Model systems in stem cell biology

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Summary

Stem cell scientists and ethicists have focused intently on questions relevant to the developmental stage and developmental capacities of stem cells. Comparably less attention has been paid to an equally important set of questions about the nature of stem cells, their common characteristics, their non-negligible differences and their possible developmental species specificity. Answers to these questions are essential to the project of justly inferring anything about human stem cell biology from studies in non-human model systems—and so to the possibility of eventually developing human therapies based on stem cell biology. After introducing and discussing these questions, I conclude with a brief discussion of the creation of novel model systems in stem cell biology: human-to-animal embryonic chimeras. Such novel model systems may help to overcome obstacles to extrapolation, but they are also scientifically and ethically contentious. ***BioEssays*** **26:1005–1012, 2004. © 2004 Wiley Periodicals, Inc.**

Introduction

Public debate over stem cell research has focused largely on scientific and ethical questions about the sources of stem cells—embryonic, fetal and 'adult' (or tissue-specific). Scientifically, there are concerns regarding differences in potency;[1,2] ethically, there are worries about the use of embryos (more specifically, human embryos) in research, in that isolating cells from the inner cell mass destroys the embryo.[2–4] This debate has been fueled by the publication of conflicting results regarding the potency of putatively tissue-specific stem cells,[5–7] and the passing of legislation limiting human embryonic stem cell research (e.g., Refs. 8 and 9; see also Ref. 10). But despite the salience of this debate, stem cell biologists have also been involved in less-well-publicized disputes over more fundamental questions about the nature of stem cells and, particularly, about the use of model systems (including chimeras) in stem cell research.

What makes a cell a stem cell, or, to put the question another way, what is the character of 'stemness'?[11] That is, what makes embryonic stem cells, neural stem cells, pancreatic stem cells and hematopoietic stem cells all 'stem cells'? What are their common characteristics? What are their fundamental, or at least non-negligible, differences? Answers to these 'definitional' questions may depend not only on the developmental stage at which, and the developmental system from which, stem cells are isolated (the sources of stem cells), but also on the organism from which they are isolated, and the techniques used for culturing the cells. If 'stemness' is species specific, we must worry about whether and if so how widely we will be able to apply to humans data generated from stem cell studies with model organisms.

These considerations are not particular to stem cell biology, of course. In developmental biology more generally, the use of model systems has generated some debate specifically related to extrapolating results, whether within or between species, from model system-based research to real world contexts.[12,13] As a bridge between model systems and real-world systems of interest (humans, typically), it is possible, and often desirable, to conduct research on non-model systems and, sometimes, to construct new models to facilitate inference making. In the particular context of stem cell research, some biologists are currently contemplating the creation of human-to-animal embryonic or fetal chimeras[14,15] or are actually creating them,[16,17] as novel model systems, providing a means potentially to bridge the inferential gap between humans and non-human model organisms—but this solution raises both scientific and ethical concerns that are themselves divisive.

What is a stem cell?

Stem cells are generally characterized as cells with the potential for self-renewal and the capacity to generate more specialized cells. Stem cells at different developmental stages appear to have different capacities for self-renewal and differentiation. The only *totipotent* cells are those removed at the pre-blastocyst stage of embryo development; these are unspecialized and so can form the embryo and placental trophoblast. Totipotent stem cells can generate any cell type; stem cell lines derived from human embryos have been shown to generate trophoblast in addition to tissue-specific cells,[18] suggesting the theoretical possibility of culturing totipotent human embryonic stem cell lines.

