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15 Systems biology, cell specificity, and physiology

Vincent Detours, Jacques E. Dumont and Carine Maenhaut

Institute of Interdisciplinary Research, Free University of Brussels,
Campus Erasme (CP602), 808 route de Lennik, B-1070 Brussels, Belgium

1. INTRODUCTION

The sequences of whole genomes published in the literature open up the possibility of knowing all the RNA and protein molecular actors involved in the physiology of various organisms and their cells and, possibly, to find the relations among these actors. Thus, the whole network of information transfer within an organism or within a cell could be defined. Simulation of such a network could then theoretically predict the behaviour of the system and its alterations in disease. Examples of such constructs flourish in the literature as general maps of the various signal transduction pathways.

With regard to such general schemes, the human mind is confronted with two opposite tendencies: (1) it rejects complexity and tends to simplify relations so as to keep schemes interpretable, e.g. it is difficult to get a feeling for the role of hundreds of proteins induced by a transcription factor and for their general effect; (2) a desire for completeness encourages the extrapolation of concepts and relations from one system to another so as to obtain an all-encompassing general picture. Both tendencies influence the picture of the proposed networks.

Following the network concept, advocates of the new ‘‘systems biology’’ have begun to exhibit, in various systems, linkages maps, and controls of signal transduction proteins deduced from genetic, gene expression, and protein interaction data (Vidal, 2001; Ge et al., 2003). Such interaction networks have helped to shape concepts of great general interest (Aldana and Cluzel, 2003), which suggest striking analogies with other non-biological networks such as the Internet. In the present short review, we argue that these specific network structures are not applicable at the

level of a single cell at a definite time. We first briefly outline the systems biology approach and then focus on its limits when it comes to investigating the multiple mechanisms through which rather general signal transduction pathways are accommodated to create the exquisite specificity of physiological cells.

2. THE EXPERIMENTAL METHODS OF SYSTEMS BIOLOGY

2.1. High-throughput methods and computational methods

Genes or protein interrelations can be studied at the level of the genome, the transcriptome, and the proteome, a.k.a. genomics, transcriptomics, and proteomics in trendy scientific jargon. These involve a range of approaches.

Comparative genomics reveals consistent association between genes during evolution, which suggests potential functional relationships. The methods are based on common phylogenetic distribution, conserved gene neighbourhood, and observed gene fusions (von Mering et al., 2003). This is pure *in silico* biology on available sequenced genomes.

Coexpression of the same RNAs or proteins in the same organisms, tissues, cell types or cell compartments (the ‘‘localisome’’) may also suggest a relation. Conversely, lack of coexpression in one system will tend to exclude a relation (Stuart et al., 2003). Because 6% of interrogated genes are ubiquitously expressed, and because each tissue expresses 30 to 40% of the whole transcriptome, the number of potential coexpression events is enormous. Some coexpression events may just reflect colocalisation on chromosomes, and have no functional significance (Dillon, 2003). However, a coexpression event observed in many different biological contexts is more likely to be biologically important, and to convey high information value (Su et al., 2002). Large scale coexpression studies have been conducted in yeast (Kemmeren et al., 2002), mouse (Zhang et al., 2004) and human (Lee et al., 2004).

Identical regulation of mRNA expression in the response of the cell to different agents, again, may reflect functional relationships. Similarly, coexpression with coordinated kinetics in response to various agents suggests functional relations. Similar morphologic responses to the suppression of genes – for instance, by the systematic use of RNA interference – has established such functional relations in Drosophila (Kiger et al., 2003).

Finally, the potential for physical interactions between proteins (the ‘‘interactome’’) may be suggested by protein structures, domain

composition, and also by their interactions in reconstituted systems, acellular preparations, or model systems such as yeast in the double hybrid methodology (Vidal, 2001; Pawson and Nash, 2003). The occurrence of such interactions in physiological cells is testable by co-immunuprecipitations. A systematic survey of proteins binding to individual tagged proteins uncovered thousands of protein complexes and protein interactions occurring *in situ* in yeast (Kumar and Snyder, 2002). The estimated false positive rate for high-throughput two-hybrid assays is 50% (Sprinzak et al., 2003). Nevertheless, new functional protein–protein interactions involving previously uncharacterised bacterial proteins have been uncovered and validated despite such a large error rate (Butland et al., 2005).

