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Perspective

Intrinsically disordered proteins and conformational noise: The hypothesis a decade later

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SUMMARY

Phenotypic plasticity is the ability of individual genotypes to produce different phenotypes in response to environmental perturbations. We previously postulated how conformational noise emanating from conformational dynamics of intrinsically disordered proteins (IDPs) which is distinct from transcriptional noise, can contribute to phenotypic switching by rewiring the cellular protein interaction network. Since most transcription factors are IDPs, we posited that conformational noise is an integral component of transcriptional noise implying that IDPs may amplify total noise in the system either stochastically or in response to environmental changes. Here, we review progress in elucidating the details of the hypothesis. We highlight empirical evidence supporting the hypothesis, discuss conceptual advances that underscore its fundamental importance and implications, and identify areas for future investigations.

INTRODUCTION

The ability of a single cell, for example the fertilized oocyte, to differentiate and give rise to myriad cellular phenotypes without any change to the DNA sequence is a striking example of phenotypic plasticity (terms indicated in blue are defined in Glossary). While terminally differentiated cells in a multicellular organism typically lose their plasticity permanently, some precursors such as stem cells, progenitor cells, and transit amplifying cells, retain it to a certain degree to replenish the population.[1] However, some terminally differentiated cells, upon malignant transformation, regain phenotypic plasticity to enhance their fitness.[2] Protists on the other hand, retain this potential throughout their lifetime since phenotypic plasticity directly influences their ability to adapt to the ever-changing environment, they live in.[3] Such adaptive changes can potentially be inherited transgenerationally both in unicellular as well as in multicellular organisms and therefore, contribute to the evolution of new species.[4–6] Thus, consequently phenotypic plasticity and hence, phenotypic switching, play a fundamental role in ecological, evolutionary, and developmental biology (eco-evo-devo), as well as in diseases such as cancer.[5,7–9]

Although well recognized and much appreciated, how a cell or an organism can switch phenotypes whether stochastically[10,11] or in response to a specific environmental input[12] leading to permanent heritable changes, is not fully understood.[8,13] Ten years ago, using malignant transformation of a normal cell as a paradigm, we proposed a hypothesis[14] postulating how the configuration of the cellular protein interaction network (PIN) which defines the phenotypic identity of a cell/organism, determines its repertoire of phenotypic plasticity. More specifically, we postulated that "conformational noise" that is noise emanating from intrinsically disordered protein (IDP) conformational dynamics which is distinct from the well-recognized transcriptional noise, guides cell fate decisions by rewiring the PIN. Because most transcription factors (83-94%)[15] are IDPs, the model posited that conformational noise is an integral component of transcriptional noise implying that IDPs may amplify total noise in the system either stochastically or in response to environmental perturbations.

IDPs typically tend to occupy hub positions in PINs and are well suited to contribute to network plasticity because they have fast interaction kinetics. Furthermore, unlike the energy landscapes of highly ordered proteins, IDP energy landscapes are rugged with many local minima separated by low-energy barriers[16] enabling stochastic fluctuations between numerous conformational states in response to subtle perturbations. Thus, we hypothesized that conformational noise and fast interaction kinetics together allow IDPs to

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interact with multiple partners to rapidly sample the network search space. This heuristic rewires the PIN and, in the process, uncovers cryptic/latent network configurations to drive phenotypic switching and hence, contributing to phenotypic heterogeneity. Further, we posited that the acquired phenotype can be either temporary so that the system can return to the original state or can be permanent and inherited transgenerationally. More importantly, in contrast to the discrete (binary) state model, we envisaged the existence of hybrid (transition) states with distinct phenotypes. To account for how the acquired information may be transmitted to the offspring, we hypothesized that since the vast majority of transcription factors and chromatin modifiers are intrinsically disordered, it is likely that the ripple effect of transcriptional noise and conformational noise emanating from IDPs could be coupled to permanent changes in the genome facilitating transgenerational information transfer[17] akin to the phenomenon of "soft inheritance."[18]

Thus, the conformational noise hypothesis not only identified conformational noise as the most fundamental in the hierarchy of biological noise, but it also provided systems level insight into the fundamental basis of nongenetic phenotypic heterogeneity which could also serve as a bet-hedging strategy by giving rise to "persisters" in the population.[19] Indeed, the involvement of IDPs in the early evolution of complex multicellularity,[20] and in sculpting the shape of the beak of Darwin's finches which respond to the pressure of natural selection,[21] are illuminating examples of how IDPs mediate epigenetic interactions between the organism and the environment and influence evolution. In fact, in the anticipation of its broader implications, we had suggested that "*Although we have focused on cancer, we believe our thesis is not restricted to cancer and may be more generally applicable to address state-switching in biology.*"[14] Nonetheless, convincing evidence supporting the key aspects of the hypothesis was lacking or scanty at best.

Over the past decade however, the hypothesis has grown in scope and although several details in terms of IDP conformational dynamics and how it impacts the energy landscape have been elucidated, many of the fundamental aspects of the hypothesis are still intact. Here, we highlight empirical evidence that supports and extends the scope of the hypothesis from cellular transformation to include embryonic development, reprogramming, transdifferentiation, lineage switching, drug resistance, and the transition of unicellular forms to multicellularity during evolution, underscoring its fundamental nature and importance in biology and medicine. We conclude with a summary of the highlights and identify aspects that warrant additional studies in the future.

EVIDENCE SUPPORTING THE CONFORMATIONAL NOISE HYPOTHESIS

Disorder is critical for intrinsically disordered protein function

Previous reports in the literature drew attention to the striking correlation between IDP expression and phenotypic plasticity.[22] However, recent studies have provided convincing experimental evidence supporting the involvement of IDPs in phenotypic switching. For example, in Molecular Dynamics simulations aimed at discerning the effect of disease-causing missense mutations (DCMMs) on intrinsically disordered regions (IDRs), three different proteins with functionally important IDRs with known DCMMs were examined.[23] The results indicated that the DCMMs attenuated the conformational heterogeneity of IDRs which is critical for their function. The studies also showed that DCMMs stabilize very few structural possibilities of IDRs either by the newly formed interactions induced by the substituted side chains or by means of restricted or increased flexibilities of the backbone conformations at the mutation sites. More importantly, the conformational preferences favored by DCMMs, did not appear to support the native functional roles of the IDRs which would lead to disease conditions.

