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MEETING REVIEW

Out of the niche: exploring unknown pathways

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ABSTRACT

In May 2014, approximately 200 stem cell scientists from all over world gathered near Copenhagen in Denmark to participate in 'The Stem Cell Niche', part of the Copenhagen Bioscience Conferences series. The meeting covered an array of different stem cell systems from pluripotent stem cells and germ cells to adult stem cells of the lung, liver, muscle, bone and many more. In addition to the stem cell niche, the meeting focused on a number of cutting edge topics such as cell fate transitions and lineage reprogramming, as well as stem cells in ageing and disease, including cancer. This Meeting review describes the exciting work that was presented and some of the themes that emerged from this excellent meeting.

KEY WORDS: Stem cells, Niche, Lineage

Introduction

'The Stem Cell Niche' meeting took place at the Novo Nordisk Favrholm Campus, a beautiful rural setting not far from Copenhagen, from 18 May to 22 May. The informal and lively environment was enhanced by a burst of summer that created the perfect atmosphere for talking science outdoors while relaxing in the Danish countryside. The meeting organizers Anne Grapin-Botton, Joshua Brickman, Palle Serup and Bo Porse from the Danish Stem Cell Centre at the University of Copenhagen had planned the program mostly according to a classic anatomical scheme: embryonic stem cells and germ cells, followed by adult stem cells in various organs as well as in cancer. Nevertheless, common problems and similar concepts were discussed, with a focus on transcriptional networks and epigenetic regulation of the stem cells, lineage tracing to follow cell fate *in vivo*, and lineage conversions either through an embryonic intermediate or directly to another cell type. Most of the presentations dealt with mammalian systems but *Drosophila*, *C. elegans* and axolotl were also represented.

Although titular for the meeting, the stem cell niche was not the feature of most talks. Nevertheless the impact of the niche was alluded to in most of the stem cell systems, recognizing the importance of extrinsic control in cell fate decisions. In this way, The Stem Cell Niche meeting was a truly comprehensive meeting, bringing together many of the elements that are fundamental to our understanding of what controls stem cells both in homeostasis and disease. Much more time and effort will be required before we reach a complete molecular, anatomical and functional understanding of different stem cell systems; however, it is clear from this meeting that significant progress is being made in this respect. In this Meeting review, we describe some of the exciting findings reported at the meeting, and apologize in advance to those colleagues whose work we cannot describe due to space restrictions.

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Pluripotent and germline stem cells

The beginning of the meeting took on a decidedly developmental flavour, with sessions focused around pluripotency and germ cells. Hotoshi Niwa [RIKEN, Centre for Developmental Biology (CDB), Japan] discussed transcriptional networks that regulate pluripotency. He suggested that there are different ways to activate the leukaemia inhibitory factor pathway, a crucial mediator of the pluripotent stem cell (PSC) state, in both permissive and non-permissive strains of mice in order to generate embryonic stem cells (ESCs). A clever approach to reduce the complexity of these networks was reported by Graziano Martello (Wellcome Trust Centre for Stem Cell Research, Cambridge, UK) who used a computational approach followed by functional validation to identify a small number of key components that interact to regulate and are indispensable for pluripotency (Dunn et al., 2014). Jacob Hanna (Weizmann Institute of Science, Rehovot, Israel) stressed the importance of understanding the stochasticity of the reprogramming process during iPSC generation. He discussed the driving and rate-limiting epigenetic steps of iPSC reprogramming and suggested a 'gas and brakes' paradigm for establishing pluripotency.

