Opinion

Cell PRESS

Feature Opinion

Cell regulation: determined to signal discrete cooperation

Toby J. Gibson

Structural and Computational Biology Unit, European Molecular Biology Laboratory, 69117 Heidelberg, Germany

Do kinases cascade? How well is cell regulation understood? What are the best ways to model regulatory systems? Attempts to answer such questions can have bearings on the way in which research is conducted. Fortunately there are recurring themes in regulatory processes from many different cellular contexts, which might provide useful guidance. Three principles seem to be almost universal: regulatory interactions are cooperative; regulatory decisions are made by large dynamic protein complexes; and regulation is intricately networked. A fourth principle, although not universal, is remarkably common: regulatory proteins are actively placed where they are needed. Here, I argue that the true nature of cell signalling and our perceptions of it are in a state of discord. This raises the question: Are our misconceptions detrimental to progress in biomedical science?

A diffuse understanding of cell regulation?

As biochemical data for cell regulatory networks accumulate they are increasingly being used to develop models of these systems. Most often, these models are based on diffusion equations derived from solution chemistry. When the law of large numbers applies, the models are deterministic; when there are few molecules in the system, the models are stochastic. Are these current approaches the best way to model cell regulation? Models that poorly fit the real system will lack predictive power. Experimental results from diverse processes throughout the cell might

Glossary

Allostery: reciprocal energetic coupling between two (or more) distinct binding events affecting the output of the ligand module. Allosteric effectors abound in the regulation of metabolic enzymes. Allosteric regulation of signalling is exemplified by the regulatory GTP-binding proteins. See Fenton [60] for discussion of a strict definition for allostery.
Cooperativity: biochemical term applied to multiple binding interactions that influence each other either positively or negatively. Allostery is a common variety of cooperativity. Preassembly of a complex to create a novel ligand-binding site (as shown in Figure 5) is a second form of cooperativity. More recently, it has been recognized that multivalent cooperativity whereby a ligand can make multiple distinct binding contacts is an important contributor to the cellular complexes making regulatory decisions [59,116]. Note, therefore, that cooperativity is used in a relaxed sense in this article. Weak interaction affinities are required for dynamic cooperative effects to be realized, regardless of the exact definition.
Deterministic: a deterministic process is one for which future evolution can be predicted. Many mathematical models are deterministic, such as those involving differential equations to solve a rate of change over time. The laws of chemistry are deterministic, provided that the law of large numbers applies.
Deterministic signalling engine: signalling engine that will always produce the same results for the same input. A computer is designed to operate as a deterministic engine (at least until the hard disk breaks).
Discrete model: a model for a system in which events occurring over time can have abrupt transitions and therefore cannot be represented in a smooth continuous framework. For cell signalling components that exhibit temporal discreteness, spatial discreteness is also expected because biochemical regulation proceeds by direct molecular interaction.
Law of large numbers: mathematical law stating that as the number of a random variable increases, the sampled mean approaches the theoretical mean. Proven by Jakob Bernoulli in 1713, the law of large numbers is particularly important in chemistry, where reaction rates are accurately predictable for macroscopic quantities of reactants.
Linear motif: abundant short regions of eukaryotic proteins (typically peptides of between 3 and 10 amino acid residues long) to which a distinct molecular function independent of the larger sequence/structure context can be assigned. Nearly always involved in regulatory processes. Some are sites of PTM and others are not. The function is almost always mediated by interactions with one or more globular domain classes.
Kinase cascade: as currently defined in the Gene Ontology ''A series of reactions, mediated by protein kinases, which occurs as a result of a single trigger reaction or compound.'' This definition is relatively innocent; the horrors of the term are implicit to the name itself.
Molecular switches: molecules that can be reversibly shifted between two or more stable states. In signal transduction, most molecular switches (e.g. G proteins) are proteins that interact with different ligand sets depending on conformational state. In future, I consider it likely that some switches will be better described as complexes of proteins, rather than as single proteins.
Natively disordered protein: segments of polypeptide that do not form autonomous stable 3D structures in the native state. Rarer in prokaryotes, but very abundant in regulatory proteins of the eukaryote cytosol and nucleus.
Reaction–diffusion models: models adapted from the laws of solution chemistry and increasingly applied in many fields. Early pioneers in biological application were Hodgkin and Huxley for electrical propagation in nerve cells and, famously, Turing for a model of how the leopard gets its spots. Models are often smoothly deterministic, but they can be stochastic. The differential equations for complex systems are hard to calculate, which is a problem in moving beyond restricted models of individual signalling pathways in isolation from the larger networks. The models assume that interactions are independent and do not take cooperativity into account.
Smooth model: a smooth process is one in which the time line can be modelled by differential equations because it lacks abrupt transitions.
Stochastic: a stochastic process is one with non-deterministic behaviour. In general the future evolution of a stochastic process cannot be reliably predicted. Therefore, stochastic models must include probability estimations as to when a specified event might occur. Brownian and diffusion calculations for intracellular components must use stochastic models when there are only a few molecules of a given type. One of the paradoxes of cell signalling is that it can be highly robust despite very low abundance of many regulatory molecules: how much does the cell leave to chance under normal working conditions?
Systems biology: field that aims to understand and predict the behaviour of the system as a whole. In contrast to the reductionism favoured by most experimental biologists, practitioners tend to adopt a synthetic philosophy. In practice, systems biologists are found among diverse topics such as quantitative data gathering and precision modelling, hypothesis generation and large-scale (omics) data acquisition allied to large-scale perturbation assays. For any nontrivial system, a computational model is essential.

Corresponding author: Gibson, T.J. (toby.gibson@embl.de).

0968-0004/$ – see front matter doi:10.1016/j.tibs.2009.06.007 Available online 8 September 2009

not fit well to these smooth deterministic models. The puzzling lack of sequence specificity of many protein-modifying signalling enzymes, the large protein complexes extracted from cells, the massively interacting hub proteins in interaction networks, the remarkably precise movements of proteins around the cell and many other observations are collectively difficult to reconcile with models derived from solution chemistry. Here I argue that control throughout the cell is governed through the assembly of molecular switches into large regulatory complexes that might be better modelled as discrete, perhaps even deterministic, signalling engines. Will you now join me on a tour of the cell so that we can examine the supporting evidence? Along the way we will see kinase gradients and scaffolds, localized protein translation, network hubs, linear motifs, allosteric regulators, microtubules, cilia, the nuclear pore and much more. It will be evident from these examples that it is cooperativity, and not diffusion, that is the key to understanding cell regulation. Consequently, there is now a need to develop appropriate discrete models and to explore their limits: when does the system relax enough to allow simple diffusion to play a meaningful role in cell regulation?

Gradients of signalling components in the cell

Powerful imaging technologies are now facilitating the design of experiments that can monitor signalling enzymes such as kinases and phosphatases in their active state in living cells. Using an antibody specific for activated mitogen-activated protein kinases (MAPKs), with both the antibody and kinase labelled with different fluorophores, Maeder *et al*. [1] harnessed Förster resonant energy transfer (FRET) with fluorescence lifetime imaging microscopy (FLIM) to study the intracellular location of yeast MAPKs under pheromone stimulation. These studies revealed a cytosolic gradient of activated Fus3 MAPK emanating from the mating projection (termed the shmoo). The gradient fitted comfortably to a classical reaction–diffusion model in which active Fus3 were steadily inactivated by soluble cytosolic phosphatases as they diffuse away from the shmoo. Reaction–diffusion models, which are smoothly deterministic (when the law of large numbers applies), are commonly used to model cell regulatory systems. Their adoption as the primary model in cell system regulation leads naturally to the expectation that kinase and GTPase gradients are widespread in the cell and fundamental to most intracellular signalling [2].

The FRET-FLIM technology was applied in a second intracellular study of an activated signalling enzyme, in this case the mammalian tyrosine phosphatase PTP1B [3]. Although a steady-state gradient could be observed in stimulated cells, this time the conclusions were rather different. In this case, a reaction–diffusion model could *not* account for the data because the gradient was remarkably robust to large parameter variations, including variation in component concentrations. The authors concluded that spatial regulation of PTP1B activity was occurring. This presents a problem for cell simulation because, when the favoured smooth deterministic models do not apply, discrete ones must be used. Although smooth models can be adapted from the laws of chemistry, this is not the case for discrete deterministic models, which might be one of the reasons why there seems to be very little effort devoted to their introduction.

A diffusion gradient might be well suited to a polarized signalling system (as with the *Drosophila* morphogens discussed later) but most other parts of the system ought not to be represented with a diffusion-based model. Much of yeast MAPK signalling is concentrated in the shmoo tip where several active kinases are in complex, suggesting that discrete modelling is required for this case. Furthermore, shuttling of Fus3 into and out of the nucleus are active processes, whereas ChIP-chip studies have shown that Fus3 is bound at the promoters of genes that it regulates [4], again implying that a discrete model is appropriate.

Trouble with kinase cascades

Notwithstanding the fact that the original evidence was fabricated [5,6], more than 10 000 PubMed abstracts include the words kinase and cascade, implying that this concept is truly a fundament of cell signalling pathways. The word cascade is redolent of linearity, stepping, irregularity and acceleration. An element of stochasticity is implied in the sense that, if a yellow bath duck is placed at the top of a water cascade, it cannot be predicted exactly where it will land. The most famous kinase cascade is the MAPK cascade, which transmits signals from the plasma membrane to the nucleus to regulate gene expression [7].

