What are the true origins of the smooth muscle cells (SMCs) present in the intimal lesions of transplant arteriosclerosis? A new study in the JCI shows that Sca-1+ cells purified from the mouse aortic root can migrate through an irradiated vein graft to the neointima of the vessel and transdifferentiate to express the early SMC differentiation marker gene SM22 (see the related article beginning on page 1258). Do Sca-1+ cells transdifferentiate into SMC-like cells, or is activation of SMC marker genes a consequence of fusion of these cells with preexisting SMCs, a possibility raised by results of studies of adult stem cells in animal models of liver regeneration (see the related article beginning on page 1266)? Or could this be bona fide transdifferentiation that recapitulates the pathologic processes in humans?

The prevailing theory of smooth muscle cell (SMC) contribution to vessel lesions is that in pathological states, such as injury and atherosclerosis, SMCs migrate to the intima from the media of the vessel (1). This theory, which has persisted for three decades, is now being challenged by results from models of vessel injury, transplant arteriosclerosis (TA) models, and human allograft studies indicating that a portion of the cells bearing SMC differentiation markers in intimal lesions may have originated from the hematopoietic system and/or circulating progenitor cells (2–5). However, these studies show variable contributions of marrow-derived cells to lesions, with increased frequency correlated to severity of medial injury or to degree of donor/allograft mismatch (6). It appears that only with necrosis of medial SMCs are bone marrow cell (BMC) investment frequencies extremely high (7), indicating that the marrow is not solely responsible for populating the intimal lesion but may represent a default pathway for SMC regeneration and vessel repair in circumstances of severe vessel wall damage.

Two seminal studies by Hu et al. provide new insights into the origin of cells populating the developing neointima in a mouse model of TA (8, 9). Using a combination of bone marrow reconstitution and vein allografts (Figure 1), they demonstrated that donor BMCs give rise to neointimal cells and smooth muscle (SM) α-actin–positive cells (2, 3), although evidence for this is somewhat controversial due to the lack of high-resolution confocal microscopic analyses showing definitive colocalization of bone marrow lineage marker genes and SM α-actin immunostaining (6). However, bone marrow reconstitution studies using donor bone marrow from SM22 promoter-LacZ–transgenic mice have shown that donor BMCs do not give rise to SM22-expressing neointimal cells (2, 3) — a surprising result, since SM22 is an early SMC differentiation marker gene (6). Donor vein graft cells also do not contribute SM22-positive neointimal cells, but host vessel–derived cells can give rise to the population of SM22-positive graft neointimal cells. However, it must be noted that the failure of donor vein graft cells to contribute to the intima appears to be unique to this allograft model, wherein there is virtual destruction of all donor vessel cells, including medial SMCs. Thus, neointimal cells that express SM22 do not appear to be bone marrow–derived but rather to have some other host-based source.

In this issue of the JCI, Hu and colleagues hypothesize that in addition to circulating progenitor cells, Sca-1+ progenitor cells that reside in the adventitia may transdifferentiate into SMC-like neointimal cells (10). These results provide fresh arguments in a key controversy in adult stem cell and progenitor cell research — i.e., that of defining the relative roles of cell fusion versus transdifferentiation.

The role of adult stem cell contribution to tissues has recently undergone a revolution and counterrevolution. Initial optimism about transdifferentiation of neuronal stem cells (11), hematopoietic stem cells (HSCs) (12), and other adult stem cell types has diminished in the face of reports of cell fusion (13) and minimal adult stem cell plasticity (14). Surprisingly, the pair of papers that began the furor over cell fusion throughout the adult stem cell field had little relevance to most cell types being studied. Terada et al. and Ying et al. demonstrated rare cell fusion events when embryonic stem cells are extensively cocultured with BMCs or neural cells, respectively (15, 16). These results, however, did lead to a healthy skepticism about the transdifferentiation potential of adult stem cells. Janus et al. developed a Cre-lox model to specifically test for fusion in their cell systems, and found none, despite the apparent transdifferentiation of BMCs into insulin-secreting pancreatic β cells (17). Some groups examining BMC transdifferentiation into hepatocytes found that their previous results were mainly due to cell fusion (13). Still others, alerted to the fusion problem, found cell fusion occurred in their systems, but at too low a frequency to account for their transdifferentiation results (18, 19).

