6.1 Taking Advantage of Small Noncoding RNA Pathways in the Mouse

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The introduction of double-stranded RNA (dsRNA) into an organism causes specific interference of gene expression termed RNA interference (RNAi). At the heart of this regulatory mechanism of gene expression are 22 nt short interfering RNAs (siRNAs) and microRNAs, both generated by the RNaseIII enzyme Dicer. Both microRNAs and endogenous siRNAs have suspected roles in diseases ranging from inappropriate cell proliferation (such as cancer) and cellular degeneration/loss of cell function (such as Fragile X). The specific mRNA targets for each small RNA are not resolved, although many targets have been proposed based on bioinformatics, but with little to no experimental evidence. We study the biological pathways that relate to the use of small noncoding RNAs, and take advantage of this pathway by designing and using novel RNAi strategies for specific gene disruption. Our studies should shed new insights into the importance of small RNAs in mammalian homeostatic and developmental processes.

6.2 Transcriptional Regulatory Complexes: A Proteomic Approach


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Transcription of eukaryotic protein-coding genes by RNA polymerase II is a complex process regulated by a large collection of transcription factors and co-regulatory complexes, including the Mediator of RNA polymerase II transcription and chromatin remodelling and modifying enzymes. Mediator is a multiprotein transcriptional coactivator that is expressed ubiquitously in eukaryotes from yeast to mammals and is required for induction of RNA polymerase II (pol II) transcription by DNA binding transcription factors. We exploited multidimensional protein identification technology (MudPIT) to carry out a proteomic analysis of the subunit composition of the mammalian Mediator complex. By comparing MudPIT data sets obtained from multiple independent Mediator preparations immunoaffinity purified through their different subunits, we have identified a set of consensus mammalian Mediator subunits. In addition, we have identified as Mediator-associated proteins the CDK8-like cyclin-dependent kinase CDK11 and the MED13-like KIAA1025 protein (MED13L), which is mutated in patients with the congenital heart defect transposition of the great arteries (TGA). We have taken a similar approach to analysis of several chromatin modifying and remodelling complexes, including the TRRAP-Tip60 histone acetyltransferase (HAT) and SRCAP and Ino80-like chromatin remodelling complexes.