

Nucleotide sequence of the replication region of the *Nostoc* PCC 7524 plasmid pDU1

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The cyanobacteria are an ancient and diverse group of prokaryotes capable of oxygenic photosynthesis and plasmids have been found in both unicellular and filamentous types. Currently, all plasmids are cryptic and only one plasmid, pUH24, from a unicellular non-nitrogen-fixing cyanobacterium, has been sequenced (1). Comparative searches (TFASTA, FASTA (2)) of pUH24 sequences did not show similarity to sequences present in the GenBank data bank (release no. 66).

Another plasmid, pDU1, from the filamentous, nitrogen-fixing cyanobacterium *Nostoc* sp. strain PCC 7524 has been used extensively in a variety of shuttle vectors (2). These vectors replicate in a limited number of filamentous cyanobacteria. The minimum region necessary for replication of this 6.28 kilobasepair (kbp) plasmid has been determined to reside on a 1.75 kbp fragment (4). Interestingly, the same study showed that a 1.3 kbp fragment was sufficient for replication in *Anabaena* sp. M-131.

The nucleotide sequence of the 1.75 kbp replication region of pDU1 is presented here. The sequenced region was isolated from the shuttle vector pRL1 (3). A large open reading frame (ORF1) is present from nucleotide position 1260 to 141. This encodes a putative protein of 373 amino acids with a molecular weight of 42,477 Daltons. A putative rho-independent transcriptional termination signal is downstream of ORF1 at nucleotide positions 33 to 60. A region of extensive dyad symmetry is located upstream of ORF1 at nucleotide positions 1512 to 1546. This region of dyad symmetry is seemingly not necessary for replication in *Anabaena* sp. M-131. The significance of this region of dyad symmetry is unclear as its presence is not always correlated with the ability of vector constructs to replicate in various cyanobacteria (4). The presence of ORF1 is correlated with the ability of shuttle vectors to replicate in cyanobacteria and thus may encode a protein involved in the plasmid's replication.

Comparative searches (FASTA, TFASTA) of the pDU1 replication region revealed no sequence similarities in the GenBank or with pUH24 sequences.

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1 CGCATGATCTGCATACGATCTCTATTGCCAAAAGCCGGACCCCTATAGGCTCTCGGTCA
61 TGCTGCACTAGTTCGTGTCGATCACTATACTGGTTGCCGAGCATTTCACGGTAAAAAAA
121 AATTCTTAAAAATGTCCTTCATATCTCGCCAGAGTGGCAACCTATTACAAAACGGTTGCC
181 TACCCGACCCGCTCGATTTCGTGAACTGGCACTGTAACAGTTGAAATGGTACTCCG
241 CGCTGCTGACATCGTTGTTGGTGAATGTTGCGCGGTAGATGTTGCACCCGATTCAT
301 GAACACCTTGTACCCACTTTGAATAATCGACCGTCAAATTCAGTCGCGTCAAATTTGTA
361 AGTGTGGGCTGTCTTTTTTGGTCCAGGGCAATGCCATCAGAAAACACAACCCGCTC
421 ACCCATAACTTGATAACCGATATCAGTTTGGTTCCAGTAAAGCCCAAAATTCAGAGCC
481 GTCATTATTCGAGCGTCCGGAGTTGATGTACTCAATTTGGCTGGCAAAGTTGAGC
541 GCGATTATGCCCAGCTGCTTTTGATGCTGCTGCACTGTCGCGCTTGTGAATACCCAACTC
601 ACAGCTGACAGCTTTTGGAGATGTAACATAGTGGATGAAATTTTGGAGACCAATATCCG
661 CGACGAACTAATGTGAAGTACACAAGTACTTCCCCCTCTGGCGGATTTAAGAGAGGATTG
721 CCTTGTCTCTTCACTAGCTCGTTCCGGTGTGGCGCTCCAAAAGTTTTCTGACTCTGG
781 TTTAAGTTGCTGTTGGCCGATAGCGGCTCTTTTGTGAAAGCTTTGTTGACTATGCC
841 AGTGGTCACTGAGCGTAAATCGCTTAACACTTGGACTAAAGGCACTACTGCAACATCACC
901 CCATCTTTTAAATTTAGGTTGTAACAACTGAAACATACCCGCAAGTAGACGGTTAT
961 CATTCCTGCTTTAAATTTTGTAGCGGGGAATGCTCCTAATTTTTTCCATCTGTAACCA
1021 ACGGTAACAGACTTATCACTACAATCTAAGAACGTCGTACTACAGGCAATGGCAATGT
1081 TAAATGACCAGACCCATCCTTATCAAGCGCTCGACACAAATACCACAACCGCGCACAAAG
1141 TTCTGACCAATGCGAGTGTGACCCGTGACCGTGAAGTCCAAAGAAATTTTCAGTTTG
1201 TAGTCCCTTGTAAAGCAGGTTAGTGATACATTTGATTTAAGCTTTCTGGGTGATCAT
1261 TTGGAAATGCTCTCAGTCCAGTACCTATGAAATGTTATTGCTTAACTGAAGTAAATAA
1321 AACTTGTAACTACACCAATTAATGATAAATCAAAGCAGCTTTTTCTGTTGGTGTGTT
1381 TGGTGTGTAACAATCTGTGTATGTTGTTTATTTAGCTTCGGTTAAGTAGCATAACA
1441 ACCCCCAAGCACTGAACTTTTTTAATAGGTAATTTAAACCTTCCATACGGCAAAATTT
1501 TCAATCAATTTGACGCCAAAGTGTGATGATCAACGTTTGAATTTTGTATTATTACT
1561 AAATACTGAAATTCGCGGTGACGCTTTTACAGATGAAATTCACGGCAAAATGTTTTTCT
1621 GCTAACTTTGCTATGTAACAAAGAACTGGCACTCGGTTATTACTAAATAAATCGGTA
1681 AAAATAACCATTAGAACCAAAAAGCAAAAACAGTACACCCCTGCGAGTTTCAAGC
1741 TTTTGCT

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Figure 1. Sequence of the plasmid pDU1 origin of replication region.

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