ticancer agents and for therapeutic use in other proliferative diseases [15] because of the central roles certain CDKs (especially isoforms 1, 2, 4, and 6) play in cell cycle regulation, which is frequently overridden in transformed cells. Other CDKs (at least isoforms 7, 8, and 9) are implicated in regulation of transcription at the level of RNA elongation, where they phosphorylate the C-terminal domain of RNA polymerase-2. Many viruses depend on host cell CDKs, often by recruiting these through virus-encoded cyclins, for their replication, and this has now led to a biomedical rationale for the application of CDK inhibitors as antiviral agents [16]. Many parasitic microorganisms also possess CDKs or CDK-like proteins; their selective inhibition may give rise to drugs against some of the most widespread human diseases, including malaria [17]. Of particular interest in connection with the Meijer et al. paper is CDK5. This kinase is highly expressed in the nervous system, where it acts on many different substrates. Of these, the microtubule binding protein tau, the β-amyloid peptides, and the neurofilament protein NF-H are of special interest because their hyperphosphorylated forms are associated with various neurodegenerative disorders, including Alzheimer's disease. Activity on such substrates appears to be due to both CDK5 and GSK-3. In fact, there exists a general association of CDK and GSK-3 activities with neuronal cell death. For application in neurodegenerative diseases, one might therefore expect neuroprotective effects with dual CDK/GSK-3 inhibitors [9].

GSK-3, of which humans have two nonredundant isoforms, α and β, that are practically identical in the kinase domain, has many other functions, notably those arising from its role in the Wnt signaling pathway. Meijer et al. use biological readouts from this pathway to demonstrate that 6-bromoindirubins do in fact behave as GSK-3 inhibitors in vitro and in vivo [3]. An additional important physiological function of GSK-3 is in the regulation of glucose to glycogen conversion. It has been demonstrated that GSK-3 inhibitors may be useful as drugs in the treatment of type II diabetes [18]. For this and other therapeutic applications of GSK-3 inhibitors, it will be important to develop selective compounds that do not possess the antiproliferative properties of CDK inhibitors.

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RNA as Multitude/RNA as One

In the configurations formed by RNA and its ions there are structural possibilities not yet realized; some are hinted at in new work on the binding of an amino acid analog.

RNA surely means different things to different biologists, but molecules are usually thought of as unitary objects, as explicit as a mountain or a building. We have been trained to think of structures in this way from the motionless, indispensable polychromes that illustrate the conclusions of structural biologists' experiments. However, the huge energetic penalty rendered for unpaired charges implies that RNA molecules are always electrically neutral. Therefore, RNAs are inevitably accompanied by ions, about one ionic charge per nucleotide phosphate. So, for many purposes, RNA is better illustrated as a fluctuating crowd of particles. Magnesium is more influential than potassium because the electrical neutrality generated by localizing a single magnesium ion frees two potassium ions, yielding an entropic ad-

