least in mutants), the *ARS317* region can be considered a "hot spot" in the yeast genome for amplification. In human tumors, there also appear to be "hot spots" of gene amplification (*12*). It is not clear whether these recurrent duplications are due to inherent instability (re-replication?) of specific origins, or are simply due to a selective advantage that duplication provides. The extent to which re-replication–driven instability explains other unusual DNA structures common in cancer cells—such as the small fragments of extrachromosomal DNA known as "double minutes"—also remains to be clarified.

References

1. D. G. Albertson, *Trends Genet.* **22**, 447 (2006).
2. B. McClintock, *Genetics* **26**, 234 (1941).
3. M. Debatisse, B. Malfoy, *Adv. Exp. Med. Biol.* **570**, 343 (2005).
4. J. J. Blow, P. J. Gillespie, *Nat. Rev. Cancer* **8**, 799 (2008).
5. B. M. Green, K. J. Finn, J. J. Li, *Science* **329**, 943 (2010).
6. S. E. Kearsey, S. Cotterill, *Mol. Cell* **12**, 1067 (2003).
7. B. M. Green, R. J. Morreale, B. Ozaydin, J. L. Derisi, J. J. Li, *Mol. Biol. Cell* **17**, 2401 (2006).
8. R. E. Palmer, E. Hogan, D. Koshland, *Genetics* **125**, 763 (1990).
9. S. S. Hook, J. J. Lin, A. Dutta, *Curr. Opin. Cell Biol.* **19**, 663 (2007).
10. Y. Tatsumi *et al.*, *J. Cell Sci.* **119**, 3128 (2006).
11. S. Gonzalez *et al.*, *Nature* **440**, 702 (2006).
12. W. W. Lockwood *et al.*, *Oncogene* **27**, 4615 (2008).

10.1126/science.1194261

NEUROSCIENCE

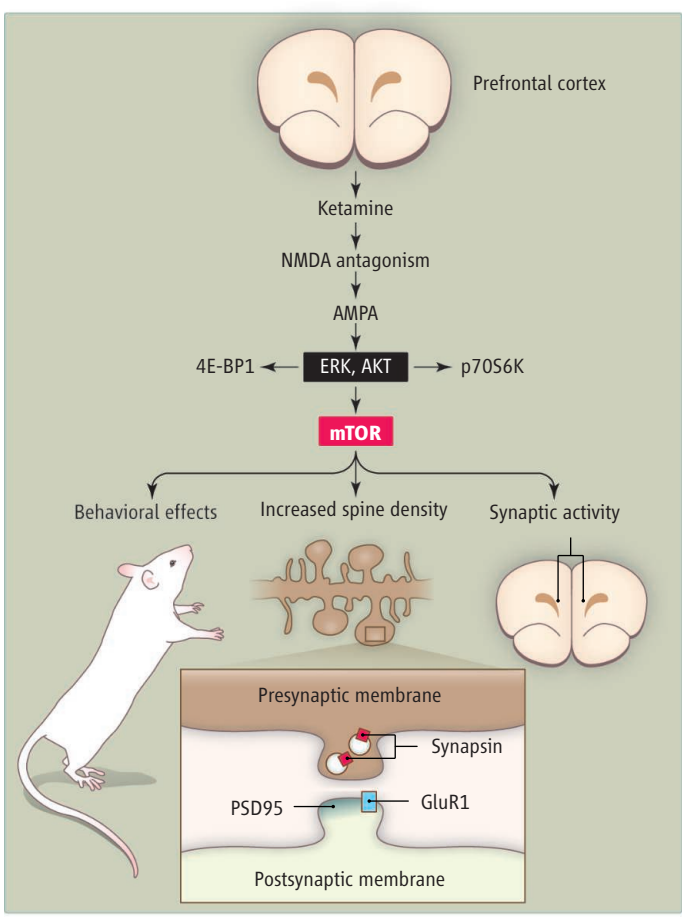
A Glutamate Pathway to Faster-Acting Antidepressants?

Activation of mTOR, a ubiquitous protein, in the prefrontal cortex could be a key goal of new drugs.

John F. Cryan and Olivia F. O'Leary

Depressive illness was described by Hippocrates in ancient Greece, but effective therapeutic agents did not emerge until the 1950s. Today, almost all antidepressant drugs in clinical use increase levels of certain neurotransmitters in the brain, in particular norepinephrine and serotonin. Although these medications are beneficial, a sizeable minority of patients remain resistant to their therapeutic effects (*1*). Moreover, in most patients, there is a delay of weeks to months before the drugs take full effect. As a result, there is an urgent need to develop faster-acting drugs (*2*–*4*).