Thus, a confusing picture has emerged for most scientists curious about the role of adult stem cells in human disease. We are left with several questions: Is transdifferentiation real or just an artefact? Is fusion a problem or is it a potential physiological mechanism to exploit? Are some tissues more susceptible to fusion than others? The liver, for instance, has proven to be highly susceptible to fusion, but, interestingly, in the fumarylacetoacetate hydrolase−/− mouse models studied, fusion of HSCs to hepatocytes corrects the enzymatic deficiency, raising the possibility that this phenomenon has therapeutic applications (12). Of note in this issue of the JCI is the work of Camargo et al., who explored the fusibility of hepatocytes with HSCs and discovered that it is specifically the myeloid lineage of these cells, rather than the stem cells themselves, that is responsible for fusion, thus raising the intriguing possibility of “fusion therapy” (20). These groundbreaking new results also emphasize the importance of understanding fusion and transdifferentiation, as they appear to play a potentially important role in several dis-
ease states that biomedical scientists have been trying to understand for decades.

Adding to the controversy over the true transdifferentiation potential of adult stem cells are the exaggerated claims made by both the scientific and the lay press regarding the relative merits of adult versus embryonic stem cells and their potential for therapeutic applications. Both avenues of research would be better served by more accurate portrayals of their science in the press, which might help to lessen the impact of partisan politics on serious consideration of the merits of either stem cell type and their potential contributions to science and human therapeutics.

In this issue of the JCI, Hu et al. provide novel evidence that may result in a more coherent picture of different cell contributions to the lesions of TA. Of major interest is the fact that Hu et al. isolated a putative SMC progenitor population from the adventitia of the aortic root with a surface phenotype of Sca-1+/c-kit+/lin− characteristic of HSCs. Further, through a series of lineage tracing studies, they demonstrated that these cells were not of hematopoietic origin, suggesting they are not HSCs that have lodged or fused in the adventitia. In culture, PDGF-BB induces expression of multiple markers of SMC differentiation, and these cells, when purified from an SM22-LacZ/ApoE−/− transgenic mouse and applied to the outside of an irradiated vein allograft, migrate to the neointima of the vessel and express SM22. These results are of interest in that they identify a potential new source of cells that contribute to neointimal formation in TA. However, several outstanding questions remain: Is this an artifact of exogenous application of the Sca-1+ cells? Does it represent fusion of these cells to SMCs? Or does this represent bona fide transdifferentiation of Sca-1+ cells from the adventitia into SMC lineages?

Figure 1
What are the true origins of the SMCs present in the intimal lesions of TA? Hu et al. previously demonstrated that donor BMCs give rise to neointimal cells (I) and that a low percentage stained with smooth muscle α-actin, suggesting transdifferentiation of BMCs to SMCs. (II) However, when SM22-LacZ bone marrow was used for lineage tracing, the neointimal cells were SM22-LacZ negative, suggesting that the BMC-derived SM α-actin-positive intimal cells were some cell type other than smooth muscle (e.g., macrophages or myofibroblasts) or were not fully differentiated SMCs (III). Interestingly, cells from the host vessel (V) but not the donor vessel (IV) can give rise to intimal cells that are SM22-positive. However, it must be noted that the failure of donor vessel cells to contribute to the intima appears to be unique to this allograft model, wherein there is virtual destruction of all donor vessel cells, including medial SMCs. Thus, in this allograft model, a key question is, what is the source of the SM22-positive intimal cells derived from the host vessel? In this issue of the JCI, Hu et al. show that Sca-1+ cells purified from the adventitia of the aortic root (VI) can migrate through an irradiated vein graft to the neointima of the vessel and express SM22. These results are of interest in that they identify a potential new source of cells that contribute to neointimal formation in TA. However, several outstanding questions remain: Is this an artifact of exogenous application of the Sca-1+ cells? Does it represent fusion of these cells to SMCs? Or does this represent bona fide transdifferentiation of Sca-1+ cells from the adventitia into SMC lineages?

