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Interstitial Contacts in an RNA-Dependent RNA Polymerase Lattice

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Catalytic activities can be facilitated by ordered enzymatic arrays that co-localize and orient enzymes and their substrates. The purified RNA-dependent RNA polymerase from poliovirus self-assembles to form two-dimensional lattices, possibly facilitating the assembly of viral RNA replication complexes on the cytoplasmic face of intracellular membranes. Creation of a two-dimensional lattice requires at least two different molecular contacts between polymerase molecules. One set of polymerase contacts, between the "thumb" domain of one polymerase and the back of the "palm" domain of another, has been previously defined. To identify the second interface needed for lattice formation and to test its function in viral RNA synthesis, we used a hybrid approach of electron microscopic and biochemical evaluation of both wild-type and mutant viral polymerases to evaluate computationally generated models of this second interface. A unique solution satisfied all constraints and predicted a two-dimensional structure formed from antiparallel arrays of polymerase fibers that use contacts from the flexible amino-terminal region of the protein. Enzymes that contained mutations in this newly defined interface did not form lattices and altered the structure of wild-type lattices. When reconstructed into virus, mutations that disrupt lattice assembly exhibited growth defects, synthetic lethality or both, supporting the function of the oligomeric lattice in infected cells. Understanding the structure of polymerase lattices within the multimeric RNA-dependent RNA polymerase complex should facilitate antiviral drug design and provide a precedent for other positive-strand RNA viruses.

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Abbreviations used: 3-D, three-dimensional; HCV, hepatitis C virus.

Introduction

The RNA replication complexes of positive-strand RNA viruses such as poliovirus, Dengue virus and hepatitis C virus (HCV) involve multiple virus- and host-encoded proteins assembled on the surface of cytoplasmic membranes. The lynchpins of such complexes are the RNA-dependent RNA polymerases, which have been shown for several such viruses to form, in addition to contacts with other

proteins, homo-oligomeric interactions both in solution[1–7] and in cells.[6–9] For the enzyme encoded by poliovirus, termed 3D polymerase, the formation of homo-oligomeric contacts correlates with cooperative RNA binding and elongation activity,[1,10–12] and mutations interfering with polymerase oligomerization interfere with virus growth.[11–15] The crystal structure of full-length monomeric 3D polymerase has been determined.[16] Like other polymerases, this structure has been likened to a right hand, and the residues that contribute to each component are shown in Fig. 1a.

Purified poliovirus polymerase forms planar sheet-like structures that can be visualized via electron microscopy (Fig. 1b and Refs. 12 and 18, reviewed in Ref. 19). To determine whether the contacts involved in lattice formation and the lattices themselves are important in the function of poliovirus 3D polymerase in viral RNA replication, we and others have sought to identify the contacts involved and to test their roles in the viral infectious cycle. All of the six tested mutations or sets of mutations in one set of potentially relevant polymerase–polymerase interactions, termed Interface I, have been shown to display growth defects (Table 1). Viruses that contained the single L342A mutation in Interface I showed dramatic temperature sensitivity, and those that contained either the single mutation L446A or the double mutation R455A/R456A gave rise to no viable virus.[11] Viruses that contained the triple mutation D339A/S341A/D349A initially reported to display only a small-plaque defect at 37 °C[14] were found to be severely temperature sensitive at slightly higher temperatures.[15] The severity of mutational effects on viral growth was found to correlate with the extent to which oligomerization was disrupted,[18] supporting the hypothesis that oligomeric contacts along Interface I are critical in viral growth.

However, the polymerase is a multifunctional protein involved in several steps in viral replication, and of the 61 total mutations or sets of mutations that have been introduced into the poliovirus 3D coding region and that have been tested for their viral phenotype, 47 have displayed temperature sensitive or lethal defects (Supplementary Table 1). Therefore, as has been pointed out,[14,20,21] mutations that disrupt Interface I might also disrupt predicted interactions with other proteins in the RNA replication complex (reviewed in Ref. 19). Furthermore, although the mutational disruption of Interface I was found to reduce polymerase activity under conditions of low RNA concentration, presumably making RNA binding rate limiting,[11,12,18] no effect of such mutations was observed when assays were performed in template excess.[14] Given the

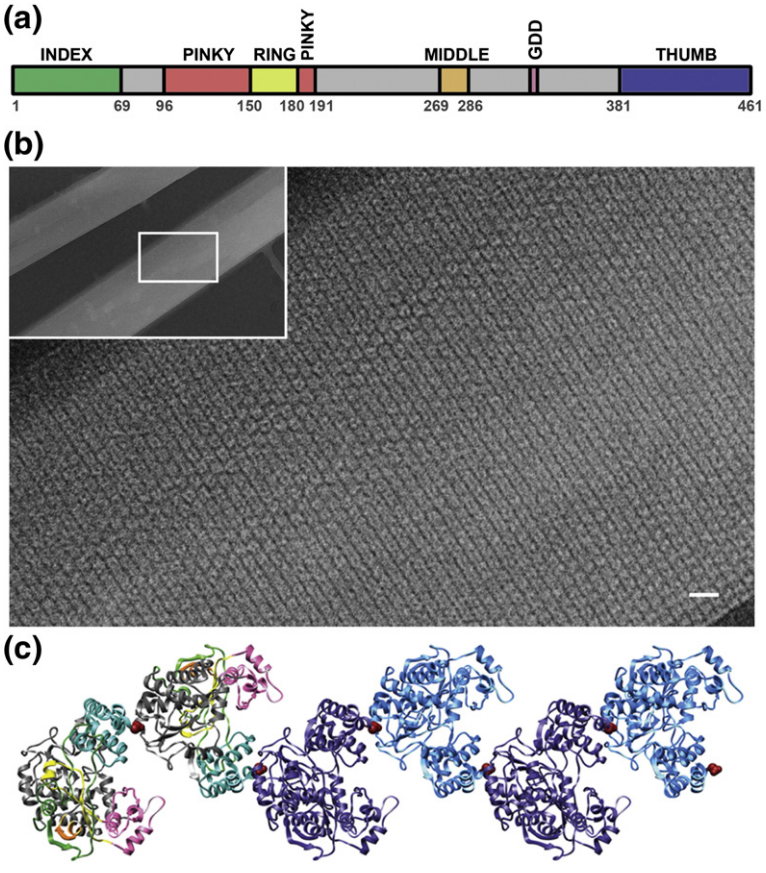

Fig. 1. Structure and oligomerization of poliovirus 3D polymerase. Structure and oligomerization of poliovirus 3D polymerase. (a) The domain structure of the 461-amino-acid poliovirus 3D polymerase is shown, with domains based on the 3-D structure of the full-length polymerase.[16] (b) Two-dimensional lattices[12] formed by purified wild-type polymerase, as shown by negative staining followed by electron microscopy. Magnification bar represents 10 nm on the enlarged image and 100 nm on the inset. (c) The polymerase–polymerase interactions shown along Interface I, a set of polymerase–polymerase interaction surfaces observed in a crystal form[17] and tested for functionality by site-directed mutagenesis.[11,12,14,15] Domains of the first two polymerases on the left are color coded as in (a). Amino acid Leu446, integral to Interface I, is shown as a red space-filling model. A single polymerase molecule is approximately 5 nm in length, width and height.