One approach has rested on the hypothesis that current drugs facilitate a gradual reorganization of the connections (synapses) and communication between neurons, perhaps by integrating new brain cells into neural networks or by increasing the turnover of synapses and their related proteins (*5*–*8*). Glutamate, the brain's major excitatory neurotransmitter, is involved in these processes. In the last decade, several clinical studies have reported that a single subanaesthetic dose of ketamine, which blocks a particular cell receptor for glutamate (the NMDA receptor), resulted in a rapid antidepressant effect within hours of administration, and that this effect could be sustained for at least 1 week (*9*, *10*). Ketamine also improved depressive symptoms in treatment-resistant patients (*10*, *11*). In addition, a single dose produced a rapid and sustained antidepressant effect in several animal models (*12*).



CREDIT: ADAPTED BY P. HUEY/*SCIENCE*

On page 959 of this issue, Li *et al.* (*13*) take a potentially important step toward identifying faster-acting antidepressants by identifying the cellular signaling pathways in the prefrontal cortex of the rat brain that are rapidly activated by ketamine. A single dose increased expression of synaptic proteins within 2 hours and increased dendritic spine density and synaptic activity within 24 hours (*12*). One of the pathways activated by ketamine was the mammalian target of rapamycin (mTOR), a ubiquitous protein kinase involved in protein synthesis and synaptic plasticity (*14*). By directly administering rapamycin, which inhibits mTOR, into the brain, they demonstrated that mTOR is required for ketamine-induced increases in synaptic proteins, dendritic spines, and some forms of synaptic activity in the prefrontal cortex. Moreover, inhibition of mTOR in the medial prefrontal cortex, a specific brain

Fast action. In rats, a single dose of ketamine was sufficient to activate the mTOR pathway in the prefrontal cortex, increasing the expression of synaptic proteins and the density of dendritic spines and inducing an antidepressant response within a day. AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; 4E-BP1, eukaryotic initiation factor 4E binding protein 1; ERK, extracellular signal–regulated kinase; GluR1, AMPA receptor subunit 1; mTOR, mammalian target of rapamycin; NMDA, *N*-methyl-D-aspartate; p70S6K, p70S6 kinase.

School of Pharmacy, Department of Pharmacology and Therapeutics, Alimentary Pharmabiotic Centre, University College Cork, College Road, Cork, Ireland. E-mail: j.cryan@ucc.ie

region thought to be affected by stress and depression (*15*, *16*), prevented ketamine from producing its antidepressant-like behavioral effects in several animal models. Notably, in one behavioral test, the animals responded to a single dose of ketamine; typically, the animals require repeated doses of current antidepressants to show a response in this test.

Li *et al*. also demonstrated that mTOR is activated by another compound, Ro 25-6981, which selectively acts on a certain type of NMDA receptor. Ro 25-6981 also increased the expression of several signaling and synaptic proteins on time scales similar to those of ketamine. Moreover, a single administration of Ro 25-6981 produced antidepressant-like behavioral effects that were also dependent upon mTOR signaling. Interestingly, the mTOR pathway was not activated by single or repeated doses of two commonly used antidepressant drugs or by electroconvulsive shock, which is one of the most effective therapies in treatment-resistant depression.

Together, these findings suggest that the rapid activation of mTOR-mediated signaling pathways may be an important and novel strategy for the rational design of fast-acting antidepressants. The exciting results also demonstrate that ketamine may be a useful tool to identify molecular mediators of rapid antidepressant effects. Neither ketamine nor mTOR, however, is the Holy Grail of rapid antidepressant therapy. Ketamine can cause symptoms that mimic psychosis and can cause cognitive impairment, limiting its potential for widespread clinical use. Because mTOR is a ubiquitous enzyme that regulates protein synthesis, treatments that fail to appropriately restrict mTOR signaling to specific times and parts of the brain could have detrimental effects in humans, such as cancer (*17*).

Although the present study demonstrates that mTOR activity, specifically in the medial prefrontal cortex, is critical to the antidepressant effects of ketamine, past studies have found that global inhibition of mTOR also has antidepressant-like behavioral effects (*18*). It remains unclear, however, whether ketamine and antidepressants alter mTOR activity in other brain regions also affected in major depression. The expression and activity of mTOR in the depressed human brain is unknown, and caution must always be taken when comparing results from rodents to humans, who have a much more elaborate frontal cortex (*15*). Clinical trials of other compounds that act on NMDA receptors are under way (*19*, *20*). As their results emerge, we now have a potential cellular mechanism to explain how these glutamate ligands induce their rapid antidepressant action. This may brighten the outlook for antidepressant drug discovery.

