Locking RNA in Motion: Mechanism of Group II Intron Inhibition

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Background: Group II introns are large self-splicing ribozymes whose conformational plasticity is essential for folding and catalysis. Although absent from the human genome, they occur in essential housekeeping genes of several human-pathogenic fungi and bacteria, making them attractive antimicrobial targets with limited host-cell toxicity. Intronistat B is a small-molecule inhibitor that binds the intron catalytic core and disrupts splicing. Crystal structures provide static snapshots of inhibitor binding to the catalytic core of the ribozyme and offer valuable insights into the bound state. However, these structures do not reveal how inhibition affects the structural rearrangements required for catalysis. Method: We used two-colour single-molecule Förster Resonance Energy Transfer (smFRET) to monitor conformational changes in individual group II intron RNA molecules. RNA structural rearrangements were followed during folding and catalysis in the presence and absence of Intronistat B, enabling direct comparison of domain-specific motions and conformational dynamics. Results: smFRET measurements revealed that Intronistat B substantially alters structural motions associated with catalytic activity. Inhibitor binding produced pronounced changes in the dynamics of conformational rearrangements, indicating that inhibition extends beyond occupation of the catalytic core and perturbs the RNA motions required to reach or maintain catalytically competent conformations. Conclusion: These results provide dynamic insight into the mechanism by which Intronistat B inhibits group II intron splicing, information not accessible through static structural methods alone. Defining how smallmolecule binding reshapes RNA conformational landscapes may support the rational development of next-generation, target-specific antimicrobial compounds