DAXX

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The Death-domain associated protein 6 (DAXX) is an evolutionarily conserved and ubiquitously expressed protein that is implicated in many cellular processes, including transcription, cellular proliferation, cell cycle regulation, Fas-induced apoptosis, and many other events.

DAXX heterochromatin histone variant H3.3 ATRX mammalian early development

1. Introduction

DAXX is a multifunctional protein present in the nucleus and cytoplasm (the cytoplasmic functions of DAXX are not discussed here). Mutations in the *DAXX* gene may be associated with some cancers [1], suggesting DAXX as one of the most important factors in maintaining the integrity of the genome. The nuclear function of DAXX is largely determined by its properties as a chaperone of the histone variant H3.3 [2][3]. DAXX-dependent deposition of H3.3 and known relations of the DAXX protein with transcription factors, epigenetic modifiers, and chromatin-remodeling proteins including the α-thalassemia/mental retardation syndrome X-linked protein ATRX [4] make DAXX a main player in chromatin silencing, mainly in the pericentromeric areas [5]. In this context, there is growing recent evidence that DAXX is an essential factor for proper development of mammalian oocytes and early embryos [6], since the processes of oocyte development during meiosis and fertilization, as well as the reprogramming of parental genomes in early embryos, are accompanied by pronounced rearrangements of the heterochromatin compartment [7][8], in which deposition of H3 variants plays an important role [9].

2. Structure and Localization

DAXX was identified in 1997 as a murine signaling protein, the C-terminal part of which specifically binds to the Fas death domain and enhances Fas-mediated apoptosis [10] by activating the Jun NH2-terminal kinase (JNK) pathway through the apoptosis signal-regulating kinase 1 (ASK1) [11]. In parallel, it has been shown that human DAXX (hDAXX), although specifically affecting Fas-mediated apoptosis, does not bind Fas and instead is found in the nucleus [12] due to the presence of two nucleus localization signals (NLS) in the molecule [13].

The structure of the DAXX molecule is now well established. A modular structure of the DAXX molecule and its main domains are depicted in Figure 1a. The molecule of DAXX exhibits six regions of sequence conservation, including a central histone-binding domain (HBD) [2], an N-terminal 4-helix bundle, and a C-terminal domain that is mostly disordered [14]. A combination of structural, biochemical, and cell-based targeting analyses of the H3.3/H4/DAXX HBD complex allowed revealing an extended fold of the DAXX HBD that envelops an H3.3/H4

dimer with seven consecutive α-helices [15][16]. The N- and C- termini of the DAXX molecule contain two SUMO-interacting motifs (SIMs: SIM-N and SIM-C, respectively), which both independently interact with a small ubiquitin-related modifier SUMO [17].