Germline stem cells are often considered the 'ultimate' stem cells, as they are able to give rise to a new generation and thus provide the continuity of life. These special cells are set aside during early embryonic development as primordial germ cells (PGCs), but understanding exactly how and when this occurs is still a major challenge for the field. Using axolotl as a model for the vertebrate tetrapod ancestor, the ancestor of all terrestrial vertebrates, Andrew Johnson (University of Nottingham, UK) discussed the timing and mechanism of PGC specification and introduced 'the last cell standing' hypothesis, which implies that the germline develops from uncommitted pluripotent cells that avoid somatic specification (Chatfield et al., 2014). Johnson described this as a basal stochastic mechanism for PGC specification in vertebrates and proposed it as a potential mechanism applicable to non-rodent mammals. The mechanism by which cells of the germline correctly transmit information to the next generation in perpetuity is also under investigation. To this end, Ruth Lehmann (New York University, USA) discussed mechanisms that mediate proper mitochondrial inheritance in *Drosophila* germline stem cells. Lehmann suggested that the mitochondria are localized posterior to germ cells through a network of proteins that anchor them in an actin meshwork, poising the mitochondria for proper inheritance. Moving on to the mouse germline, both Haifan Lin (Yale University, New Haven, USA) and Ben Simons (University of Cambridge, UK) presented data aimed at understanding how the identity of germline stem cells is maintained. Lin described the ever-increasing complexity of the transcriptional regulation of germ cells. Using mutants of a Piwi protein, he showed the effects of the piRNA pathway on mRNA turnover and implicated piRNAs in mediating the regulation of mRNAs and long non-coding RNAs by transposons and pseudogenes. Taking an entirely different

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approach, Simons explored the complex relationships among germline stem cells and spermatogonia in the mouse testis to understand how the stem cells explore cell fate. He showed that although GFRa is a self-renewal marker, NG3 is an early marker of commitment and is expressed in gonia that retain a cellular bridge after division. Intriguingly, some cells, probably in a stochastic way, break this bridge and revert to a more 'stem-like' state (Hara et al., 2014).

Lineage transitions *in vivo* and *in vitro*

Understanding how cells acquire a specific fate is a fundamental challenge not only for the stem cell community but for the broader developmental biology community as well. During development, lineage specification occurs in a stepwise manner as cells differentiate from the totipotent zygote, but the many intrinsic and extrinsic factors that control this progression are not fully understood. In the field of neural development, James Briscoe (National Institute of Medical Research, London, UK) provided evidence that FGF and WNT signalling dictates a binary lineage choices in ESCs, directing them to either an anterior neural (FGF) or a neuromesodermal (FGF+WNT) fate, which then produced either spinal cord or paraxial mesoderm cell types, respectively. He also showed how it is possible to create an appropriate dorsal-ventral pattern based on a gradient of SHH.

Differentiation is not the only process that requires transitions in cell identity. Direct lineage conversion, i.e. the transition from one somatic cell type to another, is a dramatic example of how cell fate can be reassigned, either during development or in the adult. Brigid Hogan (Duke University, Durham, NC, USA) gave a comprehensive and inspiring lecture on lineage transitions of the epithelial stem cells in the mammalian lung. The established scenario is that basal epithelial cells in the lung express stem cells markers such as p63 and NGFR, and give rise to both ciliated and secretory cells in the larger airways. Hogan expanded on this to show that, after specific types of injury, differentiated secretory cells appear able to revert to a basal cell phenotype, highlighting a previously unappreciated role of lineage conversion upon injury in the lung. The same is not true in other areas of the lung, however, as in smaller airways and in the alveoli there is an absence of basal cells. Here, it is the club cells and the type 2 pneumocytes, respectively, that play a central role in self-renewal. Together with pericytes, endothelial and interstitial cells they represent a complex niche that maintains homeostasis and can, upon specific insults, undergo fibrosis, thus preventing correct regeneration.

Ben Stanger (University of Pennsylvania, Philadelphia, PA, USA) presented what was, in our opinion, very striking and unexpected results that challenge the existing notion of a hepatic stem cell. By using different lineage tracing mice, he showed that under a variety of injury models it is the hepatocytes, and not the oval cells, that are the source of new hepatocytes and cholangiocytes (Yimlamai et al., 2014). During liver injury, therefore, new hepatocytes arise from pre-existing hepatocytes, whereas new cholangiocytes can arise from either cholangiocyte replication or hepatocyte transdifferentiation. The transdifferentiation process does not involve a transition through a foetal stage intermediate in order to generate new terminally differentiated cells, and the process is Notch dependent. In another injury setting, this time in the gut, Nicholas Buchon (Cornell University, Ithaca, NY, USA) showed that after exposure to non-lethal pathogenic bacteria, the gut lining completely delaminates and is lost. This in turn activates gut stem cells, suggesting that infection and damage cause a homeostatic loop of increased differentiation and proliferation, which requires a balance of JNK and Hippo signalling.