The Akt (also called protein kinase B, PKB) cascade is another that is intensively studied. It can be activated by several different kinases (e.g. PDK1, mTOR, ILK and MAPKAPK2) and regulates a large number of downstream proteins, subject to a number of feedback control loops [8]. However, current schematic drawings of Akt regulation look like a network and it no longer seems possible to depict this system in a fashion that reveals its cascading properties as required by received opinion. Can the two terms network and cascade accurately describe the same system?

To the detriment of progress in signalling research, it is notoriously difficult and laborious to establish kinase–substrate relationships [9]. A relatively easy experiment to perform is to overexpress a kinase and observe the newly phosphorylated proteins. However, an overexpressed kinase might enter the wrong cell compartments, is likely to be dysregulated and is no longer in balanced gene dosage with various scaffolding components with which it is otherwise associated. The same is true for *in vitro* work, in which special activating buffer conditions are used so that the kinase will phosphorylate any peptide matching its sequence specificity. For example, Ser46 of p53 is phosphorylated by HIPK2 during apoptosis [10]. HIPKs belong to the large paralogous family of proline-directed kinases that phosphorylate Ser-Pro or Thr-Pro sites [11]. This family includes the MAPKs, so how do activated MAPKs, unlike HIPK2, avoid phosphorylating p53 on Ser46 thus risking triggering apoptosis instead of growth? In part this could be achieved because MAPKs also require a docking or activating motif in the substrate [12] that is not present in p53. Likewise, the master kinase PDK1 is targeted by a docking/activation motif present in the many other AGC group kinases (a group of related kinases defined by its best

known members PKA, PKC and PKG), which it phosphorylates and activates; the same motif then works in *cis* as part of the activation system of the AGC kinase itself [13]. Tyrosine kinases seem to have little or no sequence specificity and are not known to have specific docking motifs, yet they too clearly differentiate their substrates. All through the cell there are poorly sequence-specific, post-translationally modifying (PTM) enzymes that exhibit highly specific substrate selection. The only conclusion that can be drawn is that diffusion is not the general mechanism by which kinases, acetylases and similar enzymes associate with their substrates. Why then are smooth reaction–diffusion equations often the main component of models applied to kinase signalling systems [14–18]?

Many heavily investigated kinases are bound by scaffold proteins [19]. For example, PKA, MAPKs and JNK are all scaffolded [20,21]. Scaffolds facilitate assembly of a signalling complex including the kinase itself, its upstream activators (e.g. G proteins), its substrates, its positive and negative allosteric regulators and even other kinases and phosphatases [13,20]. Because many kinases are still poorly investigated, it is not yet clear whether scaffolding is a general solution. However, the requirement to differentiate their substrates implies that this might be the rule rather than the exception. As well as affording a discrete platform for bringing kinases, regulators and substrates together, some scaffolds are actively transported along cytoskeletal filaments by motor proteins [21,22]. Therefore, at least some scaffolds do not need to diffuse to change their cellular location.

Scaffolds fit poorly into linear pathway models of cell regulation. Of course the MAPK cascade itself is only linear when taken out of context of all the other interactions that feed in and out of MAPK regulation [23]. Viewing cell signalling systems as being networked rather than as a series of pathways can provide useful insights, for example by adding power to attempts to define kinase–substrate relationships [24]. Apparently simple linear signalling pathways might often be an artefact of reductionist experimental design whereby expression of a specific gene is assayed following a single perturbation at the cell membrane, keeping all other conditions constant. From the network perspective, kinase cascades must be a highly misleading concept. Will it take another 10 000 publications before this damaging terminology is properly deprecated and falls into deserved disuse?

Modular architecture of signalling proteins

At present, biochemistry textbooks fail to give an accurate picture of the massively modular architecture of many regulatory proteins and what this allows them to get up to. The ~750-residue protein tau, best known as a hyperphosphorylated component in the neurofibrillary tangles of Alzheimer's disease, has no recognizable secondary structure in the native state and is one of the earliest proteins to have been characterized as natively disordered [25]. Tau regulates the processivity of dynein and kinesin motors along microtubules [26,27]. Being natively disordered, all ~750 of the amino acids of tau are accessible and available to interact with other proteins. Most amino acid residues in the tau sequence are conserved across mammalian species, implying that they are required for function (because in tau they do not contribute to any tertiary structure). Many conserved serine and threonine residues are candidate phosphorylation sites and it has been reported that numerous kinases phosphorylate tau [28] {see also tau entries on Phosphosite (http://www.phosphosite.org/) and Phospho.ELM [29]}. A number of phosphatases are also believed to dephosphorylate tau [28]. Four ~30-amino-acid repeats are tubulin-binding motifs and these overlap with several phosphorylation sites (Swiss-Prot:tau_human). All these sites on just this one protein create the potential for vast and convoluted phosphorylation-based regulation of motor activity.

However, tau is just one of many microtubule-associated proteins (MAPs) and end-binding proteins (EBs) that regulate microtubule-based transport systems [30,31], most of which are thought to have large regions of natively disordered polypeptide. One of the quieter bioinformatics revolutions in the present millennium has been the ability to robustly predict native disorder in proteins [32–34]. It is estimated that 25–30% of the eukaryotic proteome content is natively disordered [35,36]. It has become clear that our perceptions of protein structure have been inadequate, so that tau is not an oddity but actually a perfectly normal protein; throughout the proteome, most reported phosphorylation sites are in flexible regions of native disorder and not in structured domains [37]. Phosphorylation sites belong to the broader protein module category of linear motifs [38], short protein interaction sites that are often (but not always) modified and that usually bind by an induced fit mechanism to specific ligand domains [35]. Hundreds of instances of linear motifs belonging to >130 functional types have been collated by my colleagues in the ELM resource [39] and their association with native disorder is overwhelming [37,40–42]. Indeed, although there are certainly other functions for natively disordered polypeptide, we consider that its main role is to act as a repository for linear motifs involved in protein–protein interactions [43].

An increasing number of massively interacting proteins have been identified in protein interaction networks. These are termed hubs [44]. A frequent characteristic of hub proteins such as CBP/p300, p53 and caldesmon is that they have extensive regions of native disorder [33,45,46]. These partly unstructured hubs are always regulatory proteins (in contrast to natively folded hubs such as the cytoskeletal structural components actin and tubulin). The CBP/p300 hub is a key transcriptional regulatory protein that interacts with different sets of proteins in different transcriptional regulatory complexes [47]. Figure 1 summarizes the set of reported experimental interactions of p300 described in the STRING resource [48]. In the case of the intensively investigated hub protein p53, solved structures of complexes for a subset of the reported interactions are now available. Figure 2 provides a snapshot of the p53 interactions currently supported by structural data. Observe how efficiently the natively disordered sequence is used for regulatory purposes, even more remarkable because most of the known interaction sites – including numerous PTMs – are not shown in the figure. A striking

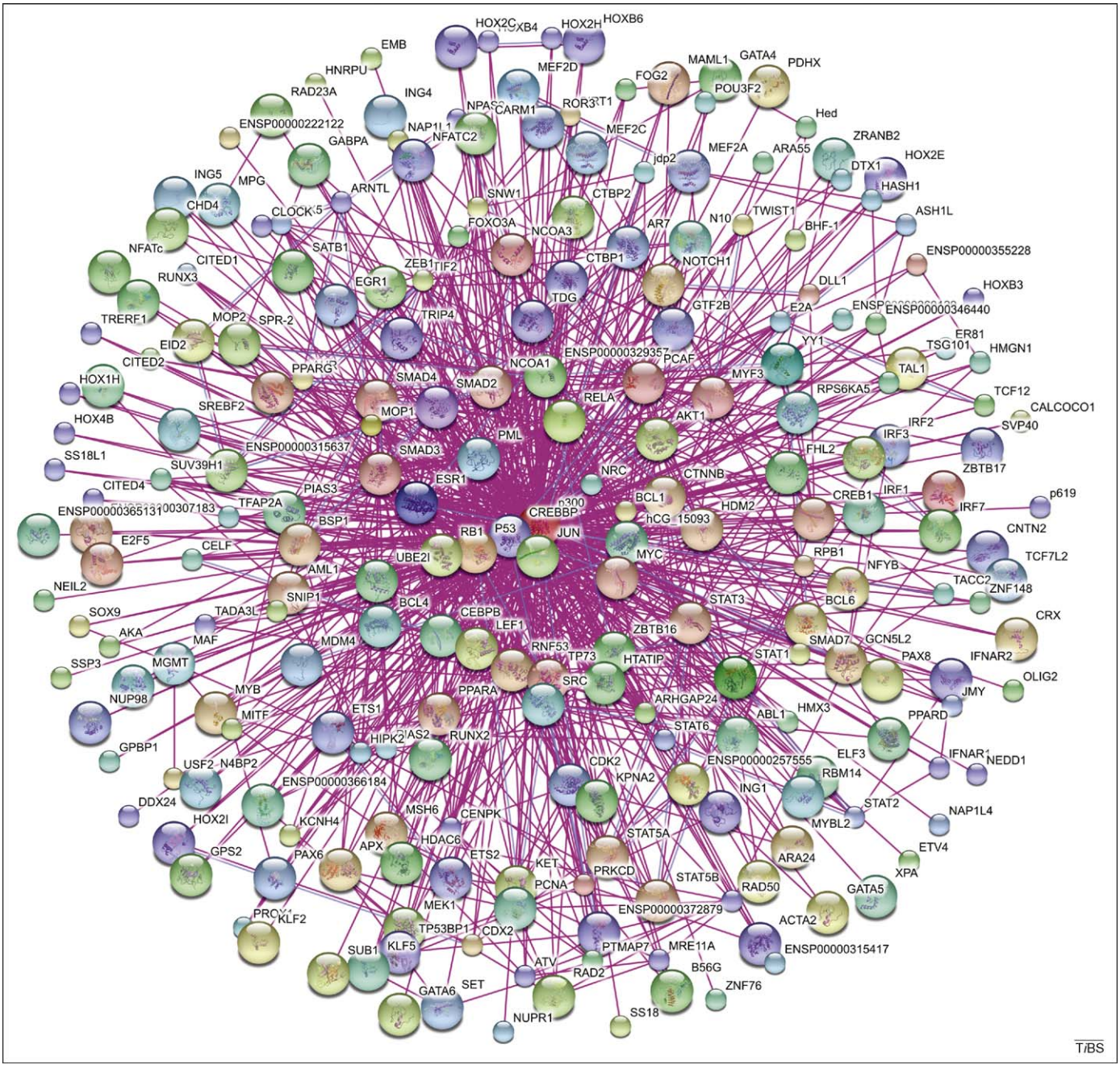

Figure 1. p300 is a gene regulatory hub protein. The figure represents the interacting proteins for which experimental data have been annotated in the STRING resource (http://string.embl.de/). p300 is represented by the almost buried red sphere at the centre. Where the network topology permits, interactors for which the strongest evidence is available are placed closer to the hub. Larger spheres indicate proteins for which some structural data are available.