After the third cell division, cells begin to specialize, and an inner cell mass (ICM) forms within the blastocyst. Cells removed from the ICM are *pluripotent*, having less, but

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DOI 10.1002/bies.20100
Published online in Wiley InterScience (www.interscience.wiley.com).

nonetheless impressive, capacity for differentiation into various cell types (though pluripotent stem cells cannot form placenta, some believe they can form the embryo). Embryonic stem cells (ES cells) are pluripotent. Some tissue-specific stem cells that are already differentiated retain *multipotent* capacity to generate cells of a still more restricted class of cell lineages; moreover, some stem cell biologists contend that putatively tissue-specific stem cells may 'dedifferentiate' and 'transdifferentiate' into other cell types (reviewed in Ref. 19; see also Ref. 20). Cells that are *unipotent*, though sometimes referred to as stem cells, should not be so described even if they retain some capacity for self-renewal, as self-renewal is a necessary but not sufficient condition for being a stem cell.

We may derive a wide variety of tissue-specific stem cells, such as neural, pancreatic, epidermal, hematopoietic, muscle, cardiac, gastrointestinal and lung stem cells. Aside from the capacity for self-renewal and differentiation, what characteristics do these tissue-specific stem cells share in common? At least four are worth noting:

- stem cells are relatively uncommon, with frequencies varying from roughly 0.0001% to roughly 5% of the total cells in a tissue—accordingly, tissue-specific stem cells may be difficult to isolate;
- stem cells cycle relatively slowly, and often we see transit amplifying (TA) cells dividing more often than stem cells;
- stem cell activity is governed by the cells' microenvironment or 'niche', comprising cell-adhesion molecules, cell–cell signals and growth factors; and
- more controversially, stem cell populations are self-maintaining, in that each stem cell division, on average, generates one stem cell and one TA cell, or each two stem cell divisions, on average, generate two stem cells and two TA cells.[1,21]

Blau et al. have recently argued that the definition of 'stem cell' is essentially functional: "rather than referring to a discrete cellular entity, a stem cell most accurately refers to a biological function that can be induced in many distinct types of cells, even differentiated cells" (p. 829) Figure 1.[20]

Blau et al.'s account is sensitive to recent studies purporting to show that differentiated, tissue-specific cells, such as bone marrow cells, are able to replenish not only the blood, but also to contribute to a wide range of additional systems: brain, heart, muscle and liver, for instance (reviewed in Ref. 20). Tissue-specific, multipotent, stem cells, or their progeny, may, but do not always, differentiate into cells that arise from all germ layers. It remains true, though, that multipotent stem cells typically produce cells within a limited range of lineages, depending on their location—for instance, within the small intestinal and central nervous systems.[1] It is clear that different stem cells, classed by developmental stage, have different capacities for self-renewal and differentiation; nonetheless, all stem cells are functional in this regard.

That said, several recent studies have purported to identify the molecular 'signature' of stem cells and their niches, and so the genetic character of 'stemness'. For instance, Ramalho-Santos et al. at Douglas Melton's laboratory at Harvard have analysed the transcription profiles of mouse stem cells at both embryonic and 'adult' stages, concluding that 230 probe sets representing some 216 genes show enriched expression in mouse embryonic, neural and hematopoietic stem cells.[11] (These genes, individually, are not uniquely expressed in stem cells; what is unique is their combined enrichment in stem cells.) Nevertheless, stem cell types can be genetically distinguished in terms of transcriptional activity, as different types of stem cells show enriched expression of different sets of genes (p. 598).[11] The overlap between transcription profiles from different stem cell types is worth noting: neural stem

