m03.o05

Structural Genomics on Human Kinases at SGC Oxford

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The Structural Genomics Consortium (SGC) is a not-for-profit organisation encompassing three sites: Universities of Oxford, Toronto and the Karolinska Institute, Stockholm. The SGC's main goals are the structure determination of proteins of medical interest and dissemination of structural data to the public domain without restrictions. The UK site focuses on three main target areas: human dehydrogenases-reductases, membrane receptor and phosphorylation dependent signalling. Last year the SGC contributed about one fifth of all novel human structures deposited in the PDB with a current submission rate of 6-8 structures per month from the Oxford site. Beyond the primary goal of increasing the number of available human protein structures, significant effort is being made to establish an infrastructure that supports the understanding of their mechanism and role in human diseases. This involves further steps from identification of potential ligands and inhibitors to determination of ligand complex structures.
The structure determination pipeline of human proteins will be outlined using the human kinases solved at the Oxford site as an example. Special aspects of this protein family will be discussed with strong emphasis on structural and crystallographic questions. Challenges in crystallisation, such as stability and crystallisability as a function of ligand binding and phosphorylation, and subsequent structure solution and refinement will be illustrated by specific examples.

m04.o01

mRNA transcription and its inhibition by RNA

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We have determined an atomic crystallographic model of the complete 12-subunit RNA polymerase II in elongation mode, with DNA and RNA in the active center cleft. From these studies has emerged a detailed three-dimensional view of mRNA elongation. We have extended this structural analysis to an elongation complex bound by the transcript cleavage factor TFIIS, which is required for polymerase escape from DNA arrest sites. A detailed model of this 550 kDa complex reveals a single tunable active site for both RNA polymerization and cleavage, and provides insights into the dynamics of the elongation complex. An additional structure of a polymerase carboxy-terminal domain (CTD) phosphopeptide bound by the 3'-RNA processing factor Pcf11 provides insights into the coupling of transcription elongation to mRNA processing. The structure of the CTD phosphatase Scp1 trapped in an intermediary enzymatic state explains polymerase dephosphorylation for recycling of the polymerase during or after transcription termination. The first structures of subunits of the multiprotein Mediator mark the beginning of a mechanistic analysis of transcriptional regulation in eukaryotes. Finally, a new structure of Pol II in complex with an inhibitory RNA provides first insights into the regulation of transcription by non-coding RNA molecules.