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Hypothesis

Autogenous translation regulation by *Escherichia coli* ATPase SecA may be mediated by an intrinsic RNA helicase activity of this protein

Eugene V. Koonin and Alexander E. Gorbalenya

Institute of Microbiology, the Academy of Sciences, Prospekt 60let Oktyabrya, 117811, Moscow and *Institute of Poliomyelitis and Viral Encephalitides, the Academy of Medical Sciences, 142782, Moscow Region, Russia*

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The seven conserved motifs typical of the helicase superfamily II have been identified in the sequences of *Escherichia coli* protein SecA, an ATPase mediating protein translocation across the inner membrane of the bacterium, and its *Bacillus subtilis* homolog Div. It is hypothesized that SecA and Div possess an RNA helicase activity and may couple ATP hydrolysis both to membrane translocation of proteins, and to hairpin unwinding in their own mRNAs, leading to the known autogenous regulation of translation.

Amino acid sequence comparison; Conserved sequence motif; Helicase; Translation regulation; Protein transport

SecA protein is an ATPase involved in the translocation of large proteins across the inner membrane of *E. coli*. SecA has been found in peripheral association with the membrane, and protein translocation mediated by SecA is thought to be coupled to ATP hydrolysis [1–4]. Similar properties have been reported for Div protein, the *Bacillus subtilis* homolog of SecA (ref. [5] and references therein). In addition, SecA has been shown to regulate autogenously the translation of its own mRNA, possibly by controlling a hairpin formation in the 5′ untranslated region [6,7]. Recently, it has been directly demonstrated that SecA binds to a sequence upstream of its own cistron in the respective polycistronic mRNA [8]. Thus SecA appears to be a bi-functional protein exerting two very different activities. In this paper we demonstrate that SecA and Div contain amino acid sequence elements characteristic of helicases and speculate that the autogenous translation regulation may be mediated by their intrinsic RNA helicase activity, and that a single ATP-binding domain may participate in both processes directed by these proteins.

In the course of systematic screening of the SWISSPROT data bank (Release 18) for the conserved motifs typical of the so-called superfamily II of DNA and RNA helicases [9], all seven of these motifs have been unexpectedly found in the SecA sequence (Fig. 1). The bank was searched sequentially for motif I ('A'), which is an identifier of a broader class of NTPases [10,11], then for motiv VI which is best conserved in this type of helicases, and then for the other, more degenerate motifs. For more precise delineation of the motifs, the program MOTIF generating a weighted frequency profile for a set of aligned sequence segments, searching for the best match in each database entry and assessing the statistical significance of the observed similarities, was used [12]. Inspection of the alignment of the amino acid sequences of SecA and Div revealed good conservation of the putative helicase motifs (Fig. 1). To further assess the significance of the similarity between SecA and Div on the one hand and the previously identified helicases on the other, their sequences were aligned using multiple alignment program OPTAL [13]. Although generation of a complete alignment was hampered by the presence of a large insert between motifs II and III in SecA and Div, highly significant alignment scores of 7.4 standard deviations (S.D.) and 11.2 S.D., respectively, were obtained upon comparison of the N- and C-terminal portions of the putative helicase domains of these two proteins with the aligned sequences of four (putative) *E. coli* helicases (DbpA, SrmB, RecQ and RecG). Recently the ATP-binding site of SecA has been localized to the N-terminal 217 amino acid residues of this protein [14]. Characteristically, the conserved motif I ('A') directly implicated in ATP(GTP) binding in numerous NTP-utilizing enzymes (reviewed in ref. [11]) lies within this segment (Fig. 1).

Abbreviations: eIF-4A, eukaryotic translation initiation factor 4A.

Correspondence address: E.V. Koonin, *present address:* National Center for Biotechnology Information, National Library of Medicine, Bldg. 38A, NIH 8600 Rockville Pike, Bethesda, MD 20894, USA. Fax: (1) (301) 480 9241.

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                    I  ("A")          Ia              II ("B")
                   !     G GKt       !! po   !       !!!!DE h
                             s                              d
UvrB(M)  (44-551) VVLMGATGSGKSA-12-TLVMVQNKTLA-261-FLLVVDESHVT- 46-
UvrB(E)  (35-544) QTLLGVTGSGKTF-12-TMVLAPNKTLA-269-GLLVVDESHVT- 46-
SrmB     (44-343) VLGSGPTQAGKTA-22-ILILTPTRELA- 61-ETLILDEADRM- 22-
DbpA     (18-311) VRVQAKTGSGKTA-18-ALVLCPTRELA- 62-NTLVMDEADRM- 22-
RecQ     (45-328) CLVVMPTGGGKSL-12-TVVVSPLISLM- 62-VLLAVDEAHCI- 26-
RecG    (292-587) RLVQGDVGSGKTL-15-VALMAPTELLA- 61-ALVIIDEQHRF- 22
                   *    ** ***       **     **       ****
SecA     (98-577) CIAEMRTGEGKTL-16-VHVVTVNDYLA- 67-HYALVDEVDSI-173-
Div      (96-530) NIAEMKTGEGKTL-16-VHVVTVNEYLA- 67-HFAVIDEVDSI-155-


