THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Synthetic biology: Rational pathway design for regenerative medicine

Citation for published version:

Davies, J 2015, 'Synthetic biology: Rational pathway design for regenerative medicine', *Gerontology*.
<https://doi.org/10.1159/000440721>

Digital Object Identifier (DOI):

[10.1159/000440721](https://doi.org/10.1159/000440721)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Gerontology

Publisher Rights Statement:

This is the peer-reviewed but unedited manuscript version of the article: The final, published version is available at <http://www.karger.com/Article/FullText/440721>

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Synthetic biology:

Rational pathway design for regenerative medicine

Jamie Davies

University of Edinburgh

Address: Prof Jamie Davies, Centre for Integrative Physiology, Hugh Robson Building, George Square, Edinburgh EH8 9XB, UK. e-mail jamie.davies@ed.ac.uk

Running title: Synthetic biology and regenerative medicine.

Abstract

Rational pathway design is the invention of an optimally efficient route from one state (eg chemical structure, state of differentiation, physiological state) to another, based on knowledge of biological processes: it contrasts with the use of natural pathways that have evolved by natural selection. Synthetic biology is a hybrid discipline of biology and engineering that offers a means for rationally designed pathways to be realized in living cells. Several areas of regenerative medicine could benefit from rational pathway design, including derivation of patient-specific stem cells, directed differentiation of stem cells, replicating physiological function in an alternative cell type, construction of custom interface tissues, and building fail-safe systems into transplanted tissues. Synthetic biological approaches offer the potential for construction of these, for example controllable ex-vivo stem cell niches, genetic networks for direct transdifferentiation from adult fibroblast to restricted stem cell without going via iPS cells, signalling pathways for realizing physiological regulation in alternative cell types, morphological modules for producing self-constructing novel 'tissues', and 'kill-switches' for therapeutically-applied stem cells. Given the potential of this approach, a closer convergence of the regenerative medicine and synthetic biology research fields seems timely.

Keywords:

stem cell, niche, differentiation, transdifferentiation, safety, synthetic morphology, diabetes, inverse pharmacology.

1) Introduction: defining key terms.

Regenerative medicine

Most readers of *Gerontology* will be familiar with the scope of regenerative medicine: the field aims to restore lost function to tissues, organs and organisms that have been damaged by trauma, disease or ageing. Researchers are currently working on several different strategies for regenerative medicine, including (i) pharmacological stimulation of repair by division of a tissue's undamaged differentiated cells or by differentiation of endogenous stem cells [1], (ii) addition of appropriately potent stem cells into damaged host tissue to directly replace damaged cells [2], (iii) addition of cells that secrete pro-regenerative signals to endogenous cells in damaged host tissue [3] and (iv) *ex corporo* construction of new tissues or organs from stem cells, followed by transplantation to the host [4]. There have been a few spectacular successes, notably regeneration of the haematopoietic system by transplanted stem cells [5] and growth of skin *ex corporo* to treat patients with serious burns [6]. In many other areas, progress has been frustratingly slow, hampered mainly by our inability to control endogenous cells as predictably as we would wish, and our inability to create from iPS cells uniform populations of cells with exactly the right potency for an application without creating many cells in unwanted states [7]. These frustrations are the main reason for a growing interest in new approaches, including those of synthetic biology.

Rational pathway design

The term 'rational pathway design' encapsulates several important engineering concepts. In its most general sense, a 'pathway' is a series of steps that lead from one state to another, together with the means of transition between these steps. The states might be chemical (eg a metabolic pathway), molecular-genetic (eg a differentiation pathway), anatomical, physiological or psychological: all of these examples are relevant to gerontology and regenerative medicine. The word 'design' implies active invention of a path, rather than making do with what has already evolved processes. Finally, the word 'rational' implies invention according to a clear plan rather than, for example, making millions of random mutations to an organism or cell in the hope that one mutation turns out to be useful.

