Crystal structure of the pre-microRNA nuclear export machinery

Mature microRNAs (miRNAs), which form a class of recently discovered non-coding RNAs, are present in a wide range of eukaryotes, fungi, plants, and animals [1]. miRNAs play important roles in the regulation of biological processes, including genetic development, cell proliferation, cell differentiation, apoptosis, transposon silencing, and antiviral defense [2]. miRNA biogenesis [3] begins in the nucleus, where capped and polyadenylated pri-miRNAs, several kilobases in length, are transcribed. The pri-miRNAs are processed by the nuclear RNase III enzyme Drosha to generate ~65 nt pre-miRNAs that form stem-loop structures containing 2-nt 3' overhangs. The pre-miRNAs are translocated from the nucleus to the cytoplasm through a nuclear pore complex by exportin-5 (Exp-5) in a RanGTP dependent manner. In the cytoplasm, the premiRNAs are further processed by the cytoplasmic RNase III enzyme Dicer, which excises a ~22-bp RNA duplex. One strand of the duplex binds to its target mRNA with imperfect complementarity, usually within the target's 3'-untranslated region, assisted by the RNA-induced silencing complex.

Exp-5 facilitates miRNA biogenesis by not only acting as the nuclear export factor for pre-miRNAs but also protecting pre-miRNAs from digestion by nucleases. The loss of Exp-5 results in the loss of cytoplasmic miRNA expression without the nuclear accumulation of pre-miRNAs. Pre-miRNA binding to Exp-5 requires GTP-bound forms of the small nuclear GTPase Ran cofactor (RanGTP). The Exp-5:RanGTP:pre-miRNA heteroternary complex formed in the nucleus is exported to the cytoplasm. We have determined the X-ray crystal structure of the Exp-5:RanGTP:pre-miRNA ternary complex at a 2.9 Å resolution [4]. Structural analysis was carried out using beamline **BL44XU**.

The crystal structure of the Exp-5:RanGTP:pre-miRNA complex, which contains full-length human Exp-5, canine RanGTP, and the human pre-miRNA-30a stem domain including the 2-nt 3' overhang, is shown in Fig. 1. The structure of Exp-5 contains 20 HEAT repeats, each comprising two antiparallel α-helices. The pre-miRNA-30a adopts a typical A-form RNA helical structure, 60 Å in length and 20 Å in diameter. The Exp-5:RanGTP:pre-miRNA complex is ellipsoidal with dimensions of 65×80×110 $Å^3$. The Exp-5:RanGTP complex forms a baseball-mitt-like structure in which the pre-miRNA is caught (Fig. 2). A tunnel-like structure at the bottom of the mitt connects the inner space of the mitt with the outer space (Fig. 2). The double-stranded stem domain of the pre-miRNA is packed into the mitt and the 2-nt 3' overhang is located in the tunnel (Figs. 3 and 4).

The 2-nt 3' overhang structure is a characteristic feature of pre-miRNA. The protruding unpaired 2-nt 3' end is inserted deep into the tunnel constructed from the loop and the inner helix of HEAT12, the loop and the N-terminal end of the inner helix of HEAT13, the N-terminal region of the inner helix of HEAT14, the C-terminal half loop, and the N-terminal region of the inner helix of HEAT15. The tunnel is 15 Å in length, and its shortest diameter is 10 Å. The inner surface of the tunnel is positively charged (Figs. 2

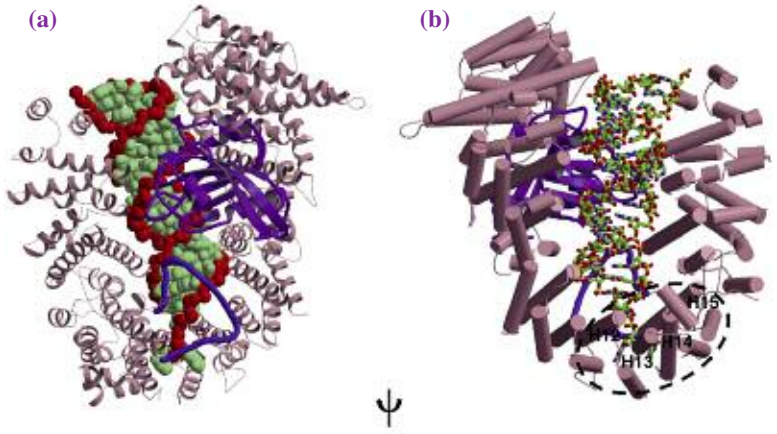

Fig. 1. Structure of the Exp-5:RanGTP:pre-miRNA-30a complex. **(a)** The structure shows human pre-miRNA-30a (red and green) bound to Exp-5 (pink) with RanGTP (purple). The long loop of HEAT15 is shown as a blue wire. In front of this view, the pre-miRNA is shielded by the long loop of Exp-5 and RanGTP. **(b)** The pre-miRNA molecule is viewed from the front open side. Exp-5 covers most of the stem moiety, and the 2-nt 3' overhang structure (circled black) has many interactions with HEAT12–15, each of which is given a letter "H" with a corresponding number.

