fragments of polyglutamine-containing huntingtin protein or amyotrophic lateral sclerosis–causing mutants in the transactivation element (TAR) DNA binding protein–43 (known as TDP-43) also inhibit mRNA export when expressed in cultured cells, suggesting that errors in nucleocytoplasmic transport may be common to multiple neurological conditions. That said, expression of mutant huntingtin in primary cultures of cortical neurons led preferentially to nuclear aggregation, which did not impair nuclear mRNA export. This is consistent with evidence that intranuclear inclusions of polyglutamine-containing huntingtin fragments are not toxic per se (*5*). In the widely used R6/2 Huntington's disease mouse model in which aggregates of a mutant huntingtin fragment accumulate intranuclearly in most neurons, Woerner *et al.* report impaired RNA export in the small proportion of neurons that accumulate aggregated huntingtin in the cytoplasm. These findings, and the consensus from analyses of human samples and most mouse models, raise the question of whether the much rarer cytoplasmic aggregates are primary contributors to toxicity in Huntington's disease, rather than the more abundant intranuclear ones.

The finding by Woerner *et al.* that cytoplasmic aggregates diminish nuclear import and/or export adds to the growing recognition that diminished nucleocytoplasmic transport may be a common component of multiple human neurodegenerative diseases, including Huntington's (*6*), Alzheimer's, amyotrophic lateral sclerosis, frontotemporal dementia, and Parkinson's, where components of the import and/or export machinery are mislocalized and found to interact with disease-associated mutant proteins. Coupled with nuclear "leakiness" that dramatically accelerates during aging (*7*), altered cytoplasmic localization offers one explanation for the age-dependence of these neurodegenerative disorders.

How nuclear import-export is inhibited in the various diseases is still unclear. Recently, expression of a hexanucleotide expansion within the *C9orf72* gene, which is the most frequently inherited cause of both amyotrophic lateral sclerosis and frontotemporal dementia, has been reported to disrupt nuclear import and/or export (*8*–*10*), but how this defect arises is not firmly established. One study identified a direct interaction between the hexanucleotide repeat–containing RNAs and Ran GTPase-activating protein (Ran-GAP), a factor required for nuclear import (*10*). Other studies implicated import inhibition by repeat associated non-AUG (referred to as RAN)–dependent translation-produced polydipeptides encoded by expansion-containing RNAs (*8*, *9*).

To this controversy, Woerner *et al.* demonstrate that nuclear and/or cytoplasmic transport defects can be attributed to a proteotoxicity caused by cytoplasmic accumulation of β-sheet proteins and their aggregates. Additionally, the recent finding that RAN translation is not restricted to diseases with noncoding region repeat expansions, but also occurs across repeats located in an open reading frame such as in Huntington's disease (*11*), provides a new perspective on potential mechanisms underlying toxicity in this disorder. A critical next step will be to determine whether RAN-encoded peptides can directly provoke nucleocytoplasmic transport defects previously reported in Huntington's disease (*6*), and whether there is compartment-selective toxicity, as now demonstrated for the β-sheet proteins.

To the unresolved, key question of how cytoplasmic accumulation of aberrant proteins and/or their aggregation provokes diminished nuclear import and/or export, it must be noted that the focus in Alzheimer's disease (*12*), Huntington's disease (*5*, *13*), and most recently in inherited amyotrophic lateral sclerosis (*14*), has reversed. An initial focus was on the large aggregates seen with conventional pathology. Most investigators have refocused on oligomeric assemblies of the misfolded protein as the most important contributors to neuronal dysfunction that leads to the characteristic disease symptoms (*15*). Seen from this prospective, location definitely matters, but the β-sheet protein aggregates (and other aggregates in the various disorders) may actually be protective, with toxicity arising from oligomeric species that are hard to detect. ■

