the complexity of the common ancestors of archaea (Csuros and Miklos, 2009) and large eukaryotic viruses (Yutin et al., 2009). It seems that the evolution of major classes of life typically begins with a turbulent phase, which leads to the emergence of a highly complex ancestor. Specific lineages then diverge from this common ancestor by one of three pathways: (1) genome streamlining, in which numerous genes are lost, the genomes shrinks, and functional redundancy decreases; (2) genome stasis, in which limited amounts of genes are lost and gained at roughly the same rate via duplication and other processes; (3) genome expansion, in which the rate of gene acquisition substantially exceeds the rate of gene loss.

Comparative genomics of free-living unicellular eukaryotes such as *Naegleria* will help to develop more detailed and confident reconstructions of the gene repertoire of the last common ancestor of eukaryotes. However, understanding the processes that led to the emergence of complex common ancestors, particularly for the eukaryotes, requires other approaches and is one of the most difficult and exciting challenges facing evolutionary biologists today.

REFERENCES

Clamp, M., Fry, B., Kamal, M., Xie, X., Cuff, J., Lin, M.F., Kellis, M., Lindblad-Toh, K., and Lander, E.S. (2007). Proc. Natl. Acad. Sci. USA *104*, 19428–19433.

Collins, L., and Penny, D. (2005). Mol. Biol. Evol. *22*, 1053–1066.

Csuros, M., and Miklos, I. (2009). Mol. Biol. Evol. *26*, 2087–2095.

Embley, T.M., and Martin, W. (2006). Nature *440*, 623–630.

Fritz-Laylin, L.K., Prochnik, S.E., Ginger, M.L., Dacks, J.B., Carpenter, M.L., Field, M.C., Kuo, A., Paredez, A., Chapman, J., Pham, J., et al. (2010). Cell, this issue.

Keeling, P.J. (2007). Science *317*, 1875–1876.

Keeling, P.J., and Palmer, J.D. (2008). Nat. Rev. Genet. *9*, 605–618.

Koonin, E.V., Fedorova, N.D., Jackson, J.D., Jacobs, A.R., Krylov, D.M., Makarova, K.S., Mazumder, R., Mekhedov, S.L., Nikolskaya, A.N., Rao, B.S., et al. (2004). Genome Biol. *5*, R7.

Mans, B.J., Anantharaman, V., Aravind, L., and Koonin, E.V. (2004). Cell Cycle *3*, 1612–1637.

Yutin, N., Wolf, Y.I., Raoult, D., and Koonin, E.V. (2009). Virol. J. *6*, 223.

Angelman Syndrome: Finding the Lost Arc

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Angelman syndrome is a neurodevelopmental disorder caused by mutations in the maternally inherited *UBE3A* gene, which encodes a ubiquitin ligase. Greer et al. (2010) now identify a UBE3A substrate called Arc that promotes endocytosis of neuronal AMPA receptors, providing insight into synaptic defects that may underlie the cognitive deficits in Angelman syndrome.

Angelman syndrome is a neurodevelopmental disorder caused by lack of a functional maternal copy of the *UBE3A* gene, which encodes a ubiquitin ligase (reviewed in Dan, 2009). Its symptoms include developmental delay, mental retardation, excessive inappropriate laughter, movement ataxia, seizures, and lack of speech. Angelman syndrome is commonly caused by the microdeletion of chromosome region 15q11-q13, which contains *UBE3A*, and to a lesser extent by mutations in *UBE3A*. When the same region on the paternal allele is deleted, the result is a different disorder called Prader-Willi syndrome, characterized by endocrine abnormalities, obesity, temper tantrums, and milder mental retardation than in Angelman syndrome.

