

Minireview

Contribution of CDP/Cux, a Transcription Factor, to Cell Cycle Progression

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Abstract CCAAT-displacement protein/Cut homeobox (CDP/Cux) was initially identified as a transcriptional repressor. However, a number of studies have now suggested that CDP/Cux is a transcriptional activator as well. Stable DNA binding activity of CDP/Cux is up-regulated at the G₁/S transition by two mechanisms, dephosphorylation by the Cdc25A phosphatase and proteolytic processing to generate a 110 kDa amino-truncated isoform, CDP/Cux p110. The generation of CDP/Cux p110 stimulates the expression of reporter plasmid containing the promoter sequences of some S phase-specific-genes such as DNA polymerase α gene, dihydrofolate reductase gene, carbamoyl-phosphate synthase/aspartate carbamoyl-transferase/dihydroorotase gene, and cyclin A gene. However, DNA binding activity of CDP/Cux is down-regulated at G₂ phase through a binding of cyclin A-cyclin-dependent kinases1 (Cdk1) to CDP/Cux. Furthermore, another CDP/Cux isoform, CDP/Cux p75, has been found to be associated with breast tumors indicating this isoform is involved in the abnormal proliferation of tumor cells. The differences in DNA binding of CDP/Cux isoforms in S and G₂ phases suggest important roles of CDP/Cux in cell cycle progression. In this review, we discuss the functions of CDP/Cux with a focus on its roles in cell cycle regulation and its possible potency leading to the cell cycle reentry of neurons.

Keywords CDP/Cux; CDP/Cux p110; cyclin A-Cdk1; DNA binding; transcriptional repressor

CCAAT-displacement protein/Cut homeobox (CDP/Cux) belongs to a family of transcription factors present in all metazoans and is involved in the control of proliferation and differentiation [1]. The first member of the family that was discovered was the *Drosophila melanogaster* Cut protein. Several lethal and viable mutations within the cut locus have been reported. One mutation preventing the function of a distant wing-specific enhancer caused the formation of truncated or cut wings. This is the phenotype that gave its name to the locus cut [2]. CDP/Cux was originally identified in vertebrates for its CCAAT displacement activity and later determined to be homologous to the *D. melanogaster* Cut protein. The human and mouse homologs are designated CDP and

Cux-1, respectively [3,4]. Later, a second *cux* gene was identified in mouse. In contrast to mouse Cux-1, which is expressed in most tissues, Cux-2 was found to be expressed primarily in nervous system tissues [5,6]. Here the term CDP/Cux is used to describe the protein in mammalian cells.

Genetic analyses in *D. melanogaster* indicate that Cut mediates phenotypic effects in a large number of tissues. Inactivation of Cux-1 by gene targeting in the mouse has revealed several phenotypes, including growth retardation, delayed differentiation of lung epithelia, altered hair follicle morphogenesis, male infertility, and a deficit in T and B cells [7,8]. In contrast to the small size of Cux-1 knockout mice, transgenic mice expressing Cux-1 showed multiorgan hyperplasia and organomegaly. Thus, from genetic studies both in *Drosophila* and in the mouse, it is clear that the CDP/Cux/Cut gene plays an important role in tissue homeostasis in many organs [9].

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CDP/Cux Gene and Proteolytic Processing

CDP/Cux gene

At the molecular level, CDP/Cux is a complex protein with four evolutionarily conserved DNA binding domains: the Cut homeodomain (HD); and the three Cut repeats (CR1, CR2, and CR3), three regions of approximately 70 amino acids that share 52%–63% amino acid identity with each other [3,10–13]. The full-length protein, referred to as CDP/Cux p200, is proteolytically processed at the G₁/S transition of the cell cycle, thereby generating the CDP/Cux p110 isoform that contains three DNA binding domains, CR2, CR3, and HD [14]. An individual Cut repeat can not bind to DNA on its own but needs to cooperate with a second Cut repeat or with the Cut homeodomain. CR1CR2 rapidly and transiently binds to DNA, whereas CR2CR3HD and CR3HD bind to DNA more slowly but stably [15]. It is predicted that CDP/Cux p110 has DNA binding properties similar to that of CR2CR3HD and CR3HD. However, CDP/Cux p200 behaves more like CR1CR2 and makes an unstable interaction with DNA, suggesting that DNA binding by CR3HD is inhibited in the context of the full-length protein [14,15].

Proteolytic processing of CDP/Cux

Specific proteolysis plays a significant role in the regulation of many basic cellular processes [16]. The control of cholesterol metabolism by proteolytic regulation of transcription factor SREBP [17], the cleavage of amyloid precursor protein that might affect the development of Alzheimer's disease [18], the proteolytic activation of Notch during development [19,20], and the intricate pathway of proteolytic cleavage of caspases and cytoskeletal proteins resulting in cell apoptosis [21–23]. In the field of transcription, proteolytic processing of transcription factors result in altering localization of these proteins or generating specific isoforms with different biochemical properties [16,17,24].

