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REVIEW ARTICLE

Promiscuity as a functional trait: intrinsically disordered regions as central players of interactomes

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Because of their pervasiveness in eukaryotic genomes and their unique properties, understanding the role that ID (intrinsically disordered) regions in proteins play in the interactome is essential for gaining a better understanding of the network. Especially critical in determining this role is their ability to bind more than one partner using the same region. Studies have revealed that proteins containing ID regions tend to take a central role in protein interaction networks; specifically, they act as hubs, interacting with multiple different partners across time and space, allowing for the co-ordination of many cellular activities. There appear to be three different modules within ID regions responsible for their functionally promiscuous behaviour: MoRFs (molecular recognition features), SLiMs (small linear motifs) and LCRs (low complexity regions). These regions allow for functionality such as engaging in the formation of dynamic heteromeric structures which can serve to increase local activity of an enzyme or store a collection of functionally related molecules for later use. However, the use of promiscuity does not come without a cost: a number of diseases that have been associated with ID-containing proteins seem to be caused by undesirable interactions occurring upon altered expression of the ID-containing protein.

Key words: interactome, intrinsically disordered, low complexity region, molecular recognition feature, small linear motif.

INTRODUCTION

It is becoming increasingly clear that only by studying proteins within the context of their interaction networks will a more complete understanding of complex cellular processes and their disease-causing malfunctions be achievable [1,2]. Substantial advances in high-throughput technologies have enabled the mapping of PPI (protein–protein interaction) networks of a few organisms in the last few years [3–9]. Although the coverage of total interactomes is only partial [10], a detailed analysis of the available protein interaction maps is a step toward a description of cellular processes at a systems level [1]. From such initial analyses, it appears that all available PPI networks display scale free topology; this topology is surprisingly pervasive, having been previously observed in social networks and the Internet [11,12]. In scale-free networks, most of the nodes have a small number of interaction partners (degree), but a small subset, the hubs, have a very large number of partners (Figure 1). This makes them robust to random node failure relative to a network whose degree distribution is random, but it also creates a vulnerability to the loss of only a few hubs. Importantly, PPI networks are highly dynamic, as their constituents are often short-lived and can be modified (e.g. phosphorylation) to shift interaction preferences [13–15].

Requiring special consideration regarding interaction networks are the proteins that harbour ID (intrinsically disordered) or natively disordered segments. ID segments lack a unique three-dimensional structure, either entirely or in parts, when expressed as autonomous units, and it is assumed that they sample a variety of conformations that are in equilibrium under physiological conditions [16–19]. Most ID segments fold, at least partially, upon complex formation [17,20], although in some cases functions of proteins have been related to the particular properties of the ID segment [21]. That it is essential to understand the interaction patterns of proteins with large ID segments becomes evident in the light of their abundance in higher eukaryotes (about 30–40 % of their proteins contain large disordered segments) [22], and the finding that proteins enriched in disorder are crucial for cellular processes such as transcription and signal transduction [23]. Of particular interest in the context of PPI networks are findings demonstrating that proteins with many or large ID segments often have the ability to bind promiscuously, with promiscuous proteins or protein segments being those that can bind to many different targets [16,24–26]. The present review focuses on recent insights gained into the promiscuous interaction behaviour of proteins with ID segments, how such behaviour relates to their functional relevance and how it might also relate to disease.

PROTEINS WITH ID SEGMENTS FORM AN INTERWOVEN NETWORK OF REGULATORY PROTEINS

From the outset of the analysis of PPI networks, it became clear that hub proteins (definitions of hubs vary, but commonly the top 20 % with respect to degree are selected [27]) must have special properties in order to interact with, in some cases, hundreds of partners [28]. Dunker et al. [16] were the first to propose that hubs may be enriched in intrinsic disorder. Subsequently, a variety of computational studies have confirmed that hubs have higher

Abbreviations used: CBP, CREB-binding protein; CREB, cAMP-response-element-binding protein; DAI, DNA-dependent activator of interferon regulatory factors; HIF1α, hypoxia-inducible factor 1α; ID, intrinsically disordered; LCR, low complexity region; MoRF, molecular recognition feature; N-WASP, neuronal Wiskott–Aldrich syndrome protein; PPI, protein–protein interaction; PQC, protein quality control; PTM, post-translational modification; RHIM, RIP homotypic interaction motif; RIP, receptor-interacting protein; Rnq1, rich in asparagine and glutamine 1; Robo2, roundabout, axon guidance receptor, homologue 2; San1, sir antagonist 1; SH3, Src homology 3; SLiM, small linear motif; Spc42, spindle pole component 42.

