



HAL
open science

Structure-function analysis of the box C/D snoRNP assembly factor Bcd1p

Benoît Bragantini, Decebal Tiotiu, Marc Quinternet, Hélène Marty, Benjamin Rothé, Bruno Charpentier, Xavier Manival

► **To cite this version:**

Benoît Bragantini, Decebal Tiotiu, Marc Quinternet, Hélène Marty, Benjamin Rothé, et al.. Structure-function analysis of the box C/D snoRNP assembly factor Bcd1p. 10th Meeting SifrARN, Mar 2016, Toulouse, France. <hal-05442328>

HAL Id: hal-05442328

<https://hal.science/hal-05442328v1>

Submitted on 5 Jan 2026

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



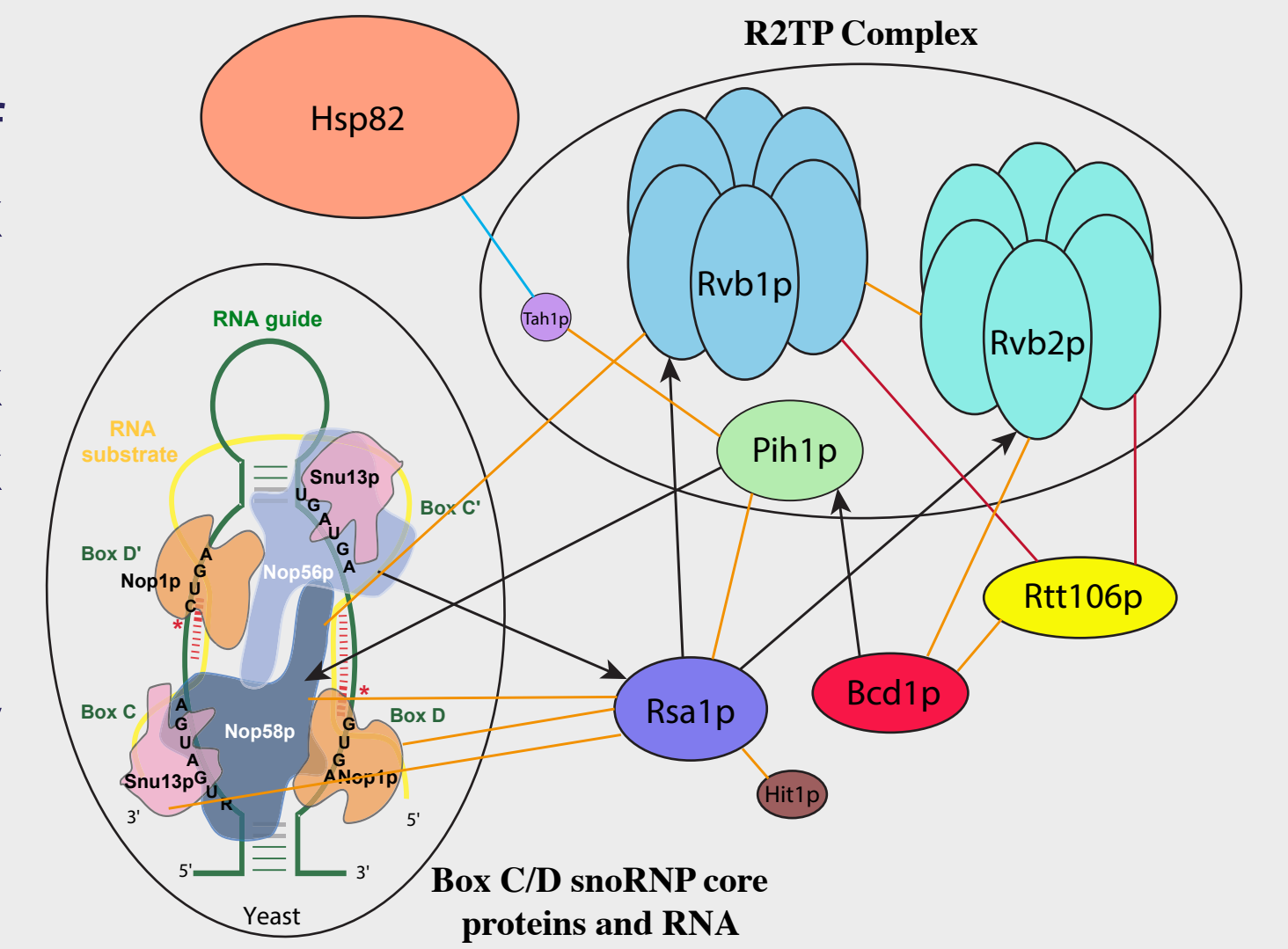
HAL Authorization

Benoît BRAGANTINI¹, Decebal TIOTIU¹, Marc QUINTERNET², Hélène MARTY¹, Benjamin ROTHÉ¹, Bruno CHARPENTIER¹ & Xavier MANIVAL¹