In the case of CDP/Cux, proteolytic processing of CDP/Cux by cathepsin L [25] generates an isoform with different DNA binding properties [14]. The resultant isoform can accelerate entry of cells into S phase, although it is not essential for cell cycle progression [26]. However, not all kinds of cathepsin L can process CDP/Cux; only the one that is devoid of a signal peptide in the nucleus can process CDP/Cux. Indeed, it has been proved that cathepsin L, which belongs to lysosomal cysteine proteases, can localize to the nucleus through a mechanism

involving translation initiation at downstream AUG sites and the synthesis of proteases that are devoid of a signal peptide [25].

Roles of CDP/Cux in Cell Cycle Regulation

Function as a transcriptional repressor

CDP/Cux was initially identified as a transcriptional repressor. It has been shown to bind to a variety of promoters or enhancer sequences of genes involved in cell differentiation, including myeloid cytochrome gp91-phox [27], dog heart myosin heavy chain [28], rat tyrosine hydroxylase [29], mouse N-CAM [4], and c-mos [30]. Overexpression of CDP/Cux revealed that it functions as a repressor of these target genes in proliferating precursor cells. In terminally differentiated cells, these target genes are induced when CDP/Cux DNA binding activity is down-regulated. The similar effects have been observed in the regulation of the osteocalcin gene. Endogenous CDP/Cux complex in osseous cells is proliferation-specific and down-regulated at the cessation of cell growth [31]. It is thus proposed that CDP/Cux functions as a transcriptional repressor that inhibits gene expression in terminally differentiated cells.

The repression of gene expression involves two distinct mechanisms: active repression through the recruitment of the histone deacetylase 1; and competition for binding site occupancy, probably through its CCAAT displacement activity [32,33].

Up-regulated DNA binding of CDP/Cux in S phase

A role for CDP/Cux in cell cycle progression, particularly at the G₁/S transition, a very important moment of the cell cycle at which the cell decides whether it should proliferate, differentiate or die, has been suggested by a number of studies [34–36]. The ability of CDP/Cux binding to DNA is cell cycle-dependent. Its binding to the ATCGAT sequence (a consensus binding site of CDP/Cux p110) increases as the cell cycle progresses into S phase [14,34]. Using the ATCGAT site as a probe, little CDP/Cux DNA binding was detected in G₀ or early G₁ phases. In contrast, strong DNA binding was observed in S phase. In NIH 3T3 cells transfected with the Myc-Cut-HA vector and then synchronized in G₀, early G₁, mid-G₁, and S phases, the 110 kDa protein was not detected in the population of cells enriched in G₀ phase. It was barely detectable in early G₁ and mid-G₁ phases, but was highly expressed in S phase. Pulse-chase labeling using NIH 3T3 cells indicates that the 110 kDa protein derives from the

200 kDa full-length CDP/Cut protein [14]. Thus the up-regulated DNA binding is due to the presence of CDP/Cux p110.

The increase in DNA binding involves two regulatory events, a specific proteolytic cleavage and dephosphorylation of the Cut homeodomain by the Cdc25A phosphatase, an important regulator of the G₁/S transition [34,37,38]. Cdc25A, one of the three members of Cdc25 homologs in human, is a tyrosine phosphatase and one of its activities is to remove an inhibitory phosphate molecule from the G₁ cyclin-dependent kinases (CDK) [39]. Many E2F-regulated genes encode proteins that are involved in DNA replication in cell cycle progression [40]. Cdc25A is also a target of E2F and can cooperate with cyclin E, another target of E2F, to induce S phase. As cells progress into S phase, a fraction of CDP/Cux p200 molecules are proteolytically processed into an amino-truncated form of 110 kDa, CDP/Cux p110 [14].

It is generally assumed that transcriptional activation requires stable DNA binding with the promoter. CDP/Cux p200 and CDP/Cux p110 show similar DNA binding affinity but very different DNA binding kinetics [15]. It is found that CDP/Cux p200 only transiently binds to DNA [14,15,35]. The presence of the N-terminal region in the full-length CDP/Cux protein inhibits its DNA binding activity and interferes with its transcriptional activation ability. Antibodies recognizing the N-terminal region of CDP/Cux p200 can enhance its DNA binding [41]. However, p110 has a slow and stable interaction with the DNA binding sequence [14]. Importantly CDP/Cux p110, but not CDP/Cux p200, is capable of stimulating expression of a reporter containing the promoter from the DNA polymerase α gene (DNA pol α). These data suggest that proteolytic processing of CDP/Cux to generate CDP/Cux p110 is an important mechanism for cell cycle regulation [14,26,35].

At the molecular level, CR3HD makes contact within the minor and major groove and wraps around the DNA, whereas CR1CR2 makes contact within the major groove only, only one side of the double helix. These differences in DNA binding are likely to explain the higher stability of the CR3HD-DNA complex compared with CR1CR2 [15]. This might further explain the molecular basis for CDP/Cux p110 binding to DNA more stably.

