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Review

Spatial and temporal organization of multi-protein assemblies: achieving sensitive control in information-rich cell-regulatory systems

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The regulation of cellular processes in living organisms requires signalling systems that have a high signal-to-noise ratio. This is usually achieved by transient, multi-protein complexes that assemble cooperatively. Even in the crowded environment of the cell, such assemblies are unlikely to form by chance, thereby providing a sensitive regulation of cellular processes. Furthermore, selectivity and sensitivity may be achieved by the requirement for concerted folding and binding of previously unfolded components. We illustrate these features by focusing on two essential signalling pathways of eukaryotic cells: first, the monitoring and repair of DNA damage by non-homologous end joining, and second, the mitotic spindle assembly checkpoint, which detects and corrects defective attachments of chromosomes to the kinetochore. We show that multi-protein assemblies moderate the full range of functional complexity and diversity in the two signalling systems. Deciphering the nature of the interactions is central to understanding the mechanisms that control the flow of information in cell signalling and regulation.

Keywords: structural biology; protein assemblies; cell signalling

1. Introduction

There is estimated to be one billion individual protein molecules present in mammalian cells at one time, of which up to 10 per cent are involved in cell signalling [1]. In this crowded environment, cell signalling and regulation have evolved to depend on multi-protein complexes that are specific and reversible in order to achieve high signal-to-noise. Structural biology is uniquely placed to provide an insight into how this is achieved. Here, we illustrate these themes by

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Dedicated to Alan L. Mackay, who has taught us so much about the assembly, organization and function of complex multi-component systems.

One contribution of 14 to a Theme Issue 'Beyond crystals: the dialectic of materials and information'.

focusing on two examples of such regulatory processes, DNA repair and mitotic checkpoint control, which comprise essential surveillance pathways that monitor and provide the time-for-error correction of key events of the cell cycle.

Non-homologous end joining (NHEJ), which is one of the two major pathways responsible for repairing double-strand breaks (DSBs) in DNA, features three main steps in vertebrates: synapsis; end processing; and end joining [2,3]. Synapsis brings the two DNA ends into close proximity and is carried out by the DNA-dependent protein kinase complex (DNA-PK), comprising the Ku70/80 heterodimer and the DNA-PK catalytic subunit (DNA-PKcs) [4,5]). End processing involves an array of nucleases to cut DNA overhangs and a polynucleotide kinase/phosphatase for preparing damaged DNA ends ready for ligation [6]. Finally, the two ends are joined by the ligase complex, including DNA ligase IV (LigIV), X-ray cross-complementation group 4 (XRCC4) and XRCC4-like factor/Cernunnos (XLF) [7,8].

The mitotic spindle assembly for the checkpoint (SAC) is the evolutionarily conserved regulatory mechanism that ensures the maintenance of genomic stability in higher organisms. The SAC controls the timely and accurate segregation of chromosomes by delaying the onset of anaphase until all chromosomes are properly bioriented and attached to the mitotic spindle. The serine/threonine kinase Bub1 is essential for the assembly of the functional inner centromere and mediates the recruitment of other checkpoint components in cells that have the checkpoint unsatisfied, while another serine/threonine kinase, BubR1, associates with unattached/incorrectly attached kinetochores and plays roles in stabilizing kinetochore–microtubule attachments and in chromosome alignment [9,10]. Together with Bub3, Mad2 and Cdc20, BubR1 forms part of the mitotic checkpoint complex (MCC) that inhibits the anaphase-promoting complex or cyclosome (APC/C) E3 ubiquitin ligase activity towards cyclin B1 and securin. The interaction of SAC kinases Bub1 and BubR1 with the protein Blinkin (also known as KNL1 and Spc105) is required for an efficient mitotic checkpoint response and links SAC signalling with the kinetochore [11,12]. This essential multi-protein complex plays a crucial role in chromosome segregation; it assembles on mitotic or meiotic centromeres and mediates the physical contact of centromeric DNA with microtubules [13,14].

The abundance of DNA breaks in cancer cells containing mitotic spindle abnormalities suggests that mitotic arrest may promote tumorigenesis and antimitotic toxicity as a result of DNA damage [15,16]. The observations that DNA damage induces a delay in the metaphase/anaphase transition, thus leading to defects in kinetochore attachment and function, and that at least one proteinaceous component of the DNA damage response pathway, 53BP1, is localized to the kinetochore during mitosis [17] suggest the possibility of a mechanism of communication between macromolecular assemblies of the DNA repair pathway and the SAC.

2. Non-homologous end joining and spindle assembly checkpoint involve complex multi-component assemblies

Both NHEJ and SAC pathways (figures 1 and 2, respectively) involve the assembly of multi-protein complexes. We begin by briefly describing the state of our knowledge on these systems.

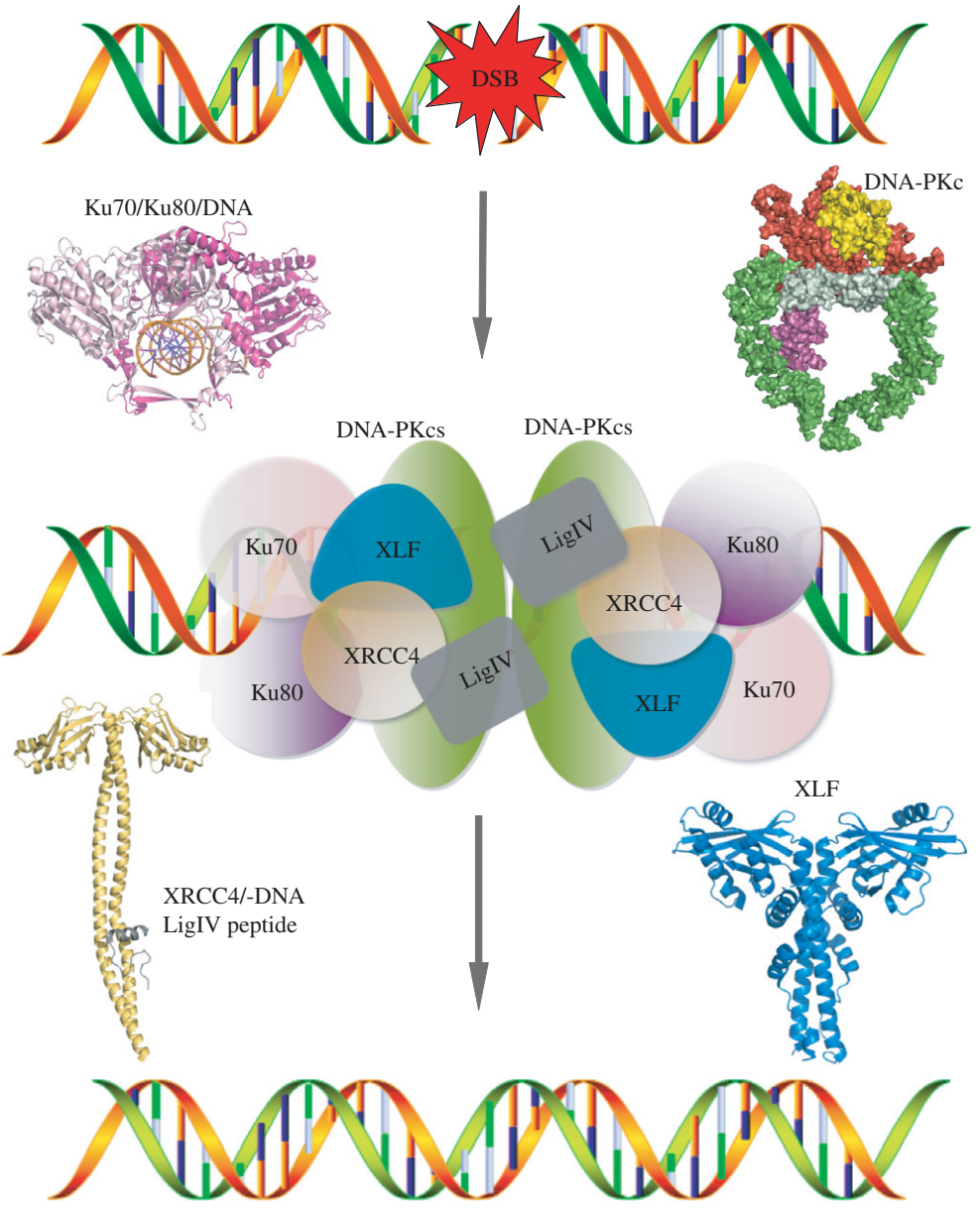

Figure 1. NHEJ pathway for DNA DSB repair. The core NHEJ protein complexes binding to DNA DSBs include: (i) Ku70/80 and DNA-PKcs for DSB recognition and DNA synapsis and (ii) XLF–XRCC4–DNA LigIV for DNA ligation. Individual crystal structures of Ku70/80 (protein data bank (pdb) code 1JEY), DNA-PKcs (pdb code 3KGV), XLF (pdb code 2QM4) and XRCC4–DNA LigIV peptide (pdb code 1IK9) are also shown. (Online version in colour.)

