Three charged amino acid residues at the extreme C-terminus of the BFK20 gp41 helicase play a role in the ATPase activity of the protein

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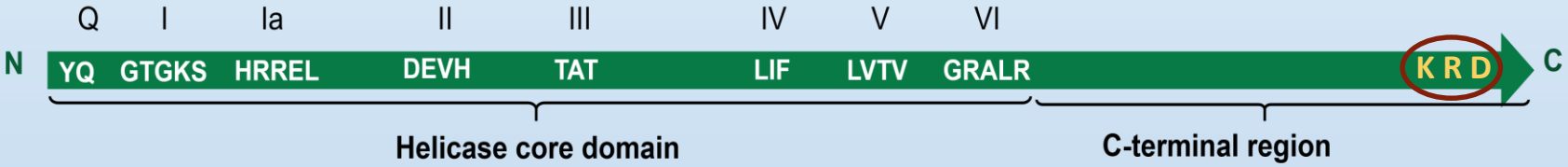
Bacteriophage BFK20 SF2 family helicase gp41



Phage helicase gp41 is composed of the helicase core domain with conserved motifs of SF2 helicases and an extended unspecified C-terminal region. Amino acids at the extreme C-terminus of the protein are important for the ATPase activity.

Bacteriophage BFK20

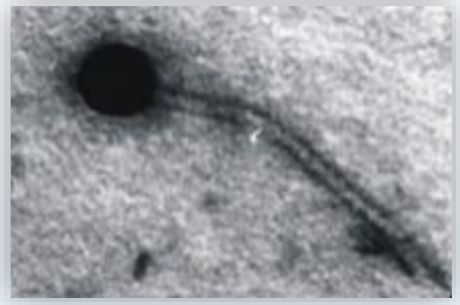


Fig. 1. Bacteriophage BFK20.

BFK20: Lytic phage of *Brevibacterium flavum* CCM 251 (Fig. 1)

Genome: 42,972 bp (updated data) linear dsDNA with 3' cohesive ends, GC content: 56.2%, 54 putative ORFs, (Bukovska et al., 2006; EMBL Acc.No. AJ278322, Fig. 2)

Replication proteins: Cassette of replication proteins: gp29 – gp46 (Fig. 3)

BFK20 genome

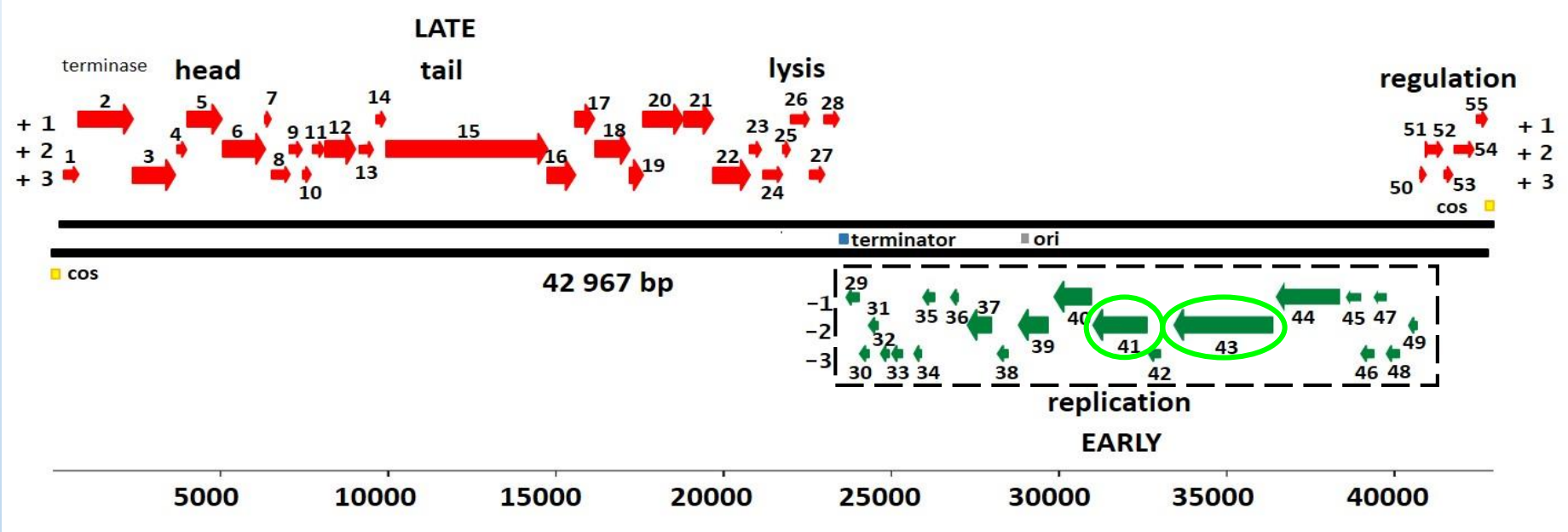


Fig. 2. Genomic organization of bacteriophage BFK20. Predicted genes are represented as colored arrows. Early genes encoding putative replication proteins are indicated in the rectangle. Helicase genes are marked by ellipses.

