APPLICATION OF MOLECULAR BIOLOGY TECHNIQUES TO ASTROBIOLOGY

RICCARDO SIDNEY GATTA[1] AND JULIAN CHELA-FLORES[2]
[1]The International Centre for Genetic Engineering and Biotechnology, New Delhi, India, and [2]The Abdus ICTP Strada Costiera 11; 34136 Trieste, Italy, and IDEA, Caracas, Venezuela

Abstract. The opportunity for direct examination of the Europan surface and sub-surface calls for a systematic and deductive approach to experimental design. To avoid the limitations of our inherent Earth-centric definition of life (Nealson *et al.*, 2002), we would be forced to examine a wide range of potential bio-signatures to guide more specific biological experiments (Chela-Flores, 2003). It is also important to look for recurring features that are important from the evolutionary history of our own biosphere (Zakon, 2002). Of the many candidate molecules, the structurally heterologous superfamily of voltage-gated cation channels is an evolutionary sensitive group of molecular structures, the single varieties of which can be easily distinguished. Implementation of the analytical aspects of this experiment would require remote control of miniaturized robotic systems. These mechanisms are under constant evolution since their uses are strongly tied to commercial, scientific and military interests. One paradigm for feasibility studies could come from data inferring the reprocessing of ice covering a Europan ocean. Reprocessing could be inducing life forms extant in the liquid water subsurface towards the ice covering, as it has already been demonstrated at the frozen surfaces of Antarctic lakes (Bhattacherjee and Chela-Flores, 2004), and as it has been suggested by analysis of the Galileo images of the surface of Europa (Greenberg *et al.*, 2002). The proposed series of experiments can be carried out *in situ* either within a submersible in the ocean beneath the ice layer, or even on the surface ice itself. Results from a preliminary examination of the environment would be used to determine the conditions necessary for sampling and pre-processing of any material of possible biological origin. Many techniques are currently available for identifying targets according to their molecular structure and their chemical-physical characteristics:
- novel sampling and isolation methods,
- specific antibodies or diabodies engineered as molecular probes,
- micro-arrays based on site-specific immobilization of complementary molecules,
- microscopy and micro-sensors for visualization/digital sampling of positive results.

New challenges will arise from the novel settings and will have to be addressed, singly, well in advance of any preliminary exercises. Moreover, a myriad of practical applications could be developed by addressing pertinent, emerging questions relating to:
- stability of sensitive organic material over a large period of time, and extreme conditions,
- maintenance of biological activity within silica-, or hydro- micropatterned biogels,
- multiplexing in a microfluidic (lab-on-a-chip) environment,
- miniaturization of analytical devices such as microscopes and their power sources.

1. Introduction

Identification of biomolecules is a common process that is approached in a concise and systematic manner. When applied to the search for life in the universe many potential targets and recognition techniques can be envisioned. These can be refined by seeking with evolutionary criteria in an environment where water is available. The problem of rational target selection is to single out known bio-signatures and evaluate their placement on an evolutionary timescale. Selection of a target based on the central dogma of molecular biology (Crick, 1958), which states that the flow of genetic information is from DNA, through RNA, to protein, is open to the idea that any of these key molecules could be, or could have once been (Woese, 2001), sources of hereditary data in some place in the universe (Crick, 1966 and 1970). The examination of samples of unknown composition yields results that are tied not only to the nature of the material under scrutiny but also to the conditions under which they are examined. The delicate pleiotropic nature of protein-protein interactions, for instance, can be permanently affected by minimal variations that would alter important conformational interactions. There are methods for the successful recovery of biological material from problematical sources (Vreeland and Rosenzweig, 2002) since insufficient levels of sterility, contamination from other sources, or less than optimal reaction conditions always lead to unreliable results (Nicastro *et al.*, 2002).