In NHEJ, the association of Ku70 and Ku80 to form a heterodimer is required for double-stranded DNA end-binding activity [18]. In the crystal structure, Ku70 and Ku80 form a pseudosymmetric heterodimer, which encircles duplex DNA [19].

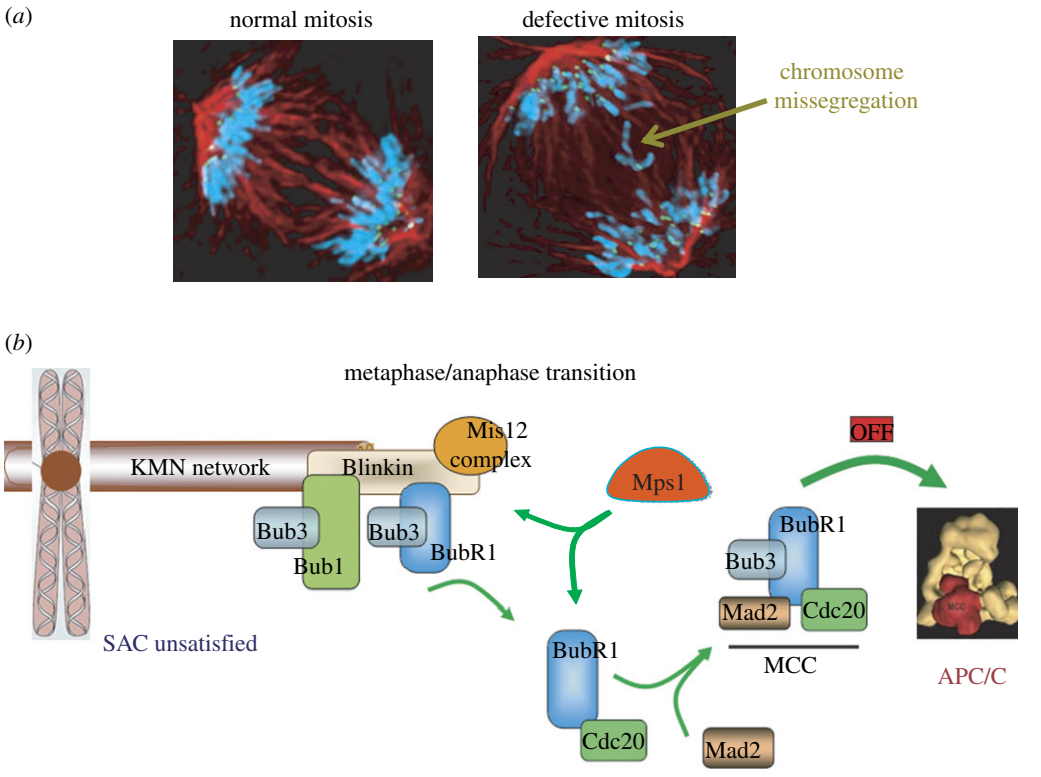

Figure 2. (*a*) Fluorescence microscopy imaging illustrating the normal and defective segregation of chromosomes. The latter condition triggers the mitotic checkpoint response. Images are found at http://dartmed.dartmouth.edu/summer09/html/discdivision.php. (*b*) The mitotic checkpoint consists of the protein components Bub1, BubR1, Bub3, Mad2, Cdc20 and Mps1, some of which are recruited to unattached kinetochores when the checkpoint is unsatisfied. Kinetochore localization of Bub1 and BubR1 is mediated by Blinkin, a central component of the kinetochore–microtubule network (KMN). Cytosolic BubR1, Bub3, Mad2 and Cdc20 associate to form the MCC, which has an inhibitory role on the anaphase-promoting complex/cyclosome (APC/C). After bipolar attachment and alignment of all chromosomes at the centre of the cell has occurred, APC/C–Cdc20 inhibition is released by silencing the mitotic checkpoint, thus allowing chromosome separation and mitotic progression from M-phase to interphase. (Online version in colour.)

No contacts with DNA bases and few interactions with the sugar–phosphate backbone are made (figure 3*a*). No large conformational changes occur in Ku70 and Ku80 on assembling around DNA other than in the C-terminal domains. One DNA face is accessible to processing enzymes that remove damaged nucleotides and fill gaps prior to ligation. These features bring the DNA helix into phase across the junction during end processing and ligation, and provide structural support to broken DNA ends.

Insights into the structures of the DNA-PKcs/Ku70/Ku80 holoenzyme and possible synaptic complexes have been derived from cryo-electron microscopy (EM) and small-angle X-ray scattering (SAXS) studies. Large conformational

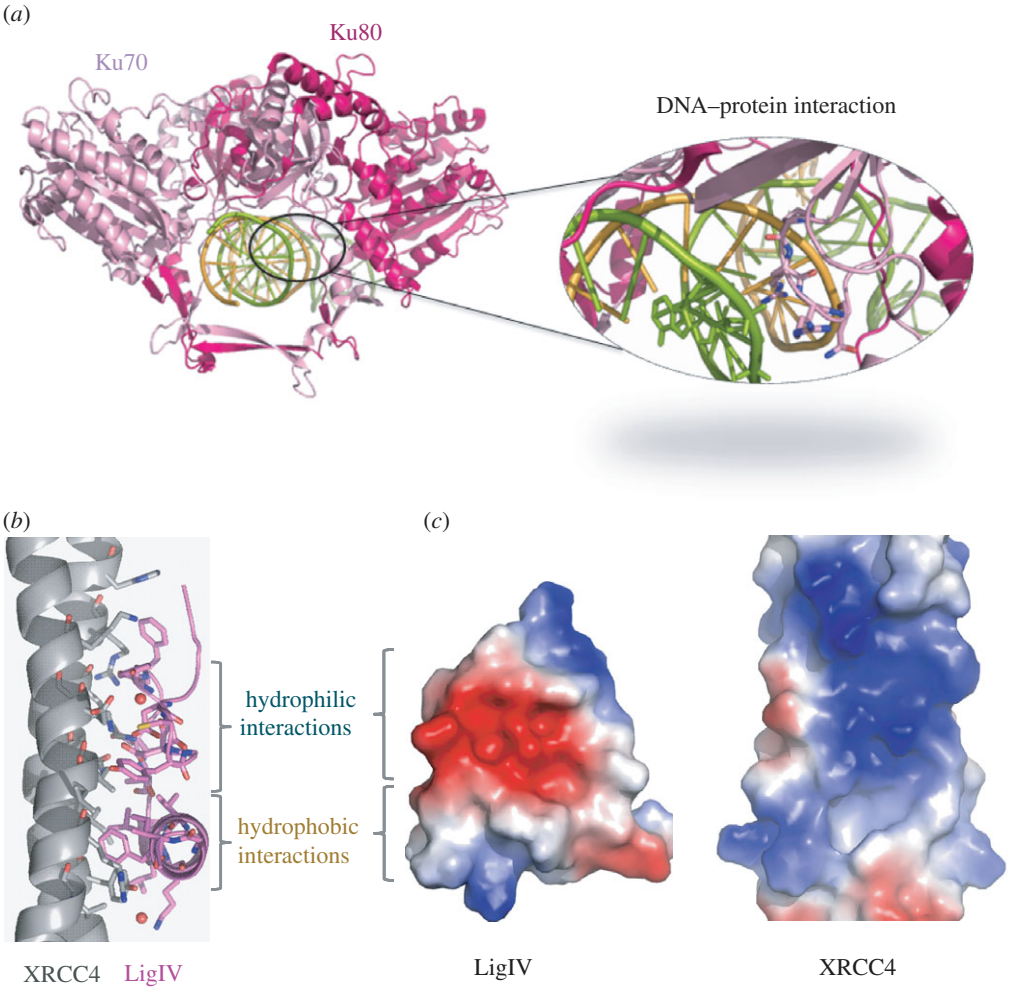

Figure 3. (*a*) Detail of the crystal structure of Ku70/80 with DNA bound. Ku70/80 heterodimer encircles duplex DNA, making no contacts with DNA bases and only a few interactions with the sugar–phosphate backbone are made. (*b*) Interactions of human DNA LigIV and XRCC4. Structures of human DNA LigIV (right) and the XRCC4 homodimer (left) are shown in a ribbon representation. Key residues for their interactions are shown. (*c*) Electrostatic surface charge of the interaction surface of the complex. (Online version in colour.)

changes occur in human DNA-PKcs when double-stranded DNA binds, suggesting that this may correlate with the activation of the kinase [20]. More recently, single-particle electron microscopy studies on human DNA-PKcs/Ku70/Ku80 holoenzyme assembled on DNA at approximately 25 Å resolution [21] have provided further evidence of large conformational changes upon Ku and DNA binding to DNA-PKcs. Moreover, a SAXS study of DNA-PKcs revealed two different modes of dimerization, either head-to-head or palm-to-palm, depending on the presence of either 40 bp hairpin or 40 bp Y-shaped DNA [22].