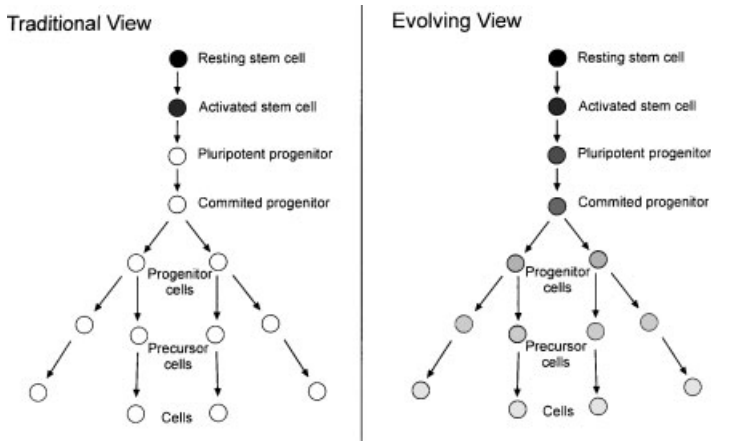

Figure 1. Two concepts of stem cells. On the left is the traditional view of stem cells, showing an irreversible loss of potency in maturing stem cells. On the right is an evolving view postulated by Blau et al. whereby stemness is a biological function that progressively degenerates over time but remains potentially recruitable within even differentiated cells in particular contexts. Redrawn with substantial modification from Fig. 7 in Blau HM, Brazelton TR, Weimann JM. 2001. The evolving concept of a stem cell: Entity or function? Cell 105:829–841, with permission of Elsevier.

cells in mice are more similar to murine embryonic stem cells than to murine hematopoietic stem cells, despite the fact that neural and hematopoietic stem cells are derived from adult mice (p. 599).[11]

In the same issue of *Science* as the Ramalho-Santos et al. study appeared, Ivanova et al. (in Ihor Lemischka's laboratory at Princeton) published on the 'molecular signature' of stem cells.[22] Using hematopoietic stem cells (HSCs) as a model, Ivanova et al. compared gene expression profiles for fetal and adult hematopoietic stem cells in the mouse; they then compared this shared HSC profile with human hematopoietic stem cells to determine genes shared between human and mouse HSCs. This group uncovered 283 genes showing enhanced expression. Despite Ivanova et al.'s interpretation of these shared genes as constituting a common stem cell 'genetic program' (p. 604),[22] it is noteworthy that there are only *six* genes shared between the sets identified by this group and by Ramalho-Santos et al.[23]

In a further study, Fortunel et al. (in Bing Lim's laboratory in Singapore) used embryonic stem cells, neural precursor cells and hematopoietic stem cells to uncover 385 expressed putative stemness genes.[23] But, comparing their results with those of the previous two studies, this third group were able to identify only *one* gene that appeared on all three lists of genes for 'stemness'! How should these results be interpreted? There are at least two options:

(1) On the one hand, there is no such thing as intrinsic stemness at the molecular level, such that perhaps stemness should be understood as a relational property between cells and their microenvironment generating the functionality of stem cells. According to this view, we might learn much from exploring the molecular 'signature' of stem cell niches, as in work in the Lemischka laboratory on the molecular profile of a candidate niche for HSCs.[24] From mouse fetal liver, Hackney et al. derived a stem cell supportive stromal cell line, AFT024, and analysed it via the techniques of functional genomics (bioinformatics and high-density array hybridization). The emergent molecular profile, they argue, points toward understanding the exogenous, microenvironmental factors determining differentiation of hematopoietic stem cells and, possibly, of all stem cells (p. 13066).[24]

(2) On the other hand, perhaps molecular stemness has been elusive to date because of confounding variables in attempts to discern it. Ramalho-Santos et al.'s criteria for 'stemness' hold, at present, only for stem cells derived from the mouse; though Ivanova et al. compare mouse with human stem cells in their attempt to discern the 'molecular signature' of stem cells and microenvironments, their focus is exclusively on hematopoietic stem cells. Meanwhile, the Melton and Lemischka laboratorie responded to the Fortunel et al. results by questioning whether those studies are "grounded in adequate definitions of SCs and, therefore, whether the data represent an analysis of gene expression in bona fide SCs" (p. 393d).[25] Interestingly, that is precisely what is in question in the search for stemness—namely, what makes a stem cell a stem cell. But notice that Fortunel et al. include neural precursor cells, which some stem cell biologists might take as a symptom of promiscuous definition of stem cells.[26] Notice as well that the cells pooled together in each of the three studies have been sourced at different stages, from different species, and, in the case of tissue-specific cells, from different tissues. These differences are precisely the sorts of variables in the face of which a universal conception of stemness would be robust; but uncovering such a conception of stemness, at least at the molecular level, appears at present to be a pipette dream.