to identify the physiological relevance of these cells and eliminate two limitations of this study. First, application of exogenous Sca-1+ cells to an irradiated vein graft represents an artificial situation in which migration may have been an experimental artifact due to lack of medial SMC contribution. To determine if such migration is physiologically relevant, a Sca-1 promoter-Cre mouse could be crossed to a ROSA-stoplox-LacZ/ApoE−/− mouse to determine if Sca-1 was ever activated in neointimal SMCs. Second, fusion of Sca-1+ cells with cells from another source, such as irradiated SMCs of the media, must be eliminated, although other groups suggest that this is an unlikely eventuality (19). A Cre-lox fusion detection system (17) could be used to quell concerns that Sca-1+ cells adopt the SMC phenotype by fusing to other SMCs rather than by transdifferentiating.
From all studies so far we are still left with the fundamental question: Do non-SMCs from the adventitia, host vessel, or bone marrow transdifferentiate to become bona fide SMCs? It is possible that these cells are expressing only a few SMC differentiation markers and/or still expressing non-SMC genes. Finally, and most importantly, we ask, are the results in these animal models relevant to the development of atherosclerosis in humans? Can these same cells be identified in humans by Sca-1 antigen? The novel and exciting studies by Hu et al. in this issue of the JCI implicate potential contributors by a putative adventitial progenitor cell population in TA and might open yet another chapter in the long quest to define origins of cells in intimal lesions and their mechanistic contributions to the pathogenesis of atherosclerosis.

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Anemia of inflammation: the cytokine-hepcidin link

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The anemia of inflammation, commonly observed in patients with chronic infections, malignancy, trauma, and inflammatory disorders, is a well-known clinical entity. Until recently, we understood little about its pathogenesis. It now appears that the inflammatory cytokine IL-6 induces production of hepcidin, an iron-regulatory hormone that may be responsible for most or all of the features of this disorder (see the related article beginning on page 1271).

In 1932, Locke et al. made the important observation that infection was associated with hypoferremia (low serum iron), providing a partial explanation for the common finding of anemia in patients with chronic infections (1). Cartwright and Wintrobe went on to show that the anemia associated with infection was indistinguishable from the anemia of inflammation, and established that hypoferremia resulted from reticuloendothelial sequestration of iron and interruption of intestinal iron absorption (2, 3). Cartwright and Lee recognized that similar findings could be induced in mice by exposure to bacterial endotoxin (4). Others correlated the anemia of inflammation with elaboration of inflammatory cytokines, and ascribed changes in iron metabolism to the effects of these cytokines (5). Cytokines have been shown to modulate the expression of iron transport and storage proteins (6), but it was not clear that these changes accounted for the abnormalities of iron homeostasis observed in the anemia of inflammation.

The roles of hepcidin and IL-6

Over the past two years, a variety of experiments have converged to establish a role for hepcidin, a liver-derived peptide regulator of iron homeostasis, as a key mediator of hypoferremia in inflammation (7–9). In an elegant report in this issue of the JCI, Nemeth, Rivera, and colleagues have elucidated an important link between inflammatory cytokines and hepcidin (10). Using both mice and humans as experimental models, they have shown that IL-6 acts directly on hepatocytes to stimulate hepcidin production. Hepcidin, in turn, acts as a negative regulator of intestinal iron absorption and macrophage iron release.