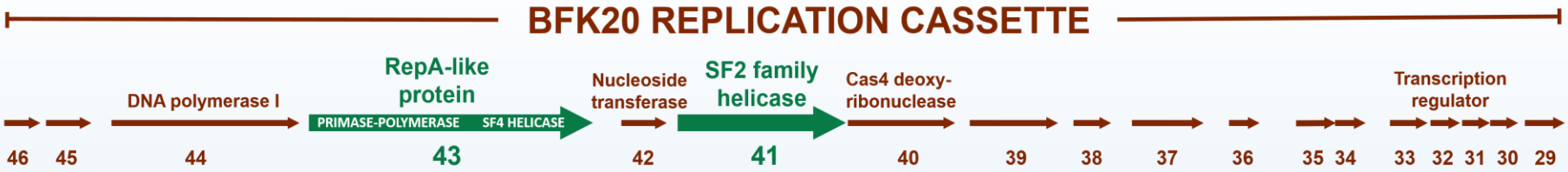


Fig. 3. BFK20 replication proteins.

BFK20 helicases

gp43: multifunctional protein with a primase-polymerase (prim-pol) domain and a helicase domain, contains conserved motifs of SF4 helicases, probably operates as a replicative helicase in BFK20 DNA replication (Halgasova et al., 2015, Halgasova et al., 2021)

gp41: DNA helicase from SF2 family, contains a helicase core composed of two RecA-like domains (Fig. 4) with SF2 helicase conserved motifs and an extended C-terminal region without defined specificity (Fig. 5).

SF2 helicase gp41

NCBI Blast Search against conserved domain database (Marchler-Bauer et al., 2017)

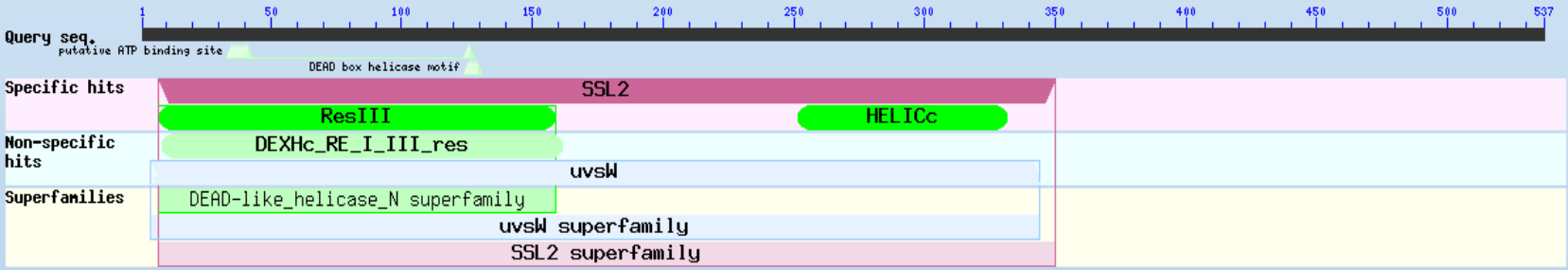


Fig. 4. Homology search on the gp41 sequence.

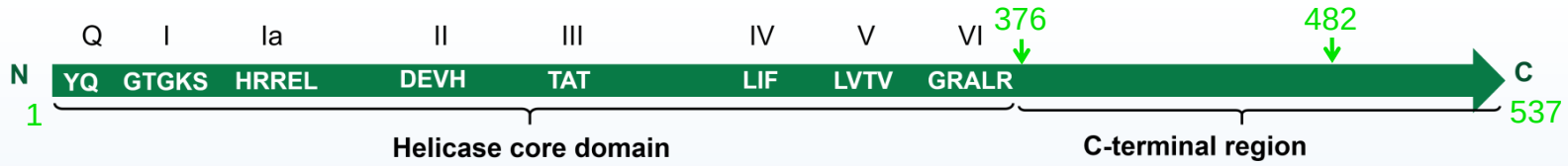


Fig. 5. Gp41 conserved motifs and extended C-terminal region.

- Wild-type like protein gp41HN has DNA-dependent ATPase, helicase and DNA-binding activities.
- Deletion of the extended C-terminal region (aa 376-537) caused a decrease in all activities (Halgasova et al., 2018). A deletion mutant without last 56 C-terminal amino acids (deleted aa 482-537) also showed only low ATPase activity both in the presence and absence of DNA (Halgasova, unpublished results). We decided to verify a function of amino acids located at the extreme C-terminus of gp41 using site directed mutagenesis.
- In this study we performed site directed mutagenesis of five selected amino acid residues in the largest helix at the extreme C-terminus of gp41: K516, R518, D520, D521 and E522 (Fig. 6).

