STRUCTURAL BIOLOGY APPROACH TO STUDY THE SPORULATION PROCESS

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Bacillus subtilis is a model organism for the study of one of the simplest cell differentiation process, called sporulation. A rich amount of genetic, biochemical and molecular biology data have been obtained during the study of this process. Recently, this study has advanced to the solving the tertiary structure of key protein regulators. This contribution focuses on the progress in protein crystallography oriented toward the understanding of sporulation mechanisms, that have been achieved in the last couple of years.

The phosphorelay is the main regulatory network in the initation of sporulation. Recently, the tertiary structures some of the components of this network were solved. The structures of response regulators Spo0F [5], Spo0A [2,3,4] and phosphotransferase Spo0B [7], together with biochemical and mutational data provide an important framework for further understanding of their biological function at the molecular level.

Structural data were also obtained from some proteins involved in activation of the first cell-type specific factor - ^{F} (structures of anti-anti- factor SpoIIAA and anti-factor SpoIIAB in the complex with ^{F}) [6,1].

The structures of only a small number from more than 100 sporulation specific proteins are known due to problems associated with the crystallization of these proteins. Structures for many of the interesting candidates remain a challenge. Among them are the membrane bound proteins and proteins with highly flexible domains.

Especially interesting for understanding of the mechanism of the transient gene expression asymmetry during sporulation would be the detailed study of sporulation septa formation by solving the crystal structure of key proteins involved in this cell division and chromosome translocation processes such as phosphatase SpoIIE, DNA translocase SpoIIIE or division protein DivIVA.

Work in authors laboratories is supported by grant 2/1004/22 from the Slovak Academy of Sciences and Wellcome Trust Project and Collaborative Research Initiative Grants (056247/Z/98/Z and 066732/Z/01/Z, respectively).

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STRUCTURAL BASIS OF PROTEIN METASTABILITY

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All information for the three dimensional structure of proteins and their functionality is encrypted within their amino acid sequences. The natural amino acid sequences of proteins have been perfected by evolution not only for their functional structure but also for a rapid and highly directional acquisition of their folded, fully functional state. An unambiguous distinction between these two possibilities is important for a clear-cut interpretation of consequences of amino acid replacements in protein engineering experiments.

The double-headed Bowman Birk serine protease inhibitor (BBI) built up from two triple stranded b-hairpin domains directed against trypsin and chymotrypsin was selected as a model protein. The double-headed arrangement of two independent subdomains facilitates the detection of long-range irregularities transmitted from the trypsin- into the chymotrypsin-inhibitory region used as a reference.

The exposed hydrophobic patches on the protein surface and a polar protein interior appear as structural peculiarities more reminiscent of the kind of structural features that have been postulated to occur in partially folded proteins rather than their folded state.

The autonomous refolding competence of the parent protein was abolished as s result of amino acid replacements, resulting in heterogeneous populations of conformers greatly differing in their activity. In contrast to the autonomous refolding competence of the parent protein, the variants require the presence of trypsin-Sepharose as a template with complementary structure in order to reach their fully active state. The fully active state of the variants attained by means of this method returns to the initial mixture of conformers upon subsequent incubation in the refolding buffer in a slow first order reaction. Therefore, the fully active state of the variants may be regarded as local energetic minima surrounded by high barriers of activa-