich and colleagues proposed a model in which NuRD is recruited to active genes required for pluripotency, where it helps to maintain a balance between acetylation and deacetylation of histones, permitting fine-tuning of gene expression. Loss of activating signals leads to an imbalance in which NuRD-mediated deacetylation is integral to decreased expression of the core pluripotency network; in the absence of functional NuRD complex, differentiation does not occur[88]. In parallel, Young and colleagues used genomic

techniques to map NuRD occupancy across the ES cell genome, concluding that CHD4 is present at many active enhancers in these cells. Presumably NuRD activity is critical for maintaining equilibrium between the acetylated and deacetylated state of histone amino termini. Importantly, NuRD-dependent histone deacetylation was required prior to demethylation of Lys4 of histone H3 by the LSD1 histone demethylase, an act critical to down regulation of enhancer function associated with ES cell differentiation[89]. Finally, an interesting study from the Fazzio and Rando groups suggested an important new determinant of NuRD complex localization in the murine ES cell system. They observed localization of NuRD at core promoters that was abolished by depletion of TET1, an enzyme responsible for generation of 5-hydroxymethyl cytosine. Biochemical analysis with recombinant proteins suggested that mouse MBD3 could specifically bind to DNA fragments containing this modified form of cytosine[90].

These observations strongly suggest that the initial models depicting NuRD as a static reinforcer of the silent state cannot explain all the biological requirements for NuRD revealed by genetic and genomic analysis. Rather, it seems more likely that transcriptional states reflect dynamic equilibrium between gene activity and repression mediated by modification status of the histone amino termini and by nucleosome positioning – chromatin properties influenced by the catalytic activities of NuRD complex. Along these lines, classic biochemical analysis of histone acetylation indicates that this modification turns over with very rapid kinetics in the active fraction of the genome[91; 92]. An emerging model[93] suggests that active genes utilize NuRD in a dynamic manner to promote turnover of acetyl groups at enhancers and possibly at promoters, an action that results in fine-tuning of gene expression (Fig. 4). Down-regulation results upon loss of activating signals with concurrent decreased local concentration of transcriptional activators and coactivators – molecules that provide enzymatic functions opposing NuRD action. Whether NuRD complex persists and reinforces a silent state once activating signals are removed remains an open question. Likewise, a requirement for ATP-dependent chromatin remodeling in this process has not been demonstrated. It should be emphasized that the data suggesting a dynamic model for NuRD function are very new and need to be further examined[93]. Nonetheless, the vast preponderance of genetic and genomic data suggests it is time to rethink models explaining NuRD function in relation to gene expression.

NuRD function in other contexts

The NuRD complex has recently been implicated in the DNA damage response in several systems. Genetic analyses in C. elegans and mice implicate egr1 and its mammalian homolog, MTA1, in the response to ionizing radiation[94; 95]. Biochemically, depletion of NuRD subunits leads to hypersensitivity to DNA damage and the accumulation of unrepaired break sites[94; 96; 97; 98]. NuRD complex rapidly accumulates in cells at sites of double-strand breaks in a manner dependent on the activity of poly(ADP ribose) polymerase, PARP[96; 98]. These data suggest that protein modification at sites of DNA damage is integral to local NuRD recruitment. Precisely what function(s) NuRD plays in DNA damage repair is currently unclear although its action seems to be required for appropriate accumulation of repair proteins including BRCA1 [94; 98].

NuRD is also essential in assembly and maintenance of higher order chromosome structures. In rapidly proliferating lymphoid cells, NuRD complex forms an unusual structure, termed a NuRD body, at pericentric heterochromatin on human chromosomes 1, 9 and 16 [99]. These structures are most abundant in S phase of the cell cycle where they colocalize with sites of active DNA synthesis and chromatin assembly. Perturbation of the central ATPase, CHD4, leads to formation of aberrant structures, loss of localization of characteristic patterns of histone modification (trimethylation of Lys9 on histone H3), and failure of HP1 isoforms to

concentrate at these regions[100]. Such cells have defects in progression through S phase, implicating NuRD in some aspect of completion of chromatin assembly in mitotic cells. Overexpression of the tandem PHD fingers of CHD4 mimics many of the actions of CHD4 depletion, including gross structural defects at pericentric heterochromatin[16], leading to the conclusion that binding of this domain to modified histone tails plays a fundamental role in guiding NuRD to appropriate sites for assembly of higher order chromatin structures during S phase.

Along these lines, the Drosophila homolog of CHD4, dMi-2, is required for proper higher order chromatin structure. Loss of function alleles of dMi-2 in larvae result in gross structural defects in polytene chromosomes. Interestingly, loss of dMi-2 is associated with an increase in condensation of these structures while overexpression is associated with a loss of condensation and an increase in nuclear volume[101]. Live imaging demonstrated that dMi-2 modulates the interaction of cohesin with chromosomes, providing molecular insights into the observed structural defects.