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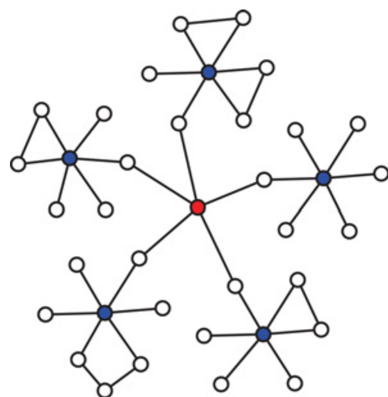

Figure 1 Intramodular and intermodular hubs in a scale-free network

Each node represents a protein in a hypothetical PPI network, whereas each edge represents an interaction. The overall topology is roughly scale free. Blue nodes represent intramodular hubs, whereas the red node represents an intermodular hub.

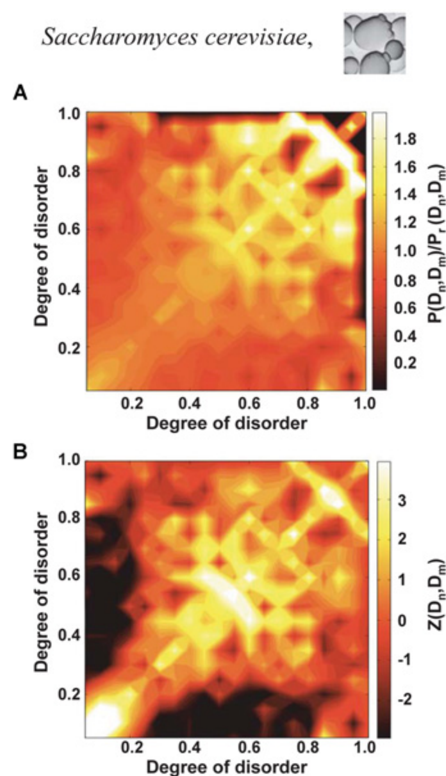

Figure 2 Correlation profiles in the yeast PPI network

(**A**) The ratio $P(D_n,D_m)/P_r(D_n ,D_m)$, where $P(D_n,D_m)$ is the probability that a pair of proteins with the degree of disorder given by D_n and D_m respectively, interact with each other in the full PPI set and $P_r(D_n ,D_m)$ is the same probability in a randomized version of the same network. The percentage of disorder D was calculated with Disopred [100]. (**B**) Z-scores for the disorder correlations: $Z(D_n ,D_m) = [P(D_n,D_m) - P_r(D_n ,D_m)]/s_r(D_n ,D_m)$, where $s_r(D_n ,D_m)$ is the S.D. of $P_r(D_n ,D_m)$ in 500 realizations of a randomized network.

levels of disorder than non-hubs [29–32]. Numerous studies have broken the hub proteins into two separate categories: those that form simultaneous stable complexes with their many interaction partners (party/multi-interface/stable hubs), and those that interact transiently with their partners at separate times (date/singlish-interface/transient hubs) [30,32–34]. Of these two hub types, only the transiently interacting hubs were found to be enriched in disorder [30,32,34]. This association between disorder and transiently interacting hubs seems to indicate an importance of ID segments in allowing proteins to interact with a large number of partners non-simultaneously. However, there is no straight correlation between the number of interaction partners of a hub and the number or percentage of disordered residues in a protein [29].

An insight into how proteins with ID segments are connected in PPI networks has been provided by a study from Shimizu and Toh [35]. Their analysis of the human PPI network revealed that interactions between wholly disordered proteins are enriched whereas those between wholly disordered and wholly ordered ones are under-represented relative to a randomized network (ordered–ordered interactions were neither enriched nor depleted). An analysis of the yeast PPI network shows a similar picture with regard to overall interactions (Figure 2). The probability that two proteins with a high disorder content are interacting with each other is increased in the yeast network compared with a randomized network. Shimizu and Toh [35] also found that the proteins involved in these interactions were significantly more likely to have some relation to phosphorylation, whether being targets or kinases themselves. Similarly, Kim et al. [34] found transient hubs to be enriched in kinase functions, and that the partners of transient hubs had significantly higher levels of disorder than the average level of disorder of proteins in the network. Patil et al. [36,37] concluded that transient hubs are more likely to interact intermodularly, allowing communication between different modules (a module simply being a highly interconnected process, like the transcription initiation machinery), whereas the stable hubs are more likely to interact intramodularly, allowing for the formation of the modules (Figure 1). Overall, these findings indicate that interactions between proteins with ID segments form an interwoven network across the proteome that allows communication between different processes in the cell.