The lineage transitions that occur between cells of a single organ system in response to injury are intriguing, but do not represent the full potential of transdifferentiation. Using *C. elegans* as a model system, Sophie Jarriault [Institute of Genetics and Molecular and Cellular Biology (IGBMC), Strasbourg, France] described at the single cell level the prototype of invariant transdifferentiation. She reported the intriguing case of a rectal cell that undergoes de-differentiation followed by a step-wise re-specification into a motoneuron (Zuryn et al., 2014). This is an irreversible process that cannot be achieved by neighbouring epithelial cells. The strict and robust nature of this cell type conversion was demonstrated by the sequential partitioning of modifiers of histone methylation. The striking conversion between two seemingly unrelated cell types that occurs naturally in some contexts has inspired many researchers to attempt to force lineage conversions to occur *in vitro*. To this end, Ihor Lemischka (Mount Sinai Hospital, NY, USA) expanded on previously published efforts in mouse to reprogram human fibroblasts into hematopoietic cells with defined transcription factors. He also showed how the information learned from this reprogramming event enabled the identification of an embryonic

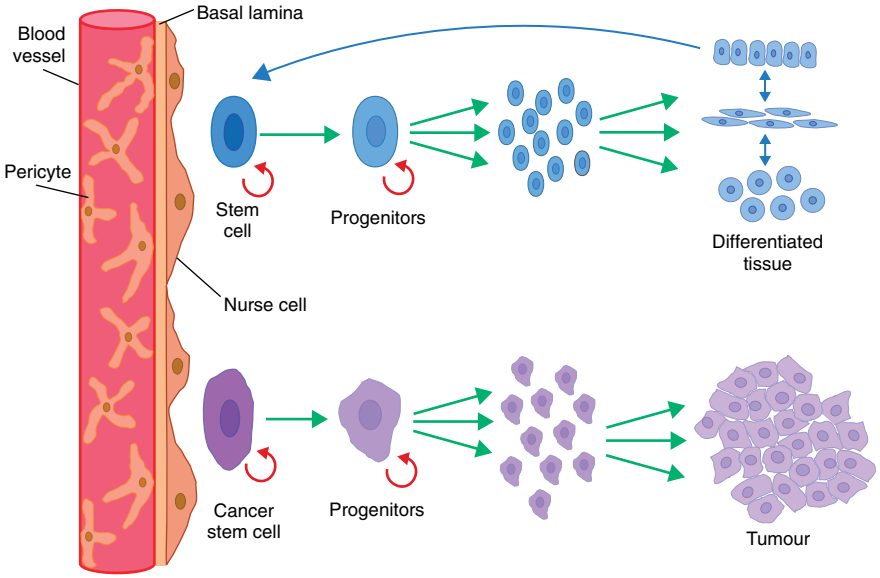

Fig. 1. The stem cell niche and beyond. The stem cell niche is unique to each tissue but is generally thought to contain a small blood vessel and its associated pericytes and basal lamina, which often provide a scaffold for the stem cell, as well as nearby nurse (or stromal) cells. During normal homeostasis, stem cells in the niche undergo an asymmetric division (red arrows) in order to both self-renew and produce a transit amplifying population of progenitors (green arrows) that proliferate and then differentiate to replace or repair normal tissues. In the case of cancer (lower panel), progenitor expansion and differentiation contributes to the growth of the tumour. Lineage conversions can also occur from fully differentiated cells (blue arrows), either to a less differentiated state or between different types of differentiated cells.

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precursor cell to hemogenic endothelium, thus demonstrating how the process of directed reprogramming could inform normal developmental processes.

The niche takes centre stage

Cell fate is profoundly influenced by the niche, the specific microenvironment in which stem cells reside. This typically consists of a basal lamina with its own ECM components, often but not always associated with a small blood vessel, as well as nurse cells that produce factors required for maintenance of 'stemness' (Fig. 1). Studying the niche involves careful examination of multiple different components, including the different types of neighbouring cells, the surrounding signalling milieu as well as physical parameters such as stiffness and mechanical strain. Matthias Lutolf (École polytechnique fédérale de Lausanne, Switzerland) described the challenging attempt to re-create the functional stem cell niche *in vitro* through a combination of advanced biomaterials, signalling molecules and cells. He showed how high-throughput analyses could provide an approach to systematically dissect the complexity of the niche in molecular terms and provide novel insights into niche-based regulation of stem cell biology (Ranga et al., 2014).