2.2. Successes

In theory, the roads outlined above to the discovery of protein–protein interactions should converge. For example, ribosomal protein genes evolved together and are expressed concomitantly whenever cells require new ribosomes. The proteins of these organelles are bound and cosegregate in the cells, and presumably such interactions should be demonstrated in acellular or in double hybrid experiments.

Network structures are emerging from protein interaction research. They already make it possible to draw very general conclusions about network structures (Alon, 2003). For instance, protein interaction networks – like metabolic pathways networks or the Internet – are scale-free. As a result, they are composed of relatively few nodes with many connections, and many nodes with few connections, constituting subsystems (modules) around the former. In such systems, the path from one node to any other is short (Bray, 2003; van Noort et al., 2004). These networks are robust. They survive massive losses of peripheral nodes. However, loss of a few highly connected nodes is lethal (Aldana and Cluzel, 2003). Such a predicted behaviour fits in well with many whole-or-nothing results of gene-knockout models. The network models generate general and even specific predictions that can be tested (von Mering et al., 2003). For example, in yeast, systematic inactivation of each gene and study of the corresponding phenotype allowed researchers to test the validity of the network connectivity. Essential genes are thought to be highly connected hubs in the network, whereas less connected genes are thought to be less essential. By identifying similar essential/secondary phenotypes for thousands of genes, von Mering et al. (2003) confirm parts of the proposed network.

However, in vertebrates, and in yeast under certain conditions, the redundancy of signal transduction proteins will often ensure the absence of a distinctive phenotype for the knockouts. One pathway may complement another for a given function. Loss of function may require concomitant suppression of all network paths for that function. Predicted network structures will incrementally improve as attempts to validate them accumulate. This exercise will be most fruitful if conducted in specific types of cells, under many well-defined sets of conditions. This research agenda is being carried out both in yeast by several groups, and in vertebrate cells by the Alliance Consortium (Gilman et al., 2002). As evolution increases complexity, the application of systems biology will certainly bring its first reliable results in evolutionary simple systems such as yeast and *C. elegans*. Data on protein expression and localisation are becoming available in both models (Huh et al., 2003; Li et al., 2004). The modest aim of proposing testable protein interactions hypotheses and of narrowing the possibilities is already a great benefit of such efforts.

2.3. Shortcomings

However, the prediction of the behaviour of a particular system in defined conditions, on the basis of protein network structure, is fraught with error. Each of the ''omic'' approaches will yield its lot of false positive and false negative results. One may wonder whether significant new molecular interactions will emerge from the background noise. The high-throughput nature of systems biology-related technologies implies that thousands of hypotheses are tested at once. Very stringent statistical significance thresholds are required. A recent study combining several high-throughput methods and selecting the interactions revealed by all of them yielded conceptually sound conclusions that were subsequently validated by tandem affinity purification tagging experiments in yeast (Jansen et al., 2003). Such success in the infancy of the field is comforting (Spirin and Mirny, 2003; Tyson et al., 2003). Cross-technology validation is increasingly applied to reduce the large number of false positives produced by noisy high-throughput methods (Detours et al., 2003).

The proposed network structures are incomplete. They do not account for the absence of some molecular actors in particular cell types. They do not account for differences in kinetics and their consequences. They do not account for concentration–action relations (which might be biphasic), nor do they account for inter- and intracellular compartmentalisation, and other essential features of cell signalling presented in the

next sections. In fact, published general signal transduction protein interaction networks are rarely applicable to the precise situation of a given cell type in a given set of circumstances.

The scale-free character of the network implies that highly connected protein nodes may be linked to dozens of other proteins which, for steric reasons, will exclude each other or interfere with each other, thus suggesting the existence of a range of different complexes whose relative importance will presumably differ among cell types.

