Experimental Standards: evaluating success in stem cell biology

I. Experiments

Experiments in scientific practice involve diverse components, lack clear boundaries, and bring new phenomena into existence.[1] Their components include materials, technologies, protocols, concepts, human interventions, and standards for success. Results are generated by elaborately contrived assemblages of heterogeneous ingredients. But exhaustive inventory of these ingredients in any particular case is difficult. Experiments do not have pre-established beginnings or ends. Their boundaries are negotiable. Practices of experimentation unfold at multiple spatio-temporal scales and diverse levels of social organization. The latter range from individual investigators or small teams, to institutes, long-running collaborations, subfields dedicated to a particular model organism or technique, extending to research initiatives of national or international scope. The results of experiments, at all these levels, are often new phenomena. Novel products of experiment alter the landscape of possibilities considered in assessing evidential and relevance relations. So experiments can transform both the world and our epistemic situation, via what Pickering calls the mangle of practice.'

These features, which make experiment an immensely productive way of gaining knowledge, also render it refractory to traditional methods of epistemic evaluation. An experiment yields evidence for or against a hypothesis only within a specific context of materials, methods, background assumptions and epistemic values. If the components and boundaries of experiments are in flux, and rapid generation of new results continuously alters the possibilities relevant for confirmation, then the relation of evidence to hypothesis is one of

[1] See Hacking 1983, Galison 1987, Rheinberger 1997, Steinle 2002, Radder 2003.

ongoing mutual adjustment rather than test. In fields driven by experiment, knowledge is produced by construction and alteration of experimental arrangements. New phenomena produced by these experiments alter the space of possibilities, inducing conceptual, evidential and technological changes. An experiment-driven field is, from the perspective of practice, a feed-forward system for producing new results, both abstract and material.

In particular cases, the ongoing mutual adjustment of experimental components, results, and hypotheses can be traced over time. The result is thick epistemic history, describing patterns in a complex, continuously changing field of experimental practices.[2] But on this approach, an evaluative or critical stance toward the field in question is difficult to ground. One can describe changes in various aspects of experimental practice, at various levels of social organization. For example, hypotheses in experiment-driven fields change rapidly, in response to new concepts and technologies as well as data. Many view such changes as epistemic progress. However, it is not obvious how to demonstrate this, if experimental arrangements, results and evidential standards are all undergoing transformation. Our sense of progress might be illusory, an artifact of hope and hype. More generally, though epistemic changes in experimental histories may be tracked and described, it is difficult to see how they can be evaluated without imposing philosophical assumptions on scientific practices.

This concern is particularly salient for the case of stem cell biology. That field is typical of experimental life science today in its interdisciplinarity, rapid turnover of hypotheses, opportunistic use of technology, and paucity of formal theories or general laws. But stem cell biology also has distinctive features that complicate the issue of epistemic evaluation.[3] General

[2] E.g., Rheinberger 1997, Creager 2002, Keating & Cambrosio 2003.
[3] For examples of the role of social, political and ethical values in stem cell biology, see Maienschein 2003, Brown et al 2006, Fox 2006, Martin et al 2008, Testa 2008, and Kraft 2009.

(i.e., non-technical) discussions of stem cell biology are dominated by themes of 'hope and hype' rather than ideals of truth, rationality or evidence. Stem cell biologists loudly and unabashedly proclaim their ambitions for a revolution in medicine, scientific stardom and profitable associations with industry. Ethical controversies, playing out differently in different national contexts, create a patchy landscape of political, bioethical and financial restrictions, which scientists must navigate to perform experiments at all. In this welter of socio-politcal values, epistemic considerations recede into the background, relegated to the esoteric workings of lab benches and the occasional unsettling production, such as human embryonic stem cells or Dolly the sheep.