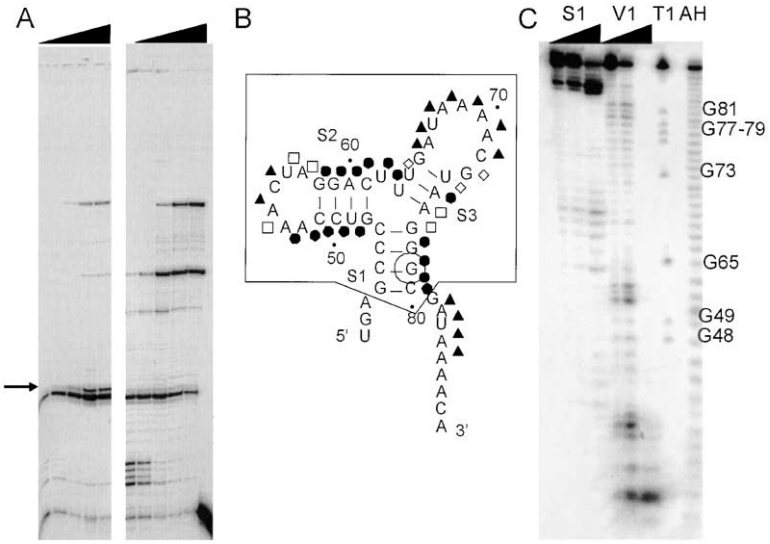

Figure 1. Secondary Structure of SHR1.48 RNA and RT Mapping of Modification Site

vantage. Even when ions cannot be seen, as they often cannot in crystallographic reconstructions, a crowd of neutralizing divalents and monovalents is still there, running in channels defined by the electric potential of the RNA [1]. Although their identities may change, electrically bound ions are "real" parts of the molecule, and their presence can be detected by techniques such as equilibrium dialysis. Ions' preference for the folded state is a major incentive for nucleic acid polymers to form compact native structures, as indicated by the results from the electrostatic calculations of Misra and Draper [2].

Associated ions are therefore essential for structural stability and are also essential to RNA function, particularly in some catalytic RNA reactions. Though once seen as exclusively metalloenzymes [3, 4], ribozymes are in fact a mixed bag. Some RNA active sites exploit the chemistry of immobilized ions, like the group I self-splicing RNA [5], whereas others, like the hairpin ribozyme, have metal-free mechanisms [6]. Regardless, ribozymes certainly draw attention to the requirement for precisely located RNA-associated ions. When a chemical reaction includes an ion, its bound state must be exquisitely specific (or a specific site and orientation must at least be attained relatively frequently). This requirement makes an infrequent circumstance disproportionately important: occasionally, a folded RNA contains such a deep negative electrostatic hole that very strongly bound water will be stripped from a bound ion, permitting better charge-charge interactions between ion and RNA. During such infrequent occurrences, RNA ligands (such as phosphate oxygens or guanine N7) enter the ions' inner coordination sphere, and the ions become a long-lived part of the RNA tertiary structure. Therefore, despite the fact that most ions are a part of a fluctuating RNA atmosphere, there is a population of key fixed ions confined by unusual electrostatic barriers and chemical coordination.

In the November issue of *Chemistry & Biology*, Sanchita Hati et al. [7] in Don Burke's laboratory at Indiana University set out to extensively explore this phenomenon by exploiting a type of extraordinarily localizable divalent ion. These ions are a group of transition metals that have extremely slow exchange rates for bound water and other ligands. Specifically, the Burke group selected an RNA that will use Ni^{2+} (with moderately slowed exchange) and Pt^{2+} (with very slow exchange) to bind a small molecule ligand, biocytin, (biocytin is biotinoyllysine) to RNA. Ni^{2+} and Pt^{2+} would be expected to slowly give up their affinity for water to join an RNA structure and then slowly release once localized in an RNA-biocytin site. These properties were readily observed: formation of RNA:Pt:biocytin is still improving at 18 hr at 52°C (pH 7), which surely must strain the stability of the RNA at the binding site. Similarly, both Ni^{2+} and Pt^{2+} complexes survived two ethanol precipitations and exhibited half-lives of hours on subsequent incubation at 25° without substrates or divalent ions.

The authors selected the RNA with the goal of isolating a molecule that reacted with the lysine moiety of biocytin to emulate the normal reaction of aminoacyl-tRNA synthetase proteins in fixing amino acids to RNA. What emerged from three different selection experiments was a molecule containing a 37 nucleotide three-helix junction (SHR1 RNA) with an unusually stringently conserved structure. Nucleic acid-metal-biocytin coordination is, unexpectedly, disrupted by variation of almost any of the nucleotides in the binding site. A unique role for nickel emerged because it was one of a cocktail of nine divalents added to the selection, fishing for RNA reactions that exploited chemistries unique to individual metals, so-called "Cheshire catalysts" [3]. In this sense, the strategy succeeded, though organic chemistry rather than bioinorganic coordination was the initial goal.