Although it has been shown that the interaction between XRCC4 and LigIV is stronger than that of XRCC4 and XLF, it remains to be established whether the XLF dimer interactions with XRCC4 dimer are maintained once the ligase has been recruited. Furthermore, mapping interaction assays have shown that XLF recruitment to DSB ends occurs through interaction with Ku70/80 only in the presence of DNA [23]. XRCC4 is dispensable for XLF recruitment to DNA ends, although it contributes to stabilizing the DNA/XLF complex [23]. Considering that both XLF and XRCC4 require a long piece of DNA for binding, the definition of the structural details of DNA association in the higher order protein complexes should provide new insights into this process.

The three-dimensional structures of binary complexes, which involve direct physical interactions between protein pairs, such as Bub1 and Bub3; Mad3 and Bub3; Mad2–Mad1; Mad2–Cdc20 and BubR1–Blinkin, provide the details of protein–protein interactions essential for SAC signalling. For instance, Bub1 and BubR1 (Mad3 in yeast) have a conserved stretch of about 40 amino acid residues that are predicted to be mainly disordered and to contain the GLE2p-binding sequence (GLEBS) motif, which is identified as the Bub3-binding motif. The crystal structures of two independent complexes formed between yeast Bub3 and peptides that mimic the GLEBS motifs of Mad3 and yeast Bub1 demonstrate that the peptides form an extensive interface along the top surface of Bub3, a single domain protein that shows a canonical WD40-repeat fold organized in seven bladed β-propellers (figure 4*a*). The interaction is essential for BubR1 kinetochore localization as a single amino acid substitution in the GLEBS motif, and the top face of Bub3 is sufficient to disrupt the interaction, thus leading to extensive defects in chromosome segregation.

Mad1, Mad2 and Cdc20 are other essential components of the SAC signalling pathway. Mad1, a 718 residue coiled-coil protein, the depletion of which severely affects the SAC in mammalian cells [24,25], forms a stable complex with Mad2 *in vitro* [25]. The crystal structure of Mad2 in complex with Mad1 residues 485–584 (pdb 1GO4) shows that Mad2 exhibits the distinctive HORMA (for *Hop*1, *R*ev7 and *Mad*2) domain, consisting of a single α/β domain organized in three layers: a central layer formed by three α-helices; a large six-stranded β-sheet on the one side; and a short β-hairpin on the other side. In the complex, the Mad1 fragment is predominantly α-helical (figure 4*b*). Binding studies suggest a conformational mechanism in which Mad1 primes the Mad2 binding site for the interaction with Cdc20 [25]. However, whether Cdc20 and Mad2 can establish direct contacts *in vivo* remains as a contentious issue.

The crystal structures of the N-terminal regions of yeast Bub1 and human BubR1, which are essential for binding Blinkin, reveal a common fold that comprises a triple-tandem arrangement of the tetratricopeptide repeat (TPR) motif [26,27]. Interactions of Blinkin with TPR Bub1 and TPR BubR1, which are essential for the recruitment of these kinases to the kinetochore, connect SAC signalling with the KMN (KNL1/Mis12/Ndc80) network [11,12,28]. Moreover, depletion of Blinkin in higher organisms by RNAi causes severe chromosomal segregation defects that resemble phenotypes characteristic of Bub1 and BubR1 protein depletion [29,30]. Detailed peptide mapping, physico-chemical, structural and functional analyses support the notion that an N-terminal Blinkin fragment is essential for binding BubR1 and that site-specific substitutions of the latter impair the mitotic checkpoint [12,31].

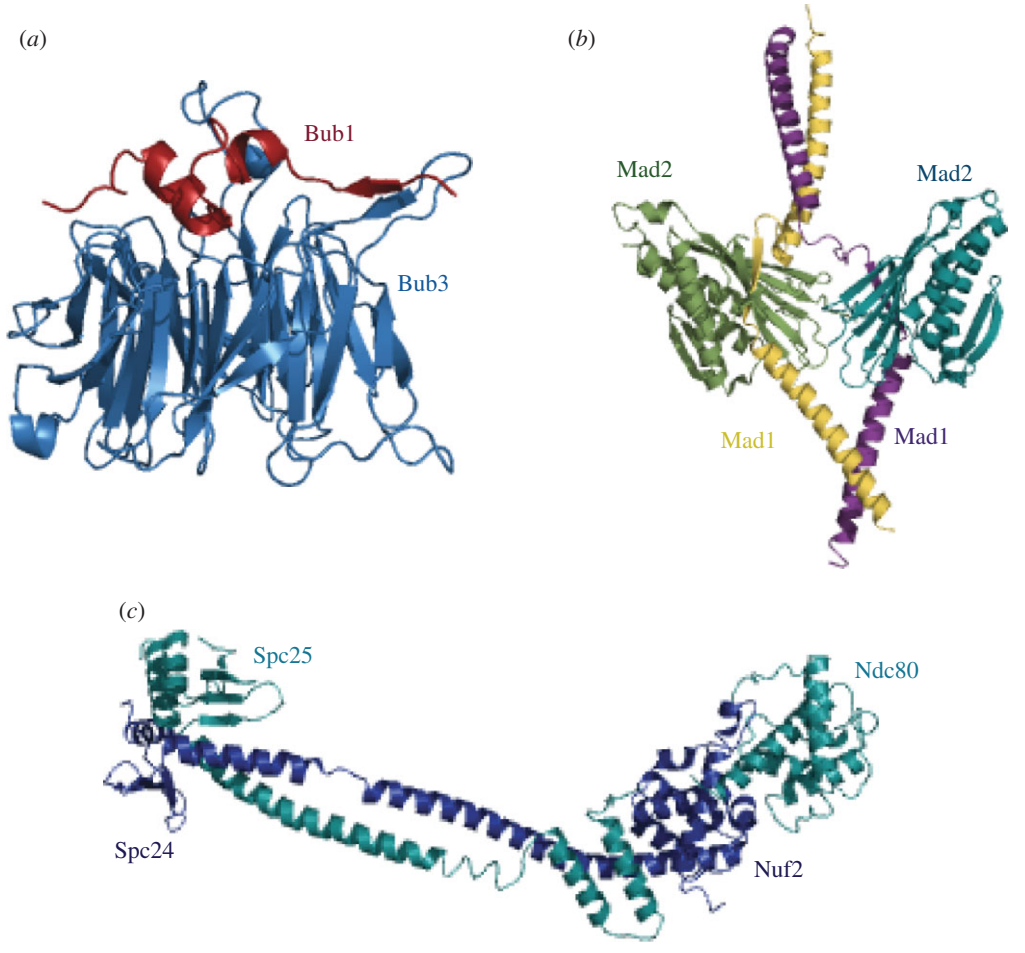

Figure 4. (*a*) Stucture of Bub3 from *S. cerevisiae* in complex with the GLEB motif of Bub1. (*b*) Structure of the Mad1–Mad2 tetrameric complex. The crystal structure shows that the two chains of Mad1 interact with Mad2 via the N-terminal coiled-coil region (pdb 1GO4). (*c*) Crystal structure of the bonsai–Ndc80 complex (pdb 2VE7). Spc24 and Spc25 have N-terminal coiled-coils that mediate inter-subunit interactions, while dimeric Ndc80–Nuf2 comprises N-terminal Calponin homology domains followed by a coiled-coil region engaged in inter-subunit interactions. (Online version in colour.)

