Confronting organised complexity

Working within the area of proteomics, with a particular focus on cancer, mitochondria and subcellular location, **Professor Jasminka Godovac-Zimmermann** discusses her far-reaching research investigating the dynamics of cellular function through a systems biology approach

To begin, could you explain why biologists need to confront organised complexity?

At the cell biology level, it is increasingly clear that the basic functional units are complex networks of interacting proteins and other molecular types, including ions, small molecules and RNA. These networks are dynamic, of non-homogeneous spatial distribution and variable composition, and have functional output that depends on dynamic inclusion/exclusion/exchange/modification of proteins and other molecules.

At the single protein level, this is increasingly shown by proteins that are involved in different functions, at different subcellular locations, and at different temporal stages of cellular state. At the level of single cell organisms, very basic functions can be spatially organised very differently. How these variable functional networks are established, maintained, exploited and evolve represents a higher level interpretation of the living state. Understanding the dynamic, organisational principles of such networks offers new prospects for applications in many fields.

Could you elaborate on how you came to study the complex organisation of subcellular biology in relationship to the energetic/integrative functions of mitochondria?

It has long been clear that the energetic properties of mitochondria are intimately connected to many facets of cellular function and a variety of diseases, even to the point where some scientists have championed the idea that cancer is primarily a metabolic disease involving energetic functions of mitochondria. We know that mitochondria

Protein diverse functions and locations

Researchers at **University College London** are using high-throughput proteomics methods to research the spatio-temporal fluxes of proteins at subcellular levels, which has implications for the understanding of cellular function

THE CONCEPTS OF biology at the cellular, organismal, ecological and evolutionary levels continue to develop rapidly. Increasingly, biologists need to confront organised complexity, of which living systems are the paramount example. Both spontaneous emergence of order and the maintenance of stability in complex systems in the face of highly variable environments need to be researched further. Complex structures are generated and maintained through energy flux, placing energy flow as one of the most important components of biological systems. In addition, diversity, whether of molecular form at the cellular level or organismal variation at the ecological level, is another crucial characteristic of biological systems.

Exploitation of the human genome in systems biology has tended to concentrate on the amounts of gene expression, although it is well-known that over 50 per cent of eukaryotic genes generate multiple transcriptional variants and a majority of proteins are subject to various kinds of functionally important post-translational modifications. It is also known that cells invest more energy in establishing and maintaining subcellular distributions and fluxes of various molecules, including proteins, than they invest in protein synthesis, but knowledge of the spatio-temporal fluxes of proteins at subcellular levels remains rudimentary.

INVESTIGATING COMPLEXITIES

Professor Jasminka Godovac-Zimmermann is leading a team of researchers at University College London which has recently established high-throughput proteomics methods that can address some of these complexities. The group aims to address the influence of diversity of molecular form and/or function, quantity (abundance), space (subcellular spatial distribution) and time (dynamic responses) at the cellular level.

are also involved in such diverse functions as programmed cell death or viral immune response. Furthermore, we know that elaborate systems exist to import proteins into mitochondria, and recently, systems that export proteins from mitochondria have also been identified. Finally, we know that mitochondria are highly dynamic organelles that change their morphology and their interactions with other subcellular organelles. Given all these punctual indications, we decided it was time to systematically investigate the dynamic characteristics of the mitochondrial proteome and how this is related to cellular function and disease.

What might account for the inertia in challenging the one gene-one protein dogma?

It is already largely accepted that the one gene gives one protein dogma is inadequate. However, because many of our common experimental tools do not deal adequately with multiple protein transcriptional/post-transcriptional isoforms or with dynamic redistribution of subcellular location, there remains the inertia that many experimental strategies essentially ignore these crucial features of cells and look instead for function at *the* location. Unconsciously we are still using the corollary to the dogma that one protein gives one location gives one function. We need instead to ask how some single gene products may be involved in coordinated, spatially distributed, dynamic responses of cells to

changes in their state and environment. For this we need to update our experimental methods.

You propose that altered nucleo-cytoplasmic shuttling may play a key role in the regulation of cell cycle arrest and in the coordination of related functions across a diversity of subcellular locations. How close are you to proving this hypothesis and what is its implication?