Now that Hati and colleagues have shown how to do it, one can easily envision generalizing their strategy to select for self-assembling RNA nanostructures. NanoRNA is an emerging field pioneered by Luc Jaeger and Eric Westhof [8], who use tetraloop-receptor interactions to build RNA structures, and extended by others [9] using kissing loops as the linking element. Given that the ultimate purpose of this work is to establish means to controllably assemble RNAs into larger functional objects, one would like to be able to either select for affinity

between RNAs or between RNA and a selected chelator in the presence of a moderately slowly exchanging metal like Ni^{2+}. The simultaneous presence of the usual mono- and divalent ions would not hamper such studies, since they will aid general RNA folding without effectively competing with slow exchanging ion at its rare binding sites. When molecules have been selected that stably bind the first (slow exchange) metal, the binding domain must be defined. This domain can then be used as a linker to join multiple RNAs in the presence of a second (very slowly exchanging) divalent, let's say Pt^{2+}, with the same coordination geometry as your first ion (both Ni^{2+} and Pt^{2+} favor square planar arrangements for their ligands). Selection, which requires reversal or disassembly of the metal-mediated complex, could be simplified using this strategy without sacrificing the ultimate production of stable multimolecular structures. If you are feeling a bit short of development time, funds, or motivation, you might well use the binding domain of SHR1 RNA, as shown above in Figure 1 (Figure 3 of Hati et al.) [7].

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RNA Sex

Recombination of genetic information is a major driving force in evolution, today catalyzed by protein enzymes. In this issue of *Chemistry & Biology*, a paper by Riley and Lehman [1] demonstrates that RNA can perform general recombination of RNA strands, thus supporting the scenario of a prebiotic RNA world.

Advances in evolution have for some time been viewed by many as a process of continuing occurrence of minor mutations under the constant scrutiny of selection pressure, blocking the amplification of deleterious mutations and favoring a few punctual improvements. Besides the apparent painful slowness of such a process, mathematical models have clearly indicated that populations under a certain number of individuals would suffer a continuous loss of fitness from mildly deleterious mutations rather than producing a series of champions [2]. Sexual recombination offers a way out, since "good" mutations can also arise in, and propagate from, strains with a number of deleterious mutations. Moreover, rather than effecting single point mutations, recombination can generate, by error or design, sequence changes to a much larger extent. Such events enlarge the complexity of a given sequence pool by leaps and bounds in comparison.

All but a few of today's replicating entities try to improve their evolutionary flexibility by employing recombination, sometimes even by borrowing genetic information from outside their species, as in the case of horizontal gene transfer in bacteria. Failure or inability to perform recombination may lead to serious drawbacks. A case in point are animal mitochondria, which have resorted to a number of complicated mechanisms to compensate for deleterious effects of slowly accumulated point mutations in the mitochondrial genome [3].

Recombination at the level of RNA has been reported for viruses [4]. Splicing of mRNAs to generate a variety of different exons from a pre-mRNA is a mechanism bearing characteristics akin to recombination, although the newly generated sequence information is not handed down to the next generation. RNA from certain organisms is capable of performing the splicing reaction without the help of cofactors, protein or other, by sequential execution of a cleavage and a ligation reaction. Incidentally, these properties present the first reported catalytic activities of RNA, the discovery of which, by Cech and coworkers in 1982 [5], was later awarded the Nobel price.

In their paper "Generalized RNA-Directed Recombination of RNA," Riley and Lehman [1] make use of the catalytic properties of such introns to catalyze a reaction resembling a metathesis reaction:

$$\text{A-B} + \text{C-D} \xrightleftharpoons[\text{catalysis}]{\text{RNA}} \text{A-D} + \text{C-B}, \qquad (1)$$

where A-B is a first RNA comprising a head and a tail part, and C-D is a second RNA composed similarly.

Based on previous observations by several other groups, the authors have turned, through proficient mo-