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Machines on Genes: Enzymes that Make, Break and Move DNA and RNA

A Biochemical Society Focused Meeting held at Robinson College, Cambridge, U.K., 12–14 August 2009. Organized and Edited by Richard Bowater (University of East Anglia, U.K.), Ben Luisi (University of Cambridge, U.K.) and Marshall Stark (University of Glasgow, U.K.).

Machines on Genes: Enzymes that Make, Break and Move DNA and RNA

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Abstract

As the vital information repositories of the cell, the nucleic acids DNA and RNA pose many challenges as enzyme substrates. To produce, maintain and repair DNA and RNA, and to extract the genetic information that they encode, a battery of remarkable enzymes has evolved, which includes translocases, polymerases/replicases, helicases, nucleases, topoisomerases, transposases, recombinases, repair enzymes and ribosomes. An understanding of how these enzymes function is essential if we are to have a clear view of the molecular biology of the cell and aspire to manipulate genomes and gene expression to our advantage. To bring together scientists working in this fast-developing field, the Biochemical Society held a Focused Meeting, 'Machines on Genes: Enzymes that Make, Break and Move DNA and RNA', at Robinson College, University of Cambridge, U.K., in August 2009. The present article summarizes the research presented at this meeting and the reviews associated with the talks which are published in this issue of *Biochemical Society Transactions*.

Introduction

The enzymes that manipulate nucleic acids perform diverse tasks that are fundamental to life, ranging from mechanical manipulations and topological trickery to molecular recognition and complex chemical transformations. Their defining substrates, DNA and RNA, are the information-carrying molecules of the cell, whose sequences of four base 'letters' encode not only the primary structures of proteins, but also a vast amount of other information such as transcription and translation start and end points, binding sites for transcriptional regulators, determinants of chromatin structure and sequences for self-recognition through regulatory RNA. Preservation of the sequence information in DNA molecules, which may be present in only one or two copies per cell, is vital if the cellular functions are to be maintained and information is to be passed to daughter cells in a stable and meaningful way. And, although RNA molecules are generally in rapid flux of synthesis and turnover and are thus more 'disposable' than DNA, their functions will be compromised if they are damaged and information is corrupted or lost prematurely. Yet, to a first approximation, nucleic acids are uniform polymers, and the structural distinctions conferred by the sequences of the bases are rather subtle. The enzymes that act on nucleic acids are therefore faced with major challenges to read, interpret and maintain the sequence information correctly. Specificity and regulation, rather than ultimate catalytic efficiency, are the hallmarks of these enzyme systems. Enzyme catalytic activity is very commonly dependent on prior assembly of large multiprotein complexes on the nucleic acid, to maximize specificity and allow integration of a number of activating or inhibitory signals. In some cases, such as replication and transcription initiation, licensing of enzyme activity in this

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way leads to ‘processive’ behaviour; that is, catalysis of many rounds of reaction. In other cases, such as in DNA recombination or RNA splicing, a large complex may be assembled to license just a single catalytic event.

Another feature of nucleic acid molecules, their extreme length, has profound consequences for their biochemistry. DNA molecules are by far the longest structures in cells, reaching several centimetres in human chromosomes. Problems due to entanglement are therefore inevitable, and are solved by a specialized class of enzymes, topoisomerases. The immensely long DNA molecules also must be stored accessibly in the cell, necessitating further sets of specialized enzymes and structural proteins. Issues of entanglement and packaging are less severe for most RNA molecules, but RNA, of course, is a key player in perhaps the most challenging process of all, the translation of the nucleic acid sequence into protein in the gene machine *par excellence*, the ribosome.

On 12 August 2009, about 110 researchers from all over the world came together in the serene modern-mediaeval surroundings of Robinson College, University of Cambridge, U.K., to find out about and discuss the latest advances in structural and mechanistic studies of the enzymes that act on nucleic acids. This Biochemical Society Focused Meeting and was a synthesis of proposals put forward by the Society’s ‘Genes’ and ‘Structure’ Theme Panels. The speaker presentations and the posters gave delegates a superb overview of the field, covering a wide spectrum of cutting-edge structural and mechanistic research. As well as the 14 talks from invited international scientists, nine delegates were selected to give oral presentations. About 50 delegates displayed posters describing their research and had the opportunity to discuss their work with all of the other participants at the sessions dedicated to this purpose. A prize for the best poster was sponsored by the journal *Biotechnology and Applied Biochemistry*, with the competition being judged by a panel of eminent senior speakers. The poster prize was awarded to Barbara Treutlein (Ludwig-Maximilians-University, Munich) for a beautifully presented poster on her studies that used single-molecule FRET (fluorescence resonance energy transfer) to analyse the movements of nucleosomes during chromatin remodelling.

The talks

The ribosome is perhaps the most sophisticated of all molecular machines. We were privileged to have two talks on ribosome structure and mechanism from world leaders in the field. Venki Ramakrishnan (MRC-LMB Cambridge) gave a *tour de force* after-dinner presentation on the first day, detailing his most recent crystallographic studies revealing allosteric mechanisms in the ribosome and their impact on translational fidelity. Ada Yonath (Weizmann Institute) described her crystallographic results with emphasis on the insights that they have provided on the mechanism and evolution of the ribosome’s peptidyl transfer activity [1].