Synthetic biology

As Justice Potter Stewart once remarked of pornography, synthetic biology is difficult to define but easy to recognize. The discipline, now over a century old [8], has many different aspects, from attempts to create life from non-living chemicals [9], to engineering metabolic pathways to optimize

production of biofuels [10], to engineering cells to express morphogenetic behaviours and create artificial 'tissues' [11]. What all of these have in common is the use of well-defined 'building blocks' (genes etc) to assemble larger-scale systems. The value of synthetic biological approaches to rational pathway design is obvious: where rational pathway design sets the aim, synthetic biology might offer the means of achieving it.

2) Opportunities for rational pathway design in regenerative medicine

Researchers in regenerative medicine are interested in a number of pathways, each of which is essentially one part of the overall pathway from the damaged/aged state to the regenerated one.

Efficient derivation of appropriately potent stem cells

At present, the most common route chosen to (attempt to) obtain stem cells appropriately potent for a regenerative application is to make induced pluripotent stem (iPS) cells from the differentiated cells of a patient or experimental animal, and then to differentiate those cells along a desired pathway, for example into neuronal or cardiac progenitor cells [12]. This includes two pathways, neither of which is particularly efficient (Fig 1a). One proceeds from the differentiated state to the iPS state, and the other generally proceeds via a recapitulation of the sequence of differentiation steps along one lineage in an embryo, for example from epiblast to mesoderm to intermediate mesoderm to nephrogenic cell [13]. The sequence is usually directed by application of a sequence of pure growth factors and similar signals to mimic the changing environment of the parts of a developing embryo that would give rise to the desired cell type in normal development. Again, and especially for cell types that arise only late in normal development (eg progenitors for kidney), this process is inefficient and typically results in a mix of desired and undesired cell types. Applying rational design principles to making these differentiation pathways more reliable might be very beneficial.

The widely used approach of making appropriately potent stem cells by de-differentiating differentiated adult cells to iPS cells, then directing them along a normal developmental pathway, is based on the evolved logic of the embryo. Theoretical treatments of genome-sized genetic networks identify cell states with basins of attraction of a complex system, and suggest that there may be many different routes into each basin [14]. Transitions from one basin to another depend on defined changes being made to the genetic network, for example the forcing 'on' of a gene by external means such as a signalling molecule. Some of the genes of the network encode signalling molecules

and receptors, and the differentiation paths available in a normal embryo are set by the interplay of signal/ receptor production by cell states, and vulnerabilities of cell states to being forced to change on receipt of signals. These models have two implications for rational pathway design. The first is that it may be possible to transition from, say, the cell state corresponding to a skin fibroblast to that of, say, a neuronal stem cell by a route that does not involve going via the iPS state but is direct [15]. Such a transition would not be available to a normal embryo because there is no signal that can change gene expression the correct way: artificial signals may well be able to do so (Fig 1b). The existence of rare transdifferentiation events, in which differentiated cells change state to that of another tissue without recapitulating embryogenesis, in both fruitflies and mammals [16], gives more weight to this idea. Natural transdifferentiation events are far too infrequent to be of practical use; the point of using artificial signals would be to make them much more reliable and/or to make sorting for the rare successes amongst thousands of failed cells much easier. The other implication of the models is that there may be some basins of attraction (= cell states [14]) that are encoded by the genome that are never visited in normal development. If we can develop signals that will lead from one normal basin to one of these, some normally unvisited cell states might be useful. To summarise, there may be scope for developing pathways for generating desired cells that do not require iPS intermediates.

Avoiding regeneration of a vulnerable structure

Some diseases result from autoimmune destruction of an important tissue; an obvious example is type I diabetes in which pancreatic beta cells are destroyed by a patient's immune system [17]. In a case like this, the idea of regenerating the missing tissue has the obvious problem that the replacement would be at a similar risk to the original; at the very least, the patient would have to be under strong immunosuppression. The actual pathway required is one that leads from a patient who cannot make adequate amounts of insulin in an appropriately regulated manner, to a patient who can. Regeneration of pancreatic beta cells does not, logically, have to be part of this pathway: making some other cell, still tolerated by the patient's immune system, produce insulin under appropriate regulation would reach the same goal without the risk of immune attack.