The compliance of a theoretical model with experimental observation does not necessarily imply its validity, because variables can often be tuned to fit virtually any desired result. It is interesting to remember at this stage that in the 1970s, simulations of the glycolysis pathway on the basis of the known properties of the involved enzymes seemed to account fully for observed behaviours (Garfinkel et al., 1970; Hess and Boiteux, 1971; Goldbeter, 1976). Later however, the discovery of fructose 2, 6 biphosphate revealed an entirely different and unforeseen regulation network of this pathway which explained its true physiological regulation (Van Schaftingen, 1987, 1993).

The systems biology approach should therefore be pursued and combined at different levels, down to the protein complexes and functional modules, and down to individual proteins in individual systems if possible. Combining the information from horizontal approaches (''omes'') and vertical approaches (one gene, several proteins, several effects for each isoform) will generate huge amounts of data that will have to be treated and integrated in comprehensive databases (''Biological Atlases'') (Vidal, 2001; Ge et al., 2003).

3. COMPLEX MOLECULAR INTERACTIONS UNDERLYING CELL SIGNALLING SPECIFICITY

Network structures published in the recent systems biology literature give a general view of the potential interactions among molecular actors. These are useful in reducing the number of biological hypotheses to be investigated, and in generating useful predictions on the overall structure of the network. However, their ability to predict the behaviour of individual pathways is limited. As a matter of fact, the molecular mechanisms used in cell differentiation to ensure specific cell regulation by the common signalling pathways are precisely the mechanisms which preclude the strict application in a given cell of general protein-interaction-based networks.

3.1. Posttranslational control of protein synthesis

Each protein is regulated quantitatively or qualitatively at transcription, translation, or posttranslation. The response of a given cell to the activation of, for instance, one specific kinase or phosphatase depends on the nature of the available substrates of these enzymes. Within one category of enzymes, the existence of different isoforms may result from the existence of different genes or from the alternative splicing of each gene, from RNA editing, or from processing of each protein. Use of different promoters for the same genes will entail differences in gene expression. Different splicings, RNA editings, or protein posttranslational processing will generate distinct protein isoforms (Cichy and Pure, 2003). Isoforms differing by only one small segment may result in opposite responses to the same signal, e.g. a phosphorylation by cAMP on one optional segment of a protein may produce an effect inverse of that of phosphorylation on another segment. TERP, a truncated estrogen receptor expressed in the pituitary, does not affect the receptor transcriptional activity, but does inhibit its repressors (Lin et al., 2003). Similarly, the truncated MDM2 inhibits MDM2 (Bartel et al., 2002). Assuming a similarity of recognition from a similarity of domain structure is dangerous. Although they lack structural similarity with the insulin receptor, LGRs of the family of glycoprotein hormone receptors are activated by insulin-like hormones (relaxin) (Hsu et al., 2002).

The number of isoforms increases with evolution. It is estimated that the ~25,000 genes in the human genome may generate more than 100,000 different proteins (Brentani et al., 2003). Many of these proteins may have different, sometimes unrelated, cell distributions, functions, affinities for ligands, and controls of expression (Pandini et al., 2003). This diversity is illustrated by cyclic nucleotide phosphodiesterases which are grouped in 12 families, sometimes with multiple genes for each family and/or multiple isoforms for each gene (Wang et al., 2003). This diversity is further compounded by the fact that a single protein may have entirely independent functions (moonlighting) (Jeffery, 2003; McKnight, 2003), and tertiary structures. For example, superoxide dismutase exists in two forms. Each one has a distinctive disulfide bridge pattern and therefore, a unique tertiary structure (Petersen et al., 2003).

3.2. A given signal may have opposite effects depending on the overall molecular state of the cell

There are countless examples of opposite results obtained by the activation of any given pathway in different cell types, or even in the same

cell in different conditions (Dumont et al., 2002). One example is the essential ambivalence of many signal transduction proteins which either promote or inhibit cell proliferation and apoptosis depending on the cell type (Sporn, 1988; Vermeulen et al., 2003). Other well-established examples are the opposite roles of TGFβ at different stages of carcinogenesis (Roberts and Wakefield, 2003) and of E2F3 on tumorigenesis of different cell types (Ziebold et al., 2003). The same cAMP signal may either be an inducer, or a repressor, depending on the state of chromatin in the promoter region (Mulholland et al., 2003). Besides the influence of intracellular protein content, the same cell may respond differently, depending on the architecture of its extracellular support (Abbott, 2003).