The ribosome is, of course, largely constructed from RNA, and its substrates are mRNA and tRNA molecules. Three talks described advances in our understanding of the RNA polymerase enzymes that make RNA. Laura Lane (University of Cambridge) presented her innovative MS-based research on the subunit architecture of eukaryotic RNA polymerases I and III, and Robert Weinzierl (Imperial College London) described novel methods for high-throughput mutational analysis, exemplified by his studies on the bridge–helix domain of archaeal RNA polymerase [2]. Nynke Dekker (Delft University) showed how magnetic bead single-molecule methods could provide insights into the mechanism of RNA polymerase, as well as the structure of supercoiled DNA.

DNA replication, transcription and other nucleic acid transactions require helicase enzymes whose function is to separate the two strands of double helices. We had two talks on the mechanisms and structures of helicases, from Dale Wigley (Cancer Research UK) who discussed the differences between helicases that progress along a DNA strand in either the $5'\rightarrow3'$ or the $3'\rightarrow5'$ direction, and Opher Gileadi (Oxford University) who presented crystal structures of the important human helicase RECQ1. Jane Grasby (University of Sheffield) described another class of enzymes that have vital roles in many DNA transactions, the flap endonucleases, and how her structural studies have provided information on the roles of the active-site metal ions and the path of the DNA strands through the enzyme complex [3].

Several talks were on subjects relating to DNA recombination. David Lilley (Dundee University) described how his recent structural studies on Holliday junction-resolving enzymes provide new insights on their mechanism and how their structural selectivity is achieved [4]. Phoebe Rice (Chicago University) showed how the activity of the DNA site-specific recombinase Sin is regulated by its dependence on the correct construction of an elaborate protein–DNA synaptic complex [5]. Femi Olorunniji (Glasgow University) presented his studies on the mechanism of DNA strand cleavage and rejoining by the serine recombinases, the group of enzymes that includes Sin [6]. Maggie Smith (Aberdeen University) discussed another member of this family, the bacteriophage ϕC31 integrase, and how discrimination between the reactions that promote integration and excision from the host genome is achieved [7]. Wei Yang (National Institutes of Health, Bethesda) focused on her cryo-electron microscopy studies, revealing the shapes of multisubunit complexes of the RAG proteins, which promote DNA rearrangements to create antibody diversity, and Nancy Craig (Johns Hopkins University, Baltimore) discussed the mechanisms of the ‘cut and paste’ transposases that mobilize transposons of the *hAT* and *piggyBac* superfamilies.

Efficient repair of damaged DNA without corruption of the genetic information is essential in all organisms. Lorena Beese (Duke University, Durham, NC) spoke about her structure-based studies on the effects of DNA lesions (mismatches and damaged bases) on replication and repair enzymes. Aidan Doherty (Sussex University, Falmer) described his work elucidating the orchestration of the enzyme-catalysed reactions in non-homologous

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end-joining, a repair process that is crucial in a wide range of organisms.

The vast lengths of DNA inside all cells must be stored in an accessible form, and the DNA must be distributed in an orderly fashion to daughter cells on cell division. David Sherratt (Oxford University) described his cutting-edge experiments, including fluorescence microscopy of living bacterial cells, to show how proteins ensure correct replication of the bacterial chromosome and segregation of chromosomes at cell division. Estelle Crozat (Oxford University) provided us with further details on how FtsK, an essential enzyme for the segregation process, is able to translocate along DNA molecules [8]. Daniela Rhodes (MRC-LMB Cambridge), kindly stepping in at short notice to replace a speaker who was unable to attend, told us about her electron microscopy and single-molecule studies revealing the structure of the 30 nm fibre, which is a key intermediate stage in the packing of nucleosomes into higher-order chromatin structures.

As noted above, topoisomerases are essential in order to separate tangled DNA molecules. Andrew Bates (Liverpool University) and Anthony Maxwell (John Innes Centre, Norwich) discussed the enigmatic role of ATP hydrolysis in the mechanisms of Type II DNA topoisomerases such as gyrase, and proposed that a previous explanation that hydrolysis is necessary for simplification of the DNA topology may not be viable. Instead, they suggested that consumption of ATP plays a safety-net role of ensuring that the strand passage steps avoid the dangerous mis-reaction of double-strand break formation [9]. This insightful proposal has parallels with energy-dependent proofreading mechanisms of other systems.

The supreme champions in specificity of nucleic acid sequence recognition are surely the restriction enzymes, whose biological function is to defend cells against invading DNA. Jacqueline Marshall (Bristol University) described the remarkable catalytic properties of Type IIB restriction enzymes, such as BcgI, which make eight DNA strand breaks in a concerted manner, following the interaction of two enzyme-bound sites [10]. Mark Szczelkun (Bristol University) showed how Type III restriction enzymes can detect the relative orientation of two distant recognition sites on DNA by helicase-driven sliding along the intervening sequence [11].

The delegates dispersed on the afternoon of Friday 14 August after 2 hectic days of world-class science. On behalf of all the participants, we thank our sponsors and all the staff at the Biochemical Society and Robinson College who helped to make this a successful and memorable meeting. Finally, we were delighted to learn, just a few weeks after the meeting, that two of our speakers, Ada Yonath and Venki Ramakrishnan, were jointly awarded the 2009 Nobel Prize for Chemistry along with Tom Steitz for "studies of the structure and function of the ribosome." We pass on our congratulations to them!

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