References and Notes

1. M. Fava, *Biol. Psychiatry* **53**, 649 (2003).
2. A. Adell *et al*., *Drug Discov. Today* **10**, 578 (2005).
3. J. F. Cryan, B. E. Leonard, *Hum. Psychopharmacol.* **15**, 113 (2000).
4. J. Mazella *et al*., *PLoS Biol.* **8**, e1000355 (2010).
5. E. Castrén, *Nat. Rev. Neurosci.* **6**, 241 (2005).
6. T. Hajszan *et al*., *Biol. Psychiatry* **65**, 392 (2009).
7. O. F. O'Leary, X. Wu, E. Castren, *Psychoneuroendocrinology* **34**, 367 (2009).
8. A. Sahay, R. Hen, *Nat. Neurosci.* **10**, 1110 (2007).
9. R. M. Berman *et al*., *Biol. Psychiatry* **47**, 351 (2000).
10. C. A. Zarate Jr., *et al*., *Arch. Gen. Psychiatry* **63**, 856 (2006).
11. N. Diazgranados *et al*., *Arch. Gen. Psychiatry* **67**, 793 (2010).
12. S. Maeng *et al*., *Biol. Psychiatry* **63**, 349 (2008).
13. N. Li *et al*., *Science* **329**, 959 (2010).
14. C. A. Hoeffer, E. Klann, *Trends Neurosci.* **33**, 67 (2010).
15. J. F. Cryan, A. Holmes, *Nat. Rev. Drug Discov.* **4**, 775 (2005).
16. D. A. Slattery, I. Neumann, J. F. Cryan, *J. Psychopharmacol.* Published online 8 June 2010; PMID: 20530589.
17. B. Shor, J. J. Gibbons, R. T. Abraham, K. Yu, *Cell Cycle* **8**, 3831 (2009).
18. C. Cleary *et al*., *Brain Res. Bull.* **76**, 469 (2008).
19. P. Skolnick, P. Popik, R. Trullas, *Trends Pharmacol. Sci.* **30**, 563 (2009).
20. Evotec AG Press Release 1 July 2010; www.evotec.com/article/en/Press-releases/EVT-101-Phase-II-study-started/2073
21. The authors thank M. Julio-Pieper for assistance with the illustration. The authors' research is funded by the European Community's Seventh Framework Programme, grant FP7/2007-2013, Grant Agreement 201714 (J.F.C.); Science Foundation Ireland, grants 02/CE/B124 and 07/CE/B1368 (J.F.C.); and Health Research Board PD/2008/26 (O.F.O.).

10.1126/science.1194313

IMMUNOLOGY

Double TIP-ping

A protein induces gene activation marks in chromatin and repairs DNA breaks to promote genetic recombination during antibody class switching.

Harinder Singh[1,2] and Ignacio A. Demarco[1]

B cells of the mammalian immune system use two different means of shuffling gene segments to generate antibodies that recognize pathogens. Unique variable regions of the heavy- and light-chain subunits that constitute an antibody are genetically encrypted through DNA recombination during B cell development (*1*). The process generates millions of naïve B cell clones, each expressing a distinct antibody whose variable portion recognizes a particular antigen of a pathogen. Upon recognizing a specific antigen, a naïve B cell differentiates into an antibody-secreting plasma cell by further shuffling gene segments that encode the constant region of the heavy chain, while holding the variable region fixed (*2*). This process, called class switch recombination, is initiated by the enzyme activation-induced cytidine deaminase (AID) (*3*). On page 917 of this issue, Daniel *et al*. (*4*) identify a dual actor in the molecular steps that precede and follow AID action, thereby enabling class switch recombination and refinement of the antibody repertoire.

Each immunoglobulin heavy-chain (IgH) constant region is associated with a cis-acting regulatory DNA sequence called a switch (S) region (see the figure), where a double-strand break (DSB) in DNA is initiated by AID. Accessibility of the S region to AID depends on its transcription. The RNA polymerase II (Pol II) complex transiently generates single-stranded DNA, the preferred substrate for AID, by unwinding helical double-stranded DNA (*2*). During B cell differentiation, transcription factors bind to promoter sequences near S regions and are thought to load AID onto RNA Pol II, allowing the former to "piggyback" into the S region (*5*). AID action at an S region involves cytidine deamination. Uracil base excision and cleavage of abasic sites by DNA repair enzymes culminates in nicks in single-stranded DNA that are converted into DSBs. DSBs activate the nonhomologous end joining pathway (NHEJ), which ligates S-region ends, yielding an allele that encodes a heavy-chain subunit with a switched constant region (*6*). These considerations led Daniel *et al*. to focus on Pax transcription activation domain interacting protein (PTIP). PTIP is implicated in NHEJ and also associates with a histone methyltransferase that functions in gene activation (*7*, *8*). The question arose as to whether PTIP might regulate class switch recombination by activating S-region transcription as well as by joining of DSBs, thereby acting before and after AID, respectively.

[1]Department of Discovery Immunology, Genentech, San Francisco, CA 94080, USA. [2]Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637, USA. E-mail: singh.harinder@gene.com