

Translocation of RNA from Parasitic Plants to their Hosts

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Introduction

Parasitic plants are among the most fascinating plant species, with several parasitic adaptations such as reduced root or leaf area and reduced photosynthetic potential. The haustorium, a specialised organ that penetrates the host plant and forms a physical and physiological bridge between the two species, is the defining trait of parasitic plants [1]. This union brings two distinct species into direct cellular touch with one another, defining the majority of their interactions. Multiple layers of plant-plant communication are involved in the host-parasite relationship, some of which are well-known while others are mostly unknown.

Chemicals that drive parasite species' seed germination and other chemicals that trigger haustorium formation in Orobanchaceae members are two examples of well-studied signal exchanges. Helicases are a kind of enzyme that plays a role in almost every phase of DNA and RNA metabolism. Despite recent advances in understanding their mode of action, the exact processes by which these enzymes link the rearrangement of nucleic acid structures to the binding and hydrolysis of ATP remain unavailable due to their low resolution [1,2].

About the Study

Understanding the cellular roles of these motor proteins requires observing specific mechanistic cycles. The RNA translocation and unwinding cycles of a hepatitis C virus helicase (NS3) monomer are tracked in real time at a resolution of two base pairs and 20 milliseconds. NS3 is a typical superfamily-2 helicase that is required for viral replication [3] and so might be a therapeutic target [4]. We show that ATP coordinates NS3's cyclic movement in discrete 11.63 base pair steps, and that real unwinding happens in smaller, faster substeps of 3.661.3 base pairs, which are likewise driven by ATP binding, implying that NS3 moves like an inchworm [5].

This ATP-coupling process is likely to apply to other non-hexameric helicases engaged in a variety of important cellular activities. The test established here should be applicable to a wide spectrum of nucleic acid translocation motors. NS3 is a critical component of the RNA replication machinery of the hepatitis C virus (HCV) and is found in a membrane-bound complex with other proteins. NS3 is a helicase activity NTPase that has been structurally described in a variety of contexts. We created a single-molecule test to track full-length NS3's mobility on its RNA substrate.

We employ optical tweezers to maintain a constant tension between two beads attached to the ends of a 60-base-pair RNA hairpin, and we measure the RNA's end-to-end distance change while it is unwound by NS3. We first

characterise the mechanical unfolding of the substrate in the absence of enzyme to lay the groundwork for interpreting enzymatic activity. At a force of 20.4 0.2 pN, the substrate unfolds.

No unfolding occurs over many minutes when the substrate is kept at a constant force below 19 pN using the instrument's force feedback system. Helicase catalyses substrate unfolding at external pressures less than 19 pN. We flow NS3 and ATP together in buffer U to follow NS3-catalyzed unwinding. The RNA substrate is then held at a constant force of 5 to 17 pN. A 30 single-stranded RNA loading site is used by NS3 to load on its substrate. The bead separation rises as NS3 unwinds the hairpin, keeping the tension on the molecule constant. At that force, the number of RNA base pairs unravelled as a function of time may be converted from the bead separation.

Because of the molecular geometry, each unravelled base pair releases two nucleotides, increasing the unwinding signal. The hairpin loop aids substrate reformation by allowing for the collection of multiple unwinding traces with each RNA substrate. Unless otherwise specified, data is taken at 22°C, 20 nM NS3, 1 mM ATP, and 17 pN. Throughout the unwinding traces acquired, a wide range of purely ATP-dependent NS3 behaviours can be seen. Sharp bursts of fast strand separation are followed by periods of steady extension as the extension grows.

Conclusion

The distances between pauses are not randomly distributed, but occur with a well-defined periodicity, according to a histogram of pairwise distances across all extensions of a particular trace. Under similar settings, a Fourier analysis of each histogram reveals an apparent unwinding step size of 11.3 bp spanning more than 100 traces.

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