Having determined that proteins with ID segments seem to occupy a central position in the interactome, the role that disorder plays in the binding process of the hubs with ID segments can be investigated. Specifically, are ID segments important in forming the interfaces with binding partners, or do they usually play a supporting role by allowing greater flexibility between structured segments (or is it really a bit of both)? ID segments can be used as flexible linkers to connect two folded domains in order to give them the conformational freedom to interact in many different configurations with partner molecules, calmodulin being a well-studied example [16]. However, a study by Patil et al. [36] looked at the relationship between the number of distinct ordered domains and the percentage of disorder in hub proteins and found that the increase in disorder that comes with an increase in the number of structured domains is more than expected if disordered segments only functioned as flexible linkers. Instead, ID segments seem to harbour the interaction region themselves, as in the fully disordered HMGA1 (high mobility group AT-hook protein 1) [16]. These regions often undergo a disorder-to-order transition upon binding to a partner [38–40]. The fact that these ID regions are folded in the complex with their partners may explain why a number of studies have found that whereas the sequence outside of the interfaces in transient hubs is enriched in disorder, the interface itself does not show such enrichment [33,34,41].

DIVERSE MECHANISMS AND SEQUENCE ELEMENTS MEDIATE PROMISCUOUS INTERACTIONS OF ID SEGMENTS

The studies performed on hub proteins in PPI networks reveal that proteins with ID regions take on roles in which promiscuity

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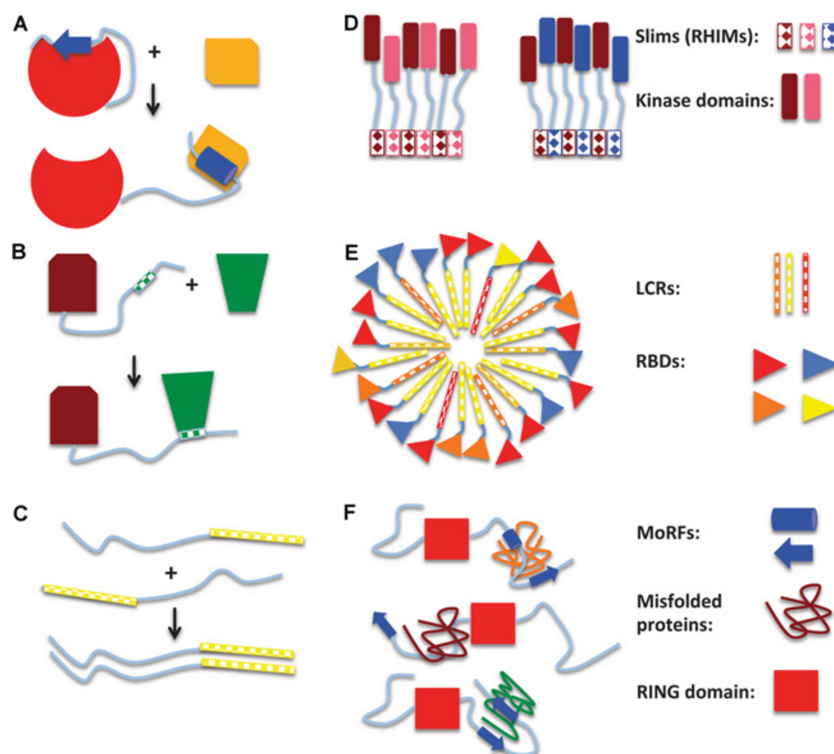

