RNAi was initially discovered as one mechanism for post-transcriptional gene silencing (PTGS) in plants. Then Fire and Mello discovered that using the sense or the antisense or the duplex RNA could silence gene expression in *C. elegans*. Can induce silencing by feeding the worms RNA or bathing them in it! For discovery, see Mello, Nature 2004.

RNAi = RNA interference operates through small RNA’s called siRNAs. Endogenous non-coding regulators are called micro RNAs or miRNA’s; they can either act like siRNAs or they can inhibit translation without destroying the mRNA, by sequestering the message.

Besides the practical implications below, there may be an entire level of regulation based on non-coding RNA (transcribed from what was thought to be junk DNA). The ncRNA has been observed to be as conserved as protein-coding genes. See Mattick, Science 2005:

Practical importance:
Can specifically knock out any gene in a temporally-controlled fashion. This allows analysis of the function of lethal deletion mutants, and it’s much more rapid than generating transgenic organisms. Can be used for large-scale screening of gene knockouts, or knocking down expression in organisms where the genetics are difficult. Also allows for removal of YFP in cell culture for biochemical analysis of the consequences, accompanied by ChIP etc to look at interacting partners. There is a lot of interest in therapeutic applications (Alnylam = ALNY), although with many of the problems associated with antisense. For example, siRNA was linked to cholesterol to get specific import into liver cells that make apolipoprotein B -> reduce overall cholesterol level (Nov. 11, 2004 Nature).
Mechanisms and consequences:

Large dsRNA is cleaved into 21-23 bp dsRNA by the Dicer complex. Then the short dsRNA is processed by the RISC complex into short single strands (via ATPase RNA helicase activity). Then the RISC complex targets destruction of target mRNA. Other complexes give sequestration or mediate silencing at the level of chromatin.

In plants and worms, the short RNA can also be amplified by RNA-dependent RNA polymerases for a longer-lasting, transmissible effect.

In mammals, long RNA induces non-specific silencing through antiviral interferon response, but this can be avoided by using short dsRNA.

Pathways, from Meister and Tuschl, Nature 2004:

![RNA silencing pathways in different organisms. Long dsRNA and miRNA precursors are processed to siRNA/miRNA duplexes by the RNase III-like enzyme Dicer. The short dsRNAs are subsequently unwound and assembled into effector complexes: RISC, RITS (RNA-induced transcriptional silencing) or miRP. RISC mediates mRNA-target degradation, miRNP's guide translational repression of target mRNAs, and the RITS complex guides the condensation of heterochromatin. In animals, siRNAs guide cleavage of complementary target RNAs, whereas miRNAs mediate translational repression of mRNA targets. miRNP's guide chromatin modification. S. pombe, C. elegans and mammals carry only one Dicer gene. In D. melanogaster and A. thaliana, specialized Dicer or DCL proteins preferentially process long dsRNA or miRNA precursors: 7mG, 7-methyl guanine; AAAAA, poly-adenosine tail; Me, methyl group; P, 5’ phosphate.]

Companies pay for good graphics, from www.alnylam.com. RNAi as a drug: It has tremendous potential, but may be hard to deliver in general.
Below: Can make *inducible knockdown-vectors* with short hairpin RNA: Transcription makes a short hairpin RNA (shRNA) which is processed to give a short dsRNA = siRNA. Can be stably transfected, expression regulated like any other inserted gene. http://www.upstate.com/features/plasmids_pathway_l p.asp?r=700

**Legend:** A double-stranded, annealed DNA oligo is generated that corresponds to the target gene mRNA sequence such that the target gene sense sequence is represented 5' of its antisense and separated by a 9 base pair "loop" region. This oligo, containing the terminator sequence, is then cloned into an expression vector that uses the human HI, RNA polymerase III-based, promoter to express the cloned sequence. The transcription is terminated by a dT5 sequence immediately 3' of the siRNA sequence. The transcript is then able to fold onto itself as the sense and antisense regions are able to base-pair. The 9 nucleotide "loop" region allows for the short hairpin RNA (shRNA) to form. Cellular ribonucleases process the shRNA into a functional, short interfering RNA (siRNA).
RNAi is involved in heterochromatin silencing

From Lippman and Martienssen. Nature Insight 2004:

Figure 2 RNAi and heterochromatic silencing. a, Silencing in S. pombe. The reverse strand of the centromeric repeats (Box 1) is transcribed in wild-type cells, but rapidly processed into siRNA by the RNAi pathway (1). The resulting siRNAs are amplified by RdRP (2), which is recruited to the repeats. siRNAs are also found in the RITS complex (3). These siRNAs are not required for the assembly of RITS but they are required for targeting. Targeting may be mediated either directly or indirectly by means of H3mK9 (red circles), which in turn probably interacts with Chp1. The role of RITS, if any, in modification of H3K9 by Clr4 (4) is not yet understood, but this leads to recruitment of another chromodomain protein Swi6, which silences the forward strand by TGS (5).

b, Silencing in Arabidopsis. Transposons and tandem repeats account for 95% of siRNA in Arabidopsis, at least some of which is processed by DCL3 and RdR2 (RNA-dependent RNA polymerase 2), although other enzymes may be involved. At least three groups of genes, whose products may form complexes, have been proposed to account for heterochromatic modifications that are guided by siRNA. The first includes DRM1 and/or DRM2, which encode redundant Dnmt3-like methyltransferases. These are required for de novo DNA methylation including non-CG methylation. The second group includes CMT3, which is required for CNG methylation and is recruited by histone H3mK9 by means of KYP and possibly other histone methyltransferases. The third group includes the Dnmt1-like methyltransferase MET1, which is required to maintain CG methylation, but may also establish methylation in the presence of siRNA. Also included in this group is the histone deacetylase HDA6, which is probably required indirectly for H3mK9. Each putative complex is thought to bind siRNA. The first two groups include at least one AGO gene, including AGO4 and AGO1. The mechanism by which siRNA accumulation depends on MET1 and DDM1 is unknown.