World of Knowledge

RNA Interference: from Serendipity to Application to Functional Genomics

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Some of the most important discoveries occurred through serendipity, i.e. accidental discovery. For instance, in 1928 Alexander Fleming observed that microbes were attenuated by invading fungus. This accidental discovery resulted in the discovery of the world's first antibiotic or bacteria killer. The discovery of RNA interference (RNAi), a form of post-transcriptional gene silencing that mimics the effect of loss-of-gene-function, also is a scientific serendipity, which involves several intriguing, quirky and surprising events. The story of RNAi from discovery to application to mammals is a fascinating process with deeper appreciation that serendipity requires an inherent talent to resolve the puzzle and thereby it proves that chance only favors for those who have a determined mind.

In 1990, Dr. Jorgensen's team (Napoli et al., 1990) attempted to create a deep purple flower color of petunia by introducing a petunia chalcone synthase (CHS) gene in pigmented petunia petals. Unexpectedly, they found that about forty-two percent of transgenic plants with the introduced CHS gene produced totally white flowers and/or chimera flowers with white and purple colors. In contrast, control plants did not exhibit such phenotypes. Interestingly, study of genomic DNA demonstrated that the introduced CHS gene always associated with novel color phenotype whereas progeny with phenotypically wild type did not. Subsequent experiments showed that the extent of purple color was strongly associated with the steady-state levels of the mRNAs produced by both the endogenous and the exogenously introduced CHS gene. Thus, the expression of CHS both in endogenous and exogenous CHS gene was co-suppressed in the white flowers. The mechanism responsible for the co-suppression of homologous genes was unclear at that time. However, the reduction of mRNA level was apparently found to be associated with the altered flower color, thus the term post-transcriptional gene silencing (PTGS) was coined for this phenomenon. Another phenomenon termed "Quelling" was reported by Romano et al. in 1992, in which they described that transient inactivation of gene expression in Neurospora crassa could be achieved by transformation with homologous sequences as seen in CSH transgenic plant. For years, no one pay much attention to these findings considering them just a bizarre natural phenomenon.

About the same time, an antisense RNA technology was developed to study gene function in Caenorhabditis elegans (C. elegans) system. In this technique, antisense RNA leads to effective and specific inhibition of gene expression in C. elegans. A group of scientists (Guo et al., 1995), for the first time demonstrated that PAR-1 was required for germline development as well as in establishing embryonic polarity in C. elegans by injecting par-1 antisense RNA into gonads of wild-type worms. However, a more effective result was obtained by treating gonads with in vitro synthesized sense RNA. This phenomenon kept scientists puzzled for years in C. elegans camp. By the year 1998, the phenomenon of PTGS had been observed in several multicellular organisms such as plant, fungus, and

C. elegans (worm). Later, the discovery that double-stranded RNA (dsRNA) triggers the cellular processes responsible for PTGS was a great scientific endeavor which fetches a Nobel Prize for it and supporting the notion that Nobel Prize always goes to those who have a determined and inquisitive mind with raising one question after another in order to reach the bottom of a matter.

In 1998, Fire and Mello (Fire et al., 1998), firstly attempted to delineate the puzzled issues of PTGS. They questioned that why (i) sense and antisense RNA preparations are each sufficient for gene function inactivation or gene interference; and (ii) interference effects can pass to next generation, even though targeted endogenous mRNAs are rapidly degraded when sense or antisense RNA introduced into C. elegans (worms) embryo. They hypothesized that RNA preparations or side products of bacteriophage RNA polymerases, employed for interference may contaminate some molecules with double-stranded character. To assess the potential involvement of double-stranded RNA (dsRNA) in PTGS, they chose unc-22 gene as a gene model to study the possibility. What they did and found are as follows (quoted from the abstract of Nature 391:806-811, 1998): “Experimental introduction of RNA into cells can be used in certain biological systems to interfere with function of an endogenous gene. Such effects have been proposed to result from a simple antisense mechanism that depends on hybridization between the injected RNA and endogenous messenger RNA transcripts. RNA interference has been used in the nematode Caenorhabditis elegans to manipulate gene expression. Here we investigate the requirements for structure and delivery of the interfering RNA. To our surprise, we found that double-stranded RNA was substantially more effective at producing interference than was either strand individually. After injection into adult animals, purified single strands had at most a modest effect, whereas double-stranded mixtures caused potent and specific interference. The effects of this interference were evident in both the injected animals and their progeny. Only a few molecules of injected double-stranded RNA were required per affected cell, arguing against stochiometric interference with endogenous mRNA and suggesting that there could be a catalytic or amplification component in the interference process.”

This elegant work done by Fire and Mello helped to demonstrate that dsRNA is the trigger of PTGS; therefore, they coined the term, RNA interference (RNAi), for this novel cellular process. In addition, they also speculated that the persistent character of RNAi could be achieved by an amplification process in the cells. RNA-dependent RNA polymerase (RdRP), which uses the antisense strand of siRNA as a primer to make more dsRNAs, was later on identified to amplify RNAi process and account for the persistency of RNAi in plants, fungi, and worms (Catalanotto et al., 2000; Dalmay et al., 2000; Mourrain et al., 2000; Sijen et al., 2001). In plants, this amplification enables RNAi-mediated gene silencing to spread through cell-to-cell transfer of dsRNAs to generate extensively resistance to viral infection. Soon it was found that dsRNAs inhibits gene expression in a sequence-specific manner which is mediated by short RNA molecules, now known as "short interfering RNA" (siRNA), with length approximately 25 nucleotides in plants (Hamilton et al., 1999) and 21-23 nucleotides in animal cells (fruit fly Drosophila melanogaster) (Hammond et al., 2000). The sequence of these siRNAs corresponds to parts of the mRNA (messenger RNA) of the silenced gene.