Figure 3 Promiscuous interaction elements of ID segments and their usage

(**A**) MoRFs are short interaction segments that undergo a disorder-to-order transition upon binding to their partner. Shown is the example of an autoinhibited protein in which a MoRF adopts a β-strand structure (blue arrow) when inhibiting the function of a domain of the same polypeptide chain (red) and a helix (blue cylinder) when binding to the partner that releases autoinhibition (orange). (**B**) SLiMs are short conserved sequence motifs which bind to a variety of targets in the proteome. A SLiM in an ID region is shown binding to a partner protein. (**C**) LCRs are regions which contain repetitive sequences or lower levels of sequence variety. The example shows two LCR-containing ID proteins interacting to form a coiled-coil. (**D**) The aggregation of SLiMs into polymers allows for local increases in concentration of the active unit, as in the case of the RIP1–RIP3 necrosome complex. (**E**) LCRs can be used to create heteromeric aggregates of proteins containing RNA-binding domains (RBDs), allowing a collection of functionally related, but not identical, RNA molecules to be localized and stored. (**F**) An example of a protein quality control member binding to multiple different misfolded proteins using different combinations of MoRFs located in its flexible ID region.

is a desired trait. The following section will now investigate the physical basis for promiscuity. Several different types of interactions have been described in the literature for ID protein segments; however, it should be noted that the lines between the categories are not distinct, and that their overlap has not been fully explored.

MoRFs (molecular recognition features)

MoRFs are short segments (usually 10–70 residues) in ID regions that undergo a disorder-to-order transition upon binding to their partner [38–40,42]. Four different categories of MoRFs have been observed: α-MoRFs, which form α-helices; β-MoRFs, which form β-strands; ι-MoRFs, which form irregular structures; and complex-MoRFs, which form a mixture of secondary structures (Figure 3A).

Because of the disorder-to-order transition, MoRFs are able to uncouple the usual link between affinity and specificity [43,44]. Although they can form structures that are highly specific to an interface, the loss of entropy that occurs upon folding allows for a balance with the gain in enthalpy, making for a relatively low affinity interaction. However, disorder-to-order transitions can still allow for high affinity, high specificity interactions. The change in enthalpy can be tuned via the size of the interface; in fact, ID segments allow for much larger interfaces and therefore much higher gains in enthalpy per residue compared with structured domains [17]. The entropy loss can be decreased by formation of so called ‘fuzzy’ complexes, complexes in which the structure is not fully defined [45–48]. In addition, the energetics of binding may be modulated by changes in the sequence context of the MoRF, leading to changes in secondary structure preferences [49] or availability of the MoRF. Indeed, it has been shown that increasing the number of charged residues in an ID sequence can lead to a transition from a molten globule to a random coil, which would increase the availability of a MoRF to its binding partners [50,51].

Two types of mechanisms have been proposed for coupled binding and folding [52,53]. In one, usually known as conformational selection, the ID segment-containing protein binds to the partner protein when it is in the process of sampling a structure that is complementary to the binding site. In the other mechanism, known as induced folding/fit, the process begins when non-specific contacts are formed with the binding partner, inducing the ID segment to fold into the correct structure as it forms more specific contacts in the binding interface. It has been shown experimentally that some systems bind with what appears to be an induced folding mechanism, the binding of the pKID (phosphorylated kinase-inducible domain) to the KIX domain of the CREB (cAMP-response-element-binding protein) transcription factor being a prime example [20]. In contrast, simulations and experimental data support a conformational selection mechanism in other systems, such as the binding of p53 to MDM2 [54,55]. Interestingly, it was recently proposed that

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subtle changes in the amino acid sequences of ID regions may result in a switch between conformational selection and induced fit, thereby providing a mechanistic choice to regulatory systems [56].

The ability of MoRFs to fold upon binding allows them to interact with a variety of differently shaped binding partners using the same [57] or different secondary structure. A clear example of the latter is provided by p53: one ID region binds to four different partners, each time with a unique secondary structure makeup [58]. Both the conformational selection and induced fit mechanisms provide potential explanations of how this may occur. In the case of conformational selection, a single MoRF might sample numerous different secondary structures, with binding partners selecting a different conformation/fold based on the structure of the binding site [55,59]. In the case of induced folding, partners could induce a different fold after the initial encounter based on the residues available for contact in the binding site. That MoRFs can bind to a variety of binding sites does not necessarily come at the cost of non-specific binding, as it may be the case that each of its binding partners simply 'reads' the sequence in a different way; in other words, the MoRF may have a discrete and limited number of ways in which a partner may form a combination of contacts with its residues [58]. More research will be needed to clarify the nature of the binding mechanisms for these chameleon sequences.

