

Controlling cellular metal homeostasis. The backbone of cellular metal homeostasis is a flow equilibrium of uptake and efflux reactions. Activity of either transport process seems to be controlled by cytoplasmic concentrations of divalent metal cations (M^{2+}). Solid lines, transport; dashed lines, regulation of transport activity; red, inhibition; green, activation. Uptake (**left**) can be understood from the structures of the Mg^{2+} uptake systems MgtE (4) and CorA (3), which also transport other divalent metal cations. Binding of Mg^{2+} to sites in cytoplasmic domains of either protein (red circles) closes the gate and prevents cation uptake at sufficient cytoplasmic concentrations (red dashed line with crosses). Similarly, the Zn^{2+} efflux gate (**right**) may be opened through binding of Zn^{2+} to sites Z2, Z3, and Z4 (green circles) in the cytoplasmic domain of YiiP (1). Thus, superfluous cytoplasmic Zn^{2+} concentrations may stimulate efflux (green dashed line ending with "+").

tems (9). Second, Zn^{2+} is taken up into most cells by highly specific Zn^{2+} uptake systems that are expressed under conditions of zinc starvation. However, other uptake systems (such as Mg^{2+} uptake systems) also transport Zn^{2+} nonspecifically. Because these nonspecific systems are regulated by their specific substrate, but not by Zn^{2+} , this may lead to superfluous zinc concentration in the cytoplasm, necessitating activated Zn^{2+} efflux.

These nonspecific uptake systems import a range of divalent metal cations until the concentration of the main substrate, such as Mg^{2+} , reaches the desired cytoplasmic concentration. Following that, the various efflux systems (for example, CDF proteins and P-type ATPases for Zn^{2+}) select and remove with high specificity all those cations that reach dangerous concen-

trations. Thus, the controlled flow of metal cations through a cellular compartment regulates not only the ambient concentration of one metal cation

but also the composition of the complete set.

The YiiP structure far increases our understanding of CDF proteins and of zinc homeostasis, but many questions remain open. YiiP transports Zn^{2+} in vitro (10), but in vivo (11), its main substrate is Fe^{2+} . Thus, YiiP is the first model not only for a transition metal transporter but also for a bacterial Fe^{2+} efflux system. However, it is currently not known which metal cation binds to which site in vivo and what consequences these binding events have on stabilization of the structure, regulation, or transport.

Answers may come from an unexpected direction. The cytoplasmic domain of YiiP forms a metallochaperone-like structure (1), and interactions between metallochaperones and transport proteins are essential

for cellular copper homeostasis mechanisms (12). No cytoplasmic zinc chaperone is known. Nevertheless, knowledge of the interaction between transport and binding processes may be the key to unraveling the mechanisms by which the cell controls zinc concentrations.

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DEVELOPMENTAL BIOLOGY

Home for the Precious Few

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Cells in multicellular organisms rarely escape the influence of other cell types. This is especially true for stem cells, which reside in tissue-specific niches, or homes. Niche cells regulate fundamental stem cell properties, including long-term survival, proliferation, and the balance between cell divisions that are self-renewing or differentiative. Simply identifying a niche can stimulate

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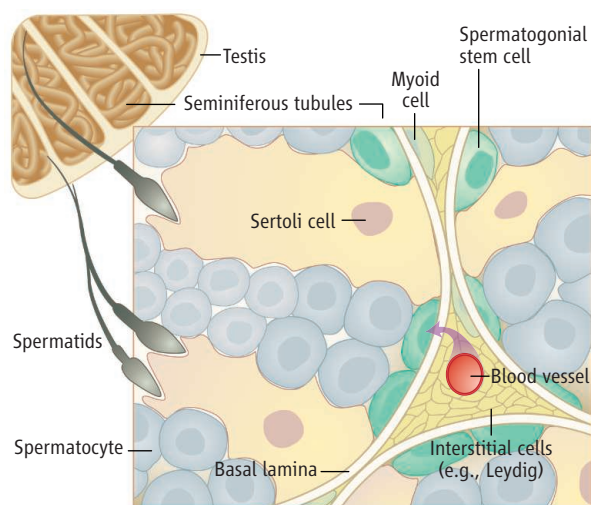
hypotheses about these properties. For instance, the identification of neural stem cells in a vascular niche (1) suggested correctly that co-culture of these stem cells with vascular endothelial cells could affect their potency (2). Similarly, in fruit fly gonads, identifying the germline stem cell niche led to the elucidation of locally acting self-renewal signals, and revealed how oriented cell divisions contribute to stem cell fate (3-6). On page 1722 in this issue, Yoshida *et al.* (7) take an exciting step toward identifying stem cell niches in the mammalian testis.

It was initially surmised that testis stem cells must exist because men make sperm for

decades. Then, in rodent models, it was observed that spermatogenesis would naturally be reestablished after a severe toxic insult. Spermatogonial stem cells were eventually identified when a preparation of cells from a donor mouse testis was shown to repopulate spermatogenesis when transplanted into the testis of a sterile recipient animal (8). As with any tissue maintained by stem cells, only a small fraction of testis cells are spermatogonial stem cells. But where is home for these precious, few cells?