As our knowledge of gene expression develops, the number of possible subcategories of cell types, and therefore cell-regulation characteristics, increases rapidly. For example, Purkinje cells, i.e. neurons with the same histology as conventional neurons, express a specific protein pattern (Zoghbi, 2003). Histologically similar endothelia in different tissues are also differentiated (Chi et al., 2003).

3.3. Proteins are localised in specific intra-cellular compartments

Compartmentalisation of proteins and signal molecules is an inherent correlate of subcellular organisation and cell polarity. The latter may be very dynamic as in chemotaxis, in which cell polarity changes with the orientation of the gradient of chemoattractants (Meili and Firtel, 2003).

The same signal may be localised or diffuse depending on the location of the synthesising and catabolising enzymes. One example is the cAMP adenylate cyclase and phosphodiesterase system (Swillens et al., 1974). If both enzymes are located on the plasma membrane, cAMP concentration is uniform in the cell. If the cyclase is on the membrane and the phosphodiesterase is in the cytosol, a decreasing gradient from the membrane to the centre of the cell is observed. The same signal may operate differently at different distances from its emission site. For example, the EGF receptor induces a very localised actin polymerisation during chemotaxis. This local activity contrasts with the body-wide EGF receptor-mediated activation of ERK (Kempiak et al., 2003).

Location of the same protein in different cell compartments or different supramolecular complexes may result in entirely distinct responses to stimulus. For example, nuclei located at the neuromuscular junction respond to stimuli by inducing genes of synaptic proteins, whereas nuclei located elsewhere in muscles respond by repressing acetylcholine receptors

(Schaeffer et al., 2001). Compartmentalisation of a protein greatly reduces the number of possible interactions. For example, scaffolding proteins JIP1 and ksr, involved in MAPK signalling, hold in a distinct supramolecular complex all the enzymes of a cascade in which each enzyme modulates its successor (Douziech et al., 2003).

Compartmentalisation must be postulated to explain how the same molecule can be used in one cell as a signal at very low concentrations, and as a metabolite at much higher concentrations. (e.g. H_2O_2 in macrophages or thyrocytes). Compartmentalisation also explains the important roles of scaffolding or anchor proteins. For instance, AKAP proteins localise cAMP-dependent protein kinase, which allows a spatially restricted interaction of cAMP and this kinase (Hulme et al., 2003), and thus of protein substrate phosphorylation.

Compartmentalisation is also a dynamically regulated process in which a protein is segregated, and consequently inactivated, in one compartment and activated after translocation in another compartment. For example, p53 is inactive in the cytoplasm, but activated in the nucleus (Nikolaev et al., 2003).

3.4. The combinatorial complexity of protein–protein interactions is huge

The response of a cell may result from the specific combination of the actors involved, which may be missed when networks are considered out of a precise physiological context. This has been especially well analysed in the control of transcription where only specific combinations of transcription factors will elicit a response. The sophistication of this combinatorial logic increases with evolution to higher living forms. It seems that the increasingly more elaborate regulation of gene expression accounts for organismal complexity (Levine and Tjian, 2003). Combinatorial variations involve the following:

- The specific combination of existing actors such as receptors, transcriptional factors, kinases, etc. may combine with an *and* control (each factors is necessary), or an *or* control (either factor is sufficient). Such regulations have been demonstrated in physiology for odour discrimination by the olfactory system. Each odour activates a specific combination of receptors, and thus of olfactory cells (Malnic et al., 1999). Similarly, different combinations of transcription factors are necessary to obtain different gene expression patterns (Ghazi and VijayRaghavan, 2000). Such combinations may partially overlap. A special case in the category is the specific addition of one factor acting as a switch to the existing

combination of factors, e.g. one general transcription factor complementing various existing combinations is necessary and sufficient to trigger an outcome specific of this combination. Induced Egr1 combined with different other transcription factors in gonadotrophs or Sertoli cells triggers the induction of different genes (Tremblay and Drouin, 1999). LHβ and αGSU, two genes needed for luteinising hormone (LH) synthesis, are activated by distinct, but partially overlapping, combinations of transcription factors (Jorgensen et al., 2004).