This interesting relationship between Angelman syndrome and Prader-Willi syndrome can be explained by the genomic imprinting of certain genes located on chromosome 15q11-q13. As a result of imprinting, some human brain regions express only the maternally derived *UBE3A*, whereas most tissues express both parental alleles. The corresponding region on murine chromosome 7 is similarly imprinted. Mice show preferential expression of maternal *Ube3a* in the hippocampus and Purkinje cells of the cerebellum. The evolutionary significance of this imprinting and expression pattern is not yet understood. It has been recognized for more than a decade that Angelman syndrome results from the loss of UBE3A function in specific brain regions, but the underlying cause of the neurological defects is little understood in molecular terms. In this issue of *Cell*, Greer et al. (2010) report an important advance with their identification of a new role for UBE3A in the regulation of synaptic transmission.

UBE3A encodes an E3 ubiquitin protein ligase containing a C-terminal HECT domain that catalyzes the ubiquitination of target proteins. Ubiquitin

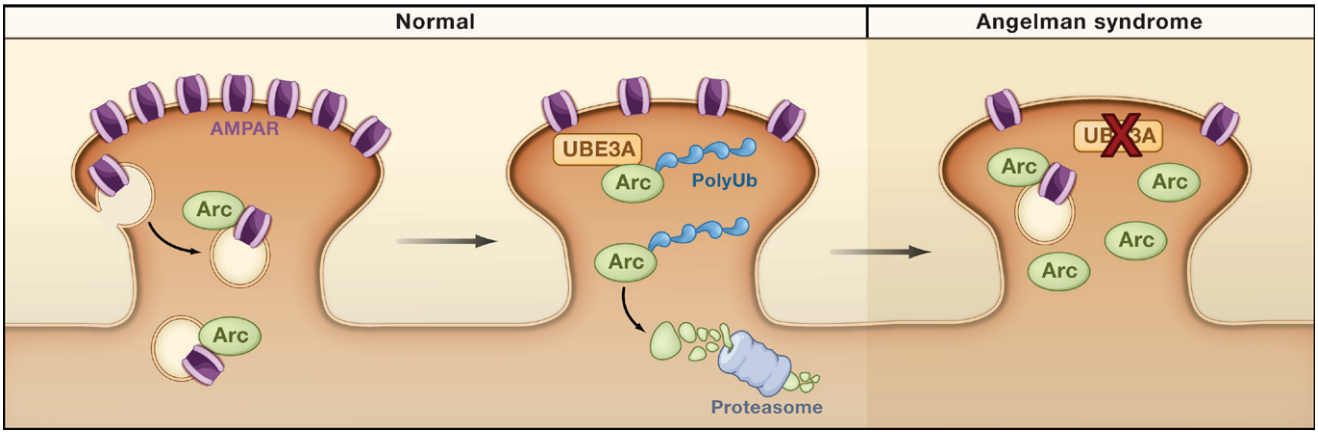

Figure 1. UBE3A, Arc, and AMPAR Endocytosis
Mutations in the *UBE3A* gene, which encodes an E3 ubiquitin ligase, are a cause of a neurodevelopmental disorder called Angelman syndrome.
(Left) A substrate of UBE3A in synapses, Arc, is transcribed rapidly after robust neuronal stimulation and promotes the internalization of AMPA-type glutamate receptors (AMPARs), resulting in a reduction in synaptic strength.
(Middle) Several hours after neuronal stimulation, newly synthesized UBE3A ubiquitinates Arc to promote its degradation by the proteasome, thus preventing further internalization of AMPARs.
(Right) A disruption in the function of UBE3A in neurons leads to increased expression of Arc and a decrease in AMPARs at excitatory synapses, which may contribute to the neurological symptoms of Angelman syndrome.

is a small protein with 76 amino acids whose C terminus can be covalently linked to a protein's lysine residues. The UBE3A protein, also called E6-associated protein or E6-AP, attaches a polyubiquitin chain to protein substrates and marks them for degradation by the proteasome. Judging from *UBE3A* point mutations found in Angelman syndrome patients, this disease can be attributed to the loss of ligase function (Cooper et al., 2004). Furthermore, UBE3A localizes to synapses, and mice lacking the *Ube3a* gene show defects in synaptic plasticity and morphology (Dindot et al., 2008, Jiang, et al., 1998, Yashiro et al., 2009). Hence, the failure to degrade certain synaptic proteins is likely to contribute to symptoms of Angelman syndrome, such as developmental and cognitive impairments.

