


Using chimaeras to study developmental processes: 28-30 Aug 2016, Edinburgh,
organisers: Jenny Nichols and Tristan Rodriguez

Sunday 28 August	
12.30 - 18.00	Registration - Foyer
12.30 - 14.00	Lunch - Centro
14.00 - 15.00	PL01 Plenary Talk - Pentland East Nicole le Douarin - College de France, Paris, France <i>Kindly Sponsored by Biology Open</i> Making chimeras in the avian embryo and using them to study development 
15.00 - 16.00	PL02 Keynote Talk - Pentland East Richard Gardner - Universities of Oxford and York, UK Investigation of cell lineage and differentiation in the early mouse embryo by blastocyst injection and reconstitution
16.00 - 16.30	Refreshment Break - Centro
16.30 - 18.00	Poster Session and Drinks - Pentland East / Centro
18.00 - 19.30	Dinner - Centro

Monday 29 August	
08.00 - 19.00	Registration - Foyer
07.30 - 09.00	Breakfast - JMCC Restaurant
Session 1 09.00 - 13.10	Lineage Tracing and Potency - Pentland East Chair: Sally Lowell
09.00 - 09.30	S01 Janet Rossant - Hospital for Sick Children, Toronto, Canada Single cell analysis of cell commitment in the preimplantation mouse embryo
09.30 - 09.45	O01 Clare Baker - University of Cambridge, Cambridge, UK Diverse embryonic and evolutionary origins for the hypoxia-sensitive cells of the vertebrate respiratory reflex
09.45 - 10.00	O02 Ben Steventon - University of Cambridge, Cambridge, UK Comparative Developmental Dynamics of Neuromesodermal Progenitors during Axis Elongation in Vertebrates

10.00 - 10.30	S02 Josh Brickman - Danstem, Copenhagen, Denmark Converting self-renewal in naïve pluripotency to naïve endoderm and back again
10.30 - 11.00	Refreshment Break and Exhibition Viewing Time - Centro
11.00 - 11.30	S03 Ginny Papaioannou - Columbia University, New York, USA The Tbx6 syndrome
11.30 - 12.00	S04 Berenika Plusa - University of Manchester, Manchester, UK Epiblast specification in mouse embryo, the role of Sox2 and Klf4
12.00 - 12.30	S05 Mike Clinton - Roslin Institute, Edinburgh, UK Using chimeras to study development of the sexual phenotype in chickens
12.30 - 12.45	O03 John West – University of Edinburgh, UK Cell lineages produced by stem cells that maintain the adult mouse corneal epithelium
12.45 - 13.00	S06 Filip Wymeersch - MRC Centre for Regenerative Medicine, Edinburgh, UK Transcriptome Analysis of Neuromesodermal Progenitors reveals Dynamic Temporal Changes in response to Wnt Signalling
13.00 - 14.00	Lunch - Centro
	Poster and Exhibition Viewing - Centro
Session 2 14.00 - 16.15	Signalling Mechanisms - Pentland East Chair: Vasso Episkopou
14.00 - 14.30	S07 Claudio Stern - University College London, London, UK A hunt for new 'organizers' uncovers the endoderm of the AIP as an organizer of the heart
14.30 - 14.45	O04 Alexander Bruce - University of South Bohemia, Czech Republic p38 mitogen-activated protein kinases (Mapk14/11) occupy a regulatory node governing entry into primitive endoderm differentiation during preimplantation mouse embryo development
14.45 - 15.15	S08 Chris Thompson - University of Manchester, Manchester, UK Coupling cell cycle oscillations, cell health and cell fate choice
15.15 - 15.45	S09 Nicola Harrison - NIAB-EMR, Kent, UK Interactions at the graft interface between rootstock and scion in apple trees
15.45 - 16.15	S10 Kim Dale - University of Dundee, Dundee, UK Regulation of cell fate choice in neuroectoderm progenitors by crosstalk between Notch and Sonic Hedgehog pathways
16.15 - 17.00	Refreshment Break and Exhibition Viewing Time - Centro
Session 3 17.00 - 18.30	Regenerative Therapy and Human Development - Centro Chair: Domingos Henrique
17.00 - 17.30	S11 Dennis Summerbell Lecture Iwo Kucinski - Gurdon Institute, Cambridge, UK The molecular signature of the loser cell status reveals key pathways regulating cell competition