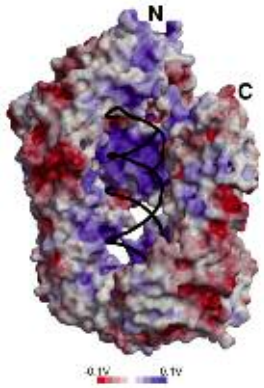

Fig. 2. Electrostatic surface potentials of Exp-5:RanGTP. The potentials are represented in a color gradient from red to blue for the vertex with potentials from –0.1 to 0.1 V. The black backbone represents the stem moiety of the pre-miRNA.

and 4(b)) and probably stabilizes the negatively charged 2-nt 3' overhang structure. The 2-nt 3' overhang structure in the tunnel is stabilized by a number of hydrogen bonds and salt bridges with amino acid residues of Exp-5, as shown in Fig. 4. Because all interactions involve atoms of the sugar-phosphate backbone, 2-nt 3' overhang recognition by Exp-5 is independent of the RNA sequence.

Although Exp-5 is an acidic protein with pI=5.6, it has localized positive charges from basic residues on the inner surface of the mitt that interact with a negatively charged double-stranded RNA (Fig. 2).

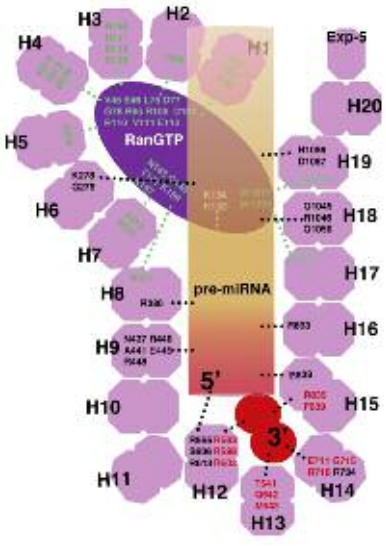

Fig. 3. Intermolecular interactions in the Exp-5:RanGTP:pre-miRNA-30a complex are shown schematically. The pre-miRNA is shown in red and light brown, where red regions interact with more basic residues of Exp-5 than light brown regions. A pink double octagon represents each HEAT repeat. Exp-5 residues shown in red are involved in the recognition of the 2-nt 3' end of the pre-miRNA, and black residues indicate interactions with the double-stranded stem of the pre-miRNA. Green residues indicate interactions between Exp-5 and RanGTP.

The interacting residues of Exp-5 are distributed broadly on the inner helices of HEAT6-19 and a loop of HEAT15 to recognize the outer phosphodiester group of the pre-miRNA stem (Fig. 3). Thus, the stem of the pre-microRNA, 45 Å in length, is roughly recognized through a broad range of positively charged inner surface residues of the Exp-5:RanGTP mitt.

Since both 3' and 5' ends of the pre-miRNA are completely shielded in the tunnel, the pre-miRNA is protected from digestion by exonucleases. Exp-5:RanGTP surrounds the pre-miRNA on four sides, protecting it from ribonuclease digestion during export from the nucleus to the cytoplasm. Thus, Exp-5:RanGTP may act as both a nuclear export carrier and a molecular stabilizer of pre-miRNAs.

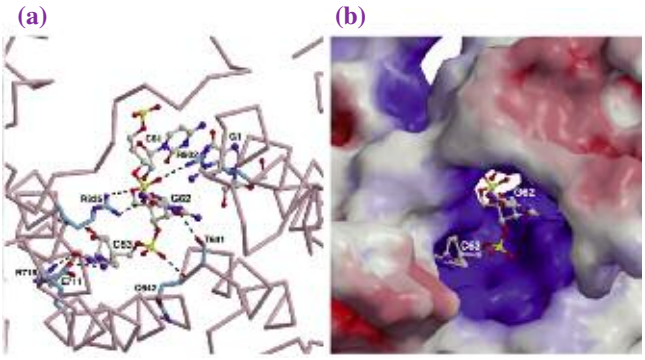

Fig. 4. Structure of the 2-nt 3' overhang of the pre-miRNA in the tunnel viewed from outside of the Exp-5 molecule. **(a)** Hydrogen bonds or salt bridges in the tunnel are represented by broken lines. Exp-5 is shown in pink for Cα, in deep blue for N, in red for O, and in sky blue for C of the side chain. RNA is shown in deep blue for N, in red for O, in yellow for P, and in gray for C. **(b)** Electrostatic potential is represented as in Fig. 2.

Eiki Yamashita[a], Chimari Okada-Jiko[b] and Tomitake Tsukihara[a,c,*]

[a] Institute for Protein Research, Osaka University
[b] Career Path Promotion Unit for Young Life Scientists, Kyoto University
[c] Department of Life Science, University of Hyogo

*E-mail: tsuki@protein.osaka-u.ac.jp

References

[1] R.C. Lee *et al.*: Cell **75** (1993) 843.
[2] V. Ambros: Nature **431** (2004) 350.
[3] V.N. Kim *et al.*: Nat. Rev. Mol. Cell Biol. **10** (2009) 126.
[4] *C. Okada, E. Yamashita, S.J. Lee, S. Shibata, J. Katahira, A. Nakagawa, Y Yoneda, T. Tsukihara: Science* ***326*** *(2009) 1275.*