Major challenges in understanding UBE3A function include devising an unbiased method to identify its substrates in neurons and deciphering its rule for substrate recognition. Greer and coworkers succeeded in tackling these issues head on by using a proteomics approach. First, they crossed hemagglutinin-tagged ubiquitin knockin mice with *Ube3a* knockout mice. By immunoprecipitating hemagglutinin-tagged ubiquitinated proteins and subjecting them to quantitative mass spectrometry, they identified proteins with decreased ubiquitination in the hippocampus of *Ube3a* knockout mice compared to wild-type mice. Next, within these identified proteins a consensus recognition motif for UBE3A was discovered and verified. This represents major progress in our understanding of UBE3A's function and provides a strategy to investigate other ubiquitin ligases.

Using the combination of mass spectrometry, bioinformatics, biochemical characterization, and *Ube3a* knockout mice, Greer and colleagues identified several new UBE3A targets, including Arc, RhoGEF, ephexin 5, and sacsin. Arc stands out as a target of interest because its importance in activity-dependent synaptic regulation has been studied extensively. The authors found that UBE3A prevents the internalization of AMPA-type glutamate receptors (AMPARs) in synaptic membranes by targeting Arc for degradation (Figure 1).

Arc is a synaptic protein that interacts with dynamin and endophilin and promotes the endocytosis of AMPARs (reviewed in Bramham et al., 2008). It is involved in several forms of synaptic plasticity, including long-term potentiation, long-term depression, and homeostatic scaling. Both its transcription and local translation in neuronal dendrites are dependent on synaptic activity. Greer et al. found that after robust synaptic activation, both *Arc* and *Ube3a* undergo MEF2-dependent transcription. *Arc* transcription is turned on more quickly (~1 hr) than *Ube3a* transcription (~6 hr). At dendritic spines, newly synthesized Arc promotes endocytosis of AMPARs, resulting in a reduction in synaptic strength in response to excessive neuronal stimulation. The delayed induction of UBE3A helps to reduce Arc levels and prevents excessive AMPAR internalization (Figure 1). In the hippocampus of *Ube3a* knockout mice, synaptic AMPAR levels are reduced, accompanied by defects in synaptic transmission. These findings suggest that experience-dependent synaptic development and activity-dependent synaptic regulation may be disrupted in Angelman syndrome.

The accumulation of Arc alone, however, probably cannot account for all of the symptoms of Angelman syndrome. Neuronal functions of other UBE3A targets are also worth exploring. Among the new targets found in this study, sacsin is also interesting because it is mutated in a neurodegenerative disorder called autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) (Takiyama, 2007). Very little is understood about sacsin's functions at this point. Furthermore, UBE3A has been found to copurify with 26S proteasomes in HEK293 cells (Wang et al., 2007) and muscle (Besche et al., 2009). Although mammalian genomes encode hundreds of E3 ubiquitin ligase

enzymes, only a few appear to interact with the proteasome. Also, a number of deubiquitinating enzymes interact with the proteasome. It is possible that E3 ubiquitin ligases and deubiquitinating enzymes associated with the proteasome can edit the ubiquitin chain as the substrate approaches the proteasome. In this way, these enzymes can modulate substrate specificity or alter the overall rate of protein degradation. If UBE3A turns out to be a component of neuronal or synaptic proteasomes, certain Angelman syndrome symptoms may also be related to altered proteasome composition in the affected neurons. This may represent an alternative model for understanding UBE3A function in the brain that counters the current notion that UBE3A is a freely diffusing E3 ubiquitin ligase with perhaps a dozen specific substrates.