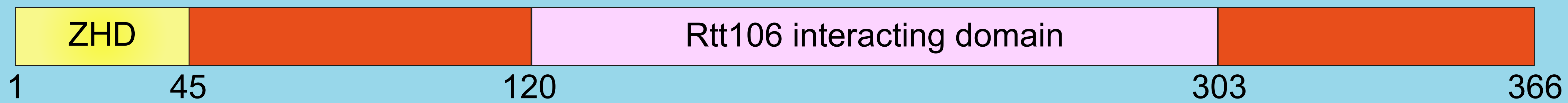
¹ Ingénierie Moléculaire et Physiopathologie Articulaires (IMoPA), UMR 7365 CNRS Université de Lorraine, Biopôle, Campus Biologie-Santé, 9 Avenue de la Forêt de Haye, CS 50184, 54505 Vandoeuvre-lès-Nancy, France.
² FR 3209 CNRS-Université de Lorraine, Bioingénierie Moléculaire, Cellulaire et Thérapeutique (BMCT), Biopôle, Campus Biologie-Santé, CS 50184, 54505 Vandoeuvre-lès-Nancy Cedex, France.

INTRODUCTION

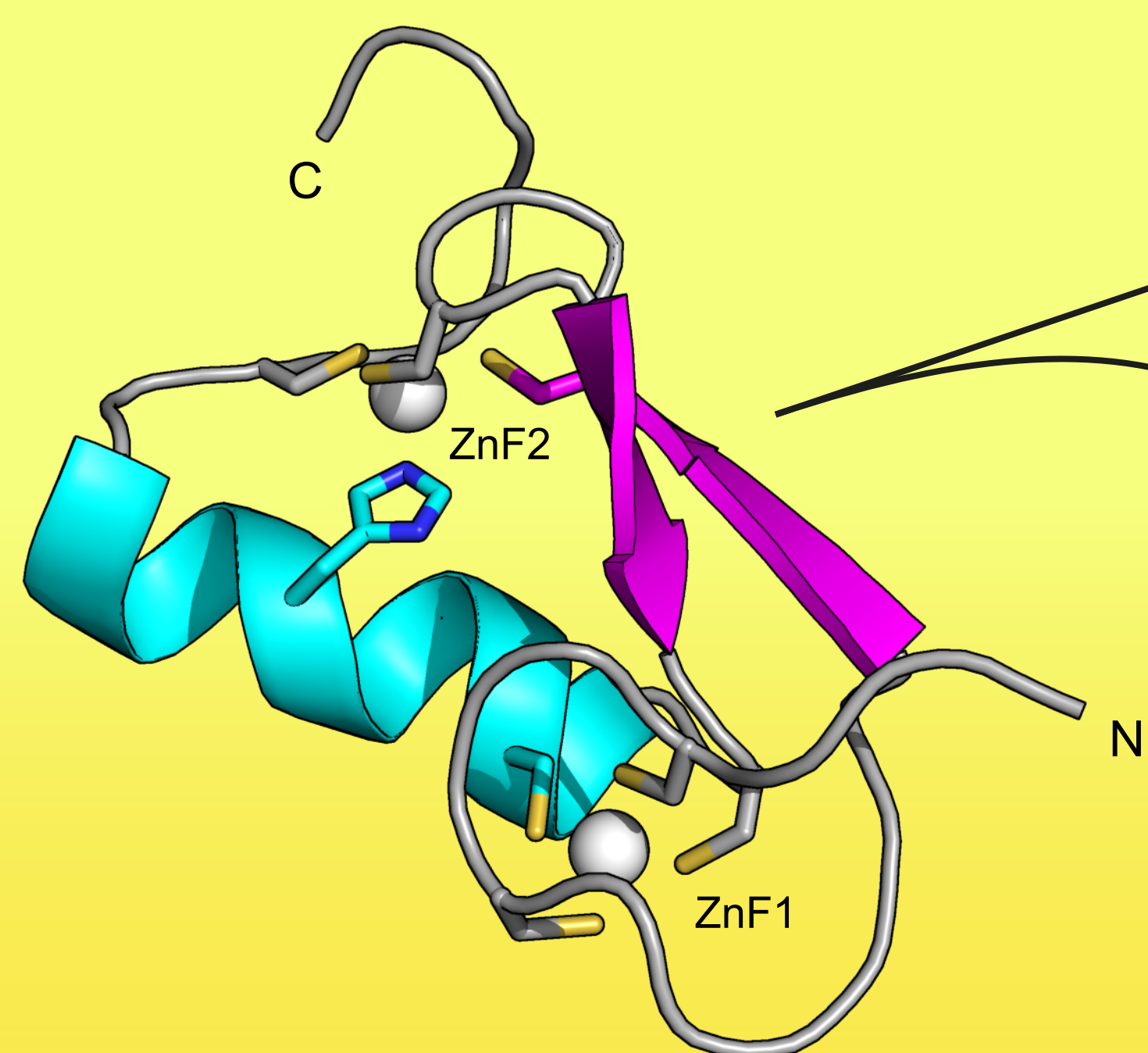
In eukaryotes, maturation of the precursor ribosomal RNA (pre-rRNA) takes place within the nucleolus, and involves two families of small nucleolar RNPs (snoRNPs): the box C/D and box H/ACA snoRNPs. Each box C/D snoRNP is composed of a specific box C/D RNA and the invariant set of four core proteins: Snu13p, Nop1, Nop56p and Nop58p (figure on right). Assembly of box C/D snoRNPs in functional entities is a complex process, which includes many quality control factors. The HSP82-interacting complex R2TP that is composed of four proteins – the AAA+ hexameric helicases Rvb1 and Rvb2, Tah1p and Pih1p – contributes to box C/D snoRNP biogenesis by interacting with the platform protein Rsa1 (Figure on the right). These factors transiently interact with the snoRNP components and are required for mature box C/D snoRNP accumulation. Here, we present structural and functional analyses for the *S. cerevisiae* assembly factor Bcd1p, also conserved in vertebrates (BCD1), which is essential for cellular viability and box C/D snoRNA stability. This protein contains in its N-terminal region a zinc finger domain from the zf-HIT family (ZHD), and is predicted to be in association with the histone chaperone Rtt106, which is important for histone deposition on DNA.