It is widely believed that RNAi is a natural and ancient defense process against dsRNA intermediates or byproducts generated by pathogens in plants and insects. However in mammalian

system, dsRNA with length greater than 30-bp interacts directly with cellular proteins, which triggers signaling pathways that lead to the expression of type I interferon (IFN) responses and the activation of non-specific RNAases. In turn, type I-IFNs induces the expression of a number of interferon-stimulated genes that possess antiviral activity in the cell resulting in a non-specific global gene silencing which lead to cell death in mammalian system. Thus IFN response obstructs the direct application of long dsRNA-mediated silencing in mammalian system. With the previous-described endeavors, Dr. Tuschl's group identified that chemically synthesized 21-nucleotide siRNA duplexes, with an overhanging 3' end, specifically suppress expression of endogenous and heterologous genes in various mammalian cell lines (Elbashir et al., 2001). Therefore, 21-nucleotide siRNA duplexes open up a new tool for dissecting gene function in mammalian cells. The use of the RNAi cellular machinery to knock down gene products has greatly accelerated the understanding of gene function. In recognition of the overwhelming importance of RNAi as a biological process and a universally applicable tool, the leading journal Science announced it "The breakthrough of the year: 2002." (Jennifer Couzin, 2002)

Subsequent biochemical studies revealed that RNAi is a remarkable pathway with intricate network of proteins to trigger the degradation of the target mRNA in order to silence the function of a gene. The detailed history and mechanism of RNAi can be found in recent reviews in Curr Top Microbiol Immunol (2008, volume 320:1-201), which is out of purview of this short essay.

Although duplexes of 21-nucleotide siRNAs with short 3' overhangs can mediate RNAi in a sequence-specific manner in cultured mammalian cells, the cost of synthetic siRNA library is high and it must be delivered into cell by transfection. However, not every cell type has good transfection efficiency for delivering siRNA, particularly in vivo. In addition, the response of the siRNA in mammals is transient due to lack of an RNAi amplification process. This limits the application of synthetic siRNA in many applications.

Meanwhile, the RNAi field is rapidly developing. During the course of studying the molecular mechanism of RNAi, scientists found that processing of siRNA and miRNA in cytoplasm is mediated by Dicer, an RNase III-like nuclease. Dicer processes precursor dsRNAs into 21-23-nucleotide siRNAs/miRNAs. Therefore, these two tiny RNAs share the same machinery for RNA processing in the cytoplasm. Strikingly, biochemical and genetic studies revealed that knockdown or mutation of Dicer lead to accumulation of approximately 70 nucleotides miRNA precursor with a shRNA (short hairpin RNA) structure both in mammals and in lower eukaryotes. This finding drives scientists to develop RNA polymerase III promoter to express shRNA in vivo; as transcription initiation site of RNA pol-III promoter is well defined, and transcription stops when polymerase encounters consecutive 4-5 Ts and terminate at the second U (T on DNA template). Thus, RNA pol-III transcripts results in uniform shRNA structure containing defined 5' and 3' ends.

There is an urgent demand for tools to carry out genome-wide genetic screens in mammalian cells. The advances in RNA interference (RNAi) technologies developed both by Biotech Company and academic institution have made this possible. Nowadays, genome-wide siRNA and shRNA libraries are commercialized by Biotech Company. On a genome-wide scale, each gene can be theoretically "silenced" and thus such libraries provide as tools to study functional genomics as well as drug discovery and therapeutic intervention in mammalian system.

References:

1. Napoli, C., Lemieux, C. and Jorgensen, R. (1990) Introduction of a chimeric chalcone synthase gene into Petunia results in reversible co-suppression of homologous genes in trans. Plant Cell 2:279-289.
2. Romano, N. and Macino, G. (1992) Quelling: transient inactivation of gene expression in Neurospora crassa by transformation with homologous sequences. Mol. Microbiol. 6:3343-3353.
3. Guo, S. and Kemphues, K.J. (1995) par-1, a gene required for establishing polarity in C. elegans embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. Cell 81:611-620.
4. Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E. and Mello, C.C. (1998) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391:806-811.
5. Mourrain P, Béclin C, Elmayan T, Feuerbach F, Godon C, Morel JB, Jouette D, Lacombe AM, Nikic S, Picault N, Rémoué K, Sanial M, Vo TA, Vaucheret H. (2000) Arabidopsis SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance. Cell 101:533-42.
6. Dalmay T, Hamilton A, Rudd S, Angell S, Baulcombe DC. (2000) An RNA-dependent RNA polymerase gene in Arabidopsis is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. Cell 101:543-53.
7. Catalanotto C, Azzalin G, Macino G, Cogoni C. (2000) Gene silencing in worms and fungi. Nature 404:245.
8. Sijen T, Fleenor J, Simmer F, Thijssen KL, Parrish S, Timmons L, Plasterk RH, Fire A. (2001) On the role of RNA amplification in dsRNA-triggered gene silencing. Cell 107:465-76.
9. Hamilton, A.J. and Baulcombe, D.C. (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 286:950-952.
10. Hammond, S.M., Bernstein, E., Beach, D. and Hannon, G.J. (2000) An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature 404:293-296.
11. Elbashir, S.M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K. and Tuschl, T. (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411:494-498.
12. Jennifer Couzin (2002) BREAKTHROUGH OF THE YEAR: Small RNAs Make Big Splash. Science 298:2296-97.