feature of these interactions is the repeated use of overlapping sequence segments, implying that many interactions are mutually exclusive and thereby operate as molecular switching mechanisms [45]. It is important to understand that these molecular switches can only work in an orderly and efficient manner in preformed complexes. A complicated switching mechanism would not be feasible if p53 and all its interactors were freely diffusing. The massive intrinsic subfunctionalization of p53 is further interdependent on its cellular location and the presence and modification status of hundreds of other interacting proteins. The corollary is that, at the biochemical level, the function of p53 cannot be simply described. From this perspective, then, it is not surprising that highly inconsistent and conflicting results are continually obtained in p53 research. This, of course, extends more generally throughout research into cell signalling, well beyond p53 research.

Thus, the great void in current textbooks is this whole interplay of abundant linear motifs contained within large segments of disordered flexible polypeptide as a platform for massive interaction, regulatory complex formation and molecular switching and therefore they fail to explain the structural basis for cell signalling and regulation. It is as though the catalytic engines of the cellular vehicle are displayed, but not the wiring diagram. If new generations of university students are still being exposed to information that is greatly misleading and is not approaching

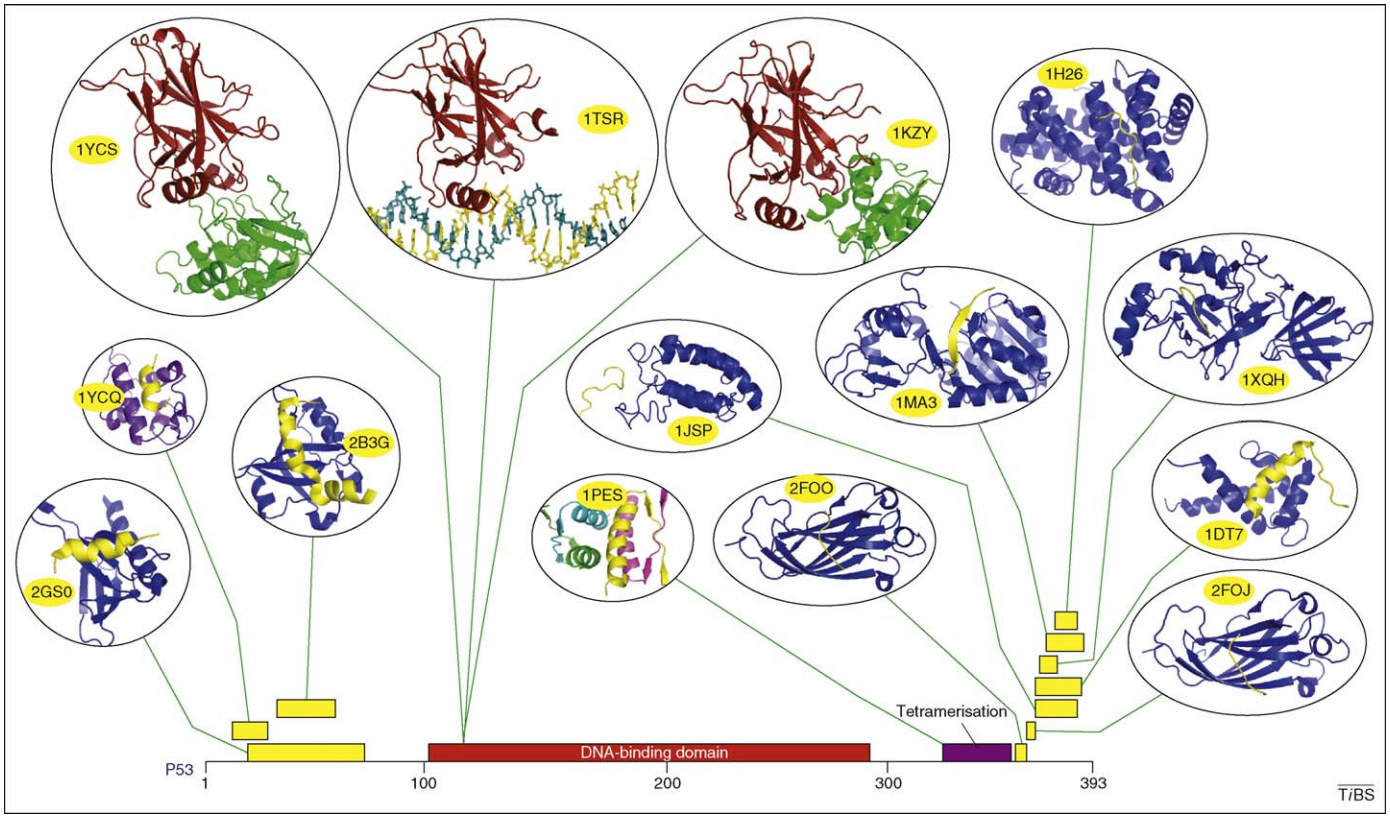

Figure 2. Molecular switching with p53. Interactions involving p53 for which structures of complexes have been determined. There are many intrinsically unstructured binding modules in p53 but only one globular module. Functional modules are indicated by blocks along the p53 sequence: the globular DNA-binding domain (red); the mutual fit tetramerization domain (purple); and induced fit linear motifs (yellow). Green lines link modules to cartoon depictions of the structures that have been determined for these modules (using the same colour scheme as above) and the interacting partners (either blue, purple, green or mixed colours) (prepared with PyMOL). The protein databank (PDB) entries for each structure are indicated in the yellow ovals. Note that most of the interacting regions of p53 overlap with other regions, both in the natively disordered regions and the DNA-binding domain, implying that mutually exclusive interactions are very common for p53. The clustered overlaps toward the C terminus are suggestive of a multi-way switch.

a state-of-the-art understanding of cell regulation, is it reasonable to expect them to design appropriate experiments as they move into the laboratory?

The human proteome has the capacity for many hundreds of thousands of phosphorylation sites and, more generally, millions of linear motifs, as well as larger induced fit polypeptide interactions. Invoking Theodosius Dobzhansky's famous statement "Nothing in biology makes sense except in the light of evolution", there is surely little evolutionary sense to having this capacity and then not using it.

Dynamic regulatory complexes

The two main locations within the cell where regulatory complexes are assembled are the cytosolic side of the membrane systems and the chromatin. For example, integrin signalling adhesion foci are large complexes with >150 known components linking the extracellular matrix to actin filaments [49]. Paxillin is regarded as a key scaffold protein interacting with many of the regulatory proteins localized on the cytosolic side of focal adhesion complexes [50]. In general, signals are received and processed at the plasma membrane, but information must be transmitted to the nucleus to regulate gene expression. In many cases, the transcription factor (TF) itself carries the information, beginning its function in a membrane-associated complex, being phosphorylated and released, entering the nucleus, binding at the promoter, transiently activating transcription, being ubiquitylated, sorted to the proteasome and destroyed. NF-κB, STATs, SMADs and β-catenin all follow this model [51–54]. Sharing the same ultimate fate as the TFs they have just phosphorylated, many transmembrane receptors are endocytosed, ubiquitylated, sorted to the lysosome and destroyed after effecting a single activation step [55].

The highly dynamic clathrin-mediated endocytic system is regulated by large highly modular proteins with extensive segments of native disorder containing a profusion of short linear motifs, including the clathrin box itself that docks regulators to the clathrin cages [56,57]. With their small buried surface areas, linear motifs tend to make low-affinity interactions, very often in the 10–50 μM range. To assemble a stable multiprotein complex, groups of linear motifs must bind cooperatively, a property that is ideal in the dynamic assembly of endocytic complexes. Low-affinity linear motifs make a fascinating analogy to sub-atomic physics, in which the weak force is sometimes regarded as much more interesting than the strong force.

