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TIME-RESOLVED CRYO-EM INVESTIGATION OF THE TRANSLATION INITIATION IN BACTERIA

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Translation initiation is a fundamental step in gene expression, ensuring precise start codon recognition and the formation of a ribosome competent for protein synthesis [1]. During this event, in bacteria, initiation factors 1 (IF1) and 3 (IF3) help to discriminate against incorrectly formed 30S initiation complex (30S IC) [2-4], and the initiation factor 2 (IF2), a GTPase, facilitates stable initiator fMet-tRNAfMet binding and promotes subunit joining [5]. However, the safeguarding role of IF1 in the ribosomal A site [3] and the regulatory function of IF2-mediated GTP hydrolysis [5,6] remain subjects of ongoing debate. Using both time-resolved cryo-EM and fast-kinetics, we describe an overall view of the translation initiation pathway involving IF1 and IF2. We demonstrate that IF1 associates with the 30S subunit and delays the formation of several inter-subunit bridges, thereby ensuring translational accuracy by allowing 50S docking only for authentic 30S initiation complexes. In addition, IF2 accelerates 30S subunit joining by anchoring its N-terminal region to the 30S subunit, thereby promoting the formation of an elongation-competent ribosome. Snapshots of these energy-driven events reveal that the major conformational changes in IF2 occur after GTP hydrolysis and inorganic phosphate (Pi) release, identifying this step as the key checkpoint that triggers IF2 rearrangement and dissociation. These findings clarify the quality control roles of IF1 and IF2 and establish the energy-driven remodelling event that sets the ribosome ready for protein production.

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L25

STRUCTURAL AND MECHANISTIC INSIGHTS INTO RIFT VALLEY FEVER VIRUS GENOME REPLICATION

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Rift Valley Fever Virus (RVFV) is an emerging zoonotic pathogen that threatens both human and animal health [1]. RVFV possesses a segmented, negative-sense, single-stranded RNA genome [1], which is replicated and transcribed by a multifunctional RNA-dependent RNA polymerase (RdRp), the L protein [2]. Functionally, the L protein employs a *de novo* internal replication strategy that is governed by viral RNA (vRNA) promoters in coordination with the L protein [2,3]. Consistent with this mechanism, previous studies have demonstrated the structural and functional conservation of the L protein across the order *Bunyavirales* [3]. Although the elongation stage of replication has been well characterized in related bunyaviruses [3], the molecular events governing the early steps of replication, particularly how vRNA promoters control replication initiation, remains poorly understood.

In this study, using an ensemble cryo-electron microscopy (cryo-EM), we captured multiple RVFV L protein replication intermediates at near-atomic resolution. These structural snapshots represent both early and late stages of genome replication, which elucidate the structural co-ordination between vRNA promoters and L protein. Together, these findings fill a fundamental gap in our understanding of vRNA promoter-mediated onset of genome replication. Given the conserved functions of the L protein across the order *Bunyavirales* [3], this work provides a broadly important framework for structure-based antiviral design targeting bunyaviral polymerases.

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STRUCTURES OF ASYMMETRIC PARTICLES OF TBEV PROVIDE INSIGHT INTO FLAVIVIRUS ASSEMBLY AND MATURATION

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Immature particles of flaviviruses are coated by a membrane decorated by spikes, each formed by three heterodimers of pre-membrane (prM) and envelope (E) proteins. Maturation requires cleavage of prM into pr and M fragments and rearrangement of the coat proteins into a smooth herringbone pattern of M-E heterodimers. Despite the global health impact of flaviviruses, their assembly and maturation are poorly understood. Here, we show that most tick-borne encephalitis virus (TBEV) particles are asymmetric and lack subsets of surface heterodimers. Transmembrane and peripheral membrane helices of prM and E induce membrane bending, which is necessary for TBEV budding into the ER membrane. Immature particles of TBEV contain incomplete spikes, providing evidence that their coats assemble directly from prM-E heterodimers. Exposure of TBEV particles to acidic pH in the Golgi complex promotes maturation. The spikes and herringbone patterns in TBEV maturation intermediates are arbitrarily oriented relative to each other rather than being aligned to one icosahedral symmetry. Furthermore, the shapes of the bare membrane areas of TBEV virions and immature particles are different. Therefore, the mature herringbone pattern assembles from a randomly oriented nucleation center by gradually adding M-E heterodimers to its edges as the

spikes disassemble and prMs are cleaved. The incompleteness of the protein coats explains how flaviviruses can be neutralized by antibodies binding to parts of E proteins inaccessible at the surface of the spiky and herringbone structures and opens possibilities for developing antivirals targeting the virus membrane.

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THE ASYMMETRY-TO-SYMMETRY TRANSITION IN COPY-OUT-PASTE-IN DNA TRANSPOSITION

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Copy-out-paste-in transposition is a widespread pathway used by bacterial insertion sequences to mobilize genetic elements, including antibiotic-resistance determinants. Unlike cut-and-paste or cointegrative mechanisms, copy-out-paste-in proceeds through a temporally separated reaction steps: an initial asymmetric single-strand transfer that generates a branched circular intermediate, subsequently resolved by host replication into a circular transposon, followed by a concerted, symmetric strand- transfer reaction that integrates the circle into a new genomic site. These steps impose mutually exclusive geometric constraints on the transpososome. Excision requires discrimination between the donor and recipient ends, as well as an asymmetric active-site configuration. In contrast, integration requires restored symmetry in order to coordinate simultaneous strand transfer. How a single autonomous transposase executes both functions without auxiliary regulators has remained a central mechanistic question. Using the ISCth4 transposase from the IS256 family as a model, we determined cryo-EM structures of two functional assemblies: an asymmetric circularization complex that enforces a single-strand transfer between transposon ends, and a symmetric integration complex that coordinates head-to-tail juxtaposed transposon ends with target DNA. Together, these findings demonstrate that the transposase assembles distinct transpososome architectures dictated by DNA substrate context explaining how a single enzyme coordinates sequential and structurally incompatible steps within the copy-out-paste-in transposition pathway.

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