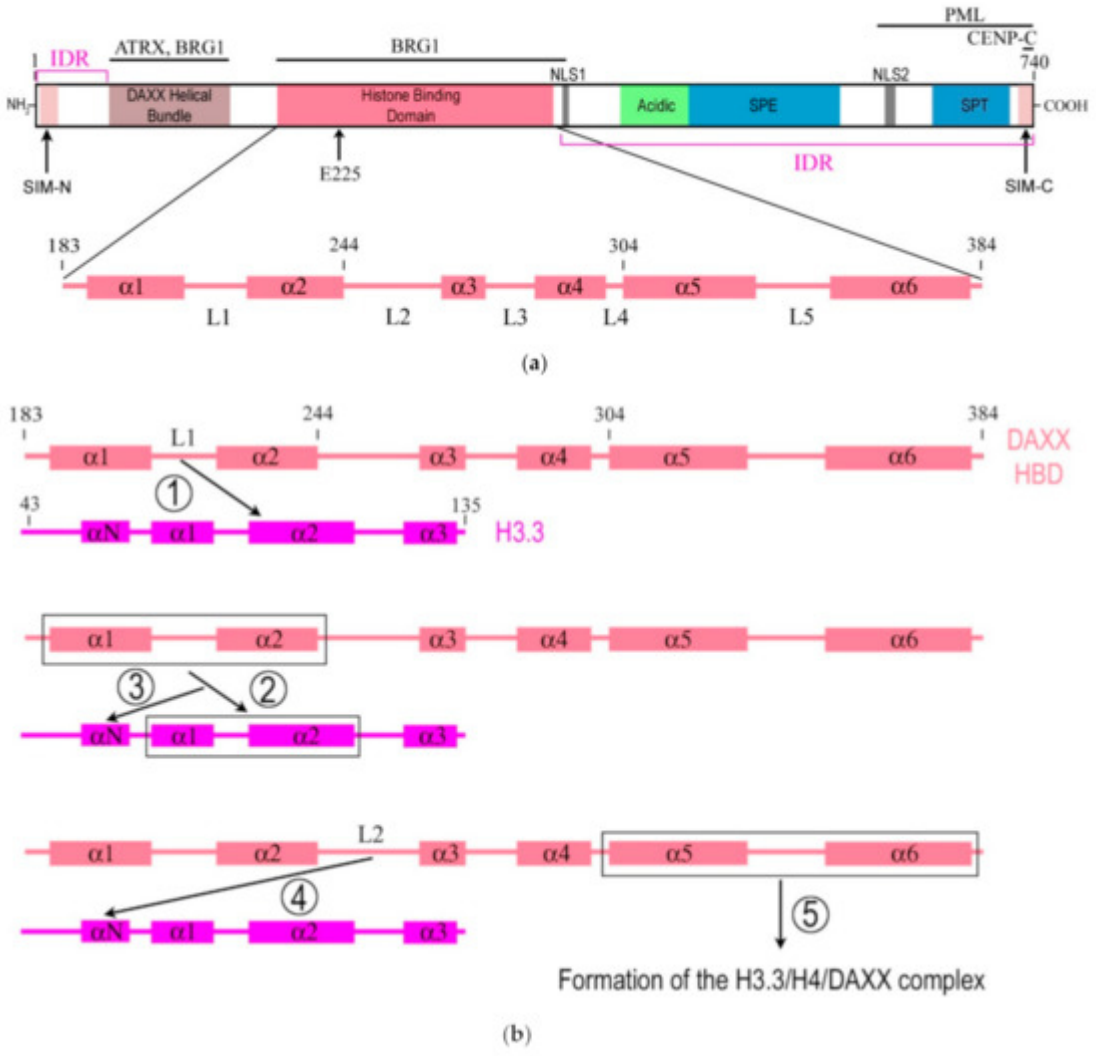

Figure 1. Simplified diagrams depicting the modular structure of the DAXX molecule (**a**) and interactions between the histone binding domain of DAXX and H3.3 during the formation of the complex H3.3/H4/DAXX (**b**). (**a**) DAXX contains two independent SUMO-interacting motifs at the N- and C-termini (SIM-N and SIM-C, respectively) [17]. The DAXX helix bundle serves as the main site for binding the α-thalassemia/mental retardation syndrome X-linked protein ATRX [18] and many other proteins that interact with the DAXX terminal region, including BRG1; another BRG1-interacting region is in the central part of DAXX [19]. The histone binding domain (HBD) is the main module involved in interactions with H3.3, a functional partner of DAXX, and the residue E225 in DAXX contributes to the specificity of this interaction. The HBD consists of six α-helices (α1–α6) and intervening loops (L1–L5); N-terminal helices (α1 and α2) are also termed 'tower' [15]. Two nucleus localization signals (NLS1 and NLS2), a Glu/Asp-rich acidic region, two segments rich, respectively, in Ser/Pro/Glu residues (SPE) and in Ser/Pro/Thr residues (SPT) are depicted. The most intrinsically disordered regions (IDRs) of the DAXX molecule are also shown. The C-terminus includes the regions to interact with the PML protein [20] and CENP-C [21], which impacts the peculiarities