Tyrosine phosphorylation of the LAT adaptor protein by the T-cell receptor results in assembly of an activated LAT–GRB2–SOS1 complex via two types of multivalent linear motif (Figure 3). Unmodified constitutive SH3 domain-binding sites cooperate with SH2 domain-binding pTyr motifs [58]. Only when the LATs are phosphorylated

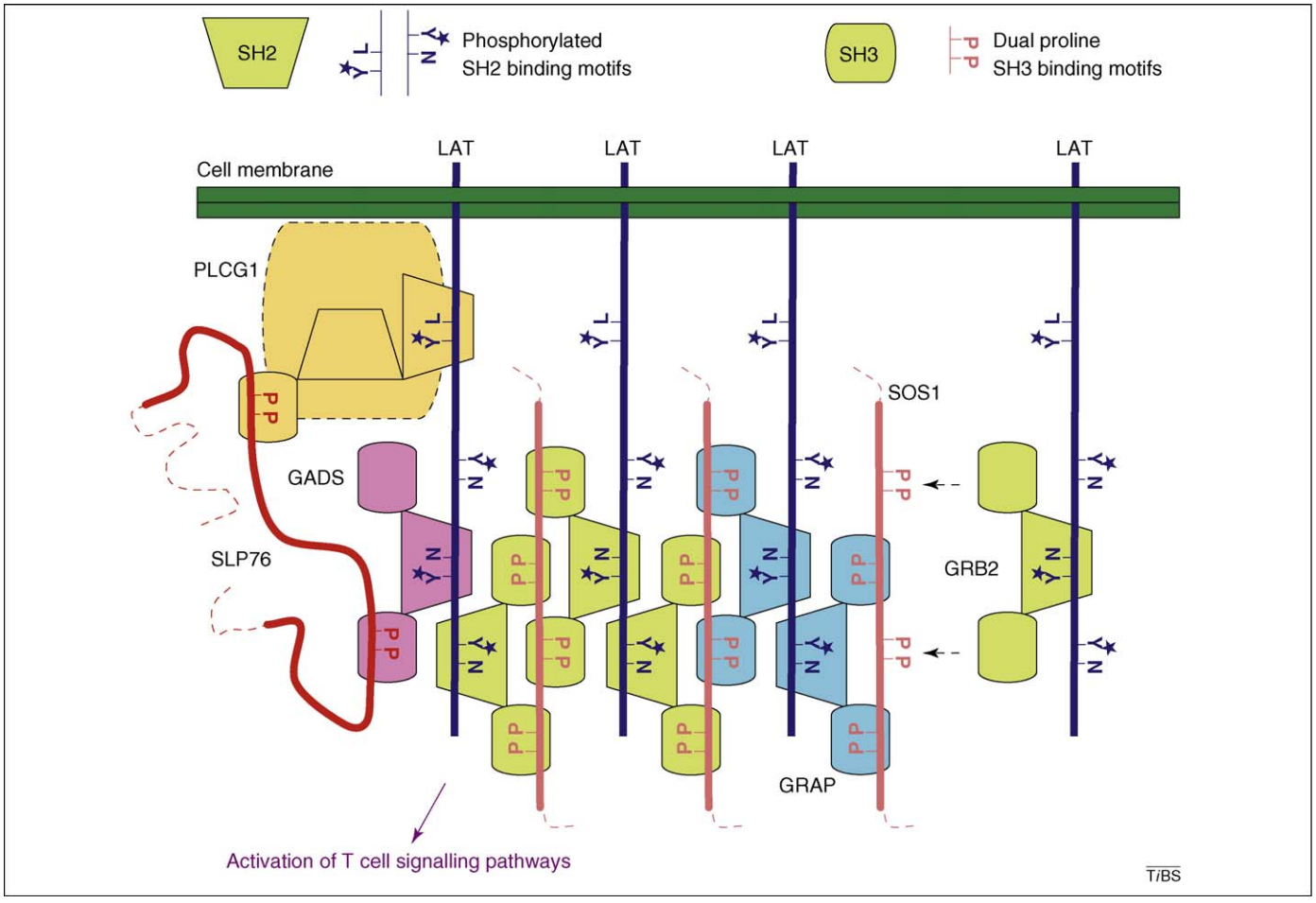

Figure 3. Simplified scheme of a dynamic complex assembled by linear motif interactions. LAT is a natively disordered membrane-anchored adaptor protein that is rapidly phosphorylated on multiple tyrosines following T-cell receptor activation. SH2 domains in GRB2 family proteins and PLCG1 bind at phosphotyrosine motifs. SH3 domains in GRB2 and paralogues bind to SOS1 (and CBL, not shown), creating large oligomerized complexes (visible as punctate clusters by cell staining). PLCG1 and GADS do not contribute to oligomerization but are shown recruiting SLP76 into the complex. LAT–GRB2–SOS1 form a platform for the recruitment of other factors in T-cell signalling (not shown). Globular domains are represented by shapes and natively disordered polypeptide segments by lines. Proteins are colour-coded. Adapted from Fig. 7 in Houtman et al. [58].

can the SH2- and SH3-containing GRB2 proteins cross-link LAT with SOS1. This key signalling complex illustrates how PTM motifs can trigger a switch to an altered regulatory state using multivalent cooperativity to generate a complex whose component proteins associate with much higher affinity than the individual motif interactions.

Cooperativity in the LAT complex seems to be a result of the increase in stability that can arise from multiple, yet independent, low-affinity interactions. As noted by Whitty, it is now necessary to recognize multivalency as a form of cooperativity [59]. Although characteristic of linear motif-based complex formation, multivalency is often found in conjunction with other forms of cooperativity such as allostery [60]. A nice example of linear motif allostery is binding of the PHD finger in *Drosophila* Pygopus to dimethylated lysine 4 on the histone H3 tail as part of Wnt signalling (Figure 4). This interaction requires prior binding of another peptide motif from the Legless protein, which stabilizes the binding cavity for the H3 tail peptide but makes no direct contacts with that peptide [61]. Linear motifs can also allosterically modulate other types of regulatory interaction. For example the LxxLL motif of nuclear receptor co-activators cooperates allosterically with steroid molecules such as androgen in the activation of target genes [62]. Another form of cooperative interaction is provided by the SCF^{Skp2} ubiquitin ligase complex, which binds the phosphodegron motif of the cell cycle kinase inhibitor $p27^{Kip1}$ (Figure 5). The phosphodegron peptide lies across the interface of two globular domains, Cks1 and Skp2 [63]; therefore, the SCF^{Skp2} complex must be preformed before the peptide can be bound and $p27^{Kip1}$ targeted for destruction, as required for the G1–S transition of the cell cycle. There could be many other ways to introduce cooperativity into binding interactions and it might be important to catalogue and classify them.

An example of a critical linear motif mutation is found in the archetypal v-Src oncoprotein. The C-terminal tyrosine (Tyr527) is mutated so that the Src SH2 domain cannot bind into the closed conformation and therefore continually drives cell replication toward cancer [64]. Other v-Src mutations are required for fully aggressive tumourigenesis. More recent evidence reveals that SH2 regulation of tyrosine kinases can be a highly cooperative process [65]. As in v-Src, lesions disrupting one functional element in a modular protein often have dominant negative phenotypes. However, an important and perhaps non-obvious

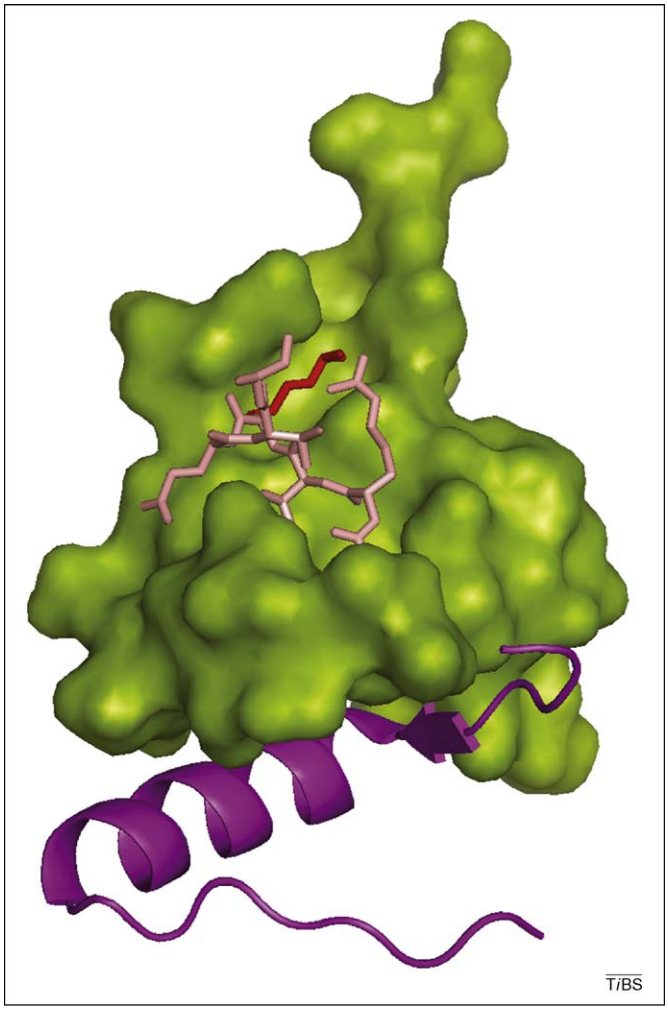

Figure 4. An allosterically regulated linear motif interaction. Methylated lysine-4 residues in histone H3 tails are markers for active promoters that are recognized by members of the PHD finger domain family. The H3-K4me2 peptide (pink sticks, red for dimethylated lysine) is bound in a groove of the Pygopus PHD finger (green surface). This interaction can only occur if a segment of the Legless protein (purple ribbon) is already bound, resulting in allosteric rearrangement of the docking groove. There is no contact between the H3 and Legless peptides, so cooperativity is indirect. The figure was prepared with PyMOL using the entry 2vpe.pdb.