Similarly, a proteome-wide comparison of the distribution of missense mutations from disease and non-disease mutation datasets revealed that, in IDRs, disease mutations are more likely to occur within short linear motifs (SLiMs) than neutral missense mutations. Further, compared to neutral missense mutations, disease mutations more frequently impact functionally important residues of SLiMs, cause changes in the physicochemical properties of SLiMs, and disrupt more SLiM-mediated interactions.[24] And in yet another example, employing a combination of a variety of computational and experimental techniques, it was shown that mutations in the IDR constituting the transactivation domain (TAD) of the tumor suppressor p53 can significantly perturb its interactions with key regulators.[25] Importantly, the authors showed that many of these mutations do not directly disrupt the known interaction interfaces but impact the disordered state of TAD domain to disrupt the interaction of p53 with its partners. More specifically, they observed that

these mutations modulated the level of conformational expansion and the rigidity of the disordered state. Taken together, the data support the idea that an IDP conformational ensemble serves as a conduit in transducing signals from cellular stimuli at the protein-protein interaction level which is one of the fundamental tenets of the conformational noise hypothesis.

Conformational noise and state switching

In biology, the term noise implies the random variation in quantities of biological molecules. Because of such noise, even isogenic cells exhibit differences in gene expression and, consequently, the resulting protein levels.[10,26,27] This is typically referred to as transcriptional noise. Translational noise is a term used to define variation due to differences in translational rates of mRNA.[28,29] In addition, noise from intrinsic promiscuity of protein-protein interactions is also considered an important source of stochastic fluctuation in cellular signal transduction.[28,30] Therefore, noise arising from various sources in the system can impact information flow that determines cell function and phenotype.

Although it is obvious from the forgoing that a cell is abuzz with noise, we postulated conformational noise as a new and important source of biological noise that needs to be appreciated. As the term itself indicates, conformational noise is defined as stochastic fluctuations in the conformational preferences of a protein, especially of IDPs or IDRs within ordered proteins that can have long-reaching effects through the amplification of transcriptional as well as signaling noise (Figure 1).

Conformational noise may harbor spatial and temporal variations of protein conformation and the two variations are distinct. For example, while spatial variation is intrinsic to the IDP sequence, temporal variation can be sensitive to environmental cues such as phosphorylation. It is well established that IDPs are typically more likely to be phosphorylated compared to ordered proteins.[31] Phosphorylation of IDPs at multiple residues is not always uniform and there can be considerable spatiotemporal differences in the extent to which the different phospho-acceptor sites are phosphorylated. Indeed, differential phosphorylation is often associated with the activation or inactivation of IDP function. Furthermore, at least in some cases, it has been observed that the kinetics of differential phosphorylation also varies.[32] Thus, while some residues are phosphorylated at a fast rate (within a few minutes), others are intermediate (phosphorylation occurs within several minutes to hours) and yet other residues are phosphorylated very slowly (take several hours). The differences in the phosphorylation kinetics could be due to the same kinase or different kinases involved in phosphorylating the various residues. More importantly, this fast/slow kinetic order can have important functional consequences; for example, in the case of the intrinsically disordered Elk-1 transcription factor, while fast phosphorylation can render the IDP functionally active, intermediate, and slow phosphorylation can render it inactive quantitatively and therefore, impact how it interacts with its partner proteins.[32]

The data on the conformational dynamics of PAGE4, a stress-response protein that plays an important role in prostate cancer, complements the spatiotemporal distinction in ensemble conformation illustrated above as well as provide further support for the conformational noise concept. PAGE4 is an IDP that functions as a transcriptional regulator to potentiate the transactivation of the AP-1 transcription factor complex.[33] The latter is a heterodimer of the oncogenes c-Fos and c-Jun and acts as a strong repressor of the androgen receptor (AR) activity in the prostate.[34,35] Phosphorylation of PAGE4 at T51 by HIPK1 and hyperphosphorylation at all eight S and T residues by CLK2, can lead to conformational changes in the PAGE4 ensemble and significantly impact its function.[36,37] Thus, while site-specific (T51) phosphorylation by HIPK1 results is the compaction of the WT PAGE4 ensemble and enables transcriptional potentiation, hyperphosphorylation by CLK2, results in unfolding the ensemble that almost resembles a random coil and attenuates its activity.[38] Furthermore, HIPK1, PAGE4, AP-1, AR and CLK2 constitute a regulatory circuit with oscillatory dynamics and can modulate the degree of androgen dependence of a PCa cell.[36] Cells with high levels of HIPK1 phosphorylated PAGE4 (i.e., activated PAGE4) are predicted to be androgen dependent. In contrast, cells with high levels of hyperphosphorylated PAGE4 (i.e., inactivated PAGE4) by CLK2 are androgen independent. Thus, consequently depending on the HIPK1/CLK2 level, PCa cells can have differing propensities for androgen dependence (Figure 2).

Nonetheless, CLK2 phosphorylates PAGE4 to varying extents.[37] While some residues are phosphorylated ~95%, other residues are only phosphorylated ~45% under the same steady-state conditions. Furthermore, it is likely that the phosphorylation of PAGE4 by CLK2 entails a spatiotemporal pattern with some

