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# Keeping things quiet: Roles of NuRD and Sin3 co-repressor complexes during mammalian development

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## Abstract

Gene inactivation studies of mammalian histone and DNA-modifying proteins have demonstrated a role for many such proteins in embryonic development. Post-implantation embryonic lethality implies a role for epigenetic factors in differentiation and in development of specific lineages or tissues. However a handful of chromatin-modifying enzymes have been found to be required in pre- or peri-implantation embryos. This is significant as implantation is the time when inner cell mass cells of the blastocyst exit pluripotency and begin to commit to form the various lineages that will eventually form the adult animal. These observations indicate a critical role for chromatin-modifying proteins in the earliest lineage decisions of mammalian development, and/or in the formation of the first embryonic cell types. Recent work has shown that the two major class I histone deacetylase-containing co-repressor complexes, the NuRD and Sin3 complexes, are both required at peri-implantation stages of mouse development, demonstrating the importance of histone deacetylation in cell fate decisions. Over the past 10 years both genetic and biochemical studies have revealed surprisingly divergent roles for these two co-repressors in mammalian cells. In this review we will summarise the evidence that the two major class I histone deacetylase complexes in mammalian cells, the NuRD and Sin3 complexes, play important roles in distinct aspects of embryonic development.

### Keywords

Development; Histone deacetylase; Epigenetics; Chromatin; Stem cells

## 1. The NuRD complex

A decade ago the first purifications were reported of an abundant histone deacetylase complex that was distinct from the best-characterised deacetylase complex at the time, namely the Sin3 complex. The analogous Mi-2, NRD, NuRD and NuRD complexes were purified from *Xenopus* egg, HeLa cell and SW13 cell nuclear extracts, respectively (Tong et al., 1998; Wade et al., 1998; Xue et al., 1998; Zhang et al., 1998). The complex has become known as the NuRD (nucleosome remodelling and histone deacetylation) complex and, as the name suggests, couples the two main chromatin-modifying activities, chromatin remodelling and histone modification.



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Independent purifications of the NuRD complex did not produce identical compositions, as there were subtle variations in each of the purifications. Nonetheless, the defining components of the complex are an Mi-2 chromatin remodelling subunit, an Mbd3 subunit and Mta subunit (Fig. 1A). Mta subunits (e.g. Mta1, Mta2 or Mta3) appear to be mutually exclusive within NuRD, possibly contributing to functional diversity of NuRD complexes (Bowen et al., 2004; Fujita et al., 2004). Mbd3 can be replaced by related protein Mbd2, forming the Mecp1 complex (Feng and Zhang, 2001; Le Guezennec et al., 2006a). The original purifications of NuRD did not identify Mbd2, indicating that Mecp1 accounts for only a small subfraction of the total NuRD in mammalian cells (Tong et al., 1998; Wade et al., 1998; Xue et al., 1998; Zhang et al., 1998). As Mbd2 has been shown to be dispensable for normal mammalian development (Hendrich et al., 2001), Mbd2 and Mecp1 will not be considered further in this review. NuRD also contains a core histone deacetylase complex comprised of Hdac1, Hdac2, Rbbp7 (formerly known as RbAp46) and Rbbp4 (formerly RbAp48). These core proteins are also found in the Sin3 complex. Additionally the presence of the Gatad2a and Gatad2b proteins (formerly kown as p66α and p66β) is often reported for NuRD purifications. While NuRD has been purified from mammalian, amphibian and insect cells, subunits of the complex have also been described in other organisms including plants and worms implying that NuRD is broadly conserved among plants and animals.

## 2. The Mi-2 nucleosome remodelling proteins

Mi-2 is a key component of the NuRD complex as it contains both the ATPase, chromatin remodelling activity and physically associates with histone deacetylases. Originally identified as an autoantigen in dermatomyositis (Seelig et al., 1995), it is the largest subunit of the NuRD complex. Mammalian genomes are capable of encoding two Mi-2 proteins: Mi-2α (encoded by the *Chd3* gene) and Mi-2β (encoded by the *Chd4* gene) (Seelig et al., 1996). The latter is the form predominantly associated with the mammalian NuRD complex (Feng and Zhang, 2001; Zhang et al., 1998), although Mi-2α has been shown to be a member of the NuRD complex in a variety of human cell lines (Le Guezennec et al., 2006a; Tong et al., 1998; Xue et al., 1998). Whether functional or cell type-specific differences exist between Mi-2α- and Mi-2β-containing NuRD complexes remains to be determined. Mi-2 proteins belong to the CHD (chromo-helicase–ATP–DNA binding) family, which is conserved from yeast to humans. Structurally, Mi-2α and Mi-2β both contain two PHD (plant homeo domain)-zinc finger domains, two chromodomains and a SWI2/SNF2-type ATPase/helicase domain, the latter being responsible for the nucleosome remodelling activity (Wang and Zhang, 2001). The chromodomains of *Drosophila melanogaster* dMi-2 have been reported to have DNA-binding ability (Bouazoune et al., 2002), but the role of the chromodomains in the mammalian Mi-2 proteins remains to be determined. Similarly, the function(s) of the PHD fingers has not yet been demonstrated.