Despite the existence of common characteristics of stem cells, the 'elusiveness' of stemness[27] suggests that we should also pay attention to some non-negligible differences between different types of stem cells and their respective biology. To date, the pluripotency of tissue-specific stem cells has not been confirmed in any species. Bone marrow hematopoietic stem cells have been the focus of much of the 'adult' stem cell potency debate,[5,7] though neural and skin cells are also of interest.[26,28] But in order that hypotheses about the potential pluripotency of tissue-specific stem cells be neither prematurely accepted nor prematurely rejected, comparative studies must be (and are being) undertaken on all relevant types of stem cells. We do not want to conclude that 'adult' stem cells as such are or are not pluripotent until we have shown this for every tissue-specific stem cell system.

Beyond the question of potency, there are some additional differences between tissue-specific stem cells. (1) Attempts to define the genetic profile of stem cells, such as those explored above, yield not only similarities but also differences between different types of stem cells at the molecular level. (2) Different methods for culturing stem cells in vitro can have different effects on their potency. (3) Blood and epidermal cells, for instance, are short lived and do not divide, and so stem cells active in blood and epidermis may function differently from those active in the brain or gastrointestinal tract. (4) While certain stem cell types may not migrate at all, hematopoietic stem cells pass through the blood stream on a daily basis.[29] (5) It is not yet clear whether all tissues and organs in fact develop from stem cells; though this has been shown in the hematopoietic system, we cannot simply assume that it will hold for all systems.

Further differences concern the specific niches of stem cells in vivo. Though stem cell microenvironments are not completely different between tissues,[21] different factors will regulate aspects of the behaviour (such as the rate and

symmetry or asymmetry of cell division) of different stem cells. At the molecular level, one might wish to compare differences (and interesting similarities) between Hackney et al.'s results regarding mouse fetal liver stem cell niches[24] and those of Lin working with the *Drosophila* gonad.[30]

Recognition of these differences should give us pause. Consider that hematopoietic stem cells have been employed as a "prototype model" for defining general biological characters of stem cells in mammals (p. 601)[22]—see also Refs. 24 and 31. But biological differences between stem cells (and their microenvironments), despite any molecular similarities (and noting molecular differences, as well), give reason to doubt that there is such a thing as a 'model' stem cell, in the prototypical or archetypal sense. Given the persistence of a range of confounding variables, the fact that no stem cell is a model for stem cells as such is to be expected. Perhaps, as with the gene concept,[32] the stem cell concept can bear a certain fuzziness without any significant side-effects. If we want to say, definitively, what counts as a stem cell and what does not, then precision is required. But stem cell research may well proceed unfettered even in the absence of a consensus stem cell concept, though to avoid confounding we must be careful to specify the developmental stage, system and source of the stem cells in question.

Stem cells in model systems

Understanding what stem cells can and cannot do is essential to the project of developing stem cell therapies in regenerative medicine. The task of understanding how to manipulate stem cells is undertaken with model systems, such as mouse, dog and monkey. It is widely recognized both that model organisms are essential to developmental research, but also that, because model organisms are highly derived research tools, research based on them may not always be appropriate sources of scientific inference; that is, model organisms may not always serve as appropriate proxies. There is thus a vigorous debate about the use of model systems in developmental biology generally, a debate that holds key lessons for stem cell biology in particular.