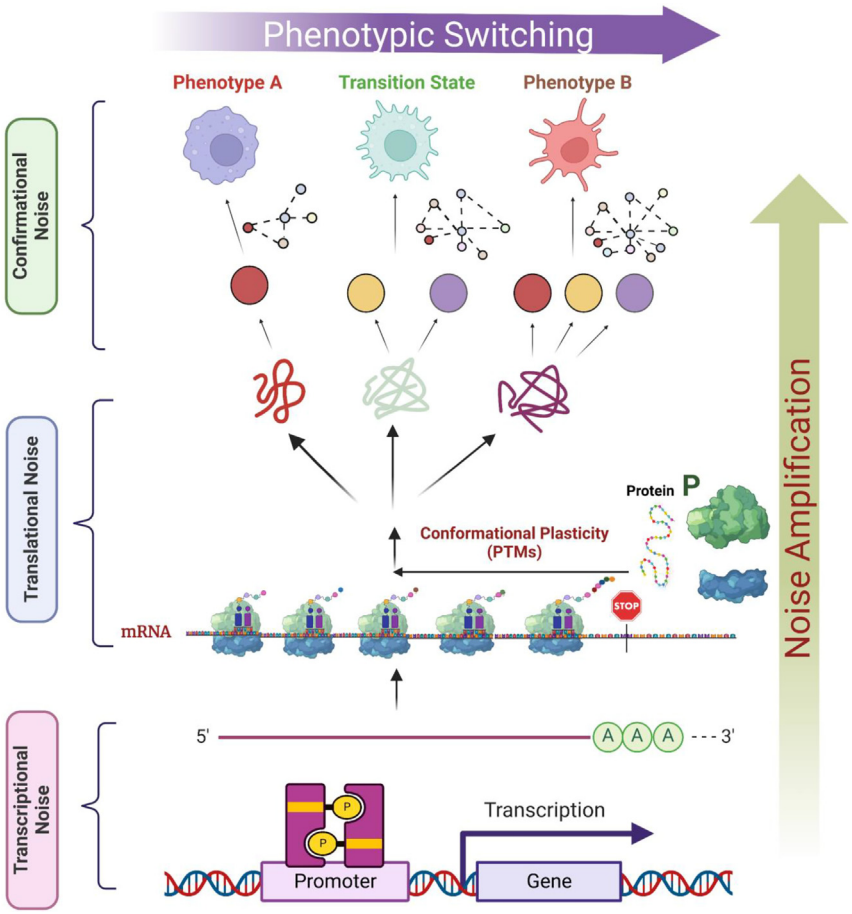

Figure 1. Conformational noise amplifies transcriptional noise, and noise due to signal transduction

Following transcription (which is subject to transcriptional noise), the mRNA is translated, and the resulting intrinsically disordered protein undergoes post-translational modifications (PTMs) such as phosphorylation for example. This can result in different conformational preferences of the modified protein (different conformations are shown in different colors) which facilitates interactions with different partners (small colored circles) resulting in rewiring of the protein interaction network. Thus, conformational noise emanating from the conformational dynamics of the conformers in the ensemble can amplify transcriptional noise to actuate phenotypic switching from A to B via an intermediary transition/hybrid state.

S/T residues being phosphorylated faster than others as is seen in Elk-1, that is phosphorylated by ERK.[32] Thus, the prevalence of the kinase(s) and phosphatases that act on a given IDP, and the residence times of the IDP ensemble in a specific conducive conformation within the vast ensemble conformational space can vary both temporally and spatially contributing to conformational noise in the system. This can give rise to non-genetic phenotypic heterogeneity in the population. Consistent with this prediction, individual cells in an isogenic population of LNCaP prostate cancer cells can have varying degrees of dependence on androgen in the medium.[39]

Several studies have shown that, much like Fisher's "lock and key" model where interaction specificity is determined by the conformations of the interactors, the partners (or targets) with which an IDP can interact with is also determined by its conformational state. However, unlike the former, IDPs are highly dynamic and therefore, can interact with multiple partners albeit with high specificity but low affinity.[40] Furthermore, as discussed above, IDP conformational dynamics is influenced by posttranslational modifications, especially phosphorylation[37,41] which in turn can have a huge impact on the functional output.[32,37,40] Collectively, these studies support the idea of conformational noise as a novel source of biological noise with implications in phenotypic plasticity. Furthermore, the observations on Elk-1 and PAGE4 show that the conformational dynamics of an IDP ensemble can be modulated spatiotemporally.

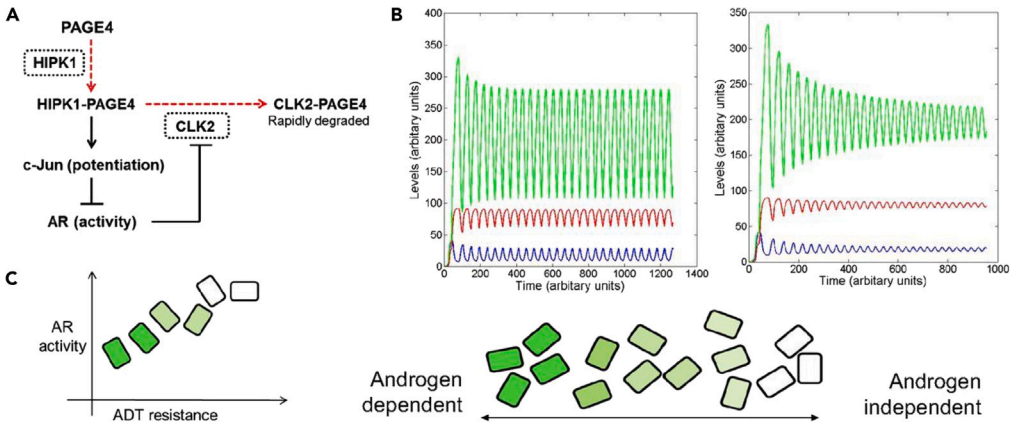

Figure 2. Modeling the PAGE4/AP-1/AR/CLK2 regulatory circuit

(A) Regulatory circuit for PAGE4/AP-1/AR/CLK2 interactions. Dashed red lines denote enzymatic reactions, and solid black lines denote nonenzymatic reactions. CLK2 and HIPK1, the two enzymes involved, are shown in dotted rectangles.

(B) Dynamics of the circuit showing sustained and damped oscillations for HIPK1-PAGE4 (PAGE4M, shown in blue), CLK2-PAGE4 (PAGE4H, shown in red), and CLK2 (shown in green).

(C) Distribution of androgen dependence for an isogenic population over a spectrum, as indicated by the shade of green. Dark green boxes denote highly androgen-dependent (i.e., ADT-sensitive) cells, and white boxes denote androgen-independent cells. Reproduced with permission from Kulkarni et al. (2017)[37] Proc Natl Acad Sci USA. 114(13):E2644-E2653.