Construction of custom tissues

One important limit of conventional stem cell-based approaches is that they lead to the construction of 'normal' tissues, in the sense of tissues that exist in the evolved developmental repertoire. Where a patient requires something abnormal, for example to ameliorate a congenital abnormality, or to

interface the nervous system with an artificial limb, non-evolved tissue forms will be needed. Surgeons have, to some extent, managed to create these at least at a coarse scale, but production of self-constructing artificial 'tissues' would be a major step forward. In analysing normal development, embryologists have shown that most structures are built by a relatively modest number of developmental mechanisms, happening to different extents and in different orders [18]. These events include cell proliferation, elective cell death, cell locomotion, chemo- and hapto-taxis, cell adhesion, epithelium-to-mesenchymal transition, mesenchymal to epithelial transition, and sheet folding. Using rational pathway design to combine activation of these natural mechanisms in new sequences might provide a pathway to self-constructing custom tissues [19].

Safety

One of the worries that haunt those who aim to introduce stem cells into a patient's body, especially if those cells have been engineered in some way, is that they will misbehave to produce, for example, a neoplasm. One desirable feature would be to include a 'kill switch': a pathway by which introduced cells could be eliminated by a physician-applied signal (eg a specific combination of drugs), should things go really wrong. Even more desirable might be a mixed population of introduced stem cells, each harbouring a different kill switch so that only a physician can eliminate only the clone that gave rise to a problem, leaving the others in place to do their job.

3) Possible synthetic biological approaches to realizing these pathways.

Better programming via the iPS route

When cells differentiate in a real embryo, they are normally in relatively small populations surrounded by a range of different cells with which they interact using bidirectional signal exchanges. In general, these signal exchanges involve feedback that controls the proliferation, self-renewal, differentiation and death of the stem cell population and balances the populations of different cell types in the embryo: the essence of so-called regulative development [20]. When experimenters try to direct the development of iPS cells in culture, they typically treat the cells with soluble factors presented at a fixed concentration in bulk medium; at the very best, this mimics only one half of the conversation with no feedback. One strategy for improving on this would be to mimic the natural environment of the embryo with a living stem cell niche. This would be

composed of cells engineered, by synthetic biological means, to receive signals from the differentiating iPS cells (ie detect their state, whether by secreted or contact-mediated influences) and to signal to them in return, using the normal signals that they would receive in an embryo. This would bring in natural feedback processes. The synthetic genetic circuits in the niche cells could also, of course, include receptors for signals provided to the system by experimenters to change from one phase of signalling to another.

Management of non-embryonic differentiation pathways

Non-embryonic differentiation pathways – that is, transition from one differentiated state to an unrelated one without proceeding via an ES/iPS cell state – rely on the induction of specific sets of transcription factors characteristic of the destination cell type. For example, forced expression of *Ascl1*, *Brn2* and *Myt1l* is sufficient to convert mouse embryonic fibroblasts into neurons [21]. The efficiency of this conversion is, however, not great, varying between 1.8 and 7.7% in runs of the experiment [21]. One possible reason for this is non-optimal and possibly variable expression level of the introduced genes. Synthetic constructs might make the process much more reliable, particularly if they include feedback to clamp each of the three transcripts to the desired level. In the longer term, when the promoter/ enhancer structures of the endogenous versions of these genes are understood better, it may be possible to design synthetic transcription-activating proteins that can be introduced into human fibroblasts to transdifferentiate them, without introducing new DNA into the cells.

Replicating function in an alternative cell type

The idea of replicating a critical physiological regulatory process, such as control of glucose uptake, in a new and safe cell type practically depends on rational pathway design. While an autonomous synthetic biological glucose regulator example has not yet been constructed in its entirety, sufficient progress has been made to illustrate the potential power of the approach. In normal physiology, the hormone glucagon-like peptide (GLP1) reduces blood glucose [22]. In a mouse model of type II diabetes, administration of GLP-1 reduces abnormally high blood glucose. Ye and colleagues [23] constructed a synthetic signalling pathway in which blue light activates melanopsin which, by signalling via protein kinase C, calcineurin and NFAT, drives the expression of GLP-1 (Fig 2). When this system is built into the human embryonic kidney 293 (HEK-293) cell line, exposure to blue light drives GLP-1 production. If these cells are transplanted subcutaneously into diabetic mice, illumination of the mice produces a controlled reduction of blood glucose after a sugary meal

[23]. The light-sensitivity opens the door to an electro-photo-biological feedback system, in which measurements of physiology can be processed electronically and fed back to a synthetic biological effector by light.

