Development of Photoswitchable, Phase Separable Biomaterials

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Abstract

Phase separation is a dynamic process that leads to the formation of biomolecular condensates. These condensates contain proteins with high concentrations of intrinsically disordered regions (IDR), which lack a fixed 3D structure. We aim to control phase separation in human cells using the LOV domain, a protein sensor that homodimerizes under blue light. We hypothesize that extant LOV proteins may show nontrivial association with intrinsically disordered regions, and these proteins with IDR regions may induce phase separation under blue light. To determine IDR percentage, we processed sequences from a database of LOV proteins through an algorithm that provides scores for likely intrinsically disordered amino acids. The distribution of these LOV proteins as a function of IDR percentage was significantly different from that of random sequences. We expressed the highest scoring proteins in human cells and monitored their ability to form condensates under blue light. These novel proteins will provide a new tool for researchers to better understand the phenomenon of phase separation.

Background

Cells are organized by phase separation, in which protein-dense liquid droplets form with resident molecules. Proteins in these droplets have high concentrations of IDR's, which lack a fixed 3-D structure and contribute to the dynamic nature of the condensate. Several extant disordered proteins also contain LOV domains, a photosensitive region that undergoes homodimerization under blue light. This experiment investigates the following questions:

Is there a relationship between LOV domain-containing proteins and intrinsic disorder?

and

Can LOV domains be used to control phase separation in human cells?

Research Methods

We began by analyzing a database of LOV-containing proteins, and processed these proteins through an algorithm to identify IDR regions. We then selected sequences for expression and transfected them into human cells.

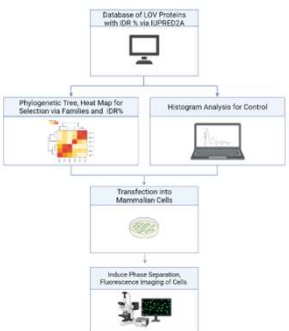

Figure 1: Experiment Flow Chart

Results

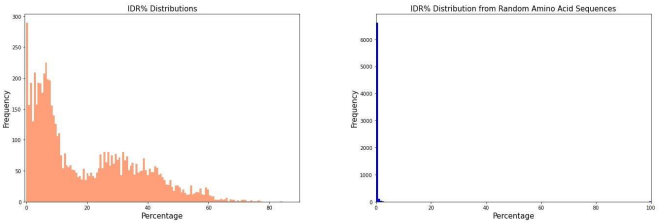

Figure 2: Histograms of LOV-domain containing proteins (left) and randomly generated amino acids (right) as a function of IDR percentage.

As seen above in Figure 2, there is significant difference between LOV domain-containing proteins and randomly generated amino acid sequences in the distribution of frequencies of proteins as a function of IDR percentage. Whereas the distribution of randomly generated amino acid sequences (right) shows exponential decay as IDR percentage increases, the distribution of LOV-domain containing proteins (left) demonstrates clusters of normal distributions at certain IDR percentages.

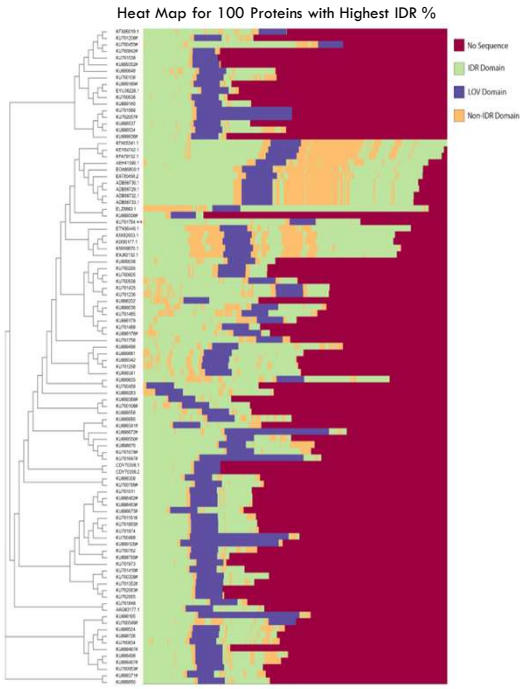

Figure 3: Heat Map of 100 LOV-domain containing proteins with Highest IDR%

In Figure 3 (above), a phylogenetic tree and heat map were generated using the sequences of the 100 LOV-domain containing proteins with the highest IDR percentages. The phylogenetic tree shows 21 distinct groups, from which one protein per group was selected for expression. The heat map, which represents the specific regions of each sequence as color-coded blocks, demonstrates a consistent pattern of non-IDR domains bordering the LOV domain region. Additionally, the placement of the LOV domain within certain closely-related sequences (top 3) is not necessarily similar, while the placement of the LOV domain within some more distantly related regions (bottom 10) shows much more similarity.

Conclusions

As shown in Figure 2 of the Results section, there is significant difference between LOV domain-containing proteins and randomly generated amino acid sequences in the distribution of frequencies of proteins as a function of IDR percentage. We conclude that there may be some nontrivial association between LOV-domain containing proteins and intrinsically disordered regions.

We have already synthesized the plasmids with the corresponding cDNA for the protein sequences selected for expression and are currently in the process of transfecting human cells with our plasmid constructs. Results from this phase of our experiment should provide insight into the viability of using LOV domain-containing proteins for photoswitchable control of phase separation in human cells. Successful transfection and reversible phase separation under blue lightin vitro would validate our hypothesis and provide a powerful for further study of phase separation.

Future Directions

Following successful transfection of the plasmid, we intend to induce phase separation in the cells using blue light. After ensuring phase separation is successfully controlled, we will analyze the diffusion kinetics using fluorescence recovery after photobleaching. We also aim to express more sequences with higher IDR percentages and from different families as per our heat map. This will allow us to create a diverse array of cell lines to observe differences in the effects of different LOV domains.

Acknowledgments

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