Phenotypic switching can give rise to transient (hybrid) states (phenotypes) that can be reversible

Contrary to prevailing wisdom, the conformational noise hypothesis envisaged a transient state that is reversible. Indeed, several recent reports have unequivocally demonstrated the existence of such transient states referred to as "hybrid" states or phenotypes. Epithelial-to-mesenchymal transition of EMT is a good case in point. Unlike the popular belief that EMT is a binary process, it is now thought of as a continuum including diverse hybrid E/M phenotypes manifested by varying degrees of transition during phenotypic switching. Indeed, detailed investigations have revealed that the cells with hybrid phenotypes co-express biomarkers characteristic of both epithelial (E) and mesenchymal (M) phenotypes. Furthermore, when compared to cells with individual E or M phenotypes, cells with hybrid E/M or M/E phenotypes display more aggressive characteristics.[42] Likewise, a recent study on breast cancer discovered that there exist multiple distinct single-cell clones that span the EMT spectrum in a population with diverse migratory, tumor-initiating, and metastatic qualities.[43]

Hybrid states are not specific to mammalian systems but are also seen in lower organisms. For example, in the yeast, a novel phenotype referred to as the "gray" phenotype was discovered that in addition to the well-known white and opaque phenotypes.[44] The three cell types differ in several aspects including cellular and colony appearances, mating competency, secreted aspartyl proteinase (Sap) activities, and virulence. Gray cells exhibit the highest Sap activity as well as the ability to cause cutaneous infections underscoring the fact that not only hybrid states exist but are also functional. More importantly, the master regulator wor1 and the Egf1 regulator that are key to state switching, are both highly intrinsically disordered underscoring the link between IDPs and phenotypic switching.

Several reports in the literature have also documented reversal of the newly acquired phenotype both within, for example in acute leukemias,[45] and across lineages[46,47] including cancer stem cells (CSCs) to non-CSCs, drug-sensitive to drug-tolerant states, and a spectrum of epithelial-hybrid-mesenchymal phenotypes.[48,49] Together, these observations provide good evidence supporting the presence of transient states and the possibility of reversal of phenotypes.

Involvement of intrinsically disordered proteins in phenotypic switching is universal

Phenotype switching plays a beneficial role in microbial populations by causing division of labor among cells or ensuring that at least some of the population survives in the event of an unexpected environmental

perturbation. In bacteria, tyrosine kinases (BY kinases) that do not appear to have any homology with their eukaryotic counterparts and leverage the ATP/GTP-binding Walker motif for autophosphorylation and substrate phosphorylation, and trpC that is essential for tryptophan biosynthesis, are involved in stochastic phenotypic switching.[50,51] And they are both IDPs. Strikingly, phenotypic switching is also seen in choanoflagellates, a group of protists that are considered as the closest living relatives of animals. A recent study showed that choanoflagellates can switch their phenotype to become ameboid by retracting their flagella and activating myosin-based motility[52] (53). Indeed, one of the proteins involved is WASP which is an IDP. Finally, emerging evidence suggests that IDPs are also involved in actuating phenotypic switching in plants.[53,54] Together, these observations illustrate the universality of the conformational noise hypothesis and its implications in evolution.

Phenotypic switching and early evolution of complex multicellularity

Multicellularity is a major milestone in the history of evolution. It is generally held that the main drivers of multicellularity were life history trade-offs between survival and reproduction in response to stress and/or predation. Indeed, several lines of evidence suggest that the cooption of ancestral pathways from unicellular organisms underlies multicellularity.[55–57] Nonetheless, how these ancient pathways were adopted to serve new functions mechanistically speaking, is not well understood. Since IDPs, by virtue of their conformational flexibility can rewire cellular PINs, the idea that they could be a source of the adaptive plasticity to give rise to multicellularity seems like an attractive proposition.

Consistent with this line of reasoning, several proteins that are thought to be important in the origin of multicellularity such as those involved in extracellular matrix expansion, cell adhesion, cell communication, cell-cycle modifications, asymmetric cell division, and cell differentiation, are IDPs.[58–60] The observations that several stress-response proteins are IDPs[21,22] and that the content of disordered residues increases in proteomes from bacteria to single-celled and multicellular eukaryotes,[61,62] lend further credence to this proposition. In support of this argument, we proposed a theoretical model showing how conformational noise can act as intrinsic noise in the PIN and therefore, how phenotypic transitions are controlled by the extent of disorder in the cell.[63]

NEW THEORETICAL DEVELOPMENTS

Though the idea of a landscape was envisioned in the original rendering, albeit with respect to IDP conformational dynamics, we subsequently perceived phenotypic switching from Waddington's epigenetic landscape metaphor perspective[64] [65] and applied dynamical systems theory to model state switching.[65–67] The height of the ridges that separate bifurcating valleys (or creodes per Waddington's terminology) in the landscape, is critical in determining cell fate. But mechanistically, how the ridge height is determined is unclear. Perhaps, the conformational noise hypothesis may shed some light on this problem if we assume that the topology or configuration of the PIN represents the high dimensional space embodying the valleys and ridges. Thus, any change in the PIN topology due to rewiring by the IDPs, would directly impact ridge height. Reprogramming of a somatic cell by the intrinsically disordered Yamanaka factors[68] is a good example of how rewiring can reorganize the rugged landscape by altering ridge height and uncovering latent attractors that specify cell fate. A model capturing the quasipotential landscape of the stochastic dynamics of a canonical gene regulatory network (GRN) that determines cell fate revealed that the asymmetry of barrier heights results in the time-directionality ("arrow-of-time") observed during differentiation lending further credence to the idea that PIN rewiring can modulate ridge height.[69]

Conclusions and future directions

It is gratifying to see how the conformational noise hypothesis that provided a simple conceptual framework for phenotypic plasticity has facilitated further progress. In retrospect, the hypothesis is paradigmatic of a systems approach across scales and connects concepts as distinct as plasticity, epigenetic interactions, protein biophysics, dynamical systems theory, network theory, ecology, and evolution. Furthermore, although additional work is needed, the evidence accumulated to date supports the notion that conformational variations of IDPs likely contribute to regulate or fine-tune the network to cause phenotypic switching. Nonetheless, there are two important points that we wish to emphasize. First, we had previously posited that conformational noise underlies the "promiscuous" nature of IDP interactions and impacts biological information transfer. Promiscuity in IDP interactions is evident when IDPs are overexpressed and can have undesirable effects such as pathological states.[70] On the other hand, conformational noise is

consequential when the IDP copy number *per se*, or the "active" conformational species from the entire ensemble, is low (even though the IDP in question is overexpressed). This distinction is critical and is well supported by the differential phosphorylation of IDPs and the spatiotemporal differences in the phosphorylation kinetics[32] both of which can have functional consequences. Therefore, developing a mathematical model that can parameterize and quantify conformational noise is imperative to strengthen the hypothesis. The second point is that GRNs are part (a subset) of the cellular PINs.