of DAXX distribution within the nucleus. The vast majority of DAXX interactions, not mentioned here, including those involved in cancer, are not depicted in the scheme, see [1] for additional reading. (**b**) DAXX is a specific H3.3 chaperone. The recognition and binding of H3.3 by the HBD of DAXX involve several folding steps [22], shown in the diagram by encircled numbers (1–5). 1—L1 of the DAXX 'tower' interacts with lateral H3.3 surface by long-range electrostatic interactions; 2–the 'tower' helices (α1 and α2) of DAXX, including portions of L1, fold onto two helical segments of H3.3 (α1 and α2), including the intervening loop (the interacting domains are ensquared); 3—the αN helix of H3.3 folds; 4—the L2 of DAXX tightly wraps around the folded H3.3 αN helix; 5—finally, the α5 and α6 helices of DAXX pack against H3.3 and H4, respectively, forming the H3.3/H4/DAXX complex. H4 interactions are not shown. For further reading and structural details regarding formation of the H3.3/H4/DAXX heterotrimer, see [15][22].

In the nucleus, SUMO-1, which modifies the promyelocytic leukemia protein (PML), is necessary for the recruitment of DAXX into the PML nuclear bodies (PML-NBs) [20], which play a role in regulation of transcription and support stability of the genome by sequestration, modification, and/or degradation of nuclear proteins [23]. The repressive function of DAXX to basal transcription is regulated by its interactions with the PML protein and is inhibited by overexpression of PML [24].

DAXX interacts with the centromere protein C (CENP-C), and this interaction is mediated by the N-terminal 315 amino acids of CENP-C and the C-terminal 104 amino acids of hDAXX [21]. At the same time, hDAXX was not found predominant in fractions obtained from partially purified mitotic chromosomes, indicating that hDAXX is either not a chromosomal protein during mitosis or that it readily dissociates from condensed mitotic chromosomes during biochemical treatments.

Importantly, DAXX interacts with the chromatin-remodeling protein ATRX—an ATP-dependent helicase, mutations in the gene of which cause the X-linked mental retardation syndrome associated with α-thalassemia [4]. ATRX and DAXX are both the components of the same ATP-dependent chromatin remodeling complex and localize to the PML-NBs. The level of these DAXX/ATRX complexes is reduced in cells of patients with ATRX syndrome [25].

DAXX interacts with the transcription activator BRG1—another chromatin-remodeling protein with an ATPase activity—and can serve as a negative regulator of several BRG1-regulated genes: either directly through BRG1 binding and/or indirectly via other factors [19]. It is possible that BRG1 and ATRX play a role in targeting of DAXX to specific chromatin regions, where DAXX performs its chromatin- and transcription-regulating functions.

3. DAXX Is an H3.3 Chaperone

Mammalian H3.3 is a variant of the major histone H3 (H3.1) that differs by only five amino acids [15]. H3.3 is deposited both at sites of active transcription with the participation of the histone cell cycle regulator A (HIRA) [26][27] and in the heterochromatic regions, including telomeres and pericentromeric zones, with participation of the

DAXX/ATRX complex [2][28][29][30][31]. As an H3.3-specific chaperone, DAXX facilitates H3.3 deposition at H3K9me3-containing heterochromatin regions.

DAXX interacts directly with the H3.3/H4 heterodimer through its highly conserved HBD, which makes extensive contacts with both H3.3 and H4. The residues E225 in DAXX and G90 in H3.3 are the main determinants of chaperone-mediated H3.3 recognition specificity [15][16]. DAXX also uses a shallow hydrophobic pocket to accommodate the small hydrophobic A87 of H3.3, whereas a polar binding environment in DAXX prefers G90 in H3.3 over the hydrophobic M90 in H3.1 [16]. DAXX can specifically associate with H3.3/H4 despite a high concentration of nearly identical canonical H3 in the cell. According to the domain-level cooperative folding model (Figure 1b), a mostly unfolded DAXX initially makes contacts near the H3.3 specificity region and then can sample a large part of the H3.3 surface before folding into place. The dynamic stability of partially folded intermediates may be responsible for the discrimination of H3.3 from other H3 variants [22].