This paper aims to bring the epistemic dimensions of stem cell experiments out of the background, and show that they can be critically evaluated. After introducing some basic concepts of stem cell biology, I set out the current "gold standard" for experimental success in that field (§2). I then trace the origin of this standard to a 1988 controversy over blood stem cells (§3). Understanding the outcome of this controversy requires attention to the details of experimental techniques, the organization of epistemic communities, and relations between the two (§4). With its resolution, a standard for experimental success was established for HSC research, which in turn serves as an exemplar for studies of other stem cells. This historical case study reveals a robust standard for experimental success in stem cell biology: to trace processes of development at the single-cell level, in the form of cell lineage hierarchies. Experiments conforming to this standard can be further critically assessed as means to the therapeutic end of stem cell research: use of stem cells to repair human organs and tissues.

2. Stem cells and "gold standards"

Stem cells are self-renewing sources for organismal development at the cellular level. They are defined by two capacities pertaining to cell division. The first is *self-renewal*: long-term production of more cells of the same type. The second is *differentiation*: production of more specialized cell types. The organs and tissues of multicellular organisms are composed of the differentiated descendents of stem cells. Small numbers of tissue-specific stem cells also persist at various sites in the adult organism, including skin, gut, bone marrow, muscle, and brain. These 'adult stem cells' function as storehouses for cell renewal in specific organs or tissues. Their pathological counterparts are cancer stem cells, the source cells that grow and multiply tumors, caricatures of organs.[4] Embryonic and fetal stem cells are artifacts of cell culture, the clonal descendants of cells extracted from organisms in early stages of development (as their monikers suggest) and cultured in vitro. Embryonic stem cells exist as cultured cell lines; they have no directly corresponding in vivo counterparts. A more recent artifact, the induced pluripotent stem cell, is the result of 'reprogramming:' introduction of genetic regulatory factors into an adult somatic cell, which induce a phenotype similar to that of an embryonic stem cell.

The above classifications are unlikely to stay fixed, but do indicate the present contours of the field of stem cell biology. Its two main branches are concerned, respectively, with adult and embryonic stem cells. The former are found in vivo, are difficult to culture outside the body, and exhibit limited self-renewal and differentiation potential. The latter are grown in culture, where they exhibit unlimited self-renewal and very broad differentiation potential. In light of these contrasts, it is unsurprising that the two branches of stem cell biology endorse different "gold standards" for experiments. The gold standard for embryonic stem cell research (now including research on induced pluripotent stem cells) is to create a cell line with the same

[4] The precise extent and characteristics of cancer stem cells are currently unknown. For many types of tumor, the existence of cancer stem cells is uncertain.

molecular features and cellular capacities as those of 'canonical' cultured embryonic cell lines The cellular capacities are unlimited cell division (self-renewal) and differentiation into all cell types of the adult organism (pluripotency); the molecular features are incompletely characterized.[5] In contrast, the gold standard for adult stem cell research is to extract cells from an adult organ or tissue, select the stem cells in the tissue using surface markers, transplant one cell immediately into a host animal, and observe self-renewal and reconstitution of the relevant tissue, organ, or cell type, for the lifespan of the animal.[6] More briefly: the avowed gold standard for embryonic stem cell research is creation of an artifact with certain (not-fully determined) features; while in adult stem cell research it is a method culminating in regeneration of some part of an animal.

Due to their demonstrated in vivo efficacy, adult rather than embryonic stem cells are the model of experimental success in the field. More specifically, the in vivo efficacy of *one* type of adult stem cell, the blood-forming or hematopoietic stem cell, is a model of experimental success. Blood stem cells (HSC) were the first non-cancerous mammalian stem cells to be isolated, and the first characterized at the molecular level. Until very recently, HSC were the only stem cells used in routine clinical practice, comprising the active ingredient, so to speak, in bone marrow transplantation. Though not the stem cells from which most is *hoped*, blood stem cells are our current exemplar of stem cells in *use*. The origins of the current gold standard for adult stem cell research, and the standard for experimental success in stem cell biology as a whole, are in HSC research. As per usual in biomedicine, research on human HSC was preceded by research on inbred mice. So, with regard to experimental success, mouse HSC research is the stem of stem cell biology.