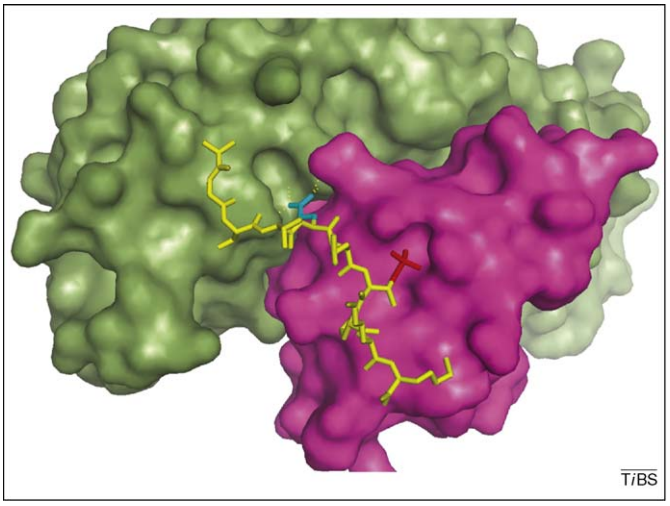

Figure 5. A linear motif binding site that spans two globular domains requiring the prior assembly of a protein complex. The cyclin regulator Cks1 (mauve) is assembled with the LRR domain of Skp2 (green), part of the SCF^{Skp2} ubiquitin ligase complex. The phosphodegron peptide of $p27^{Kip1}$ (yellow) binds across the interface of the preassembled domains. The pThr-187 phosphoryl group (red) binds in a surface depression of Cks1 and a Glu residue (cyan) binds in a depression at the Skp2–Cks1 interface, making polar interactions with both molecules: The binding energy of this residue is therefore dependent on domain dimerisation. Ubiquitylation and proteasomal destruction of the CDK inhibitor $p27^{Kip1}$ during cell cycle regulation can therefore be regulated not only by $p27^{Kip1}$ phosphorylation, but also by regulation of assembly of the SCF^{Skp2} ubiquitin ligase complex. The figure was prepared with PyMOL using the entry 2ast.pdb.

consequence of regulation by cooperative low-affinity interactions is that individual linear motifs can yield vanishingly weak phenotypes when mutated. This is well illustrated by a survey of mutations causing genetic disease: many mutations were identified in linear motif-binding globular domains, but essentially none in the motifs themselves [66]. Whereas the topic of natural selection of weak phenotypes is fascinating for evolutionists (but well beyond the scope of this article), unfortunately the likely consequence for experimentalists who cannot see an effect of a mutation will be to discount any functionality of the sequence segment. There is a need for increased awareness of how complex systems fail under stress (Box 1) and how graduated responses require buffering and fine tuning of regulatory networks.

Nuclear discretion

A large-scale survey of yeast protein expression confirmed that most TFs are present at very low levels, often less than 50 molecules per cell [67]. This finding implies that a typical TF in yeast regulates very few genes. Despite their low abundance and few binding sites, these TFs do not have difficulty in finding their target promoters and correctly regulating gene expression. If low molecule numbers lead to unreliable gene expression, natural selection should favour an increase in their numbers. Do these transcription factors find their promoters solely by diffusion through the nucleus, or would this create risks of gene expression failures, jeopardizing cell survival?

Box 1. Decision making and robustness in complex systems

For any complex system – for example, international air traffic or distributed computational networks [117] – it is important that the system can fail gracefully, at least until conditions become so extreme that the system effectively ceases to exist. In biological terms, system robustness is certainly subject to natural selection: the obvious question is which organisms never experience extreme conditions. The weak phenotypes associated with disruptions to most chaperones and many linear motif mutations [66] indicate cell systems that do indeed fail gracefully. Simple regulatory systems are likely to exhibit early catastrophic failure and therefore simple selection against catastrophic failure will favour the evolution of complex systems. The other (although interrelated) reason for complex regulation is the need to make a given decision based on multiple inputs of the cell state. Biological systems are indeed robust and this robustness is achieved through complex control mechanisms [118]. Possibly the only unifying theme underlying many very different complex cell regulatory systems is that they are cooperative. Whitty asserts that the complex molecular systems required for life could not function without cooperativity [59]. Fenton calls allostery the ''second secret of life'' [60]. These are strong statements. Do researchers who study, for example, metabolic fluxes or cell cycle regulation take a moment each day to ask themselves: ''How cooperative is my system? How can I better take account of cooperativity in my system model and my experiments?'' If not, should they?

Although actin is present, the nucleus lacks an equivalent system to the long actin and microtubule filaments of the cytosol and therefore active transport of regulatory proteins within the nucleoplasm toward their target genes seems not to be feasible. In metazoans, there are filaments in the form of the nuclear lamina below the nuclear envelope [68,69]. The importance of nuclear myosin-1 and (short) actin filaments for gene transcription has now been firmly established [70]. These filament systems are certainly important for the definition of subnuclear structure, as is the chromatin itself.

A number of subnuclear bodies have been identified, some quite recently. Besides the much larger nucleolus, there are promyelocytic leukaemia (PML) and coil (Cajal) bodies, speckles and gems, all of which are nucleoprotein complexes. The range of functions of these bodies are incompletely understood but, for example, speckles are associated with splicing small nuclear ribonucleoproteins (snRNPs), coiled bodies with RNA modification and PML bodies with SUMO-regulated gene repression [71–73]. Furthermore, transcription factories, which are sites of five or six active RNA Pol II molecules, have become rather less controversial and might be gaining general acceptance [74]. Essentially all nuclear proteins seem to be associated with large molecular complexes such as pores, chromatin modifiers, polymerase holoenzymes and RNP complexes. Even if the dynamics of nuclear protein complexes are far from understood, nuclear structure is now recognized to have discrete components that modulate gene expression.

It is also now clear that subnuclear localization is important for gene expression. Association with lamin filaments generally implies inactive chromatin and/or a TF resting place, whereas active genes have been found to be associated with nuclear pores [75,76] and nuclear pore proteins have even been found within gene regulatory complexes [77]. Thus, it is now considered that the nuclear envelope plays a key role in gene regulation [68,69,78] and indeed there is evidence linking PML bodies with nuclear pores through the SUMOylation system and the RanBP2 nucleoporin [79]. In yeast, mRNA quality control is associated with nuclear pore complexes [80]. A remarkable recent demonstration of discretely organized nuclear processes revealed that SUMOylation directs DNA damage complexes to the nuclear pores for repair [81].

There is clearly much more to be learned about nuclear organization and I would like to end this section with a prediction concerning low-abundance TFs: as each TF molecule enters the nucleus, it is retained at the nuclear pore until an appropriate chromatin segment (probably containing a target promoter) becomes available to accept it in a tightly regulated exchange reaction.

Discretionary translation of mRNA

It has been known for many years that maternal mRNAs encoding proteins important for development, such as Bicoid, Nanos and Oskar, are sorted to poles of *Drosophila* oocytes [82]. Of course, these might be considered an unusual cell type. Neurons are another unusual cell type and, although it seems obvious in retrospect that synapse proteins should be made at synapses, it took some time to convince a sceptical audience that mRNA transport down dendrites (and axons) of neurons is coupled to spatially regulated translation [83].

The good news is that in more typical cell types, research into spatial control of mRNA localization and translation has now come of age [84]. A remarkable finding is that *β-actin* is translated at the leading edge of neuronal cells under regulation of the Src kinase [85]. By contrast, actin mRNA is excluded from the set of mRNAs (encoding cytoskeletal, transport, RNA-processing and signalling proteins) that accumulate in microtubule-based projections of fibroblasts [86]. The *wingless* mRNA is localized apically in polarized cells [87]. *SGLT1* mRNA, which encodes a transporter protein, is localized apically in polarized cells [88]. *Zyxin*, *paxillin* and *talin* mRNAs localize to the focal adhesion complex [84]. The mRNAs of mitotic regulators, including cyclin B, localize to polysomes on mitotic microtubules [89]. Are these several examples indicative that localized mRNA translation is a general phenomenon? Apparently so, because of 3300 mRNAs examined in a large-scale study, 70% had spatially discrete patterns [90].

Translationally blocked mRNAs are transported from the nucleus in RNP complexes containing many proteins and mRNAs. These granules, which can be quite large and irregular (and hence have non-stoichiometric compositions), are moved by kinesin and dynein motors along microtubules and by myosin motors along actin filaments [84,91,92]. When the granules reach their terminal destination, the inhibitory proteins are removed and the mRNAs become competent for translation (see the review by Besse and Ephrussi [93] for several systems that are beginning to be understood in some detail).

Targeting translation to where proteins are needed removes any *a priori* requirement for a newly synthesized protein to diffuse to its functional location. Indeed, in many cases this would be disastrous, as clearly explained in the β-actin story [85]. It will be fascinating to investigate spatial restriction of the translation of mRNAs for low-copy-number regulatory proteins found in the cilia (which lack ribosomes).

Trouble with microtubules

The cytoskeleton provides cell shape, as well as tracks for transport and sorting of cargoes such as vesicles, mitochondria, pigment granules, intermediate filaments, RNP complexes and scaffolded signalling complexes [22,94]. The current state of knowledge presents some problems for fitting intracellular transport into the discrete and possibly deterministic theme posited here. The motors can exhibit variable processivity and often transient attachment to the filaments. Although many cargoes have been matched to individual motors on the basis of genetics and microscopy, it has been extremely hard to demonstrate direct binding interactions biochemically [95,96], implying that these are either quite weak or dynamically regulated in some unforeseen manner. It seems hard to move beyond a rather stochastic model of a ratchet and microdiffusion of cargoes. There are also issues of directionality, with some cargoes being pulled in opposite directions [97,98].

