Structure and Functional Implication of the Nanos Zinc-Finger Domain

Nanos is an RNA-binding protein involved in the development of germ cells. In combination with Pumilio, Nanos binds the 3'-UTR of an mRNA and represses its translation. Nanos has two conserved CCHC zinc-finger motifs that are indispensable for its function. We have determined the crystal structure of the zinc-finger domain of Nanos, representing the first structure of this RNA-binding protein. The structure reveals that Nanos adopts a novel fold. In addition, Nanos has a conserved basic surface that is directly involved in RNA-binding. Our results provide the structural basis for further studies to clarify the functions of Nanos.

Translational control of mRNAs is a crucial mechanism in developmental processes, including cell division, cell-fate determination and embryonic axis establishment in early embryogenesis. Most types of translational control are mediated by a sequence in the 3'-UTR and are achieved by the interaction of various regulatory factors such as RNA-binding proteins. Nanos is a highly conserved RNA-binding protein in higher eukaryotes and functions as a key regulator in translational control utilizing a 3'-UTR during the development of germ cells. The *nanos* gene was initially identified as a maternal gene crucial for posterior pattern formation in the *Drosophila* embryo. In combination with Pumilio, Nanos represses the translation of maternal *hunchback (hb)* mRNA in the early *Drosophila* embryo, thereby governing abdominal segmentation. In addition, Nanos and Pumilio have a variety of functions in the primary germ cells (PGCs). In fact, Nanos is essential for the development of PGCs. PGCs lacking Nanos or Pumilio enter mitosis prematurely, undergo apoptosis and fail to maintain stem cell identity in adults. Nanos is widespread in higher eukaryotes and comprises a non-conserved N-terminal and highly conserved C-terminal regions. The C-terminal region has conserved two CCHC-type zinc-finger motifs, which are indispensable for Nanos function. Although many studies have revealed significant functions of Nanos, neither the atomic structure of Nanos nor the structural basis of the interaction between Nanos and RNA has been reported.

We have determined the crystal structure of the zinc-finger domain of zebrafish Nanos (zNanos) at 2.1 Å resolution [1] [2]. The structure of zNanos is composed of two independent zinc-finger lobes, the N-terminal ZF1 and the C-terminal ZF2 (Fig. 1). These lobes create a large cleft (Fig. 2(a), 2(b)). The asymmetric CCHC zinc-finger of Nanos is unique and has not been observed in other CCHC zinc-finger proteins. In fact, a search for homologous structures of zNanos using the DALI server revealed no similar structure, indicating that zNanos adopts a novel fold. It has been shown that Nanos binds to RNA with no sequence specificity, suggesting that electrostatic interactions with the phosphate backbone of RNA are crucial. Consistent with this idea, calculation of electrostatic potential revealed that zNanos has a large basic region on the surface (Fig. 2(a)) comprising mostly conserved residues (Fig. 2(b)), implying that it is involved in the interaction with RNA. To evaluate this assumption, we prepared alanine mutants and tested the interaction between zNanos and ssRNA by electrophoresis mobility shift assay (EMSA). We selected K111, R123 and R141 as conserved basic residues. K111, R123 and R141 of zNanos correspond to R339, R351 and K369 of *Drosophila melanogaster* Nanos (dmNanos), respectively. Furthermore, K96, H97, R113, K126, K136 and K140 were selected as non-conserved basic residues. Those residues contribute to the positive potential on the surface (Fig. 2(a)). It has been shown

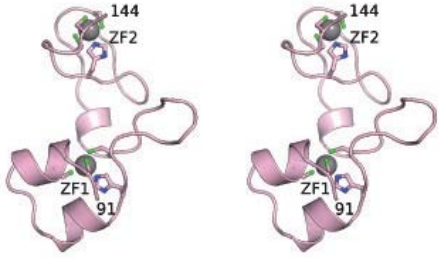

Figure 1
Structure of the zNanos zinc-finger domain, shown by a pink ribbon representation in stereo view. Only the core region, residues 91-144, is shown. The residue numbers are labeled at the N- and C-terminal ends, 91 and 144, respectively. Zinc ions in ZF1 and ZF2 are shown by gray spheres. Residues in the CCHC motifs bound to the zinc ion are shown by stick representation.

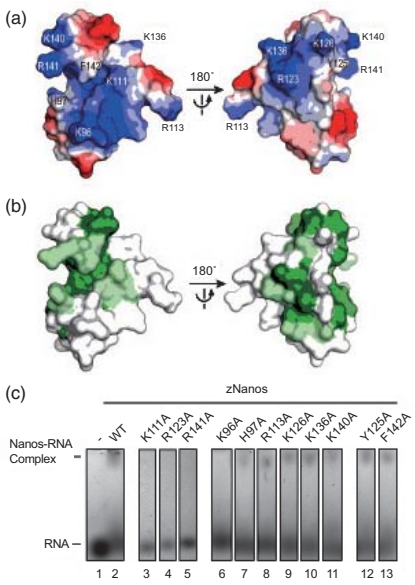

Figure 2
(a) Electrostatic potential of zNanos depicted on the molecular surface. Blue and red surfaces indicate positive and negative potential, respectively. The orientation in the left panel is the same as that in Fig. 1. Substituted residues to alanine in EMSA are indicated. (b) Sequence conservation of Nanos shown on the molecular surface of zNanos. Green and light green surfaces indicate identical and homologous residues, respectively. (c) Interaction between the zinc-finger domain of zNanos and ssRNA by EMSA. Lanes 1, 2 and 3-13 are RNA alone, RNA + zNanos wild type and RNA + zNanos mutants, respectively.

that aromatic residues are crucially involved in stacking interactions with the base moieties of ssRNA in several RNA-binding proteins. Thus, we selected Y125 and F142 as conserved and solvent-exposed aromatic residues. Initially, we confirmed that zNanos wild type binds to this RNA (Fig. 2(c), lane 2). As expected, K111A, R123A or R141A substitution significantly impaired the interaction between zNanos and the RNA (Fig. 2(c), lane 3-5). Interestingly, mutation of R339 or R351 of dmNanos, which respectively corresponds to K111 or R123 of zNanos, actually abrogates Nanos function, resulting in two strong abdominal and oogenesis *nanos* phenotypes. Thus, our results clearly reveal that those *nanos* phenotypes are attributable to defects in RNA-binding activity. Although mutation in K369 of dmNanos, which corresponds to R141 of zNanos, has not been reported in genetic studies, our results suggest that this mutation causes a strong *nanos* phenotype owing to defects in RNA-binding activity. In contrast, zNanos with a mutation in a non-conserved basic residue maintained its interaction with the RNA, except for the K96A mutant (Fig. 2(c), lane 6-11). Furthermore, the Y125A and F142A mutants also retained the binding activity. These results indicate that the electrostatic interactions between the basic residues and the phosphate backbone of RNA are crucial in the formation of the Nanos-RNA complex. K96, K111 and R141 are in close proximity and provide a large basic surface (Fig. 2(a), left panel), implying that the basic surface could be a major RNA-binding site.

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BEAMLINES

5A and AR-NW12A

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