There are several ways in which such a process could potentiate DNA repair (Fig. 1). First, because silenced chromatin is generally inaccessible to DNA-modifying enzymes, it may prevent nuclease-mediated degradation of damaged DNA ends. Second, heterochromatin-associated silencing could stop processes such as transcription from interfering with DNA repair. Third, heterochromatin-mediated condensation of the damaged DNA might facilitate the juxtaposition and subsequent ligation of the two broken DNA molecules, and could prevent these DNA ends from engaging in other recombination reactions. This third function could be particularly important *in vivo*, where damaged DNA ends might otherwise easily become irrevocably separated, and where recombination with other loci is generally undesirable.

The new data also raise the possibility that the Sir proteins target Ku to certain heterochromatic regions in the absence of DNA damage. Consistent with this idea, as is the case for inactivation of *SIR3* or *SIR4* (ref. 7), loss of yeast Ku function leads to telomeric shortening[8,9]. Thus, it will be of interest to determine whether Ku affects other Sir-dependent functions, such as tethering telomeres to the nuclear periphery and transcriptional silencing (in this regard, it is noteworthy that mammalian Ku has been shown to suppress transcription[5]). Finally, the relative inaccessibility of heterochromatin may mean that it is inherently difficult to repair DNA damage in these regions of the genome — in such chromosomal contexts, Sir-mediated targeting of Ku could be a mechanism for potentiating the DNA non-homologous end-joining system to resolve potentially lethal DNA damage. □

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RNA structure

A molecular contortionist

Jennifer A. Doudna

RNA molecules kink, bend, loop and twist themselves into a wonderful variety of shapes. These took centre stage in June, at the first meeting devoted solely to RNA structure*. Due to the abundance of biologically important RNAs and the development of technical advances to study them, RNA structural biology has now come of age. And it is clear that ribonucleic acids are capable of a level of structural complexity that was once thought to be confined to proteins.

Although RNA lacks the chemical diversity of proteins, the many conformational degrees of freedom of its phosphate backbone, along with the unique hydrogen-bonding and stacking properties of its nucleotide bases, provide ample resources for three-dimensional folding. This has been exploited by evolution — RNA molecules are integral parts of the cellular machinery for protein biosynthesis, RNA processing, chromosome end replication and protein transport (Table 1). RNA molecules are sometimes catalysts (ribozymes), and the functional diversity of this molecular jack-of-all-trades implies structural complexity.

At first glance, the principles of RNA folding seem to be simple and rather dull, because the four nucleotide building blocks readily form hydrogen-bonded base pairs and a corresponding double helix. But, unlike its DNA sibling, RNA rarely exists as a long, straight duplex. Instead, many RNA molecules fold back on themselves to produce short stretches of base-pairing interspersed with unpaired 'internal loops', bulges and terminal loops. And these loops and bulges are the key to the formation of complex RNA structures.

Internal loops, it turns out, are surprisingly ordered — the loop nucleotides make unconventional, yet specific, interactions. For example, according to the nuclear magnetic resonance (NMR) and crystal structures of the loop-E region of 5S ribosomal RNA, purine nucleotides within the 'loop' are pinched together in sheared base-pairs, the bases of one strand stacking on bases in the opposite strand (P. Moore and T. Steitz, Yale Univ.). Although the overall structure is helical, the major groove of the loop region is stapled shut by magnesium ions that bridge the phosphate backbones.

It is not known whether this dramatically distorted geometry is recognized by the ligand for the loop-E region, the L25 ribosomal protein. But other RNA internal loops change shape on binding their ligands, for example the Tat and Rev responsive-element RNAs of the human immunodeficiency virus (HIV) and an RNA that binds the small molecule theophylline (A. Pardi, Univ. Colorado). The NMR structure of the unbound tetraloop receptor — an internal loop that binds to GAAA tetraloops and is common in large RNAs — reveals a 'closed' helical conformation, containing cross-strand purine stacking that resembles the loop-E structure (J. Feigon, UCLA). When the tetraloop is docked, however, the receptor helix is opened to allow stacking of the tetraloop bases in the minor groove.