We have recently found that the distribution of numerous proteins between the nucleus and the rest of the cell is changed by cell cycle arrest. We have also shown that the abundance of key proteins involved in nucleo-cytoplasmic shuttling is changed by cell cycle arrest. The most tantalising observation is that numerous proteins with known functions elsewhere in the cell, such as mitochondria or lysosomes, are present in the nucleus and their abundance there is changed by cell cycle arrest. Some of these proteins have previously been observed in the nucleus by others, but their function there is often completely unknown.

We are now developing high-throughput methods designed to give indications of the nature of the function for proteins in the 'wrong' location compared to their presently known functions. Although we are currently concentrating on nuclear translocation, we expect that dynamic spatial translocations throughout the cell are an essential feature of cellular function.

What are the potential ramifications of proteomics in other fields such as physics, economics and sociology?

The living, complex systems that we try to study with proteomics have implications in many other fields. The nature of self-organisation and maintenance of stability in complex systems is incompletely understood, but has obvious ramifications for social organisation and in areas such as markets and economics. The technology for perturbing complex cellular systems and studying dynamic response provides us with controlled examples of complex systems so we can model their behaviour.

The investigation has involved work which suggests that multiple locations of proteins, with the possibility of participation in different functions at different locations, is a crucial, generalised characteristic of cells. This poses the question of whether the purpose and function of subcellular organelles is really understood: could they simply be a transient agglomeration of cellular components where certain events or tasks take place and where proteins come and go, as overall cellular metabolism dictates the need for different levels of specialised functions?

The researchers have adopted proteomics methods to show that at least 50 per cent of proteins detected in MCF7 breast cancer cells show multiple locations involving significant proportions of each protein, and that large numbers of the same proteins are present in multiple subcellular organelles such as nuclei and mitochondria. "Our observations are a key contradiction to the 'one protein gives one subcellular location gives one function' dogma, as we found that many proteins have multiple locations and might have different functions in different locations," Godovac-Zimmermann enthuses. "This suggests that transcription of DNA may be a warehouse phenomenon – as cells respond to altered environment, they undergo dynamic, coordinated reorganisation of functional networks that result in ordering and delivering of new cellular components from the warehouse". Spatial distribution and translocation of key proteins increasingly seems to be an essential component of cellular response and subsequent transcriptional changes.

CDC7 INHIBITORS

The cell cycle is a crucial nexus in cellular activities; changes of events in the cell cycle may be a cause of cancer. Godovac-Zimmermann's group with GH Williams and K Stoeber have elucidated the proteomics and the functional architecture of a novel origin activation checkpoint in the G1 phase of the mitotic cell cycle, which can be triggered by loss of DNA replication initiation factors such as the Cdc7 kinase. Insufficient levels of Cdc7 produce cell cycle arrest in normal cells, whereas cancer cells appear to lack this checkpoint response, leading to cell death. The differential response between normal and tumour cells at this checkpoint has led to widespread interest in the development of pharmacological Cdc7 inhibitors as novel anti-cancer agents.

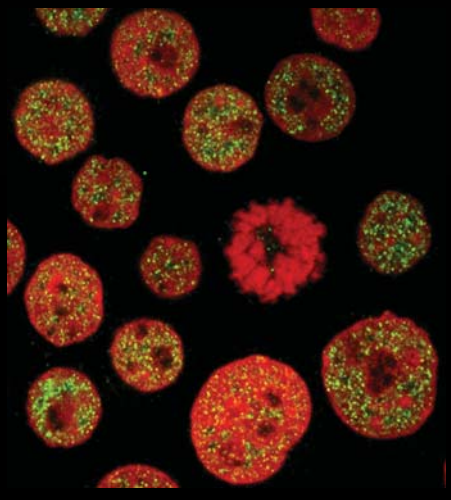

FIGURE 1. INVESTIGATING ORGANISED COMPLEXITY IN HUMAN FIBROBLASTS

PROTEOMICS IN SYSTEMS BIOLOGY – HOW DO PROTEIN FORM, ABUNDANCE, SPATIAL DISTRIBUTION AND DYNAMICS INTERACT IN COMPLEX CELLULAR FUNCTION?

OBJECTIVES

In the contexts of the general state of proteomics and our particular investigations of cancer, mitochondria and subcellular location, this research focuses on three important immediate tasks: (1) Delineation of dynamic fluxes in subcellular spatial locations of proteins; (2) Validation of subcellular location and establishment of possible functional participations by capture and characterisation of protein complexes from specific subcellular locations; (3) Exact characterisation of proteins, especially more complete MS-based characterisation of transcriptional form and modifications.

