Meeting report

DNA replication can still spring surprises

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A report of the first EMBO conference in a biennial series 'Replication and Segregation of Chromosomes', Geilo, Norway, 16-20 June 2008.

The first EMBO conference on the replication and segregation of chromosomes held recently in Norway brought together researchers from around the world. Many different techniques and organisms are being used to approach one of the oldest problems in biology: how does one organism become two? The breadth of work covered was exceptional; this report describes just a few of the most striking innovations in technology and concepts presented at the meeting, with particular reference to bacterial DNA replication.

Antoine van Oijen (Harvard Medical School, Boston, USA) amusingly described the problem of ensemble averaging in DNA replication as an alien race concluding that humans must each have one testicle and one ovary. To avoid this pitfall, his group makes single-molecule observations of the four-protein replisome of the phage T7. The copy of gp5 (the DNA polymerase) acting on the lagging strand can be washed away, allowing observation of the processivity of leading-strand replication in isolation. Measurements of processivity are possible because the passage of the replication fork on the tethered DNA causes long double-stranded (ds) DNA to be transformed into more compact single-stranded (ss) DNA. Restoring the second polymerase and adding the T7 single-strand binding protein makes the looping out of lagging strand DNA itself observable as transient changes in the length of the tethered DNA.

The archaea are apparent amalgams of eukaryotic and eubacterial traits. Their replication machinery is homologous to that of eukaryotes despite their eubacteria-like circular chromosomes; most distinctively, archaea can have multiple origins of replication in these circular chromosomes. Stephen Bell (University of Oxford, UK) described his laboratory's investigations into DNA replication in the archaeal genus *Sulfolobus*. He and his colleagues are characterizing the proteins EscrtIII, which is unique to archaea, and Vps4, which is also found in eukaryotes. These proteins are of interest as alteration in their expression results in defects in DNA segregation. Uncovering the mechanisms of DNA maintenance in the third domain of life might also shed light on more complicated eukaryotic systems.

Ever since Watson and Crick first proposed their model of DNA replication, the topological problem of overwinding the helix during its duplication has been obvious. One solution is the type I and type II DNA topoisomerases, which act to reduce the winding by passing a strand of DNA through one or two strands, respectively. Where do these topoisomerases act in relation to the replication fork? Marcelo Foiani (Fondazione Italiana per la Ricerca sul Cancro, Milan, Italy) described his group's chromatin immunoprecipitation-DNA microarray analysis of synchronized yeast cultures in early S phase. They have found that the topoisomerases Top1 and Top2, type I and type II topoisomerases, respectively, localize close to the replication forks themselves. This would suggest that topological transitions are being performed constantly during replication and within 600 bp (the resolution limit of these experiments) of the replication forks. Foiani has also begun similar work identifying the locations of replication termination in yeast and is searching for possible termination-associated factors. Intriguingly, he reported that replication forks approaching each other appear to pause before they are resolved in this system.

The understanding of the replication of bacterial chromosomes has recently experienced an upheaval. David Sherratt (University of Oxford, UK) presented the latest news on the death of the stationary 'replication factory' model of DNA replication in *Escherichia coli*, reporting the work of his colleague Rodrigo Reyes, who has fluorescently labeled many of the components of the DNA replication machinery to track the progress of the replication forks *in vivo*. The primary conclusions of these experiments is that at short

time scales the replisome assembled at each replication fork moves separately along what is assumed to be the path of the compacted DNA. Replisomes move away from each other in the general direction of opposite cell poles until near the completion of replication, when they come back together. These observations defy the possibility of a stationary replication factory from which the nascent DNA is extruded, at least in *E. coli*.

Is the factory model's epitaph premature or have we yet to appreciate the extent of variation among bacterial species? Perhaps the most controversial talk at the meeting was the description by Sigal Ben-Yehuda (Hebrew University of Jerusalem, Israel) of a novel method for illuminating the large-scale spatial details of DNA replication in *Bacillus subtilis* in real time. Her group has recently developed a method for following the incorporation of fluorescently labeled nucleotides into the DNA of growing cells. The images that result from this new technique imply that the DNA is kept in large helical loops within the cytoplasm and that these appear to spin out from presumed replication factories that remain at mid-cell. Eventually DNA loops back from the poles to fill a central channel as replication proceeds. If validated, this technique will be a major step forward in monitoring the progress of DNA replication in live cells; most importantly, such labeling techniques could be used in 'normal' non-mutant cells of any species. Caution is warranted at this stage, however, in that the nucleotide incorporation must be confirmed to be taking place at replication forks and not represent damage-induced replication or repair events.

Once the bacterial chromosome has been replicated, the two copies must be separated and accurately segregated to the daughter cells. Jeffrey Errington (University of Newcastle, UK) presented his colleague Heath Murray's most recent work on bacterial chromosome segregation in *B. subtilis*. Errington described the *Soj* gene (a *ParA* homolog), which is located in the chromosome next to *SpoOJ*, a *ParB* homolog necessary for accurate chromosome segregation in this system. *Soj* encodes an activator/inhibitor protein that controls replication initiation through interactions with DnaA, the origin initiation protein. Interactions between these neighboring proteins coordinate replication with segregation. The phenotypic characterization depended on some nice fluorescent work and mutant Soj proteins that either lack ATPase activity or cannot dimerize. One of the more interesting exchanges in the meeting came when Errington was asked, "Why does *E. coli* not use the ParAB system?" Many view *E. coli* as the prime model system for everything bacterial, but unlike many other bacteria, it does not use the ParAB system for chromosome segregation. Errington's response was clear: "I think *E. coli* is a real weirdo."

Whether *E. coli* is representative or not, Frédéric Boccard (CNRS Centre de Génétique Moléculaire, Gif-sur-Yvette, France) and his colleagues have beautifully established the presence of what appears to be a chromosome domain organizer for the terminus region in *E. coli*: the protein MatP, which binds to a 13-bp sequence (*matS*) found 23 times and only in the terminus region. In the absence of MatP there was a decrease in chromosome condensation in the terminus region as well as a lack of cohesion of the terminus after replication. Boccard's approach combines bioinformatics and genetic techniques and is revealing domains of organization in *E. coli* that satisfyingly parallel those found in *B. subtilis* and other bacteria. Altogether, the work discussed above shows that although the tasks being accomplished are similar, the mechanisms that have evolved in different bacterial species to organize and segregate genomes can vary as much as the multitude of lifestyles these organisms have adapted to.

Some would argue that there is no greater goal in life than to replicate and segregate DNA. As new techniques are brought to bear on the understanding of this most basic problem, the myriad of mechanisms revealed continue to surprise and challenge conventional wisdom.