## 3. Transcriptional regulation and the role of Mi-2β and the NuRD complex

Since its purification in 1998, numerous different functions for NuRD have been postulated, although its main function is as a transcriptional repressor complex (Denslow and Wade, 2007). As histone deacetylation is generally correlated with transcriptional repression, the presence of two Hdacs in the complex implicates it in transcriptional repression. The remodelling activity of NuRD may be required to allow access of the deacetylases to the histone tails. Indeed, histone deacetylation of nucleosomal substrates by Mi-2 complexes is stimulated by ATP hydrolysis. Importantly, the NuRD complex represents one of the most abundant forms of a deacetylase complex in amphibian eggs and cultured mammalian cells (Wade et al., 1998; Zhang et al., 1998). This implicates it as a possible general co-repressor complex.

Evidence for a repressive function of NuRD also comes from physical interaction studies. In murine lymphocytes, Mi-2β was uncovered as interacting with *Ikaros* and *Aiolos*, which are zinc finger DNA-binding factors and potent repressors required for lymphoid cell development (Kim et al., 1999). Ikaros is able to bind DNA in a sequence-specific manner and is also associated with pericentric heterochromatin in cycling T-cells, indicating that it might be involved in a more general silencing mechanism. Indeed, mammalian NuRD has been shown to interact with a variety of other transcriptional repressors (Fig. 1A) implicating it as a widely used repressor in a variety of cell types.

Mi-2β has also been implicated in transcriptional activation. Biochemical analysis revealed that the amino-terminus of Mi-2β had transcriptional activating ability in reporter assays by interacting with Brg-1 (Shimono et al., 2003). Williams et al. (2004) showed that Mi-2β is required for several steps during T cell development, playing a direct role in promoting *CD4* gene expression. This experiment defines a role for Mi-2β in gene activation in vivo. Whether this represents a NuRD-independent function of Mi-2β, or possibly implicates deacetylase function in transcriptional activation (e.g. Metivier et al., 2003; reviewed in Smith, 2008) remains to be demonstrated.

## 4. Developmental roles of the NuRD complex

Development requires the stable repression and activation of genes in different cell types. Transcriptional repression by the NuRD complex has been connected with developmental roles in numerous model systems (Table 1). In *D. melanogaster*, dMi-2 appears to function with Hb and Polycomb group proteins in HOX gene repression during embryo patterning (Kehle et al., 1998). dMi-2 is essential for embryogenesis as mutants arrest as first or second instar larvae, and is also required for germ cell development (Kehle et al., 1998). In *Caenorhabditis elegans*, mutations in the Mi-2 homologue *let-418* revealed it to be required for proper vulval development (Solari and Ahringer, 2000; von Zelewsky et al., 2000). Genetic approaches have shown that *let-418* is an essential gene and is required for larval viability. Additionally, *let-418* is required for maintenance of germline-soma distinctions in *C. elegans* as *let-418* deficient animals show inappropriate expression of germline-specific genes in somatic cells (Unhavaithaya et al., 2002). The *Pickle* (aka *Gymnos*) gene of *Arabidopsis thaliana* has been identified as a Mi-2 homologue (Eshed et al., 1999; Ogas et al., 1999). It has a similar role to *let-418*, as *Pickle* mutants express embryonic characteristics in root meristem cells (Ogas et al., 1997, 1999). Hence, *Pickle* is required to repress embryonic development to allow the transition to post-embryonic development. These studies highlight the importance of Mi-2 and NuRD in the development of various model organisms. Similarly, the involvement of the NuRD complex in mammalian development is now beginning to be understood.