Down-regulated DNA binding of CDP/Cux p110 in G₂ phase

CDP/Cux DNA binding activity is changed in different phases of the cell cycle. Phosphorylation of serine residues in the region of the Cut homeodomain reduced DNA

binding in G₁ phase, whereas increased DNA binding in S phase coincided with dephosphorylation at the same sites [34]. Similarly, DNA binding of CDP/Cux decreases as cells progress from S to G₂ phase because of the interaction between CDP/Cux and cyclin A-Cdk1, which can phosphorylate two serines in the Cut homeodomain and inhibit DNA binding activity of CDP/Cux [42].

In addition to cyclin A-Cdk1, cyclin A-Cdk2 also interacts with CDP/Cux, but it can not phosphorylate the same sites in CDP/Cux and can not inhibit DNA binding by the wild-type CDP/Cux. In actuality, cyclin A-Cdk2 might stimulate its transcriptional activity [43].

In eukaryotic cells, one cell cycle encompasses the coordination of growth, replication, and cell division processes. Cyclin-Cdk complexes are serine/threonine kinases that play crucial roles in regulating these processes. Cyclin A-Cdk1 associates with CDP/Cux *in vitro* and inhibits its DNA binding activity. Furthermore, overexpression of cyclin A-Cdk1 inhibits DNA binding of the wild-type CDP/Cux but not a mutant CDP/Cux protein in which serines 1237 and 1270 were replaced with alanine [42]. These results are in accordance with the findings that CDP/Cux DNA binding activity decrease in G₂ phase, the phase of the cell cycle when the cyclin A-Cdk1 complex becomes prominent. All of these findings indicate that cyclin A-Cdk1 complex is important for the down-modulation of CDP/Cux activity [42,43].

Interaction of CDP/Cux with cyclin A-Cdk1 complex

The interaction between cyclin A-Cdk1 and CDP/Cux results in the decrease of CDP/Cux DNA binding activity. Both the Cut homeodomain and the region encompassing the cyclin-binding motif (Cy motif) appear to be needed for efficient binding to cyclin A-Cdk1 as weak or no binding was observed with a protein (CR1HD) that contains the Cut homeodomain without the Cy motif, or with a protein that contains the carboxy terminal domain (CTD) without the Cut homeodomain in pull-down assays. In the five Cy-related sequences of CDP/Cux, only the one starting at amino acid 1298, downstream of the Cut homeodomain, was able to bind to cyclin A-Cdk1. Moreover, in the *in vitro* kinase assay, removal of the Cy sequence reduced the efficiency of phosphorylation of CDP/Cux. Yet cyclin A-Cdk1 was still able to phosphorylate a CR3HD fusion protein in which the Cy sequence had been removed. So the removal of the Cy sequence diminished but did not abolish the effect of cyclin A-Cdk1 on the activity of CDP/Cux. In another words, the Cy motif in the CTD increases the efficiency of phosphorylation by cyclin A-Cdk1 [42].

Mechanisms of Cell Cycle Regulation by CDP/Cux

CDP/Cux, a part of the histone nuclear factor D (HiNF-D)

Progression into early S phase requires induction of histone gene expression, because *de novo* synthesis of histone nucleosomal proteins is essential for the ordered packing of newly replicated DNA into chromatin [44,45].

HiNF-D is a proliferation-specific promoter factor [46] and its nuclear abundance is cell cycle-dependent in normal diploid cells [47]. Many findings support that HiNF-D can bind to the promoters of several S phase-specific histone genes, including H₄, H₃, and H₁ [48–50]. Regulation of the interaction of HiNF-D with these genes correlates with modulations in histone gene expression. In the case of H₄ gene expression, HiNF-D interacts with the domain of H₄ promoter site II and another two nuclear factors, then HiNF-P and HiNF-M stimulate the expression of H₄ [49]. A similar situation occurs in H₃ and H₁ gene expression [51]. As a complicated complex, the identification of the DNA-binding subunit of HiNF-D is essential for understanding the role of this factor in cell cycle regulation of histone gene transcription. In 1994, van Wijnen *et al.* found that HiNF-D contains CDC2, cyclin A, and a retinoblastoma (RB)-related protein. They failed to prove which is the DNA-binding subunit of HiNF-D, but the experiments showed a 76 kDa factor that might represent an intrinsic DNA-binding component of HiNF-D [51]. Two years later, the researchers identified that CDP/Cux was the DNA-binding subunit of HiNF-D/ H₄ site II and related complexes in the histone H₃ and H₁ gene [36].

In short, HiNF-D can bind to the promoters of the H₄, H₃, and H₁ genes through its intrinsic DNA-binding subunit CDP/Cux at the same time in the cell cycle when these genes are induced and function as transcriptional activators [36,46,52,53]. This fact also implies the role of CDP/Cux, as a part of HiNF-D complex, in cell cycle progression.