Perhaps the best-characterized stem cell niche is that of the bone marrow. David Scadden (Harvard University, Cambridge, MA, USA) discussed his work towards understanding the different cell types that make up the niche and how they regulate stem cell activity. By using targeted deletion of select cell types in the bone marrow, he has identified specific niches that control the heterogeneity of blood and he is continuing to elucidate the crosstalk between stem cells and their niches that mediates this control. Staying with bone, Anjali Kusumbe (Max Planck Institute, Muenster, Germany) reported recent data showing how angiogenesis and osteogenesis are coupled during postnatal bone development, and how this crosstalk is essential to maintain osteoprogenitor cells (Ramasamy et al., 2014). Kusumbe showed how the coupling of angiogenesis and osteogenesis is mediated by a specific vessel subtype in bone that can be demarcated by cell-surface expression levels of CD31 and endomucin (Kusumbe et al., 2014).

Two talks focused on myogenesis, one from Shahragim Tajbakhsh (Institut Pasteur, Paris, France) and the other from Giulio Cossu (University of Manchester, UK), highlighted the importance of the niche in regulating cell fate decisions in the muscle lineage. Dr Tajbakhsh discussed his work on asymmetric cell division and Notch-regulated quiescence of muscle stem cells (Yennek et al., 2014). Of particular relevance to this meeting was his finding that different micropatterns dictate the choice between symmetric and asymmetric segregation of DNA and transcription factors, demonstrating that extrinsic cues impinge on whether a cell divides symmetrically or asymmetrically. Interestingly, however, prospectively isolated cells engaged to divide asymmetrically will not alter their fate when they are seeded on a symmetric micropattern. Looking at foetal myogenesis, Cossu discussed the choice between smooth and skeletal muscle, as dictated by signals emanating from the endothelium (DLL4) and skeletal muscle fibres (noggin), respectively. Extrinsic signalling was also the focus of Andrea Brand's presentation (Gurdon Institute, University of Cambridge, UK), where she described the intriguing relationship between nutrients and neural stem cell quiescence, first identified in *Drosophila*. Essentially, a nutritional stimulus induces glial cells to secrete insulin/IGF-like peptides that induce NSCs to exit quiescence. The transcriptional and epigenetic changes underlying this reactivation are being investigated.

Stem cells in cancer

Although much of the meeting was focussed on how normal stem cell activity is regulated (Fig. 2), some presentations discussed how stem cells in different cancer types behave during disease progression. Peter Dirks (The Hospital for Sick Children, Toronto, Canada) gave a fascinating talk about defining and targeting the stem cell hierarchy in medullobastoma tumours in which sonic hedgehog (SHH) signalling is dysregulated. He provided convincing evidence that the SOX2+ cells within the tumour are slow cycling and that they are tumour-initiating cells. Targeting the SOX2+ population may therefore be a possible therapeutic option for this tumour type, as a SOX2+ gene expression signature correlates with a poorer prognosis (Vanner et al., 2014). In a different cancer system, Andreas Trumpp (German Cancer Research Centre, Heidelberg, Germany) is investigating how mesenchymal (MSC) and myelodysplastic syndrome (MDS) stem cells interact and form a disease unit in human bone marrow. Trumpp presented data showing that lineage-CD34+CD38− cells behave as MDS stem cells and he also provided evidence that MDS cells can instruct surrounding MSCs to shape a niche environment supporting MDS initiation, maintenance and likely progression towards a more aggressive disease (Medyouf et al., 2014). Fiona Watt (King's College, London, UK) also discussed the role of the niche in regulating cancer. She reported a novel single cell approach to study the physical cues that regulate the behaviour of squamous cell carcinoma cells compared with normal human keratinocytes. Dr Watt's group are using hydrogels of varying stiffness to study the microenvironmental interactions that trigger different outcomes via changes in signal transduction that mediate cell behaviour.