Localized regions of RNA can be dynamic, perhaps providing the basis for movement on a grand scale within large RNAs. Crosslinking of active conformers of the *Tetrahymena* self-splicing intron, for example, reveals that helices within this roughly 400-nucleotide RNA can move by as much as 60 Å (T. Cech, Univ. Colorado). In the wishbone-shaped hammerhead ribozyme (Fig. 1), movement of the helical arms is probably more limited, but it may be required to position an active-site magnesium ion close to the labile bond at the moment of cleavage (D. Herschlag, Stanford Univ.).

Metal ions are central to RNA structure and catalysis — a point that emerged as a major theme of the meeting. For example, more than 100 magnesium ions are needed for the several hundred nucleotides of ribonuclease P to be folded (C. Fierke, Duke

Table 1 **The ABCs of RNA taxonomy**

RNA ABCs	*Description*
cRNA	Ribozymes and autocatalytic molecules
gRNA	Template, or guide sequences, for editing of RNA messages
mRNA	Messenger that carries information from DNA to be translated into protein
rRNA	Essential components of the ribosome, the protein biosynthetic machinery
snRNA	Small nuclear components of the mRNA splicing machinery called the spliceosome
snoRNA	Small nucleolar RNAs that specify methylation sites in rRNA, and may have other chaperone-like functions
tRNA	Transfer molecules that carry amino acids
Other examples:	
Telomerase RNA	Chromosome end-replication template
Signal-recognition particle (SRP) RNA	Essential component of SRP, involved in protein transport in cells
Xist RNA	Required for X-chromosome inactivation during development

**RNA Structure*, Univ. California, Santa Cruz, 25–29 June 1997.

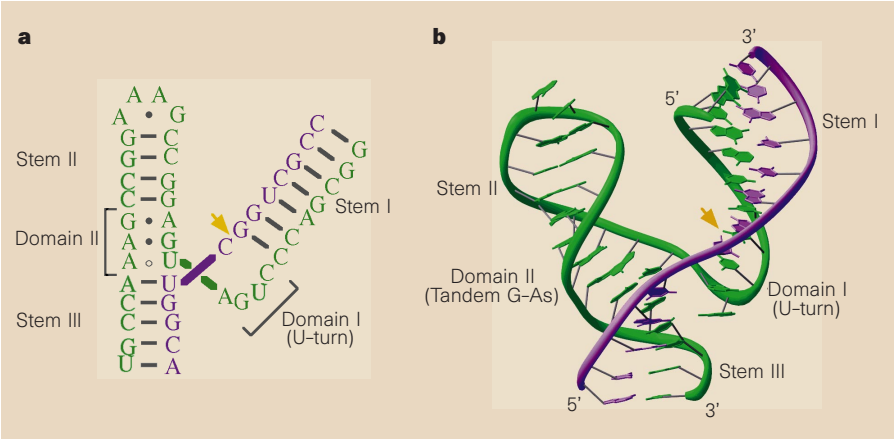

Figure 1 **The hammerhead ribozyme, found in plant-virus-like RNAs, self-cleaves at the phosphodiester bond indicated by the arrow.** a, **Secondary structure;** b, **three-dimensional shape, as revealed by X-ray crystallography.**

Univ.). And in the P4–P6 domain of the *Tetrahymena* self-splicing intron, a cluster of magnesium ions binds to phosphate oxygens and guanosine bases to form a structural core. Magnesium ions may play a similar role in the folding of a three-helix junction in ribosomal RNA (J. Williamson, MIT), and they can also bind the major groove of RNA helices near G–U base pairs. Group-II self-splicing RNAs may use this trick to position active-site metals (A. M. Pyle, Columbia Univ.).

Unique structures in RNA provide handles for proteins, yet the specificity of these interactions has been a mystery. How are transfer RNAs recognized by elongation factors and cognate tRNA synthetases, for example, at the appropriate stages during protein biosynthesis? X-ray crystallographic studies reveal that these proteins 'see' different features of tRNAs: elongation factor Tu (EF-Tu) sees the aminoacylated tRNA acceptor stem (P. Nissen, Aarhus Univ.); the histidyl tRNA synthetase recognizes a motif within the acceptor stem of tRNA-His (R. Giegé, CNRS, Strasbourg); and seryl-tRNA synthetase sees a variable hairpin loop of tRNA-Ser (S. Cusack, EMBL, Grenoble).

