Biological Physics of Molecules and Systems

Department of
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Our laboratory studies biological processes both at the molecular and cellular scales: we carry out experiments on different aspects of homologous recombination, and on genetic networks from a Systems Biology viewpoint. Homologous recombination, catalyzed by recombinases such as RecA in bacteria and Rad51 in eukaryotes, is a fundamental process in which two DNA sequences meet in a synaptic complex, and exchange genetic material, provided there is high homology between them. We attempt to answer questions such as: What are the mechanisms of search in a cell for homologous sequences? What is the efficiency of homologous recombination when the DNA molecules differ in sequence? How many base pairs are needed for a sequence comparison? How long does it take to carry out this comparison?

In addition, we study the dynamics of genetic networks in single bacterial cells and ensembles, using modern fluorescence imaging and microfluidics techniques. Currently we are focusing on three natural networks: The lambda phage genetic network responsible for the lysis-lysogeny decision, the SOS response of E. coli that is activated when DNA damage is encountered and needs to be repaired, and small RNA-controlled networks such as those involved when environmental changes in iron or oxidative agents are encountered. We characterize the timing in which different pathways are activated during the execution of a network, as well as the variation in timing and gene expression between cells in a population.

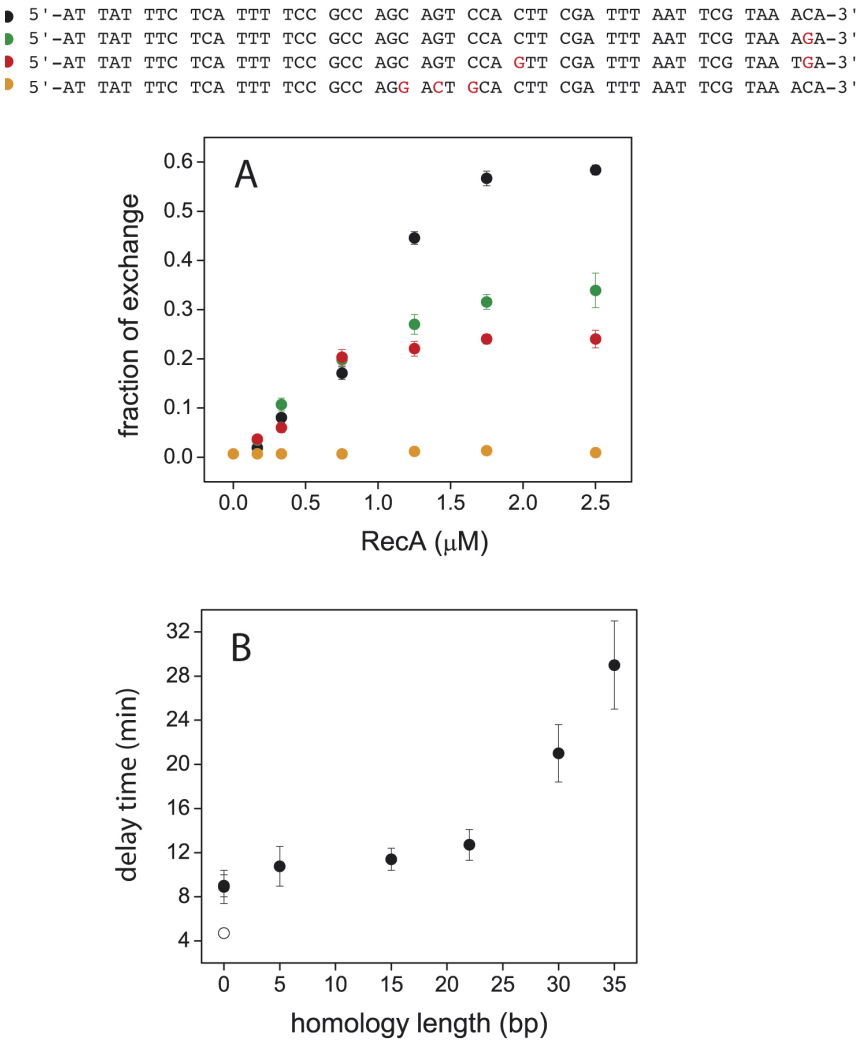

Fig. 1 *(A) Effect of the number of mismatches on the efficiency of recombination. (top) Invading strands with an increasing number of mismatches (red), yield a lower corresponding fraction of oligomers having undergone strand exchange (bottom). (B) Delay time induced in a reaction by the presence of competitor DNA sharing partial homology of variable length with the target.*

A. Homologous Recombination

Homologous recombination is one of the most fundamental biomolecular processes involving DNA. It plays a key role in the evolution of organisms from bacteria to man, generating genetic diversity by reshuffling genes in eukaryotes, and by enabling the import of new ones in prokaryotes. In an homologous recombination event several steps occur: first, a tract of DNA is bound to a recombinase. Next, this complex searches for an homologous tract along a chromosome by the formation of a synapse with the latter. When homology is found, strand exchange ensues. In order to shed light on the mechanisms of target location, we carry out experiments at the level of single molecules and ensembles, to answer a number of specific questions. For example, we use fluorescence resonance energy transfer (FRET) techniques to measure the extent to which the recombination process is sensitive to differences in sequence between the participating DNA molecules, and what is the minimal length of the DNA sequences needed

for the formation of a functional synapse. Our experiments show that the recombination process is sensitive to single sequence mismatches in its initial stages (Fig. 1A), and that about 30 base pairs are needed for a functional synapse (Fig. 1B). Together with T. Tlusty in our department, we have proposed a cascaded kinetic proofreading scheme to account for the extraordinary discrimination of homologous recombination. We are currently designing experiments to characterize better the dynamical properties of synapses and shed light on homology search mechanisms.