However, the fact that cargoes tend to be rather large means that they can bind multiple motors [99]. Therefore,

even if cargo–motor interactions are individually weak, multivalent cooperativity can again come into play. There is also evidence of motor coordination to control the bidirectionality of some cargoes [98]. Therefore, although it might be necessary to model individual motors with probabilistic models, the transport system is highly effective in moving cargoes and seems to often behave in a deterministic fashion. The control systems modulating cargo movement are known to involve many kinases and their regulators, and regulatory MAPs and EBs are highly modular proteins with extensive native disorder and linear motifs [30,100]. Therefore, these systems consist of typical signalling complexes.

Signalling ciliary subsystems have solvent insufficency

The primary non-motile cilium found on most animal cells – long regarded as a sort of useless cellular appendix – has recently become the focus of huge interest in cell regulatory research. Perhaps nothing can illuminate the discrete nature of cell regulation better than investigations into an emerging class of diseases known as ciliopathies [101–104]. In addition to their long-standing role in fluid flow and cell motility, cilia are now also recognized as major sensory devices. At least three zinc finger TFs localize to the cilia, Gli1, Gli2 and Glis2 [105,106]. Loss-of-function mutations in Glis2 can cause the inherited cystic kidney disease nephronophthisis [105]. The Hedgehog, Patched and Smoothened proteins regulate whether activating or repressing forms of Gli TFs transfer from the cilia to the nucleus [107]. Similar to the case for many other TFs, the abundance of these migratory Gli family proteins is so low that they cannot be visualized by standard histological methods unless overexpressed. There is no prospect of applying a smooth deterministic model as they journey from Patched receptors in the cilium to specific promoters in the nucleus. Yet the question arises as to whether stochastic models are ideal when these essential TFs are clearly effective in finding their gene targets: a system that is guaranteed to work is deterministic. How, therefore, could diffusion be considered to play a primary role in this migration process? Within the tightly packed cilium itself there is indeed inadequate fluid space to support macromolecular diffusion. Instead, ciliary proteins (including ciliary membrane proteins) are actively imported in large irregular complexes by kinesin motors driving up the central microtubules [108,109]. Among the wonders of intraflagellar transport (IFT), the retrograde motor (a dynamin) is itself a cargo of the anterograde kinesin and *vice versa*.

Cilia provide a unique combination of a stable organellar structure with highly dynamic transport, regulatory and signalling processes. Their exposed location outside the cell body and the associated ease of access and visualization mean that these processes can be easily studied in living cells and organisms (see, for example, the IFT movies in Ref. [110]). Many components of the IFT systems have been genetically identified, facilitating the development of schemes for the IFT cycle. In short, cilia have suddenly been identified as the organelles best suited to obtain the quality data needed to derive general principles of cell regulation applicable in systems biology models, including those for the regulation and function of microtubular transport systems.

When to be smoothly indiscrete

This article focuses on the nature of regulatory proteins, but there are of course many other chemical signals. Diffusion plays a part in intracellular signalling by calcium and potassium ions and second messengers such as NO, cAMP and cGMP. These messengers diffuse rapidly until they are bound, destroyed or exported from the cytosol. However, even these small molecules are now considered to be localized in cellular microdomains [111,112]. Words such as spatial and discrete are increasingly found in publications on calcium intracellular signalling. Furthermore, signalling downstream of second messengers tends to be transferred to protein kinases, which returns the system to the discrete protein signalling complexes.

The morphogens that specify polarity in the developing *Drosophila* egg provide some of the best-known examples of intracellular protein gradients set up by diffusion. The Bicoid transcription factor and Nanos translational repressor are synthesized at opposite poles and generate opposing gradients by diffusion through the egg [113]. These morphogen gradients are popular among cell systems modellers. Nevertheless, as a paradigm for cell regulation they might have rather a limited role, as there are likely to be rather few intracellular morphogens [114]. Bicoid shares two similarities with the Fus3 gradient introduced above. First, to make the gradient it is necessary to initially assemble a discrete complex at one end of the cell; the gradients are both used in cell polarity systems. Second, after entering the nuclei these proteins join discrete chromatin complexes to exert their gene regulatory effects.

Diffusion is always available to move molecules around the cell. Whenever diffusion is good enough for the job, the cell is likely to use this mechanism because it needs no special machinery and costs no effort. The large number of motor proteins in the eukaryotic cell is just one of the indications that unassisted diffusion is often not up to the job, however. It will be critical to define when diffusion is used and when it is not and to use appropriate models when regulation is passing through discrete sites and active transport mechanisms. The challenge for cell systems modellers will be to understand their systems well enough to mix and match discreteness versus smoothness and determinism versus stochasticity. Without this consideration, it will be difficult to develop useful models for systems involving large cooperatively assembled complexes such as T-cell activation (Figure 3) or the death-inducing signalling complex DISC [115], a key apoptotic switch (Figure 6).

Concluding remarks

In a short opinion piece it is not possible to cover the whole field of cell signalling and readers will no doubt immediately think of other regulatory complexes that could be quoted in support of the argument developed here and experimental findings that provide a counterpoint. The take-home message, however, is simple: diffusion of

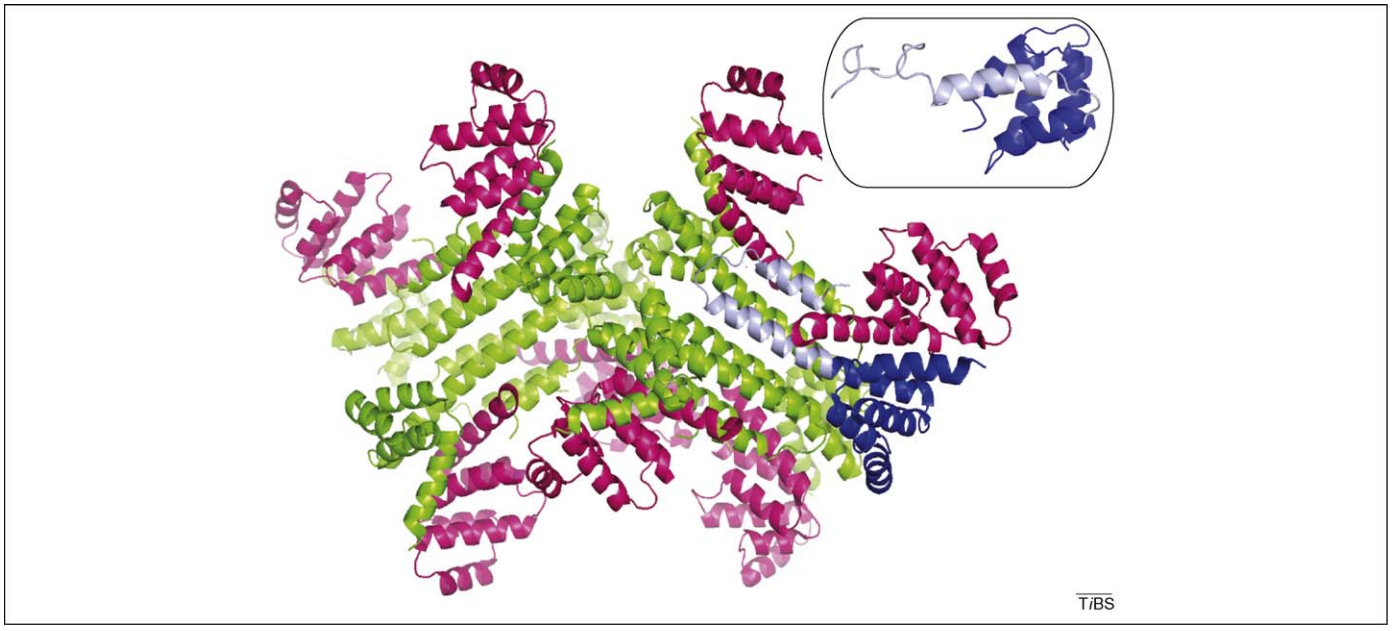

Figure 6. A large signalling complex assembled by iteration of a cooperative molecular switch [115]. The structure, consisting of 16 death domains, provides the core platform for the death inducing signalling complex (DISC), an apoptotic trigger. Death domains are composed of six α helices. The eight FADD death domains are coloured pink. Seven Fas receptor death domains are coloured green and one is coloured blue. The Fas and FADD death domains undergo conformational changes on binding so that the packing interface for the sixth helix of the Fas domain is now unfavourable. Multimerization of the Fas domains is achieved by switching the sixth helix out of the globular domain into an open conformation. It relocates to fuse with the fifth helix and, together with a new helix formed by a sequence following the death domain, it facilitates homotetramerization of the Fas domains by mutually induced fit. Light blue indicates the rearranged helices. The inset shows the closed conformation of a monomeric Fas death domain with the sixth helix in place, followed by disordered peptide. At least *in vitro*, these cooperative effects allow further assembly of DISC-like rings and ring clusters. Figure prepared with PyMOL using entries 3ezq.pdb and 1ddf.pdb.

intracellular proteins might not be very important in cell signalling. Models of the cell must reflect this reality or they will lack predictive power and provide little useful insight. Moreover, the differential equations for reaction–diffusion are computationally costly, limiting their application to small cellular subsystems.

