Ccr4–Not Complex

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This entry mainly gives an overview of the structure of the Ccr4-Not complex, its major components and their enzymatic activities. In the accompanying manuscript the biological roles of the complex is discussed in detail as well as clinical conditions associa

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1. Introduction

The yeast Ccr4–Not complex is a large (1.9-MDa) and highly conserved multifunctional assembly of proteins, involved in different aspects of mRNA metabolism. These include the repression and activation of transcription initiation, control of mRNA elongation, deadenylation-dependent mRNA turnover. The complex is also involved in ubiquitin-protein transferase activity reviewed[1][2][3][4][5]. The activation of the complex can be seen in various 'downstream effects' such as histone methylation and cell cycle regulation. Most of the original studies concentrated on the role of the Ccr4–Not complex in *Saccharomyces cerevisiae*. In yeast the complex has nine core subunits, comprising Ccr4 (carbon catabolite repression), Caf proteins (Ccr4 associated factor) (Caf1, Caf40, Caf130) and Not proteins (Not1, Not2, Not3, Not4, and Not5) as well as several less strongly associated components which are probably interacting partners[6][7] (Table 1). Not1 is the largest subunit of the complex (>200 kD) and forms a scaffold for the other components[8]. No clear function has been assigned to the Not module comprising Not2, Not3 and Not5 although it does act as a cofactor for the deadenylation activity and may be involved in interaction with the ribosome[9][10]. Genetic approaches in yeast have also demonstrated that the Not1–4 genes can globally repress RNA polymerase II activity. The mutation of these genes increases the basal expression of many genes[11]. The Ccr4-Caf1 mRNA deadenylase complex contains a 3′ exoribonuclease which is involved in removing poly (A) tails from mRNA[12][13][14][15][16]. Not4 is responsible for the second major enzymatic activity of the complex, E3 ligase-mediated ubiquitylation[17].

Complexes of a comparable size, containing the human orthologues CNOT1–CNOT9 with three additional subunits of CNOT10, Tab182 (Tankyrase 1-binding protein1, TNKS1BP1) and C2ORF29 (CNOT11), have been identified in mammals (Table 1). Four deadenylase subunits are expressed in mammalian cells. These appear to form various

heterodimers, CNOT7/CNOT6, CNOT7/CNOT6L, CNOT8/CNOT6, and CNOT8/CNOT6L. Thus, the complex contains either CNOT7 or CNOT8, suggesting they compete for binding to CNOT1[18][19]. While the E3 ubiquitin ligase Not4 is consistently present in the yeast complex, CNOT4 is not as stably associated as the other subunits in mammalian cells[18]. No orthologues of CNOT10, CNOT11, or Tab182 have been identified in yeast[20]. The CNOT3 subunit, with no specific enzymatic activity, is orthologous to two yeast subunits, Not3 and Not5. Every individual subunit appears to have a unique role with a slight overlap between some proteins[21]. The evidence in support of this includes the observation that mutations and deletions of each different subunit are responsible for different phenotypes in yeast[21].

2. Structure of the Ccr4–Not (CNOT) Complex

As with any large multi-subunit assembly of proteins considerable effort has been expended to determine the structure of Ccr4–Not. Up to the present, detailed structural information is available for the complex from four species: *Schizosaccharomyces pombe, Saccharomyces cerevisiae, Drosophila melanogaster,* and *Homo sapiens.* Limited data are also available for the thermophilic fungus *Chaetomium thermophilum* complex[9][14][16][22][23][24][25][26][27]. In all cases, it has been shown that CNOT1, the largest subunit (> 200 kD molecular mass), forms a scaffold for the complex and most, although not all, other components bind to it directly[28]. However, a number of obvious differences between the species have been identified. Firstly, there are four deadenylase components in the human complex (CNOTs 6, 6L, 7 and 8) but only two in yeast (Ccr4 and Caf1). The Ccr4-Caf1 mRNA deadenylase complex contains a 3′ exoribonuclease motif[12][13][14][15][16]. Secondly, it appears that the E3 ligase protein, CNOT4/Not4, is quite strongly associated with the complex in yeast but not in *Drosophila* or humans[17][18][29]. Thirdly, additional components have been identified in the *Drosophila* and human complexes. These are CNOT10 and CNOT11 (C2orf29). TNKS1BP1 (Tab182) also seems to be a member of the human complex[18][20][30](Table 1).

Table 1. A list of alternative names for equivalent CCR4–NOT complex subunits from *Saccharomyces cerevisiae* (baker's yeast), *Homo sapiens* (human) and *Drosophila melanogaster* (fruit fly).

