Research Roundup

Disorder for variety

Disordered regions in proteins are prime real estate for alternative splicing, say Pedro Romero, Keith Dunker (Indiana University-Purdue University, Indianapolis, IN), and colleagues find that d. The innate disorder allows protein variation that in turn increases functional diversity.

Both splicing and disorder are more common in multicellular eukaryotes than in lower organisms. Dunker wondered whether this trend might be more than coincidence. Structures are available for only five pairs of alternatively spliced isoforms but, in three pairs, the regions present in one splice form but absent in another are found within disordered regions.

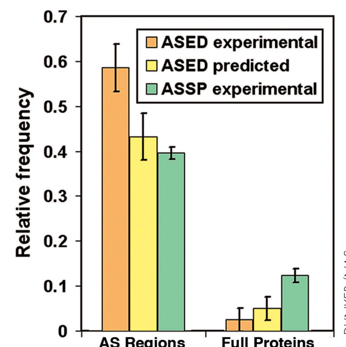
To expand the dataset, the authors compared various databases of disorder and of splicing. Indeed, alternatively spliced regions were strongly biased toward disorder. Their flexibility probably improves the odds that an addition or deletion will not impede folding and thus lead to aggregates.

Splicing in disordered regions might also increase functional diversity. Unlike structured domains, which use far-flung residues to build a single functional unit, disordered regions often use a compact and linear series of residues to create a particular functional unit. "You get more bang for your buck," says Dunker. "Just splice out ten consecutive residues, and a whole function is gone."

Disordered domains can evolve quickly, given their structural freedom, and often bind several partners. As disordered regions are commonly signaling and regulatory domains, more disorder and more splicing might have contributed to the emergence of cellular specialization. "With different splicing in different cells," says Dunker, "the signaling network becomes radically altered." Splicing out a piece of a disordered region in BRCA1, for example, eliminates p53 binding.

Testing this evolutionary hypothesis, however, will take time. To start, the group would like to work out the major signaling network differences between a pair of similar but distinct cell types, perhaps from a simple multicellular organism like the sponge, and then determine whether the changes relate to alternative splicing within disordered regions. JCB

Reference: Romero, P.R., et al. 2006. *Proc. Natl. Acad. Sci. USA*. 103:8390–8395.



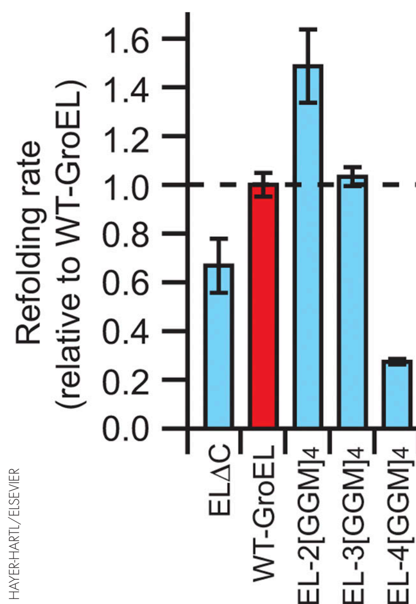
Highly disordered regions are more common in alternatively spliced regions (left) than the rest of the protein (right).

Cage folding

A well-designed chaperone cage helps proteins to fold quickly, say Yun-Chi Tang, F. Ulrich Hartl, Manajit Hayer-Hartl, and colleagues (Max Planck Institute of Biochemistry, Martinsried, Germany), probably by limiting the number of possible folding intermediates.

Bacteria's most well-studied chaperone is the GroEL nano-cage, which encapsulates a folding substrate within its walls. This cage was thought to be little more than a way to isolate substrates to prevent aggregation of slow-folding proteins. But the new findings suggest an active role, as folding rates inside the cage were up to 15-fold faster than in solution.

Folding is hastened by several cage features, including cage size. For small proteins, GroEL mutants with a smaller cavity further accelerated folding, until a point at which necessary rearrangements were spatially restricted. Confinement hastens folding by preventing those misfolded intermediates that would not fit within the cage. "The number of possible conformations," says Hartl, "is astronomically large. The cage reduces it to a subfraction of that."



Decreasing GroEL cavity size (from left to right) increases then decreases protein folding rate.

For large proteins, both smaller and larger cavities slowed folding. The cage thus seems to be evolutionarily optimized to suit its ~250 in vivo substrates. "You can improve folding rates for some with GroEL mutations," says Hayer-Hartl, "but only at the expense of other substrates."

Flexible, finger-like extensions at the bottom of the cavity were necessary for some proteins to fold quickly. The authors speculate that these mildly hydrophobic sequences gently massage misfolded states, increasing the substrate's fluidity and easing rearrangements.

Some proteins also required clusters of negative charges on the cavity wall for optimal folding. Proteins most impeded by the loss of these clusters were themselves negatively charged, so perhaps the clusters help by keeping these proteins from sticking to the cage walls.

The authors would next like to identify intermediates in spontaneous and GroEL-mediated folding events to see just how the folding landscape changes inside the cage. JCB

Reference: Tang, Y.-C., et al. 2006. *Cell*. 125:903–914.

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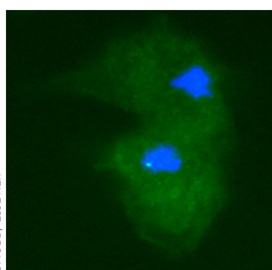
Text by Nicole LeBrasseur
lebrasn@rockefeller.edu

Genes pulse with activity

Jonathan Chubb, Robert Singer, and colleagues (Albert Einstein College of Medicine, Bronx, NY) have devised a method to view transcription inside individual cells. By removing the effects of population averaging, the method reveals transcriptional pulses of a eukaryotic developmental gene.