KEY COLLABORATORS

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The experiments combine three technologies. RNAi suppression is used to introduce a controlled, defined perturbation that reduces the cellular abundance of Cdc7, and stable isotope labelling of amino acids in cell culture (SILAC) is used to produce two types of cell populations both with, and without RNAi suppression and isotope labelling. After mixing the two types, high resolution MS proteomics is used to monitor the changes in abundance of thousands of proteins in response to Cdc7 suppression.

Bioinformatics analysis was able to identify clear changes in wide-ranging biological processes including altered cellular energetic flux and a spatially-distributed response across the mitochondria, lysosomes and the cell surface. These results provide a quantitative overview of the processes involved in maintenance of the arrested state and show that this phenotype involves active rather than passive cellular adaptation. They also highlight a diverse set of proteins responsible for cell cycle arrest and ultimately for promotion of cellular survival.

DATABASE LIMITATIONS

Screens of protein abundance by high-throughput proteomics continue to advance rapidly; they are now delivering unparalleled information regarding protein changes associated with specific cellular responses. However, there is an urgent need for better incorporation of experimental information into databases. A full description of a protein and its functions would consist of at least a five-tuplet of information: exact transcriptional form; post-translational modifications; subcellular location; function and interaction partners. There are now many cases known in which the same protein, or variants from the same gene, are known to constitute several independent five-tuplets.

Current databases usually consist of independent listings of each type of information without the integrated information of a coordinated five-tuplet. This may devalue the database for systems biology searches for functional networks or lead to uncertainty: "It is confusing when only part of the information available in the experimental literature has been incorporated into the databases," Godovac-Zimmermann reflects. "The spatial locations, interaction partners and exact transcriptional and post-translational isoforms are not measured or are not recorded." Databases need to be able to record coordinated tuplets and to deal with partial information depending on which elements of a complete tuplet are established for any particular experimental strategy. It is highly desirable therefore that many conventional biological experiments are coupled with MS-based proteomics methods that validate protein form and modifications for crucial proteins.

FUTURE GOALS

The team's next goal is to develop tools that can rapidly and completely validate tuplets for moderate numbers of candidate proteins identified by systems-wide screens. There are two important immediate tasks: validation of subcellular location and establishment of possible functional participations by capture and characterisation of protein complexes from specific subcellular locations; and exact characterisation of tuplets, especially more complete MS-based characterisation of form and modifications.

For example, it is increasingly recognised that 'cytosolic' glycolytic enzymes are also present in the nucleus. Nuclear form, modification and function have been partially characterised for a few of them (but not recorded as coordinated tuplets in databases), which turn out to be critically involved in energetic changes connected with proliferating cells and various kinds of cancers, as well as with the cell cycle.

Godovac-Zimmermann and her collaborators have now identified a substantial number of proteins that participate in specific, well-characterised mitochondrial functions, but are also present in the nucleus. In some cases their presence has also been noted by others, but their nuclear functions are often completely unknown. The next question to tackle is what they are doing there, and whether these proteins have been to, and returned from, mitochondria. In this case, their forms and processing may show characteristics of their functional history.

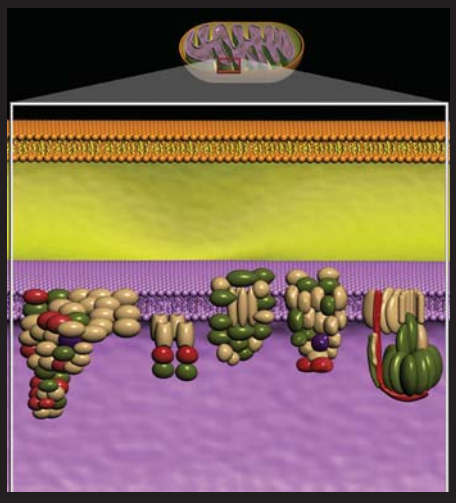

FIGURE 2. OXIDATIVE PHOSPHORYLATION PROTEINS OF THE MITOCHONDRIAL MATRIX/INNER MEMBRANE ALSO PRESENT IN THE NUCLEUS (RED/GREEN)