Characterization of functional domains of Bcd1p

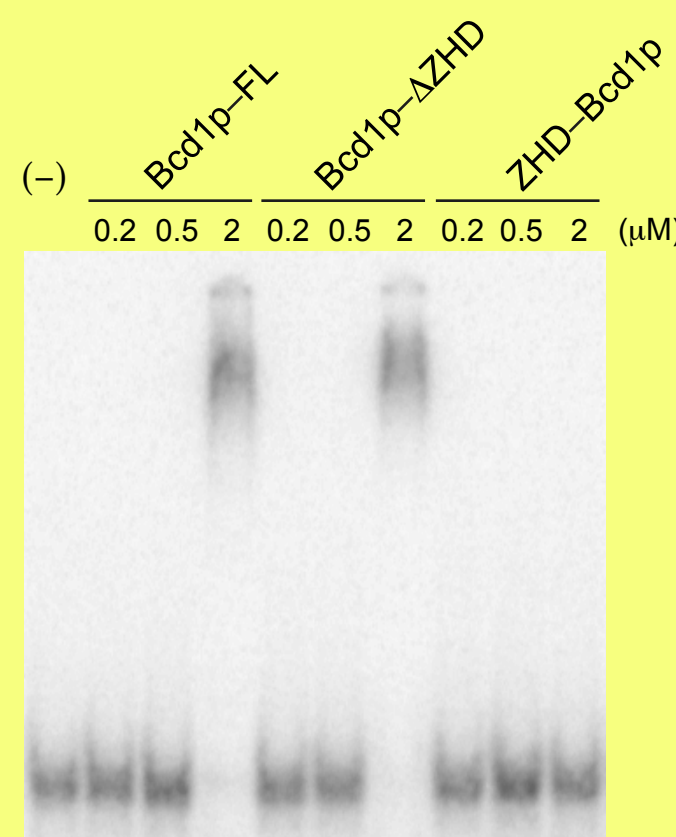


Functional significance of Bcd1p ZHD

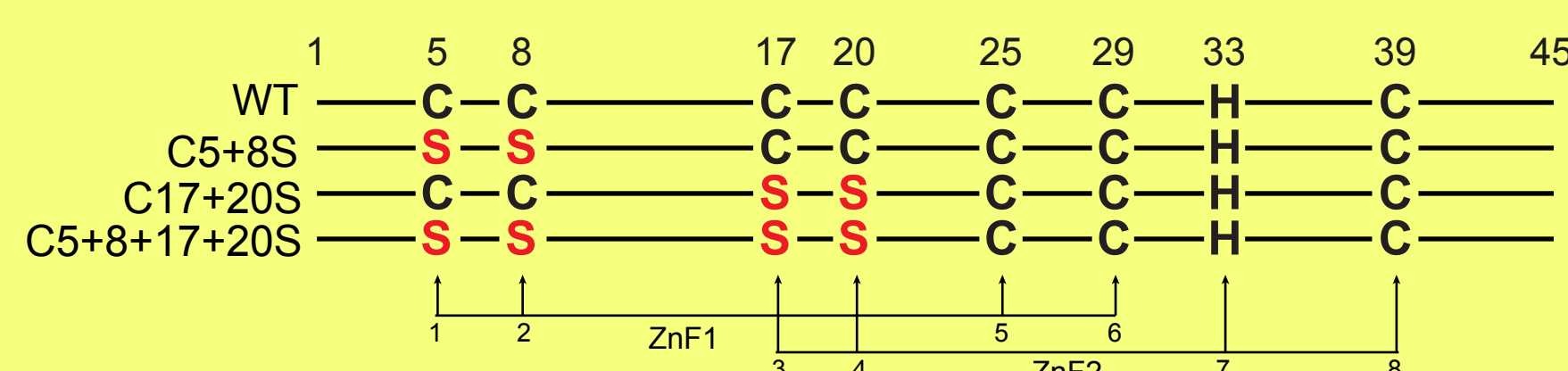


The fold of Bcd1p ZHD is typical of a member of the zf-HIT family, with 2 coordinated zinc ions