It is interesting to mention the proliferation-dependent characteristic of HiNF-D. In HL60 cells, HiNF-D activity is clearly present in growing cells but the DNA binding activity of this factor is down-regulated dramatically during differentiation [46]. HiNF-D declines during the cessation of proliferation in both ROS 17/2.8 bone tumor cells and normal diploid osteoblasts [54]. This characteristic is similar to the transcriptional repression function of CDP/

Cux, but the exact relationship is unclear.

CDP/Cux p110, stimulating the expression of S phase-specific genes

A search of the promoter database with the CDP/Cux consensus binding site revealed that the promoter sequences of the DNA pol α gene contained several putative CDP/Cux binding sites in both *D. melanogaster* and humans [35]. Using reverse transcription-polymerase chain reaction, Truscott *et al.* confirmed that DNA pol α mRNA expression was up-regulated in S phase following re-entry of NIH 3T3 cells into the cell cycle. Using NIH 3T3 cells co-transfected with a luciferase reporter plasmid containing the sequence from –1561 to +47 of the human DNA pol α gene and either an empty vector or a vector expressing CDP/Cux p110, it was found that CDP/Cux p110 had little or no effect on the expression of the DNA pol α reporter when transfected NIH 3T3 cells were allowed to grow asynchronously. In contrast, expression of the DNA pol α reporter was stimulated in the presence of CDP/Cux p110 when NIH 3T3 cells were synchronized in S phase either by thymidine blockage or by serum starvation and restimulation. Full-length CDP/Cux protein was unable to stimulate DNA pol α expression. *In vivo*, endogenous CDP/Cux p110 protein also binds to the promoter of the DNA pol α gene and this binding is altered during the cell cycle [35].

In addition to the DNA pol α gene promoter, CDP/Cux p110 could stimulate the expression of reporter plasmid containing the promoter sequences of other S phase-specific genes such as the dihydrofolate reductase, carbamoyl-phosphate synthase/aspartate carbamoyl-transferase/dihydroorotase, and cyclin A genes, although the effect was less than that observed with the DNA pol α reporter [35,43] (**Fig. 1**).

Inhibition of CDK inhibitor during G₁-S transition

Besides stimulating the expression of S phase-specific genes, CDP/Cux can repress the expression of CDK inhibitors p21 and p27, which can result in cell cycle arrest.

CDP/Cux can repress p21 by occupying the p21 promoter in a region containing the TATA box and an Sp1 binding site during S phase [34]. Similar to p21, p27 is also found to be repressed by CDP/Cux both in the early stages of nephrogenesis prior to terminal differentiation and in CMV/Cux-1 transgenic mice with ectopically expressed Cux-1 [55]. Similar to p21 promoter, there are two Sp1 sites [56] and a CCAAT site in p27 promoter [32], both targets of CDP/Cux. Thus CDP/Cux might negatively regulate the expression of p27 through binding

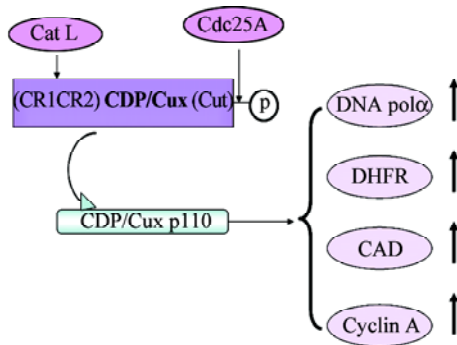


Fig. 1 CDP/Cux p110 stimulates expression of S phase-specific genes

The DNA binding activity of CDP/Cux p110 is up-regulated during S phase resulting from two events: proteolytic processing of CDP/Cux between Cut repeats CR1 and CR2 by cathepsin L (Cat L) to generate an isoform, CDP/Cux p110; and dephosphorylation of the Cut homeodomain by the Cdc25A phosphatase. The active CDP/Cux p110 can stimulate the expression of S phase-specific genes including the DNA polymerase α gene (DNA pol α), dihydrofolate reductase (DHFR), carbamoyl-phosphate synthase/aspartate carbamoyl-transferase/dihydroorotase (CAD), and cyclin A.

of these sites.

P21 is also found to inhibit proliferation cell nuclear antigen (PCNA) whose role is to confer processivity to DNA polymerase δ . As the result of CDP/Cux on p21, this inhibition is removed in S phase [34]. However, it is worthwhile pointing out one exception. In *Drosophila*, when cells are in a differentiated state, Cut is recruited to the *Drosophila* PCNA (dPCNA) promoter regions, by way of DRE as well as URE, or around these sites, and acts with other factors to switch off dPCNA gene expression [57]. This is unlike the down-regulated CDP/Cux DNA binding activity on terminal differentiation in mammalian cells and different from the situation in which CDP/Cux can activate PCNA by repressing the expression of p21 [34]. The mechanism that causes the difference between *Drosophila* and the mammalian counterpart is unclear but might be due to the various combinations of the four DNA binding domains of CDP/Cux.

