analyzed (five of seven) showed additional chromosomal abnormalities, with amplification of the chromosome 15 region harboring *Myc* observed in four of seven cases.

Normal hematopoiesis requires the precise regulation of multiple pathways whose activities fluctuate during proliferation and differentiation. Individual genes in these pathways are like 'on-off' switches that dictate the level of activity in a given pathway, and investigators hypothesize that leukemogenesis results from an absolute disruption in one or more of these switches. Such errors are usually associated with activating mutations that turn the switch on at the wrong time (*RAS*, *FLT3*) or with the creation of new chimeric proteins (Bcr-Abl, PML-RARα, RUNX1-CBFA2T1) that perturb the regulatory system. In the 1990s, however, numerous studies showed that loss of heterozygosity of some tumor suppressor genes can promote malignant transformation, suggesting that a haploinsufficient dosage effect may have a role in tumorigenesis[10,11].

Rosenbauer *et al.* expanded on this concept by showing that a dosage effect for PU.1 exists between haploinsufficient and null expression states. These results suggest that investigators must look beyond the concept of genes merely acting as on-off switches and begin considering how small fluctuations in activity impact the biology of the cell. Looking to the future, research will need to more accurately define the interactions between genes and molecular pathways to develop a clearer picture of what constitutes the 'normal' and 'abnormal' regulatory processes of cells. These investigations will necessitate the development of new quantitative approaches and improvements in existing technologies such as DNA microarrays and proteomics.

The elaborate networks controlling cellular functions have multiple checks and balances. For example, *RAS* mutations, which are constitutively active, upregulate p53 activity, resulting in apoptosis if the p53 tumor suppressor pathway remains functional[12]. Therefore, it is not surprising that multiple genetic 'hits' are necessary to promote leukemogenesis. Recently, investigators found that mutations in *FLT3* can cooperate with PML-RARα rearrangements to promote rapid development of a leukemia-like disease in a transgenic mice, whereas either genetic abnormality alone induces primarily a myeloproliferative-like syndrome[13]. These results, combined with those of Rosenbauer *et al.*, argue that initial genetic events promote an undifferentiated or preleukemic state that makes cells susceptible to additional genetic damage. The phenotype of this preleukemic state is similar to that found in myeloproliferative diseases, in which there is an expansion of the primitive hematopoietic cell compartment. Additional genetic abnormalities, like *FLT3* mutations or overexpression of c-Myc, push these cells into an aggressive leukemic phenotype. These findings are consistent with the multiple-hit theory of carcinogenesis, first proposed for solid tumors such as colon cancer[14].

The primary clinical implications from these findings are that cells in a preleukemic state retain some normal regulatory pathways. Therefore, these cells should be much more susceptible, in theory, to targeted therapies that induce preleukemic cells into a normal pathway of differentiation, and perhaps apoptosis. Once cells have evolved to a leukemic state, many of these regulatory processes have been by-passed, making targeted approaches less likely to succeed. This concept has been clearly validated in chronic myelogenous leukemia, in which the small molecular inhibitor Imatinib is effective in treating chronic myelogenous leukemia in chronic phase but has limited efficacy once the disease has progressed to blast phase, a more aggressive and overtly leukemic state[15].

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Plzf pushes stem cells

Noora Kotaja & Paolo Sassone-Corsi

The molecular mechanisms that regulate the balance between differentiation and self-renewal in spermatogonial stem cells are elusive. Two studies now show that the transcriptional repressor Plzf is an essential regulator of spermatogonial stem cell maintenance.

Among cell lineages, germ cells are unique in that they can generate a new organism. In males, germline stem cells provide a source of undifferentiated cells that allow spermatogenesis to proceed throughout the period of sexual maturity. Cells committed to differentiate enter the meiotic pathway, which comprises a unique program of gene expression and chromatin remodeling[1]. To maintain the stem cell pool, however, some germ cells must remain undifferentiated and proliferate through cyclic mitotic divisions. How does each spermatogonial stem cell decide whether to proliferate or differentiate? The molecular mechanisms controlling this delicate balance are largely unknown. New studies by F. William Buaas and colleagues and José Costoya and colleagues published in this issue[2,3] provide clues to the mechanisms required for self-renewal of spermatogonial stem cells by showing that the transcriptional repressor Plzf is required for stem cell maintenance.