Ultimately, it would be useful to build feedback into the synthetic constructs themselves, with no need for triggering by light. This has not been done for blood sugar, but it has been done for control of blood urate by urate-triggered production of a urate oxidase [24]: the engineered system has a beneficial effect in an animal model of hyperuricemia. The synthetic system involved (Fig 3) used a urate sensor from *Deinococcus* and a urate oxidase from *Aspergillus*, and operated in mammalian cells: this cross-phylum approach is typical of contemporary synthetic biology.

Custom tissues

A few years ago, my laboratory published a speculative paper that identified single driver genes that could activate common types of morphogenetic cell behaviour: the paper sketched how these genes might be built into simple synthetic modules for 'synthetic morphology' [19]. We have now built some of these modules and have shown that they function as intended, at least at the level of cell behaviour in culture [25]. Specifically, they confer on human cells controllable proliferation, apoptosis, adhesion, locomotion and formation of syncytia. The next experimental stage is the combination of cells carrying different modules to try to produce simple artificial 'tissues', and combination of the cells with normal cells to test the possibility of their making defined, designed connections. This work remains a long way from production of practical synthetic tissues, but it may turn out to be a first step towards that goal.

Safety devices

When stem cells are introduced into human recipients, especially after any genetic modification, there is a risk that they will behave pathologically, in particular by generating a neoplasm. One means of controlling this risk would be building a "kill switch" into the cells so that they can be eliminated by a specified externally-applied signal. Such a switch, using an inducible caspase to drive apoptosis, has already been built and tested in an experimental clinical trial in the field of immunology [26].

Clearly, whatever pharmacological signals are given to activate a 'kill switch' in a patient, they will have to be safe drugs. Given that clinical trials to prove the safety of drugs are so expensive, and

that if all goes well the use of a kill switch will not even be necessary, development of new drugs for this specific purpose is an unrealistic expectation. One approach to solving this problem, being explored experimentally in the author's laboratory, is 'inverse pharmacology'. In normal pharmacology, a target is identified, small molecules are screened for a desired agonist or antagonist effect, and candidates are screened for safety and practical utility until one is hopefully developed to the stage of being a fully-approved clinical drug. 'Inverse pharmacology' takes the opposite approach. The sites of action of many approved drugs on their protein targets are well understood and, in a significant number of cases, transfer of the drug-binding protein motif to another, engineered, protein should confer drug sensitivity to that protein too. An archetypal example is the use of the tamoxifen-binding domain of an estrogen receptor to the cre recombinase, resulting in a cre that enters the nucleus only when cells are treated with tamoxifen [27, 28]. Use of this general approach could produce synthetic circuits that are sensitive only to combinations of already-licensed drugs, thus avoiding the problem of additional safety testing.

It must be pointed out that all 'kill switches' have the problem that any cell that has somehow shut the kill switch system off, for example by a chromosomal part-deletion or by making the region heterochromatic, would escape. With its neighbouring cells obediently dying, an escaping cell could be at a significant competitive advantage and, especially if the problem were neoplastic growth, this could make the problem worse. Multiple kill switches may reduce the risk of this, but nothing can eliminate the risk altogether.

One final safety-related topic is the risk that the synthetic biology approach itself might carry dangers. These include neoplasia but there may be additional problems that have not been foreseen – the 'unknown unknowns' and unintended consequences that accompany all new technologies.