The standard justifications for experimental study with model systems have been well reviewed by others, especially Jessica Bolker and Scott Gilbert.[12,13,33] For present purposes, most important among these is that model systems are experimentally tractable: they yield to analysis. This may be because they are, developmentally, simple compared with other organisms.[34] Or it may be because they are *made to be analysed*.[35] Experimental research has a material culture, shaped "by the practical imperatives of choosing organisms, constructing tools, and making experiments work" (p. 3).[36]

In his 1994 book, *Lords of the Fly*,[36] Robert Kohler documents the construction of *Drosophila* as a standard laboratory creature, a model organism for transmission genetics. While eventually a "cornucopia of productive methods, concepts and problems" (p. 2)[36] for the Morgan School and others, *Drosophila* had to be *made into a model organism*. As Kohler describes in detail, "experimental creatures are a special kind of technology in that they are altered environmentally or physically to do things that humans value but that they might not have done in nature" (p. 6).[36] Manipulation through multiple generations of selection and inbreeding results in organisms dramatically distinct from their forebears, both genetically and behaviourally. Kohler thus likens them to physical instruments in the laboratory, like spectrophotometers or centrifuges—"constructed artefacts of laboratory life" (p. 88).[36] Model organisms are both selected and selectively fashioned in order to make experiments work, and use of the same experimental material facilitates replication (or disconfirmation) of results. (In current usage, a model system comprises both a model organism and the network of relationships in which it figures, both within the laboratory and between research centres.[37])

There is no doubt that experimental research with model systems is an essential component of developmental biology. But the use of model systems is not exclusively advantageous. There are, in fact, distinct liabilities associated with research based on model systems.[12,13,33,35] Model systems tend to have been "selected for their suitability to the genetic paradigm of developmental biology" and so may be insensitive to the sorts of micro- and macro-environmental wrinkles, whether exogenous or endogenous, that may contribute to or disrupt the developmental process (p. 3).[13] Accordingly, any scientific inference on the basis of model systems research must be drawn cautiously. This caveat applies widely: generalizations beyond the laboratory, and attempts to derive broad lessons about organismal development, must be independently justified. (Schaffner's philosophical study of *C. elegans* as a model system in behaviour genetics research is illustrative; see Refs. 34 and 35 for details.)

Despite sharing striking DNA sequence similarity, as well as some generic developmental processes, different creatures develop in different ways that are often environmentally context-dependent. Consequently, sometimes there simply may be no substitute for in vitro and in vivo studies in relevant non-model systems. Where the relevant non-model organism is a fish or a flower, a mouse or perhaps even a monkey, comparative data might be easily generated to either support or challenge the generalization in question. But where the relevant non-model organism is a human, for instance, the situation becomes considerably more complex, for specific ethical proscriptions attach to research on humans that do not attach to (otherwise ethically regulated) research on non-human creatures.

Given the discussion of the stem cell concept above, aspects of this general debate are clearly relevant in the particular context of stem cell research. Specifically, I have five related cautions in mind.

1. Where our models of stem cells are sourced at a particular developmental stage (embryonic, fetal, or 'adult'), we must be cautious in making generalizations to other developmental stages.
2. Where our stem cells are derived from a particular developmental system (e.g., blood, pancreas, brain, skin), we must be cautious in making generalizations to other developmental systems.
3. The existence of differences in stem cell behaviour and morphology between, say, mouse and human, indicates that we must be cautious in making generalizations across species boundaries.
4. Highly derived cell lines may be importantly different from their 'wild-type' counterparts, such that we must be cautious in making generalizations even within species.
5. It may well matter whether our studies have been conducted in vitro or in vivo, and what culture media have been used in generating and maintaining the cell lines in question.

There are, consequently, at least five variables at play here, as indicated in Table 1. Where only one of the variables matters, the situation is straightforward enough; but where two, three, or more variables matter, a degree of complexity intervenes. In these more complicated cases, a generalization can go wrong in enormous number of ways, as each variable has numerous instantiations: 'source' has three; 'system' has ten or more; and 'organism' and 'disease' comprise many more possibilities. This claim will not come as a surprise to stem cell biologists; in fact, the large number of combinatorial possibilities involved may well underwrite the commitment to determining 'stemness' *regardless of source*, system, organism, setting and culture. But as indicated in the previous section, that project has not yielded the expected results.