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[5] Thomson et al 1998, 1145.
[6] Melton & Cowan 2009, xxvii.

3. History in the blood[7]

Blood stem cell research began in the 1960s as an offshoot of radiation research, itself a response to developments in 20th century physics that culminated in the Manhattan Project.[8] In the 1950s, researchers in the US and UK discovered that mice given lethal doses of γ-radiation survive if injected with bone marrow cells from a donor of the same inbred strain. One side-effect of 'radiation rescue' was the appearance of bumps, or nodules, on the spleens of transplant recipients. In the early 1960s, Canadian researchers discovered that splenic nodules contained diverse blood cells (red blood cells, granulocytes, macrophages, lymphocytes) all descended from a single donor cell – each nodule was a clone, or colony. HSC were operationally defined as spleen colony-forming cells. Scientists then began to design experiments to isolate and characterize this elusive cell type from mouse bone marrow.

In the late 1980s, two groups claimed to have accomplished this goal: a research team of five from the Radiobiological Institute in Rijswijk, the Netherlands, led by Jan Visser (in 1984) and three members of Irv Weissman's lab at Stanford University Medical Center in California (in 1988). The two reports describe similar methods consisting of three steps: cell sorting, functional assays and microscopic observation. In the first step, bone marrow cells are sorted into populations ('subsets') by size, density, and surface phenotype. Two technical innovations of the 1970s , monoclonal antibodies and fluorescence-activated cell sorting, allowed single cells to be sorted into discrete subpopulations according to expression of specific surface molecules. Next, these subpopulations are assayed for HSC capacities. Three types of assay are described: *in vitro* cell culture, which realizes differentiation potential for a specific cell type; spleen colony

[7] Portions of this case is described in more detail in Fagan 2007, 2010.
[8] Kraft 2009.

formation after transplantation, which indicates self-renewal and multipotency; and 'radiation rescue' of irradiated hosts, which realizes the capacity for full immune reconstitution (multipotency). In the third and final step, cell populations with increased HSC capacities relative to normal bone marrow are examined by EM and light microscopy. The end result is a cell population with known physical, surface, morphological and histological characteristics, exhibiting a quantifiable degree of HSC function. Both groups argued that their method yielded all and only HSC.[9]

Despite the overall similarities, there were differences in the details. Each method used one distinctive purification step, different surface molecules for cell sorting, and, most importantly, different standards to estimate HSC purification. The Rijswijk group calculated this quantity in terms of spleen colony-forming units, a modification of the original method for detecting HSC in mouse spleens. The Stanford group, in contrast, used the rate of whole-animal radiation rescue as the standard, focusing on the in vivo effect that initiated the entire line of research. Because of these differences, the two results were not directly comparable.

Yet scientists did compare them. Controversy ensued immediately upon publication of the 1988 report. The next issue of *Immunology Today* featured an openly skeptical editorial:

> But does this represent any advance on previously published data? Are these the real stem cells? Does the report merit its widespread coverage in the newspapers or is this yet another example of indiscriminate glamourization by some sections of the press?[10]

[9] Visser et al 1984, Mulder and Visser 1987, Spangrude et al 1988.
[10] Lord and Dexter 1988, 376.

Despite these concerns, the balance of scientific opinion eventually favored the 1988 report. Widely cited (1581), it is counted among 20 major "turning points in modern immunology."[11] Generalization of its method is currently the "most rigorous assessment" of adult stem cell characteristics.[12] Its first author contributed the chapter on isolation and characterization of HSC in the main textbook of stem cell biology; its last author is currently the President of the International Society for Stem Cell Research. The 1984 report, though respected among blood cell specialists, has had much less impact (cited 107 times).[13]

4. Establishing standards

The fame of the Stanford paper is puzzling, since two reports describe similar methods and claim the same result. Neither was retracted or dismissed; and they do not contradict one another. Moreover, the 1984 paper had priority, by a four-year margin. The normal "priority rule" that the first publication of a scientific result receives the preponderance of credit was clearly violated in this case.