In previous work Nemeth, Rivera, and colleagues showed that IL-6 induced hepcidin expression in hepatic cells (9).
Here, they have replicated this effect using conditioned medium from endotoxin-treated macrophages and shown that a neutralizing antibody against IL-6 blocked hepcidin induction (10). Other inflammatory cytokines did not stimulate hepcidin production; in fact, TNF-α inhibited it.

Cytokines have all sorts of effects on cultured cells, and it was important to show that IL-6 induction of hepcidin occurred in vivo and triggered hypoferremia, as predicted. First, Nemeth, Rivera, et al. used turpentine injection to cause inflammatory abscesses in wild-type and IL-6 knockout mice and analyzed the responses (10). Wild-type mice had increased hepcidin expression and a substantial decrease in serum iron levels. In contrast, IL-6 knockout mice had no increase in hepcidin expression and no decrease in serum iron. A complementary experiment carried out in human volunteers showed that IL-6 infusion stimulated urinary hepcidin excretion within 2 hours and induced hypoferremia. Taken together, these data provide strong support for the conclusions that IL-6 is a primary inducer of hepcidin expression and that increased hepcidin expression results in hypoferremia (Figure 1). This is gratifyingly consistent with clinical observations that hypoferremia occurs very quickly after the onset of inflammation.

Earlier studies had shown that rodents with induced iron overload also had increased hepcidin expression (11, 12), presumably to try to compensate for iron excess. However, the signal to increase hepcidin expression was unknown. Here, Nemeth, Rivera, and colleagues have shown that IL-6 is not involved in the regulation of hepcidin in response to iron (10). Furthermore, their data suggest that hepcidin levels are not simply responding to increased iron stores. In human volunteers, urinary hepcidin levels were boosted soon after a single dose of oral iron, which should have no significant effect on iron stores. Perhaps the serum iron level, known to increase transiently after iron ingestion, might itself be the signal to induce hepcidin expression. Alternatively, the signal might relate to the degree of iron saturation of serum transferrin.

However, if transferrin iron saturation modulates hepcidin expression, other signals can clearly override its effects. The IL-6–mediated inflammatory induction of hepcidin does not appear to be offset by the hypoferremia it causes, at least in the short term. And mice with thalassemia intermedia (which presumably have elevated serum iron) have decreased hepcidin expression (13), as occurs in other mouse models with increased erythroid iron demand (7, 8).

In my opinion, this report from Nemeth, Rivera, et al. (10) leaves little room for doubt about the importance of hepcidin in the pathogenesis of anemia of inflammation. This was challenged by a recent report that concluded that elevated serum hepcidin levels were not useful in the diagnosis of the anemia of inflammation (14). However, that study did not provide data to support the authors’ contention that they had developed a sensitive, specific test for serum hepcidin. Furthermore, as they also pointed out, it was not clear that serum measurements were as useful as urinary hepcidin measurements. Hepcidin gene expression seems to be exquisitely sensitive to regulation, and the circulating peptide is small enough to be quantitatively filtered by the kidneys. Urine samples probably provide a better indication of recent hepcidin expression than individual serum samples.

A possible treatment?

If inflammatory induction of hepcidin causes hypoferremia, it is logical to predict that inhibition of hepcidin expression or activity would ameliorate the anemia of inflammation. Would that be advantageous? Perhaps, particularly in noninfectious inflammatory disorders. We know that patients (15) and mice (16) lacking hepcidin have increased intestinal iron absorption and increased serum iron, but this is unlikely to be harmful in the short term. However, there may be more cause for concern in patients with infections or malignancy. Decreased serum iron is believed to contribute to host defense against invading pathogens and tumor cells (17), and hepcidin itself has antimicrobial properties of uncertain importance (18). If hepcidin antagonists become available, careful clinical trials will be required to define appropriate indications for their use.