Although not explicitly stated in the original enunciation, we had implied that information regarding phenotype specification can operate across diverse timescales. Thus, information which operates over relatively short timescales may reside in the PIN topology implying that such information is nongenetic in nature and is encoded in the design principles underlying PIN configuration. Consistent with this thinking, recent work on elucidating the design principles of GRNs that guide cellular decision-making revealed that network configuration encodes information determining phenotypic plasticity.[71] But information operating over extended periods is transferred to the genome via genetic/non-genetic changes including epigenetic changes. Regardless, both forms of information were envisioned to be transferred transgenerationally.[14] More recent work demonstrated that rewiring could cause network frustration[72] which can be relieved by introducing specific mutations in the genome[73] suggesting that PIN rewiring by IDPs can produce heritable genetic/epigenetic changes. Regarding the nongenetic mechanisms, the discovery that overexpression of certain yeast prion-like IDPs resulted in traits that can be inheritable long after their expression returned to normal underscores a protein (IDP) conformation-based inheritance mechanism underlying the emergence of new traits and adaptive opportunities[74] as postulated by the conformational noise hypothesis.

The demonstration that several proteins in the green alga *Volvox carteri* that are critical to the evolution of complex multicellularity in the lineage leading to the multicellular species are IDPs that by rewiring cellular PINs facilitated the co-option of ancestral pathways for specialized multicellular functions,[20] is exciting. Providing experimental evidence supporting this observation would be a worthy endeavor in the future.

Yet another important aspect that warrants further investigation is to discern the design principles of GRNs because of which some IDPs, such as MYC for instance, can switch the phenotype of a normal cell to a malignant one yet, in concert with some other IDPs (Yamanaka factors), MYC can reprogram the same normal somatic cell into a pluripotent stem cell. Therefore, elucidating the design principles of GRNs that govern the intricate balance between robustness and plasticity, is important in understanding reprogramming as well as in aiding the design of synthetic circuits.

Advances in computational studies coupled with the advent of more sophisticated bioinformatics algorithms have enabled several studies which showed that the genomes of mammals as well as lower organisms such as yeast and bacteria, contain short opening reading frames (sORFs) that encode "micropeptides" with interesting biological functions, especially in stress response.[75–77] Remarkably, in most cases these micropeptides are intrinsically disordered.[78,79] Similarly, several proteins are now known to switch folds (also referred to as "metamorphic" proteins),[80] and a significant fraction of the proteins in the nonredundant PDB is precited to encode such metamorphic proteins that respond to cellular stimuli by remodeling their secondary structures and changing their functions.[81,82] Indeed, proteins that are charged with responding to diverse cellular signals in a tightly controlled and rapid manner (stress response), and many transcription factors associated with phenotypic switching, may be among the best examples of on-demand transformers (fold switching proteins).[83] We anticipate that many sORF-encoded micropeptides and metamorphic proteins could be involved in modulating phenotypic plasticity thus extending the IDP conformational dynamics-based paradigm advanced by the conformational noise hypothesis.

Glossary of terms

Cancer stem cells: a small subpopulation of tumor cells that are characterized by the presence of certain cell surface markers such as CD44, CD24, and CD133, and possess capabilities of self-renewal, differentiation, and tumorigenicity when implanted in a host.

Conformational noise: noise due to stochastic fluctuations in the conformational preferences of a protein, especially of intrinsically disordered proteins, or intrinsically disordered regions within ordered proteins, that can have long-reaching effects through the amplification of transcriptional as well as signaling noise.

Epigenetic interactions: a term coined by Conrad Hal Waddington to help explain the complex and dynamic interactions between the environment and the genome that ultimately shape the organism's phenotype.

Epigenetic landscape: a metaphor in developmental biology by Conrad Hal Waddington where he portrayed the landscape as an inclined surface with a series of bifurcating ridges and valleys, which represent the series of either/or fate choices made by a pluripotent cell as it differentiates over time.

Gene regulatory network: a directed graph in which regulators of gene expression e.g., transcription factors, are connected to target gene nodes by interaction edges.

Hybrid phenotype: a phenotype exhibiting characteristics of more than one well defined phenotype.

Lock and key model: proposed by Emil Fisher the model states that the active site of an enzyme precisely fits a specific substrate.

Noise: noise is the coefficient of variation $\eta x = \sigma x/\mu x$, here ηx is the noise, μx is the mean value of **x**,and σx is the standard deviation of *x*. This measure is dimensionless, allowing a relative comparison of the importance of noise without necessitating knowledge of the absolute mean.

Persisters: a subpopulation of dormant cells that form spontaneously and are highly resistant to drug treatment.

Phenotypic plasticity: the ability of an organism to switch phenotypes without involving genetic changes.

Reprogramming: the process of changing one cell fate to another, particularly in the context of converting a mature differentiated cell into a less-committed precursor.

Yamanaka factors: a set of four transcriptions factors (Oct3/4, Sox2, Klf4, c-Myc) that are used in reprogramming a differentiated somatic cell into an induced pluripotent stem (iPS) cell *in vitro*.

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AUTHOR CONTRIBUTIONS

Conceptualization, P.K. and G.R.; writing – original draft, P.K.; writing – review & editing, P.K., R.S., and G.R.; funding acquisition, G.R. and R.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Rangel-Huerta, E., and Maldonado, E. (2017). Transit-Amplifying Cells in the Fast Lane from Stem Cells towards Differentiation. Stem Cells Int. *2017*, 7602951.

2. Hanahan, D. (2022). Hallmarks of Cancer: New Dimensions. Cancer Discov. *12*, 31–46.

3. Nanjundiah, V., Ruiz-Trillo, I., and Kirk, D. (2017). Protists and Multiple Routes to the Evolution of Multicellularity. In Cells in Evolutionary Biology: Translating Genotypes into Phenotypes - Past, Present, Future, B.K. Hall and S.A. Moody, eds. (CRC Press).

4. Levis, N.A., and Pfennig, D.W. (2019). Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians. Semin. Cell Dev. Biol. *88*, 80–90.

5. Fox, R.J., Donelson, J.M., Schunter, C., Ravasi, T., and Gaitán-Espitia, J.D. (2019). Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. Philos. Trans. R. Soc. Lond. B Biol. Sci. *374*, 20180174.

6. Sommer, R.J. (2020). Phenotypic Plasticity: From Theory and Genetics to Current and Future Challenges. Genetics *215*, 1–13.