Both source and system were addressed in the previous section; while the question of the relevance of the organism was briefly mentioned, much more remains to be said. For instance, various reports suggest that murine and human stem cells differ in important ways. Moreover, it may well be the case that variations within the development of mice and of humans—such as differences in cell morphology and growth patterns in embryonic carcinoma cells—work against the scientific aim of generating generalisable results from mouse stem cell research. As it happens, embryonic stem cells cannot typically be derived from creatures other than mice, non-human primates and humans, limiting the possibility of generating comparative data from other species. But comparative data are crucial. Consider that leukemia-inhibiting factor encourages the self-renewal of murine embryonic stem cells in culture; but neither leukemia inhibiting factor nor related cytokines plays any such role in the self-renewal of human ES cells.[21,31,38]

Table 1. Possible confounding variables in stem cell research

Variable	Examples
Source	Embryonic, fetal, 'adult'
System	Embryo, heart, liver, brain
Organism	Mouse, dog, chimp, human
Setting	Genetic and epigenetic background
Culture	Substrate, media, growth factors, in vitro or in vivo

There are further interesting and non-negligible behavioural and morphological differences between the development of murine and human ES cells. Human stem cells, for instance, grow at a slower rate than murine stem cells and, while murine stem cells form spherical colonies, human stem cells form flat ones.[39] Whether these differences are inherent to the cells, or are rather an artefact of cell culture (undifferentiated human ES cells are maintained on a bed of mitotically inactivated mouse embryonic trophoblasts), and whether they may confound scientific inference, deserves further study. At any rate, morphological or behavioural differences between stem cells derived from diverse organisms (in addition to morphological and behavioural differences between organisms of different species as such) factor as potential obstacles to successful generalization across species boundaries.

To date, researchers have been unable to create mouse stem cell lines that function in all mice;[40] clearly, the situation is worse still when one crosses over to humans. Zwaka and Thomson[41] report using homologous recombination to alter human embryonic stem cells (intraspecifically) to facilitate the achievement of particular research and therapeutic aims. Of particular interest is the prospect of employing transgenic human ES cells in lieu of their mouse counterpart where the mouse 'homologue' differs from the human cell in "clinically significant ways" (p. 2),[41] such that inference difficulties may be avoided.

Regarding the genetic and epigenetic setting as a potential confounder, the National Academies report on *Stem Cells and the Future of Regenerative Medicine* makes the now-familiar point that any cell line in culture is subject to random mutation, and embryonic or other stem cell lines are no exception (p. 48).[42] Accordingly, in addition to the consideration that model systems are themselves highly derived and so possibly importantly unlike their conspecifics, the same may be true of stem cell lines in culture, although, in the latter case, this will be the result of random mutation and epigenetic effects rather than selection and inbreeding. (Note that concern for the fidelity of derived stem cell lines is heightened in a context in which various jurisdictions have invoked regulations against the derivation of new human embryonic stem cell lines. Without sufficient numbers of human ES cell lines, our capacities for justifying generalizations on the basis of non-human ES cell

lines may be hampered. And, as a function of the culture of embryonic stem cell lines as such, any such generalization will require comparison, testing and justification.)

Finally, in relation to culturing milieu, it goes almost without saying that how stem cell lines are cultured, including the substrate on which they are grown and the growth factors that are employed, make a significant difference to their potency and, accordingly, to their suitability for research and eventual therapeutic purposes. Moreover, there may be important differences between in vitro and in vivo contexts. Both in vitro and in vivo contexts are artificial, though designed for maximum isomorphism with natural contexts. Exploring a phenomenon in the laboratory may not always capture all relevant environmental variables—and may miss even some straightforward ones.[13] Laboratory research as such might not always justify extrapolation beyond the laboratory, and we must be careful to model all relevant variables. The latter task is more easily accomplished in vivo than in vitro, in that many of the developmental variables associated with an organism's ontogeny are already accounted for in the living system. Given the cautions outlined above, the particular living system utilized in vivo may well matter, for if development is different between mouse and humans (which it most certainly is) then whether we study mouse or human will make a difference to our results.