3. Weak binary interactions lead cooperatively to well-defined multi-protein complexes

Specific but low-affinity binary complexes leading to cooperative assembly of higher order signalling complexes should be advantageous for signalling [32]. If binary complexes led to signalling in the crowded environment of the cell, opportunistic interactions would likely give rise to noise. On the other hand, multi-protein systems that form cooperatively would less likely form by chance.

An example of this phenomenon is the interaction of fibroblast growth factor (FGF) with its receptor FGFR, which plays a role in cell proliferation, differentiation, survival and migration. The structure of fibroblast growth factor

receptor 2 (FGFR2) in complex with its ligand (FGF1) and heparin at 2.8 Å resolution suggests that a 2 : 2 : 1 FGF1–FGFR2–heparin decasaccharide complex may play a role in receptor activation [33]. Heparin is an analogue of the obligate secondary receptor heparan sulphate. Analyses of the complexes with gel filtration, nanospray mass spectrometry and analytical ultracentrifugation [34] demonstrate only a binary 1 : 1 FGF : FGFR complex in the absence of heparin. However, a 2 : 2 : 1 complex forms spontaneously in solution between FGF1, FGFR2 and heparin decasaccharide and less efficiently with octasaccharide. Higher order complexes, e.g. with stoichiometries 4 : 4 : 1, can be observed using mass spectrometry. This probably reflects surface clustering, which is well known to play a role in receptor function [35].

Recently, evidence has emerged for cooperative or synergistic interactions between components of the NHEJ system. Although the interaction between XRCC4 and LigIV leads to a strong 2 : 1 complex, the interactions between XRCC4 and XLF are much more cooperative. Interactions with XRCC4 led to the discovery of XLF [7]. Mutagenesis studies of XLF and XRCC4 suggested that XLF–XRCC4 interactions are mediated through relatively small regions located at the sides of the head domains and contain the helix-turn-helix structures and the β6–β7 loop [36]. SAXS structural studies of XLF–XRCC4 complexes indicated a linear model with approximately a 45° rotation angle between XRCC4 and XLF coiled-coil tails [22]. We have observed an XLF–XRCC4 concentration-dependent higher order complex formation using nano-electrospray ionization mass spectrometry. Furthermore, an 8.5 Å resolution crystal structure of XLF–XRCC4 demonstrates that XLF and XRCC4 dimers interact through their head domains and form an alternating left-handed helical structure with polypeptide coiled-coils and pseudo-dyads of individual XLF and XRCC4 dimers at right angles to the helical axis [37]. In summary, it appears that weak binary interactions between XRCC4 and XLF dimers lead to relatively stable fibres. These are likely to mediate the ligation of DNA in DSB repair through NHEJ.

Cooperative interactions essential for biological complexity also occur in the KMN subcomplexes KNL1, Mis12 and Ndc80. For instance, the crystal structure of the Ndc80 subcomplex shows that it adopts a dumbbell shape (figure 4*c*) [38–42] containing four subunits: Ndc80 (the subunit that gives its name to the entire subcomplex), Nuf2, Spc24 and Spc25. Two subcomplexes, Spc24–Spc25 and Nuf2–Ndc80, occupy opposite ends of the dumbbell (figure 4*c*) [38,40]. Cooperative interactions are exemplified by the requirement for the association of Spc24–Spc25 subunits for binding the KNL1 and Mis12 complexes [11,41,42], while the association of the Nuf2–Ndc80 subunits mediates the binding of the Ndc80 complex to microtubules [39,43,44].

4. Low structure complexity and disorder-to-order transitions

The presence of regions of low structure complexity (also referred to as regions of intrinsic local disorder) is widespread in protein molecules [45–52]. They constitute a common feature of hub proteins in interactome networks [53–58]. Indeed, neural network predictors, developed to recognize sequences that correspond to structurally disordered regions, have shown

that 35–51% of eukaryotic proteins have at least one long (i.e. <50 residues) disordered region [59], while DisProt [60], a curated database of protein disorder that provides information about intrinsically disorder proteins (http://www.disprot.org) lists 1375 disorder regions in a total of 643 proteins.

A significant correlation exists between the average predicted disorder per complex and the number of complex components [61]. Intrinsic local disorder can increase the backbone conformational entropy upon ligand binding and provide a kinetic advantage by speeding up the search for specific targets [50,62]. Furthermore, large and highly flexible interaction surfaces can assist the assembly of intertwined multimeric complexes [50]. Indeed, most hubs in protein interaction networks contain long segments of low structural complexity that engage in binding [48,57,63,64] and removal of such hub proteins has a dramatic impact on the function of the entire network [65,66].

A transition from a disorder state to a more organized one may occur upon ligand binding. This process, commonly referred to as a disorder-to-order transition has been described in multiple protein complexes. One example is $p27^{Kip1}$, the inhibitor of cyclin-dependent kinases (Cdks) and the tumour suppressor p53. Large fragments of $p27^{Kip1}$ are intrinsically unstructured with a marginal content of helix structure as shown by limited proteolysis, circular dichroism and nuclear magnetic resonance (NMR) spectroscopy analyses [67–69]. The inherent flexibility of unstructured segments is important for sequential $p27^{Kip1}$ phosphorylation by CK2 (figure 5*a*) as this post-translational modification primes $p27^{Kip1}$ for its ubiquitylation and eventual proteolytic degradation, a process required for progression through the cell cycle. Interestingly, a disorder-to-order transition takes place upon $p27^{Kip1}$ binding to cyclin A–Cdk2 (figure 5*b*), an interaction that regulates the activity of Cdk2 in a process in which $p27^{Kip1}$ residues insert into the catalytic cleft mimicking adenosine-S′-triphosphate [70]. A similar disorder-to-order transition has been observed in the interaction of N-terminal p53 with murine double minute (Mdm2) (figure 5*c*). p53 binding to DM2 results in the loss of transcriptional activity and the stimulation of ubiquitination and degradation by the proteasome. The interaction defines a largely α-helical region and involves extensive van der Waals contacts in which Phe19, Trp23 and Leu26 lying on one side of the helix play a major role [71] (figure 5*c*). As Mdm2 is often over-expressed in tumours, the Mdm2 interaction with p53 has become an interesting therapeutic target [72,73].

Individual components of the NHEJ- and SAC signalling pathways also fall in this category; they are characterized by the presence of regions of low structural complexity, some of which are disordered prior to assembly and become ordered only on binding. For example, LigIV has a tandem breast cancer gene 1 C-terminal (BRCT) domain with a linker predicted to be mostly disordered. The linker between BRCT1 and BRCT2 mediates the interaction of LigIV with XRCC4 [74,75]. This linker seems to be important for the catalytic activity of LigIV [74]. LigIV asymmetrically interacts with XRCC4 with 1 : 2 stoichiometry. This transforms the left-handed coiled-coil tail of XRCC4 into the right-handed undecad coil, making the surface of the XRCC4 interaction region flat, as a result of a kink in one of the helices in the coiled-coil (figure 3*b*,*c*) [75]. The concerted binding and folding of the previously flexible linker leads to a stable structure in which multiple and well-defined interactions are made between the ligase and the XRCC4.