7. Pigliucci, M. (2001). Phenotypic Plasticity: Beyond Nature and Nurture (The Johns Hopkins University Press).

8. Nanjundiah, V. (2020). Early thoughts on phenotypic plasticity in development and evolution. In Phenotypic Switching: Implications in Biology and Medicine, H. Levine, M. Kumar Jolly, P. Kulkarni, and V. Nanjundiah, eds. (Academic Press).

9. Lalejini, A., Ferguson, A.J., Grant, N.A., and Ofria, C. (2021). Adaptive Phenotypic Plasticity Stabilizes Evolution in Fluctuating Environments. Front. Ecol. Evol. *9*, 1–16. https://doi.org/10.3389/fevo.2021.715381.

10. Elowitz, M.B., Levine, A.J., Siggia, E.D., and Swain, P.S. (2002). Stochastic gene expression in a single cell. Science *297*, 1183–1186.

11. Ortega-Sabater, C., F Calvo, G., Dinić, J., Podolski, A., Pesic, M., and Pérez-García, V. (2022). Stochastic Fluctuations Drive Non-genetic Evolution of Proliferation in Clonal Cancer Cell Populations. Bull. Math. Biol. *85*, 8.

12. David, W. (2021). Pfennig in, 'Phenotypic Plasticity & Evolution: Causes, Consequences, Controversies (CRC Press).

13. Nanjundiah, V., and Newman, S.A. (2009). Phenotypic and developmental plasticity. J. Biosci. *34*, 493–494.

14. Mahmoudabadi, G., Rajagopalan, K., Getzenberg, R.H., Hannenhalli, S., Rangarajan, G., and Kulkarni, P. (2013). Intrinsically disordered proteins and conformational noise: implications in cancer. Cell Cycle *12*, 26–31.

15. Yruela, I., Oldfield, C.J., Niklas, K.J., and Dunker, A.K. (2017). Evidence for a Strong Correlation Between Transcription Factor Protein Disorder and Organismic Complexity. Genome Biol. Evol. *9*, 1248–1265.

16. Turoverov, K.K., Kuznetsova, I.M., and Uversky, V.N. (2010). The protein kingdom extended: ordered and intrinsically disordered proteins, their folding, supramolecular complex formation, and aggregation. Prog. Biophys. Mol. Biol. *102*, 73–84.

17. Chakravarty, A.K., and Jarosz, D.F. (2018). More than Just a Phase: Prions at the Crossroads of Epigenetic Inheritance and Evolutionary Change. J. Mol. Biol. *430*, 4607–4618.

18. Jablonka, E., and Lamb, M.J. (2008). Soft inheritance: Challenging the Modern Synthesis. Genet. Mol. Biol. *31*, 389–395.

19. Jolly, M.K., Kulkarni, P., Weninger, K., Orban, J., and Levine, H. (2018). Phenotypic Plasticity, Bet-Hedging, and Androgen Independence in Prostate Cancer: Role of Non-Genetic Heterogeneity. Front. Oncol. *8*, 50.

20. Kulkarni, P., Behal, A., Mohanty, A., Salgia, R., Nedelcu, A.M., and Uversky, V.N. (2022). Co-opting disorder into order: Intrinsically disordered proteins and the early evolution of complex multicellularity. Int. J. Biol. Macromol. *201*, 29–36.

21. Kulkarni, P., Mohanty, A., Salgia, R., and Uversky, V.N. (2022). Intrinsically disordered BMP4 morphogen and the beak of the finch: Co-option of an ancient axial patterning system. Int. J. Biol. Macromol. *219*, 366–373.

22. Niklas, K.J., Dunker, A.K., and Yruela, I. (2018). The evolutionary origins of cell type diversification and the role of intrinsically disordered proteins. J. Exp. Bot. *69*, 1437–1446.

23. Seera, S., and Nagarajaram, H.A. (2022). Effect of Disease Causing Missense Mutations on Intrinsically Disordered Regions in Proteins. Protein Pept. Lett. *29* (3), 254–267.

24. Uyar, B., Weatheritt, R.J., Dinkel, H., Davey, N.E., and Gibson, T.J. (2014). Proteome-wide analysis of human disease mutations in short linear motifs: neglected players in cancer? Mol. Biosyst. *10*, 2626–2642.

25. Schrag, L.G., Liu, X., Thevarajan, I., Prakash, O., Zolkiewski, M., and Chen, J. (2021). Cancer-Associated Mutations Perturb the Disordered Ensemble and Interactions of the Intrinsically Disordered p53 Transactivation Domain. J. Mol. Biol. *433*, 167048.

26. Norman, T.M., Lord, N.D., Paulsson, J., and Losick, R. (2015). Stochastic Switching of Cell Fate in Microbes. Annu. Rev. Microbiol. *69*, 381–403.

27. Carey, J.N., and Goulian, M. (2019). A bacterial signaling system regulates noise to enable bet hedging. Curr. Genet. *65*, 65–70.

28. Ladbury, J.E., and Arold, S.T. (2012). Noise in cellular signaling pathways: causes and effects. Trends Biochem. Sci. *37*, 173–178.

29. Engl, C. (2019). Noise in bacterial gene expression. Biochem. Soc. Trans. *47*, 209–217.

30. Azpeitia, E., Balanzario, E.P., and Wagner, A. (2020). Signaling pathways have an inherent need for noise to acquire information. BMC Bioinformatics *21*, 462.

31. Iakoucheva, L.M., Radivojac, P., Brown, C.J., O'Connor, T.R., Sikes, J.G., Obradovic, Z., and Dunker, A.K. (2004). The importance of intrinsic disorder for protein phosphorylation. Nucleic Acids Res. *32*, 1037–1049.

32. Mylona, A., Theillet, F.X., Foster, C., Cheng, T.M., Miralles, F., Bates, P.A., Selenko, P., and Treisman, R. (2016). Opposing effects of Elk-1 multisite phosphorylation shape its response to ERK activation. Science *354*, 233–237.

33. Rajagopalan, K., Qiu, R., Mooney, S.M., Rao, S., Shiraishi, T., Sacho, E., Huang, H., Shapiro, E., Weninger, K.R., and Kulkarni, P. (2014). The Stress-response protein prostate-associated gene 4, interacts with c-Jun and potentiates its transactivation. Biochim. Biophys. Acta *1842*, 154–163.

