

RNA Helicase activity of SecA protein of *Escherichia coli*

Sukyung Park* and Hyoungman Kim

Department of Biological Sciences, KAIST

SecA protein of *E. coli* is essential for the translocation of various precursor proteins across the plasma membrane. Along with it, SecA protein interacts with precursor proteins, SecY/E, SecB and is an ATPase which has multiple ATP binding sites. There is little known about the regulation mechanism of the protein translocation machinery. But SecA gene expression seems to be autogeneously regulated at the translational level in response to the degree of protein export in *E. coli* cell. It was reported that SecA protein specifically binds to the SRE (Secretion Responsive Element) region of the geneX-secA mRNA. And recently, it was found that SecA protein has 7 conserved sequences of the Helicase superfamily II. If we find SecA protein have RNA Helicase activity, it is plausible that there may be certain relationships between winding/unwinding of hairpin structure in SRE mRNA and the regulation of secA gene expression. Therefore, we decided to assess whether SecA protein really is an RNA Helicase. First, we made RNA duplexes that involve 19 base pairs and are radioactively labeled with ³²P. And their 5' and 3' ends have single-stranded regions of 3 bases relatively. Using non-denaturing polyacrylamide gel electrophoresis and autoradiography, we found that SecA protein can unwind the RNA duplex substrates into the single strands at pH 6.5 in the presence of dithiotreitol (DTT), ATP, and MnCl₂. This result led us to try to test whether SecA protein can unwind the intra-molecular hairpin structures formed in SRE mRNA. Now, we are doing experiments for this.