## 5. Role of Mbd3 in early embryonic development

The first indication that NuRD may play an important role in early cell fate decisions was provided by the observation that murine embryos lacking Mbd3 die early in embryonic development (Hendrich et al., 2001). Maternally derived Mbd3 is absent by the blastocyst stage, yet at 4.5 dpc *Mbd3* null embryos are morphologically normal and appear to have normal segregation of trophoblast, primitive endoderm and primitive ectoderm lineages, indicating that NuRD is not simply required for cell viability (Kaji et al., 2007). However by 5.5 dpc embryos lacking Mbd3 fail to develop recognisable extraembryonic ectoderm or embryonic ectoderm structures, and the few Oct4 expressing inner cell mass cells fail to expand in number and remain proximally located. The failure of Oct4 positive epiblast cells of *Mbd3* null 5.5 dpc embryos to expand does not appear to be due to increased apoptosis or a reduction in the proliferative rate of Oct4 positive cells. Rather ICM cells continue to

express both Oct4 and a marker of primitive endoderm, Gata4, as is seen in 4.5 dpc ICMs. Organised visceral endoderm also fails to form by 5.5 dpc, despite the presence of primitive endoderm at 4.5 dpc. While no extraembryonic ectoderm is detectable in Mbd3-mutant embryos, the ectoplacental cone continues to grow during post-implantation development, demonstrating that some extraembryonic lineages do not require Mbd3 function. Hence, Mbd3 is required for the development of many embryonic and extraembryonic tissues during early post-implantation development.

Ex vivo culture of *Mbd3*$^{-/-}$ ICMs revealed that Mbd3 is required for the expansion of the Oct4 expressing cell population, and no embryonic stem cell lines could be made from *Mbd3*$^{-/-}$ blastocysts (Kaji et al., 2007). Nevertheless, Mbd3null ES cells could be made by gene targeting, demonstrating that while Mbd3 function is required for ES cell derivation, it is dispensable for ES cell self-renewal (Kaji et al., 2006). The absence of Mbd3 results in a decrease in the abundance of some other subunits of the NuRD complex, including Mta1 and Mta2, implying that Mbd3 is required for the stability of other NuRD subunits. Accordingly, little, if any intact NuRD complex can be detected in Mbd3 null ES cells by immunoprecipitaiton (Kaji et al., 2006).

In the absence of Mbd3, the differentiation potential of ES cells is compromised. In a number of different contexts *Mbd3* null ES cells were shown to be able to initiate differentiation but could not commit to specific cell fates (Kaji et al., 2006). Correspondingly, *Mbd3* null ES cells were capable of LIF-independent self-renewal even after prolonged periods in culture conditions without LIF. Unexpectedly, *Mbd3* null ES cells can differentiate when treated with retinoic acid, indicating that ES cells lacking Mbd3 can differentiate in response to certain signals, and that Mbd3 is not an absolute requirement for differentiation (Kaji et al., 2006). Overall, these studies indicate that Mbd3/NuRD is required to create an epigenetic state that allows cells to commit to specific lineages in response to particular developmental cues, but is not absolutely required for differentiation.

NuRD appears to be required neither for maintenance of global histone acetylation levels (Kaji et al., 2006) nor as a global regulator of gene silencing, as only about 200 genes were found to be misregulated ≥2× in *Mbd3*$^{-/-}$ ES cells (Kaji et al., 2007; K. Kaji and B. Hendrich, unpublished). Analysis of a handful of genes mis-expressed in *Mbd3* null ES cells identified at least 11 genes that are misregulated in pre-implantation *Mbd3* null embryos. A subset of these genes were found to be inappropriately expressed in ICM cells of null embryos, while others were not appropriately silenced in the transition of ICM cells of 3.5 dpc null embryos to the early epiblast cells of 4.5 dpc embryos (Kaji et al., 2007). This indicates a role for NuRD-mediated transcriptional repression in the transition of pluripotent cells from a pre-implantation, potentially differentiation-resistant state, to that of the post-implantation embryo where cells are competent for lineage commitment. This role in silencing transcription of gene expression in pluripotent cells in vivo prior to developmental transitions is strikingly similar to the functions ascribed to the *Arabidopsis pickle* and *C. elegans let-418* genes, which were both shown to facilitate silencing of germline transcripts upon the initiation of embryogenesis.

