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Letter to the editor

Interaction between Bms1 and Rcl1, two ribosome biogenesis factors, is evolutionally conserved in zebrafish and human

Ribosomes are large RNA and protein complexes that function as the machinery for translation protein synthesis (Boisvert et al., 2007; Ben-Shem et al., 2011; Henras et al., 2015; Khatter et al., 2015; McCann et al., 2015). The eukaryotic ribosome is composed of two subunits, the 60S large subunit (LSU) and the 40S small subunit (SSU), which collectively comprise of four different ribosomal RNA (rRNA) species and more than 70 proteins (Ben-Shem et al., 2011; Henras et al., 2015; Khatter et al., 2015). The LSU contains the 28S, 5.8S and 5S rRNAs and the SSU contains the 18S rRNA. The assembly of each subunit is initiated in the nucleolus using the respective rRNAs as backbones (Ben-Shem et al., 2011; Henras et al., 2015; Khatter et al., 2015). The 28S, 18S and 5.8S rRNAs are encoded by the polycistronic 47S pre-rRNA gene, and are transcribed as a single transcript. In humans, the rDNA loci are organized in the nucleolar organizer regions (NORs), which each contains 300–400 identical repeats of the 47S pre-rRNA gene. The 47S pre-rRNA transcripts undergo sequential endonuclease and exonuclease cleavages to remove the internal transcribed spacer (ITS) and external transcribed spacer (ETS) at site A2 (Fig. S1) (Henras et al., 2015; Yoshikawa et al., 2015), resulting in the mature 28S, 18S and 5.8S rRNAs (Mullineux and Lafontaine, 2012; Wang et al., 2014). In yeast, Rcl1p, a novel type of endonuclease, plays key roles in cleaving pre-rRNAs at the A2 site (Billy et al., 2000; Horn et al., 2011). Rcl1p loading onto pre-rRNAs requires Bms1p, an interacting protein identified in a yeast two-hybrid screen using Rcl1p as the bait (Wegierski et al., 2001). Subsequently, Bms1 was demonstrated to be a GTPase (Karbstein et al., 2005) that positions the endonucleolytic activity center of Rcl1 around A2 when the Bms1-Rcl1 complex is loaded onto the pre-rRNA (Delprato et al., 2014). Thus far, most of the functional studies on Rcl1 and Bms1 have mainly been performed in yeast (Horn et al., 2011; Tanaka et al., 2011), with a few exceptions (Wang et al., 2012, 2014; Marneros, 2013). Noting the importance of the Bms1-Rcl1 complex in pre-rRNA processing in yeast, we examined the relationship between Bms1 and Rcl1 in multicellular vertebrate organisms, specifically on zebrafish and humans. Our results showed that Bms1 and Rcl1 interaction is evolutionarily conserved from yeast to these vertebrate species.

We previously reported that Bms1 is a highly conserved protein in humans, mice, zebrafish and yeast (Wang et al., 2012). Based on sequence information from GeneBank (Accession number: NP_001003865.1 for zebrafish *rcl1*, 649105902 for human *RCL1* and 40788900 for human *BMS1*), we designed specific primers and cloned full-length zebrafish *rcl1*, human *RCL1* and human *BMS1* cDNAs, using RT-PCR approach. Alignment of Rcl1 deduced protein sequences from human, zebrafish and yeast showed that zebrafish Rcl1 shares 76% and 37% identity with human and yeast Rcl1, respectively (Fig. S2). Domain analysis showed that all Rcl1 orthologs in yeast, zebrafish and humans contain an RNA 3′-terminal phosphate cyclase (RTC) domain and an RTC_insert domain (Fig. S3). Notably, the vertebrate Rcl1 proteins (zebrafish and human) contain a putative bipartite nuclear localization signal profile (NLS_BP) between the RTC and RTC_insert domains that is absent in the yeast counterpart (Fig. S3).