17.30 - 17.45	O05 Nicholas Tan - MRC Centre for Regenerative Medicine, Edinburgh, UK DamID-seq as a novel tool to reveal genome-wide transcription factor binding with limited cell numbers during mouse development
17.45 - 18.15	S12 Hideki Masaki - Stanford University, USA Apoptosis-resistant primed pluripotent stem cells can contribute to chimera formation upon injection into pre-implantation embryos
18.15 - 18.30	O06 Man Zhang - SCRUM The University of Edinburgh, Edinburgh, UK Esrrb knock-in at the Nanog locus rescues development of Nanog-null germ cells
19.30 - 00.00	Conference Dinner - Playfair Library, Old College

Tuesday 30 August	
08.30 - 13.00	Registration - Centro
07.30 - 09.00	Breakfast - JMCC Restaurant
Session 4 09.00 - 10.15	Disease Models - Pentland East Chair: Val Wilson
09.00 - 09.30	S13 Megan Davey - Roslin Institute, Edinburgh, UK Regulation of SHH in the developing avian limb bud
09.30 - 09.45	O07 Elena Lopez-Jimenez – CNIC, Madrid, Spain Oct4 regulates the expression of Hox clusters in the gastrulating mouse embryo
09.45 - 10.00	O08 Steve Pollard - MRC Centre for Regenerative Medicine, Edinburgh, UK Efficient CRISPR/Cas9-assisted gene targeting enables rapid and precise genetic manipulation of mammalian neural stem cells
10.00 - 10.15	O09 Kenzo Ivanovitch - CNIC, Madrid, Spain Analysis of early heart development by Live-Imaging in Mouse
10.15 - 10.45	Refreshment Break and Exhibition Viewing - Centro
Session 5 10.45 - 12.45	Gene Function - Pentland East Chair: Ian Adams
10.45 - 11.15	S14 Bill Skarnes - Sanger Institute, Cambridge, UK Biallelic genome editing of human iPSCs at scale
11.15 - 11.30	O10 Timothy Weil – University of Cambridge, Cambridge, UK Controlling body axis formation through the spatial distribution of mRNA and translational activators
11.30 - 11.45	O11 Vasso Episkopou – Imperial College London, London, UK Dose-dependent Smad2/3 responses are gated by selective repression of target genes

11.45 - 12.15	S16 Liz Robertson - Oxford, UK An expanding role for Blimp1 in the mammary gland
12.30	Take away lunch and delegates depart

Meeting review: BSDB/Anne McLaren Memorial Trust 2016 Autumn Meeting

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Under the sponsorship of the [Anne McLaren Memorial Trust Fund](#) and [The Company of Biologists](#), the [BSDB Autumn meeting](#) organised by [Jenny Nichols](#) and [Tristan Rodriguez](#) took place in the Pollock Halls at the University of Edinburgh. The topic this year was: **‘Chimaeras and their use in studying developmental processes and disease models’**. Chimaeras are made of cells from two or more different organisms of the same or different species. Since their first conception, chimaeras have been an essential tool to dissect cellular potential and are used to address a large number of questions in developmental biology using a variety of different model organisms, from plants to vertebrates. Read here the meeting report by [Carla Mulas](#) and [Juan Miguel Sanchez Nieto](#).

The meeting kicked off with plenary talks by Professors [Nicole le Douarin](#) and [Sir Richard Gardner](#), both responsible for key innovations using avian and mouse chimaeric embryos respectively. Their ideas and work led to remarkable discoveries that have been essential not only for our current knowledge in the area of developmental biology, but also to shed light on key biological concepts such cell fate and plasticity. Nicole le Douarin presented her earlier work on the use of chick-quail chimaeras and the realisation that their different nuclear organisation could be used as a marker to distinguish host versus graft cells.

She took the audience through the application of chimaeras to study the neural crest, revealing the large contribution of these cells to the development of anterior structures in vertebrates. This work remains relevant to the present day when mouse-human chimaeras have been used to trace neural crest in mammalian embryos ([Cohen et al. 2016](#)). Richard Gardner’s development of tools to enable injection of single cells into mouse blastocysts, and the successful development of the embryos thereafter, was essential to decipher clonal behaviour of cells during early mouse development – with important observations spanning the fields of embryology, epigenetics and embryonic stem cell biology.

Through the following two days, five sessions saw a wide range of applications of chimaeras discussed in various model systems. Overall, the talks were a mixture of traditional uses of chimaeras, recent innovations within this historical context and a broad range of other ideas and approaches – incorporating both the weird and the wonderful!

The first and largest session, *LINEAGE TRACING AND POTENCY*, focused on the use of modern labelling and imaging techniques in order to trace the descendants of specific cells, which were either labelled *in situ* or challenged by transplantation. What was particularly interesting in this session was how

“...**Claire Baker** presented work on cells responsible for sensing hypoxia in vertebrates, trying to resolve, by a combination of lineage tracing and deletion studies, whether homologous cells in different organisms share a common embryonic origin.”