An unsigned editorial in *Nature* makes the case for large-scale, comprehensive studies undertaken under "highly standardized and reproducible conditions" as the only route to generating meaningful conclusions regarding "appropriate practices, protocols and high-quality cell sources for clinical trials" eventually involving humans (p. 1).[43] The author(s) suggest(s) that National Institutes of Health funding of infrastructure for such research is inadequate, estimating that a full decade will be required in order to make good on the promises of stem cell research for human health. The suggestion is that, in order to speed things up, stem cell research requires the sort of leadership and teamwork characteristic of human genomics in the past two decades: "Perhaps it's time to start thinking about a Human Stem Cell Project" (p. 1).[43] Of course, should we choose to take such a route, we must follow the comparative approach of human genomics, as well: we'll need a mouse stem cell project, a chimp stem cell project, a macaque stem cell project and others besides.

Chimeras as novel model systems

To avoid problems of scientific inference from non-human animal models to humans, and so to bridge the inferential gap between them, some stem cell biologists are advocating the creation of human-to-animal embryonic chimeras. This objective has both basic scientific and therapeutic dimensions: in terms of potential scientific value, embryonic chimeras might be useful in understanding general principles of development, characterizing general features of stem cells, and reducing inferential difficulties from non-human models to humans; in terms of potential therapeutic value, embryonic chimeras might facilitate the study of how to make stem cells function as we need them to for therapeutic purposes, and help us to predict the outcome of stem-cell based therapies eventually to be applied in humans.

In some ways, chimera research is an extension of current research in transgenesis to generate 'humanized' animal models.[44,45] But it may also be understood as part of a continuum of techniques within developmental biology established over the past 150 years. Relevant experiments include nineteenth- and twentieth-century tissue transplants designed to discover the determinants of developmental specificity (including the work of Born on heteroplastic transplantation, especially as adopted by Harrison and Spemann),[46] Brian Hall's chorioallantoic membrane (CAM) grafting technique derived from a technique of Murray and Huxley[47] and used for understanding embryonic development,[48] and Nicole LeDouarin's work since 1969 with chick–quail chimeras in studying neural crest cell migration and other neurodevelopmental phenomena (see, e.g., Refs. 49 and 50).

Protocols for chimeric research are well established for embryonic, fetal and adult systems. Hundreds of chimeric experiments have been undertaken. To cite just two examples involving human stem cells, Ourednik et al. (in Evan Snyder's laboratory at Harvard) have transplanted human neural stem cells into the forebrain of a developing bonnet monkey in order to assess (human) stem cell behaviour in (monkey) development,[16] while Goldstein et al. (in Nissim Benevisty's laboratory at the Hebrew University, Jerusalem) have inserted human embryonic stem cells into very young (1.5–2 days) chick embryos to assess (human) stem cell differentiation in (chick) development.[17] Similar studies are currently in planning or execution stages.[15,51]

Presently, the scientific community is divided over the likely value of chimera research.[14,15] Some biologists argue that to genuinely establish the potency of (human) embryonic stem cells in vivo, we should assay the behaviour of grafted stem cells in fertile embryonic environments; since we cannot, for ethical reasons, perform such research in human embryos, non-human embryos are an appropriate proxy.[15] Through chimera research, we should be able to assess the generic properties of stem cells, and learn to manipulate them in vivo for eventual therapeutic ends. Accordingly, we may be able to sidestep the cautions identified in the previous section.

In response, some biologists contend that such research at this stage will likely prove uninformative, not least because of differences in cell cycling and lifespan[14] and the apparently species-specific nature of stem cells and their niches.[52] Moreover, there are safety concerns regarding zoonotic infection—not specific to chimera research[47,53]—and germline infiltration[2] which speak against the desirability of certain kinds of stem cell research. (The concern about germline infiltration could be neutralized by creating embryonic

chimeras only past the period of gonadal development.) Finally, it is argued that the relevant knowledge can be gained through non-chimeric research with model systems, or with interspecies adult chimeras (where cell transplants are likelier to be localized and controled); accordingly, there is no scientific rationale for proceeding with the creation of embryonic chimeras.