To determine the subcellular localization of zebrafish Bms1 and Rcl1, we generated various polyclonal antibodies against zebrafish Bms1l (rabbit and mouse) and Rcl1 (rabbit), respectively. Co-immunofluorescence revealed that the Bms1l signals were co-localized with those of Fibrillarin (Fib), a nucleolar marker for the region of dense fibrillar component (DFC), in the nucleoli of the intestinal epithelial cells of 5 dpf (day post-fertilization) zebrafish embryos (Fig. 1A). Interestingly, the Bms1l signals appeared to be distributed over a broader area than that covered by Fib (Fig. 1A). Co-immunofluorescence of Bms1l and Rcl1 showed that these two proteins were co-localized in the nucleoli of both intestinal epithelial cells (Fig. 1B) and hepatocytes (Fig. 1C) of 5 dpf zebrafish embryos. As observed in Bms1l and Fib co-immunofluorescence (Fig. 1A), the Bms1l signals had a wider distribution than those of Rcl1 (Fig. 1B and C). In the adult zebrafish liver, Bms1l also co-localized with Fib in hepatocyte nucleoli (Fig. 1D). These data suggest that the localized expression pattern of Bms1l in the nucleolus correlates well with the functional needs of ribosomal activity in a cell. The consistent observation of the larger area coverage of the Bms1l signals compared to that of Rcl1 in the embryos might suggest additional, yet to be identified, functions for Bms1l, additional to its role in pre-rRNA processing during early development.

Using commercially available antibodies against human BMS1 (HuBMS1) and human RCL1 (HuRCL1), co-immunofluorescence of HuBMS1 and Fib or HuRCL1 and Fib in human HepG2 cells showed that both HuBMS1 and HuRCL1 were localized in the nucleolus (Fig. 1E), suggesting that both Bms1 and Rcl1 are conserved nucleolar proteins in eukaryotes. Importantly, similar to previous reports of *bms1l*-morpholino injected zebrafish embryos (Wang et al., 2012), blocking Rcl1 function *via* a splicing morpholino targeting

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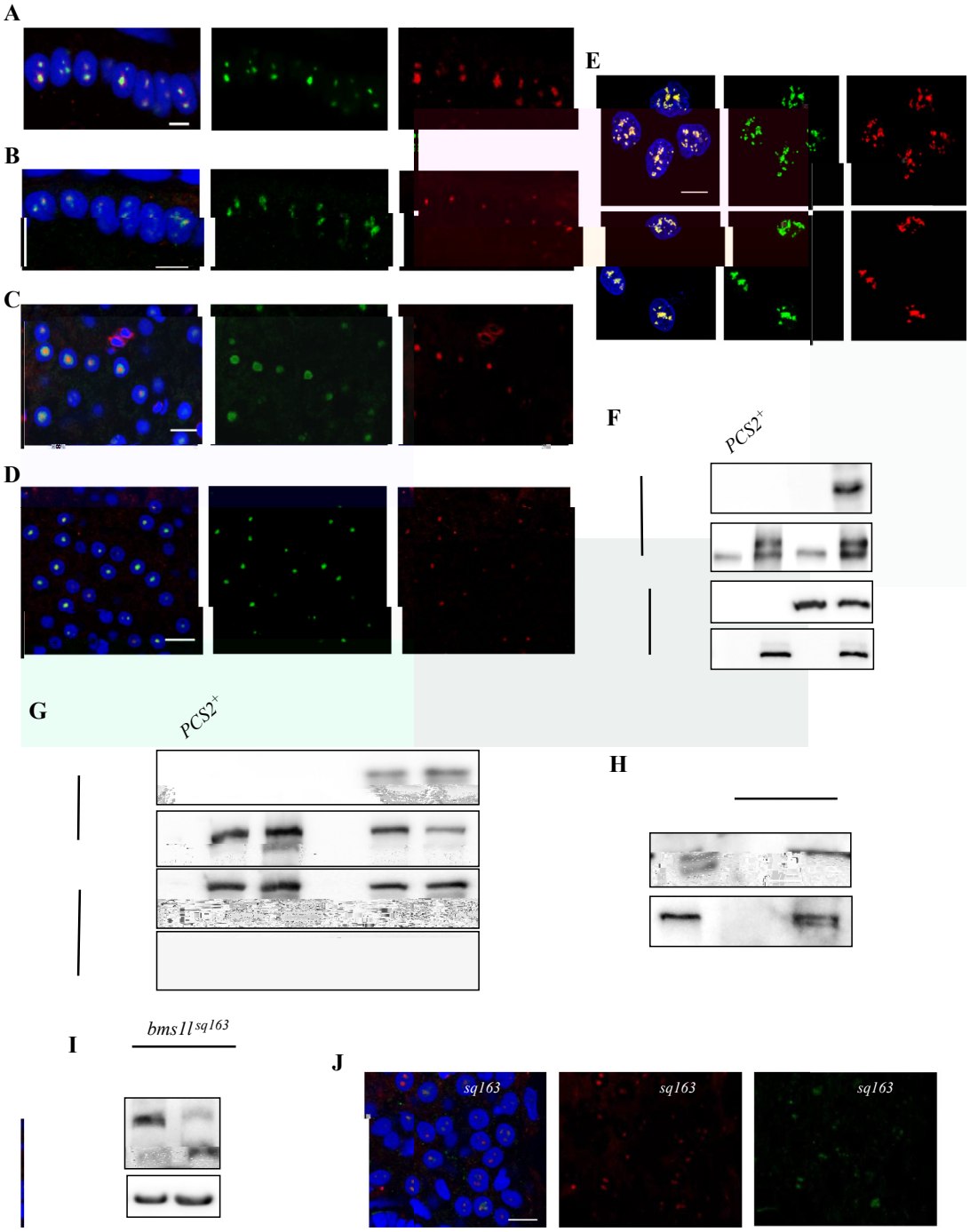