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10.1126/science.aad9872

STEM CELLS

Potency finds its niches

Adult bone marrow employs a surprisingly simple hematopoietic hierarchy

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Hematopoietic stem cells (HSCs) located atop the hematopoietic hierarchy are responsible for the lifelong production of all mature blood cells. These partly dormant, long-term self-renewing, multipotent HSCs in the bone marrow generate multipotent progenitors (MPPs) with reduced self-renewal activity before lineage determination and differentiation are initiated (*1*, *2*). For decades, it was thought that MPPs lose their multipotent capacity in a stepwise fashion, generating first a series of oligopotent and from there unipotent progenitors to finally make all hematopoietic cell types. The presence of oligopotent intermediates such as common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs) is crucial to this model because they define the path from multipotent to unipotent cells. A study by Notta *et al.* on page 139 of this issue (*3*) and another study by Paul *et al.* (*4*) provide strong evidence for the nonexistence of CMPs in human and mouse bone marrow. And on page 176 of this issue, Khan *et al.* (*5*) report a niche population in the fetal liver that maintains proliferating HSCs.

> ***"...fetal liver pericytes... promote FL-HSC cycling without differentiation..."***

In the standard textbook model, all myeloid cells (erythroid cells, megakaryocytes, granulocytes, and monocytes) are derived from oligopotent CMPs. This concept is based on the prospective isolation of CMPs by flow cytometry and their potential to generate all myeloid cell types in vitro and

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in vivo (*6*). However, because these analyses were performed at the population level, it remained possible that CMPs may contain several unipotent progenitors rather than one oligopotent population.

Notta *et al.* identified 11 distinct cell populations in human fetal liver, cord blood, and adult bone marrow by fluorescence-activated cell sorting using conventional and novel markers (cMPL/BAH1 and CD71). This analysis revealed substantial heterogeneity in MPPs, CMPs, and megakaryocyte-erythroid progenitors (MEPs). The authors then used an improved functional single-cell culture assay to address the potential of each population to give rise to granulocytes, monocytes, megakaryocytes, and erythroid cells. Evaluation of nearly 3000 single cells suggests that unipotent progenitors, rather than oligopotent progenitors such as CMPs, dominate the blood hierarchy during bone marrow hematopoiesis. By assessing the reconstitution potential of the new cell populations in mice, Notta *et al.* found that megakaryocytes were derived directly from HSCs or MPPs, thereby obviating a lineage route via oligopotent CMP and MEP intermediates. This agrees with mouse data demonstrating the presence of stem cell–like megakaryocyte progenitors within the HSC compartment, which rapidly generate new platelets in response to stress (*7*, *8*).

Paul *et al.* (*4*) used single-cell transcriptomic analysis of more than 2700 mouse myeloid progenitors. They clustered each cell according to its expression of 3461 genes, revealing 19 progenitor subpopulations displaying a homogeneous expression signature. Using known lineage-specific gene networks, the authors found that each of the identified myeloid progenitor subpopulations was transcriptionally primed toward one of at least seven myeloid fates. For example, isolation of classically defined MEPs led to a homogeneous erythrocyte progenitor spectrum with no significant megakaryocyte expression signature. This agrees with the idea that these are instead derived from HSCs or MPPs (*3*). Single-cell transcriptome analysis failed to detect cells such as CMPs that express multiple lineage-specific genes or transcription factors that promote different fates (*3*, *4*), in line with recent studies (*9*, *10*).

