A Molecular Mechanism of Transcriptional Activation by a Mouse Testis-specific Long Noncoding RNA, *Tesra*

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Many functional long noncoding RNAs (lncRNAs) are expressed in the mouse testis, but detailed molecular mechanisms of their action are poorly understood. We identified *Tesra*, a mouse testis-specific lncRNA, at the mouse *Prss/Tessp* locus and found that it is localized in both germ cells and Leydig cells. In this study, based on the knowledge that lncRNAs often regulate the expression of neighboring genes, we tested the possibility that *Tesra* regulates expression of the *Prss/Tessp* genes and investigated a molecular mechanism underlying it. We first performed the chromatin isolation by RNA purification (ChIRP) assay and found that *Tesra* bound to chromatin region of the *Prss42/Tessp-2* promoter in germ cells from the testis. Moreover, the overexpression of *Tesra* increased the *Prss42/Tessp-2* expression level and its promoter activity in the *in vitro* cell culture system. Thus, the *Prss42/Tessp-2* gene, which is specifically expressed in spermatocytes, is likely to be a target of *Tesra*. To reveal a molecular mechanism underlying the regulation of the *Prss42/Tessp-2* gene by *Tesra*, we searched for *Tesra*-binding proteins by the Ribotrap assay and the mass spectrometry analysis. One candidate protein, polypyrimidine tract binding protein 2 (PTBP2), was expressed in correlation with both *Tesra* and *Prss42/Tessp-2* during testis development. Since both *Tesra* and PTBP2 are known to be localized in the nucleus of spermatocytes and our RNA immunoprecipitation (RIP) assay revealed the interaction between PTBP2 and *Tesra* in the nucleus of testicular germ cells, we next investigated the involvement of PTBP2 in the gene regulation by *Tesra*. We established the *in vitro* reporter gene assay system in which the expression of *Tesra* was induced by the Tet-on system and thereby, *Prss42/Tessp-2* promoter activity was enhanced to drive the luciferase gene. The addition of doxycycline to this system induced the *Tesra* expression and increased luciferase activity, and the knockdown of PTBP2 significantly decreased luciferase activity. Lastly, we confirmed that PTBP2 bound to chromatin at the *Prss42/Tessp-2* promoter in germ cells from the testis by the chromatin immunoprecipitation (ChIP) assay. Taken together, our results suggest that *Tesra* activates transcription of the *Prss42/Tessp-2* gene in spermatocytes and that PTBP2 contributes to the *Prss42/Tessp-2* transcriptional activation by *Tesra*. Because the *Prss42/Tessp-2* gene is involved in the regulation of meiotic progression, *Tesra* may be an

important regulator of spermatogenesis.