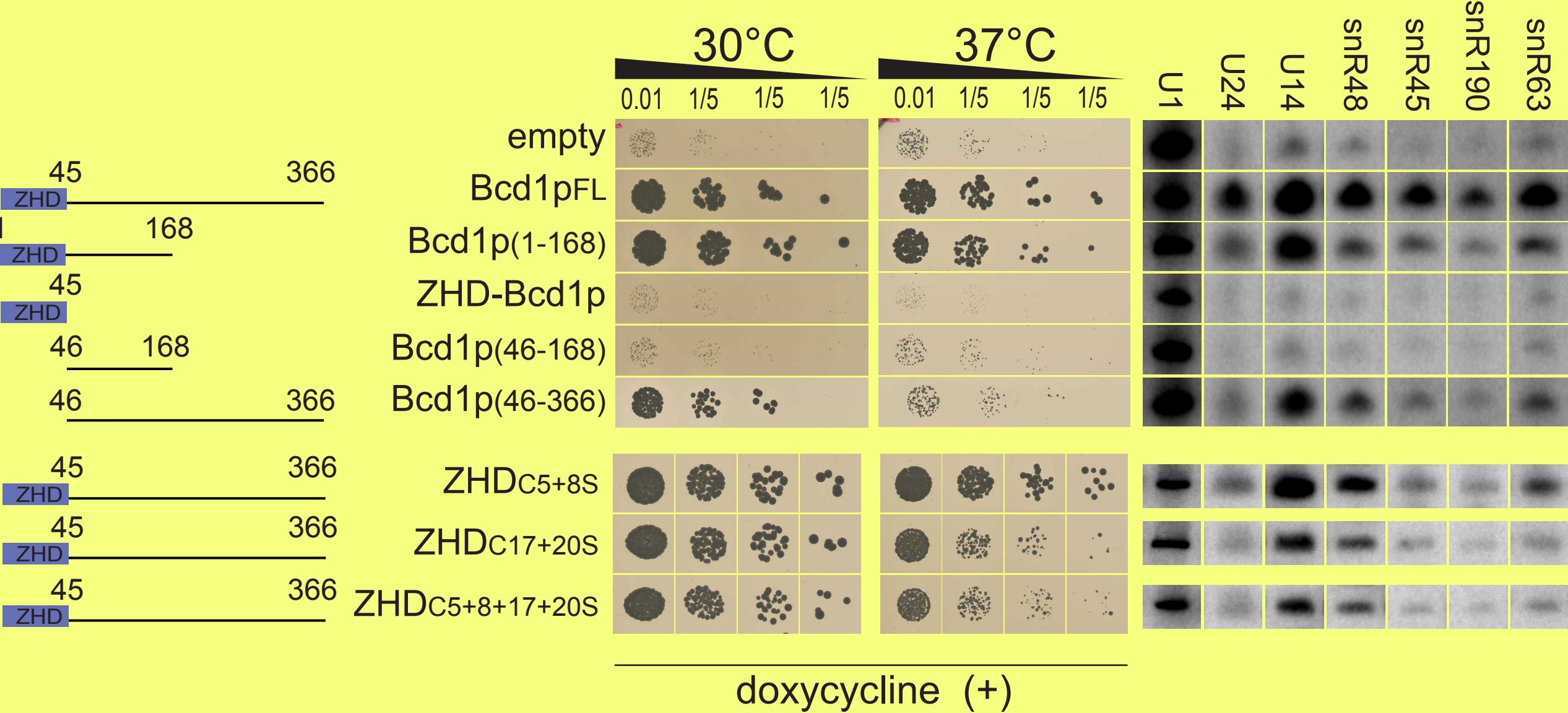
Bcd1-ZHD is not an RNA binding domain



Design of 3 mutants disrupting coordination with the 1st, 2nd or both zinc ions

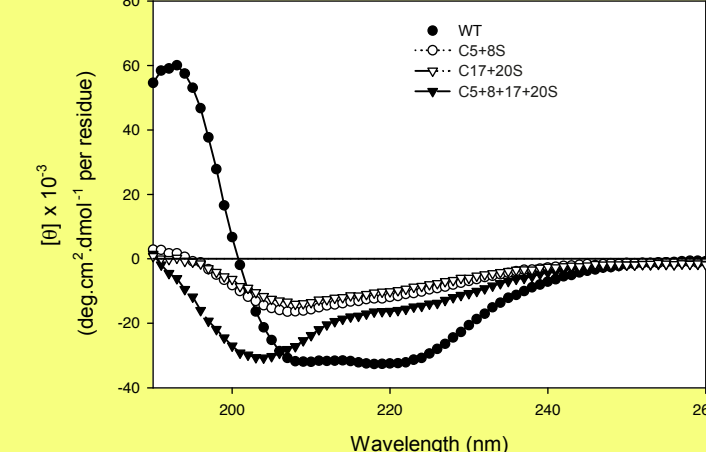


Tests on yeast growth and box C/D snoRNA level



Functional analysis of Bcd1p domains and mutants of ZHD. Complementation tests with different fragments or mutants of Bcd1 in *tetO₂::BCD1* yeast strain (R1158) in presence of doxycycline and associated northern blot analysis of box C/D snoRNA levels (at 30°C). Fragments or mutants are represented on the left.

Mutants of ZHD are defolding the protein.