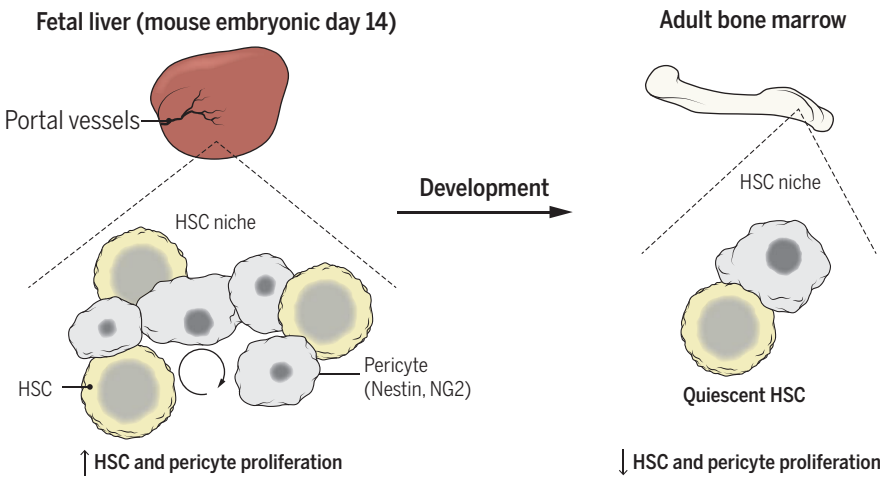
The findings of Notta *et al.* and Paul *et al.* suggest that either CMPs represent a highly transient cell state, or that such oligopotent cells do not exist, at least in mouse or human bone marrow. Instead, Notta *et al.* suggest a simpler two-tier model for the human bone marrow: a top tier containing multipotent cells such as HSCs or MPPs, and a bottom tier composed of committed unipotent progenitors. However, the progenitors identified during homeostasis appear to give rise to only one lineage on the basis of their expression signature, whereas transplanted megakaryocyte-erythroid progenitors reconstituted megakaryocytes and erythroid cells in mice (*4*). Thus, the potential of stem cells or their progenitors may be much broader in a stressed environment compared to what they actually do in the unperturbed system.

Whether an adult HSC remains dormant, self-renews, or differentiates toward a specific lineage is regulated in conjunction with its microenvironment (niche). Several distinct niches exist in the adult bone marrow, including cell types such as arteriolar pericytes [expressing Nestin and neural/glial antigen 2 (NG2)], leptin receptor–expressing pericytes, and megakaryocytes (*11*, *12*). During hematopoiesis, HSCs in the fetal liver (FL-HSCs) are highly proliferative, and in contrast to the ones in the bone marrow, lineage differentiation arises from them via oligopotent progenitors (*4*). However, the niche cells that promote FL-HSC expansion during embryonic and fetal growth were unknown.

Khan *et al.* report that murine portal vessels in the fetal liver also contain Nestin- and NG2-expressing pericytes (see the figure). These cells sustain the highly proliferative FL-HSCs (*5*). The authors found that FL-HSCs are located in close proximity to the pericytes and that these niche cells, rather than other fetal liver components, are sufficient to maintain FL-HSCs in vitro. Khan *et al.* also show that deletion of NG2-expressing cells (98% of which are Nestin-expressing pericytes) in mice reduces the numbers and proliferative status of FL-HSCs.

Although Khan *et al.* demonstrate the importance of Nestin-expressing pericytes for maintaining proliferative FL-HSCs, similar cells located in the bone marrow maintain HSC quiescence (*11*). To uncover this difference, Khan *et al.* performed comparative transcriptome analysis, revealing no differential expression of stem cell–maintaining signaling molecules. Instead, there was enrichment for cell cycle processes and vessel development in fetal liver pericytes, which suggests that niche and FL-HSC expansion are linked.

Why do FL-HSCs leave this environment in the liver around the moment of birth? Khan *et al.* show in mice that ligation of the umbilical inlet at birth causes drastic hemodynamic changes in portal vessel blood flow, leading to loss of Nestin-expressing pericytes and consequent disruption of the portal vessel stem cell niche. Consequently, hematopoiesis is driven from the liver into adult bone marrow niches, which subsequently promote a dormant HSC state in the adult organism (*1*). The potential of fetal liver pericytes to promote FL-HSC cycling without differentiation may be used to develop complex niches in the culture dish for clinical applications, such as the expansion of HSCs derived from cord blood. ■



From one niche to another. HSCs and niche pericytes jointly expand in the fetal liver. At birth, the fetal liver niche collapses. HSCs then move to the bone marrow, where niche pericytes and HSCs become quiescent.

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10.1126/science.aae0325

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Science **351** (6269), 126-127.
DOI: 10.1126/science.aae0325