Although recent advances in genomics have allowed us to pinpoint the cause of hereditary neurological disorders like Angelman syndrome to a single gene like *UBE3A*, it has proven difficult to understand the functional consequences of the gene mutation or deletion. The same can be said for parkin, an E3 ubiquitin ligase linked to autosomal recessive juvenile Parkinsonism. This reflects our general lack of understanding about how the ubiquitin-proteasome system contributes to the maintenance and regulation of the neuronal proteome. A broad-scale approach, like that taken by Greer et al., can elucidate elements both upstream and downstream of the gene of interest, positioning the mutation in a functional synaptic context where the link to the disease may be clarified.

REFERENCES

Besche, H., Haas, W., Gygi, S., and Goldberg, A. (2009). Biochemistry *48*, 2538–2549.

Bramham, C.R., Worley, P.F., Moore, M.J., and Guzowski, J.F. (2008). J. Neurosci. *28*, 11760–11767.

Cooper, E.M., Hudson, A.W., Amos, J., Wagstaff, J., and Howley, P.M. (2004). J. Biol. Chem. *279*, 41208–41217.

Dan, B. (2009). Epilepsia *50*, 2331–2339.

Dindot, S.V., Antalffy, B.A., Bhattacharjee, M.B., and Beaudet, A.L. (2008). Hum. Mol. Genet. *17*, 111–118.

Greer, P.L., Hanayama, R., Bloodgood, B.L., Mardinly, A.R., Lipton, D.M., Flavell, S.W., Kim, T.-K., Griffith, E.C., Waldon, Z., Maehr, R., et al. (2010). Cell, this issue.

Jiang, Y.H., Armstrong, D., Albrecht, U., Atkins, C.M., Noebels, J.L., Eichele, G., Sweatt, J.D., and Beaudet, A.L. (1998). Neuron *21*, 799–811.

Takiyama, Y. (2007). Cerebellum *6*, 353–359.

Wang, X., Chen, C.F., Baker, P.R., Chen, P.L., Kaiser, P., and Huang, L. (2007). Biochemistry *46*, 3553–3565.

Yashiro, K., Riday, T.T., Condon, K.H., Roberts, A.C., Bernardo, D.R., Prakash, R., Weinberg, R.J., Ehlers, M.D., and Philpot, B.D. (2009). Nat. Neurosci. *12*, 777–783.

A Penetrating Look at Stochasticity in Development

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In recent work published in *Nature*, Raj et al. (2010) use single mRNA molecule quantification to show that variation in gene expression in *Caenorhabditis elegans* increases in mutants displaying incomplete penetrance. They find that a bimodal response is triggered when noisy expression of an upstream regulator crosses a critical threshold.

Robustness to genetic and environmental variation is an essential feature of all biological systems. In recent years, it has become possible to address the molecular mechanisms that ensure the reproducible outcomes of biological processes. These mechanisms often break down in mutant conditions, leading to variable outcomes. In genetic terms, penetrance refers to the proportion of individuals of a particular mutant genotype displaying a mutant phenotype. In a paper that recently appeared in *Nature*, Raj and colleagues examine mutant backgrounds of the nematode *Caenorhabditis elegans* to explore the role of variable gene expression in incomplete penetrance (Raj et al., 2010). Their analysis provides evidence that mutant conditions increase noise in gene expression. They further show that the downstream genes in affected pathways respond to these variable inputs at certain thresholds and that chromatin has a role in modulating variability of gene expression.

Several groups have evaluated well-defined genetic circuits with new and highly quantitative methodologies to derive fundamental principles about the nature of these circuits and of gene regulation in general (for instance, Cağatay et al., 2009; Gregor et al., 2007; Maamar et al., 2007; Mangan et al., 2003; Süel et al., 2006, 2007). Working in a similar vein, Raj and colleagues have now sought to address the mechanisms controlling incomplete penetrance using a tech-