## 6. Role of Gatad2a in murine development

Gatad2a and 2b were not identified in the initial mammalian NuRD purifications, however it now appears that these zinc finger proteins are *bona fide* NuRD components (Brackertz et al., 2002; Le Guezennec et al., 2006a). The single *Drosophila* homologue of these proteins was independently identified in a genetic screen for modifiers of Wnt signalling, and homozygous mutant flies displayed metamorphosis defects consistent with misregulation of ecdysone mediated transcription (Kon et al., 2005). In mice, *Gatad2a* null embryos are

capable of implantation but die at around 10.5 dpc (Marino and Nusse, 2007). Homozygous embryos have a range of defects detectable from 7.5 to 10.5 dpc including delayed development, smaller size, abnormal tissue development, failure to turn and increased amounts of apparent necrotic tissues. *Gatad2a* null ES cells were found to be viable and capable of normal differentiation in embryoid bodies (Marino and Nusse, 2007). Thus while Gatad2a is not required for early lineage decisions, a degree of redundancy during these early developmental decisions with Gatad2b remains a possibility.

## 7. Mi-2β and somatic stem cells

Recently evidence has emerged for an important role of Mi-2β in somatic stem cells. By inducing deletion of a conditional *Chd4* allele in the haematopoietic system, Yoshida et al. (2008) provided evidence that Mi-2β plays important roles in both homeostasis and lineage choice of haematopoitic stem cells in vivo . Further, this group has provided extensive evidence for Mi-2β function in lymphopoiesis (Williams et al., 2004; Yoshida et al., 2008). By crossing these same conditional *Chd4* mice with a keratin 14-Cre transgenic line, the Georgopoulos lab also found that Mi-2β functions during transition stages of normal skin development. Mi-2β appears to be required in the conversion of ectodermal progenitor cells to become basal cells, and the subsequent reprogramming of basal cells to follicle progenitors and later, matrix stem cells (Kashiwagi et al., 2007). This requirement for a NuRD component during transition stages of development observed in the epidermis is reminiscent of the role of Mbd3 in developmental transitions during development of pluripotent cells in vivo. However some evidence exists that some of the functions attributed to Mi-2β during haematopoiesis does not involve the NuRD complex (Williams et al., 2004; Yoshida et al., 2008). Whether a constitutive *Chd4* deletion would reveal similar functions during peri-implantation development as did the Mbd3-knockout mice, or possibly a more severe phenotype, consistent with Mi-2β functioning both within and independently of NuRD, remains to be seen.

## 8. The mammalian Sin3 complex

The other class I histone deacetylase-containing complex present in mammalian cells, the Sin3 complex, was first described in yeast as a general repressor of transcription via histone deacetylation (Vidal and Gaber, 1991; Vidal et al., 1991). The mammalian homologues of the yeast Sin3 protein, Sin3a and Sin3b, serve as co-repressor scaffold proteins that physically bridge connections between histone deacetylases and sequence-specific transcription factors (Fig. 1B), thereby bringing histone deacetylase activity within proximity of target genes (for further review see Silverstein and Ekwall, 2005). Repressive Sin3/Hdac complexes have also been described in worms (Choy et al., 2007), flies (Tsai et al., 1999), amphibians (Vermaak et al., 1999), and plants (Hill et al., 2008), suggesting this mechanism of co-repression is widespread throughout metazoans.

The highly conserved domains shared by Sin3a and Sin3b are the Hdac interaction domain (HID), required for Hdac1 and Hdac2 recruitment via the Suds3 protein (Laherty et al., 1997) (Fig. 1B; Table 2), and four paired amphipathic helix (PAH) domains that directly bind diverse transcriptional repressors (Brubaker et al., 2000; Le Guezennec et al., 2006b; Sahu et al., 2008), Although Sin3a and Sin3b complexes share many core components, transcription factor binding partners, and gene targets in mammalian somatic cells both paralogs are independently required for mouse embryonic development (Cowley et al., 2005; Dannenberg et al., 2005; David et al., 2008), indicating that the functions of Sin3a and Sin3b cannot be completely redundant. However genetic evidence for some redundancy of the proteins was provided by the observation that deletion of *Suds3* results in cell death due

to defects in centromere function (David et al., 2003), whereas neither Sin3a nor Sin3b is essential for cell viability (see Table 1).