34. Sato, N., Sadar, M.D., Bruchovsky, N., Saatcioglu, F., Rennie, P.S., Sato, S., Lange, P.H., and Gleave, M.E. (1997). Androgenic induction of prostate-specific antigen gene is repressed by protein-protein interaction between the androgen receptor and AP-1/c-Jun in the human prostate cancer cell line LNCaP. J. Biol. Chem. *272*, 17485–17494.

35. Tillman, K., Oberfield, J.L., Shen, X.Q., Bubulya, A., and Shemshedini, L. (1998). c-Fos dimerization with c-Jun represses c-Jun enhancement of androgen receptor transactivation. Endocrine *9*, 193–200.

36. He, Y., Chen, Y., Mooney, S.M., Rajagopalan, K., Bhargava, A., Sacho, E., Weninger, K., Bryan, P.N., Kulkarni, P., and Orban, J. (2015). Phosphorylation-induced Conformational Ensemble Switching in an Intrinsically Disordered Cancer/Testis Antigen. J. Biol. Chem. *290*, 25090–25102.

37. Kulkarni, P., Jolly, M.K., Jia, D., Mooney, S.M., Bhargava, A., Kagohara, L.T., Chen, Y., Hao, P., He, Y., Veltri, R.W., et al. (2017). Phosphorylation-induced conformational dynamics in an intrinsically disordered protein and potential role in phenotypic heterogeneity. Proc. Natl. Acad. Sci. USA *114*, E2644–E2653.

38. Lin, X., Kulkarni, P., Bocci, F., Schafer, N.P., Roy, S., Tsai, M.Y., He, Y., Chen, Y., Rajagopalan, K., Mooney, S.M., et al. (2019). Structural and Dynamical Order of a Disordered Protein: Molecular Insights into Conformational Switching of PAGE4 at the Systems Level. Biomolecules *9*, 77.

39. Terada, N., Shiraishi, T., Zeng, Y., Aw-Yong, K.M., Mooney, S.M., Liu, Z., Takahashi, S., Luo, J., Lupold, S.E., Kulkarni, P., and Getzenberg, R.H. (2014). Correlation of Sprouty1 and Jagged1 with aggressive prostate cancer cells with different sensitivities to androgen deprivation. J. Cell. Biochem. *115*, 1505–1515.

40. Wright, P.E., and Dyson, H.J. (2015). Intrinsically disordered proteins in cellular signalling and regulation. Nat. Rev. Mol. Cell Biol. *16*, 18–29.

41. Bah, A., and Forman-Kay, J.D. (2016). Modulation of Intrinsically Disordered Protein Function by Post-translational Modifications. J. Biol. Chem. *291*, 6696–6705.

42. Subbalakshmi, A.R., Ashraf, B., and Jolly, M.K. (2022). Biophysical and biochemical attributes of hybrid epithelial/mesenchymal phenotypes. Phys. Biol. *19*, 025001.

43. Brown, M.S., Abdollahi, B., Wilkins, O.M., Lu, H., Chakraborty, P., Ognjenovic, N.B., Muller, K.E., Jolly, M.K., Christensen, B.C., Hassanpour, S., and Pattabiraman, D.R. (2022). Phenotypic heterogeneity driven by plasticity of the intermediate EMT state governs disease progression and metastasis in breast cancer. Sci. Adv. *8*, eabj8002.

44. Tao, L., Du, H., Guan, G., Dai, Y., Nobile, C.J., Liang, W., Cao, C., Zhang, Q., Zhong, J., and Huang, G. (2014). Discovery of a "white-gray-opaque" tristable phenotypic switching system in candida albicans: roles of non-genetic diversity in host adaptation. PLoS Biol. *12*, e1001830.

45. Dorantes-Acosta, E., and Pelayo, R. (2012). Lineage switching in acute leukemias: a consequence of stem cell plasticity? Bone Marrow Res. *2012*, 406796.

46. Mu, P., Zhang, Z., Benelli, M., Karthaus, W.R., Hoover, E., Chen, C.C., Wongvipat, J., Ku, S.Y., Gao, D., Cao, Z., et al. (2017). SOX2 promotes lineage plasticity and antiandrogen resistance in TP53- and RB1-deficient prostate cancer. Science *355*, 84–88.

47. Ku, S.Y., Rosario, S., Wang, Y., Mu, P., Seshadri, M., Goodrich, Z.W., Goodrich, M.M., Labbé, D.P., Gomez, E.C., Wang, J., et al. (2017). Rb1 and Trp53 cooperate to suppress prostate cancer lineage plasticity, metastasis, and antiandrogen resistance. Science *355*, 78–83.

48. Wölfl, M., Rasche, M., Eyrich, M., Schmid, R., Reinhardt, D., and Schlegel, P.G. (2018). Spontaneous reversion of a lineage switch following an initial blinatumomab-induced ALL-to-AML switch in MLL-rearranged infant ALL. Blood Adv. *2*, 1382–1385.

49. Jain, P., Duddu, A.S., and Jolly, M.K. (2022). Stochastic population dynamics of cancer stemness and adaptive response to therapies. Essays Biochem. *66*, 387–398.

50. Grangeasse, C., Nessler, S., and Mijakovic, I. (2012). Bacterial tyrosine kinases: evolution, biological function and structural insights.

Philos. Trans. R. Soc. Lond. B Biol. Sci. *367*, 2640–2655.

51. Shimizu, Y., Tsuru, S., Ito, Y., Ying, B.W., and Yomo, T. (2011). Stochastic switching induced adaptation in a starved Escherichia coli population. PLoS One *6*, e23953.

52. Brunet, T., Albert, M., Roman, W., Coyle, M.C., Spitzer, D.C., and King, N. (2021). A flagellate-to-amoeboid switch in the closest living relatives of animals. Elife *10*, e61037.

53. Covarrubias, A.A., Cuevas-Velazquez, C.L., Romero-Pérez, P.S., Rendón-Luna, D.F., and Chater, C.C.C. (2017). Structural disorder in plant proteins: where plasticity meets sessility. Cell. Mol. Life Sci. *74*, 3119–3147.

54. Hsiao, A.S., Wang, K., and Ho, T.H.D. (2020). An Intrinsically Disordered Protein Interacts with the Cytoskeleton for Adaptive Root Growth under Stress. Plant Physiol. *183*, 570–587.

55. Prochnik, S.E., Umen, J., Nedelcu, A.M., Hallmann, A., Miller, S.M., Nishii, I., Ferris, P., Kuo, A., Mitros, T., Fritz-Laylin, L.K., et al. (2010). Genomic analysis of organismal complexity in the multicellular green alga Volvox carteri. Science *329*, 223–226.