These recent findings suggest that NuRD action is not strictly limited to gene regulation. It impacts multiple fundamental chromatin-related processes from local histone modification properties to the global folding of chromosomes. In the future, the application of new tools emerging from the exciting structural analysis of NuRD subunits and their functional domains promises to revolutionize how we think about the functions of this enzyme in chromosome biology.

Association with disease

The catalytic ATPase subunits of NuRD, CHD3 and CHD4, were identified as autoantigens in a class of idiopathic inflammatory myopathies[102], dermatomyositis, where the autoantigen was termed Mi-2. Autoantibodies against Mi-2 are associated with a very characteristic clinical presentation including specific types of skin lesions. Patients with Mi-2 autoantibodies tend to have a more favorable prognosis, respond to steroid therapy, and have a lower incidence of cancer when compared to other dermatomyositis patients[102]. As CHD3 and CHD4 are nuclear proteins, it is likely that autoantibodies to these species are a marker of disease, rather than drivers of pathology.

The CHD3/4 proteins are expressed at higher levels in muscle tissue collected from dermatomyositis patients than from control subjects[103]. In these patients, elevated expression of Mi-2 is primarily localized to nuclei within myofibers exhibiting perifascicular atrophy or features of myofiber regeneration. In an experimental system of muscle injury and regeneration, Mi-2 expression increased dramatically following muscle injury and remained elevated for up to 12 days[104]. Further study in a cell culture model of muscle differentiation revealed high level expression of Mi-2 in myoblasts which declines as these cells are differentiated. Depletion of Mi-2 by RNA interference led to accelerated differentiation[104]. These findings, prompted by the utility of Mi-2 autoantibodies as a clinical marker of disease, suggest that Mi-2 protein(s) are expressed at high levels in immature and/or regenerating muscle nuclei where they may play a role in regulating differentiation.

NuRD action is linked to both cancer and to aging. Connections of NuRD to cancer have been recently reviewed[7] and will not be considered in detail here. In a landmark report, Misteli and colleagues[105] took advantage of the unique properties of cells derived from patients with the premature aging disorder Hutchinson-Gilford Progeria syndrome to probe the intersection of NuRD with aging. The authors observed that patient-derived cells were characterized by decreased expression of multiple NuRD subunits, concurrent with accumulation of defects in higher order chromatin structures – particularly at pericentric

heterochromatin. Depletion of these same NuRD subunits in primary fibroblasts elicited responses similar to the defects observed in HGPS. Comparison of primary cells from young versus elderly subjects confirmed that NuRD subunit abundance decreased during normal aging. These results suggest that NuRD function declines with age, perhaps contributing to the chromosomal defects that accumulate with age.

Concluding Remarks

Over the past decade NuRD-mediated signaling pathways have been studied extensively and some of the NuRD components have been examined in detail. It becomes increasingly clear that the activity of NuRD depends on the precise composition of the complex as well as on the interactions of individual subunits with various non-NuRD partners. Although we have begun gathering information on the structures of domains within the individual subunits, the overall mechanism by which all subunits interact with their ligands and are assembled into the complex and recruited to specific genomic sites remain unclear. The intermingled action of and cross-talk between subunits not only enhance the overall affinity of the complex, but can also provide a high degree of specificity for a particular gene locus. Furthermore, combinatorial assembly of various paralogs of each subunit represents an additional mechanism for altering the chromatin-targeting capability of NuRD. Many uncertainties remain in understanding the biology of NuRD: old models are no longer adequate to explain all the data, and it has become apparent that NuRD is not strictly devoted to a single task. The continued accumulation of structural, biochemical and cell biology data will no doubt drive the generation of new tools and hypotheses to investigate the biological function of this imperative regulator of chromosomes.