Saccharomyces Cerevisiae	***Homo Sapiens***	***Drosophila Melanogaster***	***Function***
NOT1/CDC39	CNOT1	NOT1	**Scaffold**
NOT2/CDC36	CNOT2	ReginaNOT2	**Unknown but contributes to stabilization of the complex and RNA substrate recruitment**

NOT3	Not present	Not present	Unknown but contributes to stabilization of the complex and RNA substrate recruitment
NOT5	CNOT3	NOT3	Interaction with ribosomes
NOT4/MOT2/SIG1	CNOT4	NOT4	Ubiquitin E3-ligase activity
CCR4	CNOT6, CNOT6L	Twin, CCR4	Deadenylase
CAF1	CNOT7, CNOT8	P0P2, CAF1	Deadenylase
CAF40	CNOT9 (RQCD1)	RCD1	Transcriptional cofactor
Not present	CNOT10	NOT10	Unknown but contributes to stabilization of the complex and RNA substrate recruitment
Not present	CNOT11 (C2orf29)	NOT11	Unknown but contributes to stabilization of the complex and RNA substrate recruitment
CAF130	Not present	Not present	Unknown

Not present	**TNKS1BP1 (Tab182)**	**Not present**	**Multifunctional**

The complex, of approximately 1.9M molecular weight in yeast, is L-shaped with two arms of approximately equal length (180–190Å) and a hinged region present in the centre[22][24].

The complex is assembled on the NOT1 backbone. How the other components associate has been determined using in vitro protein binding studies, crystallography, and electron microscopy. Binding sites for the other components of the complex on CNOT1 appear to consist of α-helical domains. A number of structural sub-complexes have been delineated-these comprise the deadenylase module (Ccr4 and Caf1 in yeast, CNOT6/CNOT6L and CNOT7/CNOT8 in humans), the NOT4 (Not4) the E3 ligase module and the 'Not module' (Not2 and Not5 in yeast, CNOT2 and CNOT3 in humans). The binding sites of additional subunits have also been mapped-thus, metazoan CNOT10 and CNOT11 bind to the N-terminal domain of CNOT1 and CNOT9 (Caf40 in yeast) binds to the central region of CNOT1[16][20][25][31] (Figure 1). The C-terminal region of CNOT1 forms a rigid structure, which comprises two perpendicular stacks of HEAT-like repeats. CNOT2 and CNOT3 each contain SH3-like Not box domains which provide dimerization sites. CNOT2/CNOT3 binds to the CNOT1 C-terminal region[23][28] (Figure 1). The C-terminal binding site of CNOT1 comprises 10 HEAT repeats which contain helix A-turn-helix B motifs. The structures of the C-terminal complexes have been determined for the yeast and human proteins. CNOT2/Not2 and CNOT3/Not5 form a heterodimer through their Not-box motifs. A region of CNOT3/Not5 interacts with HEAT repeats 1–5 through hydrophobic and polar amino acids and a region of CNOT2/Not2 is spread across Not1 from HEAT repeats 9 and 10 and binding to repeats 4–6[9][23]. Although no specific functions have been determined for this C-terminal module it is linked to the stability of the complex as a whole and the recruitment of mRNAs [9]. The C-terminal complex associates with synthetic ribonucleotides, such as poly(U) RNA, in vitro with a binding site comprising structural elements from Not1, Not2 and Not5[9]. Boland et al. have also shown that an intact C-terminal module of the Drosophila CCR4–NOT complex is required for optimal mRNA degradation [23]. Furthermore, the CNOT2/CNOT3 heterodimer can stimulate the deadenylase activity of the complex [23]. Recent evidence indicates that yeast Not5 directly associates with the ribosome and, together with Not4, plays an important role in the regulation of mRNA half-life (Section 3)[10].

CNOT4 (Not4) is an evolutionarily conserved E3 ubiquitin ligase[17][29]. It contains a RING domain, a linker region which tends to form a coiled-coil, an RNA recognition motif (RRM) domain, and a C3H1-type zinc finger domain (ZNF)[31]. These motifs are all present within the conserved N-terminal region of human CNOT4. The C-terminal region of metazoan Not4 is variable in sequence but contains a conserved Caf binding motif (CBM) through which it binds directly to Caf40 (CNOT9)[31]. Flanking sequences to the CBM are involved in interaction with Not1 as well as assisting with Caf40 binding. This motif is not present in the yeast Not4 proteins although a Not1 binding site has been described[9]. It is not fully clear why yeast Not4 associates strongly with the full complex whereas the human and *Drosophila* proteins do not. However, it has been shown that the C-terminal region of human CNOT4 (Not4-C) is able to bind to the complex[31]. It has been suggested that the N-terminal region, therefore, somehow

prevents Not4-C from interacting in the human complex. This could be explained by possible post-translational modifications or additional binding partners[31]. As well as binding to Caf40/CNOT9 the Not4-C also interacts with the C-terminal HEAT region of CNOT1, with a relatively slight contribution from CNOT2 and CNOT3[9][31] (Figure 1). A number of substrates for ubiquitylation by CNOT4 have been identified and these are discussed elsewhere in this review (Section 4). Significantly, CNOT4 is required for optimal deadenylation activity by the full human CNOT complex, although a short C-terminal peptide will substitute for the whole protein[31].