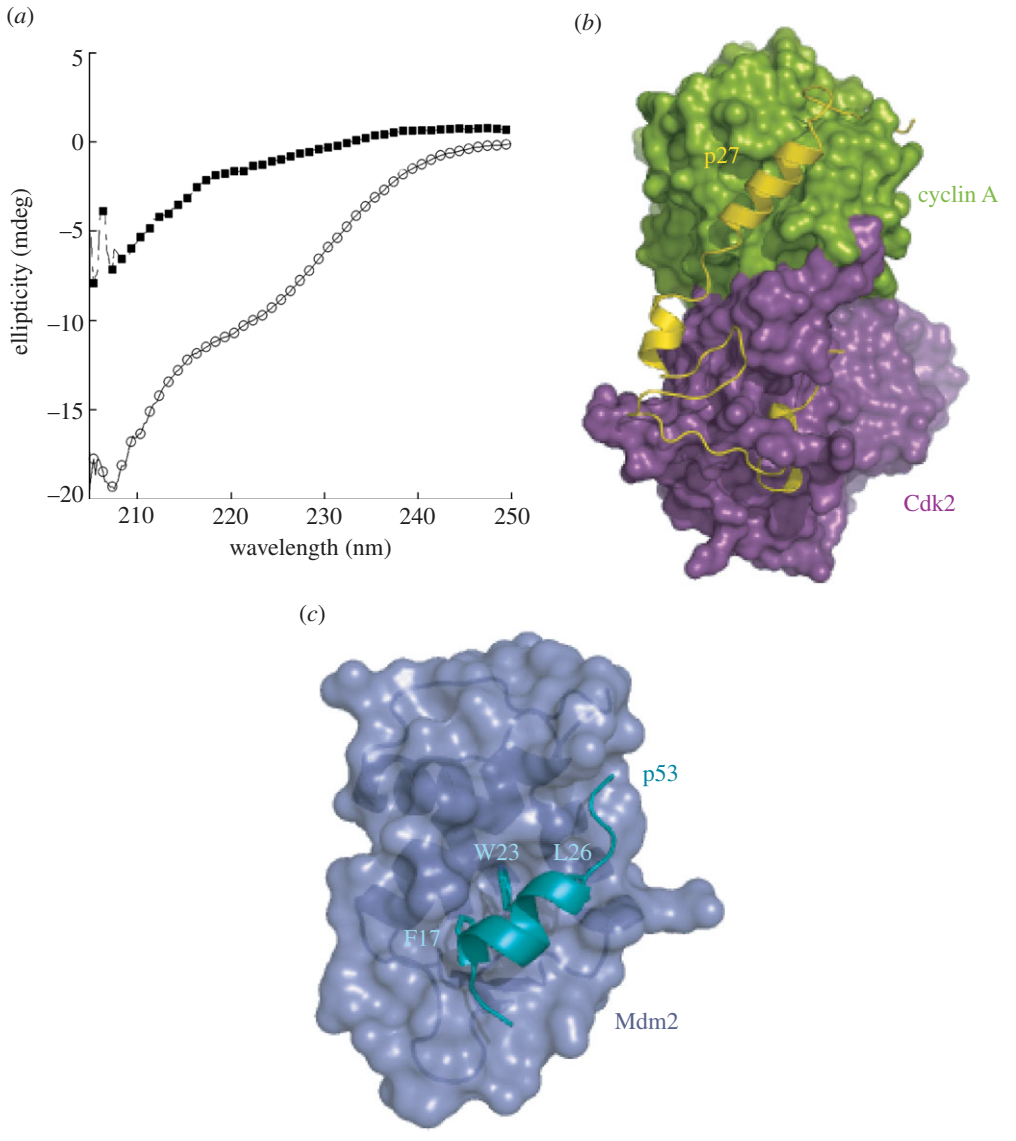

Figure 5. (*a*) Far-UV circular dichroism shows that a disorder-to-order transition occurs upon $p27^{Kip1}$ phosphorylation by CK2 (open circles, p27WT; filled squares, p27-P). (*b*) $p27^{Kip1}$ binding to Cdk2–cyclin A and C. A disorder-to-order transitions has been observed in p53 binding to Mdm2. (Online version in colour.)

In SAC signalling, several disorder–order transitions have been observed. For instance, Bub1 and Mad3 GLEBS motifs in complex with Bub3 involve a transition from a predominantly disordered (unbound) to a more ordered (Bub3-bound) state. A similar situation is observed on the interaction between N-terminal Blinkin and BubR1 where Blinkin undergoes an important folding transition upon binding [31]. The disorder-to-order transition opens the possibility that Bub1 and BubR1 binding to N-terminal Blinkin induces

conformational changes that affect the interaction of this kinetochore protein with other ligands such as Aurora B and protein phosphatase 1, thus conferring directionality to the flow of information encoded in this signalling pathway [76].

5. Variation of assemblies over time and in space

The remodelling of macromolecular assemblies in time and space has evolved as a successful strategy that allows sequential obligate steps and the increase of selectivity with a minimal margin for errors. This highly versatile and dynamic nature of remodelling of macromolecular assemblies constitutes a great challenge for their structural characterization, and often requires a multi-disciplinary strategy that combines SAXS, EM, X-ray crystallography, NMR and other biochemical, biophysical, molecular and cellular approaches. In order to understand the temporal organization of the NHEJ repair system as a whole, knowledge of the order of interactions during the assembly of the DNA-PKcs/Ku70/Ku80/DNA ternary complex and the LigIV/XRCC4/XLF/DNA quaternary complex will be essential. The classical sequential model [77–79] suggests that Ku70/80 and DNA-PKcs, which have higher DNA-binding affinity compared with LigIV/XRCC4/XLF, most probably form the DNA-PKcs/Ku70/Ku80/DNA ternary complex first. For the following LigIV/XRCC4/XLF/DNA complex formation, the order and dynamics of protein assembly are still to be determined. The initial interaction between XRCC4 and XLF is relatively weak, but as we have seen earlier, they undergo strong cooperative interactions to form helical structures. Whether these structures are compatible with retention of the XRCC4 interactions with LigIV remains to be determined.

Post-translational modifications (acetylation, phosphorylation, ubiquitylation, etc.) have a significant impact on protein function as they can affect protein stability, turnover, reversibility, sub-cellular localization and/or the hierarchical order of assembly/disassembly [80,81]. For instance, in NHEJ signalling, phosphorylation of DNA-PKcs induces a large conformational change, sufficient to open the gap in the ring and provide access to or release from DNA [82]. The C-terminal domain of Ku80 (Ku80CTD) has been shown to be flexible and to extend in solution to the benefit of recruitment of DNA-PKcs, suggesting that the interacting of Ku80 with DNA-PKcs occurs on both sides of DSBs [22]. Although phosphorylation of LigIV, XRCC4 and XLF by DNA-PKcs does not interfere greatly with the core functions of these proteins, their differential phosphorylation probably regulates the correct spatial arrangement of the higher order complexes by altering the relative binding affinities of various protein–protein or protein–DNA interactions. Further studies should aim to characterize these complexes temporally as well as spatially.

Some experimental studies have suggested a two-phase NHEJ protein-binding model [23]. This has been investigated by Li & Cucinotta [83], who have proposed a mathematical model describing the biochemical reaction network. They suggest that, if a DSB repair pathway independent of DNA-PKcs exists, then the sequential model is indistinguishable from the two-phase model where after binding of Ku70/Ku80 with DNA, the other NHEJ components can all be recruited to DNA sites and then rearrange and change interaction modes according to the process required.

Knowledge of the recruitment hierarchy among the kinetochore subunits should eventually provide clues about the mechanisms mediating kinetochore function and its regulation by the SAC. However, the specific roles of a large number of proteins that participate in the assembly and regulation of the kinetochore remain to be established. For instance, Ohta and collaborators have combined stable-isotope labelling by amino acids in cell culture mass spectrometric techniques with bioinformatics analyses to identify chromosomal proteins in the context of intact chromosomes [84]. This large-scale quantitative mass spectrometry study allowed the identification of 4029 mitotic chromosome-associated proteins, of which 562 were previously uncharacterized; there is clearly much remaining of the complexity of these systems to define.

An important consideration in SAC signalling is that the link made by the KMN network to connect the centromere to the microtubule fibre of the mitotic spindle must be strong enough to sustain the pulling forces during anaphase. At the same time, it must be sufficiently dynamic to permit the polymerization–depolymerization of the plus ends of microtubules and respond efficiently to the regulatory mechanisms that enable chromosomes to align at the metaphase plate prior to anaphase. Thus, a number of properties are required to ensure the flow of information in order to signal both correct and incorrect attachment of chromosomes, to enable a weakening of microtubule binding when there is a need to correct the attachment, and to delay anaphase until proper attachment has occurred.

The mode of assembly and regulation of macromolecular complexes of NHEJ and SAC checkpoint signalling pathways are reminiscent of Mackay's early ideas on the physical meaning of hierarchy in complex structures [85], which recalls Bernal's definition of life as 'a partial, continuous, progressive, multi-form and conditionally interactive, self-realization of the potentialities of atomic electron states' [86]. As the use of high-throughput experimental techniques, together with molecular and computational methods of systems biology, begin to give insights into the dynamic architecture of the interactome, we will learn more of the exquisite complexity of interactions between thousands of protein components participating in finely tuned regulatory pathways essential to all eukaryotes. Definition of genotype–phenotype associations at different levels of complexity, i.e. genes, RNA transcripts, peptides, proteins, metabolites and environmental factors, that characterize cellular networks can only be understood in the light of evolution and should expose the mechanisms and principles underlying the regulation of complex biological systems [87–89].