Recently, the use of epigenetic modifiers for cancer therapy has come into play clinically. Maarten van Lohuizen (Netherlands Cancer Institute, Amsterdam, The Netherlands) presented data that suggest that these types of therapies may have important consequences for tumour progression and treatment options. Using a model of glioblastoma that overexpresses polycomb group genes such as Bmi1 and Ezh2, van Lohuizen showed that although knock down of Ezh2 can reduce tumour growth and extend lifespan, ultimately the tumour returns in an even more aggressive form. He suggested that DNA repair genes are induced upon knockdown, and that this may abrogate the efficacy of commonly used chemotherapeutic agents. For this reason, caution should be taken when using epigenetic modifiers in combination therapies, although this approach may still be efficacious if a DNA repair inhibitor is used prior to the other therapies. Continuing with the theme of epigenetics, Matthias Hebrok (University of California, San Francisco, USA) presented his work on the mechanisms

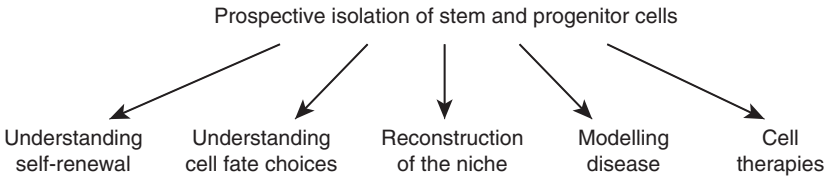

Fig. 2. Identification and isolation of stem cells and their niches. Prospective isolation of stem cells or reprogramming of differentiated cells enables the study of mechanisms underlying self-renewal, fate choice and differentiation. It also allows reconstruction of the niche *in vitro*, which is crucial for understanding stem cell regulation and also for clinical applications, such as disease modelling and expansion of cells and tissue for cellular therapies.

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mediating pancreatic ductal adenocarcinoma (PDA), which has one of the poorest prognoses of all cancers. PDA can be divided into several subtypes, including pancreatic intraepithelial neoplasia (PanIN) and intra-ductal papillary mucinous neoplasia (IPMN) with PanIN having the worst outcomes. Using transgenic models, Dr Hebrok demonstrated that the epigenetic regulator Brg1 plays a role in specification of progenitor cells for distinct subsets of PDA. Elimination of Brg1 in duct cells promotes IPMn-PDA formation, but inhibits PanIN-PDA from acinar cells (von Figura et al., 2014).

Towards the clinic: using stem cells to understand ageing and disease

One of the exciting applications of stem cell research is as a platform to model different types of diseases, predominantly via induced pluripotent stem cell (iPSC) technology but also using adult stem cells. In addition, there is also growing recognition of the role of stem cells in ageing, and understanding how this process is regulated has important implications for the potential treatment of age-related disease. Gerald de Haan (European Research Institute for the Biology of Aging, Groeningen, The Netherlands) is continuing to use a bar-coding technology to investigate the properties of aged hematopoietic stem cells. He presented data that suggest it is not the niche itself that causes age-related defects in HSC transplantation but that the process is intrinsic to the stem cells. In addition, he showed that there is a very heterogeneous distribution of HSC clones throughout the skeleton under normal homeostatic conditions, but that this disappears upon mobilization (Verovskaya et al., 2014).

One particular focus of age-related disease is neurodegeneration, which is responsible for a number of different diseases such as Parkinson's, Huntington's and Alzheimer's disease, to name a few. However, these late-onset diseases have been particularly difficult to model *in vitro* because directed differentiation from PSCs tends to produce immature cells. Lorenz Studer (Memorial Sloan Kettering Cancer Center, NY, USA) presented a novel and fascinating potential solution to this problem in his work using human iPSC-derived dopaminergic neurons to model Parkinson's disease. He reported a way to 'induce' ageing in cells by modifying them to express progerin, the mutant protein that causes progeria, an accelerated ageing disorder. The progerin-expressing iPSC-derived dopaminergic neurons more closely recapitulated the pathology of Parkinson's disease, thus opening a window for molecular studies and therapeutic modelling.