The differential impact would not be surprising if there had been, at some point between 1988 and the present day, a consensus of HSC researchers that 1988 method yielded "the real stem cells" while the 1984 method did not. But there was no such consensus. Instead, within a year, there was broad agreement in the HSC community (including the Rijswijk and Stanford groups) that neither method yielded all and only HSC. This 'negative consensus' has persisted for thirty years, robust to all the technological advances and experimental refinements during that interval: *There is no widely agreed-upon procedure for isolating all and only HSC.* So, mere

[11] Special issue of *Immunological Reviews* (July 2002).
[12] Melton & Cowan 2009, xxvii.
[13] Citation counts from Web of Science (Nov 2009).

months after the HSC controversy began, it was over. A consensus, including the Stanford and Rijswijk groups, emerged that *neither* method yielded "the real stem cells," but a mixture of HSC and blood progenitor cells. [14]

Sociological factors are another possible explanation for the outcome of the HSC episode. The 1984 paper was published in the *Journal of Experimental Medicine*, the 1988 paper in *Science* – a more prestigious and widely read journal. Furthermore, Weissman drew additional attention to his result by holding a press conference, while the Rijswijk group did not alert the media. It is possible that the relative prestige of US and European science, or of the types of institutions involved, contributed to the difference. Determining which, if any, of these prima facie plausible explanations is correct is a task for empirical sociologists. My concern is not to show that one or another sociological explanation of the HSC episode is correct, but to show that, whatever sociological factors were in play, experimental methods and results also played a crucial role. The implications of a *purely* sociological explanation are unwelcome. If experimental methods and results did not play a decisive role in the establishment of stem cell biology's standards of experimental success, then the drivers of stem cell research are merely hope, hype and media attention. The HSC case cannot be dismissed as atypical or minor. As noted above, it provides the exemplar or model for isolation of other stem cells, setting the current standard in stem cell biology. The HSC episode was a turning point in the development of those standards.

A more satisfactory understanding of the HSC case emerges if we consider how each of the two groups participated in its wider scientific community. The Rijswijk group belonged to a community of medically-trained hematologists (blood cell experts), the Stanford group to the

[14] Spangrude 1989.

rising community of cellular immunology (experts on cells of the immune system). The hematologists formed a global network with centers in Toronto, Melbourne, Rijswijk, Manchester, and the Eastern US. Beginning with the spleen colony assay, these centers 'differentiated:' inventing new assays, variations on the original spleen colony theme. There was a division of labor among them. From the late 1960s, the role of the Rijswijk group was to isolate HSC. Other groups focused on the specific blood cell lineages, the biochemistry of regulatory factors in blood cell development, and the concept of a stem cell niche.

The Rijswijk group used a classic hematological approach: classify bone marrow cells into 'subsets' using "well-defined" cell properties (size, density, morphology, surface molecules, and more or less sensitivity to a given drug), then localize HSC to one 'subset.' They focused on physical and chemical properties of bone marrow cells, using classic methods of cell biology (density centrifugation and electron microscopy) to distinguish cell types. New technologies were added to existing techniques as they became commercially available. But the basic strategy remained the same: systematically survey the published literature for ways of non-lethally tagging blood cells, and test every available antibody or chemical marker for correlation with increased spleen colony formation. It was a lucid approach: all cells have some size, density, morphology, surface phenotype, and more or less sensitivity to a given drug. The trick was to find what combination of these general cell properties correlated with the ability to form spleen colonies. The markers themselves, however, were diverse, based on general properties of cells and available biochemical tags. Visser et al's ever-lengthening protocol for isolating HSC was the concatenation of fortuitous discoveries gleaned from the wider community. Their key bioassays measured colony-formation in spleen and on agar. In the late 1970s and early 1980s,

this standard for measuring HSC function was increasingly questioned in the wider community of hematologists.

