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MEETING REVIEW

Programming and reprogramming the brain: a meeting of minds in neural fate

Magdalena Götz[1,2,3] and Sophie Jarriault[4,*]

ABSTRACT

In early April 2017, over 130 delegates met in Munich, Germany, to discuss the latest research in the development and reprogramming of cells of the nervous system. The conference, which was organised by Abcam and entitled 'Programming and Reprogramming the Brain', was a great success, and provided an excellent snapshot of the current state of the field, and what the challenges are for the future. This Meeting Review provides a summary of the talks presented and the major themes that emerged from the conference.

KEY WORDS: Brain, Cellular plasticity, Cellular reprogramming, Developmental programme, Central nervous system

Introduction

For two days in April 2017, organizers Paola Arlotta (Harvard University, USA), Benedikt Berninger (Mainz University, Germany) and Marisa Karow [Ludwig-Maximilian University of Munich (LMU), Germany] brought together scientists from around the world with a shared interest in understanding how cells of the nervous system are made. The conference, which was entitled 'Programming and Reprogramming the Brain', was hosted by Abcam and held at the Biomedical Center of the LMU in Munich, Germany. The topic of this meeting was particularly timely given the fast acceleration of the field and the close interplay between programmes identified in development and their use to instruct given cell types or entire organs. It was therefore both exciting and very fruitful to bring together scientists with a focus on pure developmental biology as well as directed differentiation and reprogramming. This Meeting Review summarises the new and exciting findings that were presented at the meeting, ranging from how neuronal diversity is programmed *in vivo* – either during development or in the natural transdifferentiation of cells into neurons – to the forced reprogramming of various cell types *in vitro* and *in vivo*. These two sides of the neuronal coin encircle the central question of how similar or different the developmental and reprogramming programs actually are (for discussion of this, see Masserdotti et al., 2016; Morris, 2016). Although there is still some way to go in unravelling the relationship between these processes, it was clear from this meeting that they are sufficiently similar to each benefit immensely from the findings of the other.

[1]Physiological Genomics, Biomedical Center Ludwig-Maximilians-University Munich, 82152 Planegg/Martinsried, Germany. [2]Institute for Stem Cell Research, Helmholtz Center Munich, 85764 Neuherberg, Germany. [3]Excellence Cluster of Systems Neurology (SYNERGY), 82152 Planegg/Martinsried, Germany. [4]Institut de Génétique et de Biologie Cellulaire et Moléculaire (IGBMC), Department of Development and Stem Cells CNRS UMR7104, INSERM U964, Université de Strasbourg, 1 rue Laurent Fries, 67404 Illkirch CU Strasbourg, France.

*Author for correspondence (sophie@igbmc.fr)

S.J., 0000-0003-2847-1675

Programming the brain

When using the term programming, a long-standing issue is apparent, namely the extent to which intrinsic mechanisms 'program' cell fate versus the extent to which the external environment modifies it. This is particularly relevant in the brain, where cellular diversity is extremely high, and models for the mechanisms that instruct this diversity range from stochastic decisions to intrinsically fixed lineages. As ontogeny mediates phylogeny, these mechanisms are also key to consider in a species-specific context as the extent to which they contribute may vary accordingly.

Clonal analysis, i.e. the tracing of the progeny of a single neurogenic progenitor to determine whether certain cell types all share a common ancestor, has been constrained by technical limitations. Indeed, following the entire progeny of a single progenitor is rather challenging in complex brains with long-distance migration. Gordon Fishell (New York University Langone Medical Center, USA) presented his research on the lineage of interneurons, a population notorious for its dispersal, using a DNA-barcoded library of viral vectors. Although particular interneuron subtypes originate from distinct parts of the ganglionic eminence, the extent to which they derive from the same ancestor is presently disputed (Sultan et al., 2016; Mayer et al., 2016). Following up on published data that the position of clonally related neurons is indeterminant (Mayer et al., 2016; Sultan et al., 2016), new single-cell sequencing data revealed no obvious differences in gene expression within interneuron progenitors. Instead, a common set of ground state identities appear to be shared across progenitors, independent of their origin. This work suggested that the diversity of GABAergic projection and interneurons could be attributable to a small set of 'cardinal' genetic determinants.