Fig. 1. Bms1 and Rcl1 interaction is conserved in zebrafish and humans. **A**–**D**: Co-immunofluorescence of Fibrillarin (green) and Bms1l (red) (**A, D**) or Bms1l (green) and Rcl1 (red) (**B, C**) in 5 dpf WT zebrafish gut epithelial cells (**A, B**), hepatocytes (**C**), and adult zebrafish liver (**D**). DAPI (blue) is used to stain the nuclei. **E**: Co-immunofluorescence (Co-IP) of Fibrillarin (Fib) and HuBMS1 (upper panels) or Fib and HuRCL1 (lower panels) in cultured human HepG2 cells. **F**: Co-IP analysis. Western blot of HA-HuBMS1 (by the anti-HA antibody) and HuRCL1-Myc (by the anti-Myc antibody) in protein samples eluted from agarose beads conjugated with HA antibodies. The 293T cells were transfected with the plasmids as indicated for 60 h. Co-IP was performed using agarose beads conjugated with HA antibodies (Ab HC: antibody heavy chain). **G**: Co-IP analysis. Western blot of Bms1l (Bms1l-WT) and Bms1l^{sq163} (Bms1l-163) using anti-Bms1l antibodies (rabbit origin), and Rcl1-Myc using anti-Myc antibodies in protein samples eluted from beads captured with Bsm1l antibodies (mouse origin). The 293T cells were transfected with the plasmids as indicated for 60 h. **H**: Co-IP analysis. Western blot of endogenous Bms1l using an anti-Bms1l antibody (mouse origin) and endogenous Rcl1 using an anti-Rcl1 antibody. Total protein extracts from adult zebrafish livers were used in the Co-IP using protein A + G beads conjugated with Bsm1l antibody (rabbit origin). **I**: Western blot of Bms1l and Bms1l^{sq163} in the WT siblings and *bms1l*^{sq163} homozygotes at 5 dpf obtained from the progenies of *bms1l*$^{sq163/+}$ × *bms1l*$^{sq163/+}$. N.S., non-specific band. β-actin is used as a loading control. **J**: Co-immunofluorescence of Rcl1 (red) and Bms1l^{sq163} (green) in the liver of *bms1l*^{sq163} mutant (*sq163* MU) of 5 dpf zebrafish embryos.

the junction between intron 3 and exon 4 of *rcl1* (Rcl1-MOspl) resulted in aberrant pre-rRNA processing in zebrafish embryos (Fig. S4). These results suggest an evolutionary conservation of these two proteins in ribosome biogenesis in eukaryotes.