“...**Virginia Papaioannou's** tour de force on her analysis of T-box genes and their importance as an example of harnessing knowledge from the study of development to understand the human condition.”

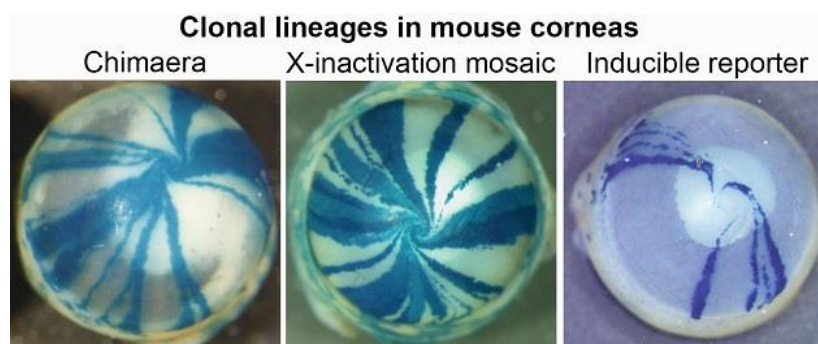
“In the **SIGNALLING MECHANISMS** session that followed, the speakers covered the influence and relevance of signalling pathways regulating cell fate choice.”

universal the application of chimaeras is in different organisms and at different developmental stages. For example, **Clare Baker** presented work on cells responsible for sensing hypoxia in vertebrates, trying to resolve, by a combination of lineage tracing and deletion studies, whether homologous cells in different organisms share a common embryonic origin. Both **Janet Rossant** and **Berenika Plusa** used chimaeras as tools to assess the changes in cellular potential within the mouse pre-implantation embryo as it undergoes the first two cell fate decisions. Janet explained the correlation between the plasticity of cells from the inner cell mass (ICM) and Hippo signalling pathway combining classic chimaera experiments with single cell sequencing technologies to probe deeper into questions of cell fate and cell potency in the pre-implantation mouse embryo. Berenika discussed the different roles of Sox2 and Klf4 in regulating the fate choice that ICM cells make between epiblast and primitive endoderm. Staying with the early mouse embryo, **Josh Brickman** argued the importance of specific nutrients in the media to support the maintenance of the naïve pluripotent and endodermal states as well as increasing the efficiency of chimaera formation. After this talk came another highlight of the meeting, **Virginia Papaioannou's** tour de force on her analysis of T-box genes and their importance as an example of harnessing knowledge from the study of development to understand the human condition.

From here we moved back to avian models, where **Mike Clinton**, used

mixed-sex chimaeras in chickens to study how the host influences the grafted tissue. He investigated how sex identity is specified, showing that somatic cells possess an identity which is cell autonomous. To round off the session, two speakers illustrated the enormous power of chimaeras and lineage labelling to uncover the boundaries for cell fate determination and cell plasticity, **John West** in the adult mouse cornea and **Filip Wymeersch** for the neuromesodermal progenitors.

In the **SIGNALLING MECHANISMS** session that followed, the speakers covered the influence and relevance of signalling pathways regulating cell fate choice. **Claudio Stern's** hunt for a new universal organiser's signature opened probably the most diverse session of the conference. **Alexander Bruce** presented his work, in which he identified p38 as a regulator of primitive endoderm differentiation in the early mouse embryo (**Thamodaran and Bruce, 2016**). **Chris Thompson**, conversely, used *Dictyostelium* as a model system to interrogate how genetically uniform systems can break symmetry and undergo differentiation. In this session there was also exciting insight provided by plant chimaeras. **Nicola Harrison** discussed the implications of the technique of grafting in apple trees and how our understanding of this process may affect the quality of the product and the yield of the crops. **Kim Dale**, the last speaker of this session, presented her work on how Notch amplifies Shh signalling pathway in the neural tube regulating the cell fate of neuro-ectodermal progenitors (**Stasiulewicz et al. 2015**).



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“Throughout the last two sessions, DISEASE MODELS and GENE FUNCTION several speakers discussed the advances in modern techniques and their applications to developmental biology and potential regenerative therapies.”