Meaningful differences between cells do not always lie at the transcriptional level. Focusing on one transcription factor that is well-known as a master regulator of the telencephalic interneuron lineage, Noelia Urban (The Francis Crick Institute, London, UK) presented as yet unpublished work on the deletion of *Huwe1*, the main regulator of Ascl1 protein stability, in the developing ganglionic eminence. In the absence of Huwe1, Ascl1 protein persisted in later stages of the lineage, into the basal or intermediate progenitors, which then continue to amplify the neuronal lineage for longer. Intriguingly, a different phenotype was observed in the context of adult neurogenesis, whereby increased persistence of Ascl1 interfered with return to quiescence, thus depleting the pool of adult neural stem cells (NSCs) (Urban et al., 2016). Given that different levels of Ascl1 play a key role in regulating aspects of cell cycle entry and progression, it is surprising that this function has not been observed in reprogramming experiments that use Ascl1 (Wapinski et al., 2013; Masserdotti et al., 2015).

A round of exciting presentations dealt with the *ex vivo* production of 3D neural cell cultures and their self-patterning into different brain cells. Paola Arlotta presented a fascinating new

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organoid system in which the 3D structures are cultured much longer than nine months, achieving mature neurons that develop spines, together with more mature glial cells at six months (Quadrato et al., 2017). Excitingly, the different tissues in the organoids appeared to be akin to many different brain regions including the retina, and neuronal signals could be elicited by light stimulation. One approach to assessing the composition and variation among organoids is by comparison of single-cell sequencing data with progenitors and neurons from the developing human brain. To this end, both Barbara Treutlein (Max Planck Institute Leipzig, Germany) and Paola Arlotta reported an impressive similarity at the transcriptional level between cells derived from cerebral organoids and those derived from human foetal brain. Although there was significant variation in the quantitative composition, for example relatively fewer basal radial glial cells were present in the organoids, the identity of the cells and their differentiation pathways appeared to be comparable (Camp et al., 2015).

An interesting issue in programming the brain is the extent to which species-specific programs exist – in particular the existence of so-called 'human-specific' aspects. The first speaker to address this question was Silvia Cappello (Max Planck Institute of Psychiatry, Munich, Germany), who presented a comparison of phenotypes that result from mutations in the planar polarity genes *Fat4* and *Dchs1* in human and mouse models. Analysis of cerebral organoids derived from patients with mutations in these genes revealed slower neuronal migration accompanied by destabilisation of microtubules, which eventually resulted in ectopic positioning of neurons closer to the ventricle. Comparison with mouse models (Cappello et al., 2013) highlighted a species-specific effect, whereby some phenomena were only observed in mouse and not human organoids, such as the effect of Fat4 and dachsous on proliferation. This highlights the importance of modelling human disease phenotypes using patient cell-derived cerebral organoids, but also its limitations, as often certain regions of the brain, for example the telencephalon, are not generated and neuronal maturation is not always achieved.

Brain organoids are particularly useful in studying the differences in human NSCs compared with NSCs derived from chimpanzees. This was highlighted in work presented by Barbara Treutlein, who showed live-imaging of the human-specific lengthening of prometaphase-metaphase (Mora-Bermúdez et al., 2016). Using similarly comparative approaches to highlight developmental strategies specific to humans and their evolution, both Pierre Vanderhaeghen (Université Libre de Bruxelles, Belgium) and Johan Jakobsson (Lund University, Sweden) presented their research concerning the identification of genomic events that correlate with human-specific characteristics. Using a transcriptomic approach targeted at the human foetal cortex combined with an analysis to identify newly duplicated hominid-specific genes, Vanderhaeghen reported the identification of over 60 such genes putatively involved in human corticogenesis. He further described how some of these hominid-specific genes might control the cell cycle properties and thereby the expansion potential of human cortical progenitors.