Conclusions

Despite the tremendous complexity of the stem cell niche, it is clear from this meeting that various aspects are beginning to be understood and, in our opinion, the hypothesis that the niche regulates most, if not all, of the key functions of stem cells will likely be confirmed in the near future. Another take home message of the meeting is that it may be unwise to look for 'universal' anatomical and functional features of the niche, as all tissues are different and the diversity of tissue-specific stem cells may well be reflected in their niche. Efforts to deconstruct and rebuild the niche in order to understand its composition, function and how this varies between different tissues continues to be a major focus in stem cell biology, one that will likely reap rewards in terms of understanding the control of stem cells in the years to come. The authors of this Meeting review and indeed most, if not all, of the participants left the meeting with the high hopes of returning in a few years time to learn about the advances in niche stem cell biology that will undoubtedly occur in the meantime.

Competing interests

The authors declare no competing financial interests.

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References

Chatfield, J., O'Reilly, M.-A., Bachvarova, R. F., Ferjentsik, Z., Redwood, C., Walmsley, M., Patient, R., Loose, M. and Johnson, A. D. (2014). Stochastic specification of primordial germ cells from mesoderm precursors in axolotl embryos. *Development* **141**, 2429-2440.

Dunn, S.-J., Martello, G., Yordanov, B., Emmott, S. and Smith, A. G. (2014). Defining an essential transcription factor program for naive pluripotency. *Science* **344**, 1156-1160.

Hara, K., Nakagawa, T., Enomoto, H., Suzuki, M., Yamamoto, M., Simons, B. D. and Yoshida, S. (2014). Mouse spermatogenic stem cells continually interconvert between equipotent singly isolated and syncytial states. *Cell Stem Cell* **14**, 658-672.

Kusumbe, A. P., Ramasamy, S. K. and Adams, R. H. (2014). Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. *Nature* **507**, 323-328.

Medyouf, H., Mossner, M., Jann, J.-C., Nolte, F., Raffel, S., Herrmann, C., Lier, A., Eisen, C., Nowak, V. and Zens, B. et al. (2014). Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. *Cell Stem Cell* **14**, 824-837.

Ramasamy, S. K., Kusumbe, A. P., Wang, L. and Adams, R. H. (2014). Endothelial Notch activity promotes angiogenesis and osteogenesis in bone. *Nature* **507**, 376-380.

Ranga, A., Gobaa, S., Okawa, Y., Mosiewicz, K., Negro, A. and Lutolf, M. P. (2014). 3D niche microarrays for systems-level analyses of cell fate. *Nat. Commun.* **5**, 4324.

Vanner, R. J., Remke, M., Gallo, M., Selvadurai, H. J., Coutinho, F., Lee, L., Kushida, M., Head, R., Morrissy, S. and Zhu, X. et al. (2014). Quiescent sox2(+) cells drive hierarchical growth and relapse in sonic hedgehog subgroup medulloblastoma. *Cancer Cell* **26**, 33-47.

Verovskaya, E., Broekhuis, M. J. C., Zwart, E., Weersing, E., Ritsema, M., Bosman, L. J., van Poele, T., de Haan, G. and Bystrykh, L. V. (2014). Asymmetry in skeletal distribution of mouse hematopoietic stem cell clones and their equilibration by mobilizing cytokines. *J. Exp. Med.* **211**, 487-497.

von Figura, G., Fukuda, A., Roy, N., Liku, M. E., Morris IV, J. P., Kim, G. E., Russ, H. A., Firpo, M. A., Mulvihill, S. J. and Dawson, D. W. et al. (2014). The chromatin regulator Brg1 suppresses formation of intraductal papillary mucinous neoplasm and pancreatic ductal adenocarcinoma. *Nat. Cell Biol.* **16**, 255-267.

Yennek, S., Burute, M., Théry, M. and Tajbakhsh, S. (2014). Cell adhesion geometry regulates non-random DNA segregation and asymmetric cell fates in mouse skeletal muscle stem cells. *Cell Rep.* **7**, 961-970.

Yimlamai, D., Christodoulou, C., Galli, G. G., Yanger, K., Pepe-Mooney, B., Gurung, B., Shrestha, K., Cahan, P., Stanger, B. Z. and Camargo, F. D. (2014). Hippo pathway activity influences liver cell fate. *Cell* **157**, 1324-1338.

Zuryn, S., Ahier, A., Portoso, M., Redhouse White, E., Morin, M. C., Margueron, R. and Jarriault, S. (2014). Sequential partitioning of histone methylation and demethylation activities determines the robustness of natural transdifferentiation. *Science* (in press) doi:10.1126/science.1255885.