proteins bound hypophosphorylated isoforms, they were not efficient at stimulating ubiquitination. Thus, phosphorylation may play additional roles following substrate binding. Furthermore, binding of phosphorylated Sic1 may be more complex and additional positively charged residues distributed on the surface of Cdc4 might serve as additional phosphoacceptors. The situation might be even more complex in vivo because homologs of Cdc4 have been found to form oligomers (Kominami et al., 1998; Wolf et al., 1999). Nonetheless, by perturbing the CDP-Cdc4 interface, the authors strongly support the idea that the single CPD binding site is critical for setting the hexameric phosphorylation threshold for Sic1 binding. Thus, they illustrate the theory that binding of a polyvalent ligand to a single receptor site can create cooperative binding and an ultrasensitive transition.

The CPD-Cdc4 structure will certainly allow more detailed tests of how Cdc4 counts phosphorylation sites and may provide clues to the binding specificity of other WD40 containing F box proteins, including the highly studied βTrCP. Even so, the remaining questions of how substrates, once bound, are presented to the SCF ubiquitin ligase, the potential role of oligomerization, and the mechanism of ubiquitin chain assembly will keep us busy for more time to come!

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Visualizing Genetic Influences on Human Brain Functions

Egan and colleagues (2003), in this issue of *Cell*, integrate genetics and functional brain imaging by showing that variation in the human brain-derived neurotrophic factor (BDNF) gene is associated with variation in episodic memory ability and in hippocampal neurochemistry and function.

Over the last decade, two of the most exciting frontiers of human biology have been genetics and functional brain imaging. Genes that influence human behavior must logically do so through their effects in the brain at the level of neuronal functioning. The bridges from gene to brain to mind have, however, been studied by indirect inferences, such as twin studies that compare similarities in brain structures and mental abilities between unrelated people, dizygotic twins, and monozygotic twins (e.g., Thompson et al., 2001). These interesting studies, however, are limited by much-debated assumptions about heritability estimates and by an absence of specification of genetic and molecular mechanisms.

In a pioneering study that integrates genetics and functional imaging of the human brain, Egan et al. (2003) have linked genetic variation in humans to variation in both memory ability and hippocampal function. The hippocampus, located bilaterally in the medial temporal lobes, is essential for the formation of long-term memory in animals (Squire, 1992) and humans (Gabrieli, 1998). In humans, damage to the hippocampus and adjacent structures results in global amnesia, the inability to form new memories for events (episodic memory) and facts (semantic memory) despite otherwise intact mental abilities. The neural mechanisms underlying hippocampal plasticity have been investigated in detail, and long-term potentiation (LTP) has arisen as the predominant model of hippocampal learning mechanisms. In vivo and in vitro animal studies have shown that the BDNF protein plays an important role in hippocampal LTP, and this suggests that genetic variation associated with BDNF may affect hippocampal LTP and, thus, memory function.

Egan et al. (2003) divided subjects on the basis of a common, single nucleotide polymorphism that alters the amino acid sequence in the pro-region of the human BDNF gene. Subjects were divided into three BDNF alleles varying by a valine (val) to methionine (met) substitution. The met/met group demonstrated inferior performance on a test of episodic memory for short stories compared to the other two groups (val/val and val/met). Two in vivo imaging measures of the hippocampus were utilized to further compare differences between individuals with the val/met and met/met alleles. One measure involved proton magnetic resonance spectroscopic imaging (MRSI), which provides a measure of intracellular neurochemical integrity. MRSI revealed that val/met heterozygotes had lower levels of hippocampal NAA, an intracellular marker of neuronal function, than did val/val homozygotes.

The second brain measure utilized functional magnetic resonance imaging (fMRI), which provides a mea-

sure of regional brain activation underlying mental operations invoked by task performance. In this case, the task was an "N-back" working memory test. In a 2-back version of the N-back test, subjects saw a stream of individually presented digits and responded each time the current digit was identical to that seen two trials previously (e.g., in the series 1, 3, 2, 2, 4, 1, 4, 3, 1, 2, 1, they would respond to the second appearance of the "4" and the last appearance of the "1"). This is a challenging task because subjects must memorize each digit, compare it to the two prior trials in memory, and constantly update two trials in mind as each next digit appears. Activation during this 2-back task was compared to a much easier condition in which subjects simply identified the currently presented digit. This is not a classic task of long-term memory and hippocampal function and is usually used to examine working (current) memory and frontal-lobe function. However, prior studies from this group found an inverse relation between frontal and hippocampal activation for the more demanding task–as frontal activation increased, hippocampal activation decreased (Meyer-Lindenberg et al., 2001). This may reflect selective allocation of frontal-lobe resources for this difficult task. In any case, these findings indicate, counterintuitively, that reduced hippocampal activation is optimal for this task. The fMRI measure revealed, in two cohorts, that while the val/val homozygotes had the expected reduction of hippocampal activation during the 2-back task, the val/met individuals showed an abnormal increase in hippocampal activation for the more difficult condition. Together, these findings suggest that genetic variation in BDNF (in particular met substitution) has consequences for human long-term memory through its effects on hippocampal function. Egan et al., in a remarkable example of integrative research, also demonstrated in vitro that that the val to met substitution results in differences in intracellular distribution and activity-dependent secretion of BDNF. These studies, in a single paper, pursue the neural basis of human memory from molecule to mind.

The direct link of genetic variation to memory performance, hippocampal neurochemistry, and hippocampal function is an exciting advance. Only two other papers, both from the same group, have made such direct links from genes to brain function. In one study, variation in one gene (COMT) influenced working memory and prefrontal activation (Egan et al., 2001). In the other study (Hariri et al., 2002), variation in the serotonin transporter gene was correlated with differential response to fearful facial expressions in the amygdala, which is known to have a critical role in fear and anxiety. These two studies demonstrate that functional brain imaging in humans is unexpectedly sensitive for detecting genetic effects on brain functions. Indeed, in two studies, functional brain measures were more sensitive to genetic variation than were behavioral measures.

Despite the importance of these studies, many aspects of these findings remain to be fully understood. In the study of the serotonin transporter gene, there were no behavioral differences between the groups with different alleles, despite thorough and thoughtful behavioral measurement. Thus, there were differences in genetics and brain function, but no evidence as to how either difference influences behavior or experience. In the current study, the met/met group that had inferior scores on story recall did not have inferior scores on a test of memory for a list of words. Intriguingly, there is some convergent evidence from studies of patients with Alzheimer's disease that the hippocampus may have a greater role in recall for stories than in recall for word lists (Wilson et al., 1996). More perplexing, however, is that the genetic variation that influenced episodic memory scores (which differentiated the met/met group) was different than that which influenced hippocampal neurochemistry and function (which differentiated the val/met and val/val groups; there were too few met/met individuals for the imaging study). Perhaps imaging tasks that more directly measure episodic memory will help align behavioral and brain findings in the future. Finally, the authors thoughtfully note that the magnitudes of the effects of the BDNF genotypes were small, which is not unexpected for complex mental and brain functions that are likely to have polygenic influences. This study, however, opens up an exciting new horizon of research in which memory, brain function, and genes may be related to one another with remarkable specificity, and their relations visualized via imaging genomics.

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