Fluorescence microscopy as a research tool in bacterial cell biology

Immunofluorescence methods, together with GFP and digital imaging, are providing a revolutionary new view of the subcellular organization of bacteria, as **Jeff Errington** describes.

▼ Technician using a fluorescence microscope. *CC Studio / Science Photo Library*

Bacterial cells are remarkable in many ways. They carry out a huge and diverse range of biochemical reactions and are capable of growing in an amazing range of environmental conditions. One secret of their success lies in the adaptability that comes from their rapid growth and division. This comes, in turn, from their small size and simple architecture, compared with cells of higher organisms. When the electron microscope was developed in the mid-20th century, and applied to bacteria, it confirmed the notion that bacterial cells are simple. There was no sign of the complex, membrane-bound organelles that characterize eukaryotes. However, there is a sense in which these experiments did a disservice to bacteria, because they emphasized all of the things they were missing – cytoskeleton, membrane-bound organelles, particularly a nucleus. Meanwhile, some bacteria, such as *Escherichia coli* and *Bacillus subtilis*, became great experimental work-horses because of their rapid and easy growth and excellent genetics. Many scientists began to view them essentially as 'bags of enzymes' – wonderful for biochemical studies and as tools for molecular biology, but of little interest from the perspective of cellular organization.

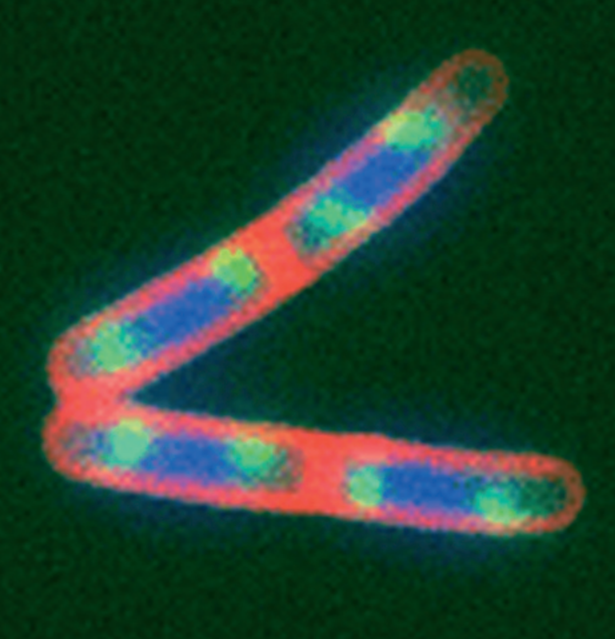
This view changed dramatically in the mid-1990s when the renewed application of immunofluorescence methods to bacteria, together with the advent of green fluorescent protein (GFP) and digital imaging, provided a revolutionary new view of the subcellular organization of bacteria. These developments founded what was effectively a new field of 'bacterial cell biology' and provided a powerful suite of methods and approaches with which to probe fundamental aspects of the workings of bacterial cells.

Powerful new technologies

Fluorescence microscopy had been applied sporadically to bacteria in various ways for 30 years or more. However, in the early 1990s it entered the mainstream with the successful application of immunofluorescence methods to intracellular proteins in bacteria, followed soon after by the harnessing of GFP. At around the same time, the development of affordable CCD cameras with high sensitivity and spatial resolution, together with digital image processing packages, made it much easier to capture the faint fluorescence from bacterial cells.

Immunofluorescence methods essentially involve the use of a fluorescently labelled antibody to identify the location of the target protein. A major advantage of the method is that it can be done with completely wild-type cells, provided a specific antibody is available. In principle, the location of the fluorescent antibody reports the position of the native protein. Alternatively, the cells can be genetically engineered to add a short (~10 aa) epitope tag to the target protein, allowing it to be detected by any of a number of readily available monoclonal antibodies. The main disadvantage of this method arises from the need to get large antibody molecules into the cell. To achieve this, the cells need to be permeabilized. Since this would normally result in destruction of the cells, they first need to be gently fixed. But this is tricky because too much fixation makes the cells impenetrable. Therefore, the whole process is technically demanding. Inevitably, the problems of access of the fluorescent label and degradation of cellular structure mean that artefacts and even complete failure of detection are not uncommon. Finally, since the cells are obviously killed by the process, artefacts can arise from cell death and there is no question of being able to follow movement or changes in localization over time.