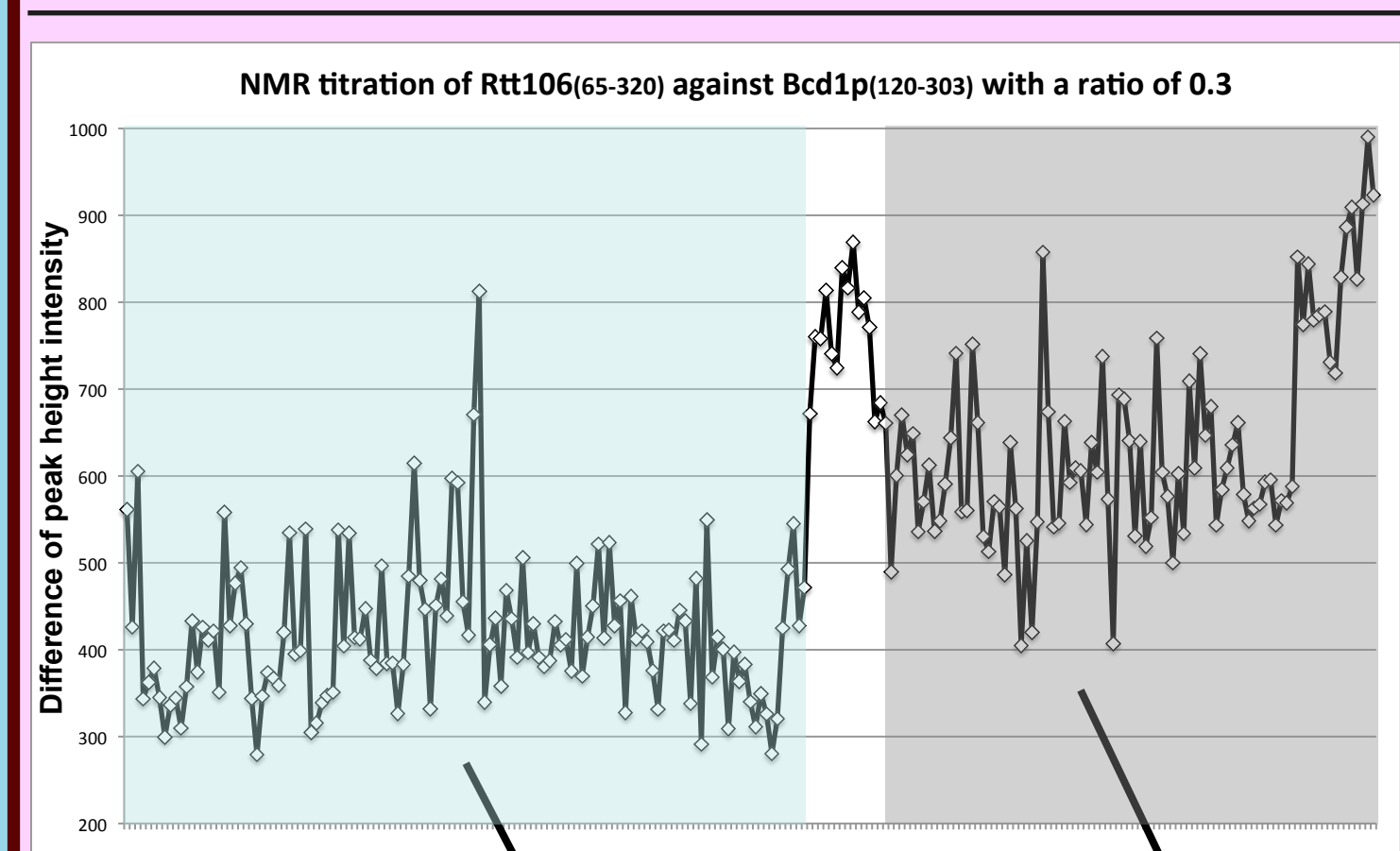