DAXX- and ATRX-mediated H3.3 chromatin assembly is required for many functions including H3K9 tri-methylation at pericentromeres, endogenous retroviral (ERV) sequences, imprinted genes, intragenic methylated CpG islands and telomeres in embryonic stem cells (ESCs) [32][33][34][35]. The function of DAXX as a H3.3 chaperone is mediated by its interaction with the constitutive centromeric protein CENP-B, which serves as a kind of ‘beacon’ for H3.3 incorporation [36]. The interaction of DAXX with CENP-B and the association of DAXX with centromeres are SUMO-dependent and require two SIMs of the DAXX molecule, enabling DAXX binding to SUMOylated proteins, such as PML [36]. Depletion of SUMO-2, but not SUMO-1, decreases the interaction between DAXX and CENP-B and impairs the accumulation of DAXX and H3.3 at centromeres, which proves the different functions of the SUMO paralogs in the H3.3 deposition processes [36]. DAXX-mediated H3.3 deposition is repressed by the PML protein, and PML-NBs coordinate this process [37]. Specifically, PML plays a role in the routing of H3.3 to chromatin and in the organization of megabase-size heterochromatic PML-associated domains (PADs). Loss of PML impairs the heterochromatic state of PADs by shifting the balance of H3 methylation from K9me3 to K27me3. In addition, this alters the ATRX/DAXX-dependent, but not HIRA-dependent, deposition of H3.3 in PADs [38].

DAXX not only regulates the deposition of H3.3, but is one of the leading factors in maintaining the global heterochromatin landscape, since loss of DAXX can seriously affect the subnuclear organization in general. In the absence of DAXX, there are changes in H3K9me3-enriched heterochromatin domains, including the loss of a typical chromocenter structure and the loss of overlap between non-nucleolar and perinucleolar compact chromatin and H3K9me3. In addition, the structural integrity of nucleoli and the organization of rDNA are disrupted [39].

DAXX is important for the regulation of the cell cycle, mitosis, and cytokinesis. A decrease in the S-phase duration accompanied by an increase in the number of cells with double nuclei, micronuclei, and nuclear blebs was observed in DAXX$^{-/-}$ cells [36][40], indicating mitotic segregation defects. DAXX can be involved in the control of mitosis in particular through the regulation of Aurora-A kinase and cyclin B stability, including through the interaction of DAXX with the mitotic checkpoint protein RASSF1 [41][42]. DAXX plays an important role in the regulation of centromeric heterochromatin activity. Knockout of DAXX reduces the association of ATRX with centromeres and significantly increases chromosomal instability [36][40]. Accumulation of DAXX in centromeric and

pericentromeric chromatin regions is enhanced by stress, including heat shock, as shown for several cancer cell lines [43], suggesting that DAXX-containing complexes are useful in maintaining the normal epigenetic landscape of heterochromatin.

4. DAXX is Essential for Normal Development of Mammalian Oocytes and Embryos

DAXX demonstrates a significant role in global transformations of the nucleus during late oogenesis and early embryogenesis of mammals, but mostly, its function, which could impact on development, is still elusive. However, loss of DAXX in oocytes and embryos affects many nuclear processes (Figure 2).