SLiMs (short linear motifs)

SLiMs, also known as ELMs (eukaryotic linear motifs) or just LMs (linear motifs), are short conserved sequences (usually no longer than ten residues) found mostly in ID regions that form interfaces to partner proteins [60] (Figure 3B). In contrast with MoRFs, the definition of SLiMs is based on sequence rather than structure, and overall they seem to be smaller; however, there appears to be overlap between the two types of ID interaction modules [61–63]. Many SLiMs also undergo disorder-to-order transitions upon binding to their partner [60,64]. It might be expected that specificity would be hard to achieve with such short motifs. Nevertheless, changes in only one or two residues of the SLiM targets of certain SLiM binding domain families seem to provide a level of specificity to the binding [65,66]. In addition, the structures formed by the SLiMs do not solely consist of residues from the core conserved motif; conserved flanking sequences also form part of the interface, contributing to about 20 % of the overall binding energy [67,68].

Proteins can increase their number of binding partners by having SLiMs distributed throughout their ID segments, the scaffolding ID region of RNase E being one example [69]. An analogous situation is found in many stable hub proteins, where a large number of ordered domains are linked together to give a protein the ability to bind many partners [36]. However, SLiMs offer the advantage of providing more flexibility between binding regions, as well as requiring fewer residues between them to allow simultaneous binding of the partners.

LCRs (low complexity regions)

LCRs, also known as low complexity sequences or low complexity domains, are sequences in which a low level of sequence information is encoded; it is usually quantified using a concept from information theory known as Shannon's entropy [70] (Figure 3C). LCRs may take the form of highly repetitive sequences or sequences with only a few different types of amino acids. LCRs were found to be significantly depleted in the PDB when compared with a protein sequence database; in the cases where an LCR was present in a structure, it was usually found to be in an unstructured region [71–73]. Poly-Q (polyglutamine) sequences are one of the most well-studied LCRs because of their implication in a number of neurodegenerative diseases [74]. In monomeric form, these sequences do not show any significantly populated conformational states; however, they are prone to forming aggregates with amyloid structure [75]. Coiled-coils, especially those whose sequences are predicted to contain disordered regions, have been found to be enriched in LCRs [76,77]. Callaghan et al. [78] found that the C-terminal domain of RNase E, an endoribonuclease, had low sequence complexity and was mostly unstructured, but did contain a coiled-coil region that functioned to bind structured RNAs.

Using data from yeast PPI networks, Coletta et al. [70] found that proteins containing LCRs generally had more binding partners than those without. Lukatsky et al. [79] found that diagonally correlated sequences, sequences in which residues of the same amino acid type are more likely to be located in clusters, were significantly enriched in ID regions. Furthermore, they performed computational studies and found that sequences with such diagonal correlations were more likely to have higher levels of binding promiscuity [80].

FUNCTIONAL RELEVANCE OF PROMISCUOUS ID SEGMENTS

The cell utilizes the aforementioned promiscuous ID interaction segments in numerous ways. In the present review, we focus on three functions: their use in assembling dynamic, macromolecular structures, their role as interaction switches in regulation and signalling, and their role as recognition elements in the PQC (protein quality control) system.

Assembly of dynamic macromolecular structures

The RIP1 (receptor-interacting protein 1)–RIP3 complex is required in a process known as programmed necrosis [81]. In a recent paper, it was found that the RIP1–RIP3 complex has a cross-β-amyloid core structure [82]. Furthermore, SLiMs, referred to here as RHIMs (RIP homotypic interaction motifs), located in an ID segment of RIP1 and RIP3, are key in forming the functional protein aggregate that mediates programmed cell necrosis (Figure 3D). It also seems that the kinase activity of each of the substituents of the complex is required for complex formation, indicating the process is controlled via phosphorylation [81]. Importantly, the RHIMs are also found in other proteins, such as the cytoplasmic DNA sensor DAI (DNA-dependent activator of interferon regulatory factors) and the Toll-like receptor signalling adapter TRIF [TIR (Toll/interleukin-1 receptor) domain-containing adaptor protein inducing interferon β] [83]. In both of these cases, the RHIM functions to mediate interactions with the RIP1–RIP3 complexes. At least for the interaction between DAI and RIP1, the formed complex has been shown to be filamentous and amyloid-like in nature [81]. These findings suggest that promiscuous interactions of the RHIMs enable the formation of heteromeric aggregates that bring different signalling proteins together in order to allow signal integration and transmission.