Concluding remarks

This mini-review has necessarily been largely speculative, because the field of mammalian synthetic biology is young [29]: its ideas are far more numerous than achievements. Nevertheless, the rational pathway approach to designing new biological mechanisms to meet regenerative challenges may turn out to be faster than using only evolved paths. At present, the synthetic biology and the regenerative medicine communities are largely separate. It may, at the very least, be wise to use conference invitations to bring them together and see what ideas and potential solutions to clinical problems emerge.

Figures

Figure 1: Natural and rational pathways for stem cell generation. (a) In the naturally inspired pathway common in contemporary regenerative medicine research, an adult cell such as a fibroblast is turned into an induced pluripotent stem cell (iPSC) and a population of these cells is differentiated into a desired cell type through the same sequence of intermediate cell types that is produced during normal embryogenesis. Many of these intermediate cell types are themselves multipotent, and unwanted cell types are commonly produced along with the desired one. (b) In induced transdifferentiation, an adult cell is treated with a set of transcription modulators to take its transcriptional state to the one of the desired cell type without the need to proceed via the iPSC stage.

Figure 2: The glucose-regulating opto-biological system of Ye and colleagues [23]. Melanopsin, not normally present in HEK cells, is activated by blue light and transduces a signal via phospholipase C (PLC), protein kinase C (PKC), calmodulin and calcineurin to the transcriptional activator NFAT, which activates an engineered promoter-gene complex to drive the production of glucagon-like peptide 1 (GLP-1).

Figure 3: The urate-regulating synthetic circuit of Kemmer and colleagues [24]. A urate transporter, a urate sensitive DNA binding protein, and an engineered promoter-operator-gene complex, are introduced into cells. With no urate, the DNA-binding protein binds the operator site in the DNA and inhibits the transcription of urate oxidase. With urate present, it binds urate instead, freeing the operator and allowing the urate oxidase to be transcribed. Following translation, the enzyme destroys urate, returning the system to its starting condition.

References

1. Aggarwal S, Moggio A, Bussolati B. (2013) Concise review: stem/progenitor cells for renal tissue repair: current knowledge and perspectives. *Stem Cells Transl Med.* 2(12):1011-9.
2. Li L, Black R, Ma Z, Yang Q, Wang A, Lin F. (2012) Use of mouse hematopoietic stem and progenitor cells to treat acute kidney injury. *Am J Physiol Renal Physiol.* 302(1):F9-F19.
3. 25752945 Huang W, Lv B, Zeng H, Shi D, Liu Y, Chen F, Li F, Liu X, Zhu R, Yu L, Jiang X. (2015) Paracrine Factors Secreted by MSCs Promote Astrocyte Survival Associated with GFAP Downregulation After Ischemic Stroke Via p38 MAPK and JNK. *J Cell Physiol.* 2015 doi: 10.1002/jcp.24981 (epub ahead of print)
4. Davies JA, Chang CH. (2014) Engineering kidneys from simple cell suspensions: an exercise in self-organization. *Pediatr Nephrol.* 29(4):519-24.
5. Annaloro C, Onida F, Lambertenghi Delilieri G. (2009) Autologous hematopoietic stem cell transplantation in autoimmune diseases. *Expert Rev Hematol.* 2(6):699-715.
6. Gardien KL, Middelkoop E, Ulrich MM. (2014) Progress towards cell-based burn wound treatments. *Regen Med.* 9(2):201-18.
7. Morales EE, Wingert RA. (2014) Renal stem cell reprogramming: Prospects in regenerative medicine. *World J Stem Cells.* 6(4):458-66.
8. Leduc S (1912) *La Biologie Synthétique. Etudes de Biophysique, 1912* (Ed. A Poinat), Paris.
9. Luisi PL (2006) *The emergence of life: from chemical origins to synthetic biology.* Cambridge University Press, Cambridge (UK).
10. 23659832 Wen M, Bond-Watts BB, Chang MC. (2013) Production of advanced biofuels in engineered *E. coli*. *Curr Opin Chem Biol.* 17(3):472-9.
11. Davies JA. (2008) *Synthetic morphology: prospects for engineered, self-constructing anatomies.*