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An epigenetic connection

Plzf (promyelocytic leukemia zinc-finger) belongs to the POK (POZ and Krüppel) family of transcriptional repressors. In addition to nine Krüppel-type sequence-specific zinc fingers, Plzf contains a conserved POZ

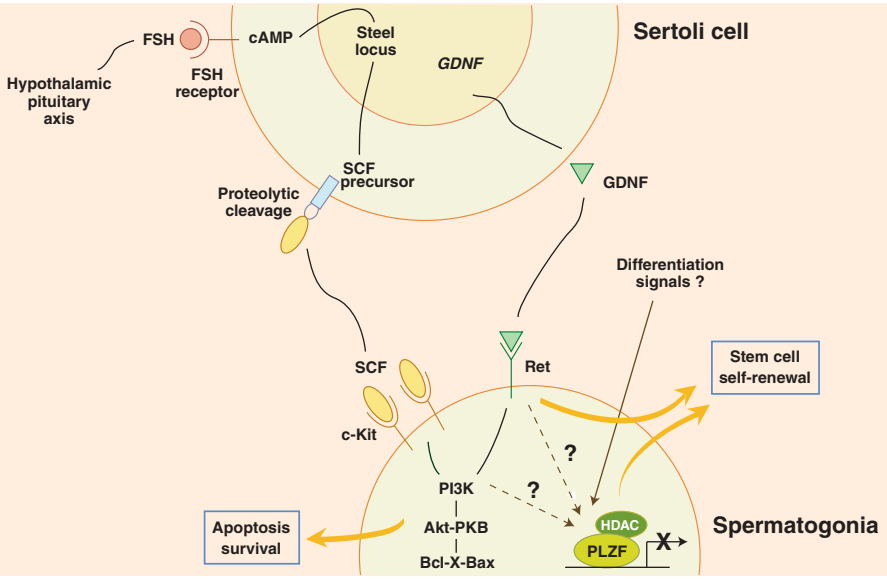

Figure 1 Some known signaling cascades that control spermatogonial cell fate, and that could converge on Plzf, are schematically represented. Sertoli cells produce SCF, the natural ligand of c-Kit, a tyrosine kinase receptor located on the surface of spermatogonia. Sertoli cells produce SCF when induced by follicle stimulating hormone (FSH), a pituitary hormone released in response to hypothalamic signals. SCF binding to c-Kit elicits dimerization of the receptor and consequent activation of the phosphoinositide 3-kinase (PI3K) pathway, which in turn triggers the Akt-PKB kinase. This cascade directly regulates the apoptosis-survival decision, as Akt-PKB regulates proteins of the Bcl2-Bax family. The SCF-c-Kit pathway is paralleled by the GDNF-Ret system. GDNF is released by Sertoli cells and binds to the oncoprotein Ret, which is also coupled to the PI3K-PKB signaling pathway.

(poxvirus and zinc finger) domain in its N terminus. This domain, common to several zinc finger–containing transcription factors, mediates protein-protein interactions and allows POZ domain proteins to participate in various differentiation pathways, including hematopoiesis, adipogenesis, hippocampal neurogenesis, osteoclastogenesis and muscle differentiation.

Earlier studies highlighted the role of Plzf in regulating differentiation. First, Plzf is expressed in early, but not differentiated, hematopoietic cells[4], suggesting that Plzf is involved in stem cell maintenance. In addition, Plzf regulates genes involved in cellular proliferation and differentiation (cyclin A2, *Myc* and *Hox* genes). Finally, targeted deletion of Plzf in mice disrupts patterning of the limb and axial skeleton[5]. These features are reminiscent of the luxoid mouse, a mutant described 50 years ago for its limb abnormalities and recessive skeletal phenotype. The studies of Buaas *et al.* and Costoya *et al.* now show that luxoid mutants and Plzf-null mice share similar defects in sperm production that are due to an inability of spermatogonial stem cells to self-renew. Consistent with these shared phenotypes, Buaas *et al.* show that the luxoid mutation results from a frameshift mutation in *Zfp145*, which encodes Plzf[2].