Acknowledgments

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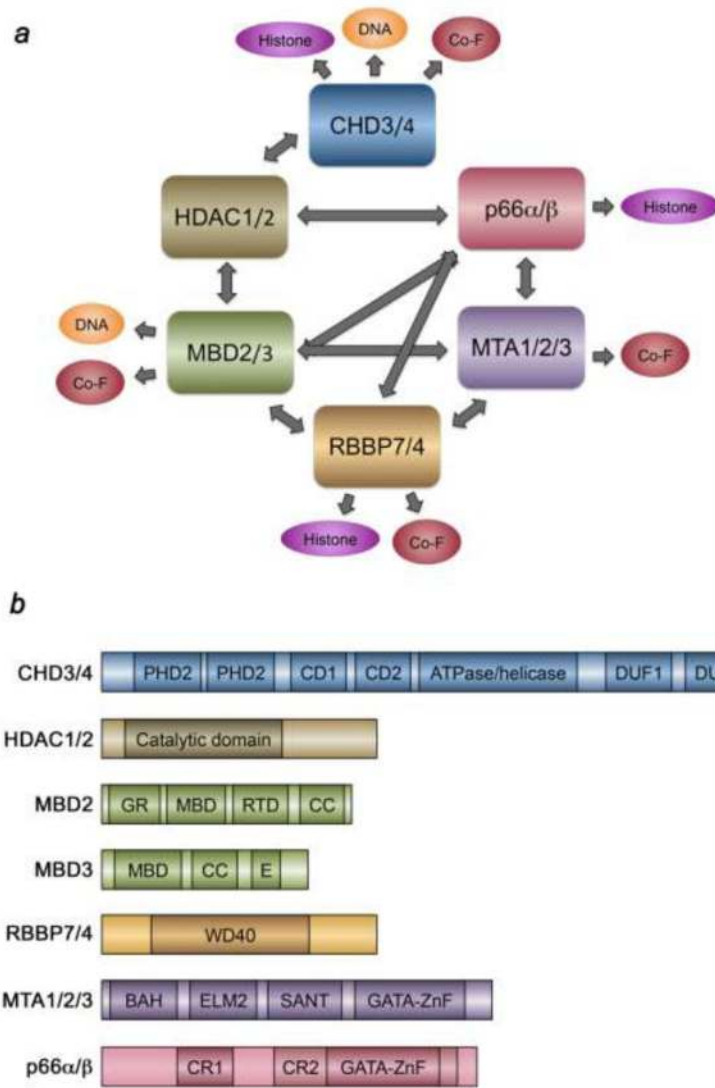
Figure 1. The NuRD complex and its core components
(a) Interactions between subunits and binding of the individual subunits to histones, DNA and other proteins or cofactors (Co-F). Co-F of CHD3/4: hunchback, Tramtrack69, KAP1, NAB2, RFP, E7, ROR Ikaros, Ki-1/57 and CGI-55; of MBD2: MIZF and FAK; of RBBP7/4: FOG-1, BCL11B and BRCA1; of MTA: BCL-6, BCL11B, oestrogen receptor NRIF3, MAT1, MICoA, LMO4 and FOG-1. (b) Domain architecture of each subunit.
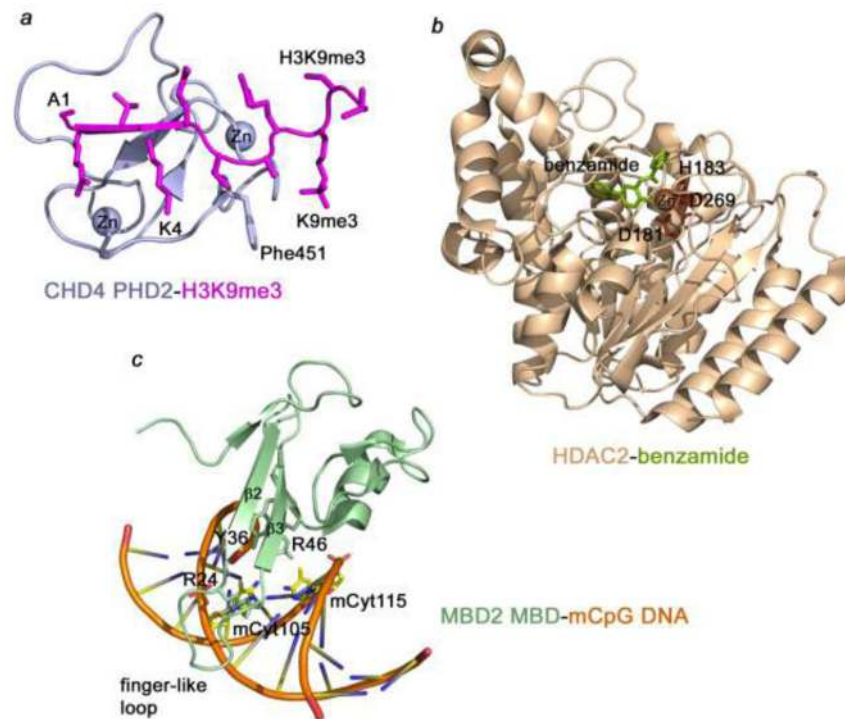
Figure 2. The structural basis for ligand binding by the NuRD components CHD4, HDAC2 and MBD2