## 9. Sin3 binds repressors in diverse developmental pathways

Mammalian Sin3a and Sin3b were initially identified through their interactions with Mxd1 (aka Mad)-Max and Mxd1-Mxi1 heterodimeric complexes, which bind to and repress Myc target genes, thereby blocking Myc-induced proliferation and promoting cellular differentiation (Ayer et al., 1995; Laherty et al., 1997; Schreiber-Agus and DePinho, 1998). Subsequent purifications of Sin3 complexes from numerous mammalian cell types have revealed that they are recruited by a wide range of repressors, many of which, as with Myc/Mxd1 network proteins, control the critical processes of cell cycle progression, arrest, and exit throughout development. Notable examples of Sin3 interactors that promote cellular differentiation via cell cycle exit include Rb (Lai et al., 2001) and E2F family members (Rayman et al., 2002) that control the G1/G0 restriction point in many cell types (Halaban, 2005) and Hbp1 which blocks proliferation by repressing targets of the Wnt/β-catenin pathway (Sampson et al., 2001; Swanson et al., 2004). In a contrasting role, Sin3a and Hdac1/2 have been shown to interact with Dach1 to repress CDK inhibitor targets of Six6, thereby driving proliferation during organogenesis (Li et al., 2002). Furthermore, the tumour suppressor p53 requires Sin3/Hdac complexes to block cell cycle progression and induce apoptosis in response to genotoxic stress (Lagger et al., 2003; Murphy et al., 1999).

Sin3/Hdac complexes have also been shown to interact with a variety of developmentally important factors that are not directly involved in cell cycle control. For example, Sin3/Hdac recruitment is required for repression by Rest/Nrsf (Grimes et al., 2000; Huang et al., 1999; Nomura et al., 2005), which silences neuronal gene expression programmes in non-neuronal tissues (Chong et al., 1995; Schoenherr and Anderson, 1995) and fetal cardiac genes in adult cardiac myocytes (Bingham et al., 2007). It has also been proposed that the unliganded nuclear hormone receptors thyroid hormone receptor (TR) and retinoic acid receptor (RAR) can repress their targets by recruiting co-repressor supercomplexes containing Sin3, Hdacs, and the closely related co-repressors NCoR and Smrt (Alland et al., 1997; Heinzel et al., 1997; Nagy et al., 1997). Further evidence for this mechanism of repression is provided by genetic and physical interaction studies in *Drosophila* showing that ecdysone receptor (EcR)-mediated repression requires recruitment of both dSin3 and the NCoR/Smrt orthologue Smrter (Tsai et al., 1999). As a final example, Ikaros and Aiolos, which are crucial for proper proliferation and differentiation throughout B- and T-lymphocyte development, recruit Sin3a/b, Mi-2β, and Hdac1/2, all of which are required for complete repression by Ikaros (Koipally and Georgopoulos, 2002; Koipally et al., 1999).

In addition to possessing Hdac activity, Sin3 complexes have been found to associate with other chromatin-modifying factors in mammalian cells, such as Swi/Snf complex subunits (Sif et al., 2001), and the histone methyltransferase Eset (Yang et al., 2003). Furthermore, Sin3a complexes have been shown to possess potent histone deacetylase-independent transcriptional repression activity (Laherty et al., 1997; Shi et al., 2006; Vermeulen et al., 2006). Taken together, all of these interactions in diverse contexts link Sin3-mediated repression to many critical roles in mammalian development.

## 10. Sin3a is required for peri-implantation development, MEF proliferation, and T-cell differentiation

The essential role for Sin3a in mouse development is evident from the absence of homozygous null pups born from *Sin3a*$^{+/-}$ intercrosses (Cowley et al., 2005; Dannenberg et al., 2005). Analysis of early embryos from *Sin3a*$^{+/-}$ matings revealed that all genotypes are

present at E3.5, but null embryos cannot be detected at E6.5. Instead, null embryos appear to die shortly after implantation leaving behind empty decidua, indicating that they survive until at least embryonic day 4.5–5 and are capable of implantation. At this time in development, cell cycle dynamics in the epiblast are relatively stable compared to the profound changes in regulation observed after E6.5 (White and Dalton, 2005; White et al., 2005). Also at this time, however, signals from the pluripotent ICM begin to stimulate proliferation of the trophectoderm (Kunath et al., 2004). Therefore, it is possible that in the developing embryo a lack of repression by Sin3a is lethal due to a trophoblast defect rather than an epiblast defect. Nevertheless, E3.5 null blastocysts appear morphologically normal but show severely retarded proliferation when cultured ex vivo, indicating a requirement for Sin3a in the ICM as well as the trophectoderm (Cowley et al., 2005).