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in liver and intestine implicate abnormal hepcidin and Cybrid I expression in mouse hemochromato-
mRNA expression in thalassemic mice. Br. J. Haem-

**Hold the antioxidants and improve plasma lipids?**

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Intrahepatic proteolysis is a major determinant of secretion of ApoB-contain-
ting lipoproteins into plasma. Stimulation of post-ER presecretory
proteolysis (PERPP) of ApoB by n-3 polyunsaturated fatty acids has been
found to result in reduced secretion of VLDL particles by hepatocytes. A new
study has shown that this stimulation is promoted by pro-oxidant condi-
tions that result in increased hepatic lipid hydroperoxide content (see the
related article beginning on page 1277). Conversely, PERPP is suppressed by
antioxidants and by saturated fatty acids, which are not susceptible to lipid
peroxidation. Hence reduction of oxidative stress may have the unexpected
side effect of increasing plasma lipid levels.

Dietary fats with differing fatty acid com-
position can influence plasma lipid levels
by modulating hepatic production and
clearance of lipoproteins (1), as well as by
altering activity of choleseterol ester transfer
protein (2). Suppression and stimulation of
hepatic LDL receptor activity are major
determinants, respectively, of the effects of
saturated and polyunsaturated fatty acids
on plasma LDL clearance (1), but the mech-
anisms for effects of specific fatty acids on
hepatic lipoprotein production are less well
understood. This is in large part due to the
multiple influences of fatty acids on pro-
cesses that regulate hepatic lipid produc-
tion and storage, and processing of ApoB
in conjunction with lipoprotein synthesis and
secretion (3).

Fatty acids are critically involved in
hepatic lipoprotein production pathways
that help maintain hepatic cholesterol
homeostasis and the ability to respond
to energy and other metabolic needs. Tri-
glycerides influence a critical early step in
secretion of ApoB-containing lipoproteins,
namely the cotranslational binding of lip-
ids to ApoB in the ER mediated by micro-

Nonstandard abbreviations used: ER-associated deg-
radation (ERAD); microsomal triglyceride transfer pro-
tein (MTP); post-ER presecretory proteolysis (PERPP);
thiobarbituric acid–reactive substance (TBARS).

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Serum hepcidin in clinical specimens. Br. J. Haema-
tol. 122:996–1000.
peptide hepcidin is associated with severe juvenile
expression and severe tissue iron overload in
upstream stimulatory factor 2 (USF2) knockout
2001. Hepcidin, a urinary antimicrobial peptide syn-

Oxidative stress and modulation of pathways for hepatic
lipoprotein secretion

The direct relation of the number of unsaturated bonds to lipid peroxidation
is consistent with the lack of a suppressive effect of saturated fatty acids on plasma
triglyceride levels. In contrast, a saturated fatty acid, myristic acid, was found to
stimulate secretion of ApoB-containing

Post-ER presecretory proteolysis and
hepatic lipid hydroperoxide content

Recently, Fisher et al. have identified
another degradative process that can mod-
ulate hepatic secretion of more mature
ApoB-containing lipoproteins (4). They
have found that inhibition of hepatic
ApoB secretion by n-3 polyunsaturated fatty
acids occurs via activation of this
process, which has been designated post-
ER presecretory proteolysis (PERPP) (4).
In this issue of the JCI, Pan, Fisher, and
colleagues have now shown, using several
lines of evidence, that this effect is medi-
ated by fatty acid peroxidation, and that it
also occurs with n-6 polyunsaturated fatty
acids (5). Moreover, the finding that ApoB
degradation is stimulated by pro-oxidant
conditions and inhibited by antioxidants
raises the question of whether variation in
intrahepatic oxidative stress contributes to
physiologic and/or pathologic modulation
of ApoB-containing lipoprotein metabo-
lism. There is indeed abundant evidence
that consumption of supplements of lon-
ger-chain n-3 marine-derived polyunsatu-
rated fatty acids (eicosapentaenoic and
docosahexanoic acids) can lower plasma
triglyceride and VLDL levels in humans
(6). Recently, it has been shown that popu-
lation variation in dietary intake of a plant-
derived n-3 polyunsaturated fatty acid,
linolenic acid, is significantly associated
with plasma triglyceride levels, indepen-
dent of other nutrients, including longer-
chain n-3 polyunsaturated fatty acids (7).
As Pan et al. point out, however, in their
study linolenic acid suppressed ApoB secre-
tion from rat hepatoma cells to a greater
extent than could be accounted for by the
relation of secretion to intrahepatic lipid
hydroperoxide content as assessed by levels
of thiobarbituric acid–reactive substances
(TBARSs) (5). It should be noted, howev-
er, that TBARSs do not represent the full
spectrum of products of lipid peroxidation,
such as F2-isoprostanes. Moreover, despite
the capacity for peroxidation of both n-3
and n-6 polyunsaturated fatty acids, the
latter have minimal and generally non-
significant effects on plasma triglyceride
levels in humans (8). Hence increased lipid
peroxidation of n-3 fatty acids does not
fully explain the effects of these fatty acids
on hepatic ApoB-containing lipoprotein
secretion. Other effects, such as reduced
lipid synthesis, may also play a role.