Transcription was visualized by inserting upstream stem loops into an endogenous developmentally regulated *Dictyostelium* gene called *dscA*. The nascent transcripts were then detected by a GFP fusion protein that binds to the RNA stem loops.

Single-cell analyses revealed discontinuous transcription, or pulses, rather than smooth, uniform expression. Determining the pulse mechanism will require more investigation, but possibilities include reversible chromatin modifications or variations in transcription factor binding.


CHUBB/ELSEVIER

GFP reveals a spot of dscA transcription (light blue).

Pulsing would provide exquisite regulatory control, suggests Singer. "A burst that makes a lot of message might overshoot the amount of protein needed. That can have deleterious effects." Better to turn on for a flicker, wait to equilibrate, then cycle back on if more protein is needed.

dscA is induced as *Dictyostelium* form fruiting bodies. This increase did not come from stronger or more frequent pulses, but rather from more pulsing cells. The new recruits were often clustered, suggesting either that the cluster is seeing the same developmental stimulus or that expressing cells tell their neighbors to join in. Whether constitutive genes also pulse remains to be determined. JCB

Reference: Chubb, J.R., et al. 2006. *Curr. Biol.* 16:1018–1025.

Signaling from keratins

Being supportive is just not enough for a keratin. Seyun Kim, Pauline Wong, and Pierre Coulombe (Johns Hopkins University, Baltimore, MD) find that Keratin 17 (K17) also takes on signaling duties for cell growth during the wound response.

Keratins are a family of structural proteins that form intermediate filaments. Coulombe's group had found that embryos lacking K17 did not heal wounds properly. They now find that these problems do not stem from structural issues.

Wound-adjacent epithelial cells usually turn on K17, grow larger, reduce adhesion, and polarize before migrating into the wound site. This marked cell growth was noticeably stunted, however, in K17 mutants, and the authors traced this defect to a block in mTOR-activated protein synthesis.

At least one known contributor to mTOR activation, the 14-3-3σ adaptor protein, was found with cytoplasmic K17 filaments in wild-type keratinocytes. In cells lacking K17, however, 14-3-3σ was mostly nuclear. In the cytoplasm, 14-3-3σ might bring various components of the mTOR pathway to K17, though the exact mechanism of translational up-regulation by the keratin is unclear.

Coulombe has also found that keratins delay apoptosis in the hair follicle. Such signaling functions, he says, "might be the missing link towards accounting for why keratins exhibit such exquisite cell- and context-specific regulation. Do we need 50 different keratins just for a mechanical function? Probably not." JCB

Reference: Kim, S., et al. 2006. *Nature.* 441:362–365.

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Virus squeezed shut

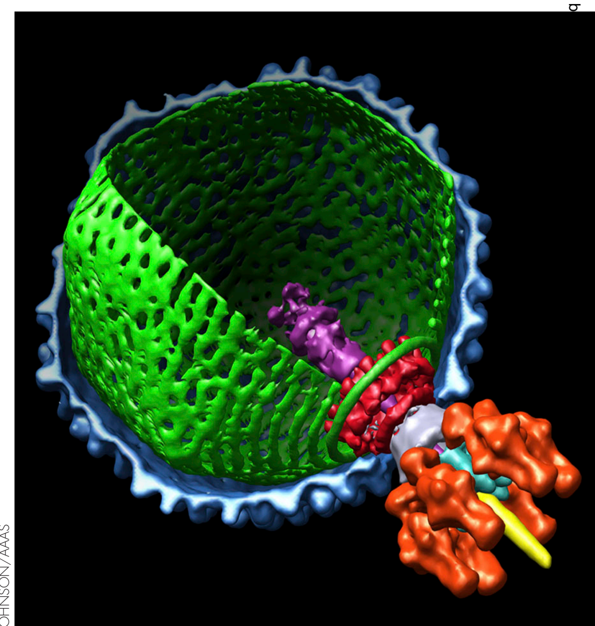
The DNA in a fully packed viral capsid squeezes a pressure sensor, as revealed by images from Gabriel Lander, John Johnson (Scripps Research Institute, La Jolla, CA), and colleagues. The embrace triggers the end of DNA packaging.

Each particle of the p22 bacteriophage, a herpes cousin, contains a single copy of its genome. The DNA is pumped as a long concatamer into one end of the capsid through a portal of gp1 proteins. After one genome enters, the concatamer is cleaved and the portal plugged shut. In gp1 mutants, too much DNA is let in, but just how the wild-type portal senses full capacity was unknown.

Johnson's group viewed the fully assembled portal using automated electron microscopy, which identified items of interest systematically, thus retrieving ten times as many images as in manual reconstructions. The higher resolution data revealed features of the intact viral particle that were previously hidden, including the gp1 portal. "Everything we dreamed of seeing was staring us in the face," said Johnson.

The portal had previously been seen as an isolated entity, but it looked much different in the DNA-filled virion. A ring of DNA wrapped around the portal "sort of like a belt," said Johnson. "It looks like the DNA is squeezing the portal and changing its conformation. This probably signals to the outside, 'we are full of DNA.'" The pumps then know to cut the DNA. JCB

Reference: Lander, G.C., et al. 2006. *Science.* doi:10.1126/science.1127981.


JOHNSON/AAAS

In a full virion, a ring of DNA (green) squeezes the gp1 portal (red) into a new conformation.