Gene 492 (2012) 42–53

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Review

The Ccr4–Not complex

Martine A. Collart *, Olesya O. Panasenko

Dpt Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, 1211 Geneva 4, Switzerland

ARTICLE INFO

Article history:
Accepted 29 September 2011
Available online 15 October 2011

Received by Meghan Jendrysik

Keywords:
Gene expression
Transcription
Deadenylation
Ubiquitination
Chaperone

ABSTRACT

The Ccr4–Not complex is a unique, essential and conserved multi-subunit complex that acts at the level of many different cellular functions to regulate gene expression. Two enzymatic activities, namely ubiquitination and deadenylation, are provided by different subunits of the complex. However, studies over the last decade have demonstrated a tantalizing multi-functionality of this complex that extends well beyond its identified enzymatic activities. Most of our initial knowledge about the Ccr4–Not complex stemmed from studies in yeast, but an increasing number of reports on this complex in other species are emerging.
In this review we will discuss the structure and composition of the complex, and describe the different cellular functions with which the Ccr4–Not complex has been connected in different organisms. Finally, based upon our current state of knowledge, we will propose a model to explain how one complex can provide such multi-functionality. This model suggests that the Ccr4–Not complex might function as a "chaperone platform".

Contents

Abbreviations: EEP, endonuclease-exonuclease-phosphatase; RNase, ribonuclease; TAF, TBP-associated factor; HAT, histone acetyl transferase; CTD, C-terminal domain; CPF, Cleavage and polyadenylation factor; TFIID, Transcription factor II D; TFIIS, Transcription factor II S; RNR, Ribonucleotide reductase; NAC, Nascent polypeptide associated complex; 3′UTR, 3′Untranslated region; AGO, Argonaute; PUF, Pumillo family.

* Corresponding author. Tel.: +41 22 3795476; fax: +41 22 3795702.
E-mail address: martine.collart@unige.ch (M.A. Collart).

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doi:10.1016/j.gene.2011.09.033

1. Introduction

The Ccr4–Not complex is a unique, essential, and still rather enigmatic multi-subunit complex that is conserved across the eukaryotic kingdom and acts at the level of many different cellular functions to regulate gene expression (for previous reviews see (Collart, 2003; Denis and Chen, 2003; Collart and Timmers, 2004)). Over the last decade the multi-functionality of this complex has been truly tantalizing. In yeast it consists of 9 core subunits: Ccr4, Caf1, Caf40, Caf130, and Not1-5 that exist in at least two distinguishable forms of 1 and 2 MDa (Bai et al., 1999; Liu et al., 1998). Complexes of a similar size containing the human orthologs CNOT1–CNOT10 have been identified in human cell extracts (Gavin et al., 2002; Morel et al., 2003). The smaller form is likely to consist only of the core subunits, while the larger form might correspond to multiple species of the core complex interacting with other cellular factors.

Genome-wide studies have demonstrated that the different subunits have very specific and only partially overlapping roles in the expression of the genome (Azzouz et al., 2009b; Cui et al., 2008; Mittal et al., 2011). Consistently, different mutant phenotypes have generally been associated with mutations or deletions of the different subunits (for review see Collart, 2003). Hence, the reason for the association of these different subunits in a single complex, and the role of the complex for the function of the different subunits has been difficult to grasp. It is today still mostly elusive.

Most of our initial knowledge about the Ccr4–Not complex stemmed from studies in yeast, but an increasing number of reports on this complex in other species are emerging. We will first go over what we know about the structure and composition of the complex, concentrating on *Saccharomyces cerevisiae* and to a lesser extent human. We will then describe the different cellular functions with which the Ccr4–Not complex has been connected in different organisms. Finally based upon the current state of our knowledge we suggest that the essential function of this conserved complex might be that of a “chaperone platform”.

2. Description of the CCR4–Not complex

2.1. Structure of the Ccr4–Not complex

As mentioned above 9 core subunits define the Ccr4–Not complex in yeast, and homologs of these subunits exist in most cases in the other eukaryotes (Fig. 1). There are some exceptions. For instance, no gene with similarity to *CAF130* could be identified in *Drosophila*, but all the other subunits, with the exception of Not4, are associated in a stable complex (Temme et al., 2010). Another example concerns Not3 and Not5. The yeast proteins share 44% identity within their N-terminal coiled-coil domains, and in both human (Albert et al., 2000) and *Drosophila* (Temme et al., 2004) there is only one gene encoding a protein with a domain homologous to this yeast N-terminal domain, which has been called CNOT3. In human this unique gene is nevertheless alternatively spliced to produce a long or short protein (CNOT3L and CNOT3S). At present no data confirms that this gene is the functional homologue of yeast *NOT3* rather than that of yeast *NOT5*. In yeast the two genes are not entirely redundant and the deletion of *NOT5* has a much more severe phenotype than that of *NOT3* (Oberholzer and Collart, 1998). The two genes may have originated by gene duplication. Gene duplication and divergence have certainly expanded the *CCR4* and *CAF1* genes in plants, flies, worms and mammals (Dupressoir et al., 2001).