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On day two, the *REGENERATIVE MEDICINE & HUMAN DEVELOPMENT* session was started with a talk by **Iwo Kucinski**. Iwo was awarded the first Dennis Summerbell Lecture Prize, and he presented his work deciphering signalling pathways favouring the elimination of unfit cells in the process of cell competition. Interestingly Iwo showed that unfit cells, identified by a variety of means, show a number of common signalling changes that are not detrimental for viability when surrounded by other unfit cells, but that trigger their elimination when in a competitive environment with fitter cells. **Nicholas Tan** presented a novel strategy (DNA Adenine-Methyltransferase Identification sequencing) to identify genome-wide transcription factor binding targets within single embryos or with samples that have only 1000 cells and **Man Zhang** discussed the importance of ESSRb for Nanog function. Unfortunately, Hiro Nakauchi could not attend the meeting but **Hideki Masaki** flew in from Japan to present his own work in collaboration with the Nakauchi lab. Very interestingly, they observed that primed pluripotent stem cells with acquired resistance to apoptosis can contribute to chimaeras when injected into blastocysts, a process that does not occur when attempted with wild-type primed pluripotent cells. These experiments suggest that not only the pluripotency status of the cells is important for efficient chimaera formation but also their apoptotic threshold, thus providing an avenue for efficient chimaera generation with cells with restricted developmental potential.

Throughout the last two sessions, *DISEASE MODELS & GENE FUNCTION* several speakers discussed the advances in modern techniques and their applications to developmental biology and potential regenerative therapies. For instance, **Stephen Pollard** and **Bill Skarnes** outlined CRISPR-based approaches to generate genetically modified adult and embryonic stem cells while **Ben Steventon** and **Kenzo Ivanovich** demonstrated beautiful applications of live imaging to study the development of

neuro-mesodermal progenitors during axis elongation and the early stages of mouse heart development, respectively. **Vasso Episkopou** presented her work analysing how Arkadia modulates the levels of TGFb signalling during early mouse development and **Elena Lopez-Jimenez** discussed how Oct4 is not only a pluripotency factor, but can provide positional information by regulating the Hox cluster. To complement these approaches, **Megan Davey** gave a fascinating insight into how chick chimaeras can tell us not only about the signalling inputs that pattern the vertebrate limb, but also shed light into the evolutionary origins of our five digit structure. The grandiose finale of the meeting was **Liz Robertson**, who discussed the importance of Blimp1 in the control of mammary gland development and homeostasis. Interestingly she showed how important Blimp1 is for the organization of this epithelial tissue, providing new insight into the regulation of mammary gland tissue integrity ([Ahmed et al. 2016](#)).

Throughout the meeting there were many fond tributes to **Anne McLaren**, who, amongst her many other accomplishments, previously organised a chimaera-themed meeting in the early 90s. A great scientific atmosphere was created during all the poster sessions that ran throughout the breaks and during the evenings, where everybody had the chance to present their work, learn, discuss and network. Reflecting the beauty of developing systems, chimaera and embryo-inspired artwork was on display and available for purchase, designed by **Mia Buehr** [[LINK](#)] and **Aurora Lombardo** [[LINK](#)].



Overall, it was a brilliant and diverse meeting that took the audience literally through time, from the earlier discoveries and innovations presented by the keynote speakers, towards the current state, where modern techniques are allowing a new generation of developmental biologists to explore deeper into development and disease by using chimaeras.

Acknowledgements

We would like to thank the meeting organisers and sponsors, especially the Anne McLaren Memorial Trust Fund and the Company of Biologists. We apologize to all the speakers and references that are not mentioned directly owing to space limitations.

References

Ahmed M.I., Elias S., Mould A.W., Bikoff E.K. and Robertson E.J. (2016) The transcriptional repressor Blimp1 is expressed in rare luminal progenitors and is

essential for mammary gland development. *Development* 143: 1663-1673 [doi: [10.1242/dev.136358](https://doi.org/10.1242/dev.136358)]

Cohen M.A., Wert K.J., Goldmann J., Markoulaki S., Buganim Y., Fu D. and Jaenisch R. (2016). Human neural crest cells contribute to coat pigmentation in interspecies chimeras after in utero injection into mouse embryos. *PNAS* 113. 1570-75 [doi: [10.1073/pnas.1525518113](https://doi.org/10.1073/pnas.1525518113)]

Stasiulewicz M., Gray S.D., Mastromina I., Silva J.C., Björklund M., Seymour P.A., Booth D., Thompson C., Green R.J., Hall E.A., Serup P. and Dale J.K. (2015) A conserved role for Notch signaling in priming the cellular response to Shh through ciliary localisation of the key Shh transducer Smo. *Development* 142: 2291-2303 [doi: [10.1242/dev.125237](https://doi.org/10.1242/dev.125237)]

Thamodaran V and Bruce A.W. (2016) p38 (Mapk14/11) occupies a regulatory node governing entry into primitive endoderm differentiation during preimplantation mouse embryo development. *Open Biol.* [doi: [10.1098/rsob.160190](https://doi.org/10.1098/rsob.160190)]