The early lethal phenotype in *Sin3a*$^{-/-}$ embryos prompted functional studies of conditional *Sin3a* deletion in somatic cells (Cowley et al., 2005; Dannenberg et al., 2005). As suggested by the interaction data implicating Sin3 in the regulation of proliferation and cell cycle progression, loss of Sin3a in mouse embryonic fibroblasts (MEFs) results in profound growth arrest, apoptosis, and G2/M arrest. This type of arrest is also observed upon Sin3 depletion in *Drosophila* cells (Pile et al., 2002), and coincides with unscheduled DNA replication followed by a functional S-phase checkpoint. Interestingly, the observed apoptosis and arrest in *Sin3*$^{-/-}$ MEFs are independent of p53 despite pronounced upregulation of the primary p53 target p21$^{Cip1}$, as these phenotypes were not alleviated by loss or functional inactivation of p53.

Consistent with the observed phenotypes, transcriptional profiling revealed significant de-repression of numerous genes controlling cell cycle progression (including especially the G2/M transition), DNA replication and repair, as well as known and predicted targets of E2F, Rb, p53, and Myc upon loss of Sin3a. Substantial overlap exists between the affected processes in MEFs and the results of a similar experiment in *Drosophila* (Pile et al., 2003). This de-repression appears to be the result of local changes in chromatin structure rather than a global change in histone acetylation, although the deletion of Sin3a did cause a general delocalization of the heterochromatic protein HP1α throughout the nucleus when compared to wild-type MEFs (Cowley et al., 2005; Dannenberg et al., 2005).

The observation that some older *Sin3a*$^{+/-}$ mice developed enlarged spleens with an unusually high CD4/CD8 positive T-cell ratio prompted an investigation into the role of mSin3a in T-cell development (Cowley et al., 2005). Deletion of Sin3a using a T-cell-specific cre led to decreased thymic cellularity and reduced number of circulating T-cells. This was found to be due, at least in part, to an apparent partial block at the beta-checkpoint leading to reduced proliferation consistent with a cell cycle defect. Together these genetic studies in mice indicate that Sin3A-mediated transcriptional repression is important for maintaining proliferative potential in a wide variety of cell types.

## 11. Sin3b is required at late stages of development

The two mammalian Sin3 paralogs are both widely expressed and highly homologous, and biochemical evidence for distinct Sin3a- and Sin3b-containing co-repressor complexes is scarce. Nevertheless, the fact that *Sin3a*$^{-/-}$ embryos are not rescued by the presence of Sin3b provides clear genetic evidence for distinct functions for these two proteins. Indeed, recently Sin3b was also demonstrated to be essential for embryogenesis in mice, although the consequences of Sin3b deletion are not manifested until after midgestation (David et al., 2008). Notably, *Sin3b*$^{-/-}$ embryos show defects in erythrocyte and granulocyte maturation similar to those seen in embryos deficient for known Sin3 interactors E2F4 and Mxd1 (Mad) (Foley et al., 1998; Humbert et al., 2000; Rempel et al., 2000), thereby providing evidence

that Sin3b functions to promote E2F4- and Mxd1-dependent terminal differentiation of these lineages. Similarly, *Sin3b*$^{-/-}$ embryos were found to display defects in skeletal development reminiscent of those shown to be dependent upon Rb, Rbl1 (p107) and/or Rbl2 (p130) (Cobrinik et al., 1996), thus establishing a genetic link to the Rb family of proteins. Finally, a further link to E2F4 and the Rb family of repressors was provided by evidence that Sin3b mutant cells display defects in G0/G1 restriction point control (Dannenberg et al., 2000; David et al., 2008; Gaubatz et al., 2000; Sage et al., 2000).