So the Rijswijk group's standard diverged from that of the wider HSC community. This occurred because that community was differentiated with respect to its methods. This diversity, along with geographic distance, made coordination of results difficult. Rijswijk's functional assays were specific to Rijswijk. Elsewhere, however, hematologists were fitting their diverse results together, however, and coming up with unsatisfying results. As early as 1974, hematologists had questioned the identification of HSC with spleen colony-forming cells, due to inconsistencies in results from different laboratories. Gradually, the simple identification of HSC with spleen colony-forming cells was replaced with a "three-tiered model" of HSC, lineage-committed progenitor cells, and mature blood cells. Colony-forming cells could be the first, second or a mixture of the two, depending on experimental conditions. Once recognized as an ambiguous indicator of HSC, spleen colony-formation was replaced by a new defining standard: radiation rescue of cell transplant recipients. But the shift was slow and uneven, because the hematological community shared little beyond an interest in blood cells and a historical tie to the spleen colony assay. The Rijswijk group retained the old standard later than most.

The Stanford group participated in the immunology community in a quite different way. They were part of a large laboratory, which formed its own community within the larger context of cellular immunology. In the 1970s, there were two distinct immunology communities at Stanford's Medical Center: one biochemical, focused on Ab-Ag binding; the other investigating immune cells and genetics. The latter community was closely knit by formal and informal discussion groups, which fostered interactions between diverse laboratories and departments.

Stanford was a major center for immunology, and regular visits by prominent researchers linked it with the wider international field. Within this disciplinary context, the Weissman laboratory was distinguished by an emphasis on immune cell development, but not unified by a single aim or technique. The majority of members studied the development of immune function in cells from diverse lymphoid organs (blood, thymus, lymph nodes) using various methods (molecular genetics, biochemistry, cell biology, surgery, developmental biology). Collaborations emerged *in situ*, and were encouraged both within and outside the laboratory.

The Weissman lab did, however, have a strong methodological norm: to work with single cells or pure cell populations, emulating biochemical rigor. Assays that worked at the single cell level were the standard for in vitro functional assays, cell sorting, and in vivo radiation rescue. This 'single-cell' standard was consistent with wider community norms in immunology. Working to this standard, members of the Weissman lab collaborated with one another, and with other immunology groups, to work out developmental pathways for immune cells. In the mid-1980s, three of these lineage projects converged on a single cell population. A striking experimental result, produced by post-docs sharing materials from their different projects, knit these previously distinct lines of inquiry into a single coordinated search for the blood stem cell. Further collaborations within the laboratory, with other Stanford groups, and with a West German immunology lab, yielded a new experimental arrangement in 1988. Though the controversial report had only three authors, its result emerged from decades of experimental work by dozens of researchers at Stanford and beyond.

The outcome of the HSC controversy can now be explained. Both groups used similar methods to isolate cells they identified as HSC. But the two methods involved different forms of participation in a wider epistemic community. The Stanford group was a center of continuous,

cumulative collaboration aimed at understanding interrelated pathways of immune cell development at the single-cell level. The 1988 method was a specific refinement of a general method deployed throughout this collaborative network. The Rijswijk group, in contrast, interacted with the widely-distributed hematology community only to gather sorting criteria for bone marrow cells into 'subsets;' their 'downstream' method and standards were local.

The community perspective brings out a further contrast: the two methods implicitly describe different *models* of HSC. The model in the Rijswijk method (so to speak) was a cytophysical profile of colony-forming cells, correlating a complex of properties (size, density, morphology, surface phenotype, drug-sensitivity) with HSC capacities. But these criteria were based on general properties of cells and available biochemical tags; a concatenation of fortuitous discoveries gleaned from the wider community. The cell population profiled in this way was linked to HSC only via functional assays increasingly viewed as ambiguous in the field. In contrast, the model in the Stanford method is of blood cell development. The Weissman group's markers tracked what was then known about developmental pathways in different blood cell lineages, and related these to the HSC surface phenotype. By focusing on single cells in this coordinated system of bioassays, they characterized HSC as the unique stem of a unified model of blood cell development.