Rather, the cell is to a large extent regulated by highly discrete yet dynamic protein complexes in which individual proteins can make remarkable numbers of interactions. The primary material for these interactions are short accessible linear peptide motifs, many of which are regulated by PTM, whereas others are not. Cooperative interactions dictate the stability and status of these cellular signalling engines. A property of such cooperative low-affinity interactions is likely to be the ability to perform discretely deterministic signalling. Systems modellers might welcome this because, freed of costly differential equations, they should be able to develop computationally efficient models for many parts of cell regulation: high-level modelling might in some cases be as simple as flicking switches. Cooperativity is fundamental: it enables order to arise from the chaos of the spaghetti-like strands of thousands of different natively disordered polypeptide sequences. There is no dictator in cell regulation, no first among equals, no master regulator, no top-down system of governance. The time has come to acknowledge that the cell is anarcho-syndicalist: Homage to Catalonia.

Acknowledgements

I thank many colleagues and friends in the ELM Team, at EMBL and elsewhere who were so willing to discuss the nature of cell regulation, helping me to refine the ideas presented here. This article is dedicated to Matti Saraste, who would have understood.

References

1 Maeder, C.I. *et al.* (2007) Spatial regulation of Fus3 MAP kinase activity through a reaction-diffusion mechanism in yeast pheromone signalling. *Nat. Cell Biol.* 9, 1319–1326
2 Stelling, J. and Kholodenko, B.N. (2009) Signaling cascades as cellular devices for spatial computations. *J. Math. Biol.* 58, 35–55
3 Yudushkin, I.A. *et al.* (2007) Live-cell imaging of enzyme-substrate interaction reveals spatial regulation of PTP1B. *Science* 315, 115–119
4 Pokholok, D.K. *et al.* (2006) Activated signal transduction kinases frequently occupy target genes. *Science* 313, 533–536
5 Racker, E. and Spector, M. (1981) Warburg effect revisited: merger of biochemistry and molecular biology. *Science* 213, 303–307
6 Racker, E. (1981) Warburg effect revisited. *Science* 213, 1313
7 Seger, R. and Krebs, E.G. (1995) The MAPK signaling cascade. *FASEB J.* 9, 726–735
8 Vivanco, I. and Sawyers, C.L. (2002) The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat. Rev. Cancer* 2, 489–501
9 Berwick, D.C. and Tavare, J.M. (2004) Identifying protein kinase substrates: hunting for the organ-grinder's monkeys. *Trends Biochem. Sci.* 29, 227–232
10 Cecchinelli, B. *et al.* (2006) Ser58 of mouse p53 is the homologue of human Ser46 and is phosphorylated by HIPK2 in apoptosis. *Cell Death Differ.* 13, 1994–1997
11 Roux, P.P. and Blenis, J. (2004) ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol. Mol. Biol. Rev.* 68, 320–344
12 Bardwell, L. (2006) Mechanisms of MAPK signalling specificity. *Biochem. Soc.Trans.* 34, 837–841
13 Gold, M.G. *et al.* (2006) Lining the pockets of kinases and phosphatases. *Curr. Opin. Struct. Biol.* 16, 693–701
14 Arcak, M. and Sontag, E.D. (2008) A passivity-based stability criterion for a class of biochemical reaction networks. *Math. Biosci. Eng.* 5, 1–19
15 Chiam, K.H. and Rajagopal, G. (2007) Oscillations in intracellular signaling cascades. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* 75, 061901
16 Elf, J. and Ehrenberg, M. (2004) Spontaneous separation of bi-stable biochemical systems into spatial domains of opposite phases. *Syst. Biol.* 1, 230–236

17 Georgiev, N. *et al.* (2006) Reaction-diffusion modeling ERK- and STAT-interaction dynamics. *EURASIP J. Bioinform. Syst. Biol.* 85759
18 Naka, T. *et al.* (2006) Compensation effect of the MAPK cascade on formation of phospho-protein gradients. *Biosystems* 83, 167–177
19 Pawson, T. and Scott, J.D. (1997) Signaling through scaffold, anchoring, and adaptor proteins. *Science* 278, 2075–2080
20 Malbon, C.C. (2005) G proteins in development. *Nat. Rev. Mol. Cell. Biol.* 6, 689–701
21 Morrison, D.K. and Davis, R.J. (2003) Regulation of MAP kinase signaling modules by scaffold proteins in mammals. *Annu. Rev. Cell Dev. Biol.* 19, 91–118
22 Schnapp, B.J. (2003) Trafficking of signaling modules by kinesin motors. *J. Cell Sci.* 116, 2125–2135
23 Pimienta, G. and Pascual, J. (2007) Canonical and alternative MAPK signaling. *Cell Cycle* 6, 2628–2632
24 Linding, R. *et al.* (2007) Systematic discovery of *in vivo* phosphorylation networks. *Cell* 129, 1415–1426
25 Mandelkow, E. *et al.* (1995) On the structure of microtubules, tau, and paired helical filaments. *Neurobiol. Aging* 16, 347–354
26 Dixit, R. *et al.* (2008) Differential regulation of dynein and kinesin motor proteins by tau. *Science* 319, 1086–1089
27 Vershinin, M. *et al.* (2007) Multiple-motor based transport and its regulation by Tau. *Proc. Natl. Acad. Sci. U. S. A.* 104, 87–92
28 Stoothoff, W.H. and Johnson, G.V. (2005) Tau phosphorylation: physiological and pathological consequences. *Biochim. Biophys. Acta* 1739, 280–297
29 Diella, F. *et al.* (2008) Phospho.ELM: a database of phosphorylation sites – update 2008. *Nucleic Acids Res.* 36, D240–D244
30 Akhmanova, A. and Steinmetz, M.O. (2008) Tracking the ends: a dynamic protein network controls the fate of microtubule tips. *Nat. Rev. Mol. Cell. Biol.* 9, 309–322
31 Drewes, G. *et al.* (1998) MAPs, MARKs and microtubule dynamics. *Trends Biochem. Sci.* 23, 307–311
32 Dosztanyi, Z. *et al.* (2007) Prediction of protein disorder at the domain level. *Curr. Protein Pept. Sci.* 8, 161–171
33 Dunker, A.K. *et al.* (2005) Flexible nets. The roles of intrinsic disorder in protein interaction networks. *FEBS J.* 272, 5129–5148
34 Receveur-Brechot, V. *et al.* (2006) Assessing protein disorder and induced folding. *Proteins* 62, 24–45
35 Diella, F. *et al.* (2008) Understanding eukaryotic linear motifs and their role in cell signaling and regulation. *Front. Biosci.* 13, 6580–6603
36 Lobley, A. *et al.* (2007) Inferring function using patterns of native disorder in proteins. *PLoS Comput. Biol.* 3, e162
37 Fuxreiter, M. *et al.* (2007) Local structural disorder imparts plasticity on linear motifs. *Bioinformatics* 23, 950–956
38 Miller, M.L. *et al.* (2008) Linear motif atlas for phosphorylation-dependent signaling. *Sci. Signal.* 1, ra2
39 Puntervoll, P. *et al.* (2003) ELM server: a new resource for investigating short functional sites in modular eukaryotic proteins. *Nucleic Acids Res.* 31, 3625–3630
40 Dinkel, H. and Sticht, H. (2007) A computational strategy for the prediction of functional linear peptide motifs in proteins. *Bioinformatics* 23, 3297–3303
41 Perrodou, E. *et al.* (2008) A new protein linear motif benchmark for multiple sequence alignment software. *BMC Bioinformatics* 9, 213
42 Davey, N.E. *et al.* (2009) Masking residues using context-specific evolutionary conservation significantly improves short linear motif discovery. *Bioinformatics* 25, 443–450
43 Russell, R.B. and Gibson, T.J. (2008) A careful disorderliness in the proteome: sites for interaction and targets for future therapies. *FEBS Lett.* 582, 1271–1275
44 Han, J.D. *et al.* (2004) Evidence for dynamically organized modularity in the yeast protein–protein interaction network. *Nature* 430, 88–93
45 Dyson, H.J. and Wright, P.E. (2005) Intrinsically unstructured proteins and their functions. *Nat. Rev. Mol. Cell. Biol.* 6, 197–208
46 Dosztanyi, Z. *et al.* (2006) Disorder and sequence repeats in hub proteins and their implications for network evolution. *J. Proteome Res.* 5, 2985–2995
47 Goodman, R.H. and Smolik, S. (2000) CBP/p300 in cell growth, transformation, and development. *Genes Dev.* 14, 1553–1577
48 Jensen, L.J. *et al.* (2009) STRING 8 – a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Res.* 37, D412–D416
49 Zaidel-Bar, R. *et al.* (2007) Functional atlas of the integrin adhesome. *Nat. Cell Biol.* 9, 858–867
50 Deakin, N.O. and Turner, C.E. (2008) Paxillin comes of age. *J. Cell Sci.* 121, 2435–2444
51 Cadigan, K.M. and Nusse, R. (1997) Wnt signaling: a common theme in animal development. *Genes Dev.* 11, 3286–3305
52 Ghosh, S. *et al.* (1998) NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* 16, 225–260
53 Heldin, C.H. *et al.* (1997) TGF-β signalling from cell membrane to nucleus through SMAD proteins. *Nature* 390, 465–471
54 Kisseleva, T. *et al.* (2002) Signaling through the JAK/STAT pathway, recent advances and future challenges. *Gene* 285, 1–24
55 Raiborg, C. *et al.* (2003) Protein sorting into multivesicular endosomes. *Curr. Opin. Cell Biol.* 15, 446–455
56 Dafforn, T.R. and Smith, C.J. (2004) Natively unfolded domains in endocytosis: hooks, lines and linkers. *EMBO Rep.* 5, 1046–1052
57 Owen, D.J. (2004) Linking endocytic cargo to clathrin: structural and functional insights into coated vesicle formation. *Biochem. Soc.Trans.* 32, 1–14
58 Houtman, J.C. *et al.* (2006) Oligomerization of signaling complexes by the multipoint binding of GRB2 to both LAT and SOS1. *Nat. Struct. Mol. Biol.* 13, 798–805
59 Whitty, A. (2008) Cooperativity and biological complexity. *Nat. Chem. Biol.* 4, 435–439
60 Fenton, A.W. (2008) Allostery: an illustrated definition for the 'second secret of life'. *Trends Biochem. Sci.* 33, 420–425
61 Fiedler, M. *et al.* (2008) Decoding of methylated histone H3 tail by the Pygo–BCL9 Wnt signaling complex. *Mol. Cell* 30, 507–518
62 Baek, S.H. *et al.* (2006) Ligand-specific allosteric regulation of coactivator functions of androgen receptor in prostate cancer cells. *Proc. Natl. Acad. Sci. U. S. A.* 103, 3100–3105
63 Hao, B. *et al.* (2005) Structural basis of the Cks1-dependent recognition of p27 (Kip1) by the SCF (Skp2) ubiquitin ligase. *Mol. Cell* 20, 9–19
64 Superti-Furga, G. and Gonfloni, S. (1997) A crystal milestone: the structure of regulated Src. *BioEssays* 19, 447–450
65 Filippakopoulos, P. *et al.* (2008) Structural coupling of SH2-kinase domains links Fes and Abl substrate recognition and kinase activation. *Cell* 134, 793–803
66 Kadaveru, K. *et al.* (2008) Viral infection and human disease – insights from minimotifs. *Front. Biosci.* 13, 6455–6471
67 Ghaemmaghami, S. *et al.* (2003) Global analysis of protein expression in yeast. *Nature* 425, 737–741
68 Bridger, J.M. *et al.* (2007) The nuclear lamina. Both a structural framework and a platform for genome organization. *FEBS J.* 274, 1354–1361
69 Dorner, D. *et al.* (2007) Nucleoplasmic lamins and their interaction partners, LAP2α, Rb, and BAF, in transcriptional regulation. *FEBS J.* 274, 1362–1373
70 Ye, J. *et al.* (2008) Nuclear myosin I acts in concert with polymeric actin to drive RNA polymerase I transcription. *Genes Dev.* 22, 322–330
71 Bernardi, R. and Pandolfi, P.P. (2007) Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies. *Nat. Rev. Mol. Cell. Biol.* 8, 1006–1016
72 Handwerger, K.E. and Gall, J.G. (2006) Subnuclear organelles: new insights into form and function. *Trends Cell. Biol.* 16, 19–26
73 Trinkle-Mulcahy, L. and Lamond, A.I. (2008) Nuclear functions in space and time: gene expression in a dynamic, constrained environment. *FEBS Lett.* 582, 1960–1970
74 Carter, D.R. *et al.* (2008) Transcription factories. *Biochem. Soc.Trans.* 36, 585–589
75 Dillon, N. (2008) The impact of gene location in the nucleus on transcriptional regulation. *Dev. Cell* 15, 182–186
76 Heessen, S. and Fornerod, M. (2007) The inner nuclear envelope as a transcription factor resting place. *EMBO Rep.* 8, 914–919
77 Mendjan, S. *et al.* (2006) Nuclear pore components are involved in the transcriptional regulation of dosage compensation in *Drosophila*. *Mol. Cell* 21, 811–823