## 12. Conclusions

The NuRD and Sin3 complexes are abundant in mammalian cells, and both have been linked to repression by a number of different sequence-specific transcription factors. Contrary to what might have initially been expected, neither appears to be functioning as a 'global' transcriptional repressor. Genetic analyses in mammals are now revealing that, like in flies, worms, and some plants, NuRD is particularly important for developmental transitions. Work with mice and cell lines mutant for NuRD components has indicated that this co-repressor complex plays an important role in maintaining stem cell homeostasis and lineage choice in both the haematopoietic system and in skin, and during maturation and lineage commitment of pluripotent cells in early embryos. In contrast Sin3a and Sin3b complexes appear to be used by the cell to control cell cycle progression and proliferation, terminal differentiation of some somatic cell types, and possibly centromere function. Hence NuRD and Sin3 provide a mechanism for the cell to apply one silencing mechanism, namely histone deacetylation by class 1 deacetylases, to two different ends. However it is worth keeping in mind that most studies of NuRD and Sin3 function have been directed towards studying deacetylation of chromatin, while the non-histone targets of this deacetylation, and the effects that may have upon transcription, remain largely ill-defined (for review see Smith, 2008). Defining the targets of NuRD- and Sin3-mediated chromatin modification and protein deacetylation, and the proteins that target these actions, will allow us to further elucidate the molecular pathways controlling lineage commitment and control of proliferation during mammalian development, and inevitably, in cancer.

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## Abbreviations

| | |
|---|---|
| **Hdac** | histone deacetylase |
| **ESCells** | embryonic stem cells |
| **ICM** | inner cell mass |
| **dpc** | days post coitum |
| **MEF** | mouse embryonic fibroblast |

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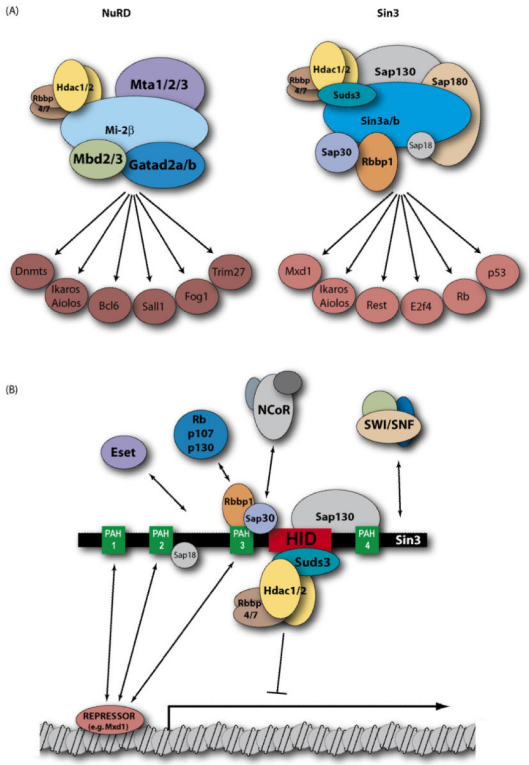


**Fig. 1.**

(A) Schematic of the NuRD and Sin3 co-repressor multiprotein complexes in mammalian cells. The individual components of the complexes are indicated above, with arrows indicating associations with transcriptional repressors. Each complex contains both Hdac1 and Hdac2 proteins and both Rbbp4 and Rbbp7 proteins. NuRD may contain both Gatad2a and Gatad2b, but Mta1, Mta2 and Mta3 are mutually exclusive within NuRD, as are Mbd2 or Mbd3. Similarly Sin3 complexes contain either Sin3a or Sin3b. Ikaros and Aiolos have been reported to associate with both complexes. “Dnmts” refers to reports that some NuRD components can interact with Dnmt1, Dnmt3a and Dnmt3b. (B) The co-repressor Sin3 serves as a scaffold, physically coupling class I histone deacetylases (via the Hdac-interaction domain, red) to sequence-specific transcriptional repressors (via PAH domains, green). In this way Sin3 can be recruited to diverse promoter sequences in many different cell types. The specificity and functionality of the complex are further increased by incorporating adaptor proteins that can recruit additional repressors and chromatin-modifying complexes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)




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## Table 1

Role of the NuRD and Sin3 complex subunits and their respective homologs in development of different model organisms, inferred from genetic (e.g. mutation or deletion) experiments