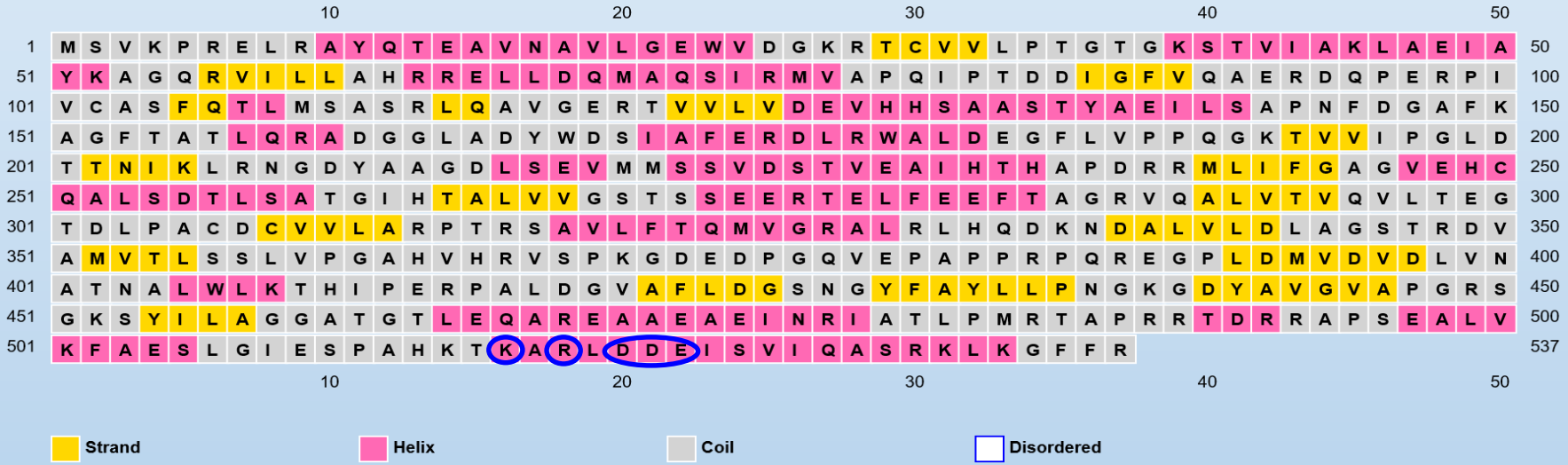


Fig. 6. PSIPRED analysis of gp41, mutated amino acids are marked by ellipses.

Strategy

- Cloning of mutated *orf41* with changed codons for selected amino acids into the pET28a+ vector and transformation of recombinant plasmids into expression host E. coli BL21(DE3).
- Expression of point mutants gp41K516A, gp41R518A, gp41D520A, gp41D521A and gp41E522A, which have selected amino acids replaced by an alanine. The recombinant proteins contain N-terminal His-Tag sequences located similarly to wild type-like protein gp41HN.
- Isolation of gp41 mutants by immobilized cobalt affinity chromatography and testing of their ATPase activity in the presence and absence of DNA using modified plate version of the colorimetric method of Lanzetta et al. (1979). Comparison of the results to those measured for wild type-like protein gp41HN.

Results

Wild-type like gp41HN and gp41 mutants after isolation were verified by SDS-PAGE (Fig. 7).

