

The History of RNAi and MicroRNA Discovery

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MicroRNAs belong to one of the classes of non-coding RNAs. Non-coding RNAs are functional RNAs that do not translate into protein. They comprise: transfer RNA (tRNA); ribosomal RNA (rRNA); small nucleolar RNA (snoRNA); microRNA (miRNA); small interfering RNA (siRNAs); small nuclear RNA (snRNA); piwi-interacting RNA (piRNA), and long ncRNA. To date, there are approximately 1881 pre-miRNAs and 2588 mature human miRNAs have been identified (miRBase, June 2014). MicroRNAs are about 19-25 nucleotides in length and are now known to have important post-transcriptional roles in almost every cellular process in eukaryotes. These processes include the regulation of developmental timing and signalling pathways, apoptosis, metabolism, myogenesis and cardiogenesis, brain development, and human pathologies like viral diseases, genetic disorders and cancer (Shi et al. 2008).

THE DISCOVERY OF RNA INTERFERENCE

Fire and colleagues first reported that the injection of double stranded RNA

caused the degradation of mRNA encoded by the gene *unc-22* of the small nematode *Caenorhabditis elegans*. They termed this effect RNA interference (RNAi) and showed that it was sequence specific and effective at concentrations far lower than the target *unc-22* mRNA, indicating the involvement of amplification effect in this process (Fire et al. 1998).

At the same time, David Baulcombe's group (Hamilton & Baulcombe 1999) discovered posttranscriptional gene silencing (PTGS) in plants. Both viral infection and transgenic expression in plants can induce PTGS, which targets both cellular and viral mRNA. It was inferred that PTGS operates through the generation of small RNA molecules of 21 to 25 nucleotides, which are now known as small interfering RNAs (siRNAs). Consequently, work on both animals and plants together revealed a highly conserved mechanism of RNA interference that had evolved at least in part for combating viral infection.

Subsequently, Philip Sharp group was able to recapitulate RNA interference in cell free extracts from *Drosophila* cells, which led to the establishment of three phases of the RNA interference

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reaction: cleavage of a long dsRNA into shorter dsRNA segments by Dicer; the loading of single stranded RNA into the RISC (RNA-induced silencing complex) and the targeting and degradation of mRNA by this complex (Tuschl et al. 1999). Subsequently mutations of *C.elegans* that confirm resistance to RNA interference were found to disrupt genes that encoded components of RISC (Fire 2007). Eventually, Andrew Z. Fire and Craig C. Mello became the recipients of Nobel Prize in Physiology and Medicine in 2006 (www.nobelprize.org). While David C. Baulcombe, together with Victor R. Ambros and Gary B. Ruvkun received the Albert Lasker Award for Basic Medical Research (www.laskerfoundation.org).

THE DISCOVERY OF MICRORNAS

Lee et al. (1993) identified two overlapping transcripts of the *lin-4* gene of *C. elegans*, of approximately 22 and 61 nts that inhibited the expression of *lin-14* through complementarity to the 3' untranslated region (UTR) of *lin-14* mRNA. The 61 nucleotides molecule can also fold into a double-stranded "hairpin". They suggested that *lin-4* inhibits translation of *lin-14* through an antisense RNA-RNA interaction.

Subsequently, it was shown that *lin-4* and a second gene *let-7* acted in a sequential stage specific expression pattern that regulates the timing of *C.elegans* development. The *let-7* gene encodes a 21-nucleotide RNA that is complementary to the 3' UTR of genes *lin-14*, *lin-28*, *lin-41*, *lin-42* and *daf-12*. *Let-7* is expressed at the adult

but not embryonic stage. *Let-7* was also identified in humans, fruit flies, chickens, frogs, zebrafish, molluscs and sea urchins and the binding site in its target was conserved in some of these organisms (Pasquinelli et al. 2000). In 2001, there were discovery of large number of similar small RNA molecules, referred to as microRNAs, in *C.elegans* and subsequently mouse (Lee & Ambros 2001).

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