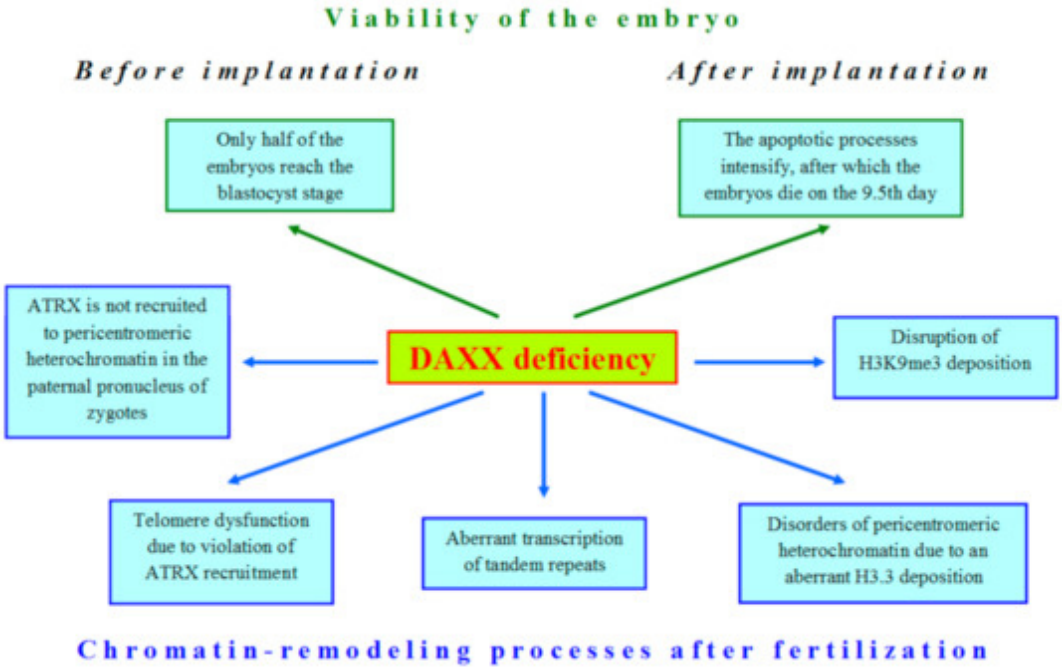

Figure 2. The deficiency of DAXX violates many cellular processes in early development of mammals.

The first attempts to shed light on the role of DAXX in embryonic development began immediately after the identification of this protein, when DAXX-deficient mice were obtained [44]. The authors expected to find a hyperproliferative disorder in development of these mice, but the result was unexpectedly opposite: mutations in the DAXX gene led to extensive apoptosis. Only 50% of DAXX-deficient embryos can reach the blastocyst stage [45]. After implantation, DAXX$^{-/-}$ embryos are developmentally retarded and completely disintegrated by the 12.5 day of gestation [40][44]. Since DAXX is an H3.3 chaperone, the disruption of the developmental program in DAXX-deficient embryos is not surprising due to the significant role of H3.3 in oogenesis and embryogenesis [46][47]. The whole-transcriptome analysis of metaphase II (MII) oocytes allowed revealing expression of DAXX and other H3.3 chaperones, such as HIRA, in mouse oogenesis [48]. However, DAXX cannot compensate normal H3.3 deposition in HIRA-depleted oocytes [49]. Similar results were also obtained for mouse ESCs [30].

In oocytes, DAXX predominantly localizes to the pericentromeric heterochromatin (PCH) regions, as shown by analysis of the nuclear localization of DAXX and centromeric proteins [50]. DAXX deposition in PCH is significantly reduced in fully-grown GV (germinal vesicle stage) oocytes deficient in the heterochromatin protein 1β (HP1β), also known as the Chromobox Protein Homolog 1 (CBX1), and completely abrogates in oocytes deficient of the methyltransferase SUV39h2, in which the PCH regions lack both H3K9me3 and HP1β [45]. After fertilization, DAXX is revealed in the pronuclei of zygotes, initially in close association with atypical nucleoli [45][51][52], also known as the so-called nucleolus precursor bodies (NPBs), which serve as major organizing structures for heterochromatin in mammalian zygotes and early embryos [53]. The experiments, when the atypical nucleoli were removed from immature oocytes (enucleolation), have shown that the components of the maternal “nucleolus” are critically important for the deposition of DAXX in zygotes [51]. The absence of maternal “nucleoli” affects the dynamics of the S phase, and DAXX is no longer detected in enucleolated zygotes, suggesting that DAXX is not stably associated with maternal DNA.