Phosphorylation of CDP/Cux in G₂ phase

Sequence analysis of CDP/Cux reveals the presence of 23 potential C phosphorylation sites, SerPro, or ThrPro. Only two sites, Ser-1237 and Ser-1270 of the Cut homeodomain, are situated within or close to a DNA binding domain. These two sites are also in close proximity to the putative Cy site at position 1301–1303. The findings above are in accordance with the fact that phosphorylation within a region encompassing the Cut homeodomain was pre-

viously shown to correlate with inhibition of DNA binding. In a mutant assay, mutation of serine 1270 with alanine only slightly reduced the level of phosphorylation, whereas mutation of serine 1237 had a greater effect. Mutation of the two serines at the same time further reduced the level of phosphorylation. So serine residues 1237 and 1270 represent major and minor sites, respectively, of phosphorylation by cyclin A-Cdk1 [42] (Fig. 2).

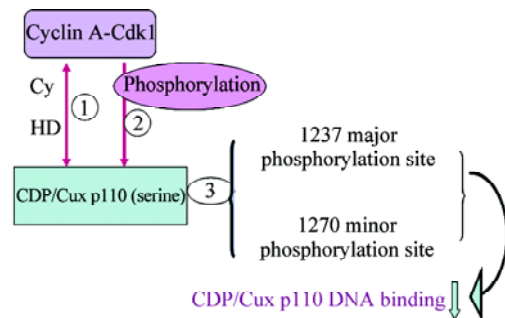


Fig. 2 CYCLIN A-CDK1 represses activity of CDP/Cux p110

Both the Cut homeodomain (HD) and the cyclin-binding motif (Cy motif) are needed for the interaction between cyclin A-Cdk1 and CDP/Cux. Phosphorylation of Ser-1237 and Ser-1270 in the HD by cyclin A-Cdk1 results in down-regulated DNA binding activity of CDP/Cux p110.

Possible Function of CDP/Cux in Cancers

From the research outlined above we can conclude that CDP/Cux contributes to cell proliferation and, especially, cell cycle progression in S phase. We could speculate that this function might stimulate the growth of some cancers. It has been reported that a majority of uterine leiomyomas expressed a higher level of CDP/Cux protein. In particular, the proteolytically processed isoforms of CDP/Cux, including CDP/Cux p110 and CDP/Cux p100, were expressed at a higher level in leiomyomas [58].

Apart from CDP/Cux p110, there is another isoform, CDP/Cux p75, that is detected in many breast tumor cell lines and breast tumors. It was found that its expression was activated in breast tumor cell lines and in primary human breast tumors. Like CDP/Cux p110, CDP/Cux p75 can localize to the nucleus, repress the P21^{WAF1/CIP1} reporter, and stimulate expression of the DNA pol α reporter. Studies indicated higher expression of p75 in invasive carcinoma is associated with a more diffused growth pattern [59]. The oncogenic potential of CDP/Cux p75 was further studied by Cadieux *et al.* with p75 transgenic mice. In their experiment, they found that transgenic mice

overexpressing CDP/Cux p75 developed a myeloproliferative disease-like myeloid leukemia. Thus increased p75 expression might play a causative role in the neoplastic process [60].

Here we noted that the identification of a CDP-related 76 kDa DNA binding subunit of HiNF-D, detected by van Wijnen *et al.* [36] is most likely related to the CDP/Cux p75, although there is no evidence to prove this idea and they seem to have different functions in different cell lines. However, at least, both of them should be devoid of the CR1 repeat.

Summary

In summary, in addition to being a transcriptional repressor, CDP/Cux might also act as a transcriptional activator. It is believed that proteolytic processing of CDP/Cux by cathepsin L in the nucleus generates the CDP/Cux p110 isoform at the beginning of S phase [25]. In other words, cathepsin L in the nucleus triggers the activation of CDP/Cux. CDP/Cux p110 can stimulate the promoters of DNA pol α gene and other S phase-specific genes. CDP/Cux p110 is also a part of HiNF-D. Taken together, these observations strongly suggest CDP/Cux might play an important role in cell cycle progression.

An abortive cell cycle attempt might be involved in neuronal apoptosis [61]. In recent studies, we found that cathepsin L could relocate to the nucleus of dopaminergic neurons on intoxication with 6-hydroxydopamine. This might cause a re-entry of dopaminergic neurons into cell cycle, suggesting that the activation of cathepsin L probably contributes to apoptosis of dopaminergic neurons. These observations have opened a new field for elucidating the pathogenic mechanisms in Parkinson's disease and warrant further investigation.