B. Systems Biology of Bacterial Genetic Networks in Single Cells

Systems Biology constitutes a new framework to understand study how complex genetic networks work as a whole. We study a number of genetic networks in bacteria, in order to elucidate how the configuration of the networks endows them with their characteristic properties and function. Our studies, conducted at the level of single cells, allow us to probe non-genetic individuality in cell populations, and how they react to different stimuli.

One of the networks we have studied is the SOS response, following exposure of *E. coli* cells to UV irradiation, which induces DNA damage. This network consists of about 40 genes organized in different pathways of repair such as nucleotide excision repair, recombinational repair and mutagenenesis. How are these pathways coordinated? When are different genes activated and shut off? What are the differences in the response for small and large amounts of DNA damage? We address these questions by studying the dynamics of the SOS response at the level of single cells in real time, using GFP fusions to gene promoters in the network. Analysis of real-time images shows that the corresponding promoter activity of RecA and other SOS is temporally modulated, the modulations appearing at the same time in all cells with high precision, irrespective of the time at which each cell underwent cell division. The network is thus endowed with an internal mechanism for measuring time.

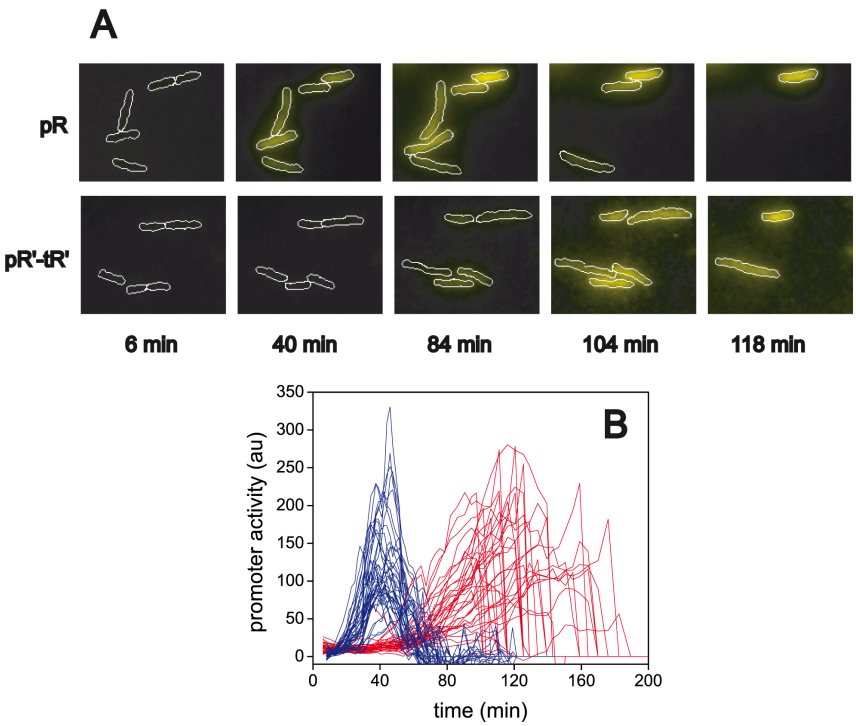

Fig. 2 *Induction of individual lysogens following irradiation with 20 J/m2. (A) Snapshots of cells harboring the pR-GFP (top panels) and pR'-tR'-GFP (bottom panels) reporter plasmids undergoing induction taken at the times shown after irradiation. Some cells lyse and disappear from the field of view. (B) pR (blue) and pR'-tR' (red) promoter activity profiles of individual cells as a function of time.*

Another system we study is the interaction of the lambda phage with *E. coli* cells. Lambda phage is a paradigm system to study how a biological decision is made, an issue relevant to development. Much about fundamental biomolecular processes was discovered through the study of the lambda phage. Lambda phage belongs to a class of viruses called temperate, meaning that after attacking a bacterial cell such as *E. coli*, the phage must decide between two fates: lysis or lysogeny. When the lytic pathway is chosen, the virus utilizes bacterial resources to replicate many times, until the cell bursts (lyses) with new viruses that can go and infect new cells. Alternatively, the viral genome can insert itself into the bacterial genome (the prophage state), stay dormant by repressing viral genes that lead to lysis and replicating passively with the bacterial genome. The lysogenic state is highly stable but DNA damage can result in an irreversible switch into the lytic pathway (induction). We have followed the dynamics of both lytic and lysogenic pathways in real time upon infection of cell ensembles, and upon induction of lysogenic cells, both in ensembles and in individual cells, using promoter-GFP fusions. Fig. 2 shows cells expressing GFP from a fusion to thepR and pR'-tR' promoters, after induction of the lytic pathway by irradiation with UV light. Cells gradually become more fluorescent, and suddenly disappear from the field of view by undergoing lysis. We study how timing fluctuations in the occurrence of early events give rise to larger timing fluctuations of later events along the induction genetic cascade, and how this reflects upon the cascade's architecture.

Currently we are focusing on the study of networks in which small, non-coding RNA molecules (sRNA) play a leading regulatory role. In contrast to the more familiar transcriptional control of gene expression by proteins, such as activators and repressors, control by

sRNAs takes place post-transcriptionally, and may increase or decrease expression by affecting translation and mRNA stability respectively. About 60 sRNAs have been identified in *E. coli.* We are studying the dynamical behavior of sRNA-controlled networks in *E. coli*, focusing on their dynamics and noise properties. Gene expression is monitored in real time at the level of individual cells by means of GFP fusions both to promoters and proteins in the networks, and microfluidics techniques.

Selected publications

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A. Amir, O. Kobiler, A. Rokney, A. B. Oppenheim and J. Stavans (2007) Noise in timing of gene activity in a genetic cascade, Mol. Sys. Biol. 3, 71

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