A similar mechanism seems to play a key role in the assembly of RNA granules, but with LCRs taking the place of SLiMs. RNA granules are membraneless organelles composed of proteins and RNA [84]. Generally, RNA granules are known to be used for greater control over the fate of specific mRNAs. A variety of signalling pathways have been described in controlling

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their formation, with PTMs (post-translational modifications) appearing to be a prevalent method of control [85]. It was noted that LCRs were present in a number of the proteins found in the granules [85,86], and a recent set of studies from the McKnight group has shed light on their function [87,88]. McKnight and co-workers were able to produce RNA granule-like assemblies and demonstrate that the LCR regions were necessary for the formation of these assemblies (Figure 3E). Furthermore, when the LCR from a purified member of the assemblies was present at a high enough concentration, a reversible phase transition to a highly dynamic hydrogel was observed. The hydrogel was capable of binding to the LCRs of other members of the isolated assemblies (heterotypic trapping). The structure of the LCR hydrogel had characteristics of cross-β-amyloids, but, because of the reversibility and dynamism, was not nearly as stable to SDS denaturation as the yeast prion-like fibril tested. McKnight and co-workers also found a three residue repeat sequence and, by phosphorylating the tyrosines of the repeat sequence, were able to control the formation of the hydrogel. However, it remains to be seen whether these amyloid-like structures form *in vivo* [89].

Interestingly, sol–gel transitions have also recently been observed as the result of interactions between proteins with several instances of the same SLiM and partners harbouring multiple copies of the corresponding binding domains (multivalent proteins). Li et al. [90] found that a sharp phase transition is observed when oligomers containing multiple copies of the SH3 (Src homology 3) domain and its proline-rich motif ligand suddenly begin to form macroscopic polymers. At the critical concentration, highly dynamic protein based-droplets are formed. Importantly, when experimenting with the NCK–nephrin–N-WASP (neuronal Wiskott–Aldrich syndrome protein) complex, which contains multiple copies of the same interaction partners, the same sorts of dynamic droplets were able to be formed. The actin polymerizing activity was found to be increased significantly upon formation of the dynamic droplets, indicating a functional relevance of the transition.

Hence, these results suggest that at least some LCRs and SLiMs allow the signalling and regulatory proteins that harbour them to move in and out of heteromeric macromolecular assemblies by reversible formation of either amyloid-like polymers or protein-based droplets.

Signalling and regulatory switches

Promiscuous ID segments also play an important role as interaction switches that are used, for instance, to integrate signals [91]. In cells, signals are often integrated via networks of proteins controlled by PTMs [92]. This mechanism of signal transmission requires that the signalling protein bind to the modifying enzyme as well as the physiological target, a seemingly prime application of a promiscuous ID segment. A good example is found in the case of HIF1α (hypoxia-inducible factor 1α), which plays a key role in the hypoxic response pathway by acting as an on/off switch [17]. A MoRF in HIF1α mediates binding to its physiological effector, CBP (CREB-binding protein) and p300, adopting a helical structure when in complex [17]. That same MoRF is also able to bind to an enzyme that hydroxylates one of its conserved aspargine residues; this impairs binding to CBP and p300 in normoxic cells, interfering with the hypoxic response [93]. ID binding regions can also act as switches when more stable interactions are formed between them and their binding partners. An example can be found in the tuning of actin polymerization. The recently characterized nephrin–NCK–Robo2 (roundabout, axon guidance receptor, homologue 2) complex inhibits the polymerization of actin in certain filtration cells of the kidney, opposite to the effect of the nephrin–NCK–N-WASP complex. NCK uses the same three SH3 domains to bind SLiMs in N-WASP and Robo2, leading to a competition between the proteins in forming the complex, allowing actin polymerization levels to be fine-tuned through control of the relative concentrations of these two proteins [94].

We recently observed that autoinhibition of proteins is frequently achieved with the help of ID regions containing promiscuous interaction elements [95]. These ID regions in autoinhibitory proteins can act as switches that activate or inhibit the protein. In the inhibited state, the ID region binds to the functional domain or interaction region of the same protein, causing the function to be impaired. Through the use of PTMs, partner binding or proteolysis, inhibitory contacts can be released to restore functionality [95]. For instance, calmodulin-dependent kinases are autoinhibited by an ID segment that contains a MoRF [95] (Figure 3A). The autoinhibitory ID segment prevents ATP from binding by interacting with a region near to its binding site. The autoinhibition is relieved when the ID segment binds to calmodulin itself, during which the MoRF forms a short α-helix [96].