In the case of the spliceosomal proteins U1A and U2B′′, specificity for RNA loops that differ by just one nucleotide is conferred by the interaction of U2B′′ with a second protein, U2A′ (K. Nagai, MRC). Single-stranded-RNA binding proteins, including U1A, U2B′′ and the MS2-phage coat protein, use a β-sheet motif to recognize splayed nucleotides in the RNA (F. Allain, UCLA; K. Nagai; L. Liljas, Uppsala Univ.). But a double-stranded-RNA binding domain from *Xenopus* grabs its substrate by interacting with two successive minor grooves along one face of the RNA duplex (S. Schultz, Univ. Colorado). Localized distortion widens the major groove of the target RNA, and a network of ordered water molecules lines the protein–RNA interface.

The emerging themes of RNA structure — stable loops, major- and minor-groove interactions, modular motifs and metals — indicate that it may one day be easier to model and predict RNA folds than to predict protein structure. New and modified algorithms for identifying secondary structures in RNA sequences are already used. Moreover, a database of structural models and corresponding biochemical data for the 30S ribosomal subunit is now available on the World Wide Web (http://www-smi.stanford.edu/projects/helix/riboweb.html) (R. Altman, Stanford Univ.).

Modelling of RNA tertiary structures has proven more difficult. Although a model of the HIV Rev-binding element compared favourably to an NMR structure (F. Leclerc, Univ. Montreal), a model of the ribosomal A-site (S. Harvey, Univ. Alabama) was quite different from the NMR structure of this region (J. Puglisi, Univ. California, Santa Cruz). Models of the hammerhead and other larger ribozymes continue to spark new ideas about catalytic mechanisms (E. Westhof, CNRS).

One of the most exciting directions of RNA structural biology is now to understand how large RNAs, and RNA–protein complexes, work. Chemical cross-linking and phylogenetic analyses have been used to model group-II self-splicing introns (F. Michel, CNRS), ribonuclease P RNA (N. Pace, Univ. California, Berkeley) and the ribosome (H. Noller, Univ. California, Santa Cruz). But a detailed understanding of these RNAs will require structures at atomic resolution.

Tantalizing images of ribosome crystals were presented during the final hour of the meeting (H. Noller and C. Wilson, Univ. California, Santa Cruz; T. Steitz). So when the RNA structural biologists reconvene in two years, honoured guests may include, among other RNA behemoths, the ribosome itself — an exciting prospect indeed. □

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100 YEARS AGO

It has long been my conviction that we study animals too much as dead things. We name them, arrange them according to our notions of their likeness or unlikeness, and record their distribution. Then perhaps we are satisfied, forgetting that we could do as much with minerals or remarkable boulders. Of late years we have attempted something more; we now teach every student of Zoology to dissect animals and to attend to their development.... But the animals set before the young zoologist are all dead; it is much if they are not pickled as well. When he studies their development, he works chiefly or altogether upon continuous sections, embryos mounted in balsam, and wax models. He is rarely encouraged to observe live tadpoles or third-day chicks with beating hearts. As for what Gilbert White calls the *life and conversation of animals*, how they defend themselves, feed, and make love, this is commonly passed over as a matter of curious but not very important information; it is not reputed scientific, or at least not eminently scientific. – L. C. Miall

From *Nature* 26 August 1897.

50 YEARS AGO

In facing realities we must agree that the world is not yet at peace, neither is it free from the threat of further conflagration, perhaps even on a world-wide scale. It follows that though every effort towards world peace must be made, it would be foolish during present troublesome times to fall into a state of lethargy and unpreparedness. So some scientific research, especially that dealing with certain aspects of atomic energy, must even now, and perhaps for some time to come, be carried on under the ban of offical secrecy. Feeling is high these days, and concern is often expressed that military secrecy might take the place of former industrial interests in slowing up the free flow of ideas which are essential to the smooth progress of scientific endeavour. But at present men of science must face the fact that some secrecy is inevitable, and this makes it all the more imperative, therefore, that they themselves should strive to bring before each other and above all before the general non-scientific public the many beneficent aspects of science.

From *Nature* 30 August 1947.