6. Closing remarks

The functions of eukaryotic cells depend upon the organization of macromolecular assemblies. Such organization is a structural theme that occurs widely in the regulation of signalling pathways. Regions of low structural complexity play essential roles in this process as they allow greater selectivity in molecular recognition and will tend to increase signal-to-noise ratio. Further selectivity is gained by the involvement of multiple components in systems regulating DNA DSB repair by NHEJ recombination and SAC signalling. A spatial and temporal understanding of NHEJ and SAC signalling should provide insights into the mechanism of these critical cellular processes.

The combination of structural biology of macromolecular assemblies, together with systems biology approaches, should lead to a more holistic understanding of the cell and provide insights into how defects in molecular interactions can lead to cellular misfunction. Databases that enable the utilization of the available structural information on protein interactions with small molecules in combination with sequence and molecular interaction data [90,91] are likely to be central to the design of useful chemical tools for dissecting these complex pathways and for providing a basis for drug discovery in the coming years.

In this review, we have demonstrated the importance of crystal structures for the derivation of knowledge of macromolecular interactions and architecture, but we have also shown how we can go 'beyond crystals' in order to infer molecular and cellular function in space and time. We have observed how information and structure interact: information defines structure but material structure and organization in turn define information.

References

1 Milo, R., Jorgensen, P., Moran, U., Weber, G. & Springer, M. 2010 BioNumbers—the database of key numbers in molecular and cell biology. *Nucleic Acids Res.* **38**, D750–D753. (doi:10.1093/nar/gkp889)

2 Lieber, M. 2004 The mechanism of vertebrate nonhomologous DNA end joining and its role in V(D)J recombination. *DNA Repair* **3**, 817–826. (doi:10.1016/j.dnarep.2004.03.015)

3 Lieber, M. R. 2010 The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Ann. Rev. Biochem.* **79**, 181–211. (doi:10.1146/annurev.biochem.052308.093131)

4 Grawunder, U., Wilm, M., Wu, X., Kulesza, P., Wilson, T. E., Mann, M. & Lieber, M. R. 1997 Activity of DNA ligase IV stimulated by complex formation with XRCC4 protein in mammalian cells. *Nature* **388**, 492–495. (doi:10.1038/41358)

5 Sibanda, B. L., Chirgadze, D. Y. & Blundell, T. L. 2010 Crystal structure of DNA-PKcs reveals a large open-ring cradle comprised of HEAT repeats. *Nature* **463**, 118–121. (doi:10.1038/nature08648)

6 Jilani, A., Ramotar, D., Slack, C., Ong, C., Yang, X. M., Scherer, S. W. & Lasko, D. D. 1999 Molecular cloning of the human gene, PNKP, encoding a polynucleotide kinase 3′-phosphatase and evidence for its role in repair of DNA strand breaks caused by oxidative damage. *J. Biol. Chem.* **274**, 24 176–24 186. (doi:10.1074/jbc.274.34.24176)

7 Ahnesorg, P., Smith, P. & Jackson, S. P. 2006 XLF interacts with the XRCC4–DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell* **124**, 301–313. (doi:10.1016/j.cell.2005.12.031)

8 Buck, D. *et al.* 2006 Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. *Cell* **124**, 287–299. (doi:10.1016/j.cell.2005.12.030)

9 Lampson, M. A. & Kapoor, T. M. 2005 The human mitotic checkpoint protein BubR1 regulates chromosome-spindle attachments. *Nat. Cell Biol.* **7**, 93–98. (doi:10.1038/ncb1208)

10 Elowe, S. Dulla, K., Uldschmid, A., Li, X., Dou, Z. & Nigg, E. A. 2010 Uncoupling of the spindle-checkpoint and chromosome-congression functions of BubR1. *J. Cell Sci.* **123**, 84–94. (doi:10.1242/jcs.056507)

11 Kiyomitsu, T., Obuse, C. & Yanagida, M. 2007 Human Blinkin/AF15q14 is required for chromosome alignment and the mitotic checkpoint through direct interaction with Bub1 and BubR1. *Dev. Cell* **13**, 663–676. (doi:10.1016/j.devcel.2007.09.005).

12 Bolanos-Garcia, V. M. *et al.* 2011 Structure of a Blinkin-BUBR1 complex reveals an interaction crucial for kinetochore-mitotic checkpoint regulation via an unanticipated binding site. *Structure* **19**, 1691–1700. (doi:10.1016/j.str.2011.09.017)

13 Tanaka, T. U. 2010 Kinetochore–microtubule interactions: steps towards bi-orientation. *EMBO J.* **29**, 4070–4082. (doi:10.1038/emboj.2010.294)
14 Bloom, K. & Yeh, E. 2010 Tension management in the kinetochore. *Curr. Biol.* **20**, R1040–R1048. (doi:10.1016/j.cub.2010.10.055)
15 Jackson, S. P. & Bartek, J. 2009 The DNA-damage response in human biology and disease. *Nature* **461**, 1071–1078. (doi:10.1038/nature08467)
16 Dalton, W., Nandan, B., Mandayam, O., Moore, R. T. & Yang, V. W. 2007 Human cancer cells commonly acquire DNA damage during mitotic arrest. *Cancer Res.* **67**, 11 487–11 492. (doi:10.1158/0008-5472.CAN-07-5162)
17 Falck, J., Coates, J. & Jackson, S. P. 2005 Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature* **434**, 605–611. (doi:10.1038/nature03442)
18 Feldmann, E., Schmiemann, V., Goedecke, W., Reichenberger, S. & Pfeiffer, P. 2000 DNA double-strand break repair in cell-free extracts from Ku80-deficientw cells: implications for Ku serving as an alignment factor in non-homologous DNA end joining. *Nucleic Acids Res.* **28**, 2585–2596. (doi:10.1093/nar/28.13.2585)
19 Walker, J. R., Corpina, R. A. & Goldberg, J. 2001 Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* **412**, 607–614. (doi:10.1038/35088000)
20 Boskovic, J., Rivera-Calzada, A., Maman, J. D., Chacón, P., Willison, K. R., Pearl, L. H., & Llorca, O. 2003 Visualization of DNA-induced conformational changes in the DNA repair kinase DNA-PKcs. *EMBO J.* **22**, 5875–5882. (doi:10.1093/emboj/cdg555)
21 Spagnolo, L., Rivera-Calzada, A., Pearl, L. H. & Llorca, O. 2006 Three-dimensional structure of the human DNA-PKcs/Ku70/Ku80 complex assembled on DNA and its implications for DNA DSB repair. *Mol. Cell* **22**, 511–519. (doi:10.1016/j.molcel.2006.04.013)
22 Hammel, M., Yu, Y., Fang, S., Lees-Miller, S. P. & Tainer, J. A. 2010 XLF regulates filament architecture of the XRCC4·ligase IV complex. *Structure* **18**, 1431–1442. (doi:10.1016/j.str.2010.09.009)
23 Yano, K. & Chen, D. J. 2008 Live cell imaging of XLF and XRCC4 reveals a novel view of protein assembly in the non-homologous end-joining pathway. *Cell Cycle* **7**, 1321–1325. (doi:10.4161/cc.7.10.5898)
24 Dobles, M., Liberal, V., Scott, M. L., Benezra, R. & Sorger, P. K. 2000 Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein MAD2. *Cell* **101**, 635–645. (doi:10.1016/S0092-8674(00)80875-2)
25 Luo, X., Tang, Z., Rizo, J. & Yu, H. 2002 The MAD2 spindle checkpoint protein undergoes similar major conformational changes upon binding to either MAD1 or CDC20. *Mol. Cell* **9**, 59–71. (doi:10.1016/S1097-2765(01)00435-X)
26 Bolanos-Garcia, V. M. *et al.* 2009 The crystal structure of the N-terminal region of BUB1 provides insight into the mechanism of BUB1 recruitment to kinetochores. *Structure* **17**, 105–116. (doi:10.1016/j.str.2008.10.015)
27 D'Arcy, S., Davies, O. R., Blundell, T. L. & Bolanos-Garcia, V. M. 2010 Defining the molecular basis of BubR1 kinetochore interactions and APC/C-CDC20 inhibition. *J. Biol. Chem.* **285**, 14 764–14 776. (doi:10.1074/jbc.M109.082016)
28 Bolanos-Garcia, V. M. & Blundell, T. L. 2011 BUB1 and BUBR1: multifaceted kinases of the cell cycle. *Trends Biochem. Sci.* **36**, 141–150. (doi:10.1016/j.tibs.2010.08.004)
29 Cheeseman, I. M., Hori, T., Fukagawa, T. & Desai, A. 2008 KNL1 and the CENP-H/I/K complex coordinately direct kinetochore assembly in vertebrates. *Mol. Biol. Cell* **19**, 587–594. (doi:10.1091/mbc.E07-10-1051)
30 Przewloka, M. R., Zhang, W., Costa, P., Archambault, V., D'Avino, P. P., Lilley, K. S., Laue, E. D., McAinsh, A. D. & Glover, D. M. 2007 Molecular analysis of core kinetochore composition and assembly in *Drosophila melanogaster*. *PLoS ONE* **2**, e478. (doi:10.1371/journal.pone.0000478)
31 Simpson, P. J., Cota, E. & Bolanos-Garcia, V. M. 2012 ^{1}H, ^{13}C and ^{15}N resonance assignments of the kinetochore localisation domain of BUBR1, a central component of the spindle assembly checkpoint. *Biomol. NMR Assign.* **6**, 115–118. (doi:10.1007/s12104-011-9355-9)