The CNOT9 (Caf40) subunit binds to a central CNOT1 coiled coil domain (termed CN9BD or DUF3819). This is adjacent to the CNOT1 MIF-4G (middle portion of eIF4G) region (Figure 1). The human CNOT9 monomer contains six armadillo repeats forming a solvent-accessible, positively-charged cleft 21–22 Å wide[32]. Armadillo repeats are normally involved in protein-protein interactions but it has been shown that CNOT9 can also bind certain oligonucleotides in in vitro assays[32].

The deadenylase module associates with the central HEAT repeat-containing MIF-4G region of Not1. Thus, Caf1 (CNOT7) binds to Not1 through pre-existing structural motifs, made up of conserved hydrophobic residues. This allows full access for RNA molecules to the active site on Caf1[15][16]. The leucine rich repeat (LRR) domain of Ccr4 (CNOT6L) binds to Caf1. Caf1 interacts with Ccr4 via a surface formed by a long loop and an α-helix. This region of Caf1 undergoes a localized conformational change compared to the unbound structure[16]. In humans, two additional deadenylase components are present in the complex, CNOT6 and CNOT8, and it seems likely that they bind to CNOT1 in a comparable way to CNOT6L and CNOT7 (Figure 1).[32][33][34][35][36][37][38][39][40]

One of the obvious differences between the yeast and metazoan CNOT complexes is the presence of CNOT10 and CNOT11 (C2orf29) in the latter complex. Human and *Drosophila* CNOT11 binds to the N-terminal region of CNOT1 and CNOT10 binds to it[20]. The area of Not1 between the MIF-4G region and the N-terminal region comprises thirteen HEAT domains. The Not1 a.a.154–753 structure has been shown to contain antiparallel helices assembled side by side to form an elongated molecule. It has been suggested that these form structures ideal for protein–protein interactions within the complex or for binding of other molecules[16]. It should be noted, however, that this structure was determined for yeast CNOT1 which has no CNOT10 and 11 orthologs. Although CNOT10 and 11 do not appear to have any enzymatic activity of their own, their presence stimulates deadenylation through stabilization of the RNA substrate. It has been suggested that Caf40 (CNOT9) is the major enhancer of deadenylation, probably due to its proximity to the exonucleases, but if this is not available, CNOT10 and CNOT11 can compensate[25].