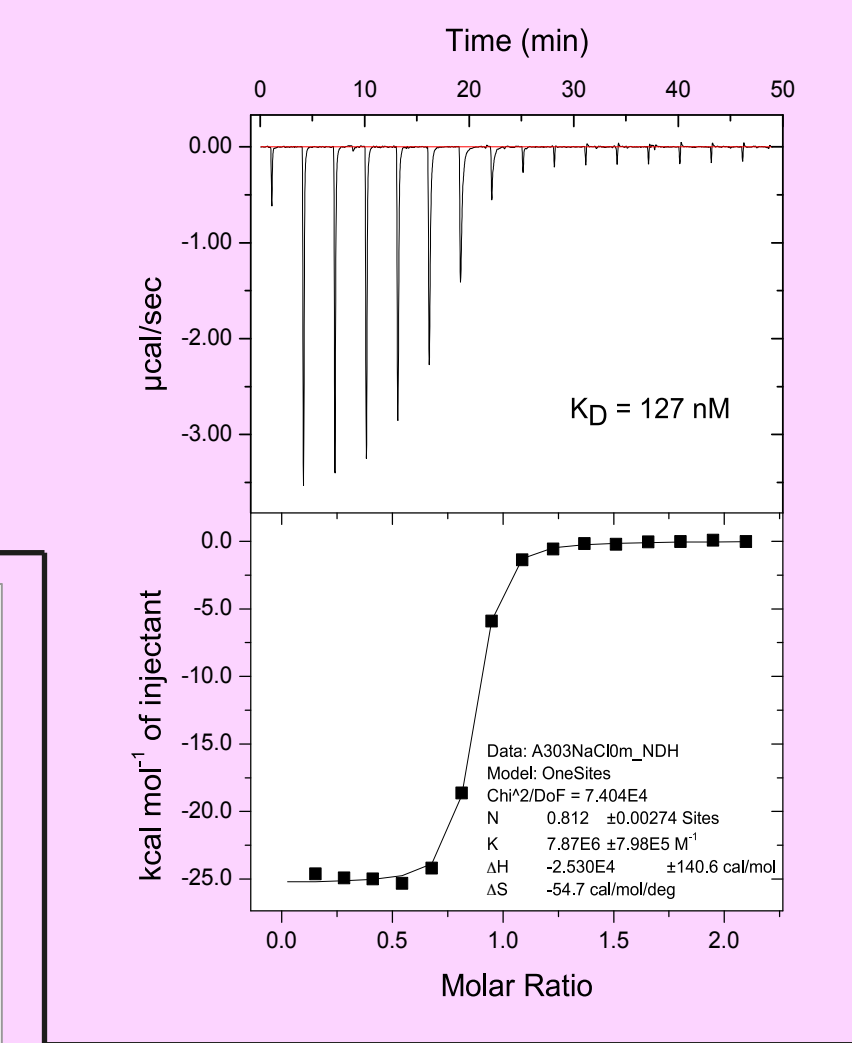