            III          IV
         ! !tat        !f  s o  ^
            sqt         y  t
UvrB(M)  VYLSATP- 51-VLVTTLTKRMA- 37-
UvrB(E)  IYVSATP- 53-VLVTTLTKRMA- 37-
SrmB     LLFSATL- 59-SIVFVRKRERV- 37-
DbpA     LLFSATW- 56-CVVFCNTKKDC- 37-
RecQ     MALTATA- 54-GIIYCNSRAKV- 37-
RecG     LIMTATP- 59-CTLIEESELLE- 43
            ****        *    *
SecA     AGMTGTA- 61-TISIEKSELVS- 32-
Div      AGMTGTA- 61-TVAVETSELIS- 32-


                 V                     VI
           !!! to!   q o!           o  q  GR  R
                 s    s                n
UvrB(M)  FDVIVGINLLREGLDLPEVSLVA-15-SLIQTIGRAAR
UvrB(E)  FDVLVGINLLREGLDMPEVSLVA-15-SLIQTIGRAAR
SrmB     VNVLVATDVAARGIDIPDVSHVF-10-TYLHRIGRTAR
DbpA     ARVLVATDVAARGLDIKSLELVV-10-VHVHRIGRTAR
RecQ     LQIVVATVAFGMGINKPNVRFVV-10-SYYQETGRAGR
RecG     LHLLVATTVIEVGVDVPNASLMI-11-QLHQLRGRVGR
           * *****   * **              *   ** **
SecA     AAVTIATNMAGRGTDIVLGGSWQ-46-IDNQLRGRSGR
Div      GAVTIATNMAGRGTDIKLGEG---16-IDNQLRGRSGR
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Fig. 1. Conserved motifs in the eubacterial helicases of superfamily II. The motifs are designated after ref. [9]. The consensus pattern of conserved amino acid residues derived for the entire set of superfamily II helicases (after ref. [9], with minor modifications); upper case, invariant residues; lower case, partially conserved residues. Asterisks: positions where identical or similar residues are found both in SecA and Div, and in at least four of the six shown sequences of eubacterial (putative) helicases. The grouping of amino acid residues by physico-chemical similarity was as follows: 1, G,A; 2, S,T; 3, D,E,N,Q; 4, K,R; 5, I,L,V,M; 6, F,Y,W. 'A' and 'B' are for the two conserved motifs of the purine NTP-binding pattern [10,11]. UvrB(E), UvrB(M), helicases involved in the repair of ultraviolet-damaged DNA in *E. coli* and *Micrococcus luteus*, respectively; DbpA, DEAD box protein A, an *E. coli* putative helicase with unknown function; SrmB, *E. coli* RNA-dependent ATPase involved in ribosome biogenesis; RecQ, *E. coli* helicase involved in DNA repair; RecG, putative *E. coli* helicase involved in recombination; Div, *Bacillus subtilis* protein required for cell division, sporulation and secretion of extracellular enzymes. The sequences were from Swissprot data bank, except for RecG [19] and Div [5].

These findings strongly suggest that SecA and Div may possess an RNA and/or DNA helicase activity. It is tempting to speculate that an RNA helicase activity might be involved in local unwinding of RNA required for autogenous regulation of translation. The RNA helicase activity of eIF-4A, one of the best studied members of superfamily II, mediates unwinding the secondary structure in 5′-proximal untranslated regions of mRNAs during translation initiation [15]. Moreover, the yeast homolog of eIF-4A, Tif1/Tif2 protein, has been implicated in the regulation of translation of specific mRNAs [16]. However, SecA is distinct from these RNA helicases in that it has been shown to repress, not to enhance, its mRNA translation; moreover, this repression is abolished when the ATPase activity of SecA is eliminated either genetically, or biochemically [6,7,17]. It can be speculated that a specific hairpin is required for efficient initiation of SecA cistron translation, and its unwinding by the SecA helicase leads to a decrease in the translation level.

These findings raise the exciting possibility of involvement of a single protein, SecA(Div), in two superficially unrelated NTP-consuming processes, i.e. duplex unwinding in RNA and protein membrane translocation. Moreover, coupling of each of these processes to ATP hydrolysis may be secured by one and the same ATP-binding domain. This hypothesis is compatible with the failure to uncouple the activities of SecA in membrane translocation and in translation regulation in a recent extensive mutagenesis study [18].

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