The Weissman group's model conformed to and elaborated the three-level hierarchy widely-accepted in the hematology community at the time. This allowed the two communities to merge, establishing single-cell assays and reconstitution of animal parts (i.e., the immune system) as the gold standard for HSC research. Experiments conforming to this standard further elaborated the cell lineage hierarchy stemming from HSC. This was a collective enterprise, undertaken not by a single research team or lab but by an inclusive experimenting community.

The new, expanded HSC community had ties to both predecessors. Visser and Weissman helped constitute it, traveling worldwide to argue for their groups' respective methods before audiences of blood and immune cell experts. Groups investigating HSC with new tools of molecular biology critiqued both methods. All this led to the eventual consensus that HSC had not yet been isolated. Various groups (including those at Rijswijk and Stanford) began working to characterize more finely-grained cell populations from mammalian bone marrow.

Though both groups contributed to the new consensus, it was the Weissman group's method, and the hierarchical model of cell development implicit within it, which provided the standard for HSC research. And this model and method were quickly extrapolated to other experimental studies of stem cells: from mouse to human HSC, from blood to brain, gut, skin, muscle, liver, pancreas, the enteric nervous system, from normal to cancerous development (leukemia, colon cancer, breast cancer, prostate cancer). HSC served as an exemplar and a basis for comparison – a model system. The basic method was adapted, with numerous alterations, to each new experimental context. Results from other systems in turn 'fed back' to further refine the HSC model, further elaborating the simple three-level hierarchy to an intricately-tiered structure of lineages. This process was well underway when human embryonic stem cells were created in 1998. The epistemic community of adult stem cell research grew as a reticulated network of experimental models and methods, linked by complex relations of similarity and difference to one another and to embryonic stem cells. But in all this variety and revision, there is a robust standard for experimental success: trace processes of development at the single-cell level, in the form of cell lineage hierarchies.

5. Evaluating experiments

Experiments in stem cell biology can be critiqued in terms of this robust standard. Moreover, the field's therapeutic aim adds another evaluative dimension. The clinical aim of stem cell biology is to cure injuries and pathological conditions by introducing cells or cell products into human bodies. The robust experimental standard discussed above suits this aim. Experiments that meet it contribute to hierarchical models of cell development that predict the pathways taken by single cells. But it is not enough that experimental methods and results mesh with our aspirations and hopes. The therapeutic aim of stem cell research gives particular significance to predictions about what cells will do when let loose in human bodies. It is these hypotheses which most need critical evaluation. Before using experimentally-based models of cell development to predict the effects of cells in vivo, it is crucial to distinguish between features of these models that reflect our interventions or aspirations, and those that reflect 'cell intrinsic' pathways or stable features of physiological environments. Close attention to experimental methods is required to make this distinction. So critical evaluation of experiments in stem cell biology must attend to the details of experimental practice. But the case study above narrows the focus of this attention considerably. Experiments can be evaluated for fit with the robust single-cell standard, and as means to the therapeutic end of cell therapy. Though stem cell biology remains in flux, with new standards grounded in systems biology now becoming established, the HSC case shows that criticism of experiments in stem cell biology is possible. Its results provide a starting point from which to explicate additional standards and norms, as the field continues to transform itself.

6. Summary and conclusion

Several features of experiment problematize epistemic evaluation: diverse components, unclear boundaries, and transformative potential. This paper has shown how to gain critical purchase on

an experimental field which exhibits these features to a high degree: stem cell biology. A historical approach emphasizing the details of experimental methods, organization of epistemic communities, and relations between the two, reveals a robust standard for experimental success in stem cell biology: delineate hierarchies of development at the single cell level. This critical historical approach, including future hopes and clinical values, reveals stable standards for evaluating experiments in stem cell biology which are grounded in scientific practice rather than philosophical assumptions. Since stem cell biology is a hard case (§2), this critical approach should apply to other experiment-driven sciences as well, though the specific standards explicated here likely will not.

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