Maintaining the focus on human CNS development, Jakobsson presented work on the role of transposable elements in human neural progenitor cells, asking whether and how acquired DNA might have impacted and co-evolved with brain development. Transposable elements make up more than half of the human genome, with around 8% attributable to endogenous retroviruses (ERVs), inherited from retroviral infections. The ERV repertoire is highly divergent among species, and most ERVs entered the human genome during primate evolution. Jakobsson discussed how in human neural progenitor cells, ERVs act as a docking platform for the repressor TRIM28. Besides the ensuing repression of these ERVs, the local formation of a TRIM28-dependent heterochromatic environment negatively impacts on the expression of nearby genes. Their data suggest a model whereby a repressor system that includes TRIM28 might have initially been involved in protecting against mobile elements and aberrant expression from retroviral DNA elements, and might have evolved into a co-regulatory role to control the expression of nearby protein-coding genes (Brattås et al., 2017).

Genome regulation was also the focus of a presentation from Florian Noack (Calegari lab, Center for Regenerative Therapies Dresden, Germany), who presented new data on the changes in the methylome during cortical development from NSCs and progenitors to neurons. Taking advantage of recent clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 technology, Noack used dCas9 fused to the active domain of the Tet enzymes to manipulate single sites in the genome. He showed how activation of a regulatory site at a key candidate gene could produce a measurable phenotype, highlighting the importance of this regulatory mechanism for specific genes. His work was an example of how recent developments in gene editing technology have provided better tools to target individual sites in the genome. However, the beauty of CRISPR/Cas9 technology is not only that it allows engineering of specific epigenetic marks at individual sites, but also that it facilitates genome-wide screens, providing a new technology for forward genetic screens in precise contexts in mammals. Along this line, Randall Platt (ETHZ, Basel, Switzerland) gave some fascinating examples of success with such screens (Platt et al., 2014; Joung et al., 2017) and provided new insights into ongoing screens and the possibilities of this exciting technology in the neural field. From these two talks alone, it was clear that concepts and technologies move fast in this ever-accelerating field, providing excellent tools to dissect fate specification and reprogramming as the basis of cellular identity in development as well as repair.

Programming adult NSCs

Neurogenesis continues in the mammalian adult brain at specific locations, owing to the existence of a set of slow-dividing adult NSCs. Important questions are when and how the pool of adult NSCs is formed during development, and how homogenous the population actually is. Sven Falk (Götz lab, LMU, Munich, Germany) presented data showing how the fate of two populations of embryonic neural progenitors is greatly influenced by the orientation of the cleavage plane. Randomisation of spindle orientation favours the production of short neural progenitors (SNPs), which lack a basal process, at the expense of radial glial cells (RGCs), which will give rise to the adult NSCs. This phenotype correlates with a later decrease in NSCs, providing a mechanistic basis for how and when their number is determined. Of note, the role of spindle orientation in determining the number of RGCs, and later the number of NSCs, is restricted to a specific time window in development, embryonic day 14-15, and a specific neurogenic region, the lateral ganglionic eminence, which generates most of the adult NSCs (Falk et al., 2017).

François Guillemot (The Francis Crick Institute, London, UK) examined how the pool of adult NSCs is maintained over time in the postnatal dentate gyrus (Urban et al., 2016). The transcription factor Ascl1 was found to play a key role both in the activation of quiescent stem cells into dividing stem cells, as well as in the return to

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quiescence of the progeny of activated stem cells. These functions are regulated through post-translational control of the Ascl1 protein by Id4, or via the modulation of its half-life by the E3-ubiquitin ligase Huwe1 in both instances. His data call for a model in which two populations of adult NSCs co-exist. The first is a resting population, which results from a recent cell division and is more prone to divide again. This population requires Huwe1 activity for long-term maintenance. The second is a larger pool of dormant NSCs that are less likely to enter cell cycle and that express higher levels of Id4. This points to the importance of the intrinsic cellular context, a theme that re-appeared in the later sessions on reprogramming.