A twist in this story is that Plzf probably influences the epigenetic program of spermatogonial cells. Although direct proof is not given in these studies[2,3], previous work showed that the POZ domain of Plzf recruits members of the mammalian Polycomb family, such as BMI1 (refs. 6,7). Polycomb proteins maintain stable and heritable repression of several developmental genes. Recruitment of BMI1 by Plzf results in the subsequent recruitment of histone deacetylases[8], thereby linking epigenetic modifications to transcriptional control. Thus, it is reasonable to hypothesize that Plzf-dependent histone deacetylases impose specific chromatin remodeling events that contribute to the decision between differentiation and self-renewal in spermatogonial stem cells.

The reports by Buaas *et al.* and Costoya *et al.* highlight the importance of epigenetic modifications and transcriptional regulation as key mechanisms for stem cell maintenance. As several POZ transcriptional regulators function in cellular differentiation, POZ-dependent epigenetic modifications may have a more common role in directing stem cell behavior. This possibility becomes more promising when one considers that the totipotency of germ cells may be epigenetically regulated through DNA methylation.

Signaling and self-renewal

The behavior of germ cells in the seminiferous tubules is largely controlled by the surrounding somatic Sertoli cells. In mice, GDNF (glial cell line–derived neurotrophic factor) produced by Sertoli cells regulates cell fate decisions in undifferentiated spermatogonia[9]. Studies in fruit flies identified the importance of somatic cell EGF (epidermal growth factor) receptor and Raf activity in determining the differentiation capacity of stem cells[10,11]. Furthermore, maintenance of the stem cell population in fruit flies seems to be secured by activation of the JAK-STAT pathway in germ cells by the unpaired ligand produced by somatic cells of the testis[12]. In flies, the translational repressor Nanos is also essential for conserving stem cell status[13], and the centrosomal protein centrosomin and the tumor suppressor APC (adenomatous polyposis coli) determine the fate of male germline stem cells by regulating mitotic spindle orientation during asymmetric cell division[14].

Despite progress in understanding the mechanisms by which external signals regulate stem cell renewal, little is known about the cell-autonomous factors that control this process in mammalian germ cells. Thus, Plzf is a notable example of a cell-autonomous germ cell factor required for spermatogonial stem cell maintenance. But it remains to be seen how Plzf interacts with known signaling pathways, particularly in response to Sertoli-derived signals such as GDNF and stem cell factor (SCF; **Fig. 1**).

Stem cells and cancer

The involvement of Plzf in cancer brings additional importance to its identification as a regulator of stem cell differentiation. Chromosomal translocations that fuse Plzf and RARα (retinoid acid receptor α) are associated with acute promyelocytic leukemia[15]. The ability of Plzf to control stem cell maintenance may be perturbed in acute promyelocytic leukemia, thus providing leukemic progenitors with a proliferative advantage. The Plzf interaction partner BMI1 is required for maintenance of both hematopoietic and leukemic stem cells[6,7]. Understanding the molecular programs controlling stem cell self-renewal will have an additional impact on development of anticancer therapies.

The unique role of germ cells in transmitting the genome from one generation to the next emphasizes the importance of studying germline stem cells. The multilevel regulatory mechanisms governing germline stem cell self-renewal and differentiation are beginning to be uncovered. The further use of mouse models will provide crucial insights into the

genetic and epigenetic components that constitute the complex molecular network underlying stem cell biology.

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Huntingtin aggregates ask to be eaten

Carson C Thoreen & David M Sabatini

A new study identifies a protective role for cellular aggregates in Huntington disease by showing that aggregates promote the clearance of mutant protein by activating autophagy through the inhibition of mTOR. This challenges the common view that they are possibly innocuous but probably harmful to the host cell.