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1253
Lipoproteins from liver cells in conjunction with protection from a proteolytic process consistent with PERPP (9). Interestingly, the secreted lipoproteins under these conditions were denser than VLDLs and were more triglyceride-depleted than those secreted in the presence of albumin or oleic acid. This suggests that differences in conformation or composition of newly synthesized ApoB-containing lipoproteins can alter their exposure to PERPP in such a way that smaller, triglyceride-depleted particles formed under the influence of saturated fatty acids (SFAs) protect the smaller particles from PERPP, leading to increased secretion. Peroxidation products of n-3 polyunsaturated fatty acids (n-3 PUFA-ox), and perhaps also n-6 polyunsaturated fatty acids (not shown), increase PERPP, leading to decreased secretion of larger VLDLs and hence lower levels of their catabolic products, including small LDL particles.

Many questions remain as to the nature and physiologic importance of the PERPP system, the properties of lipoproteins that affect their susceptibility to PERPP, the mechanisms by which dietary fatty acids can affect this susceptibility, and the means by which oxidation products can suppress PERPP. It will be particularly challenging to assess the extent to which these mechanisms may influence metabolism of ApoB-containing lipoproteins in humans. However, clues may be found in evidence that levels and distribution of LDL subspecies can serve as markers for intrahepatic pathways that result in formation of different hepatic secretory products (10) (Figure 1).

We have found that increased intake of saturated fatty acids is associated with higher levels of larger, more buoyant LDL particles (11). The concentrations of large, buoyant LDL particles are inversely related to plasma VLDL levels (12). Lipoprotein kinetic studies in humans have indicated that reduced plasma triglyceride and VLDL levels are correlated with the extent of direct secretion of IDLs and LDLs into plasma (13). Hence, one might surmise (Figure 1) that increased intake of cholesterol-raising saturated fatty acids increases direct hepatic secretion of IDLs and large LDLs, a process consistent with the evi-
Dysbindin-1 and schizophrenia: from genetics to neuropathology

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The gene encoding dysbindin-1 has recently been implicated in susceptibility to schizophrenia. In this issue of the JCI, Talbot et al. show that, contrary to expectations, dysbindin-1 is located presynaptically in glutamatergic neurons and is reduced at these locations in schizophrenia (see the related article beginning on page 1353). Further studies of dysbindin-1 and the proteins with which it interacts can be expected to throw light on the pathogenesis of schizophrenia.

Nonstandard abbreviations used: hippocampal formation (HF).

Conflict of interest: The authors have declared that no conflict of interest exists.

