Methods in Molecular Biology – "Archaea: methods and protocols"

Primer for 'Progress and challenges in archaeal molecular biology'

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In the late 1950s the discovery and formulation of the 'Central Dogma of Molecular Biology' rocked the world of science when it provided a cogent framework of the molecular reality underlying biology [1]. Through the structural basis of the DNA double helix and nucleobase complementarity [2], it became possible to rationalise biological phenomena like heredity, genetics, the principle of semiconservative DNA replication, DNA template-dependent transcription and RNA template-encoded protein synthesis, translation. Previously unexplained phenomena involving acidic substances in the cell were suddenly not mysterious any longer, and we started to understand how biological information was related to the traits of an organism – the connection between genotype and phenotype. The early characterisation of the DNA structure was followed up by an ever more detailed investigation into the molecular mechanisms of life. This is the reason why Molecular Biology like no other discipline has changed the way we understand life.

Twenty years later, in the late 1970s, another ground-breaking discovery changed the way we think about the evolution of life on earth: the Archaea were recognised as life forms that were distinct from their bacterial and eukaryotic cousins [3]. The notion of a third domain of life that included features from the other two was as revolutionary as it was preliminary. Revolutionary, because it postulated that the molecular machines driving the central dogma of molecular biology were eukaryote-like but acted in the context of a prokaryotic cell with a genome and operon structure akin to bacteria (see Chapter 1 for details). Preliminary, as it turned out that the phylogenetic similarity of archaea and eukaryotes was even stronger than originally anticipated, in as much that they are now considered by many to be sister groups of the same

domain of life [4]. In other words: 'We are all Archaea!' This is a simple and clear message that not only resonates with our students but also with the general public, and importantly with funding agencies that now widely acknowledge the validity of Archaea as model systems in addition to their extraordinary value for the discovery of new biology. The cultivation of Asgård archaea, which are assumed to be the closest relatives to the ancestors of eukaryotes, is still a formidable challenge [5] (see Chapter 1 for details). However, the Asgård sequence space has entered the field of molecular biology and Asgårdian proteins can be produced in recombinant form, and their structure and functional properties can be studied on a par with proteins from extant Archaea and eukaryotes (e. g. [6-8]).

Substantial changes in our understanding of life are increasingly driven by multiscalar and multidisciplinary approaches and large research teams, and the key challenges of the future include developing new disciplines that fill the gaps between the scales in biology. This includes e. g. electron tomography [9] that bridges structural biology and cell biology, or ChIP-exo [10] that bridges structural biology and functional genomics. In addition, it calls for methods that integrate disparate data obtained from different disciplines, which requires time and resources spent on developing new and improved bioinformatics tools [11]. Even though public funders and charities like to emphasise their aspiration for 'transformative' and 'leap frogging' break-through science when emphasising their remit, the mainstay strategy and reality of modern research is still to carefully build on and expand the existing knowledgebase, step-by-step. As we appreciate the 'bigger picture' provided by systems biology, our progress in understanding biology goes hand in hand with developing new experimental protocols in molecular biology. This chapter, 'Progress and Challenges in Archaeal Molecular Biology', reflects that we have started to embrace the global aspects of archaeal molecular biology, and the collection of methods will hopefully enable more research teams to adopt the new protocols in their research portfolio.

A rigorous biochemical, molecular biological and structural characterisation in vitro plays a pivotal role in nailing down the detailed function of a molecule. However, it cannot capture the cellular complexity in vivo, the way that molecular machines are coupled, coordinated and regulated, a view that can be provided by systems biology. In the following part of the book,

leading scientists describe detailed protocols at the interface of molecular and systems studies, including functional genomics such as global occupancy of transcription- (see Chapter 13 & 19) and translation machineries (see Chapter 14), transcriptome- (see Chapter 15), proteome- (see Chapter 16, 17 & 18) and metabolome- (see Chapter 21) analyses, as well as ways of harnessing the power of the central dogma for the production of recombinant proteins and chemical compounds at scale (see Chapter 23), and metal nanoparticles (see Chapter 22).

I wish you best of luck and great success with your future projects!

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