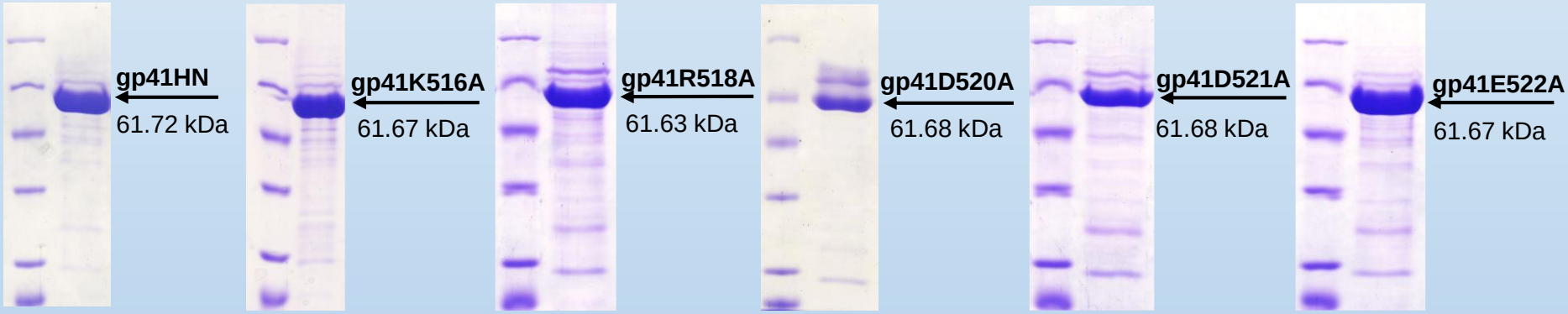
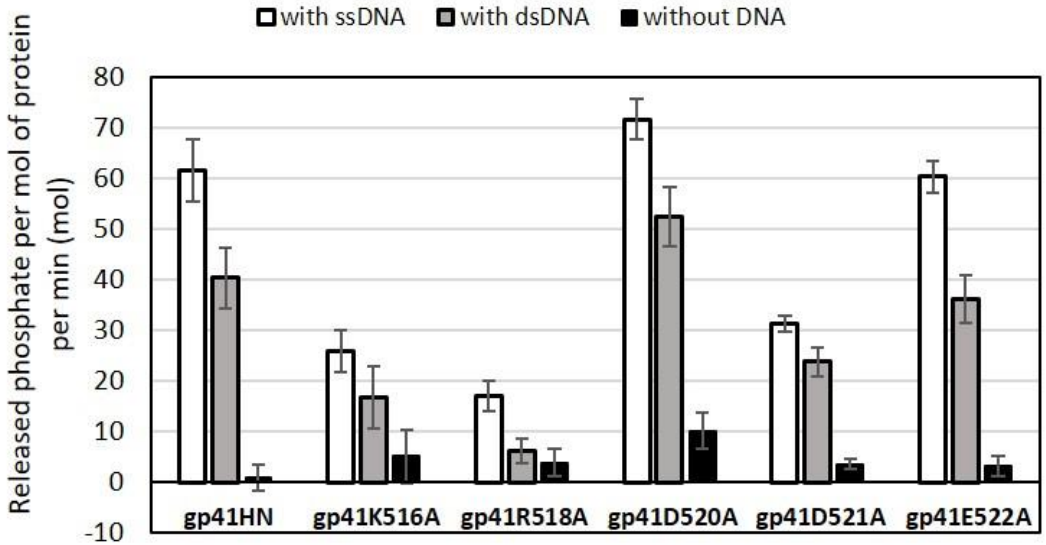


Fig. 7. Wild-type like gp41HN and gp41 point mutants with corresponding molecular weights.

ATPase activities of gp41 point mutants

- ATPase activity rates of individual mutant proteins in comparison with gp41HN are shown in Fig. 8.
- Mutant proteins gp41K516A, gp41R518A and gp41D521A show significantly lower ATPase activities in the presence of both single- and double stranded DNA than gp41HN.
- Mutants gp41D520A and gp41E522A show comparable levels of ATPase activities as gp41HN.

Fig. 8. ATP hydrolysis rate

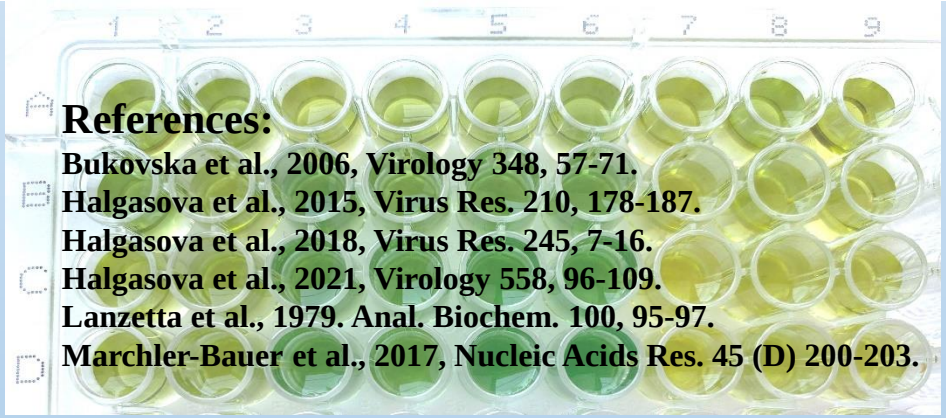


gp41HN - wild type-like protein

gp41K516A, gp41R518A, gp41D520A, gp41D521A, gp41E522A - point mutants with selected amino acids replaced by an alanine

Summary and conclusions

- The results indicate that three charged amino acids at the extreme C-terminus of the gp41 helicase are important for its ATPase activity, and probably they are necessary for the function of this phage helicase.
- The work emphasizes the role of accessory domains in helicases function.



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