As in somatic cells, H3.3 deposition in mammalian oocytes and embryos involves DAXX in the complex with ATRX. Moreover, both these proteins could operate in different complexes and assemble on chromatin with different kinetics [45][54]. The ATRX protein is necessary to recruit DAXX to PCH during meiotic prophase I. In the absence of ATRX, the DAXX protein fails to associate with the PCH regions of oocytes at the GV stage, despite of these regions contain H3K9me3, a mark of repressed chromatin [50]. In contrast with somatic cells in which the association of DAXX with ATRX at the PCH regions exists only for a brief period at the S phase [25][40], the DAXX/ATRX complex remains in association with these heterochromatin regions while an oocyte grows [50].

After fertilization, a clear colocalization of DAXX and ATRX initially revealed already at stage PN0 in the maternal pronucleus (mPN) and at stage PN2 in the paternal pronucleus (pPN), i.e., before replication, but thereafter, the DAXX level reduces rapidly in maternal PHC [45]. Importantly, the character of functional interactions between DAXX and ATRX after fertilization is fundamentally different in mPNs and pPNs, indicating a known asymmetry of the pronuclei in mammalian zygotes [45]. In the mPN, DAXX colocalizes with ATRX in the PCH regions of decondensed chromosomes containing H3K9me3 and HP1β. However, in the pPN, these chromatin regions contain only DAXX but not ATRX. It was also demonstrable that DAXX is necessary for ATRX recruitment to the PCH regions in paternal but not maternal pronuclei. In contrast, ATRX is required in the mPN to recruit DAXX, as shown in experiments with ATRX-depleted oocytes [45].

In mouse early embryos, DAXX-mediated H3.3 deposition is required for chromosome stability. The DAXX protein was shown to regulate repression of the Polycomb Repressive Complex 1 (PRC1) target genes in oogenesis and early embryogenesis. However, PRC2 and H3K27me3 do not serve as key determinants of DAXX recruitment and function in mouse zygotes [45]. The role of DAXX in maintaining the genome integrity is also evident during the period of global DNA demethylation that occurs after fertilization [55] and is accompanied by an increase in the recruitment of the DAXX/ATRX complex to tandemly repeating sequences, including retrotransposons and telomeres [33]. Knockdown of DAXX/ATRX in cells with hypomethylated DNA increases the aberrant derepression of tandem repeat transcription and expands telomere dysfunction. The DAXX/ATRX complex also suppresses H3K9 tri-methylation during mouse embryogenesis, interacting with the methyltransferase SUV39h [33].

As an H3.3 chaperone, DAXX is probably involved in remodeling of pericentromeric heterochromatin—the main place where H3.3 is deposited in oocytes and embryos. It has been shown that the mutation K27R in the H3.3 molecule results in the aberrant accumulation of pericentromeric transcripts, HP1 mislocalization, dysfunctional chromosome segregation, and developmental arrest [56]. Together with ATRX, the DAXX protein is also involved in repression of telomeric sequences. A central role for DAXX in association of the DAXX/ATRX complex with telomere/subtelomere regions was confirmed in experiments using mouse ESCs knocked out for DNA methyltransferases [33]. Loss of ATRX has a minor effect on the telomeric localization of DAXX, but knockdown of DAXX severely compromised the ability of ATRX to localize to telomeres. As DAXX and ATRX are localized in the telomeric regions of embryonic chromosomes, it cannot be excluded that in embryogenesis, DAXX and ATRX are involved in the Alternative Lengthening of Telomeres (ALT), especially since mutations in the *DAXX* gene have been described in patients with telomerase-negative pancreatic neuroendocrine cancers [57].

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