32 Blundell, T. L., Bolanos-Garcia, V., Chirgadze, D. Y., Harmer, N. J., Lo, T., Pellegrini, L. & Sibanda, B. L. 2002 Asymmetry in the multiprotein systems of molecular biology. *Struct. Chem.* **13**, 405–412. (doi:10.1023/A:1015888617329)

33 Pellegrini, L., Burke, D. F., von Delft, F., Mulloy, B. & Blundell, T. L. 2000 Crystal structure of fibroblast growth factor receptor ectodomain bound to ligand and heparin. *Nature* **407**, 1029–1034. (doi:10.1038/35039551)

34 Harmer, N. J., Ilag, L. L., Mulloy, B., Pellegrini, L., Robinson, C. V. & Blundell, T. L. 2004 Towards a resolution of the stoichiometry of the fibroblast growth factor (FGF)–FGF receptor–heparin complex. *J. Mol. Biol.* **339**, 821–834. (doi:10.1016/j.jmb.2004.04.031)

35 Robinson, C. J., Harmer, N. J., Goodger, S. J., Blundell, T. L. & Gallagher, J. T. 2005 Cooperative dimerization of fibroblast growth factor 1 (FGF1) upon a single heparin saccharide may drive the formation of 2:2:1 FGF1:FGFR2c:heparin ternary complexes. *J. Biol. Chem.* **280**, 42 274–42 282. (doi:10.1074/jbc.M505720200)

36 Malivert, L. *et al.* 2010 Delineation of the Xrcc4-interacting region in the globular head domain of cernunnos/XLF. *J. Biol. Chem.* **285**, 26 475–26 483. (doi:10.1074/jbc.M110.138156)

37 Wu, Q., Ochi, T., Matak-Vinkovic, D., Robinson, C. V., Chirgadze, D. Y. & Blundell, T. L. 2011 Non-homologous end-joining partners in a helical dance: structural studies of XLF–XRCC4 interactions. *Biochem.Soc. Trans.* **39**, 1387–1392.

38 Ciferri, C. *et al.* 2005 Architecture of the human Ndc80-Hec1 complex, a critical constituent of the outer kinetochore. *J. Biol. Chem.* **280**, 29 088–29 095. (doi:10.1074/jbc.M504070200)

39 Ciferri, C. *et al.* 2008 Implications for kinetochore-microtubule attachment from the structure of an engineered Ndc80 complex. *Cell* **133**, 427–439. (doi:10.1016/j.cell.2008.03.020)

40 Wei, R. R., Sorger, P. K. & Harrison, S. C. 2005 Molecular organization of the Ndc80 complex, an essential kinetochore component. *Proc. Natl Acad. Sci. USA* **102**, 5363–5367. (doi:10.1073/pnas.0501168102)

41 Joglekar, A. P. & DeLuca, J. G. 2009 Chromosome segregation: Ndc80 can carry the load. *Curr. Biol.* **19**, R404–R407. (doi:10.1016/j.cub.2009.04.014)

42 Wan, X., *et al.* 2009 Protein architecture of the human kinetochore microtubule attachment site. *Cell* **137**, 672–684. (doi:10.1016/j.cell.2009.03.035)

43 Cheeseman, I. M., Chappie, J. S., Wilson-Kubalek, E. M. & Desai, A. 2006 The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. *Cell* **127**, 983–997. (doi:10.1016/j.cell.2006.09.039)

44 Wei, R. R., Al-Bassam, J. & Harrison, S. C. 2007 The Ndc80/HEC1 complex is a contact point for kinetochore-microtubule attachment. *Nat. Struct. Mol. Biol.* **14**, 54–59. (doi:10.1038/nsmb1186)

45 Dunker, A. K., Garner, E., Guilliot, S., Romero, P., Albrecht, K., Hart, J., Obradovic, Z., Kissinger, C. & Villafranca, J. E. 1998 Protein disorder and the evolution of molecular recognition: theory, predictions and observations. *Pac. Symp. Biocomput.* 473–484.

46 Dyson, H. J. & Wright, P. E. 1998 Equilibrium NMR studies of unfolded and partially folded proteins. *Nat. Struct. Biol.* **5**, 499–503.

47 Dyson, H. J. & Wright, P. E. 2002 Coupling of folding and binding for unstructured proteins. *Curr. Opin. Struct. Biol.* **12**, 54–60. (doi:10.1016/S0959-440X(02)00289-0)

48 Dyson, H. J. & Wright, P. E. 2005 Intrinsically unstructured proteins and their functions. *Nat. Rev. Mol. Cell Biol.* **6**, 197–208. (doi:10.1038/nrm1589)

49 Fong, J. H., Shoemaker, B. A., Garbuzynskiy, S. O., Lobanov, M. Y., Galzitskaya, O. V. & Panchenko, A. R. 2009 Intrinsic disorder in protein interactions: insights from a comprehensive structural analysis. *PLoS Comput. Biol.* **5**, e1000316. (doi:10.1371/journal.pcbi.1000316)

50 Meszaros, B., Tompa, P., Simon, I. & Dosztanyi, Z. 2007 Molecular principles of the interactions of disordered proteins. *J. Mol. Biol.* **372**, 549–561. (doi:10.1016/j.jmb.2007.07.004)

51 Tompa, P. & Fuxreiter, M. 2008 Fuzzy complexes: polymorphism and structural disorder in protein–protein interactions. *Trends Biochem. Sci.* **33**, 2–8. (doi:10.1016/j.tibs.2007.10.003)

52 Gsponer, J. & Babu, M. M. 2009 The rules of disorder or why disorder rules. *Prog. Biophys. Mol. Biol.* **99**, 94–103. (doi:10.1016/j.pbiomolbio.2009.03.001)

53 Dosztanyi, Z., Chen, J., Dunker, A. K., Simon, I. & Tompa, P. 2006 Disorder and sequence repeats in hub proteins and their implications for network evolution. *J. Proteome Res.* **5**, 2985–2995. (doi:10.1021/pr060171o)

54 Dunker, A. K., Cortese, M. S., Romero, P., Iakoucheva, L. M. & Uversky, V. N. 2005 Flexible nets: the roles of intrinsic disorder in protein interaction networks. *FEBS J.* **272**, 5129–5148. (doi:10.1111/j.1742-4658.2005.04948.x)

55 Haynes, C., Oldfield, C. J., Ji, F., Klitgord, N., Cusick, M. E., Radivojac, P., Uversky, V. N., Vidal, M. & Iakoucheva, L. M. 2006 Intrinsic disorder is a common feature of hub proteins from four eukaryotic interactomes. *PLoS Comput. Biol.* **2**, e100. (doi:10.1371/journal.pcbi.0020100)

56 Kim, P. M., Sboner, A., Xia, Y. & Gerstein, M. 2008 The role of disorder in interaction networks: a structural analysis. *Mol. Syst. Biol.* **4**, 179. (doi:10.1038/msb.2008.16)

57 Schlessinger, A., Liu, J. & Rost, B. 2007 Natively unstructured loops differ from other loops. *PLoS Comput. Biol.* **3**, e140. (doi:10.1371/journal.pcbi.0030140)