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Schizophrenia is a common, severely disabling, mental disorder (1), and understanding its etiology and pathogenesis is one of the most important challenges facing psychiatry. Despite great endeavor, achieving this has proven difficult given the absence of a diagnostic neuropathology or biological markers, and few clear insights have emerged. However, there is currently a consensus that the disorder is, at least in part, neurodevelopmental (2). At the structural level, there are reductions in the neocortex and neuronal size that are widespread but not uniform, with temporal lobe structures, particularly affected (3). These changes in turn probably result from alterations in synaptic, dendritic, and axonal organization (3). At the functional level, accumulating evidence also implicates altered glutamate
regulator of G protein signalling 4 (RGS4), present in glutamate synaptic vesicles, regulates expression of synthesis, or degradation and is a component of the postsynaptic density (PSD). NRG1 is present in glutamate synaptic vesicles, regulates expression of N-methyl-D-aspartate receptors (NMDARs), activates ErbB4 receptors, which colocalize with NMDARs, and interacts with the PSD. G72 interacts with DAAO, which oxidizes D-serine, an endogenous modulator of NMDARs. RGS4 is a negative regulator of G protein–coupled receptors, especially the metabotropic glutamate receptor 5 (mGluR5), via its effects on the G protein Gα. Figure modified with permission from The Lancet (7).

Figure 1
Schematic representation of a glutamatergic synapse. Several genes have been implicated in susceptibility to schizophrenia that can potentially impact on glutamate (Glu) synaptic function including dysbindin-1, neuregulin 1 (NRG1), G72, D-amino acid oxidase (DAAO), and regulator of G protein signalling 4 (RGS4). Dysbindin-1 may influence VGlut T-1 expression, synthesis, or degradation and is a component of the postsynaptic density (PSD). NRG1 is present in glutamate synaptic vesicles, regulates expression of N-methyl-D-aspartate receptors (NMDARs), activates ErbB4 receptors, which colocalize with NMDARs, and interacts with the PSD. G72 interacts with DAAO, which oxidizes D-serine, an endogenous modulator of NMDARs. RGS4 is a negative regulator of G protein–coupled receptors, especially the metabotropic glutamate receptor 5 (mGluR5), via its effects on the G protein Gα. Figure modified with permission from The Lancet (7).

Presynaptic reductions in dysbindin-1 in schizophrenia
In this issue of the JCI, Talbot and colleagues have tested this hypothesis by examining dysbindin-1 protein levels in human brain (14). Their findings provide several novel and important insights. First, while they confirmed fairly widespread neuronal distribution of dysbindin-1 and β-dystrobrevin, they also showed presynaptic localization of dysbindin-1 but not β-dystrobrevin, suggesting that dysbindin-1 plays a presynaptic role in the HF that is independent of β-dystrobrevin and the dystrophin glycoprotein complex. Second, they found high levels of dysbindin-1 in the cells providing the intrinsic glutamatergic pathways of the HF and an inverse correlation with VGlut T-1, the main vesicular glutamate transporter present in the HF. This supports a relationship between dysbindin-1 and glutamate neurotransmission and suggests an effect on VGlut T-1 expression, synthesis, or degradation in the HF. Third, they found a significant reduction in dysbindin-1 expression at these hippocampal presynaptic sites in two separate populations of patients with schizophrenia. If correct, these findings suggest that, in the HF at least, dysbindin-1 may influence schizophrenia risk through presynaptic mechanisms that are independent of the dystrophin glycoprotein complex.

The authors have tried hard, by careful matching of cases and controls, to minimize the potential impact of many of the possible confounding variables that bedevil neurotransmission in addition to classical hyperdopaminergic explanations (4).

The most robust body of evidence regarding etiology comes from genetic epidemiological studies, which show that individual differences in liability are predominantly genetic, with heritability estimates around 80% (5). The most common mode of transmission is probably oligogenic, polygenic, or a mixture of the two (5), but the number of loci, the disease risk conferred by each, the extent of genetic heterogeneity, and the degree of interaction among loci are unknown. As for other common genetic disorders, the small effect on susceptibility conferred by any given locus makes identifying susceptibility genes by positional genetics difficult. However, as sample sizes and hence power have increased, convincing linkages to a number of chromosomal regions have emerged (6). Moreover, systematic examinations of several of these linkage regions have produced replicated evidence implicating specific schizophrenia susceptibility genes (6, 7).