(a) Solution structure of the CHD4 PHD2 finger in complex with a H3K9me3 peptide (PDB: 2L75). (b) Crystal structure of HDAC2 in complex with a small molecule inhibitor N-(4-aminobiphenyl-3-yl)benzamide (PDB: 3MAX). (c) Solution structure of the MBD module of MBD2 bound to a methylated DNA fragment, derived from the Ⓧglobin promoter (PDB: 2KY8).
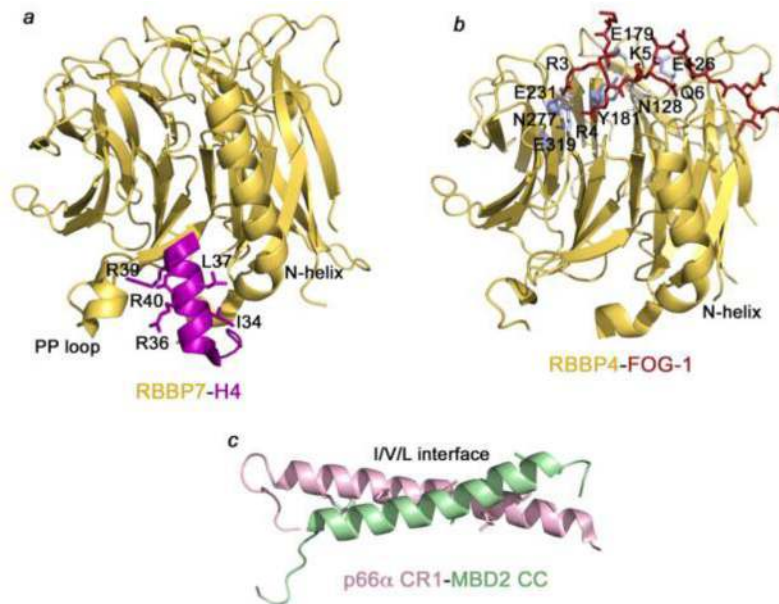
Figure 3. The structural basis for ligand binding by the NuRD components RBBP7/4 and p66
(a) Crystal structure of RBBP7 in complex with a H4 peptide (PDB: 3CFS). (b) Crystal structure of RBBP4 in complex with a FOG-1 peptide (PDB: 2XU7). (c) Solution structure of CR1 of p66 bound to the CC domain of MBD2 (PDB: 2L2L). Hydrophobic Ile, Val and Leu residues forming the coiled-coil interface are shown.
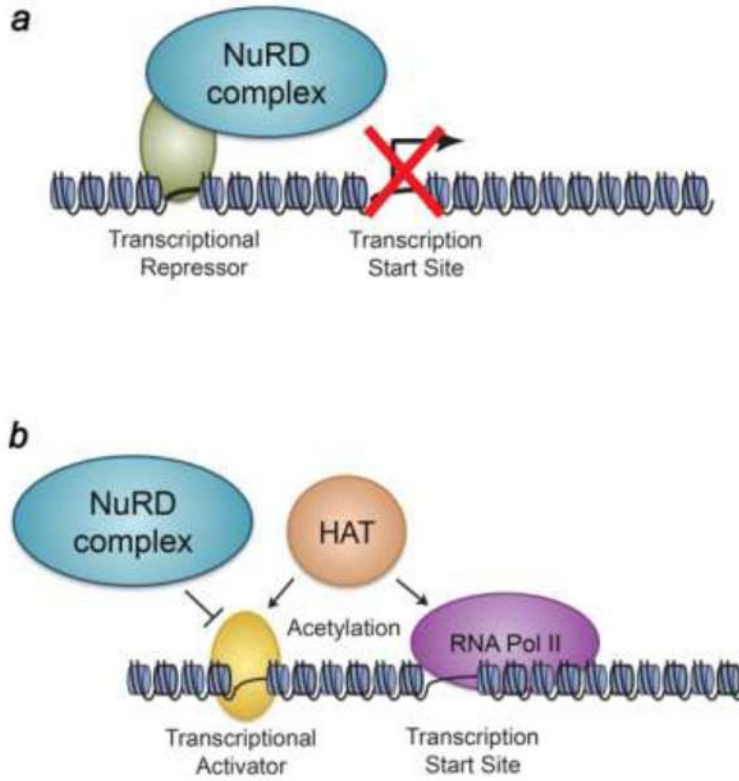
Figure 4. Models depicting NuRD complex recruitment and function
(a) The classic, static model of NuRD action shows association of the NuRD complex with inactive genes. Recruitment is typically depicted as resulting from direct interaction between a NuRD subunit and a transcriptional repressor. (b) A dynamic model for NuRD function shows NuRD as acting to maintain dynamic equilibrium of histone acetylation at an active gene, resulting in fine-tuning of gene expression.