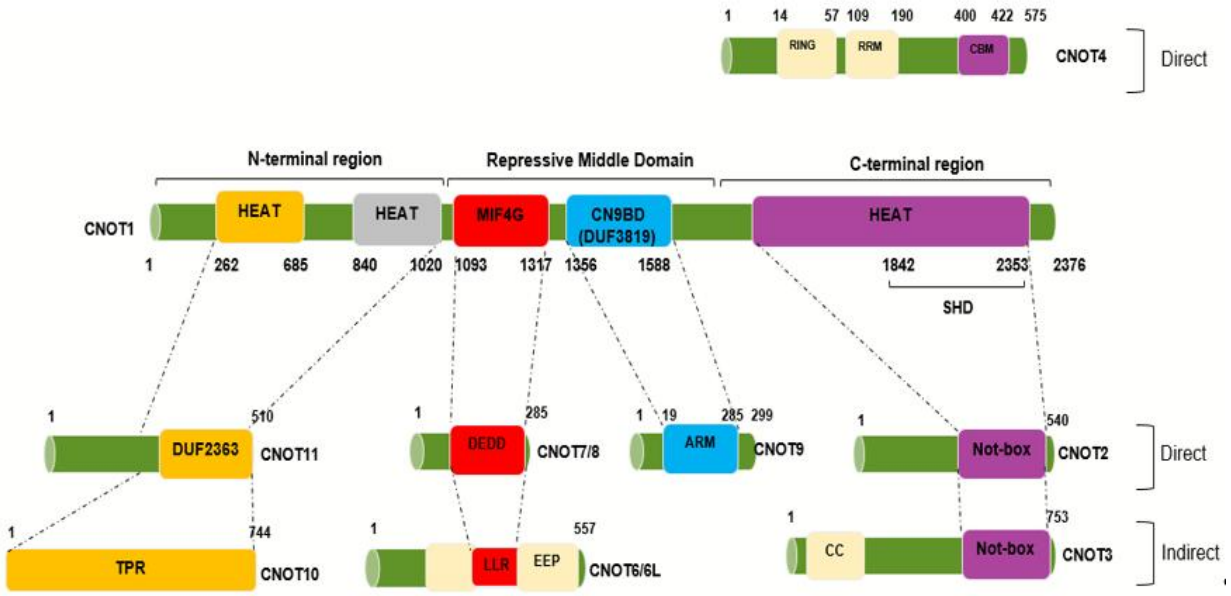

Figure 1. Human CNOT1 interaction regions with the other CNOT subunits. Interaction map obtained from negative-stain electron microscopy showing the interaction sites between CNOT1, acting as a scaffolding platform, and the other CNOT subunits. The human homologue, CNOT1, encompasses 2376 amino acids and is 20% identical (32% similar) to its yeast counterpart. Different colors represent the different regions as shown above. The human Tab182 interacting site has not been mapped yet. Negative on TATA-less (NOT), Middle domain of eukaryotic initiation factor 4G (MIF4G), Domain of uncharacterized function (DUF3819 and DUF2363), Exonuclease-endonuclease-phosphatase (EEP)-DNase I-like domains, Death effector domain-containing protein (DEDD)-RNase D-like domains, Leucine-rich region (LRR)-RNase D-like domains, Superfamily Homology Domain (SHD), Armadillo (ARM) Repeat Domain, predicted coiled-coil domain (CC), Predicted domain consisting of α-helical TPR-like repeats (TPR), CAF40-binding motif (CBM) (Adapted from[25][31][33][34].

Depletion of CNOT1 results in the destabilization of the whole complex and degradation of some other subunits such as CNOT2, CNOT6L, CNOT7, and CNOT9, but not CNOT3, in HeLa cells[35]. Although, CNOT1 has no enzymatic activity, as far as is known, the importance of its scaffolding function for the deadenylase activities of the CNOT complex cannot be overstated. It has been shown that in CNOT1-depleted HeLa cells the level of CHOP mRNA increased and the cells undergo caspase-4 activation causing ER stress-mediated apoptosis, indicating CNOT1 is essential for viability and cell proliferation[35]. In addition, CNOT1 depletion in HeLa cells reduces the deadenylase activities and decreases the level of P-body formation, where mRNA decay is thought to take place[35].

What are considered to be the major constituents of the CNOT complex are described above. However, a number of other proteins have been routinely found to associate with the complex[36][6][7]. It appears that these components are not integral but are required for routine functions. For example, the BTG/Tob complex binds to CNOT7 and has a role in the recruitment of mRNAs[37]. Consequences of the interaction have been reported variously to be either activation or repression of deadenylase activity[38][39][40]. A large number of other RNA binding proteins (for example, Nanos2, Pumilio, Smaug and Tristetrapolin) act as adaptor proteins and interact with the CNOT complex

and cause the suppression of target mRNAs[36]. Recent evidence suggests that Puf3 and Zfs1 associate with the complex and are critical in mRNA substrate selectivity as well as enhancing RNA binding [41]. Puf3 can distinguish between RNAs of very similar sequence and can therefore facilitate the ability of the Ccr4–Not complex to regulate the level of expression of particular target proteins[41] [41]. Interaction of the complex with GW182 (a component of miRISC) through CNOT1 plays a role in miRNA-induced deadenylation[42][43][44]. The core subunit, CNOT1, has also been shown to associate with the translational repressor and decapping activator, the DEAD box protein DDX6[45][46][47] (Section 3). DDX6 plays a role as a translational repressor in different pathways, including mRNA storage in erythropoiesis and microRNA mediated gene silencing[47]. Other proteins shown to interact with the CNOT complex include Bag-of-marbles, which represses the expression of specific mRNAs and binds to CAF40 (CNOT9), HIPK family kinases, which associate with the CCR4–NOT components CNOT2 and CNOT3 and phosphorylate the complex, the transcription factor EBF1, which binds CNOT3 and yTAFI, a core component of TFIID, which binds Not1[48][49][50][51]. A number of these are discussed in more detail in the following sections.

Interestingly, it has been shown that the Ccr4–Not complex strongly associates with the YTH domain of nucleus-specific RNA binding subunit, Mmi1 (meiotic mRNA interceptor 1), close to the nuclease module, in *Schizosaccharomyces pombe*[24]. In fission yeast, Mmi1 is essential for viability. It represses the expression of transcripts and increases the deadenylation of target RNAs in in vitro assays[8].

In the accompanying manuscript, the role of the Ccr4-Not complex in various biological processes is discussed in detail as well as its links to various clinical conditions.

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