A common thread connecting various neurodegenerative diseases is the accumulation of insoluble protein aggregates in and around neurons. In Huntington disease, these aggregates almost certainly result from the expansion of glutamine repeats in the huntingtin protein, perhaps altering its structure and enabling it to form cellular aggregates. Although there is considerable disagreement as to whether the aggregates are harmful to cells, mounting evidence suggests that clearing them through the autophagy pathway can reduce cell death[1]. Autophagy is a conserved process in plant, fungal and animal cells and is generally thought to recycle cytoplasmic components when cells are starved for nutrients and, under harsher conditions, act as an alternate mechanism for programmed cell death[2]. Research has traditionally focused on its destructive role, but there is an increasing awareness that it has a protective function in human diseases such as cancer[3]. In this issue, Brinda Ravikumar and colleagues (page 585) report that cells containing huntingtin aggregates protect themselves by clearing mutant protein through the autophagic pathway[4]. The aggregates themselves seem to have a key role in inducing autophagy by sequestering and suppressing mTOR, a negative regulator of the autophagic pathway.

When mutant huntingtin is expressed, the first 100–150 residues of the protein, including the polyglutamine repeats, are cleaved off and act as the toxic entity[5]. It is unclear how the fragments cause disease, but there is a well-established correlation between the length of the polyglutamine repeats and disease progression, including the formation of insoluble aggregates. The debate over whether the aggregates themselves are toxic is based largely on the observation that cell death in the brain does not always correlate with the presence of aggregates[6]. There is a growing agreement, however, that aggregates are present but are too small to be detected by commonly used microscopy or filtration techniques[6]. Recent *in vitro* experiments also support the idea that they are toxic, as injecting aggregates directly into cells recapitulates the effects of expressing mutant huntingtin[7].

Consuming aggregates

There is suggestive evidence that autophagy acts as a protective mechanism by degrading mutant huntingtin. Although huntingtin aggregates are resistant to cytosolic proteases, they do not seem to be permanent, as their presence depends on the continued expression of the mutated gene[6,8]. Mutant huntingtin can be taken up and degraded by autophagic vacuoles[6,9]. Additionally, treating cells with rapamycin, an FDA-approved immunosuppressant that also induces autophagy by inhibiting mTOR, substantially enhanced the clearance of aggregates and

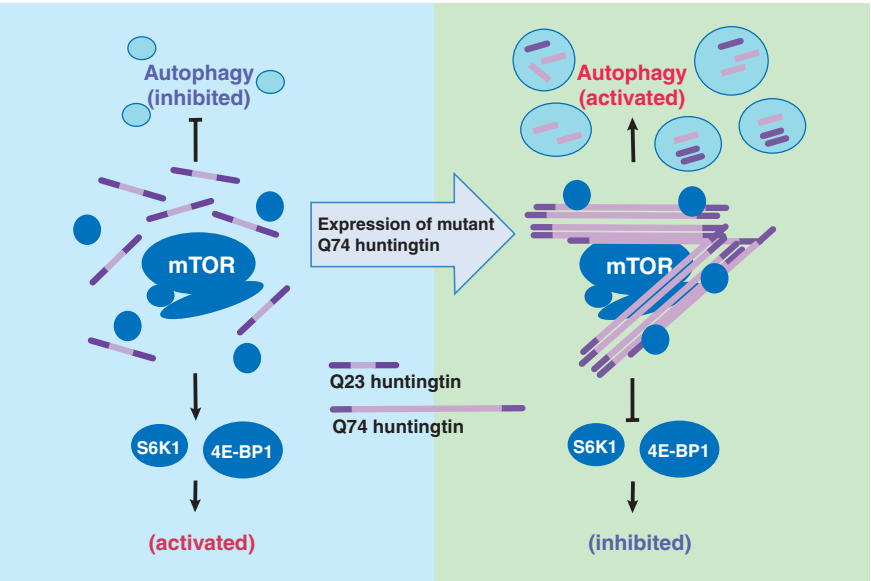

Figure 1 The mTOR pathway stimulates cellular growth in reponse to nutrients and growth factors by enhancing protein synthesis and inhibiting autophagy. The pathway functions normally in cells expressing the Gln_{23} huntingtin fragment (Q23) but is inhibited by the aggregates that form in cells expressing the Gln_{74} fragment (Q74).

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