78 Akhtar, A. and Gasser, S.M. (2007) The nuclear envelope and transcriptional control. *Nat. Rev. Genet.* 8, 507–517
79 Saitoh, N. *et al.* (2006) *In situ* SUMOylation analysis reveals a modulatory role of RanBP2 in the nuclear rim and PML bodies. *Exp. Cell Res.* 312, 1418–1430
80 Skru Ny, M. *et al.* (2009) An endoribonuclease functionally linked to perinuclear mRNP quality control associates with the nuclear pore complexes. *PLoS Biol.* 7, e8
81 Nagai, S. *et al.* (2008) Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science* 322, 597–602
82 Ephrussi, A. *et al.* (1991) Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. *Cell* 66, 37–50
83 Giuditta, A. *et al.* (2002) Axonal and presynaptic protein synthesis: new insights into the biology of the neuron. *Trends Neurosci.* 25, 400–404
84 Gilligan, P.C. and Sampath, K. (2008) Reining in RNA. Workshop on intracellular RNA localization and localized translation. *EMBO Rep.* 9, 22–26
85 Huttelmaier, S. *et al.* (2005) Spatial regulation of β-actin translation by Src-dependent phosphorylation of ZBP1. *Nature* 438, 512–515
86 Mili, S. *et al.* (2008) Genome-wide screen reveals APC-associated RNAs enriched in cell protrusions. *Nature* 453, 115–119
87 Simmonds, A.J. *et al.* (2001) Apical localization of wingless transcripts is required for wingless signaling. *Cell* 105, 197–207
88 Pedder, C.M. *et al.* (2008) Targeting of transcripts encoding membrane proteins in polarized epithelia: RNA–protein binding studies of the *SGLT1* 3′-UTR. *Biochem. Soc. Trans.* 36, 525–527
89 Blower, M.D. *et al.* (2007) Genome-wide analysis demonstrates conserved localization of messenger RNAs to mitotic microtubules. *J. Cell Biol.* 179, 1365–1373
90 Lecuyer, E. *et al.* (2007) Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. *Cell* 131, 174–187
91 Hirokawa, N. (2006) mRNA transport in dendrites: RNA granules, motors, and tracks. *J. Neurosci.* 26, 7139–7142
92 Muller, M. *et al.* (2007) Directional mRNA transport in eukaryotes: lessons from yeast. *Cell Mol. Life Sci.* 64, 171–180
93 Besse, F. and Ephrussi, A. (2008) Translational control of localized mRNAs: restricting protein synthesis in space and time. *Nat. Rev. Mol. Cell. Biol.* 9, 971–980
94 Ross, J.L. *et al.* (2008) Cargo transport: molecular motors navigate a complex cytoskeleton. *Curr. Opin. Cell Biol.* 20, 41–47
95 Hirokawa, N. and Takemura, R. (2005) Molecular motors and mechanisms of directional transport in neurons. *Nat. Rev. Neurosci.* 6, 201–214
96 Li, J.F. and Nebenfuhr, A. (2008) The tail that wags the dog: the globular tail domain defines the function of myosin V/XI. *Traffic* 9, 290–298
97 Gross, S.P. (2004) Hither and yon: a review of bi-directional microtubule-based transport. *Phys. Biol.* 1, R1–11
98 Welte, M.A. (2004) Bidirectional transport along microtubules. *Curr. Biol.* 14, R525–R537
99 Gross, S.P. *et al.* (2007) Cargo transport: two motors are sometimes better than one. *Curr. Biol.* 17, R478–R486
100 Steinmetz, M.O. and Akhmanova, A. (2008) Capturing protein tails by CAP-Gly domains. *Trends Biochem. Sci.* 33, 535–545
101 Adams, M. *et al.* (2008) Recent advances in the molecular pathology, cell biology and genetics of ciliopathies. *J. Med. Genet.* 45, 257–267
102 Badano, J.L. *et al.* (2006) The ciliopathies: an emerging class of human genetic disorders. *Annu. Rev. Genomics. Hum. Genet.* 7, 125–148
103 Hildebrandt, F. and Zhou, W. (2007) Nephronophthisis-associated ciliopathies. *J. Am. Soc. Nephrol.* 18, 1855–1871
104 Marshall, W.F. (2008) The cell biological basis of ciliary disease. *J. Cell Biol.* 180, 17–21
105 Attanasio, M. *et al.* (2007) Loss of GLIS2 causes nephronophthisis in humans and mice by increased apoptosis and fibrosis. *Nat. Genet.* 39, 1018–1024
106 Haycraft, C.J. *et al.* (2005) Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. *PLoS Genet.* 1, e53
107 Uhlenhaut, N.H. and Treier, M. (2008) Transcriptional regulators in kidney disease: gatekeepers of renal homeostasis. *Trends Genet.* 24, 361–371
108 Blacque, O.E. *et al.* (2008) Intraflagellar transport: from molecular characterisation to mechanism. *Front. Biosci.* 13, 2633–2652
109 Scholey, J.M. (2008) Intraflagellar transport motors in cilia: moving along the cell's antenna. *J. Cell Biol.* 180, 23–29
110 Blacque, O.E. *et al.* (2004) Loss of *C. elegans* BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. *Genes Dev.* 18, 1630–1642
111 Martin, A.C. and Cooper, D.M. (2006) Layers of organization of cAMP microdomains in a simple cell. *Biochem. Soc. Trans.* 34, 480–483
112 McCarron, J.G. *et al.* (2006) Ca2+ microdomains in smooth muscle. *Cell Calcium* 40, 461–493
113 Driever, W. and Nusslein-Volhard, C. (1988) A gradient of bicoid protein in *Drosophila* embryos. *Cell* 54, 83–93
114 Kornberg, T.B. and Guha, A. (2007) Understanding morphogen gradients: a problem of dispersion and containment. *Curr. Opin. Genet. Dev.* 17, 264–271
115 Scott, F.L. *et al.* (2009) The Fas-FADD death domain complex structure unravels signalling by receptor clustering. *Nature* 457, 1019–1022
116 Smock, R.G. and Gierasch, L.M. (2009) Sending signals dynamically. *Science* 324, 198–203
117 Gribble, S.D. (2001) Robustness in Complex Systems. *Eighth Workshop on Hot Topics in Operating Systems* 2001, 0021
118 Lehar, J. *et al.* (2008) High-order combination effects and biological robustness. *Mol. Syst. Biol.* 4, 215