Evidence that DTNBP1 is a susceptibility gene for schizophrenia
Currently, the best-supported susceptibility gene is DTNBP1 (e.g., refs. 8–10), which encodes dysbindin-1 and is located within chromosome 6p22.3. Dysbindin-1 is a 40–50 kDa protein that binds both α- and β-dystrobrevin, which are components of the dystrophin glycoprotein complex. The possibility is indirectly supported by evidence for as yet unknown cis-acting polymorphisms affecting DTNBP1 expression in human brain (13).
studies of postmortem brain. Most patients with schizophrenia have taken psychotropic medication for many years, and postmortem material from drug-naive patients is extremely difficult to obtain. Talbot and colleagues showed that dysbindin-1 and VGLUT1-T1 levels were not correlated with antipsychotic drug dose in the month before death and provide evidence against an effect of chronic drug treatment from studies of chronic haloperidol administration to mice (14). However, as the authors acknowledge, the validity of drug-treated mice as a control is open to question, and also no mention is made of matching of cases and controls for agonal state. Given the difficulties confirming previous postmortem findings in schizophrenia, it is now imperative that multiple independent replications be obtained. From the genetic perspective, it would also strengthen the mechanistic case of Talbot and colleagues if disease-associated single nucleotide polymorphisms or haplotypes can be linked to effects on gene expression. Here, ethnic variation in risk haplotypes and possible allelic heterogeneity within populations might prove problematic.

**Dysbindin-1 and the pathogenesis of schizophrenia**

These findings raise a number of important issues. First, what is the presynaptic function of dysbindin-1? A possible clue comes from recent evidence implicating it as a member of a protein complex involved in the trafficking of lysosome-related organelles in association with the proteins mup1, pallidin, and cappuccino (15). As Talbot et al. point out, lysosomes are found presynaptically in the HF, and reduced lysosomal trafficking might result in elevated VGLUT1-T1 (14). However, further studies are required to identify dysbindin-1 interactors and to understand the control of DTNBP1 expression in brain. In addition, analysis of the mouse mutant sandy (ady), which, in its homozygous form, expresses no dysbindin-1 protein due to a large deletion in *Dtnbp1*, and of conditional knockouts might also be expected to throw light upon the function of dysbindin-1 in brain. Second, Talbot and colleagues studied a restricted number of brain regions, particularly the HF. Dysbindin-1 is widely expressed in brain (11), and many lines of evidence suggest widespread involvement of different brain areas in the structural and functional abnormalities of schizophrenia (3). There is now a need to determine whether reduced dysbindin-1 levels are found in other brain areas and whether the association with glutamate is generalized or specific to the relatively restricted population of hippocampal regions implicated by Talbot and colleagues. Interestingly, Talbot and colleagues report regional variation in the distribution of the two dysbindin isoforms studied: the HF and cerebral cortex contained both 50 and 40 kDa isoforms while the cerebellar cortex contained only the 50 kDa variant, implying that there are differences in their transcriptional control.

The identification of a potential role for dysbindin-1 in glutamate transmission is of considerable interest. Evidence for glutamate dysfunction in schizophrenia is accumulating from both basic and clinical research (4). Moreover, a case can be made that other susceptibility genes identified on the basis of positional genetic studies, such as those encoding Neuregulin 1, G72, D-amino acid oxidase, and regulator of G protein signalling 4, may have convergent effects upon glutamate synapses (Figure 1), although the links are speculative and several caveats remain (7).

Finally, if these findings are confirmed, they will indicate the potential power of positional genetics to home in on novel mechanisms. There are other linkages in schizophrenia that are as strong as that to 6p22.3, indicating the location of other susceptibility genes (6). On the basis of Talbot and colleagues’ work (14) we can expect their identification to yield further crucial advances.

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