pocket of a paclitaxel molecule [10], whereas Q292E mutation is located in the helix H9 near the M loop. The mutation at 292 is particularly interesting because it is located apparently outside of the paclitaxel binding site. The most likely explanation is that, at 292, the presence of an extra-positive ionic charge due to glutamine (instead of glutamic acid) induces a distortion in the M loop, thereby hampering the entry of paclitaxel (and epothilones) into the pocket of the binding site. As a matter of fact, this mutation is linked to an extremely high degree of drug resistance (around 400-fold) and greatly reduced binding of [^{3}H]paclitaxel as compared to the drug-sensitive parental cells.

Consequently, the work of Kavallaris and her collaborators provides novel insights into drug-target interactions in microtubules. It is now essential to understand which of the reported drug-resistance mechanisms actually occurs in clinical settings so that we can focus our efforts on the generation of new and efficacious molecules as well as therapeutic strategies to overcome drug resistance.

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RNA as a Transcriptional Activator

Two recent reports demonstrate that in vivo selection can isolate novel RNAs that activate transcription when tethered to a gene promoter. This highlights the structural plasticity that allows RNA to fulfill many functions normally carried out by proteins.

Eukaryotic transcription factors are modular proteins that are generally comprised of a sequence-specific DNA binding domain (DBD) tethered to a transcriptional activation (AD) or repression domain (RD). Extensive structural diversity can be found in the various motifs utilized for both DNA binding and activation/repression. DBDs are very well characterized, and numerous high-resolution structures are now available for zinc finger, helix-turn-helix, leucine zipper, winged helix, and other types of DBDs [1]. The molecular details underlying base-sequence specificity have been unraveled for many of these DBDs. While activation and repression domains are also well characterized at the level of amino acid sequence composition and character, structural studies lag far behind the DBDs, and the precise mechanisms leading to gene activation or repression in many cases have not yet been elucidated. Activation domains are thought to be responsible for the recruitment of coactivators, components of the basal transcription machinery, or enzymes for chromatin modification (chromatin remodeling factors and histone-modifying enzymes) [2]. Common targets may be shared between different transcriptional activators, but many activation domains share little sequence homology to one another.

The chemical biology community has long sought to devise methods to control gene expression by targeting specific DNA sequences with novel peptides, oligonucleotides, or small molecules [3–5]. Natural or synthetic DBDs have been tethered to peptides derived from potent viral activators [6–9], and fully synthetic peptides have been fused to natural DBDs for gene activation [10, 11]. In another approach, peptide libraries

have been screened by phage display methods for sequences that will bind to particular coactivators. An excellent example of this is reported by Frangioni et al., who screened phage libraries for peptides that bound CBP/p300 [12]. The selected peptides then can be fused to DBDs to affect gene activation. Perhaps the most surprising finding of these studies is the diversity of workable solutions to the problem of gene activation, particularly in the role played by the AD.

Two pioneering groups have taken a novel approach toward generating synthetic activators where the activation domain is composed entirely of RNA [13, 14]. In the June issue of *Chemistry & Biology*, David Liu and colleagues present the results of a functional screen for RNA activators in yeast. Three separate components are required for this method (see Figure 1 in Buskirk et al. [14]). First, selection depends upon a reporter plasmid harboring the consensus binding site for the LexA DBD (the LexA operator), a minimal promoter, and a selectable marker such as the *HIS3* gene (or β-galactosidase for quantitation). Second, an expression plasmid encoding a fusion of the LexA DBD to the coat protein of the MS2 RNA bacteriophage is introduced into the same cells, and third, a plasmid encoding a random sequence of 40 or 80 nucleotides (N_{40} or N_{80}) fused to additional RNA-coding sequences and two copies of the MS2 RNA hairpin-coding sequence (this hairpin RNA binds the MS2 coat protein). This entire expression system is driven by a yeast RNA polymerase III promoter. These plasmids are introduced into yeast cells, which are then selected for growth on media lacking histidine. To stabilize the random RNA region from intracellular degradation, this region is embedded within a larger RNA sequence that is known to have a stable secondary structure. In order to activate transcription, the LexA-MS2 fusion protein must first bind to the LexA operator and then recruit MS2 RNA linked to an activating RNA sequence. Successful RNAs will then recruit the transcription apparatus, resulting in strong transcription of the reporter gene. Expression of the *HIS3* gene product allows survival of yeast cells on selection media lacking histidine and recovery of RNA sequences that activated transcription for further study. Potent activating RNAs were indeed isolated, some of which activated transcription at levels comparable to natural ADs, such as yeast Gal4 and herpes virus VP16.

In a first round of screening, about 0.2% of the clones from the N_{40} library passed initial selection and rescreening (compared to only 0.01% of the N_{80} library), and, remarkably, one clone actually activated transcription more than 10-fold relative to a Gal4-positive control. Control experiments indicated that tethering of the activating RNA to the reporter template via the MS2 protein-RNA interaction is essential for gene activation. When the sequences of the selected RNAs were determined, a variety of structural motifs could be predicted based on computer modeling. This suggests that either various RNA structures can serve as activators by targeting common components of the transcription apparatus or that multiple protein targets can be bound by various RNA structures.

Random mutations (at a level of 20% per base) were introduced into one of the most potent activator RNA sequences, and this RNA was subjected to an additional round of selection under more stringent conditions, yielding 32 unique sequences. One of these activated transcription 53-fold stronger than the Gal4 control AD and only 2-fold weaker than the highly potent VP16 AD. Thus, evolution generated an RNA activator comparable to one of the most efficient known natural protein ADs. In another recent report by Ptashne and colleagues [13], a random sequence loop of only 10 nucleotides attached to an RNA stem was used for selection, and far lower levels of gene activation were found compared to the larger 40 nucleotide sequence used in the present study [14]. Additional experiments by Liu et al. [14] compared the sequences of the evolved RNAs, showing that several structural elements in the selected RNA could play key roles in transcriptional activation. Lastly, mutagenesis of one of the most potent RNA activators revealed the important structural components of this RNA required for activation, most notably the importance of base-paired regions of the RNA.

While the molecular identity of the targets of the selected activating RNAs has yet to be determined, it is reasonable to speculate that these RNAs bind their targets (coactivators or general transcription factors) with comparable affinities to natural transcriptional ADs. Once further investigation has identified these target molecules, it will be of interest to compare the mechanisms by which the natural protein activators and the RNAs bind the same or similar targets. Successful identification of RNA transcriptional activators, along with aptamers and ribozymes, illustrates the vast structural and functional diversity available to RNA, perhaps even rivaling the diversity found in protein structures.

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