12. Keller GM. (1995) In vitro differentiation of embryonic stem cells. *Curr Opin Cell Biol.* 7(6):862-9.
13. Takasato M, Er PX, Becroft M, Vanslambrouck JM, Stanley EG, Elefanty AG, Little MH. (2014) Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. *Nat Cell Biol.* 16(1):118-26.
14. Kauffman SA (1993) *The origins of order.* Oxford University Press, Oxford.
15. Tsunemoto RK, Eade KT, Blanchard JW, Baldwin KK. (2015) Forward engineering neuronal diversity using direct reprogramming. *EMBO J.* 2015: e201591402.
16. Slack JM, Tosh D. (2001) Transdifferentiation and metaplasia--switching cell types. *Curr Opin Genet Dev.* 11(5):581-6.
17. Morran MP, Vonberg A, Khadra A, Pietropaolo M. (2015) Immunogenetics of type 1 diabetes mellitus. *Mol Aspects Med.* 42:42-60.
18. Davies JA (2013) *Mechanisms of Morphogenesis (2nd Edition).* Academic Press, London.
19. Davies JA. (2008) Synthetic morphology: prospects for engineered, self-constructing anatomies. *J Anat.* 212(6):707-19.
20. Angerer LM, Angerer RC. (1999) Regulative development of the sea urchin embryo: signalling cascades and morphogen gradients. *Semin Cell Dev Biol.* 10(3):327-34.
21. Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Südhof TC, Wernig M. (2010) Direct conversion of fibroblasts to functional neurons by defined factors. *Nature.* 463(7284):1035-41.
22. Holz GG 4th, Kühtreiber WM, Habener JF. (1993) Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). *Nature.* 361(6410):362-5.

23. Ye H, Daoud-El Baba M, Peng RW, Fussenegger M. (2011) A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. *Science*. 332(6037):1565-8.
24. Kemmer C, Gitzinger M, Daoud-El Baba M, Djonov V, Stelling J, Fussenegger M. (2010) Self-sufficient control of urate homeostasis in mice by a synthetic circuit. *Nat Biotechnol*. 28(4):355-60.
25. Cachat E, Liu W, Hohenstein P, Davies JA (2014) A library of mammalian effector modules for synthetic morphology. *J Biol Eng*. 8(1):26.
26. Molldrem JJ, Lu S. (2014) A GVHD kill switch helps immune reconstitution. *Blood*. 123(25):3849-50.
27. Danielian PS, Muccino D, Rowitch DH, Michael SK, McMahon AP. (1998) Modification of gene activity in mouse embryos in utero by a tamoxifen-inducible form of Cre recombinase. *Curr Biol*. 8(24):1323-6.
28. Li N, Yousefi M, Nakauka-Ddamba A, Jain R, Tobias J, Epstein JA, Jensen ST, Lengner CJ. (2014) Single-cell analysis of proxy reporter allele-marked epithelial cells establishes intestinal stem cell hierarchy. *Stem Cell Reports*. 3(5):876-91
29. Aubel D, Fussenegger M. (2010) Mammalian synthetic biology--from tools to therapies. *Bioessays*. 32(4):332-45.

Glossary

Cre recombinase: an enzyme that causes recombination between LoxP sites introduced into the genome: depending on the orientation of the LoxP sites, Cre can be used to remove a gene flanked by LoxP sites, or to reverse its direction.

Feedback: control of a process by its own output, for example control of a heater by a room thermostat, or control of insulin production by plasma glucose.

Genetic network: any network in which the activity of individual genes determines whether other genes in the network are transcribed.

Inverse pharmacology: engineering of proteins to make them controllable by already known and established drugs.

Kill switch: a cellular system designed to kill the host cell on command, eg by a drug.

Synthetic biology: engineering new complex functions into living systems (also, engineering life from non-living components, though this sense is not discussed in this article).

Synthetic morphology: a sub-discipline of synthetic biology dedicated to engineering novel morphogenetic (= anatomy-creating) processes into cells.

Transdifferentiation: the differentiation of one cell type into another without proceeding via a pluripotent (= early embryo-like) intermediate state.