References

- Nepveu A. Role of the multifunctional CDP/Cut/Cux homeodomain transcription factor in regulating differentiation, cell growth and development. *Gene* 2001, 270: 1–15
- Jack J, Dorsett D, Delotto Y, Liu S. Expression of the cut locus in the *Drosophila* wing margin is required for cell type specification and is regulated by a distant enhancer. *Development* 1991, 113: 735–747
- Neufeld EJ, Skalknik DG, Lievens PM, Orkin SH. Human CCAAT displacement protein is homologous to the *Drosophila* homeoprotein, cut. *Nat Genet* 1992, 1: 50–55
- Valarche I, Tissier-Seta JP, Hirsch MR, Martinez S, Goridis C, Brunet JF. The mouse homeodomain protein Phox2 regulates Ncam promoter activity in concert with Cux/CDP and is a putative determinant of neurotransmitter phenotype. *Development* 1993, 119: 881–896
- Quaggin SE, Heuvel GB, Golden K, Bodmer R, Igarashi P. Primary structure, neural-specific expression, and chromosomal localization of Cux-2, a second murine homeobox gene related to *Drosophila* cut. *J Biol Chem* 1996, 271: 22624–22634
- Iulianella A, Vanden Heuvel G, Trainor P. Dynamic expression of murine Cux2 in craniofacial, limb, urogenital and neuronal primordia. *Gene Expr Patterns* 2003, 3: 571–577
- Luong MX, van der Meijden CM, Xing D, Hesselton R, Monuki ES, Jones SN, Lian JB *et al.* Genetic ablation of the CDP/Cux protein C terminus results in hair cycle defects and reduced male fertility. *Mol Cell Biol* 2002, 22: 1424–1437
- Sinclair AM, Lee JA, Goldstein A, Xing D, Liu S, Ju R, Tucker PW *et al.* Lymphoid apoptosis and myeloid hyperplasia in CCAAT displacement protein mutant mice. *Blood* 2001, 98: 3658–3667
- Ledford AW, Brantley JG, Kemeny G, Foreman TL, Quaggin SE, Igarashi P, Oberhaus SM *et al.* Deregulated expression of the homeobox gene Cux-1 in transgenic mice results in downregulation of p27(kip1) expression during nephrogenesis, glomerular abnormalities, and multiorgan hyperplasia. *Dev Biol* 2002, 245: 157–171
- Andres V, Chiara MD, Mahdavi V. A new bipartite DNA-binding domain: Cooperative interaction between the cut repeat and homeo domain of the cut homeo proteins. *Genes Dev* 1994, 8: 245–257
- Harada R, Dufort D, Denis-Larose C, Nepveu A. Conserved cut repeats in the human cut homeodomain protein function as DNA binding domains. *J Biol Chem* 1994, 269: 2062–2067
- Aufiero B, Neufeld EJ, Orkin SH. Sequence-specific DNA binding of individual cut repeats of the human CCAAT displacement/cut homeodomain protein. *Proc Natl Acad Sci USA* 1994, 91: 7757–7761
- Harada R, Berube G, Tamplin OJ, Denis-Larose C, Nepveu A. DNA-binding specificity of the cut repeats from the human cut-like protein. *Mol Cell Biol* 1995, 15: 129–140
- Moon NS, Premdas P, Truscott M, Leduy L, Berube G, Nepveu A. S phase-specific proteolytic cleavage is required to activate stable DNA binding by the CDP/Cut homeodomain protein. *Mol Cell Biol* 2001, 21: 6332–6345
- Moon NS, Berube G, Nepveu A. CCAAT displacement activity involves CUT repeats 1 and 2, not the CUT homeodomain. *J Biol Chem* 2000, 275: 31325–31334
- Vogel JL, Kristie TM. Autocatalytic proteolysis of the transcription factor-coactivator C1 (HCF): A potential role for proteolytic regulation of coactivator function. *Proc Natl Acad Sci USA* 2000, 97: 9425–9430
- Brown MS, Goldstein JL. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 1997, 89: 331–340
- Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB *et al.* Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 1999, 286: 735–741
- De Strooper B, Annaert W, Cupers P, Saftig P, Craessaerts K, Mumm JS, Schroeter EH *et al.* Presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature* 1999, 398: 518–522
- Schroeter EH, Kisslinger JA, Kopan R. Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. *Nature* 1998, 393: 382–386
- Salvesen GS, Dixit VM. Caspases: Intracellular signaling by proteolysis. *Cell* 1997, 91: 443–446
- Muzio M. Signalling by proteolysis: Death receptors induce apoptosis. *Int J Clin Lab Res* 1998, 28: 141–147
- Patel T, Gores GJ, Kaufmann SH. The role of proteases during apoptosis. *FASEB J* 1996, 10: 587–597