Besides the translational regulation of Ascl1 and its impact on progenitor development and NSC quiescence, translational control has emerged as an important theme in NSC differentiation as well. Ana Martin Villalba [German Cancer Research Center (DKFZ), Heidelberg, Germany] launched a systematic transcriptomic analysis of adult NSCs and their progeny to compare ribosome-associated mRNAs with the total mRNA pool using a RiboTag approach (Llorens-Bobadilla et al., 2015). These data showed that the regulation of translation efficiency is highly dynamic during the generation of newborn olfactory bulb interneurons. Her work suggests that a translational-based regulatory mechanism might operate in different stem cell populations, as it resonates with the mammalian target of rapamycin complex 1 (mTORC1)-dependent G-alert state that has been observed in muscle stem cells (Rodgers et al., 2014).

Cellular identity and reprogramming: erasing the past to make way for the future

Decades of work have shown that cells can naturally be reprogrammed or can be forced to do so, most famously by using one or several transcription factors. When induced experimentally, the identity of the chosen transcription factor(s) determines the final identity of the cell. But how is the initial cellular identity erased during transcription factor-induced reprogramming and what makes some cells more amenable to it? Several talks highlighted the importance of somatic transcription factor depletion to improve reprogramming efficiency. Kathrin Plath (University of California, Los Angeles, USA) presented her work investigating how pluripotent reprogramming factors, such as Sox2 and Oct4 (POU5F1), which have been shown to act as transcriptional activators (Wang et al., 2011; Hammachi et al., 2012), mediate the erasure of differentiated cell identity. The transition during pluripotent reprogramming from murine embryonic fibroblasts (MEFs) to induced pluripotent stem cells (iPSCs) was analysed at four distinct time points, using genome-wide approaches to map the binding of the Oct4, Sox2, Klf4 and Myc (OSKM) transcription factors, as well as the histone modification landscape, chromatin accessibility and the transcriptomic profile of the cells. Surprisingly, these data suggested that the OSK factors are important not only for the activation of the pluripotency enhancers in a stepwise fashion, but also to switch off the somatic (MEF) enhancers, both via direct binding to them and via an additional genome-wide mechanism that is yet to be elucidated. Cooperative OSK binding rather than individual pioneer-like transcription factor binding appeared to be key at each step in murine cells. This was correlated with a redistribution of somatic transcription factors from the somatic enhancers to transient ones, as well as with the timing of pluripotency enhancer selection (Chronis et al., 2017). Continuing with the induced pluripotency theme, Baris Tursun (Max Delbrück Center for Molecular Medicine, Berlin, Germany) interrogated the molecular basis of the resistance to transcription factor-induced direct reprogramming by conducting genetic screens in *Caenorhabditis elegans*. These led to the identification of the FACT ('facilitates chromatin transcription') complex as a barrier to induced reprogramming in several tissues of the worm, including the germline. Interestingly, knockdown of FACT complex activity during iPSC reprogramming appeared to enhance the process. Although FACT has mostly been described as a positive modulator of transcription (Orphanides et al., 1998; Mason and Struhl, 2003; Birch et al., 2009), analysis of chromatin accessibility in human fibroblasts and in the worm suggests that it acts as a barrier to reprogramming through a transcriptional repressive activity. Thus, similar reprogramming barrier mechanisms are used during induced pluripotent and direct reprogramming, even in distant species.

Oliver Hobert (Columbia University, NY, USA) discussed his efforts to examine how the molecular mechanisms involved in establishing a terminal differentiated identity, and the memory of it, could impact the efficiency of direct reprogramming. In an impressive large-scale effort, his team identified the specific combinations of transcription factors needed for terminal differentiation of most neuronal classes of *C. elegans* (Hobert, 2016). Termed 'terminal selectors', these factors endow neuronal cells with a specific neuronal subtype identity, rather than initially establishing a neuronal fate and gross morphology. Overexpression of such terminal selectors cannot impose a new identity on other cell types, including neurons, at least not after embryonic development, when cells express other terminal selector genes (Zuryn et al., 2012; Patel and Hobert, 2017). However, Hobert showed how, in loss-of-function mutants removing *unc-3* activity, which is the terminal selector of A/B type motor neurons, ectopic cells expressing ASE neuron markers were obtained after overexpressing the ASE terminal selector CHE-1. This result could be repeated using other terminal selector mutants and suggests that terminal selectors are not only important to trigger terminal differentiation but also to restrict aberrant expression of other programmes, possibly via an H3K9 methylation-based mechanism (Patel and Hobert, 2017).