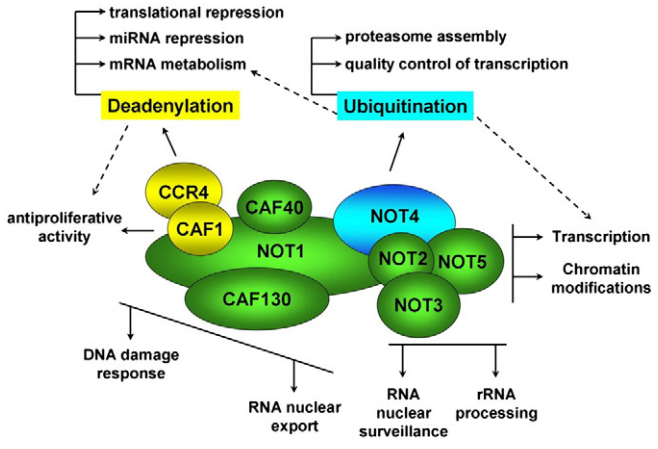

Fig. 1. Scheme of the Ccr4–Not complex. Different subunits of the Ccr4–Not complex are grouped in color codes according to their function: subunits of the complex responsible for deadenylation activity (Ccr4 and Caf1) are indicated in yellow and that for ubiquitination activity (Not4) in blue. The other subunits of the complex are in green.

Although the composition of the Ccr4–Not complex has been known for quite some time, not much structural information is available. Some insight into the global shape of the complex purified by the Not1 subunit and after cross-linking on a glycerol gradient, was recently provided by electron microscopy (Nasertorabi et al., 2011). An L-shaped complex with 2 arms of similar length was described. Nevertheless, it is still too early to say how this shape relates to its function. Otherwise, conserved domains and/or motifs present within the different subunits have been described as summarized in several reviews and manuscripts (Albert et al., 2000; Collart, 2003; Collart and Timmers, 2004; Denis and Chen, 2003). This contributed to reveal two different enzymatic activities associated with the Ccr4–Not complex, namely ubiquitination and deadenylation (Fig. 1). Ubiquitination is an activity provided by the Not4 subunit, a RING E3 ligase (blue subunit in Fig. 1). The first clue was obtained by the isolation of E2 enzymes as two-hybrid partners for the human Not4 (CNOT4) RING finger domain (Albert et al., 2002). Subsequently a solution structure of this C_4C_4 RING domain of CNOT4 was defined by heteronuclear NMR and ubiquitin ligase activity could be demonstrated *in vitro* (Albert et al., 2002; Hanzawa et al., 2001). In human and fly cells, CNOT4 is not a stable subunit of the complex (Jeske et al., 2006; Lau et al., 2009) in contrast to the situation in yeast, where its interaction domain with the rest of the complex has been mapped to its C-terminus (Panasenko and Collart, 2011).

The second enzymatic activity is deadenylation (Tucker et al., 2001). This activity is provided by the Ccr4 subunit in yeast, but by the orthologs of both Ccr4 and Caf1 in other eukaryotes (see yellow subunits in Fig. 1). Ccr4 has an exonuclease domain that belongs to a large endonuclease–exonuclease–phophatase (EEP) family of proteins, and it also defines a sub family of RNases. It is a 3′ exoribonuclease with a preference for poly(A) substrates (Chen et al., 2002) and is active as a monomer. A high-resolution 3-D structure of the nuclease domain of a human ortholog, CNOT6L, has been obtained (Wang et al., 2010). Caf1 links Ccr4 to the rest of the Ccr4–Not complex and contributes importantly to deadenylation (Tucker et al., 2002). It has itself a RNaseD domain from the DEDD superfamily (named after conserved catalytic Asp and Glu residues in 3 exonuclease motifs) that however does not contribute to deadenylation in yeast and has a small amino acid insertion in the active site not conserved in other species. Yet yeast Caf1 has 3′-5′ exonuclease activity *in vitro* (Daugeron et al., 2001). The first structure of an RNAseD domain was that of *S. cerevisiae* Caf1 (Thore et al., 2003), and subsequently a crystal structure for the *Schizosaccharomyces pombe* Caf1 with a fully active DEDD site, was solved at high resolution (Jonstrup et al., 2007). Selectivity of Caf1 for poly(A) and its distributive behavior could be attributed to specific conserved side chains in the active site (Andersen et al., 2009). Yeast has single Caf1 and Ccr4 proteins, human have CNOT7 and CNOT8 (also called hCaf1 and hPop2) as orthologs of Caf1, and CNOT6 or CNOT6L for Ccr4. While these four deadenylases are all associated with the Ccr4–Not complex, only one DEDD/Caf1-type subunit, and one EEP/Ccr4-type deadenylase