2. Molecular Techniques

Structural information for the identification of life could be sought in membrane composition (channels, peptidoglycans, lipids, chirality) or in the form of genetic information (DNA, RNA…). The lack of available information about non-terrestrial macromolecules, however, makes it difficult to seek life through molecular probing of these components, though all can be analysed with specific assays: sugars (DeAngelis, 2002), proteins (Zakon, 2002) and regulatory machinery of gene expression (Conant and Wagner, 2003). Molecular subtyping methods can seek differences in control of fatty acid (Tornabene *et al.*, 1980), protein or nucleic acid (Woese and Fox, 1977) biosynthesis. Many evolutionarily conserved biomolecules could serve the purpose of attempting to ground a universal tree of life. Families of proteins where there are conserved structural elements, or domain-specific features (Marck and Grosjean, 2002), are thought to lead to ancient origins or even to a last universal common ancestor (LUCA) (Ouzounis and Kyrpides, 1996). The link between structure and function is apparent in the conservation throughout evolution of families of proteins that perform essential tasks (Ruta *et al.*, 2003). Ion channels belong to a large family of related genes that regulate vital functions. The simplest channels are found in all kingdoms of life. Ion channels consist of assemblies of subunit components (Hille, 2001) and thus share aspects of their membrane topologies (Miller, 2000). However, diverse ion specificities (Jeziorski *et al.*, 2000) and methods of functional regulation make ion channels ideal targets for probes seeking differentiation by evolutionary criteria (Harte and Ouzounis, 2002) with, for example, semisynthetic libraries (Braunagal, 2003) that lend themselves to rational design and chemical synthesis. In order to assure the correct interpretation of image data obtained *in loco*, it is important to sample data at a sufficiently high resolution so that any further

enhancement does not alter the acquired data. Light microscopy can utilize illumination sources that can be easily varied, such as with filtered short wavelength radiation that can cause fluorescent substances to produce emission spectra (Kain *et al.*, 1995). Green fluorescent protein (GPF) has been used as a specific reporter gene for ion channel expression (Marshall *et al.*, 1995). Confocal (laser scanning) microscopy (CLSM) offers a further advantage of being able to increase spatial resolution. Multi-photon microscopy uses short pulses of low energy, infra-red, light to excite a restricted cross-section within the sample without the need for confocal apertures. This increases the photostability of fluorescent molecules (Geddes *et al.*, 2003), though a recently developed alternative to organic molecules for immunocytochemical imaging is quantum dot technology. The higher quantum yield and stability of quantum dots could solve the major problem associated with a large amount of parallel assays and with the long latency period between assay set-up and performance (Tokumasu and Dvorak, 2003). They can be combined into highly specific bioconjugates for studying genes or proteins in applications that are not envisioned with traditional organic dyes and fluorescent proteins (Medintz *et al.*, 2003). Nuclear magnetic resonance (NMR) can be used to detect 3 dimensional (3D) placements of unmarked individual atoms, even in extremely low magnetic fields (McDermott *et al.*, 2002). Analytical systems such as scanning probe microscopes already enable direct visualization and manipulation of individual macromolecules (Malayan and Balachandran, 2001): a key interest in process miniaturisation (PIM) in the field of molecular diagnostics (Brush, 1999). A new generation of scientific instruments is in development that can be adapted to the context of planetary exploration. Novel power sources and remote robotic control would guarantee the completion of a mission even in unforeseen circumstances. Microfabricated devices have already been adapted for transcript expression profiles of genes related to ion channels, with the ability to identify changes down to channel subunit level. Biochips are used that permit electrophoretic separations and highly specialized applications of molecular biology. Though several different matrices and protocols are available for microarrays, storage periods remain very limited (Angenendt *et al.*, 2002). Biomimetic systems apply novel production methods and materials for creating surface moieties similar to those using proteins or nucleic acids that are made by bio-systems. The transport of fluids through nanoscopic conduits (Drexler, 1994) will allow single molecules of DNA to be analysed. It has been a long established goal to guide complex sequences of actions in simple nanoscale systems in order to create more intricate patterns (Deamer and Branton, 2002). Alternative biogels are being derived from a marine sponge, *Tethya aurantia*, that produces a protein group call silicateins responsible for biosilicate formation under benign conditions. Molecular-scale channels are essentially entirely interfaced with no bulk fluid; thus, a complete understanding and control of interfacial chemistry on the nanometer scale to obtain a stable microfluidic network cannot be underestimated (Kim *et al.*, 2001). The challenge of finding life in the universe is driving research to develop more efficient scientific instruments that will not fail to benefit applications in every field, and in every biosphere that may be found to exist.

3. References

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