Work presented by Moritz Mall (Stanford University, USA) also emphasised the importance of efficiently switching off or preventing the expression of several somatic programmes during reprogramming. Mall has examined the role of the pan-neuronal zinc finger protein Myt1l, which features in the three-factor cocktail used to directly reprogramme MEFs into neurons. Although Myt1l bound to similar sites in both MEFs and neurons, it alone was not sufficient to elicit neuronal reprogramming. Structure-function experiments suggested that Myt1l acts as a transcriptional repressor, which is consistent with its interaction with Sin3B (Mall et al., 2017). Transcriptomic data suggest that many genes and possibly somatic expression programmes, including genes representing a fibroblast signature, are downregulated by Myt1l, and that neural genes are impoverished in Myt1l binding sites. Together, these data suggest that Myt1l facilitates neurogenesis and induced reprogramming by repressing non-neuronal expression programmes.

Why are certain cells amenable to reprogramming, whereas others are not? To address this issue, Sophie Jarriault (Institute of Genetics and Molecular and Cellular Biology, Strasbourg, France) presented her lab's efforts in investigating how the competence to reprogramme is promoted in a natural context, using the transdifferentiation of a rectal cell named 'Y' into a motor neuron named 'PDA' in *C. elegans*. The unpublished data supported a model in which a transient Notch signal is necessary and sufficient to endow a cell with the ability to reprogramme. However, this

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Notch signal can only be interpreted in a permissive intrinsic cellular context, and during a defined time window. Outside of this opportunity window, Notch signalling can exert the opposite effect – that is, to block reprogramming – by modulating the balance between the reinforcement of the initial identity and the triggering of its erasure. This work underscores the importance of investigating the dynamics of cellular processes *in vivo*, as important variables in the local environment and the developmental timing can transform the cellular context and hence affect reprogramming, and offers clues as to the mechanisms that govern it.

Reprogramming endogenous brain cells

The ability to produce new neurons via forced reprogramming has opened up tremendous possibilities, in particular for cellular therapies. Such forced conversions can be performed *in vitro*, but also, and advantageously, *in vivo*. For both approaches, however, there are many experimental variables to consider, not least of which is the extent to which the proliferative status of the starting cell impacts the final identity of the reprogrammed cell. Other important issues include reprogramming efficiency, especially in absence of a lesion, as well as how new neurons can be coaxed to integrate into the existing neural network in a functional manner. An exciting series of talks on endogenous neural reprogramming brought important answers to these questions, underscoring the quick pace of progress in this area.

Neuronal replacement therapy has long been attempted using transplants of foetal neurons, but the extent to which these neurons can fully and adequately integrate into pre-existing circuitry remains unknown. Magdalena Götz (LMU and Helmholtz Center Munich, Germany) has recently demonstrated that this is indeed possible by transplanting foetal neurons into the adult murine visual cortex and monitoring both brain-wide input and output and the functional receptive field properties (Falkner et al., 2016). These exciting new data show that neuronal replacement therapy and the re-establishment of neuronal circuitry is theoretically feasible. But can this be achieved by direct neuronal reprogramming? Götz discussed how her group was able to attain more than 90% efficiency in reprogramming proliferating glial cells after a stab wound injury into pyramidal neurons, some showing deep layer specification (Gascón et al., 2016). She also showed unpublished data showing that non-proliferating astrocytes were converted at a similarly high rate into pyramidal neurons. Intriguingly, this approach worked well for astrocytes in some regions but not others, highlighting the importance of the starting cell type and even region-specific subtypes of the same cell type. Consistent with this theme, Guillermina Lopez-Bendito (CSIC, Alicante, Spain) found that reprogramming of cultured astrocytes from the thalamus resulted in the generation of thalamic neurons using the same transcription factors that instruct cortical neurons when expressed in cortical astrocytes *in vivo* or *in vitro* (Gascón et al., 2016; Heinrich et al., 2010). To develop this approach towards a therapeutic avenue to improve sensory deprivation, Lopez-Bendito is currently using transcriptomic profiling of thalamic nuclei to identify which transcription factors could be used to reprogramme somatic cells to a thalamic identity (Gezelius et al., 2016).

