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Authentic interdomain communication in an RNA helicase reconstituted by expressed protein ligation of two helicase domains

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Keywords expressed protein ligation, helicase, interdomain communication, RNA-dependent ATPase RNA helicases mediate structural rearrangements of RNA or RNAâ"protein complexes at the expense of ATP hydrolysis. Members of the DEAD box helicase family consist of two flexibly connected helicase domains. They share nine conserved sequence motifs that are involved in nucleotide binding and hydrolysis, RNA binding, and helicase activity. Most of these motifs line the cleft between the two helicase domains, and extensive communication between them is required for RNA unwinding. The two helicase domains of the Bacillus subtilis RNA helicase YxiN were produced separately as intein fusions, and a functional RNA helicase was generated by expressed protein ligation. The ligated helicase binds adenine nucleotides with very similar affinities to the wild-type protein. Importantly, its intrinsically low ATPase activity is stimulated by RNA, and the Michaelisâ"Menten parameters are similar to those of the wild-type. Finally, ligated YxiN unwinds a minimal RNA substrate to an extent comparable to that of the wild-type helicase, confirming authentic interdomain communication.

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