- 24 Aza-Blanc P, Ramirez-Weber FA, Laget MP, Schwartz C, Kornberg TB. Proteolysis that is inhibited by hedgehog targets Cubitus interruptus protein to the nucleus and converts it to a repressor. *Cell* 1997, 89: 1043–1053
- 25 Goulet B, Baruch A, Moon NS, Poirier M, Sansregret LL, Erickson A, Bogoy M *et al.* A cathepsin L isoform that is devoid of a signal peptide localizes to the nucleus in S phase and processes the CDP/Cux transcription factor. *Mol Cell* 2004; 14: 207–219
- 26 Sanaregret L, Goulet B, Harada R, Wilson B, Leduy L, Bertoglio J, Nepveu A. The p110 isoform of the CDP/Cux transcription factor accelerates entry into S phase. *Mol Cell Biol* 2006, 26: 2441–2455
- 27 Skalnik DG, Struss EC, Orkin SH. CCAAT displacement protein as a repressor of the myelomonocytic-specific gp91-phox gene promoter. *J Biol Chem* 1991, 266: 16736–16744
- 28 Andres V, Nadal-Ginard B, Mahdavi V. Clox, a mammalian homeobox gene related to *Drosophila* cut, encodes DNA-binding regulatory proteins differentially expressed during development. *Development* 1992, 116: 321–334
- 29 Yoon SO, Chikaraishi DM. Isolation of two E-box binding factors that interact with the rat tyrosine hydroxylase enhancer. *J Biol Chem* 1994, 269: 18453–18462
- 30 Higgy NA, Tarnasky HA, Valarche I, Nepveu A, Van der Hoorn FA. Cux/CDP homeodomain protein binds to an enhancer in the rat c-mos locus and represses its activity. *Biochim Biophys Acta* 1997, 1351: 313–324
- 31 van Gurp MF, Pratap J, Luong M, Javed A, Hoffmann H, Giordano A, Stein JL *et al.* The CCAAT displacement protein/cut homeodomain protein represses osteocalcin gene transcription and forms complexes with the retinoblastoma protein-related protein p107 and cyclin A. *Cancer Res* 1999, 59: 5980–5988
- 32 Mailly F, Berube G, Harada R, Mao PL, Phillips H, Nepveu A. The human Cut homeodomain protein can repress gene expression by two distinct mechanisms: Active repression and competition for binding site occupancy. *Mol Cell Biol* 1996, 16: 5346–5357
- 33 Li S, Moy L, Pittman N, Shue G, Aufiero B, Neufeld EJ, Le Leiko NS *et al.* Transcriptional repression of the cystic fibrosis transmembrane conductance regulator gene, mediated by CCAAT displacement protein/Cut homolog, is associated with histone deacetylation. *J Biol Chem* 1999, 274: 7803–7815
- 34 Coqueret O, Berube G, Nepveu A. The mammalian Cut homeodomain protein functions as a cell-cycle-dependent transcriptional repressor which downmodulates P21^{WAF1/CIP1/SDH1} in S phase. *EMBO J* 1998, 17: 4680–4694
- 35 Truscott M, Raynal L, Premdas P, Goulet B, Leduy L, Berube G, Nepveu A. CDP/Cux stimulates transcription from the DNA polymerase α gene promoter. *Mol Cell Biol* 2003, 23: 3013–3028
- 36 van Wijnen AJ, van Gurp MF, de Ridder MC, Tufarelli C, Last TJ, Bimbaum M, Vaughan PS *et al.* CDP/Cut is the DNA-binding subunit of histone gene transcription factor HiNF-D: A mechanism for gene regulation at the G₁/S phase cell cycle transition point independence of transcription factor EF2. *Proc Natl Acad Sci USA* 1996, 93: 11516–11521
- 37 Jimno S, Suto K, Nagata A, Igarashi M, Kanaoka Y, Nojima H, Okayama H. Cdc25A is a novel phosphatase functioning early in the cell cycle. *EMBO J* 1994, 13: 1549–1556
- 38 Blomberg I, Hoffmann I. Ectopic expression of Cdc25A accelerates the G₁/S transition and leads to premature activation of cyclin E- and cyclin A-dependent kinases. *Mol Cell Biol* 1999, 19: 6183–6194
- 39 Draetta G, Eckstein J. Cdc25 protein phosphatases in cell proliferation. *Biochim Biophys Acta* 1997, 1332: M53–M63
- 40 Vigo E, Muller H, Prosperini E, Hateboer G, Cartwright P, Moroni MC, Helin K. CDC25A phosphatase is a target of E2F and is required for efficient E2F-induced S phase. *Mol Cell Biol* 1999, 19: 6379–6395
- 41 Truscott M, Raynal L, Wang YF, Berube G, Leduy L, Nepveu A. The N-terminal region of the CCAAT displacement protein (CDP)/Cux transcription factor functions as an autoinhibitory domain that modulates DNA binding. *J Biol Chem* 2004, 279: 49787–49794
- 42 Santaguida M, Ding QM, Berube G, Truscott M, Whyte P, Nepveu A. Phosphorylation of the CCAAT displacement protein (CDP)/Cux transcription factor by cyclin A–Cdk1 modulates its DNA binding activity in G₂. *J Biol Chem* 2001, 276: 45780–45790