Maria Pereira (Parmar lab, Lund University, Sweden) emphasised how the environmental and cellular context influences the reprogramming process. Using an Ascl1, Lmx1a and Nurr1 (Nr4a2) (Caiazzo et al., 2011) reprogramming cocktail to induce direct reprogramming of NG2 (Cspg4)-positive oligodendrocyte progenitor cells, she observed that dopaminergic neurons were obtained from postnatal NG2 glia *in vitro*, whereas other neuronal types, including GABAergic parvalbumin-positive interneurons, are generated when targeting this population in the adult striatum *in vivo*. This underscores the importance of *in vivo* studies to determine the specific neuronal identities achieved by reprogramming, especially as many aspects of neuronal identity, such as long-distance axonal output and input from far-distant brain sites can be accessed only *in vivo*. In line with this, Benedikt Berninger presented work on the influence of starting cell type and extracellular context in direct reprogramming, this time looking at young postnatal versus adult proliferating glial progenitors. Berninger's work was surprising in that it showed that a single neurogenic factor was not sufficient for the successful neuronal reprogramming of even young postnatal proliferating glial cells *in vivo*, whereas they could readily be reprogrammed by only Neurog2 or Ascl1 *in vitro* (Heinrich et al., 2010). *In vivo*, the best reprogramming results even at this early postnatal stage were achieved in combination with Bcl2 as described for reprogramming in the adult brain (Gascón et al., 2016). However, the youngsters still had an advantage – namely, injury is apparently not required, whereas it seems to be required in the adult brain, at least for NG2 glia (Heinrich et al., 2014).

One of the key, unresolved issues regarding neural reprogramming is how closely the transition from somatic cell into neuron recapitulates natural developmental pathways. Marisa Karow found that Ascl1 or Sox2 alone fail to reprogramme human pericytes derived from the adult brain, but that the combination of both factors together is effective. To understand why this is the case, Karow performed transcriptomic analysis and found that neurogenic factors are not activated by either of the two transcription factors on their own, but only when they are expressed together. Single-cell sequencing unravelled a striking heterogeneity among pericytes, with only one subclass being amenable to reprogramming by Ascl1 and Sox2. Interestingly, the pericytes that failed to reprogramme showed signs of a diversion to a partial muscle identity, as observed for MEFs (Treutlein et al., 2016). The transcriptional analyses suggest that successful pericyte reprogramming starts with the loss of the pericyte expression programme, followed by expression of a transient set of so-called 'switch genes' upon which early and late neuronal genes are expressed. These intriguing switch genes could represent the establishment of a brief progenitor-like state as they are found expressed in progenitors during development but not in neurons. Thus, Karow's data may suggest that such a step-wise reprogramming process encompasses – at least partially – the deployment of a developmental-like programme to allow re-differentiation.

Concluding remarks

Our ability to manipulate cell fate – programming and reprogramming the brain as it were – depends much on our understanding of how neuronal cells are specified during development and after injury in the adult. It was clear from this meeting that cellular context is a crucial determinant of cell fate, and influences not only which cells can be reprogrammed, but also the factors used, the efficiency at which it occurs and sometimes the final identity adopted. Differences across species are expected, as well as between the *in vitro* and *in vivo* setting. Yet this meeting highlighted a number of common themes – for example, the importance of erasing previous cellular identity for efficient neuronal reprogramming, which appears to be true regardless of species. Another common theme was the presence and nature of barriers to reprogramming, and the step-wise transcriptional changes that proceed in several natural or induced reprogramming

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settings. Understanding the molecular basis that underpins the basic science of programming and reprogramming is of fundamental importance when it comes to translating this approach to the clinic, whether for disease modelling, drug screening or cell therapies. Although there is much that we still do not understand, it is both exciting and extremely encouraging to see the progress that this field has made in recent years, and thus it is with great anticipation that we look forward to future meetings on this theme.

Competing interests

The authors declare no competing or financial interests.

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