To determine whether human or zebrafish Bms1 and Rcl1 form a complex as in yeast, we transfected 293T cells with a *pCS2*$^{+}$*-HA-BMS1* or *pCS2*$^{+}$*-RCL1-Myc* plasmid alone or with a mix of *pCS2*$^{+}$*-HA-BMS1* and *pCS2*$^{+}$*-RCL1-Myc* plasmids to express HA-tagged (at

the N-terminal) HuBMS1 (HA-HuBMS1) and Myc-tagged (at the C-terminal) HuRCL1 (HuRCL1-Myc), and performed co-immunoprecipitation assays with total protein lysates extracted 60 (hpt) hours post transfection. Our results showed that HuBMS1 and HuRCL1 formed a complex (Fig. 1F). Similar analysis with Myc-tagged (at the C-terminal) zebrafish Rcl1 (Rcl1-Myc) or zebrafish Bms1l without a tag (Bms1l-WT) alone or together in 293T cells showed that zebrafish Bms1l could interact with Rcl1 (Fig. 1G) in human cells. To determine whether the two proteins could interact with each other endogenously, we extracted total protein from adult zebrafish liver and analyzed similarly with Co-IP assay. Western blot analysis revealed that the endogenous Rcl1 was co-immunoprecipitated with Bms1l (Fig. 1H), thereby confirming conservation of the interactions between Bms1 and Rcl1 across species.

We previously reported that the *bms1l*^{sq163} mutation is caused by a T to A substitution converting the amino acid L^{152} to Q^{152} in the GTPase domain of the protein (Wang et al., 2012). A western blot analysis of total protein extracted from 5 dpf wild type (WT) siblings and *bms1l*^{sq163} homozygous mutant embryos showed that the Bms1l^{sq163} mutant protein remained expressed, albeit at a lower level than in the WT siblings (Fig. 1I). Co-immunofluorescence of Bms1l^{sq163} and Rcl1 revealed that the Bms1l^{sq163} mutant protein could co-localize with Rcl1 in the nucleoli of the hepatocytes at 5 dpf (Fig. 1J). Furthermore, Rcl1-Myc was co-immunoprecipitated with the Bms1l^{sq163} mutant protein when they were co-expressed in 293T cells (Fig. 1G), suggesting that although the L^{152} residue is essential for the biological function of Bms1l, the substitution mutation does not affect its interaction with Rcl1. This finding is consistent with structural analysis of the yeast Rcl1-Bms1 complex in which the GTPase domain has been shown to be dispensable for Rcl1 and Bms1 interaction but essential for Rcl1 accession to the A2 site (Delprato et al., 2014). Taken together, our data show that the interaction between Bms1 and Rcl1, two SSU biogenesis factors, is evolutionarily conserved in eukaryotes. The L^{152} to Q^{152} in the Bms1 GTPase domain in *bms1l*^{sq163} does not affect the Rcl1-Bms1 interaction but is essential for the biological function of the complex.

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Supplementary data

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References

Ben-Shem, A., Garreau de Loubresse, N., Melnikov, S., Jenne, r.L., Yusupova, G., Yusupov, M., 2011. The structure of the eukaryotic ribosome at 3.0 Å resolution. Science 334, 1524–1529.

Billy, E., Wegierski, T., Nasr, F., Filipowicz, W., 2000. Rcl1p, the yeast protein similar to the RNA 3′-phosphate cyclase, associates with U3 snoRNP and is required for 18S rRNA biogenesis. EMBO J. 19, 2115–2126.

Boisvert, F.M., van Koningsbruggen, S., Navascues, J., Lamond, A.I., 2007. The multifunctional nucleolus. Nat. Rev. Mol. Cell Biol. 8, 574–585.

Delprato, A., Al Kadri, Y., Perebaskine, N., Monfoulet, C., Henry, Y., Henras, A.K., Fribourg, S., 2014. Crucial role of the Rcl1p-Bms1p interaction for yeast pre-ribosomal RNA processing. Nucleic Acids Res. 42, 10161–10172.

Henras, A.K., Plisson-Chastang, C., O'Donohue, M.F., Chakraborty, A., Gleizes, P.E., 2015. An overview of pre-ribosomal RNA processing in eukaryotes. RNA 6, 225–242.

Horn, D.M., Mason, S.L., Karbstein, K., 2011. Rcl1 protein, a novel nuclease for 18 S ribosomal RNA production. J. Biol. Chem. 286, 34082–34087.

Karbstein, K., Jonas, S., Doudna, J.A., 2005. An essential GTPase promotes assembly of preribosomal RNA processing complexes. Mol. Cell 20, 633–643.