The modular approach to protein partner binding afforded by short ID regions also simplifies rewiring of signalling and regulatory protein-interaction networks at the transcriptional level. Recent studies by Buljan et al. [97] and Ellis et al. [98] found that the subset of alternatively spliced exons that were present only in specific tissues were enriched for ID regions; these ID regions themselves were enriched in PTM sites and conserved MoRFs. A similar study by Weatheritt et al. [99] discovered that, in addition, the alternatively spliced exons are enriched for SLiMs. Importantly, Buljan et al. [97] observed that proteins containing tissue-specific exons occupy central positions in interaction networks and display distinct interaction partners in the respective tissues. Hence, changing the combinatorial use of promiscuous ID regions via alternative splicing allows for time and tissue specific rewiring of the protein-interaction network. It is clear that alternative splicing on structured domains can also be used to change interaction potential [100], but splicing within a structured region is arguably subject to more constraints in order to preserve functionality and prevent misfolding. The advantage of ID regions in rewiring networks can be observed on an evolutionary time scale as well. Mosca et al. [101] found that interactions involving ID segment-containing proteins were less conserved between organisms, and that these changes were not just because of the lower levels of evolutionary constraint; it seemed that this lack of conservation was due to selective pressure acting on newly formed interactions.

In more general terms, SLiMs, MoRFs and their corresponding binding domains constitute a finite set of building blocks that can be used in combination to create complex signalling pathways that contain switches or other regulatory elements to permit integration of signals from multiple sources.

Target recognition in protein quality control

Recent results indicate that the promiscuous binding behaviour of ID segments is also exploited in PQC systems to recognize substrates. Recently, small, ATP-independent and highly promiscuous chaperones have been identified that are activated upon stress-induced order-to-disorder transitions [102,103]. For instance, the *Escherichia coli* protein HdeA is found to be fully structured under physiological conditions, but enters a disordered state at low pH and begins to display chaperone

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activity [104]. Specifically, it will bind to other unfolded proteins and prevent their aggregation during the stress conditions, as well as during the refolding period [105]. Importantly, Jakob, Bardwell and co-workers showed that HdeA adopts different conformations when bound to different substrates [104]. This finding is consistent with the idea that PQC systems need to be able to recognize a diverse range of shapes and sizes to accommodate the diversity in structure of unfolded and misfolded proteins. Other ID proteins, such as α-casein, β-casein and LEA (late embryogenesis abundant) dehydration proteins, have been shown to act like chaperones and prevent aggregation [103]. It was proposed that these proteins engage in more transient interactions with their targets that sterically inhibit formation of aggregates; as they do not encourage folding, they are alternatively referred to as molecular shields [106].

Parts of the ubiquitin–proteasome system have also been shown to rely on ID regions for partner recognition. The yeast nuclear PQC ubiquitin ligase San1 (sir antagonist 1) was found to recognize substrates via intrinsically disordered N- and C-terminal regions that contain conserved MoRFs (Figure 3F) [107]. It is likely that San1 is able to recognize a variety of misfolded proteins via the combinatorial use of different MoRFs that are embedded in flexible ID regions. Importantly, the MoRFs themselves may fold differently, depending on the shape, size or residue composition of the target and thereby enable the recognition of a heterogeneous set of targets. In a recent collaboration between the Mayor laboratory and our own it was revealed that the targets of the PQC systems after heat shock are enriched in long ID regions [108]. Furthermore, the ID regions themselves appear to be enriched in SLiMs and LCRs relative to an average ID region.

Further work is required to elucidate whether and how other PQC proteins that are enriched in ID segments [109] and their targets exploit the promiscuous binding potential of MoRFs, SLiMs and LCRs to recognize each other and, if so, which combinations of the interaction-mediating elements they use.