Bcd1p N-terminal part is required for box C/D snoRNA accumulation. ZHD of Bcd1 provides the stability of the full-length protein, mostly at high temperature.

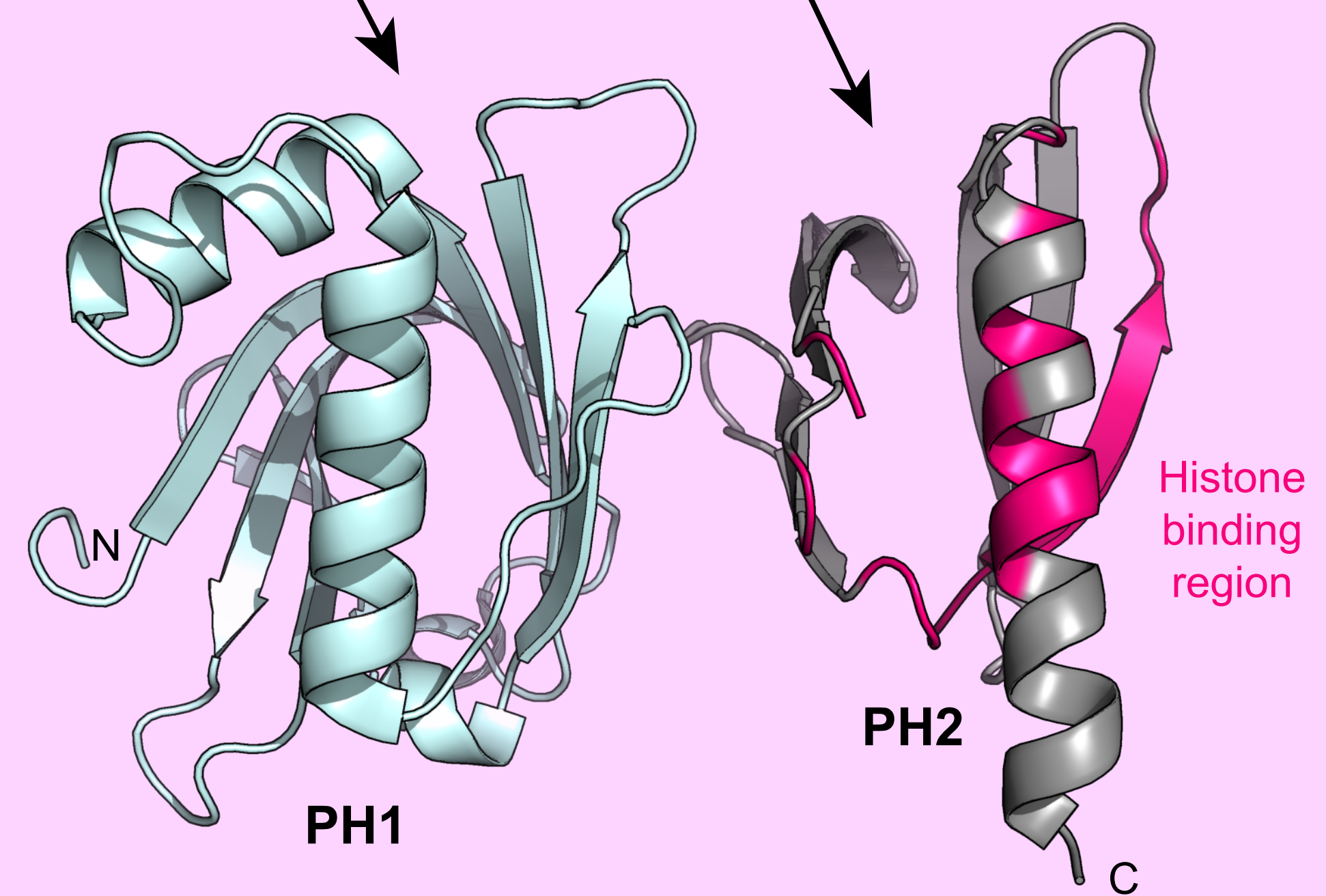
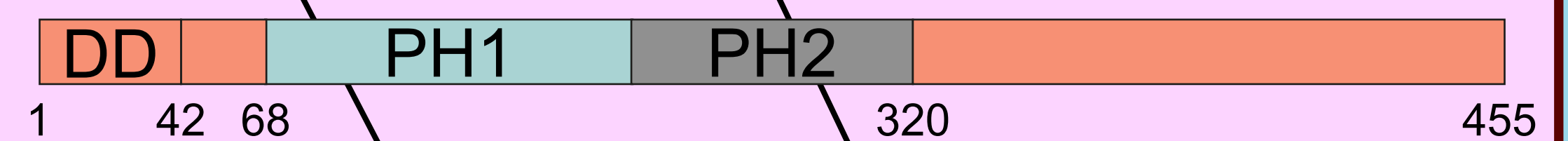
Bcd1p and the histone chaperone Rtt106

Direct interaction between these two proteins

ITC experiment between Bcd1p(120-303) and Rtt106(65-320) performed at 20°C without salt. The calculated K_d is 127 nM.



Peak height difference between free and bound states from NMR titration of Rtt106(65-320) by Bcd1p(120-303) with a ratio of 0.3 (plotted on the graph for each residue). More a residue is impacted by the binding of Bcd1p, lower is the corresponding value.



Ribbon representation of Rtt106(65-320) X-ray structure (from Su *et al.*, 2012; PDB 3TVV). The magenta colored regions correspond to important residues for the interaction with histone H3. The PH1 domain is colored in light blue and the PH2 in gray.

Bcd1p interacts with the PH1 domain of the histone chaperone Rtt106.

CONCLUSION

Only the N-terminal end of protein Bcd1 spanning amino acid residues 1 to 168 and including the zinc finger domain (ZHD) is necessary for box C/D snoRNA accumulation. This minimum domain for Bcd1p functionality seems to be important for the stability of the protein, and more particularly during heat stress, as shown by the loss of growth at 37°C with the ZHD mutants. The NMR structure of the ZHD shows a typical zinc finger fold from the zf-HIT family. Bcd1p is able to interact with the histone chaperone Rtt106. The Bcd1p region spanning amino acid residues 120 to 303 and thus overlapping the minimum functional domain interacts with Rtt106p. Our data indicate that the first PH (Pleckstrin Homology) domain of Rtt106 seems to bind Bcd1p, while the histone H3-interacting region is known to be located in the second PH domain. These data suggest a non-mutually exclusive interaction of Bcd1p and histone H3 with Rtt106. We are currently trying to obtain the 3D structure of the free Bcd1p(120-303) fragment by NMR and the complex Rtt106-Bcd1p by X-ray diffraction. This could bring us essential elements to understand the function of the protein Bcd1.