56. Brunet, T., and King, N. (2017). The Origin of Animal Multicellularity and Cell Differentiation. Dev. Cell *43*, 124–140.

57. Niklas, K.J., and Newman, S.A. (2020). The many roads to and from multicellularity. J. Exp. Bot. *71*, 3247–3253.

58. Cheng, Q., Pappas, V., Hallmann, A., and Miller, S.M. (2005). Hsp70A and GlsA interact as partner chaperones to regulate asymmetric division in Volvox. Dev. Biol. *286*, 537–548.

59. Olson, B.J.S.C., Oberholzer, M., Li, Y., Zones, J.M., Kohli, H.S., Bisova, K., Fang, S.C., Meisenhelder, J., Hunter, T., and Umen, J.G. (2010). Regulation of the Chlamydomonas cell cycle by a stable, chromatin-associated retinoblastoma tumor suppressor complex. Plant Cell *22*, 3331–3347.

60. Desvoyes, B., and Gutierrez, C. (2020). Roles of plant retinoblastoma protein: cell cycle and beyond. EMBO J. *39*, e105802.

61. Kastano, K., Erdős, G., Mier, P., Alanis-Lobato, G., Promponas, V.J., Dosztányi, Z., and Andrade-Navarro, M.A. (2020). Evolutionary Study of Disorder in Protein Sequences. Biomolecules *10*, 1413.

62. Gao, C., Ma, C., Wang, H., Zhong, H., Zang, J., Zhong, R., He, F., and Yang, D. (2021). Intrinsic disorder in protein domains contributes to both organism complexity and clade-specific functions. Sci. Rep. *11*, 2985.

63. Kulkarni, P., Bhattacharya, S., Achuthan, S., Behal, A., Jolly, M.K., Kotnala, S., Mohanty, A., Rangarajan, G., Salgia, R., and Uversky, V. (2022). Intrinsically Disordered Proteins: Critical Components of the Wetware. Chem. Rev. *122*, 6614–6633.

64. Waddington, C.H. (1957). The Strategy of the Genes (Geo Allen & Unwin).

65. Mooney, S.M., Jolly, M.K., Levine, H., and Kulkarni, P. (2016). Phenotypic plasticity in prostate cancer: role of intrinsically disordered proteins. Asian J. Androl. *18*, 704–710.

66. Jia, D., Jolly, M.K., Kulkarni, P., and Levine, H. (2017). Phenotypic Plasticity and Cell Fate Decisions in Cancer: Insights from Dynamical Systems Theory. Cancers *9*, 70.

67. Kulkarni, P. (2020). Intrinsically Disordered Proteins: Insights from Poincaré, Waddington, and Lamarck. Biomolecules *10*, 1490.

68. Xue, B., Oldfield, C.J., Van, Y.Y., Dunker, A.K., and Uversky, V.N. (2012). Protein intrinsic disorder and induced pluripotent stem cells. Mol. Biosyst. *8*, 134–150.

69. Wang, J., Xu, L., Wang, E., and Huang, S. (2010). The potential landscape of genetic circuits imposes the arrow of time in stem cell differentiation. Biophys. J. *99*, 29–39.

70. Vavouri, T., Semple, J.I., Garcia-Verdugo, R., and Lehner, B. (2009). Intrinsic protein disorder and interaction promiscuity are widely associated with dosage sensitivity. Cell *138*, 198–208.

71. Hari, K., Ullanat, V., Balasubramanian, A., Gopalan, A., and Jolly, M.K. (2022). Landscape of epithelial mesenchymal plasticity as an emergent property of coordinated teams in regulatory networks. Elife *11*, e76535.

72. Tripathi, S., Kessler, D.A., and Levine, H. (2020). Biological Networks Regulating Cell Fate Choice Are Minimally Frustrated. Phys. Rev. Lett. *125*, 088101.

73. Fitzgerald, D.M., Hastings, P.J., and Rosenberg, S.M. (2017). Stress-Induced Mutagenesis: Implications in Cancer and Drug Resistance. Annu. Rev. Cancer Biol. *1*, 119–140.

74. Chakrabortee, S., Byers, J.S., Jones, S., Garcia, D.M., Bhullar, B., Chang, A., She, R., Lee, L., Fremin, B., Lindquist, S., and Jarosz, D.F. (2016). Intrinsically Disordered Proteins Drive Emergence and Inheritance of Biological Traits. Cell *167*, 369–381.e12.

75. Galindo, M.I., Pueyo, J.I., Fouix, S., Bishop, S.A., and Couso, J.P. (2007). Peptides encoded by short ORFs control development and define a new eukaryotic gene family. PLoS Biol. *5*, e106.

76. Guerra-Almeida, D., and Nunes-da-Fonseca, R. (2020). Small Open Reading Frames: How Important Are They for Molecular Evolution? Front. Genet. *11*, 574737.

77. Khitun, A., Ness, T.J., and Slavoff, S.A. (2019). Small open reading frames and cellular stress responses. Mol. Omics *15*, 108–116.

78. Schlesinger, D., and Elsässer, S.J. (2022). Revisiting sORFs: overcoming challenges to identify and characterize functional microproteins. FEBS J. *289*, 53–74.

79. Erady, C., Boxall, A., Puntambekar, S., Suhas Jagannathan, N., Chauhan, R., Chong, D., Meena, N., Kulkarni, A., Kasabe, B., Prathivadi Bhayankaram, K., et al. (2021). Pan-cancer analysis of transcripts encoding novel open-reading frames (nORFs) and their potential biological functions. NPJ Genom. Med. *6*, 4.

80. Bryan, P.N., and Orban, J. (2010). Proteins that switch folds. Curr. Opin. Struct. Biol. *20*, 482–488.

81. Porter, L.L., and Looger, L.L. (2018). Extant fold-switching proteins are widespread. Proc. Natl. Acad. Sci. USA *115*, 5968–5973.

82. Kim, A.K., and Porter, L.L. (2021). Functional and Regulatory Roles of Fold-Switching Proteins. Structure *29*, 6–14.

83. Artsimovitch, I., and Ramírez-Sarmiento, C.A. (2022). Metamorphic proteins under a computational microscope: Lessons from a fold-switching RfaH protein. Comput. Struct. Biotechnol. J. *20*, 5824–5837.