- 43 Santaguida M, Nepveu A. Differential regulation of CDP/Cux p110 by cyclin/Cdk2 and cyclin A/Cdk1. *J Biol Chem* 2005, 280: 32712–32721
- 44 Schumperli D. Cell-cycle regulation of histone gene expression. *Cell* 1986, 45: 471–472
- 45 Osley MA. The regulation of histone synthesis in the cell cycle. *Annu Rev Biochem* 1991, 60: 827–861
- 46 van Wijnen AJ, Wright KL, Lian JB, Stein JL, Stein GS. Human H4 histone gene transcription requires the proliferation-specific nuclear factor HiNF-D. Auxiliary roles for HiNF-C (Spl-like) and HiNF-A (high mobility group-like). *J Biol Chem* 1989, 264: 15034–15042
- 47 Holthuis J, Owen TA, van Wijnen AJ, Wright KL, Ramsey-Ewing A, Kennedy MB, Carter R *et al.* Tumor cells exhibit deregulation of the cell cycle histone gene promoter factor HiNF-D. *Science* 1990, 247: 1454–1457
- 48 van Wijnen AJ, Ramsey-Ewing AL, Bortell R, Owen TA, Lian JB, Stein JL, GS Stein. Transcriptional element H4-site II of cell cycle regulated human H4 histone genes is a multipartite protein/DNA interaction site for factors HiNF-D, HiNF-M, and HiNF-P: Involvement of phosphorylation. *J Cell Biochem* 1991, 46: 174–189
- 49 van Wijnen AJ, van den Ent FM, Lian JB, Stein JL, Stein GS. Overlapping and CpG methylation-sensitive protein–DNA interactions at the histone H4 transcriptional cell cycle domain: Distinctions between two human H4 gene promoters. *Mol Cell Biol* 1992, 12: 3273–3287
- 50 van den Ent FM, van Wijnen AJ, Lian JB, Stein JL, Stein GS. Cell cycle controlled histone H1, H3, and H4 genes share unusual arrangements of recognition motifs for HiNF-D supporting a coordinate promoter binding mechanism. *J Cell Physiol* 1994, 159: 513–530
- 51 van Wijnen AJ, Aziz F, Graña X, De Luca A, Desai RK, Jaarsveld K, Last TJ *et al.* Transcription of histone H4, H3, and H1 cell cycle genes: Promoter factor HiNF-D contains CDC2, cyclin A, and an RB-related protein. *Proc Natl Acad Sci USA* 1994, 91: 12882–12886
- 52 Wright KL, Dell’Orco RT, van Wijnen AJ, Stein JL, Stein JS. Multiple mechanisms regulate the proliferation-specific histone gene transcription factor HiNF-D in normal human diploid fibroblasts. *Biochemistry* 1992, 31: 2812–2818
- 53 el-Hodiri HM, Perry M. Interaction of the CCAAT displacement protein with shared regulatory elements required for transcription of paired histone genes. *Mol Cell Biol* 1995, 15: 3587–3596
- 54 van den Ent FM, van Wijnen AJ, Last TJ, Bortell R, Stein JL, Lian JB, Stein GS. Concerted control of multiple histone promoter factors during cell density inhibition of proliferation in osteosarcoma cells: Reciprocal regulation of cell cycle-controlled and bone-related genes. *Cancer Res* 1993, 53: 2399–2409
- 55 Ledford AW, Brantley JG, Kemeny G, Foreman TL, Quaggin SE, Igarashi P, Oberhaus SM *et al.* Deregulated expression of the homeobox gene Cux-1 in transgenic mice results in downregulation of p27(kip1) expression during nephrogenesis, glomerular abnormalities, and multiorgan hyperplasia. *Dev Biol* 2002, 245: 157–171
- 56 Minami S, Ohtani-Fujita N, Igata E, Tamaki T, Sakai T. Molecular cloning and characterization of the human p27^{kip} gene promoter. *FEBS Lett* 1997, 411: 1–6
- 57 Steo H, Hayashi Y, Kwon E, Taguchi O, Yamaguchi M. Antagonistic regulation of the *Drosophila* PCNA gene promoter by DREF and Cut. *Genes Cells* 2006, 11: 499–512
- 58 Moon NS, Zeng WR, Premdas P, Santaguida M, Berube G, Nepveu A. Expression of N-terminally truncated isoforms of CDP/Cux is increased in

- human uterine leiomyomas. *Int J Cancer* 2002, 100: 429–432
- 59 Goulet B, Watson P, Poirier M, Leduy L, Bérubé G, Meterissian S, Jolicoeur P *et al.* Characterization of tissue-specific CDP/Cux isoform, p75, activated in breast tumor cells. *Cancer Res* 2002, 62: 6625–6633
- 60 Cadieux C, Fournier S, Peterson AC, Bedard C, Bedell BJ, Nepveu A. Transgenic mice expressing the p75 CCAAT-displacement protein/Cut homeobox isoform develop a myeloproliferative disease-like myeloid leukemia. *Cancer Res* 2006, 66: 9492–9501
- 61 Liang ZQ, Wang X, Li LY, Wang Y, Chen RW, Chuang DM, Chase TN *et al.* Nuclear factor-kappaB-dependent cyclin D1 induction and DNA replication associated with N-methyl-D-aspartate receptor-mediated apoptosis in rat striatum. *J Neurosci Res* 2007, 85: 1295–1309

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