58 Singh, G. P., Ganapathi, M. & Dash, D. 2007 Role of intrinsic disorder in transient interactions of hub proteins. *Proteins* **66**, 761–765. (doi:10.1002/prot.21281)

59 Dunker, A. K. Brown, C. J., Lawson, J. D., Iakoucheva, L. M. & Obradoviæ, Z. 2002 Intrinsic disorder and protein function. *Biochemistry* **41**, 6573–6582. (doi:10.1021/bi012159+)

60 Sickmeier, M. *et al.* 2007 DisProt: the database of disordered proteins. *Nucleic Acids Res.* **35**, D786–D793. (doi:10.1093/nar/gkl893)

61 Hegyi, H., Schad, E. & Tompa, P. 2007 Structural disorder promotes assembly of protein complexes. *BMC Struct. Biol.* **7**, 65. (doi:10.1186/1472-6807-7-65)

62 Sigalov, A. B., Zhuravleva, A. V. & Orekhov, V. Y. 2007 Binding of intrinsically disordered proteins is not necessarily accompanied by a structural transition to a folded form. *Biochimie* **89**, 419–421. (doi:10.1016/j.biochi.2006.11.003)

63 Kim, P. M., Lu, L. J., Xia, Y. & Gerstein, M. B. 2006 Relating three-dimensional structures to protein networks provides evolutionary insights. *Science* **314**, 1938–1941. (doi:10.1126/science.1136174)

64 Dunker, A. K., Oldfield, C. J., Meng, J., Romero, P., Yang, J. Y., Chen, J. W., Vacic, V., Obradovic, Z. & Uversky, V. N. 2008 The unfoldomics decade: an update on intrinsically disordered proteins. *BMC Genomics* **9**(Suppl. 2), S1. (doi:10.1186/1471-2164-9-S2-S1)

65 Albert, R. 2005 Scale-free networks in cell biology. *J. Cell Sci.* **118**, 4947–4957. (doi:10.1242/jcs.02714)

66 Albert, R., Jeong, H. & Barabasi, A. L. 2000 Error and attack tolerance of complex networks. *Nature* **406**, 378–382. (doi:10.1038/35019019)

67 Tapia, J. C., Bolanos-Garcia, V. M., Sayed, M., Allende, C. C. & Allende, J. E. 2004 Cell cycle regulatory protein p27KIP1 is a substrate and interacts with the protein kinase CK2. *J. Cell Biochem.* **91**, 865–879. (doi:10.1002/jcb.20027)

68 Bienkiewicz, E. A., Adkins, J. N. & Lumb, K. J. 2002 Functional consequences of preorganized helical structure in the intrinsically disordered cell-cycle inhibitor p27(Kip1). *Biochemistry* **41**, 752–759. (doi:10.1021/bi015763t)

69 Kriwacki, R. W., Hengst, L., Tennant, L., Reed, S. I. & Wright, P. E. 1996 Structural studies of p21Waf1/Cip1/Sdi1 in the free and Cdk2-bound state: conformational disorder mediates binding diversity. *Proc. Natl Acad. Sci. USA* **93**, 11 504–11 509.

70 Russo, A. A., Jeffrey, P. D., Patten, A. K., Massagué, J. & Pavletich, N. P. 1996 Crystal structure of the p27Kip1 cyclin-dependent-kinase inhibitor bound to the cyclin A–Cdk2 complex. *Nature* **382**, 325–331. (doi:10.1038/382325a0)

71 Kussie, P. H. Gorina, S. Marechal, V., Elenbaas, B., Moreau, J., Levine, A. J. & Pavletich, N. P. 1996 Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science* **274**, 948–953. (doi:10.1126/science.274.5289.948)

72 Madhumalar, A., Jun, L. H., Brown, C. J., Lane, D. P. & Verma, C. 2009 Design of a novel MDM2 binding peptide based on the p53 family. *J. Cell Cycle* **8**, 2828–2836. (doi:10.4161/cc.8.17.9516)

73 Cheok, C. F., Verma, C. S., Baselga, J. & Lane, D. P. 2011 Translating p53 into the clinic. *Nat. Rev. Clin. Oncol.* **8**, 25–37. (doi:10.1038/nrclinonc.2010.174)

74 Grawunder, U., Zimmer, D. & Lieber, M. R. 1998 DNA ligase IV binds to XRCC4 via a motif located between rather than within its BRCT domains. *Curr. Biol.* **8**, 873–876. (doi:10.1016/S0960-9822(07)00349-1)

75 Sibanda, B. L., Critchlow, S. E., Begun, J., Pei, X. Y., Jackson, S. P., Blundell, T. L. & Pellegrini, L. 2001 Crystal structure of an Xrcc4-DNA ligase IV complex. *Nat. Struct. Biol.* **8**, 1015–1019. (doi:10.1038/nsb725)

76 Bolanos-Garcia, V. M., Nilsson, J. & Blundell, T. L. In press. The architecture of the BubR1 tetratricopeptide tandem repeat defines a protein motif underlying mitotic checkpoint-kinetochore communication. *BioArchitecture* **2**. (doi.org/10.4161/bioa).

77 Mahaney, B. L., Meek, K. & Lees-Miller, S. P. 2009 Repair of ionizing-induced DNA double-strand breaks by non-homologous end-joining. *Biochem. J.* **417**, 639–665. (doi:10.1042/BJ20080413)

78 Sharma, S. & Raghavan, S. C. 2010 Non-homologous end joining in cell-free extracts. *J. Nucleic Acids* **2010**, 389129. (doi:10.4061/2010/389129)

79 Ochi, T., Sibanda, B. L., Wu, Q., Chirgadze, D. Y., Bolanos-Garcia, V. M. & Blundell, T. L. 2010 Structural biology of DNA repair: spatial organisation of the multicomponent complexes of nonhomologous end joining. *J. Nucleic Acids* **2010**, 621695. (doi:10.4061/2010/621695)

80 Pawson, T. & Nash, P. 2003 Assembly of cell regulatory systems through protein interaction domains. *Science* **300**, 445–452. (doi:10.1126/science.1083653)

81 Seet, B. T., Dikic, I., Zhou, M. M. & Pawson, T. 2006 Reading protein modifications with interaction domains. *Nat. Rev. Mol. Cell Biol.* **7**, 473–483. (doi:10.1038/nrm1960)

82 Perry, J. J., Cotner-Gohara, E., Ellenberger, T. & Tainer, J. A. 2010 Structural dynamics in DNA damage signaling and repair. *Curr. Opin. Struct. Biol.* **20**, 283–294. (doi:10.1016/j.sbi.2010.03.012)

83 Li, Y. & Cucinotta, F. A. 2011 Modeling non-homologous end joining. *J. Theor. Biol.* **283**, 122–135. (doi:10.1016/j.jtbi.2011.05.015)

84 Ohta, S. *et al.* 2010 The protein composition of mitotic chromosomes determined using multiclassifier combinatorial proteomics. *Cell* **142**, 810–821. (doi:10.1016/j.cell.2010.07.047)

85 Mackay, A. L. 1969 The structure of structure—some problems in solid state chemistry. *Chimia* **23**, 433–437.

86 Bernal, J. D. 1967 *The origin of life*, p. xv. London, UK: Weidenfeld and Nicolson.

87 Dobzhansky, T. 1964 Biology, molecular and organismic. *Am. Zool.* **4**, 443–452.

88 Caetano-Anollés, G. 2010 *Evolutionary genomics and systems biology*. Hoboken, NJ: Wiley-Blackwell.

89 Castrillo, J. I. & Oliver, S. G. 2011 Yeast systems biology: the challenge of eukaryotic complexity. In *Yeast systems biology: methods and protocols*, vol. 759 (eds J. I. Castrillo & S. G. Oliver), 1st edn, ch. 1. Humana (MiMB Series). New York, NY: Springer.

90 Higueruelo, A. P., Schreyer, A., Bickerton, G. R., Pitt, W. R., Groom, C. R. & Blundell, T. L. 2009 Atomic interactions and profile of small molecules disrupting protein–protein interfaces: the TIMBAL database. *Chem. Biol. Drug Des.* **74**, 457–467. (doi:10.1111/j.1747-0285.2009.00889.x)

91 Lee, S. *et al.* 2009 Structural interactomics: informatics approaches to aid the interpretation of genetic variation and the development of novel therapeutics. *Mol. Biosyst.* **12**, 1456–1472. (doi:10.1039/b906402h)