In mice, putative spermatogonial stem cells were initially identified histologically, as type A single (A_s), undifferentiated cells. A_s cells gen-

erate a transit-amplifying pool of interconnected daughter cells, A_{pr} (paired) and A_{al} (aligned), which mature into more differentiated germ cells. Recent studies have determined that genes expressed in the A_s , A_{pr} , and A_{al} cells act autonomously to regulate spermatogonial stem cell self-renewal (9, 10). Despite this progress, the specific identity of bona fide spermatogonial stem cells is still in question. Are some or all A_s cells—or even some of the transit-amplifying cells—spermatogonial stem cells? Yoshida and colleagues (11) recently combined a creative labeling method with a transplantation/repopulation assay to follow spermatogonial stem cells in normal mouse testes and in testes (depleted of resident spermatogonial stem cells) that were recipients of transplanted cells. Remarkably, these two analyses gave different results, suggesting that the stem cell population is not



At home, in small narrow places. Spermatogonial stem cells localize to interstitial regions between seminiferous tubules in the mouse testis. This implies that interstitial cells and branching blood vessels secrete factors (arrow) that influence stem cell fate.

homogeneous. Instead, their data suggest that the A_s , A_{pr} , and A_{al} cells exhibit differing degrees of stem cell potential, with the highest potential perhaps in the A_s cells. Spermatogonial stem cell potential is revealed by the degree of niche occupancy. In normal testes, niches are generally occupied by stem cells with the highest potential for self-renewal. But in recipient testes depleted of resident spermatogonial stem cells, transplanted cells can fill unoccupied niches, even if the cells have a low potential for self-renewal.

Unlike in fruit flies and worms, however, the mammalian seminiferous tubules—sites in the testis where spermatozoa mature—do not offer an obvious anatomical niche. Although spermatogonial stem cells are in direct contact with constituents of the

tubules—Sertoli cells (that nourish developing spermatogonia) and the basal lamina that surrounds each tubule—no functional sub-compartment has been identified among the vast area delimited by these components. Glial cell line-derived neurotrophic factor (GDNF), secreted by Sertoli cells, affects spermatogonial stem cell behavior (12), but whether this factor is made by all or only a subset of Sertoli cells is uncertain. Additionally, a receptor subunit for this factor is selectively enriched on undifferentiated gonial cells (13), which is not consistent with a simple model in which GDNF determines spermatogonial stem cell fate.

So, where is home for resident spermatogonial stem cells? Yoshida *et al.* use heroic three-dimensional reconstructions and live imaging (up to 4 days) of the mouse testis to draw inferences about the niche. Of necessity, the authors followed undifferentiated spermatogonia, and not “pure” stem cells, but they analyze the data exhaustively and interpret it with great care. They conclude that these primitive cells are not randomly positioned around or along a seminiferous tubule, but rather are enriched at defined positions, usually where the interstitial area just outside the tubule is filled with various cell types and, curiously, branching blood vessels (see the figure). Although an association with the interstitium had been suggested for spermatogonial stem cells, the external vasculature had not been implicated (14). In addition, although spermatogonial migration had been previously proposed, it had never been visualized live.

Now, Yoshida *et al.* see that once small chains of A_{al} cells are produced, they migrate along the inside surface of the basal lamina. Later, these cell groups divide to form larger chains of differentiating spermatogonia. The extent of this process had not been appreciated until now.

The position of spermatogonial stem cells near the interstitium implies the existence of secreted factors that must act over a distance to influence the niche. Factors emanating from interstitial cells must traverse a cellular and biochemical barrier (the myoid cell layer surrounding seminiferous tubules, and the basal lamina upon which myoid cells are situated; see the figure) before they can gain access to the niche compartment. Interstitial factors—perhaps testosterone from Leydig cells in the interstitium or unknown factors

from the blood vessels—could act directly on the spermatogonial stem cells. Alternatively, a relay might be engaged, whereby signals act on myoid or Sertoli cells, which in turn act on the spermatogonial stem cells.

Regardless of the mechanism, the limited number of active niches suggests strongly that all Sertoli cells cannot be equal. Perhaps, as the present work suggests, only Sertoli cells near interstitia that harbor a branched vasculature can support spermatogonial stem cells. To investigate this possibility, the authors grafted a tubule segment, labeled with green fluorescent protein, from a donor testis into an unmarked host testis. They found that undifferentiated cells were enriched near the realigned host vasculature. Despite the intriguing association of other progenitor cells with vasculature (1), it remains unclear whether vascular branches in the mouse testis generate the niches (where spermatogonial stem cells are found), or whether stem cells, under hypoxic stress, cause nearby vessels to branch.

Spermatogonial stem cells make only spermatozoa. However, cells cultured from mouse testes have been coaxed toward multipotency and even pluripotency (15, 16). These cultures were enriched (and not “pure”) in spermatogonial stem cells, so precisely which cells were manipulated is questionable. The location of the niche might suggest new candidate signals that influence stem cell behavior, leading to the eventual purification and efficient expansion of spermatogonial stem cells in culture. That, in turn, might lead to the coaxing of human spermatogonial stem cells toward pluripotency, yielding a ready source of cells untainted by ethical concerns associated with deriving embryonic stem cells.

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