Despite a long history of proving valuable in developmental biology more generally, it is too soon to tell whether the creation of embryonic chimeras in stem-cell biology will indeed bridge the inferential gap between humans and non-human animals. It is at least possible that such research will instead teach us about the biology of chimeras, and not about the biology of either donor or host. In such a case, chimeras would themselves embody the systemic limitations of all model organisms, and we would then need to bridge inferential gaps between chimeras and their progenitors—we might dub this state of affairs 'Xenopus's paradox'.[1]

As with transgenic research, chimera research is morally controversial. Françoise Baylis and I published a Target Article on crossing species boundaries in stem cell biology in *The American Journal of Bioethics*,[54] and as the editor noted in his introductory essay, no other *AJOB* article to date had ''occasioned as much interest'' as our piece.[55] Perusing the responses of our two dozen commentators—who disagree as much with each other as with our arguments—suggests that there is much more than science at stake in the debates over chimera research. Whether an unfavourable moral response to embryonic chimeras is justified remains to be demonstrated.[54] At the very least, it is clear that providing a scientific rationale for building chimeras will not alone be sufficient for permitting the practice. But a strong scientific rationale for embryonic chimera research, based on relevant preliminary work with adult hosts and comparative research with a variety of model systems, should certainly factor into rational ethical and policy deliberation.[55]

Conclusion

Public and scientific debate about stem cell research and the derivation of stem cell lines should not be limited to the developmental-stage specific source of the cells (embryo, fetus or adult). We must also attend to possible differences *between* the organisms that we study, possible differences between the systems *within* those organisms on which we focus, and possible differences between stem cells *beyond* the issue of their source. Standardization of criteria and techniques may help to clarify what counts as a *bona fide* stem cell and stem cell line. But the almost irresistible project of defining 'stemness' must not be allowed to blind us to the differences between different sorts of stem cells. That is, even if we do arrive at a robust definition of stem cells, it will still be important to continue to specify the developmental stage, system and organismal source of the cells in question. To this end, comparative stem cell research with model systems is both unavoidable and critically important. It is also subject to the same concerns as have arisen regarding the use of model systems in developmental biology generally. While some stem biologists are advocating the use of chimeras both to avoid ethical concerns about using human embryos in research and to facilitate extrapolation to humans across species boundaries, such research remains both scientifically and ethically controversial. In resolving this controversy, we must carefully consider the full range of developmental variables, lest our hope for stem cell therapies amount merely to so much hype.

[1]Credit for this delightful pun is due to Eric Juengst (personal communication, 12 April 2003). The reference is to Xeno's paradoxes of motion whereby, if the Tortoise is given a head start, fleet Achilles will never pass the slower Tortoise: by the time Achilles reaches the Tortoise's starting point (say, 100m), the Tortoise will have proceeded further (say, 10m), and by the time Achilles reaches that point, the Tortoise will have proceeded further still (say, 1m), and so on *ad infinitum*—while the distance between them grows smaller, it never disappears. The same may be true of the inferential gap across species boundaries.

Acknowledgments

I am grateful to members of the Model Systems Strategic Research Network and the Novel Genetic Technologies Research Team for discussion of prior drafts of this paper. Additionally, the editor and two referees for this journal provided immensely helpful comments, as did my audiences at the Stem Cell Network annual general meeting, Vancouver, British Columbia, 20 September 2003, and at the Southwest Colloquium on History and Philosophy of Life Sciences, University of Texas at Austin, 5 March 2004. As ever, I thank Gillian Gass for her keen criticism; I also thank Mary Sunderland for valuable research assistance. My research is currently funded through a New Investigator Award and operating grant from the Canadian Institutes of Health Research, and an operating grant from the Stem Cell Network, a member of the Networks of Centres of Excellence (NCE) programme.

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