Promiscuous interactions of ID segments in disease

It is clear that interactions mediated by promiscuous ID segments have to be regulated. As discussed previously, PTMs, especially phosphorylation, are commonly used to determine interaction specificity of promiscuous motifs. In addition, time- and location-specific expression are likely to contribute to the regulation of the interactions [110]. A number of studies have also shown that proteins with a high percentage of ID regions, and particularly those that harbour SLiMs, are tightly regulated at transcriptional, post-transcriptional, translational and post-translational levels, resulting in a high turnover rate and low abundance of these proteins [111–115]. On the basis of these findings, it has been proposed that the tight regulation of proteins with ID segments may contribute to signalling fidelity by ensuring that they are available in appropriate amounts and not present longer than needed [114]. In other words, the tight control of synthesis and degradation may reduce the risk of non-functional interactions that are mediated by these regions.

Proteins with long ID segments have been associated with several human disease conditions [116]. For instance, overexpression of the ID Stathmin has been linked to cancer, whereas the overexpression of tau, ataxin-1, α-synuclein and huntingtin is associated with various neurodegenerative disorders [117,118]. These findings and the observed tight regulation seem to indicate that altered interactions of promiscuous elements in ID segments are an essential factor in the pathophysiology of diseases caused by the overexpression of these proteins. Indirect evidence for a link between promiscuous interaction elements in ID segments and disease pathogenesis comes from the analysis of high-throughput genomic and proteomic data. Vavouri et al. [113] identified factors that are associated with dose-sensitivity of genes in yeast, i.e. whether a gene is harmful to the yeast cell when overexpressed. They found that intrinsic disorder of proteins is an important determinant of dose-sensitivity, particularly when associated with the presence of SLiMs. Importantly, this property of proteins also has a strong association with dose-sensitive human oncogenes.

As a consequence of these findings, key questions emerge about the mechanisms of non-functional promiscuous interactions that lead to disease. A recent study by Treusch and Lindquist [119] has provided some insight into how non-functional interactions of the entirely disordered yeast prion Rnq1 (rich in asparagine and glutamine 1) can lead to cytotoxicity in quite a specific manner. When the yeast prion Rnq1 is overexpressed in the presence of its amyloid form, the spindle pole body component Spc42 (spindle pole component 42), which Rnq1 does not normally interact with, is sequestered into the insoluble protein deposit, causing mitosis to come to a halt. Importantly, it was found that when overexpressing Rnq1 with a previously discovered single residue mutant that induces cell-cycle arrest in the absence of the amyloid form, Spc42 was still sequestered. This result indicates that the non-functional interactions between Spc42 and Rnq1 that cause cytotoxicity are non-amyloid in nature.

However, amyloid-like interactions of proteins with promiscuous ID regions in functional aggregates may also be prone to failure upon perturbation [115]. By increasing the aggregation propensity or concentration of such proteins, other members of functional aggregates may be sequestered at levels high enough to disrupt cellular function. A recent paper provides an example in the case of the previously discussed RNA granules. Kim et al. [120] studied a protein involved in RNA granule formation and discovered that by mutating a single residue in the prion-like domain, which was predicted to increase aggregation potential, they were able to significantly increase formation of stress granules. The mutation was found to be involved in a series of related neurodegenerative diseases, including ALS (amyotrophic lateral sclerosis), implicating the excessive formation of aggregates capable of heterotypic trapping as a disease mechanism. Consistent with this idea, Olzscha et al. [121] found that amyloid-like cytotoxic protein aggregates sequester many pre-existing and newly synthesized proteins. Importantly, the functionally heterogeneous group of sequestered proteins share some distinct properties: they are large in size and are enriched in ID segments.

CONCLUSIONS

MoRFs, SLiMs and LCRs enable many proteins to interact promiscuously within the proteome. Such promiscuous behaviour is exploited by signalling and regulatory systems through the use of functional aggregates, switching mechanisms and the dynamic rewiring of the connections within these systems. These roles have caused proteins with extensive ID regions to be favoured as hubs in the interactome, where it can be seen that they allow connections between major cellular processes. One may speculate that without promiscuity it seems unlikely that the current level of functional complexity in many higher eukaryotic organisms could have been achieved, as complex organisms need adjustable regulatory networks for different cellular environments, but have a finite number of regulators due to the spatial and energetic

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constraints of cells. However, the use of promiscuous interaction elements may come at the price of the necessity for an elaborate proteostasis machinery that ensures fidelity in interactions, and the risk of unwanted interactions that, when proteostasis fails, can lead to significant detrimental phenotypic changes.

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