- The specific combination of posttranslational modifications on a given protein may determine activity or location. For example, the regulation of p53 expression is controlled by a phosphorylation code, each phosphorylation site having a functional meaning (Webley et al., 2000).
- The sequence of the molecular events is essential. The expression of a gene depends successively on the nature of its responsive element (RE), on the transcription factor(s), on the opening or closing of the chromatin, DNA methylation, specific histone phosphorylation, methylation, or acetylation, etc. (Schreiber and Bernstein, 2002). Expression also depends on the qualitative or quantitative modulation of the transcription factor by posttranslational modification or on allosteric agent binding, on their complement of coactivators and corepressors, etc. (Holstege et al., 1998; Yamamoto et al., 1998). Studies on estrogen receptor-α targets have revealed a sequential and combinatorial assembly of dozens of transcription factors controlling the timing of promoter activation, supporting the concept of a ''transcriptional clock'' (Metivier et al., 2003).

3.5. Effects of signals depend on their timing

In addition to the sequence of molecular events, the duration and/or rhythmicity of the signal may specify qualitatively the response. Bimodal-signal processing characteristics with respect to stimulus duration have been demonstrated for NFκB, the main transcription factor involved in inflammation (Hoffmann et al., 2002). With regard to rhythmicity, high-frequency calcium pulses account for cardiac rhythmic contractions while sustained intracellular calcium concentrations and calcineurin activation are responsible for heart hypertrophy (Vega et al., 2003). Egg to embryo transition is driven by differential responses to Ca^{++} oscillation frequency (Ducibella et al., 2002) Other phenomena typically overlooked by systems biology approaches include:

- The quantitative variations of stimulus strength, which may result in the activation of distinct cascades, sometimes with opposite effects.

An example is the opposite effects of the same MCSF signal but at different concentrations in cell proliferation and ERK activation (Rovida et al., 2002). The TNF receptor activates both an apoptotic and a survival cascade (Micheau and Tschopp, 2003). Estrogen receptors α or β have opposite effects in response to estrogen (Weihua et al., 2003). The final effect reflects the balance between the responses of both receptors to estrogen.

- The quantitative differences of expression by quantitative or qualitative modulation of synthesis or degradation: cyclin, which trigger cell cycle and DNA synthesis, are regulated both at the transcriptional level, and at the level of their degradation following ubiquitinylation (Reed, 2003).
- Both positive and negative specific modulators, which may act on the expression of proteins at various levels, on the synthesis or degradation of mRNAs, on proteins intracellular signal molecules, on posttranslocational control of proteins, or directly on the activity of proteins. (Dumont et al., 2002).

4. CONCLUDING REMARKS

Evolution increases complexity. The number of protein isoforms increases considerably from simple organisms like *C. Elegans* to man. If different isoforms have evolved to carry on specific functions, one would expect specificity to increase also with complexity, possibly at the price of a loss of efficiency. In the case of the duplication of follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH), and of their receptors from their common ancestral genes, a great increase in specificity allowing a complete separation of the function of the two signalling systems entails a decreased affinity (Smits et al., 2003). Similarly, analysis of protein domain interactions in yeast suggests system-wide negative selection optimising specificity in a network (Zarrinpar et al., 2003). Thus, evolution increases both the number of possible actors at many steps of signal transduction cascades and also confers them with interaction specificity, which allows the same cascades to produce different regulation patterns in different cell types.

Systems biology and physiology are not antagonistic, but complementary. The former will allow the formulation of general concepts of regulation and provide the catalogue of possible molecular interactions to be considered in the study of any given model. Physiology and cell biology will define what makes any cell type at a given time in its history

specific, and allow detailed predictions on behaviour in normal or pathological conditions.

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