| Gene | Organism | Developmental role | References |
|---|---|---|---|
| Hdac1/2 | | | |
| *Hda-1* | *Caenorhabditis elegans* | Embryonic viability, vulval development | Shi and Mello (1998), Solari and Ahringer (2000) |
| *Rpd3* | *Drosophila melanogaster* | Embryonic segregation, groucho-mediated transcriptional repression | Chen et al. (1999), Mannervik and Levine (1999) |
| *Hdac1* | *Mus musculus* | Embryonic viability (die at ~9.5 dpc), control of proliferation | Lagger et al. (2002), Montgomery et al. (2007) |
| *Hdac2* | *M. musculus* | Body size? | Montgomery et al. (2007), Zimmermann et al. (2007) |
| Rbbp4/7 | | | |
| *Lin-54* | *C. elegans* | Embryonic viability, vulval development | Lu and Horvitz (1998), Shi and Mello (1998), Solari and Ahringer (2000) |
| *Rba-1* | *C. elegans* | Embryonic viability, vulval development | Shi and Mello (1998), Solari and Ahringer (2000) |
| Mi-2 | | | |
| *Pickle* | *Arabidopsis thaliana* | Repression of embryonic and meristem genes | Eshed et al. (1999), Ogas et al. (1999) |
| *let-418* | *C. elegans* | Larval viability, vulval development, maintenance of germline-soma distinctions | Solari and Ahringer (2000), Unhavaithaya et al. (2002), von Zelewsky et al. (2000) |
| *dMi-2* | *D. melanogaster* | Hox repression, larval and germ cell viability | Kehle et al. (1998) |
| *Chd4* | *M. musculus* | Haematopoiesis, epidermal development, peri-implantation development | Kashiwagi et al. (2007), Williams et al. (2004), Yoshida et al. (2008), Costello and Hendrich (unpublished) |
| Mta1/2 | | | |
| *Egl-27* | *C. elegans* | Embryonic patterning, Hox regulation, Wnt signalling, vulval development | Ch'ng and Kenyon (1999), Herman et al. (1999), Solari and Ahringer (2000), Solari et al. (1999) |
| *Egr-1* | *C. elegans* | Embryonic patterning, Hox regulation, vulval development | Ch'ng and Kenyon (1999), Solari and Ahringer (2000), Solari et al. (1999) |
| *Mta2* | *M. musculus* | Embryonic development and viability | Lu et al. (2008) |
| Mbd3 | | | |
| *dMbd2/3* | *D. melanogaster* | Stability of peri-centric heterochromatin, suppressor effect on peri-centric position effect variegation | Marhold et al. (2004) |
| *Mbd2/3* | *C. elegans* | Morphological defects (post-embryonic) | Gutierrez and Sommer (2004) |
| *Mbd2* | *M. musculus* | None detected | Hendrich et al. (2001) |
| *Mbd3* | *M. musculus* | Lineage commitment of pluripotent cells, silencing of pre-implanation genes | Kaji et al. (2006), Kaji et al. (2007) |
| Gatad2a/b | | | |
| *Simj* (*p66*) | *D. melanogaster* | Embryonic viability, Wnt signalling | Kon et al. (2005) |
| *Gatad2a* (p66) | *M. musculus* | Pleiotrophic effects post-gastrulation, silencing of a few TE genes | Marino and Nusse (2007) |

| Gene | Organism | Developmental role | References |
|---|---|---|---|
| Sin3 | | | |
| *Sin3a* | *M. musculus* | Peri-implantation development, cell proliferation | Cowley et al. (2005), Dannenberg et al. (2005) |
| *Sin3a* | *D. melanogaster* | Embryonic viability, Wnt signalling, cell cycle progression | Pennetta and Pauli (1998), Pile et al. (2002), Tsai et al. (1999) |
| *Sin3b* | *M. musculus* | Embryonic viability, terminal differentiation | David et al. (2008) |
| *Suds3* (*Sds3*) | *M. musculus* | Centromere function, cell viability | David et al. (2003) |
| *Arid4a* (*Rbbp1*) | *M. musculus* | Genomic imprinting? | Wu et al. (2006) |
| *Arid4b* (*Sap180*) | *M. musculus* | Peri-implantation development | Wu et al. (2006) |

Alternate names for genes are given in parentheses. Modified and updated from Solari and Ahringer (2000).

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**Table 2**

Sin3 complex components and their proposed functions

| Component | Function |
|---|---|
| Sin3a/b | Scaffold |
| Suds3 (mSds3) | Mediates Hdac1/2 interaction with Sin3 |
| Rbbp4/7 (RbAp48/46) | Mediates Hdac1/2 interaction with Sin3 and acetylated histones |
| Hdac1/2 | Histone deacetylase activity |
| Sap30 | Mediates interaction with NCoR, p33ING1b, and Rb family proteins |
| Sap130 | Contributes to repression |
| Arid4a (Rbbp1) | Mediates interaction with Rb family proteins |
| Arid4b (Sap180) | Contributes to repression |
| Sap18 | Mediates interaction with hedgehog signalling targets |

Alternate names for proteins are given in parentheses.