GFP is an intrinsically fluorescent protein derived from a jellyfish, *Aequoria victoria*. The big advantage of GFP labelling is that it can be done in native, unperturbed cells. Moreover, the cells can be imaged repeatedly, offering the possibility of collecting optical sections through cells, or obtaining a time lapse series. Although GFP has had a revolutionary effect on research across biology (of which more below) there are some caveats and limitations to its use. The main disadvantage arises from the fact that the cells need to be genetically engineered in some way, to produce a fusion of GFP and the target protein. Many, perhaps most, fusion



◀ *Bacillus subtilis* cells carrying a GFP fusion to the Spo0J chromosome segregation protein. This protein sticks to regions that flank the origin of replication of the circular chromosome and forms spots or 'foci' (green) that mark the origin region. The spots duplicate soon after chromosome replication begins, as soon as there are two copies of the origin region. These then move rapidly apart towards opposite poles of the cell. The chromosome (blue), occupies most of the cell and the foci lie at each end of the DNA mass. The cell outline is stained red with a membrane dye. Two pairs of cells are shown. The cells are about 2 µm long.
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proteins are not completely wild-type in behaviour, because adding the 27,000 Da GFP moiety can mess up the target protein's activity in any one of a number of different ways. It is therefore important to check that the fusion is functional, by testing for its ability to sustain the function of the normal gene, where possible. If the protein is not fully functional, any pattern of localization should be treated with caution. In our experience the two most common problems that arise are: first, aggregation of the GFP fusion to form 'inclusion bodies', often in the form of a single bright spot located near the pole of the cell; second, loss of a specific pattern because of saturation, due to the fusion protein being overexpressed relative to the native protein.

A good way to mitigate being misled by this kind of problem is to construct GFP fusions to both the N and C terminus of the target protein. Sometimes only one of them is functional, and it is not uncommon for the two fusions to give different results! In our experience with hundreds of fusions, the N-terminal fusions have a slightly better success rate, which is unfortunate because they are somewhat more difficult to construct (the *gfp* gene has to be placed between the coding and upstream regulatory sequence of the gene).

Although GFP is often used as a reporter of subcellular localization, it can also be used as a reporter of the timing or cell-specificity of gene expression. Fusing *gfp* to the regulatory sequences of one's favourite gene provides a way of identifying where and when the gene gets turned on. This can be very informative in multicellular situations, such as in biofilms, or to study genes turned on under any kind of stochastic control.

A myriad of patterns

GFP fusions have now been made to many thousands of bacterial proteins. An ever-increasing multitude of different localization patterns has been described. Some of the results have been of a unifying nature. For example, it is now clear that transcription occurs in the core of the cell and translation in the periphery, just as in eukaryotes, even though there is no nuclear membrane demarcating these zones in bacteria. DNA replication occurs in specialized 'replication factories', just as in eukaryotes. Localization experiments have also been crucial in demonstrating that bacteria have all three elements of the eukaryotic cytoskeleton, tubulin (FtsZ), actin (MreB) and intermediate filament proteins (crescentin), with critical roles in the cell cycle and cell morphogenesis. Among the surprises have been the discovery of proteins that rapidly oscillate, from pole to pole, or round and round the cell, and which are involved in mapping the size and shape of the cell for the purposes of DNA equipartition or accurate selection of the mid-cell site for cell division.

Overall, the results show that bacteria have an astonishing and completely unexpected level of complexity. The patterns being distinguished today probably represent the tip of the iceberg, because resolution is currently confined by the physical limitations of fluorescence in the visible light range. A greater level of sophistication probably awaits discovery when emerging technologies that can overcome the current limits of resolution become available to bacterial cell biologists.

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Further reading

Ausmees, N., Kuhn, J.R. & Jacobs-Wagner, C. (2003). The bacterial cytoskeleton: an intermediate filament-like function. *Cell* 115, 705–713.

Errington, J. (2003). Dynamic proteins and a cytoskeleton in bacteria. *Nat Cell Biol* 5, 175–178.

Jones, L.J., Carballido-Lopez, R. & Errington, J. (2001). Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell* 104, 913–922.

Lemon, K.P. & Grossman, A.D. (1998). Localization of bacterial DNA polymerase: evidence for a factory model of replication. *Science* 282,1516–1519.

Lewis, P.J., Thaker, S.D. & Errington, J. (2000). Compartmentalization of transcription and translation in *Bacillus subtilis*. *EMBO J* 19, 710–718.

Lutkenhaus, J. (1991). FtsZ ring structure associated with division in *Escherichia coli*. *Nature* 354, 161–164.

Moller-Jensen, J. & Lowe, J. (2005). Increasing complexity of the bacterial cytoskeleton. *Curr Opin Cell Biol* 17, 75–81.

Raskin, D.M. & de Boer, P.A. (1999). Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*. *Proc Natl Acad Sci U S A* 96, 4971–4976.

Wang, X., Possoz, C. & Sherratt, D.J. (2005). Dancing around the divisome: asymmetric chromosome segregation in *Escherichia coli*. *Genes Dev* 19, 2367–2377.